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Hand-rearing protocol and comparison of growth rates in parent-reared versus hand-reared offspring: a case study in *Callicebus cupreus*

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Abstract

The European Endangered Species Programme (EEP) for coppery titi monkeys (*Callicebus cupreus*) has a total population of around 90 individuals, living in zoos, that has experienced low reproductive rates and relatively high neonatal mortality. Bristol Zoo Gardens (BZG) housed a pregnant breeding female in 2017, who died during parturition from shock secondary to uterine prolapse. To ensure the infant's survival, it was hand-reared. The aim of this publication is to report the first detailed hand-rearing protocol for *Callicebus* and to compare the growth rates of two hand-reared infants against seven parent-reared titi monkeys by collating weight information using the zoo animal database, Zoological Information Management System (ZIMS). Day had a significant effect on the weight of both hand-reared and parent-reared titi monkeys ($F_{(1,57)}$=919.3, P<0.001, np^2=0.942). Parent-reared titi monkeys had significantly higher growth rates (457.9±9.3) compared to the hand-reared (390.7±11.1) titi monkeys ($F_{(1,57)}$=19.804, P<0.001, np^2=0.258). Despite the differences in infant growth rates between rearing strategies, the hand-rearing protocol was considered successful due to the infant being the first hand-reared coppery titi monkey to survive to adulthood without any nutritional deficiencies. Parent-rearing is still recommended, and hand-rearing should only be selected when strictly necessary and if recommended by the EEP Coordinator. In such circumstances, an early introduction to the parent or conspecifics is extremely important for the infant's development. Further research is needed to develop a hand-rearing protocol that considers increased milk quantity and earlier reintroduction procedures to improve hand-reared coppery titi monkey growth and development in captivity.

Background

The coppery titi monkey (*Callicebus cupreus*) is one of 34 primates of the genus *Callicebus*, originating from regions of Brazil and Peru. Classified as Least Concern on the International Union for the Conservation of Nature's (IUCN) red list, they are the only *Callicebus* species commonly found in zoos and serving as important ambassadors for education and research (Botelho et al. 2012). They live in small family groups that consist of a monogamous breeding pair and their offspring (Mason 1974). Titi monkeys are highly social, making physical contact for up to 47% of their activity budget, with breeding pairs engaging in grooming or tail intertwining (Freeman et al. 2014; Ragen et al. 2015). Pair compatibility is considered an important factor in titi monkey husbandry (Fernandez-Duque et al. 1997) and has been associated with reproductive success (Spence-Aizenberg et al. 2016; E. Lewis, personal communication, 3 January 2018). *Callicebus* species are one of few primates that exhibit biparental care of offspring, whereby males are almost solely responsible for transporting, grooming, sharing food, and playing with infants (Spence-Aizenberg et al. 2016). Pairs who are not compatible may fight rather than mate during oestrus, and stress during pregnancy has been associated with reduced reproductive success (Johnson et al. 1991; E. Lewis, personal communication, 3 January 2018).

Titi monkeys are seasonal breeders in the wild and oestrus cycles last from 17 to 21 days during which mating can occur (Valeggia et al. 1999). Females give birth to a single offspring that is dependent on the dam's milk until 4–6 months old. During this time, dams will undergo a relatively prolonged

period of anovulation following the birth, during which offspring slowly develop (Allman et al. 1998), staying with their family group until approximately 2 years old. Mature female offspring will not breed while they remain with the family group (Valeggia et al. 1999; Veiga et al. 2013).

