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Management and Population Status of Kihansi Spray Toad *Nectophrynoides asperginis* in Captive Breeding Facilities in Tanzania

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Abstract

Kihansi spray toad *Nectophrynoides asperginis* was discovered in 1996 in Kihansi gorge. The toad was declared extinct in the wild in 2009 due to habitat alteration. Before its extinction, 499 individuals were flown to the United States of America zoos in 2000 for captive breeding. In 2010, the University of Dar es Salaam, and in 2011 the Kihansi captive breeding facilities were established in Tanzania. The intention was to breed, and reintroduce the toad back to the natural environment in the gorge. The founder populations for the two facilities were sourced from the USA zoos. This study addresses captive management practices, and population status of the toad at the two facilities from 2013 to 2019. Toad counts were carried out once every two months. There was a significant increase in the number of toads over the years. The increase was associated with suitable management practices. Success in captive breeding at the facilities led to the removal of 3,236 individuals from the University for restocking the Kihansi facility and for reintroduction, and 1,273 individuals from Kihansi facility for reintroduction. The establishment of the two facilities played a major role in both the *ex-situ* and *in-situ* conservation of the toad.

Keywords: Kihansi spray toad, extinction, captive breeding, population, reintroduction

Introduction

Kihansi spray toad history

Kihansi Spray Toad *Nectophrynoides asperginis* was discovered in Kihansi gorge that is located in south-central Tanzania in an area of less than 4 Ha in 1996 (Poynton et al. 1998). The toad is an ovoviviparous amphibian species in the family Bufonidae that gives birth to live new ones (Channing et al. 2006, Lee et al. 2006). It depends on sprays of water in form of mists for its survival. No other known amphibian species lives in such conditions. The habitat where the toad was discovered was put under pressure following the construction of the Kihansi hydroelectric power dam that led to the diversion of over 90% of the water flowing down the gorge in 2000 (Hirji and Davis 2009). The diversion of water caused the spray wetlands in the gorge to become considerably drier (LKEMP 2004, Rija et al. 2011).

The population of Kihansi Spray Toad (KST) in the gorge during its discovery was estimated at 20,000 individuals (Norplan 2002, Channing et al. 2006). After the diversion of the water the number of toads decreased due to the desiccation of habitats in the gorge caused by the diversion of water. This necessitated the establishment of the artificial sprinklers in 2001 to imitate the natural sprays. Initially, the sprinklers lead to the increase in the number of toads but suddenly between 2003 and 2004, the number of toads decreased sharply. No toad was sighted or heard in the gorge after they

disappeared (LKEMP 2004, Lee et al. 2006, Krajick 2006). Following the disappearance of the toad in the gorge it was declared extinct in the wild in 2009 (Channing et al. 2009). The decline and disappearance of the toads in the gorge was linked to a number of factors including habitat alterations caused by the construction of the hydropower facility (LKEMP 2004, Lee et al. 2006, Krajick 2006), an outbreak of chytrid fungus (Weldon 2006) and pesticides (Lee et al. 2006, Rija et al. 2011). The decline and the extinction of the toad in the wild could also be associated with processes that regulate population size including density dependent processes. Juliano (2007) pointed out clearly that when the density of a certain population rises above equilibrium level there are changes in the processes that produce decreases in birth rates or increases in death rates.

Captive breeding programme

Captive breeding programme for the Kihansi spray toad was initiated in 2000 after the Kihansi hydropower project came into operation. This programme was initiated to serve as an assurance population for the toad following the alterations of the natural habitat in Kihansi gorge. A total of 499 toads from the gorge were flown to the United States of America zoos for captive breeding. In USA zoos the toads were housed at Bronx zoo, USA before they were distributed to other five zoos including Detroit, Oklahoma, Toledo, Maryland and Buffalo. The number of toads in all zoos in the USA decreased to less than 70 individuals by February 2004 (Lee et al. 2006). All the toads at Oklahoma zoo died, and the remaining individuals at Maryland and Buffalo zoos were sent to Bronx zoo, and those at the Detroit zoo were sent to Toledo zoo (Lee et al. 2006). The deaths were linked to diseases, parasitic infections and husbandry challenges.

