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Reintroduction of the Kihansi Spray Toad *Nectophrynoides asperginis* Back to its Natural Habitat by Using Acclimatizing Cages

Charles A. Msuya[1*] and Nassoro Mohamed[2]
[1]*Department of Zoology and Wildlife Conservation. University of Dar es Salaam. P. O. Box 35064, Dar es Salaam, Tanzania.*
[2]*Kihansi Catchment Conservation and Management Project, National Environment Management Council, Tanzania.*
[*]*Corresponding author, e-mail: cmsuya@uccmail.co.tz; Co-author e-mail: nassudsmzoology@gmail.com*

Abstract

The Kihansi Spray Toad (*Nectophrynoides asperginis*) is considered to be extinct in the wild. Captive breeding populations exist in Bronx and Toledo zoos in USA and in two captive breeding facilities in Tanzania. Efforts to reintroduce the species back to its natural habitat at Kihansi Gorge wetlands have become a long process. Both *ex-situ* and *in-situ* experiments have revealed promising outcomes but when the toads are freely (hard) released in the wetlands they disperse widely and detection becomes difficult. Cages for acclimatising the toads before hard release have been constructed in two of the Kihansi Gorge spray wetlands. Factors such as density dependence, predators, food availability and diseases have been identified of concern to successful reestablishment of the species in its natural environment. The use of large cages (60 m^2), close monitoring and partial control of the factors as a new approach has shown promising results at present and for future reintroduction processes of the Kihansi Spray Toad.

Keywords: Kihansi Spray Toad, Reintroduction, Hard release, acclimatizing cages.

Introduction

Kihansi Spray Toad (KST) is an ovoviviparous amphibian species in the family Bufonidae. This endemic species to Kihansi Gorge was discovered in 1996 inhabiting a unique habitat of wetlands created by spray from the falls of the Kihansi River, within the Udzungwa Mountains in Tanzania (Poynton et al. 1998). Diversion of Kihansi River for hydropower production in 1999 greatly reduced the flow of water from the initial 16 m^3/s to less than 2 m^3/s (NORPLAN 2002). This caused desiccation of the wetlands and altered vegetation composition of the unique habitat resulting into encroachment of the KST predators and forest plants to the wetlands (LKEMP 2004, Channing et al. 2006). In attempt to rescue the declining population of KST in-situ, a total of 499 toads were taken for captive breeding in USA (Bronx and Toledo zoos). In addition to that, an artificial sprinkler system was installed in 2001 in three wetlands; Upper Spray Wetland (USW), Lower Spray Wetland (LSW) and Mid-Gorge Spray Wetland (MGSW) to mimic natural conditions and help restore the habitat (NORPLAN 2002, Mtui et al. 2008). Environmental conditions improved and the KST population recovered quickly but the population declined dramatically in the year 2003 until 2004 when it was confirmed no individual toad existed in the Kihansi Gorge wetlands (Msuya 2004). Various factors attributed to the decline, including habitat alterations and desiccation, chytrid fungus, pesticides, and potential predators such as safari ants and crabs (Carey and Alexander 2003, Corn 2005, Channing et al. 2006). The Kihansi Spray Toad was then declared extinct in the wild by the IUCN in 2009 (IUCN 2009). Fortunately, the spray toad survived and was prolific in captivity in USA and in facilities constructed in Kihansi and Dar es Salaam, Tanzania, and therefore inspired attempts to reintroduce the species back to its natural habitat in 2012. The Upper Spray Wetland has been the main focus of reintroduction due to its relatively big size and had historically hosted about 89% of the spray toad population (Poynton et al. 1998, World Bank 2002). In October 2012, 2000 Kihansi Spray toads were freely reintroduced in the Upper Spray Wetland (hard release). Succeeding reintroductions were carried out in the USW in March 2013 (1500 toads), February 2015 (1483 toads) and July 2016 (700 toads). There has always been a shortage of toads available in captive breeding facilities to meet the demand for experiments and releases in the wild.