The European Endangered Species Breeding programme (EEP) for titi monkeys is limited by a small population and a relatively high neonatal mortality rate (Veiga et al. 2013); in the last 12 months, over a quarter of births in European zoos have led to mortality and research has yet to investigate the causes of this (ZIMS 2018). The small population makes it more vital for pairs to be matched based on genetics rather than on pair-compatibility traits, such as animal temperament and experience (Valeggia et al. 1999; E. Lewis, personal communication, 3 January 2018). This may contribute to the limited breeding success in zoos, as once paired, it can take individuals months or years to become settled with one another and if a transfer is required, this is dependent on the availability of another genetically suitable individual (ZIMS 2018; Safina 2018). Titi monkeys are naturally very shy animals and react to novelty, such as changes to their environment, more cautiously than do other New World primates (Hennessy et al. 1995; Mayeaux and Mason 1998). They can be, therefore, difficult to keep in captivity without provision of adequate captive conditions (Jones 1968; L. Bugg, personal communication, 4 February 2018; Poole 1991). The impact of specific variables in zoos, such as noise and visitor numbers, on titi monkeys, and specifically how this may impact breeding success in captivity, is unknown.

Hand-rearing genetically important offspring is a growing conservation strategy in zoos (Quintard et al. 2017). Parent-rearing is always recommended, and hand-rearing should be applied only when strictly necessary and if recommended by the EEP Coordinator. The decision to hand-rear is made on an individual basis and must consider the practicalities of hand-rearing as well as the need to maintain good welfare for the individuals involved. Early introduction of the infant to parents or conspecifics is necessary during hand-rearing to maximise the chances of the infant being accepted by the group; this is extremely important for the infant's development (Quintard et al. 2017). Hand-rearing is considered a last resort because hand-reared individuals, especially primates, tend to exhibit poor social skills. For example, hand-reared primates have previously performed abnormal repetitive behaviours and have suffered with vitamin deficiencies with long-term consequences (Novak et al. 2006; Hosey et al. 2013; Bogart et al. 2014). There is a need to better understand how to house and breed titi monkeys in captivity to ensure a sustainable population of coppery titi monkey (*Callicebus cupreus*), and to respond to when more endangered titi monkey populations will require conservation measures such as ex-situ breeding programmes (L. Bugg, personal communication, 4 February 2018; Wallace et al. 2006).

To date, only two coppery titi monkey infants have been successfully hand-reared past weaning and only one individual has survived to 2 years of age. No previous reports have been published which detail the milk formula, feeding or socialisation protocols used in hand-rearing this species. The aim of this report is to describe the protocol employed at Bristol Zoo Gardens (BZG) for hand-rearing a coppery titi monkey in a zoo environment and to compare the growth rates of hand-reared versus parent-reared titi monkeys to evaluate the effectiveness of the hand-rearing protocol used.

Action

Individuals

A male titi monkey infant was hand-reared in 2017 at Bristol Zoo Gardens by a team of keepers following the death of the mother due to shock secondary to uterine prolapse during parturition. The infant was raised following protocols detailed in the callitrichid hand-rearing guidelines and a short document with infancy weights provided by a zookeeper who had previously hand-reared a coppery titi monkey at Shaldon Zoo in 2011 (Baker 2017; EAZA 2010). At the time of rearing, no published information was available detailing the nutritional requirements of infant coppery titi monkeys. The hand-rearing protocol was initiated at parturition; the first feed was given by placing the infant on the deceased dam to obtain 0.1 ml of colostrum. From the second feed onwards, the infant was hand-reared by BZG mammal keepers. Weight data were collected from Day 1 until Day 322 by weighing the infant on scales. From Day 1 until Day 60 the infant was placed on a monkey soft toy and weighed daily at 0900. Once the infant was mobile, he was encouraged to step onto the scales for milk feeds and weighed opportunistically. At Day 140, weighing was reduced to once every 2 weeks. Weight data for the second hand-reared individual based at Shaldon Zoo was obtained using the zoo animal database, Zoological Information Management System (ZIMS). Shaldon Zoo adopted a similar rearing approach to BZG over the first week of infant care; however, after this the rearing strategy for the second hand-reared individual is unknown. Weight data for seven parent-reared titi monkey offspring from other zoological collections were also collected using ZIMS.