Efforts to solve the challenges encountered, including the mitigation plan agreed by the Government of Tanzania and the World Bank led to the increase in the number of toads in the USA zoos (Lee et al. 2006). By 2008, there were 500 toads in Bronx and Toledo zoos (IUCN 2007) and by 2010 the population was over 6,000 individuals (Khatibu et al. 2011). Following successes in captive breeding of the toad in the USA zoos efforts were initiated to establish breeding facilities in Tanzania for the intention of releasing the captive bred toads back to the wild environment in Kihansi gorge. Two facilities were constructed, one at the University of Dar es Salaam (UDSM KST captive breeding facility) in 2010 and another at Kihansi, Morogoro (Kihansi KST captive breeding facility) in 2011.

The aims of establishing the two facilities in Tanzania were to ensure the long-term *ex-situ* conservation of the toad and to have assurance population for supporting the reintroduction of the species into the wild. As of October 2019 there were four KST captive breeding facilities in the world including the Bronx and Toledo zoos in the USA, and the UDSM and Kihansi in Tanzania. The first batch of 100 individuals was received at the UDSM facility on 11th August 2010 from Bronx zoo. This batch was used to test for the suitability of the UDSM facility for breeding the toad using locally available resources and expertise. The second batch of 200 individual toads from Toledo zoo was received at the UDSM facility on 25th February 2011 for testing the suitability of the substrates in the Kihansi gorge for reintroduction of the KST back to the original habitat. The third batch of 500 individual toads was received on 11th May 2012 at the UDSM facility for captive breeding. The toads currently maintained at the UDSM facility were the generations of these 500 toads.

Since the establishment of the UDSM and Kihansi captive breeding facilities in Tanzania only a few studies have documented the biology and conservation status of the toad in the facilities (see Makange et al. 2014, Rija et al. 2014, Nahonyo et al. 2017). No detailed study has addressed the population status, husbandry and management challenges

encountered for the nine years of raising the KST in captivity in Tanzania. The study further describes the role of the facilities for reintroduction of the toad in their natural habitat in the gorge. The information generated will form a basis for informed management and conservation of Kihansi spray toad in captivity.

Materials and Methods

Description of the captive breeding facilities

The Kihansi spray toad (KST) captive breeding facility at the University of Dar es Salaam (UDSM) is located between Botany and Geology departments' buildings at S 06°46.743', E 039°12.304'. It is a concrete structure that measures approximately 12 m long x 6 m wide with seven compartments. The first compartment houses a standby generator, the second is the entrance verandah that is also used for propagating crickets and springtails (insects that are a source of food for the toads), and the third is a culture room for fruit flies (the main food of the adult toads), that is also used as an office. The fourth compartment is a shower room for biosecurity reasons, and the fifth is a preparatory room before entering the two rooms used to breed the toads. The preparatory room is furnished with water filtration system for filtering water, water storage tanks for storing filtered water and mist pumps for pumping the filtered water to the mist nozzles for spraying the toads. The sixth and the seventh rooms hold the terraria that are used to breed the toads. Each terrarium is constructed of transparent glasses and is furnished with mist nozzles, ultraviolet bulbs for light and heat source, live plants for the toads to attach, and water outlet for taking out the sprayed water. Each terrarium has a width of 30 cm, the length of 30 cm and the height of 40 cm (i.e., volume of 36,000 cm^3) with a capacity of holding 50 individual toads. The UDSM facility has a capacity of holding approximately 3,000 toads at the current settings.

The facility at Kihansi is located at Mlimba ward in Morogoro region, southern Udzungwa Mountains at S 08°37.562', E 035°50.799'. It is a concrete structure with three partitions: the first one is used to hold the toads, the second is used to propagate fruit flies and springtails and the third one is used to breed the toads. The Kihansi captive breeding facility has a potential to accommodate approximately 5,760 toads, i.e., 50 toads per terrarium at the current setup.

Each toad room in the two facilities was installed with two air conditioners (one is a standby air conditioner in case of breakdown of the other) to keep the rooms cool. The toads do well in cool microclimate with temperature ranging from 16 to 26 °C (Lee et al. 2006). Water filtration systems in the two facilities remove toxic substances from the water that are potentially harmful to the toads. These substances include ammonia, nitrates, chlorine and heavy metals, all of which may harm the toads (see de Wijer et al. 2003, Adlassnig et al. 2013). In addition, each facility was installed with reserve water tanks to keep water, and a standby generator as a source of power during power cut from the national grid. Bio-security protocols were enforced in the facilities prior to entering the insect and toad breeding rooms to reduce the possibility of spreading diseases and disease causing microbes to the facilities. These protocols included showering, dressing proper clothes (laboratory coats and shoes), and sterilising feet in the troughs located at the door entrances.

