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# Captive husbandry, reproduction, and fecundity of the golden mantella (*Mantella aurantiaca*) at the Mitsinjo breeding facility in Madagascar

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**Abstract.** We provide an account of maintaining a captive population of the Critically Endangered mantellid frog *Mantella aurantiaca* at a breeding facility near Andasibe, Madagascar, reporting novel observations on behaviour, fecundity, reproduction, temperature tolerance, age at maturity, and survivorship. In April of 2012, 25 breeding groups were established from founder stock collected at three natural breeding sites located on the footprint of the Ambatovy nickel and cobalt mine. Over a two-year period, 469 breeding events were recorded. Breeding activity was highly seasonal and aligned with average monthly temperatures, with peak breeding activity observed during the austral summer months of December and January. An average of 7 egg clutches per female was recorded over the two years, with the mean clutch size being 74 eggs (193 max/24 min). Tadpoles completed metamorphosis between 53 and 139 days, with 441 individuals from 22 clutches of eggs surviving to one year of age. Males were recorded vocalizing 4 months after completing metamorphosis, and the first fertile eggs were produced at 11 months. Reproduction in the $F_1$ generation was captured on video and we provide a detailed description of this behaviour, including an observation of males 'pulsating' femoral glands on the dorsum of a female during reproduction. Based on these data and observations, we discuss the importance of record keeping for captive amphibians, potential conservation implications of creating new breeding sites for reintroducing *M. aurantiaca*, as well as the advantages of running captive breeding programmes within the native range of a species.

Key words. Amphibia, Anura, captive breeding, ex situ conservation, reproductive behaviour, Andasibe, Mantellidae, mining, biodiversity offset, species reintroduction.

## Introduction

In recent years, amphibians have been highlighted as a particularly suitable group of animals for captive breeding programmes and reintroductions (Griffiths & Pavajeau 2008, Browne et al. 2011). Establishing captive survival assurance colonies of highly threatened species as a short-term solution to prevent their extinction has been advocated by numerous zoological institutions and conservation organisations, especially in response to the threat of infectious diseases (McGregor & Zippel 2008, Pavajeau et al. 2008, Zippel et al. 2011). To this end, we developed a captive breeding facility near the village of Andasibe, Madagascar, specifically for the region's local hyper-diverse amphibian fauna. The project was launched in early 2011 as part of the country's national amphibian conservation strategy known as the Sahonagasy Action Plan (Andreone et al. 2012, Edmonds et al. 2012).

So far, no modern amphibian species extinctions have been detected in Madagascar (Andreone et al. 2008), although more thorough surveys are needed, especially given the recent news that the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) is widespread throughout the country (Bletz et al. 2015). DNA barcoding has revealed potentially more than 500 endemic amphibian species, many of which have yet to be described (Perl et al. 2014). Still, they continue to be under tremendous threat from anthropogenic activities, most notably habitat loss.

Deforestation is the most pressing threat (Andreone et al. 2005). Only 10–15% of the original forest cover is left on

the island and deforestation continues at an alarming rate (Harper et al. 2007). Addressing habitat loss by protecting key areas for priority species is clearly the best approach to take. However, in certain situations, the rapid and imminent loss of amphibian habitat requires ex situ conservation action. Familiar examples elsewhere in the world include captive breeding programmes for *Chirixalus romeri* (Banks et al. 2008), *Hyperolius pickersgilli* (Visser 2012, Tarrant 2013), and *Nectophrynoides asperginis* (Lee et al. 2006).

*Mantella aurantiaca* is one of nine frog species in Madagascar assessed as Critically Endangered by the IUCN Red List, with habitat degradation being considered the main threat to the species' survival (Vences & Raxworthy 2004). It is well known due to its aposematic orange-red colouration and presence in the international pet trade, despite its having an extremely restricted distribution. Only 26 localities are reported for the species, all of which are confined to a small area of heavily fragmented humid forest around seasonally flooded ponds to the northeast and southwest of the city of Moramanga, east-central Madagascar (Randrianavelona et al. 2010). In recent years, additional localities have been discovered, but mainly within the same tiny geographic area as those already previously known (Piludu et al. 2015).

Following a conservation needs assessment, the Amphibian Ark prioritised *M. aurantiaca* as a species in need of ex situ assistance to assure its survival (Johnson 2008). Although there are captive populations at zoological institutions outside of Madagascar, these consist of individuals sourced via the pet trade from unknown localities, and most are maintained informally without proper biosecurity and population management practices in place. To reduce the risk of introducing foreign pathogens to wild populations and to involve local people and government in the conservation of their native species, it is recommended that captive assurance colonies be established within the native range of the species (Gagliardo et al. 2008). For this reason, a captive colony of *M. aurantiaca* was established at our breeding facility in Andasibe, Madagascar, to help mitigate the environmental impact of the nearby Ambatovy nickel and cobalt mine. In this paper, we detail our work towards maintaining a captive assurance colony of *M. aurantiaca* and expand the existing knowledge of its life history through observations made in captivity.

## Materials and methods

### Collection and acclimation of founder stock

In February of 2012, 99 male and 55 female *M. aurantiaca* were collected from two breeding ponds (known as MP7 and MP8) located ~50 metres apart on the footprint of the Ambatovy mine (Tab. 1). An additional three males and five females were collected in April from a third site (MP5) ~160 metres southeast from the other two. Collection took place during the day and was conducted by technicians of the NGO Madagasikara Voakajy and the Ambatovy Project. Frogs were confined in either transparent bags or ventilated plastic cups overnight and then transported by Mitsinjo 15 km southeast to the captive breeding facility on the following day. The facility itself had been set up a year earlier, specifically for the purpose of maintaining captive amphibian populations, was constructed from brick, cement, and a corrugated zinc roof, and measures 185 $m^2$ (Fig. 1).

Upon arrival, each individual frog was weighed, photographed and assigned a unique identification number. For the first 60 days, we housed the frogs individually or in male–female pairs in an isolated quarantine room. At 20 day intervals, each individual had their physical condition examined and were weighed using an Ohaus TAJ402 digital scale. We used an "All in/All out" entry and exit protocol as described by Pessier & Mendelson (2010). Following the quarantine period, the frogs were assigned to 25 different breeding groups and transferred to terraria. These were set up in a room that also held terraria accommodating a variety of local species from the Andasibe area, but otherwise isolated from these on different shelves with a separate drainage system. The technicians servicing the *Mantella* colony were different from those servicing the other species to further reduce biosecurity risks.