Hand-rearing protocol

Teats used for bottle-feeding were classic kitten hand-rearing teats. The substitution milk was a SMA Infant Milk formula and 4.3 g was used and mixed with 90 ml water for the total daily feed. The daily formula, divided into portions needed for each meal, were prepared at 0800 daily and then stored in the refrigerator. Before each feed, the portion to be fed was warmed, and any leftovers were discarded after each feed. Syringe-feed intake was recorded daily in ml. After each feed, the infant was gently stimulated in the perineal region with a humid cotton bud, to stimulate urination and defecation.

Nutrient and energy intake

Based on a 100 ml prepared feed, of which 12.536% is dry matter, the formula was composed of: 280 kj (67 kcal), 3.6 g fat, 1.5 g saturates, 2.1 g unsaturates, 7.1 g carbohydrates, 7.1 g sugars, 0.4 g fibre, 1.25 g protein. Table 1 shows the average quantity fed

Table 1. Guidelines on the quantity of milk formula fed during hand-rearing.

Day (D)	Average quantity fed (ml) per feed	Quanitity fed (% of body weight) in a day (24 hours)
D1 to D2	0.97	23
D3to D4	1.55	23.6
D5 to D10	1.87	18.6
D11 to D18	3.37	32.6
D19 to D28	4.73	34.2
D29 to D42	5	23.9
D43 to D59	5.25	14
D60 to D99	7.12	12.3
D100 to D133	13.2	8.4

Table 2. Changes in the number of milk syringe-feedings in a 24-hour day throughout the hand-rearing period.

Day (D)	Number of milk syringe-feeds consumed daily
D1	8
D2 to D10	12
D11 to D12	10
D13	11
D14 to D18	8
D19 to D28	7
D29	5
D30	6
D31	4
D32 to D33	5
D34	6
D35	5
D36	6
D37 to D42	5
D43 to D44	4
D45 to D46	3
D47 to D48	4
D49 to D58	3
D59	4
D60 to D99	3
D100 to D126	2
D127 to D133	1

Table 3. Changes in the amount of solid food offered per day with age.

Age (days)	Adult diet percentage (%)	Food type	Amount (g)	Dry matter (DM) (%)
133	50	Trio munch pellet	69	90
		Marmoset jelly	22	44
		Starchy vegetables	138	18.33
		Watery vegetables	138	13.58
		Leafy vegetables	138	7.44
		Live food	9	35.625
		Browse	Ad-lib	42.102
231	70	Trio munch pellet	96.6	90
		Marmoset jelly	30.8	44
		Starchy vegetables	193.2	18.33
		Watery vegetables	193.2	13.58
		Leafy vegetables	193.2	7.44
		Live food	12.6	35.625
		Browse	Ad-lib	42.102
367	90	Trio munch pellet	124	90
		Marmoset jelly	40	44
		Starchy vegetables	248	18.33
		Watery vegetables	248	13.58
		Leafy vegetables	248	7.44
		Live food	14	35.625
		Browse	Ad-lib	42.102
541	100	Trio munch pellet	138	90
		Marmoset jelly	44	44
		Starchy vegetables	276	18.33
		Watery vegetables	276	13.58
		Leafy vegetables	276	7.44
		Live food	16	35.625
		Browse	Ad-lib	42.102

(ml) per feed from Day 1 to Day 133 and its equivalent in body %. The number of milk feeds given each day from Day 1 to Day 133 is provided in Table 2. The quantity of milk substitute fed to the infant varied according to the infant's development over the week and also how much the infant would take.

SMA Infant milk formula was fed to the infant every 2 hours until Day 11, when the number of feeds were gradually reduced, and then until Day 133 when the infant was weaned. Weaning food was offered at intervals and combined with reduced milk feeds (Table 2); 1–2 ml of baby rice from Day 27, steamed vegetables (10 g) including courgette, carrots, peppers and sweet potato from Day 37, marmoset jelly (5 g) from Day 55, trio munch pellet (2 g) from Day 80, and solid vegetables (ad lib) from Day 93. From Day 133, a juvenile diet was offered over four separate feeds a day (Table 3) and dietary supplements were provided (Table 4).