The spray toads once hard released in the wetlands disperse widely and therefore their detection becomes difficult and their fate cannot be established (Tuberville and Buhlmann 2014, Ngalason et al. 2015). It was therefore important to design acclimatizing enclosures/cages that would limit dispersion and control some factors that may have attributed to poor survivorship of the released toads population. The main objective of the study was to successfully reintroduce the Kihansi Spray Toad to its natural habitat through controlling some of the known causes of population decline after reintroductions. Acclimatizing cages were then constructed and several factors were taken into consideration. The factors in consideration included; size of cage that could allow population density between 17 and 23 animals per square meter (the historic density of KST in USW before diversion of water); potential predators such as crabs, snakes, spiders, driver ants, birds and other frogs by screening and removing most of them from cages; food availability by placement of cages in identified sites with high density of prey and other food items and by feeding the toads prior to their release; disease prevalence by swabbing some animals for chytrid test and collecting faecal for gut microbes overload analysis and treating when necessary; natural disasters by placement of acclimatizing cages in areas not likely to be affected by landslides and strong natural spray from the falls. The 60 m^2 size cages were designed to allow observers to walk in and conduct experiments freely without causing much damage to the cage environment and the released toads. Acclimatising the animals is meant to be a compromise and intermediate step between life in captivity where there is husbandry and wild where natural life exists.

Materials and Methods

Cage design and construction

Three cages were designed with the aim of controlling the area of dispersal of the released Kihansi Spray Toads and to minimize predation pressure. Two cages (Cage No.1 and Cage No. 2) were constructed in the Upper Spray Wetland (USW) and Cage No. 3 in the Lower Spray Wetland (LSW). Construction work started in early October 2016 and lasted for 6 weeks. The size of each cage is 10 m length × 6 m width × 2 m height, covering an area of 60 square meters. Cages were designed to accommodate 1000 animals and allow researchers to walk inside (Plate 1). Cage frames are made from ¾ inches galvanized steel water pipes fixed with “flexible joints” easy to set and dismantle when the need arises. Cage walls and roof are covered with aluminium wire mesh. The first 1/2 m of height is enclosed by a fine aluminium wire mesh of 1/16 inches to avoid toad lets from getting out while 1/8 inches aluminium wire mesh covers the entire 2 m height and roofing. The wire mesh prevents the toads from escaping and predators from entering, while allowing the preys for KST to enter. Three walkways with stepping stones were constructed, one inside cage along the centre and two outside on both sides of the cage to minimize stamping on delicate soil and vegetation.

Plate 1: Acclimatizing cage 1 in Kihansi Gorge Upper Spray Wetland.

Habitat stabilization in cages

Construction of cages had observable negative impacts on habitat, especially vegetation and soil which provide microhabitat for toads and micro-invertebrates food for KST. It was necessary to leave the cage environment to stabilize for at least 2 weeks, to allow for vegetation recovery and invertebrates population to stabilize. Search and removal of potential predators (crabs, spiders, snakes and other amphibians) continued during construction and stabilization of cages. Special funnel traps for crabs were designed and made locally from 1/8th inch aluminium wire mesh and five traps were set in each cage. Large and small crabs were hand collected or trapped and were frequently removed from the cages before introducing the toads.

Introducing spray toads in cages and monitoring

The first batch of 892 spray toads from Toledo Zoo was received in the Kihansi Gorge on 28th October 2016, of which 25% of adult toads were marked with a red elastomer colour to help identifying the cohorts and source of the animals. Animals were kept in temporary cages inside the wetland and were fed with fruit flies *Drosophila* sp for 3 hours prior to release to the acclimatizing cages. This helped to minimize deaths associated with starvation due to inexperience in hunting wild food in their first and second day after release. A second batch of 100 toads and third batch of 969 toads from Bronx zoo were introduced in cage 2 in the USW on 19th November 2016 and 11th December 2016, respectively. Twenty five percent of adult toads of the second and third batches were marked with orange and green elastomers, respectively.