Assessment of Kihansi spray toad population in the facilities

Toad counts in the terraria in the two facilities were carried out once after every two months throughout the study period from February 2013 to October 2019. Total count method was used in which all toads in the terraria were counted. It involved removing all the toads from each terrarium and temporarily holding them in perforated dell cups, count them, clean the terrarium and

thereafter return the toads to the same enclosure. During terrarium cleansing the UV lights and mist pumps were switched off to avoid short circuit. Adult Kihansi spray toads were relatively big with mustard-yellow colour with dark-brown lateral lines, whereas the juveniles were relatively small and dark-grey in colour. During toad counting, information on the conditions of the toads and records of toads found dead was obtained. In addition, ambient temperature and humidity in the rooms were measured by using indoor Onset Hobo data loggers at an interval of one hour.

Data analysis

Toad count records in the two facilities after every two months during the study period were summarised using descriptive statistics. A test by Kolmogorov-Smirnov indicated the data to meet the assumptions of parametric distribution ($p > 0.05$), thus one-way analysis of variance (ANOVA) was conducted to test for the differences in toad counts between different months and years. The statistical analyses were performed by Graph pad package (Graph Pad Software Inc, USA) and set to alpha = 0.05.

Results

Kihansi spray toad population trend at the UDSM captive breeding facility

This study shows the trend of Kihansi spray toad population at the UDSM captive breeding facility from 2013 to 2019. The initial population was 535 individual toads in February 2013 and the highest number recorded was 3,005 in February 2016. There was a steady and a significant increase in the mean number of toads as shown in Figure 1 ($F = 67.518$, $p < 0.05$). There was no increase in the number of toads at the facility after 2016 (Figure 1) but was maintained at between 2,000 and 2,500. The number of toads maintained at the facility was limited by the carrying capacity of the facility. All extra individual toads above 2,000 were removed and used either to restock the Kihansi facility or for the release in the natural environment in the spray wetlands in Kihansi gorge. As of October 2019 the total number of toads from the UDSM facility used to restock the Kihansi facility was 903 and those used for reintroduction in the spray wetlands was 2,333 (Table 1). The monthly mean number of individuals of the KST at the UDSM facility ranged from 1,454 in April to 1,877 in February (Figure 2). These results suggested more births at the beginning of the year. The monthly comparison of the number of toads at the facility showed no significant difference ($F = 0.120$, $p > 0.05$).

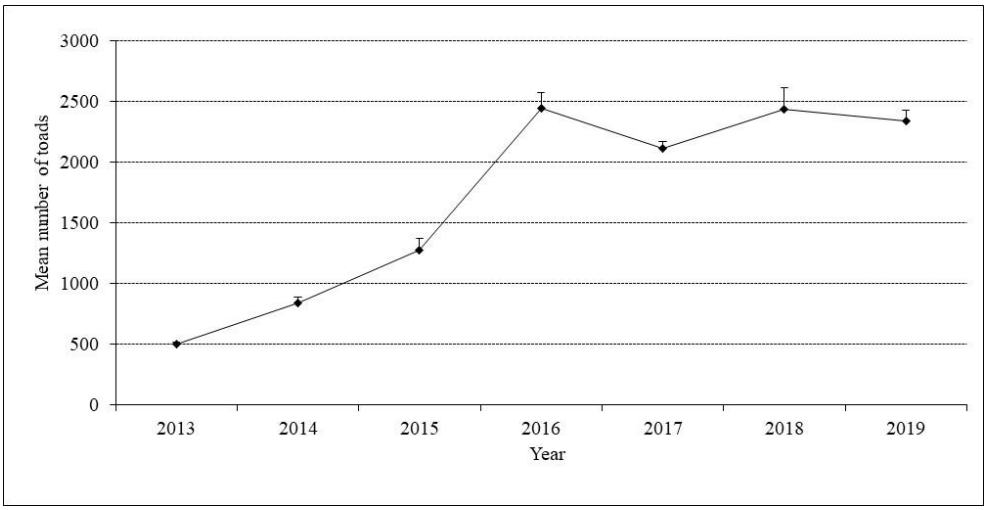

Figure 1: Population trend (Mean ± SE) of Kihansi spray toads at the UDSM captive breeding facility during different years.