Most breeding groups were intentionally composed as male-biased to help promote breeding activity (Tab. 2). The minimum group size was three males and two females and the maximum six males and three females. The PopFrog Population Management Tool (www.popfrog.org) and Amphibian Ark Population Management Guidelines (Schad 2007) were used to determine how many breeding groups needed to be established to maintain the genetic viability of the captive population for the duration of the breeding programme, supposing that each collection site represented a separate conservation unit given that the genetic diversity of the frogs at each site had not been analysed and it was not clear whether or not migration was taking place between sites at the time of collection.

Figure 1. Overview of the Mitsinjo captive breeding facility near Andasibe, Madagascar.

Table 1. Site name, location, date and number of individuals of *Mantella aurantiaca* collected per locality.

| Site | Date of Collection | # Males | # Females | Latitude | Longitude |
|---|---|---|---|---|---|
| MP7 | 8 February 2012 | 46 | 29 | 18°49'28.1" S | 48°20'09.5" E |
| MP8 | 8 February 2012 | 53 | 26 | 18°49'27.7" S | 48°20'11.2" E |
| MP5 | 11 April 2012 | 3 | 5 | 18°49'32.5" S | 48°20'00.7" E |

Table 2. Sex ratios of the 25 breeding groups of *Mantella aurantiaca* formed from wild-caught founder specimens.

| Group | # Males | # Females |
|---|---|---|
| MP5-A | 3 | 5 |
| MP7-A | 6 | 3 |
| MP7-B | 4 | 2 |
| MP7-C | 2 | 4 |
| MP7-D | 3 | 3 |
| MP7-E | 3 | 3 |
| MP7-F | 4 | 2 |
| MP7-G | 4 | 2 |
| MP7-H | 4 | 2 |
| MP7-I | 4 | 2 |
| MP7-J | 4 | 2 |
| MP7-K | 4 | 2 |
| MP7-L | 4 | 2 |
| MP8-A | 5 | 3 |
| MP8-B | 4 | 2 |
| MP8-C | 4 | 2 |
| MP8-D | 5 | 2 |
| MP8-E | 4 | 2 |
| MP8-F | 6 | 2 |
| MP8-G | 4 | 2 |
| MP8-H | 6 | 3 |
| MP8-I | 3 | 2 |
| MP8-J | 4 | 2 |
| MP8-K | 4 | 2 |
| MP8-L | 4 | 2 |

## Captive environment

Housing during quarantine made use of glass terraria with screen covers that measured 45 × 25 × 25 cm, or plastic boxes of similar size (Fig. 2). We provided a substrate of locally-sourced sphagnum moss and leaf litter, which was cleaned and partially replaced every 20 days. Permanent terraria to house breeding groups measured 60 × 40 × 45 cm and were constructed from glass with ventilation on top (Fig. 3). Their bottoms were furnished with a plastic drain to allow excess wastewater to drain into a network of PVC plastic pipes below.

Initially we used a substrate of synthetic foam rubber into which holes were cut and potted plants were inserted, as well as a small ceramic water bowl about 2 cm deep. Three terraria were alternatively set up with a substrate of large pea gravel (ca 1–2 cm in diameter). In March of 2013, the substrate of all terraria was changed to gravel, as we found it easier to maintain than foam. To provide cover and visual barriers for the frogs, we scattered leaf litter collected from the surrounding forest over the substrate. The leaf litter covered at least 50% of the surface in most terraria. Additionally, a coconut hut was provided in each terrarium for shelter. All organic materials used within the terraria were thoroughly rinsed with water and dried in the sun for several days or weeks before entering the facility.

The terraria were serviced daily, and during this time we counted all individual frogs, assessed their health and condition, and searched for eggs. We sprayed the terraria with water to prevent waste matter from accumulating and maintain an appropriately moist environment. Once every 25 days, we removed all animals and items from their respective terraria and rinsed the substrate and glass with water, using a razor blade to remove mineral deposits, bacterial build-up, and other films from the sides of the enclosure.

Temperature was allowed to fluctuate with the climate outside since the captive population was maintained within the native range of the species. No artificial heating or cooling devices were used, however, an alarm was

Figure 2. Housing during the 60-day quarantine period made use of plastic boxes or small terraria for individual frogs or male-female pairs, set up with locally-sourced sphagnum moss and leaf litter substrate.

triggered when temperatures inside the building reached 26°C, at which time we would turn on two large oscillating fans to help circulate air and prevent potential overheating. Temperatures were recorded in two terraria with LogTag-TRIX-8 data loggers set to take readings every 66 minutes. We also placed two of these data loggers in the field at the *M. aurantiaca* locality called "Torotorofotsy 3" by Bora et al. (2008) near Menalamba in the Torotorofotsy Wetland. This site was located ca 8 km NNW of the breeding facility and ca 7 km SSE of the collection sites at the mine, with the data loggers being hidden at ground level and tied to tree stumps in the forest.

Lighting was provided with standard T8 fluorescent light bulbs timed to run for 9–10 hours every day. Additional ambient light from windows provided a total photoperiod of 12–13 hours per day, depending on season. We rotated fluorescent bulbs that produced UV-B radiation (ZooMed ReptiSun® 10.0 or ExoTerra ReptiGlo® 5.0) over the enclosures for several hours every day between December and April, when the frogs were most active and likely to be exposed. UV-B lighting was provided to enable the frogs to metabolise calcium from their diet, as has been documented as an important husbandry regimen for other frog species (Antwis & Browne 2009, Verschooren et al. 2011). The bulbs were positioned approximately 30 cm above the substrate on top of the screen covers and rarely were used for more than three days per month per terrarium.

Figure 3. Terraria housing breeding groups of adult frogs, set up with gravel substrate, leaf litter, potted plants, and connected to a PVC pipe drainage system to facilitate cleaning and maintenance.