Housing protocol

On Day 1, the infant was removed from its mother and placed on a cotton plush toy (20 cm high) used as a surrogate in an incubator. From Day 1 to Day 9, the incubator was transported with keepers offsite to enable 2 hourly feeds throughout the night and kept in an off-show facility during the day at BZG. From Day 9, the infant, within the incubator, was placed in one area of the sire's enclosure that the sire could see into but could not gain access. At parturition, the temperature in the incubator was set at 30°C and decreased slowly by one degree each week, until Day 28. It was then reduced by 0.5°C each day until 21°C was reached. At this stage, the infant was increasingly active and placed in an indoor satellite cage (60x60x80 cm of mesh) every day (22°C) inside one part of the sire's enclosure, returning to the incubator only for the night (24°C). Throughout the hand-rearing process, the infant was exposed to daylight hours between 0700–1900 and the humidity level was maintained between 40–60%.

Inside the satellite cage were three pieces of bamboo (1.5 cm diameter) for branching, a water dish and enrichment (ropes, larger bamboo, hammocks) which were changed throughout the rearing period according to the behavioural development of the infant. From Day 12, the satellite cage was placed within one area of the sire's enclosure, 60 cm under UV-B light for 2 hours daily to expose the infant to 80–100 µW/cm² UV-B to prevent vitamin D^3 deficiency. As the infant became more active, he was allowed out of the satellite cage into half of the sire's enclosure to which the sire did not have access and put back in the satellite cage for 2 hours of UV-B exposure and for feeding.

Table 4. Supplements offered from Day 133.

Supplement	Amount given (ml)	Administration method
Zolcal D	1 ml per kg bodyweight, three times weekly	Syringed onto vegetables
Nutrobal	1 pinch per kg bodyweight, three times weekly	Dusted on live food
Samylin (small breed)	0.25 g daily during winter only	Dusted on vegetables

The satellite cage was removed at 4 months when the infant was integrated with his sire and therefore the infant was exposed to the same environmental conditions as the sire: temperature (22°C), humidity (40–60%), UV-B exposure (between 0900–1700 daily) and light levels 0700–1900.

Socialisation protocol

No socialisation protocols existed for hand-reared titi monkeys, so the protocol employed was devised using callitrichid guidelines as guidance. The hand-reared infant at BZG was kept in visual, olfactory and auditory contact with the sire from Day 9 in a satellite cage in the sire's enclosure. During this time, the sire showed interest in the infant by vocalising at him and sitting in close proximity to his cage. Due to an unusual incident where the infant got his finger stuck in part of his enclosure furnishing, he required a finger amputation procedure and attempts to put the infant back with his sire began when the infant was strong enough to move around and feed himself at 3 months old. The introductions continued for 2 weeks, when the infant was given access to the sire's enclosure by opening the infant's cage door during the day and shutting the infant back in the cage at night. The first encounters show the sire sitting near the infant, watching him intently, but with no attempt to pick up or carry the infant. The infant also approached the sire cautiously; both vocalised to each other and ate in close proximity to one another. After a few days, the infant became more confident around the sire and was seen taking food out of the sire's hand; they also displayed physical contact by sleeping together every night and occasionally during play. At 4 months old, these positive social behaviours continued between the sire and infant so that the infant's satellite cage was eventually removed, and the infant was permanently left in with his sire.

Statistical analysis

A linear regression model was used to analyse the growth of titi monkeys up to 344 days old. An analysis of covariance (ANCOVA) was carried out under two models using SPSS version 25. A first model analysed the weight variance of the titi monkeys according to age only, whereas a second model analysed the weight variance of the infants by the interaction between their age and rearing methods.

Consequences

Growth rates of hand- versus parent-reared

Day had a significant effect on the weight of both hand-reared and parent-reared titi monkeys ($F_{(1,57)}$=919.3, P<0.001, np^2=0.942). When Day was controlled for, the parent-reared titi monkeys had significantly higher growth rates (457.9±9.3) compared to the hand-reared titi monkey (390.7±11.1) ($F_{(1,57)}$=19.804, P<0.001, np^2=0.258), although both displayed a linear growth rate (Figure 1).