Table 1: Total number of toads removed from the UDSM captive breeding facility for restocking the Kihansi facility and for reintroduction in the Kihansi gorge

Date	Toads sent to Kihansi facility	Toads sent to Kihansi Gorge
Apr-2016	703	0
Jul-2016	0	700
Dec-2016	0	150
Mar-2017	200	0
May-2018	0	210
Oct-2018	0	300
Jan-2019	0	950
Apr-2019	0	23
Total	**903**	**2333**

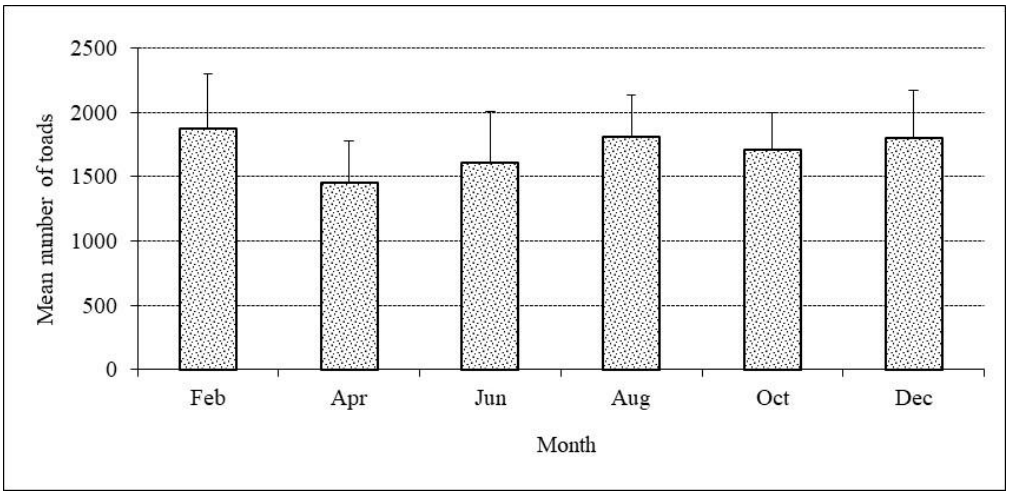

Figure 2: Mean number (± SE) of Kihansi spray toads at the UDSM facility during different months.

Kihansi spray toad population trend at the Kihansi captive breeding facility

The initial population size at the Kihansi captive breeding facility at the beginning of the study period was 348 individuals in February 2013 and the maximum number of toads recorded was 2,815 in April 2019. The facility contributed 1,273 toads for the reintroduction in the Kihansi gorge (Table 2). The trend shows the mean number of toads at the facility decreased to 170 individuals in 2015 before increasing significantly to its maximum in 2019 ($F = 76.363$, $p < 0.05$, Figure 3). The highest number of toads at the facility was recorded in April although the difference between months was not significant ($F = 0.4756$, $p > 0.05$, Figure 4).

Table 2: Total number of toads removed from the Kihansi captive breeding facility for reintroduction in the Kihansi gorge

Date	Toads sent to Kihansi Gorge
May 2018	106
July 2018	215
July 2018	252
January 2019	300
June 2019	400
Total	**1273**

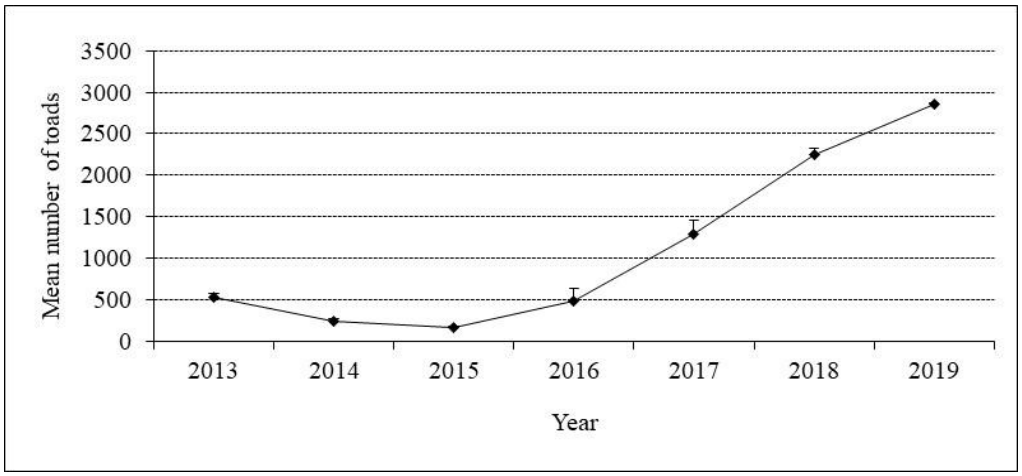

Figure 3: Population trend (Mean ± SE) of Kihansi spray toads at the Kihansi captive breeding facility during different years.