## Captive diet

The frogs were fed a diet of locally sourced invertebrates farmed at the facility. Adults were fed at least twice weekly and sometimes as often as daily. Fruit flies (*Drosophila* sp.) and five different species of crickets (*Gryllodes sigillatus*, *Gryllus bimaculatus*, *Malgasia marmorata*, and two *Modicogryllus* sp.) made up the bulk of the diet, with between five and 20 food items offered per frog at each feeding session. Additionally, collembolans were introduced to the terraria 2–4 times per month.

We dusted the feeder fruit flies with a powdered vitamin and mineral supplement (Repashy Calcium Plus®) prior to their being fed to the frogs to assure that nutritional requirements were met and to help immobilize the flies. Additionally, we provided the crickets with a varied diet including carrots, zucchini, sweet potatoes, plums, apples, and other fruits and vegetables as available by season, in addition to ground, dried atyid shrimp (locally called 'patsamena') prior to their being fed to the frogs. Starting in August of 2013, the feeder animals were also dusted with Repashy Super Pig®, a carotenoid supplement, 3–4 times per month.

## Husbandry of larvae and juveniles

When eggs were located, we attempted to identify the female that laid them by examining the colouration and body structure of the thinnest female in the terrarium and comparing it to reference photographs. Body profile, especially head structure, slight variation in ventral colouration, and the shape of the flash marks between the joints of the limbs were key features used to distinguish individuals. We allowed eggs to develop for three days within the terrarium before removing them. On the third day, we counted all eggs and assessed how many were fertile. We then left the eggs to develop further on top of moist sphagnum moss above ~5 mm of water in a 900 ml plastic container until they hatched. To prevent the captive population from growing beyond the capacity of the breeding facility and because receptor ponds and suitable habitat for releases had not yet been identified or created, we used a diluted solution of ethanol or MS-222 to stop the development of surplus clutches once we had counted the number of eggs and assessed how many were fertile.

Up to 30 larvae from the same clutch of eggs were kept in aquaria measuring 45 × 25 × 25 cm without filter systems. The aquaria were left bare with no substrate or objects inside. Water was sourced from a tap providing unfiltered water directly from a nearby stream in Andasibe National Park. For the first 1–2 weeks, the water level was main-

tained at between 2 and 4 cm. We then increased the water depth to between 15 and 20 cm (~16–22 litres of total water volume) once the tadpoles began to actively move about (Gosner [1960] stages 23–25), at which point we started feeding them and exchanging their water daily. Between 50–80% of the water was replaced every day. Additionally, we mechanically removed leftover food and waste using a fine fish net. A variety of foods were used in a scheduled daily rotation including powder spirulina, ground 'patsamena' shrimp, TetraMin Tropical Flake, Aquafin Professional Red Flake, and Sera Micron brand fish foods. We used this variety of foods with the idea that it could potentially help avoid nutritional deficiencies in larvae. Water was tested weekly for pH, ammonia, nitrite and nitrate with a colourmetric test kit, with the latter three normally remaining at 0 or below 0.25 ppm and pH ranging from 6.0 to 6.8.

Once the tadpoles reached Gosner stages 41–42 we transferred them to 900 ml ventilated plastic containers with ~1–2 cm of water covering the bottom and several dead leaves protruding from the water surface. When the tail was fully resorbed, we added a substrate of moist sphagnum moss to the container. Up to four individual frogs were housed per container. Only frogs from the same clutch of eggs were housed together and we labelled each container with an identification code to avoid mix-ups.

We counted all individual juvenile frogs in each container daily and fed them either collembolans or fruit flies dusted with a nutritional supplement. Additionally, we sprayed each container with water to rinse waste from the sides and maintain a moist and humid environment. At least once per week, we replaced the moss or washed it with water. Newly hatched crickets were added to the diet of juveniles after they had reached 2–3 weeks of age.

At 6–8 weeks after completing metamorphosis, we transferred juvenile frogs to glass terraria measuring 50 × 25 × 40 cm in groups of between 6 and 40 individuals from the same egg clutch. The terraria were set up with a bottom substrate of pea gravel, sphagnum moss, leaf litter, PVC plastic pipe shelters and live plants. At between eight months and one year of age, when the frogs were close to adult size, we divided the offspring into smaller groups and housed them in terraria measuring 60 × 40 × 45 cm and set up in the same manner as those for the adults.

## Results

### Weight upon acclimation

Female frogs on average weighed more than males, with a mean weight of 1.19 g, compared to males that had a mean weight of 0.68 g (Fig. 4). The minimum weight recorded for a female upon arrival was 0.82 g and the maximum 1.72 g, while for males the minimum was 0.45 g and the maximum 1.05 g.

### Reproductive events, seasonality and fecundity

All 25 breeding groups established from wild-caught founder stock produced fertile eggs, with a total of 469 clutches recorded during the two-year period from April of 2012 through March of 2014. Of these, 436 clutches (93%) were fertile. Concerning the 33 infertile egg clutches, 17 were produced soon after breeding groups were assembled during the winter, between June and September of 2012.

The maximum number of clutches produced by a single female in the period from April of 2012 through March of

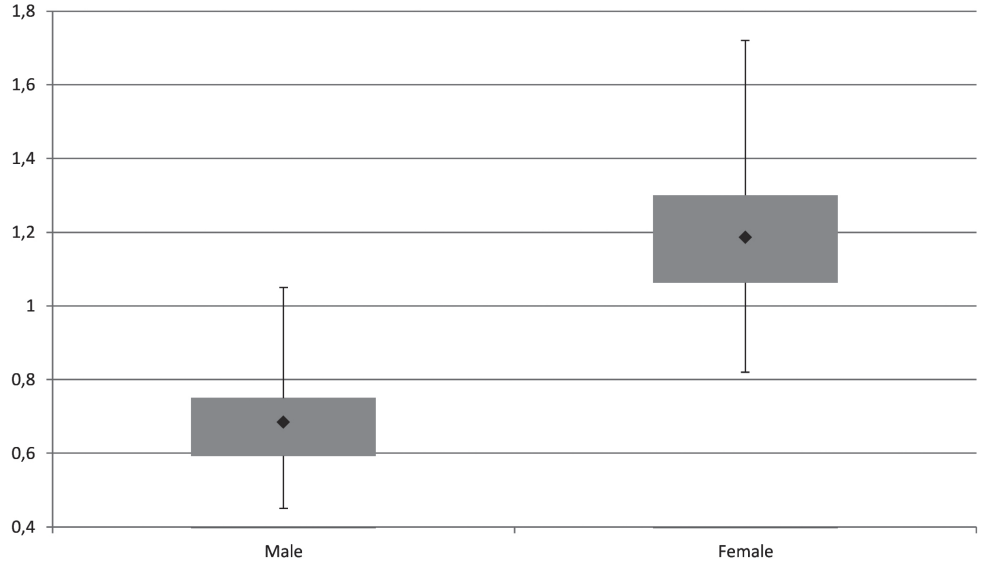


Figure 4. The weight in grams of wild-caught founder *Mantella aurantiaca* upon arrival at the breeding facility. The black diamond is the mean for each sex.