Day and night monitoring were conducted for seven days after release; thereafter monitoring frequency was reduced to every three days for the following three weeks. After four weeks of close monitoring, the number of the toads in cages continued to be checked once every two weeks. Monitoring protocols involved 2 observers inside cage walking along middle walkway then to the outside to observe animals that could not be spotted from the inside middle walkway. Counting of the toads was conducted for at least one hour. Number of toads observed was often higher during the night than day; therefore after seven days of close monitoring, the protocol was restricted to nights only. Microclimate conditions, mainly temperature and relative humidity were automatically recorded by using a HOBO mobile Data logger Version 1.4.2, Model MX1101 mounted in water protection container placed low in vegetation about 15 cm from ground inside cages.

Disease diagnosis

A small sample of toads was collected randomly in each cage and individuals were placed in deli cups for overnight and faecal specimens were collected the following morning. Direct faecal analysis technique was employed by mixing faecal debris with a drop of dilute saline solution on a microscope slide and examined by using a Bresser LCD microscope.

Predator control

Entrance of predators to cages was controlled by wire mesh fence of the cage and careful operation of cage entry doors. Some fresh water crabs trapped inside cages during construction and those manoeuvring to enter through dug tunnels were trapped and removed from cages by using locally designed funnel traps. Five traps were set per cage and different baits were used, including dried beef, dried fish and occasionally maize flour cake.

Data analysis

Monitoring data for the KST is presented as the number of individuals counted per sampling effort. Sampling effort is calculated as the time spent searching in hours multiplied by the number of persons involved (Timed Constrained Searching). The difference in number of toads observed between day and night was determined by two sample unpaired t-test performed using Statistical Package Service and Solution (SPSS) v 20 software (International Business Machines Corporation, IBM).

Results

Micro-climate (temperature and relative humidity)

Generally, the Kihansi Gorge environment was very dry between October 2016 and February 2017 with ambient temperature ranging between 25 °C and 34 °C. However, temperature in the wetlands was cooler due to spray and wind. A minimum temperature of 16.15 °C was recorded around midnight, while day temperature rose to a maximum of 31.84 °C in some days (Table 1). Day time temperature in the USW was rising above 20 °C starting from 9.00 hrs and started to drop below 20 °C at around 17.00 hrs. The KST remained deep in vegetation, with temperature cooler by one or two degrees in day hours and came out at night when it was dark and cool. Relative humidity was constant at 100% during nights, and ranged between 58.40% and 99.99% during the day.

Table 1: Summary statistics of air temperature and humidity in the USW cage No.1. Source: Hobomobile MX 1101 data logger set (28 Oct–17 Jan 2017).

Parameter	Samples	Interval (hrs)	Minimum	Maximum	Average	Std Dev
Temperature (°C)	970	2	16.15	33.52	21.44	3.09
Relative humidity (%)	970	2	58.40	99.99	97.14	6.75

Status of the released Kihansi Spray Toads

The rate of detecting the toads was high on the first day of release as most animals were on the move to discover better spots to hide. From day two up to day six, the toads were poorly seen on vegetation possibly due to movement of observers during the day and night counts. Detection of toads increased from day seven and thereafter when the toads were getting acclimatized to the new environment and when the frequency of observation was reduced to once every 3 days and then once every two weeks. A total of 108 and 117 toads (equivalent to 54 and 56 toads per person-hour) were observed during the day and night, respectively within 24 hours after release. In cage 2, the total number of toads observed during day and night surveys within 24 hours after release were 110 (55 toads per person-hour) and 255 (128 toads per person-hour), respectively. Most of the Kihansi Spray Toads remained in dense vegetation cover during the day and ascended to the top during the night. Higher numbers of toads were recorded during the night in both cages (Figure 1 and Figure 2). There was significant higher number of toads observed during the night surveys than during the day surveys in cage 1(Unpaired t-test, $t = 3.684$, $df = 12$, $p = 0.0031$) and cage 2 (Unpaired t-test, $t = 14.642$, $df = 12$, $p < 0.0001$). Therefore, to minimize disturbance and stress to the toads, it was decided that only night surveys should be conducted.