Growth pattern

Both hand-reared and parent-reared titi monkeys displayed a linear growth rate; however, parent-reared titi monkeys showed significantly higher growth rates. This may be attributed to a variety of causes: natural parental investment allows titi monkey offspring access to a healthy amount of milk with natural nutritional consistencies of colostrum and fat levels. The replacement milk formula used may differ nutritionally to the dam's formula. A dam's milk naturally alters over time, but this is difficult to fully describe or to replicate when hand-rearing as detailed data are not available for the nutritional content of mother's milk in coppery titi monkeys.

Both the nutritional content of the milk and the feeding protocol used may have affected the growth rates of hand-reared individuals. There were no feeding recommendations available for titi monkey infants, so the feeding protocol developed was based around callitrichid hand-rearing guidelines. The guidelines suggest to start at a feed every 2 hours, whereas parent-reared individuals would be fed on an ad-lib basis; these differences may account for differences in growth rates, as the dam may have been offering

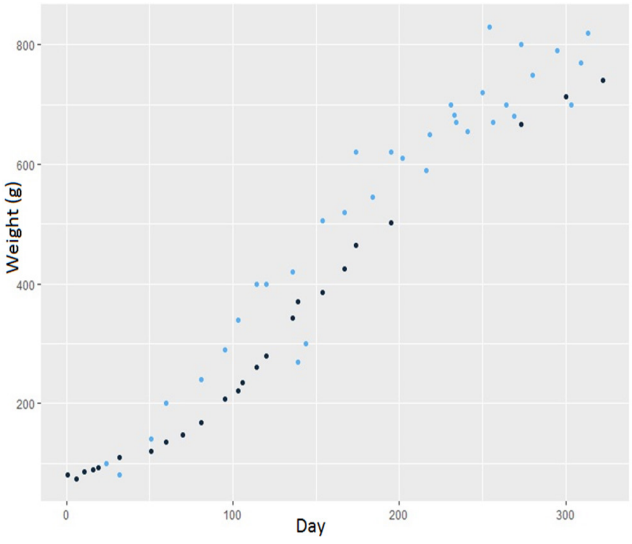

Figure 1. Growth curves of hand-reared (black, n=2 and parent-reared (blue, n=7) titi monkeys.

higher quantities of milk. Additionally, the callitrichid husbandry guidelines recommend feeding up to 20% the individual's body weight. From Day 10 of the hand-rearing process, keepers realised feeding 20% of the individual's body weight was potentially hindering growth, as the infant still appeared hungry after feeds. Keepers therefore started to offer up to 25% of the individual's body weight, if the infant was still showing signs of hunger, for example, mouthing at the syringe. Based on the growth rates presented from Day 1 to Day 322 of development, future hand-reared titi monkeys could be trialled at a slightly higher feed level relative to the animal's body weight, but this should be very closely monitored given that the infant in the current study continued to grow and survive. This recommendation coincides with the hand-reared infant's 6-month health check where he was given a body condition score of 3/9. Lower growth rates in hand-reared offspring may have also been affected by health problems rather than, or in addition to, nutritional differences in milk formula and feeding protocol. During the hand-rearing process, there were periods of ill–health, including weakness following a finger amputation, dehydration, constipation, diarrhoea and signs of aspiration which may negatively affect growth rate if infants are unwell and less willing to feed.

Socialisation

The socialisation protocol shows unique insight into reintroducing a hand-reared male coppery titi offspring to his sire after dam mortality as this information has not been previously published. Attempts to put the infant back on the sire began at 3 months with visual, olfactory and auditory contact from Day 9. Although formal statistical analysis was not appropriate, the results suggest that like other primate species, the sooner the infant is put back in its natal group, the more likely they are to accept the offspring. Mixing the infant at 3 months, however, still allowed both the infant and sire to develop a social bond which was demonstrated through social behaviours including eating together, sleeping together, play and sitting in close proximity to one another. The infant also learnt a range of new vocalisations after being mixed with his sire and they would call to one another. However, in the presence of keepers the infant was people-focused.