2014 was 16, while the average per female was 7. Two females were not identified as producing eggs. Clutch size ranged from 24 to 193 eggs, with a mean of 74 ± 22, n = 461. This excludes two clutches that we counted as comprising more than 230 eggs but which we suspect constituted more than one clutch laid simultaneously by more than one female, as well as an unusual find of only 3 fertile eggs , possibly representing leftover eggs from a clutch removed from this terrarium previously that same week. Additionally, the numbers of eggs in six different clutches were simply recorded as "unknown".

Breeding events and activity increased between the months of October and March, which is similar to the time of year the species has been recorded breeding in the wild (Ramilijaona et al. 2004). The months from April through September saw little to no reproduction, while the months of December and January were the most productive, especially during the second year in captivity when this seasonality was particularly evident (Fig. 5).

The increase in activity during the austral summer corresponds to the seasonal variation in temperature (Fig. 6). Over a period of 12 months, the maximum temperature

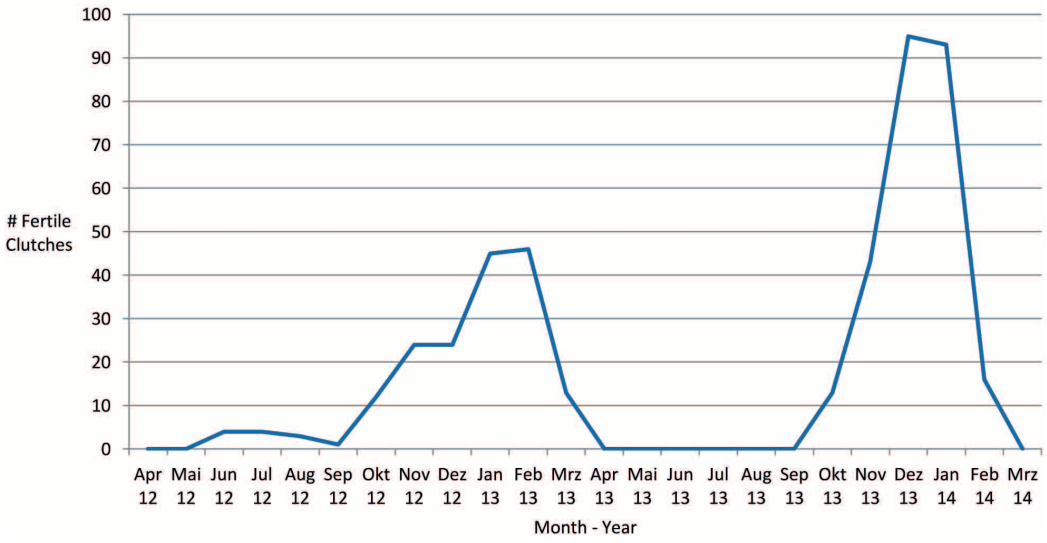


Figure 5. Seasonality of breeding events showing the number of fertile clutches per month from all 25 breeding groups established from wild-caught founder specimens.

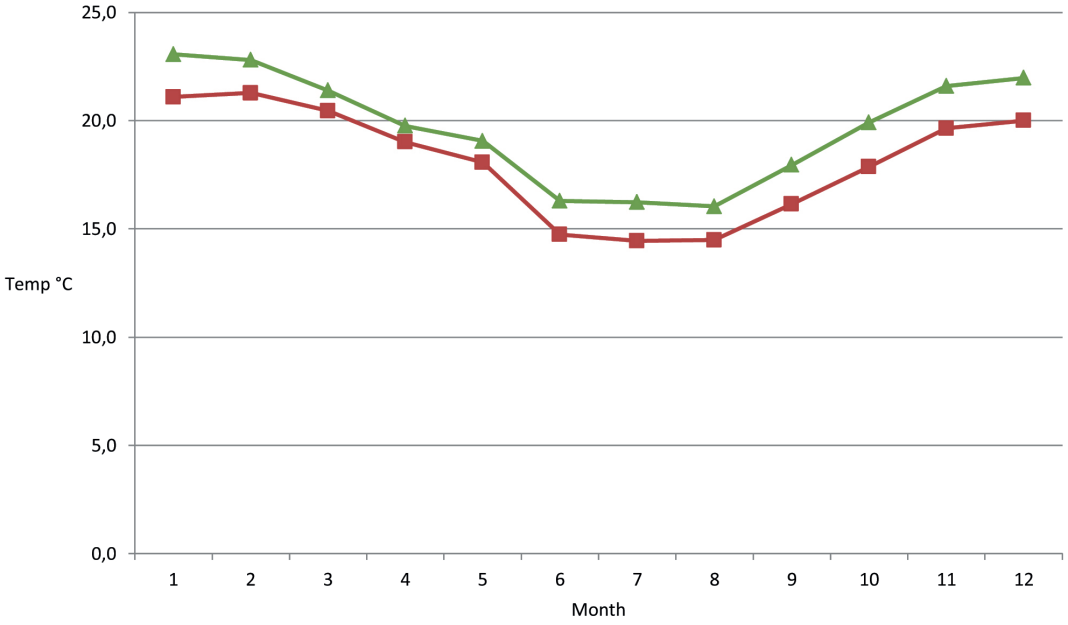


Figure 6. Average monthly temperature for keeping *Mantella aurantiaca* in captivity (green/triangles) and in situ at a breeding site in Torotorofotsy Wetland (red/squares) recorded for one year between November of 2012 and November of 2013.