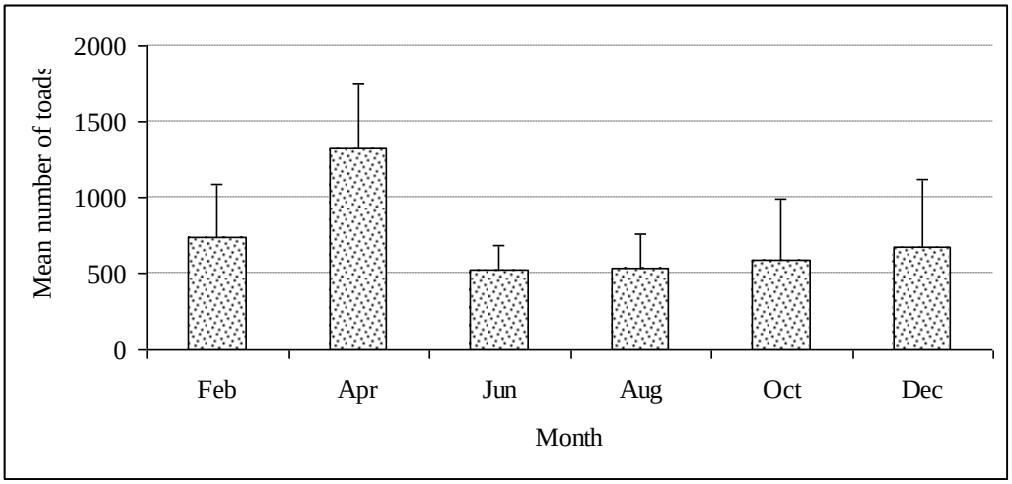

Figure 4: Mean numbers (± SE) of Kihansi spray toad at Kihansi captive breeding facility during different months.

The role of the UDSM facility in holding the toads on transit from the USA zoos to Kihansi gorge

Since its establishment in 2010, the UDSM captive breeding facility played a major role of receiving and temporarily holding Kihansi spray toad populations from the USA zoos that were on transit to the natural environment in the gorge. As of October 2019, 12,800 toads were received and temporarily kept at the UDSM facility before being transported and released in Kihansi gorge spray wetlands (Table 3). The toads on transit were fed and acclimatized for at least three days before they were transported and released in the gorge. The maximum amount of time the toads on transit were kept at the facility was two months.

Table 3: Toads received from the USA zoos and retained at the UDSM captive breeding facility before being transported to Kihansi gorge for re-introduction

Date	Total number of toads received	Source
August 11, 2010	100	Bronx and Toledo zoos
February 25, 2011	200	Bronx and Toledo zoos
May 11, 2012	500	USA zoos
June 23, 2012	1,000	Bonx and Toledo zoos
October 19, 2012	2,000	Bonx and Toledo zoos
March 2013	2,000	Bonx and Toledo zoos
January 23, 2015	1,500	Bonx and Toledo zoos
August 24, 2016	1,100	Toledo zoo
November 10, 2016	1,000	Bronx zoo
May 3, 2017	600	Toledo zoo
April 2018	1,000	USA zoos
December 2018	800	USA zoos
May 2019	1,000	USA zoos
Total	**12,800**	

Kihansi spray toad population management at the UDSM and Kihansi captive breeding facilities