Based on these introductions, it is recommended that hand-reared coppery titi monkey offspring should be mixed back with their natal groups as soon as the infant is able to access to food to maximise the chances of the natal group accepting the offspring; in this case, acceptance by the sire is required, to carry and care for the offspring as his own. This could be achieved in the future by training the sire to station train to allow keepers access to the sire, to feed the infant on the sire's back. This would need to be assessed on a case-by-case basis depending on the health of the offspring and the temperament of the male, and, if deemed suitable, training should begin from the day hand-rearing begins. Based on this protocol, initial numbers of feeds can be dropped from 12 every 2 hours, to eight every 3 hours, from 2 weeks of age. From this point, the sire's behaviour towards the offspring and the sire's training should be assessed and when keepers have confidence that the sire trusts the keepers in close proximity during station training, they could then trial mixing the offspring.

Blood results

At 6 months old, the hand-reared individual at BZG received its first health check by vets (Table 5).

Alkaline phosphatase is high compared to tests on other primates; however, 1,309 U/L is still considered within the normal range for a growing animal. All other blood results are within expected result range. Hand-reared individual was body scored 3/9 (moderately underconditioned).

Table 5. Blood results of hand-reared individual at six months old (R. Killick, personal communication, 13 January 2018; ZIMS 2018).

Test	Result	Expected result (based on closes species match)
White blood count (WBC)	$4.7 * 10^9$ cells/L	2.0-9.3
Red blood count (RBC)	$5.39 * 10^{12}$ cells/L	3.85-6.98
Haemoglobin (HGB)	13.7g/dl	9.0-17.6
Hematocrit (HCT)	42%	28.3-58.1
Neutrophil	46%	20.1-76.0
Lymphocytes	48%	22.0-77.5
Monocytes	5%	0.2-8.0
Alkaline phosphatase (ALKP)	1,309 U/L	279
Calcium (Ca)	2.39 mmol/L	2.4
Phosphorous (Phos)	2.49 mmol/L	2.77

Discussion

This paper provides the first evidence-based practice of hand-rearing the coppery titi monkey in captivity. In this case, parent-reared titi monkeys had significantly higher growth rates compared to hand-reared individuals; however, hand-reared individuals still displayed a linear growth rate and continued to grow throughout hand-rearing and post weaning. Veterinary health checks performed at 6 months old confirmed blood results to be within normal range for the species, including white blood cell count (WBC), red blood cell count (RBC), haemoglobin (HGB), hematocrit (HCT), neutrophil, lymphocytes, monocytes, alkaline phosphatase (ALKP), calcium (Ca) and phosphorus (Phos) following the above hand-rearing protocol with no nutritional deficiencies. Based on this finding, it is advised that the protocols described in this report have the potential to be used in future for successful hand-rearing titi monkeys. However, from the growth rate recorded and 3/9 body score evaluation we suggest to trial offering daily feeds at 25% of the individual's body weight compared to the 20% currently recommended for hand-rearing callitrichids (Schiffmann et al. 2017; R. Killick, personal communication, 13 January 2018). Further research with a bigger sample size is needed to determine the cause of differences in growth patterns in hand-reared versus parent-reared coppery titi monkeys in captivity, and to ascertain whether the difference will have a negative impact on individuals overall. The socialisation protocol employed highlights the importance of early reintroduction to the parent or conspecifics for good development of the infant in line with socialisation protocols for other hand-reared primate species (Quintard et al. 2017). This case study was based predominantly on one individual, so future work should seek to apply this protocol to other instances of hand-rearing. Development of an appropriate species-specific hand-rearing protocol will contribute to successful hand-rearing of infants in the future if necessary and if recommended by the EEP Coordinator. Future investigation is needed to explore the causes of neonatal mortality in captive coppery titi monkeys, as is research to identify requirements for breeding success in captivity to help eliminate the need for hand-rearing. Nevertheless, this protocol documents the first detailed account for hand-rearing *Callicebus* and can serve as a guideline for future cases.

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