Table 3. Egg location and fertility for breeding events from the 25 groups of wild-caught founder specimens between April of 2012 and March of 2014.

| Location | # Fertile clutches | # Infertile clutches | Total clutches |
|---|---|---|---|
| Exposed on substrate | 117 | 5 | 122 |
| Under dead leaves or in coconut hut | 104 | 5 | 109 |
| In pot of plant | 180 | 5 | 185 |
| Scattered in multiple locations | 15 | 1 | 16 |
| Unknown / not recorded | 20 | 17 | 37 |
| Total | 436 | 33 | 469 |

recorded in the terrarium was 28.2°C, the minimum was 12.5°C, and the average was 19.8°C. We did not observe muscle spasms, heat stress, or related health issues in response to warm temperatures, as reported in captivity by other authors (Staniszewski 2001, Ralph 2014), even during periods during which the temperature repeatedly rose to above 26°C every day for more than a week.

Numerous locations served as sites for oviposition, including out in the open directly on the gravel or foam substrate (26.0% of all clutches), hidden under leaf litter or in the coconut hut (23.2%), buried in the moss or gravel of a plant pot (39.4%), or scattered about the terrarium (3.4%). A summary of oviposition sites is provided in Table 3.

## Development of offspring

It was usually apparent whether or not eggs were fertile after three days following oviposition. We allowed 22 of the 436 fertile egg clutches to develop, resulting in the hatching of 809 tadpoles. We culled excess fertile clutches because no sites had been established for releasing stock at the time, and allowing all to develop would have exceeded the resources available at the breeding facility. Tadpoles hatched from their eggs at between 2 and 15 days after being discovered in the terrarium, with the average being 8 days. The tadpoles completed metamorphosis within 53–139 days after emerging from eggs. As noted by other authors (Zimmermann 1992, Walker 2005), juvenile *M. aurantiaca* were brown in colour with a distinct black facemask, as opposed to the uniform orange-red colouration of adults (Fig. 7). The majority of individuals only assumed their full adult colouration after one year or more (Fig. 8).

Vocalization was observed as early as at an age of four months after completing metamorphosis, before individuals were fully grown. We recorded the first fertile eggs from the $F_1$ generation at 11 months after completing metamorphosis. In total, the $F_1$ generation produced 266 clutches between November of 2013 and March of 2014, with clutch sizes of 20–149 eggs (mean 62 ± 25, n = 244). Excluded here are two clutches with counts of >200 eggs that we suspect consisted of more than one clutch laid simultaneously. 211 (79%) of the 266 egg clutches from the $F_1$ generation were fertile.

## Survivorship and mortality

Out of 809 tadpoles hatched, 637 survived and completed metamorphosis (78.7%). Most of the mortality events occurred during the final stages of metamorphosis when tadpoles were moved to plastic containers with shallow water (see above). The timing of adding moss to the containers at this stage was critical to prevent metamorphs from drowning, and it would be worthwhile experimenting with other rearing techniques at the end of metamorphosis to minimise mortality.

Deaths in the $F_1$ generation after metamorphosis were almost exclusive to within the first two months of age (183 of 196 individuals that died). Of the 637 individuals that completed metamorphosis, 441 survived to one year of age. Concerning the 162 wild-caught founder specimens, there were only six deaths in the two-year period after collection. Two were due to desiccation following escapes, one of which occurred while still in quarantine, and two were due to trauma suffered within the terrarium. Necropsies were performed on the two unexplained mortalities by a visiting

Figure 7. *Mantella aurantiaca* at less than three weeks after completing metamorphosis, showing the typical black facemask and brown dorsal colouration of juveniles.

veterinarian from the San Diego Zoo Institute for Conservation Research, but no cause of death could be identified.

Health issues included a possible bacterial infection, abnormal pigmentation, rectal prolapse, rostral abrasions, ocular discolouration, and physical trauma sustained from contact with items within the terrarium. All these frogs recovered within 1–4 months while kept isolated in separate terraria without treatment other than a topical triple antibiotic ointment used to treat rostral abrasions and a sugar-water solution applied to individuals that had suffered a rectal prolapse. We also observed on several occasions that individual frogs became stuck between the drainage slots of the net-style plant pots we used in terraria, however these individuals fully recovered after having been carefully dislodged by hand.

## Breeding behaviour

We observed oviposition events on numerous occasions. In general, we observed multiple males in a terrarium courting a gravid female one to two days prior to oviposition. Courting involved vocalization (often while in contact with the female), stroking the female with the forearms, grasping the female around the head in a manner that resembled cephalic amplexus, and sitting on the head of the female while rubbing the femoral glands on her head or dorsum. Oviposition took place most often during the morning in hidden locations, and when observed did not involve an amplectant hold, but rather one or more males in contact with a female but without grasping or holding on to her.

On 8 December 2013, at approximately 10:00 a.m., two of us (SSS & ET) captured a spawning event on video (Fig. 9). The individuals observed were representatives of the $F_1$ generation. The main stages of the process were recorded as follows: two males were discovered sitting on top of a gravid female in a plant pot with their hind legs splayed and extended outwards. Their femoral glands were noted to be in direct contact with the dorsum of the female. After retrieving the camera and returning to the terrarium, the female began depositing eggs. One male vocalized intermittently, and its abdomen and limbs pulsated up and down on top of the female at a rate of slightly less than one pulse per second. After 15 minutes, the male that pulsated and vocalized shifted his position so that its vent was aligned with that of the female and at this point appeared to fertilize the eggs, while the other male remained immobile facing to one side. At 19 minutes, the female changed position and moved out from under the males while continuing to expel eggs. Both males remained positioned over the eggs with their legs splayed. At 23 minutes, the male that had not vocalized or moved changed position and came in contact with the female again, and one minute later, the other male left. At 25 minutes, the remaining male moved away, no longer touching the female, but continuing to stay in the plant pot, nosing the gravel, eggs, and moss with his head for another 4 minutes, and digging several small holes before finally leaving. The female appeared to deposit several more eggs without either male present and finished completely by timestamp 36 minutes. She then sat at the edge of the plant pot and used her hind legs to wipe the sides of her body, exiting the pot at 41 minutes.