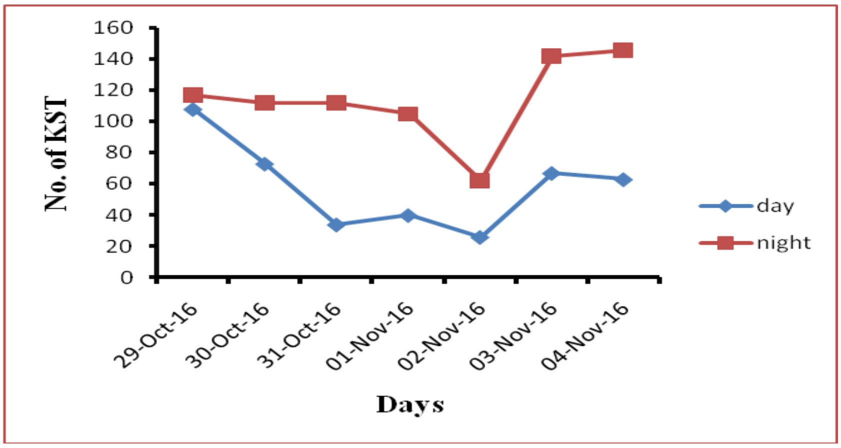

Figure 1: Number of toads detected during seven days (day and night surveys) in cage 1.

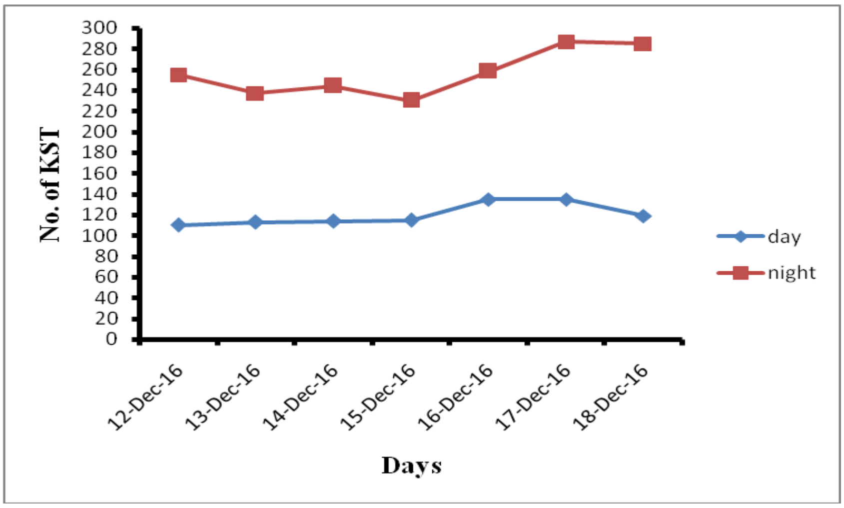

Figure 2: Number of toads detected during seven days (day and night) surveys in cage 2.

By using acclimatizing cages, it was possible to follow closely the trends of toads and the associated factors hindering their re-establishment in the wetlands. In cage 1, the number of toads detected increased to 247 (124 toads per person-hour) after 2 weeks and then it gradually declined when the animals were not treated for gut parasites overload (Figure 3). In cage 2, the number of toads observed over one month remained stable after early treatment of gut parasites with 0.001% of 1% ivermectine (Figure 4).