Daily activities at the two facilities include maintenance of suitable conditions in the enclosures, and rooms, and feeding the toads. Air conditioning system was used to keep the rooms cool at all the time. The temperature in the rooms ranged from 14.9 °C to 24.6 °C although in the enclosures the temperature may vary with gradient, being relatively warmer near the ultraviolet bulb. The lowest temperatures were recorded at between 04:00 and 06:00 hours and maximum temperatures were recorded between 13:00 and 17:00 hours, and during temporary periods of electric power cuts. Mist nozzles were connected to the mist pumps by water tubes that were used to spray water in form of mist to the toads. Relative humidity in the toad rooms ranged from 37.75 to 92.03% with an average of 62.70%. Relative humidity in the enclosures could be slightly higher due to the presence of mist from the mist nozzles. The toads were fed after every one day by feeder insects including fruit flies, pinhead-size crickets and springtails. These insects were bred at the facility in which the fruit flies were harvested 10 days after preparing the culture. The pinhead-sized crickets were harvested 10 days after incubating the eggs in an incubator and the springtails were harvested 14 days after setting the medium. The harvested insects were dusted with mineral and vitamin supplements before feeding the toads. The newborns were fed with springtails, and the juveniles, and adults were fed with fruit flies and pinhead-size crickets. During feeding the mist pumps were switched off to allow the toads to feed for one hour before they were switched on.

No medical related issues were observed at the UDSM facility although at the Kihansi facility there was an outbreak of chytridiomycosis, a fungal disease caused by chytrid fungus *Batrachochytrium dendrobatidis* in two occasions, one in 2012 and another in 2016. During the 2012 disease outbreak, 304 toads died whereas 45 toads died in 2016. Other major challenges at both facilities include electric power cutoff, failure of air conditioning system and water cut off from municipal supply. The standby generator and the water storage tanks installed at the facilities served the purpose of alleviating the problems.

Discussion

KST population trend at the UDSM and Kihansi facilities

The study showed a steady increase in the number of toads at the UDSM facility to its maximum in the year 2016. The mean number of toads did not increase after 2016, but was maintained at between 2,117 and 2,436 toads. The number maintained at the facility was determined based on the carrying capacity of the enclosures used to breed the toads. The UDSM facility at its current set up (as of October 2019) can hold only up to 3,000 toads in a closed population where there is no immigrating or emigrating animals. The major determinants of the population size are therefore the birth and death rates. In such situations, the population changes exponentially until the environment establishes a constant limit to the growth of populations (Price 1999, Turchin 2001). The concept of exponential growth can be clearly elucidated by ecological models that show that initially the population grows exponentially but the rate gets smaller and smaller as population size approaches its maximum due to limited resources (Hixon 2008). The initial population size at the facility was 535 toads but due to births it increased to a maximum number of 3,005. Ecological models show that growth rates get small when the population carrying capacity is approached or reached. To avoid getting to slower growth rates, all extra toads above 2000 individuals were taken either to the Kihansi facility for restocking or to Kihansi gorge for release (Table 1). The removal of all extra individuals in the cages could have contributed to the growth trend we observed at the UDSM facility. Maintenance of the population size at an optimum level prevents processes that may act to regulate population as reported elsewhere (see Klein 1968, Hixon 2008). These processes among others, include parasitism, intra-specific resource competition, intra-specific interference, and other social interactions (Juliano 2007).

At the Kihansi facility, the trend showed the decrease in mean numbers of toads from 2013 to 2015 before the population increased to its maximum in 2019. Despite these increases the facility has not been able to attain its maximum carrying capacity due to some captive breeding challenges including the outbreak of chytridiomycosis at the facility (Makange et al. 2014), and captive breeding challenges. These challenges are thought to have contributed to the decrease in the number of toads observed between 2013 and 2015. Pathogens feature prominently among other factors as causal processes that are affecting population dynamics (Juliano 2007).

Monthly comparison of the numbers of the toads indicated that the population size at the UDSM facility was highest in February (3,005 individual toads) followed by the other months. These results suggested that more births occurred in between December and February. The results coincide with those at Bronx zoo in which the peak births occurred between December and April (Lee et al. 2006). The same trend was observed at Kihansi facility in which the highest population size was recorded in February followed by April and other months. A study on the reproductive conditions of another species in the same genus, i.e., a robust forest toad (*Nectophrynoides viviparus*) also indicated that a large proportion of births occurred after December (Ngalason 2010).

Conclusion and recommendations

This study assesses the population status and management practices at the UDSM and Kihansi captive breeding facilities. The number of toads at the UDSM facility increased over time to its population carrying capacity of 3,000 toads. At Kihansi facility, the highest number attained was 2,815, which is below its carrying capacity. The findings indicated that the Kihansi spray toad population increased steadily when the number of individuals was above 500. For the sharp increase in the population size at the

facilities, a certain number of individual toads should be maintained, preferably above 1,000. The study calls upon the expansion of the UDSM facility to ensure the supply of suitable number of individuals of the toad for reintroduction.

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