Figure 8. Individuals from the $F_1$ generation aged between 11 and 13 months after completing metamorphosis with uniform orange-red adult colouration, shown here feeding on collembolans.

## Discussion

*Mantella aurantiaca* has been kept and propagated in captivity since at least the mid 1960's and early 1970's (Audy 1973, Mudrack 1974, Oostveen 1978). Most of these published accounts, however, are based on single breeding events or from a single group of animals. While the species has been maintained by numerous zoological institutions throughout the world since then, quantitative data from these experiences are missing or have largely remained unpublished.

Having quantitative information on fecundity, survivorship, age at sexual maturity, and other basic life history traits are crucial to being able to accurately assess the extinction risk of a species or develop models that may reveal insights into its population ecology. In the case of *M. aurantiaca*, it could be useful to explore the effects of collection for the international pet trade more thoroughly or assess the potential impact of emerging infectious diseases with such models.

From our results, in terms of captive management, we can identify the importance of temperature and seasonal variation for reproduction. Other authors have also observed the significance of providing an extended cool period for captive-kept *Mantella* species (Gagliardo 2009, Staniszewski 2001). These benefits were maximised by running the breeding programme within the native range of the species so that the frogs were exposed to natural climate variation throughout the year without supplemental heating or cooling.

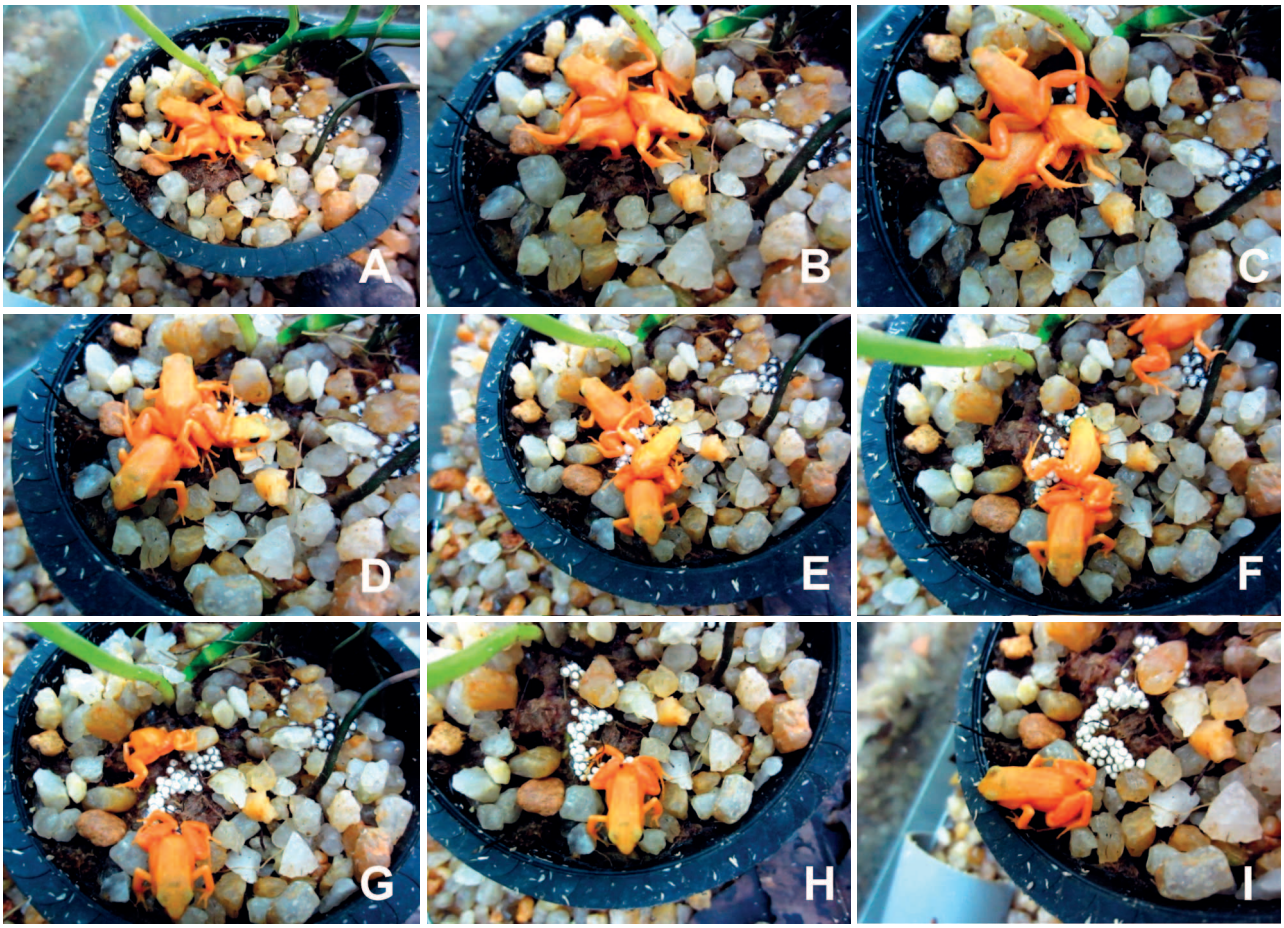


Figure 9. Snapshots taken from the breeding event recorded on video on 8 December 2013, illustrating details of mating behaviour. Note that the egg clutch visible in the background to the right in some of the images was laid the previous day by a different female. A) 0 min. 0 sec. – Two males are perched on top of a gravid female with their legs splayed and femoral glands in contact with her dorsum; B) 1 min. 48 sec. – Male 1 (on the left) pulsates his femoral glands against the dorsum of the female at a rate of around 1 pulse/sec.; C) 6 min. 14 sec. – The female expels eggs while male 1 continues sporadic 'pulsating'; D) 16 min. 13 sec. – Male 1 has shifted his position and is now positioned directly over the eggs, parallel to the female but facing in the opposite direction, and appears to be fertilizing the eggs; E) 22 min. 39 sec. – Male 1 has moved off of the female and resumed a normal position while male 2 continues to keep his femoral glands in contact with the female; F) 24 min. 23 sec. – Male 1 exits the pot while male 2 begins digging in the substrate surrounding the eggs; G) 28 min. 28 sec. – Male 2 investigates the site and digs next to the eggs before leaving the female; H) 32 min. 53 sec. – The female appears to lay several more eggs and rubs her hind limbs over and under her flanks; I) 39 min. 39 sec. – The female continues to sit near her egg clutch, occasionally flicking her hind limbs over and under her body before exiting the pot at 41 min. 05 sec.