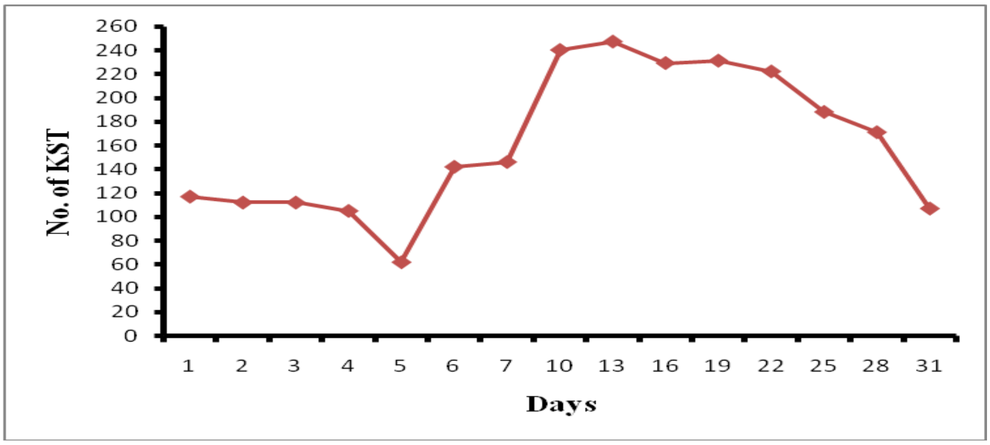

Figure 3: Number of toads detected over the period of one month after release in cage 1 (28th October 2016 to 28th November 2016).

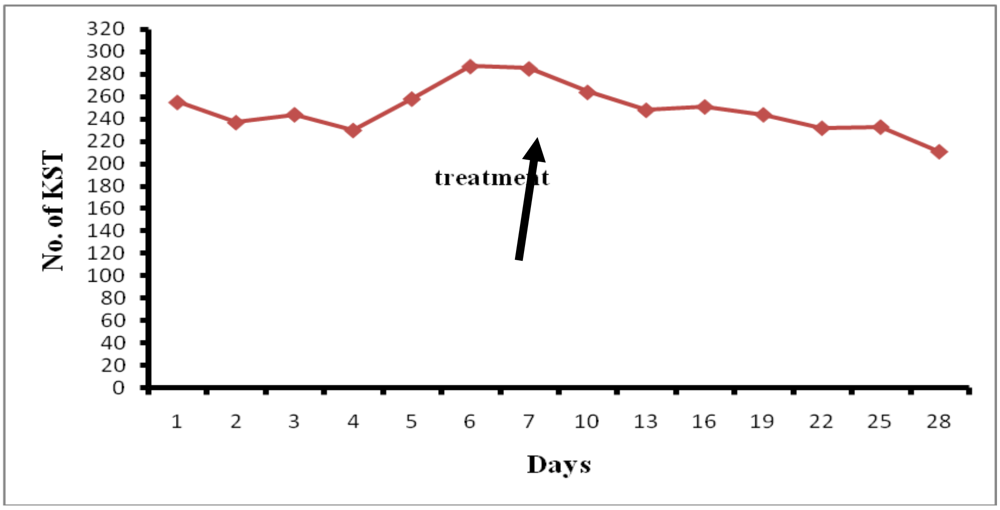

Figure 4: Number of toads detected over the period of one month after release in cage 2 (12th December 2016 to 08th January 2017).

Potential predators of Kihansi Spray Toad

Several potential predators of the Kihansi Spray Toad including fresh water crabs (*Potamonaetus* sp) and house snakes were removed from the cages during construction. However, it was not possible to remove all the predators; some hid in dense vegetation matrix during construction and were not spotted. The removal of predators from the cages is ongoing exercise requiring day and night checks. One house snake *Lamprophis fulliginosus* was removed from cage 1 during construction and another one was collected in cage 2 and dissected just to find out that gut contents did not have remains of KST. Tree frogs, including a spiny reed frog, *Afrixalus fornasini*, spotted reed frog *Hyperolius puncticulatus* and Mitchell's reed frog *Hyperolius mitchelli* (Plate 2) were also spotted and removed from cage 1. *H. mitchelli* males were calling intensively from mid-March till mid-June in the USW and at least 10 animals were picked and one egg clutch deposited on a leaf of *Brillantansia* *madagascariensis* was removed from cage 1. Several Southern Torrent frogs *Arthroleptides yakusini* were found clinging on the outside of fence of cage 1 and on vegetation near cage 1. A flock of slender-billed starling *Onchognathus tenuirostris* were seen perching on the sprinkler lines very close to the wetland near cage 2 and were observed searching on wetland vegetation for food.