Our observations of mating behaviour confirmed the lack of a traditional amplexus, as has been noted for all representatives of the subfamily Mantellinae (Glaw & Vences 2007). However, we noticed that males stayed in contact with the female for a period of time while mating even if they would not grasp her. The possible role of pheromonal communication, as noted in the mantellid *Mantidactylus betsileanus* by Poth et al. (2012), during courting and mating in *M. aurantiaca* would be interesting to investigate, as clearly the pulsating and rubbing of their femoral glands by mating males on the dorsum of the female plays a prominent role in this species' reproduction.

With continued habitat loss threatening the species, restoring and creating additional breeding sites is a necessary conservation option. With regard to the outlook of the breeding programme, it is important that project stakeholders collaboratively conduct in a timely matter a risk assessment regarding reintroduction to determine whether to release captive stock at created sites. In hindsight, it would have been advantageous if this had been done before starting the breeding programme and we recommend that future organisations considering establishing a survival assurance colony do this in advance of collecting founder stock, if at all possible.

Lastly, the success of this breeding programme in the range country of the species must be pointed out. While there have been discussions regarding developing infrastructure and the capacity to implement captive breeding programmes within Madagascar (Andreone et al. 2006, Furrer 2008, Mendelson III & Moore 2008), there continues to be a push within the international zoo community to export species for husbandry research or survival assurance colony purposes. This is not only sometimes unnecessary, but may even be wasteful, because the potential resources currently used to establish breeding programmes at zoos abroad would in fact go much farther in Madagascar. With a dedicated team of individuals and long-term financial support, we believe it would be possible to replicate our experience with *M. aurantiaca* in Madagascar with species assessed as in need of ex situ conservation action. On that note, we hope to see the outlook towards amphibian breeding programmes with Malagasy amphibian species shift away from exportation and towards building capacity in-country.

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### References

Andreone, F., J. Cadle, N. Cox, F. Glaw, R. A. Nussbaum, C. J. Raxworthy, S. N. Stuart, D. Vallan & M. Vences (2005): Species review of amphibian extinction risks in Madagascar: Conclusions from the Global Amphibian Assessment. – Conservation Biology, **19**: 1790–1802.

Andreone, F., A. I. Carpenter, J. Copsey, A. Crottini, G. García, R. K. B. Jenkins, J. Köhler, N. H. C. Rabibisoa, H. Randriamahazo & C. J. Raxworthy (2012): Saving the diverse Malagasy amphibian fauna: where are we four years after implementation of the Sahonagasy Action Plan? – Alytes, **29**: 43–57.

Andreone, F., N. C. Carpenter, L. du Preez, K. Freeman, S. Furrer, G. García, F. Glaw, J. Glos, D. Knox, J. Köhler, J. R. Mendelson, V. Mercurio, R. A. Mittermeier, R. D. Moore, N. H. C. Rabibisoa, H. Randriamahazo, H. Randrianasolo, N. R. Raminosoa, O. R. Ramilijaona, C. J. Raxworthy, D. Vallan, M. Vences, D. R. Vieites & C. Weldon (2008): The challenge of conserving amphibian megadiversity in Madagascar. – PLoS Biology, **6**: 943–946.

Andreone, F., V. Mercurio & F. Mattioli (2006): Between environmental degradation and international pet trade: conservation strategies for the threatened amphibians of Madagascar. – Natura – Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano, **95**: 81–96.

Antwis, R. & R. Browne (2009): Ultraviolet radiation and vitamin $D_3$ in amphibian health, behavior, diet and conservation. – Comparative Biochemistry and Physiology, **154**: 184–190.

Audy, F. (1973): *Mantella aurantiaca*. – Aquarama: Revue Aquariophile Trimestrielle, **7**: 56–57.

Banks, C. B., M. W. N. Lau & D. Dudgeon (2008): Captive management and breeding of Romer's tree frog *Chirixalus romeri*. – International Zoo Yearbook, **42**: 99–108.

Bletz, M. C., G. M. Rosa, F. Andreone, E. A. Courtois, D. S. Schmeller, N. H. C. Rabibisoa, F. C. E. Rabemananjara, L. Raharivololoniaina, M. Vences, C. Weldon, D. Edmonds, C. J. Raxworthy, R. N. Harris, M. C. Fisher & A. Crottini (2015): Widespread presence of the pathogenic fungus *Batrachochytrium dendrobatidis* in wild amphibian communities in Madagascar. – Scientific Reports, **5**: 8633, DOI: 10.1038/srep08633.

Bora, P., R. Dolch, R. Jenkins, O. Jovanovic, F. C. E. Rabemananjara, J. Randrianirina, J. Rafanomezantsoa, L. Raharivololoniaina, O. Ramilijaona, N. Raminosoa, R. Randrianavelona, A. Raselimanana, B. Razafimahatratra, T. Razafindraibe & M. Vences (2008): Geographic distribution of three species of Malagasy poison frogs of high conservation priority: *Mantella aurantiaca, M. crocea* and *M. milotympanum*. – Herpetology Notes, **1**: 39–48.

Browne, R., K. Wolfram, G. García, M. Bagaturov, & Z. J. J. M. Pereboom (2011): Zoo-based amphibian research and conservation breeding programs. – Amphibian and Reptile Conservation, **5**: 1–14.

Edmonds, D., J. C. Rakotoarisoa, R. Dolch, J. Pramuk, R. Gagliardo, F. Andreone, N. H. C. Rabibisoa, F. C. E. Rabe-

mananjara, S. Rabesihanaka & E. Robsomanitrandrasana (2012): Building capacity to implement conservation breeding programs for frogs in Madagascar: Results from year one of Mitsinjo's amphibian husbandry research and captive breeding facility. – Amphibian and Reptile Conservation, **5**: 57–69.

Furrer, S. (2008): Madagascar amphibian conservation in Zoo Zürich, Switzerland. – pp. 301–308 in: Andreone, F. (ed.): A Conservation Strategy for the Amphibians of Madagascar. – Monografie XLV. Museo Regionale di ScienzeNaturali, Torino.

Gagliardo, R., P. Crump, E. Griffith, J. Mendelson, H. Ross & K. Zippel (2008): The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. – International Zoo Yearbook, **42**: 125–135.

Gagliardo, R. (2009): Mantellas – Association of Zoos and Aquariums, Amphibian Biology, Conservation, and Management Training Program, Specialty Taxa Husbandry Monograph.

Glaw, F. & M. Vences (2007): A field guide to the amphibians and reptiles of Madagascar. Third edition. – Cologne, Vences & Glaw Verlag.

Gosner, K. L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification. – Herpetologica, **16**: 183–190.

Griffiths, R. & L. Pavajeau (2008): Captive breeding, reintroduction, and the conservation of amphibians. – Conservation Biology, **22**: 852–861.

Harper, G. J., M. K. Steininger, C. J. Tucker, D. Juhn & F. Hawkins (2007): Fifty years of deforestation and forest fragmentation in Madagascar. – Environmental Conservation, **34**: 325–333.

Johnson, K. (2008): Amphibian Ark Species Prioritization – Madagascar: *Mantella aurantiaca*. – https://aark.portal.isis.org/.

Lee, S., K. Zippel, L. Ramos & J. Searle (2006): Captive-breeding programme for the Kihansi spray toad *Nectophrynoides asperginis* at the Wildlife Conservation Society, Bronx, New York. – International Zoo Yearbook, **40**: 241–253.

McGregor, R. & K. Zippel (2008): Can zoos and aquariums ensure the survival of amphibians in the 21stcentry? – International Zoo Yearbook, **42**: 1–6.

Mendelson, J. R. III & R. Moore (2008): Amphibian conservation at the global, regional, and national level. – pp. 15–20 in: Andreone, F. (ed.): A Conservation Strategy for the Amphibians of Madagascar – Monografie XLV. Museo Regionale di ScienzeNaturali, Torino.

Mudrack, W. (1974): Haltung und Zucht des Goldfröschchens, *Mantella aurantiaca* Mocquard, 1900. – Das Aquarium, **8**: 160–161.

Oostveen, H. (1978): Persoonlijkeervaringen met het rode kikkertjeuit Madagaskar, *Mantella aurantiaca*. – Lacerta, **36**: 51–55.

Pavajeau, L., K. Zippel, R. Gibson & K. Johnson. (2008): Amphibian Ark and the 2008 year of the frog campaign. – International Zoo Yearbook, **42**: 24–29.

Perl, R. G. B., Z. T. Nagy, G. Sonet, F. Glaw, K. C. Wollenberg & M. Vences (2014): DNA barcoding Madagascar's amphibian fauna. – Amphibia-Reptilia, **35**: 197–206

Pessier, A. P. & J. R. Mendelson (2010): A manual for control of infectious diseases in amphibian survival assurance colonies and reintroduction programs. – IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, MN, USA.

Piludu, N., N. Dubos, J. H. Razafimanahaka, P. Razafindraibe, J. C. Randrianantoandro, & R. K. B. Jenkins (2015): Distribution, threats and conservation of a Critically Endangered amphibian (*Mantella aurantiaca*) in eastern Madagascar. – Herpetology Notes, **8**: 119–123.

Poth, D., K. C. Wollenberg, M. Vences & S. Schulz (2012): Volatile amphibian pheromones: marcrolides from mantellid frogs from Madagascar. – Angewandte Chemie International Edition, **51**: 2187–2190.

Ralph, J. (2014): Introducing the beautiful Mantellas. – Practical Reptile Keeping, **60**: 28–32.

Ramilijaona, O. R., N. Raminosoa, D. Rakotondravony, F. C. E. Rabemananjara, P. Bora & J. T. Razafindrabe (2004): Donnéessur les grenouilles du genre *Mantella* spp. endémique de Madagascar. – Proceedings of the CITES Twenty-second meeting of the Animals Committee, Lima (Peru), 7–13 July 2006. Université d'Antananarivo Département de Biologie Animale.

Randrianavelona, R., H. Rakotonoely, J. Ratsimbazafy & R. K. B. Jenkins (2010): Conservation assessment of the critically endangered frog *Mantella aurantiaca* in Madagascar. – African Journal of Herpetology, **59**: 65–78.

Schad, K. (2007): Amphibian Population Management Guidelines. – Proceedings of the Amphibian Ark Amphibian Population Management Workshop, 10–11 December 2007. – San Diego, California, USA. – Available at http://www.amphibianark.org.

Staniszewski, M. (2001): Mantellas. – Chimaira Press, Frankfurt, Germany.

Tarrant, J. (2013): Jumping in to frog conservation: A new program for the Endangered Wildlife Trust, South Africa. – FrogLog, **107**: 57–58.

Vences, M. & C. J. Raxworthy (2004): *Mantella aurantiaca*. – IUCN Red List of Threatened Species. Version 2013.1 – Available at http://www.iucnredlist.org.

Verschooren, E., R. Brown, F. Vercammen & J. Pereboom (2011): Ultraviolet B radiation (UV-B) and the growth and skeletal development of the Amazonian milk frog (*Trachycephalus resinifictrix*) from metamorphosis. – Journal of Physiology and Pathophysiology, **2**: 34–42.

Visser, I. (2012): Ex-situ amphibian conservation in Southern Africa. – FrogLog, **103**: 17–19.

Walker, C. (2005): Management Guidelines for the Welfare of Zoo Animals – Golden Mantella. – The British and Irish Association of Zoos and Aquariums (BIAZA).

Zimmermann, H. (1992): Nachzucht und Schutz von *Mantella crocea*, *Mantella viridis* und vom madagassischen Goldfröschchen *Mantella aurantiaca*. – Zeitschrift des Kölner Zoo, **35**: 165–171.

Zippel, K., K. Johnson, R. Gagliardo, R. Gibson, M. McFadden, R. Browne, C. Martinez & E. Townsend (2011): The Amphibian Ark: A global community for ex situ conservation of amphibians. – Herpetological Conservation and Biology, **6**: 340–352.

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