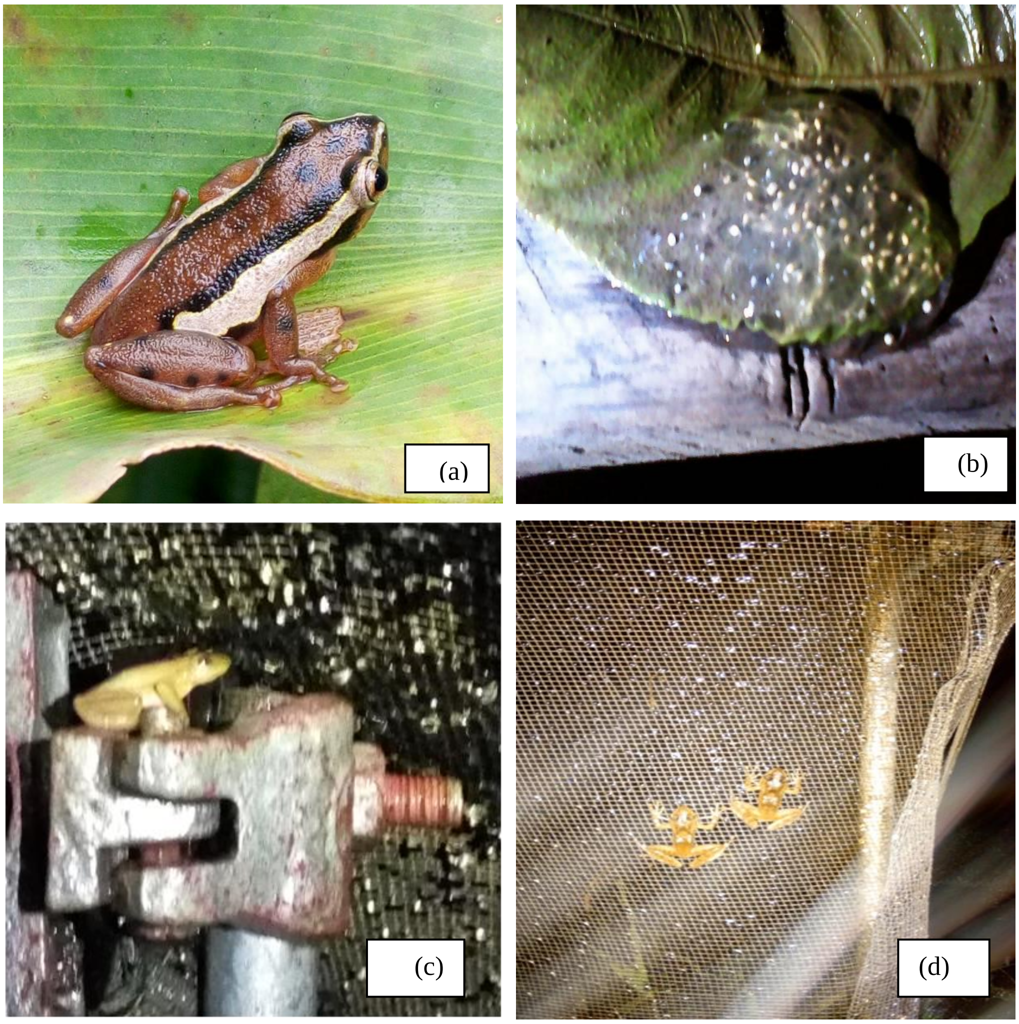

Plate 2: Species of amphibian observed in cages (a) Mitchell's reed frog *Hyperolius mitchelli*, (b) Eggs of *Hyperolius mitchelli* deposited on a leaf *Brillantansia madagascariensis*, (c) Spiny reed frog, *Afrixalus fornasini* in cage 1, and (d) Torrent frogs *Arthroleptides yakusini* clinging on outside of cage 1 fence.

There may be two species of fresh water crabs in Kihansi Gorge spray wetlands that have not yet received serious taxonomic attention. The small forms are purple in colouration and are aggressive in hunting for the KST in the wetlands. The larger forms have brown carapace and orange pereopods and are common in the gorge forest as well as the wetlands. The larger forms are easily caught in traps. In one of the crab traps, the larger form was recorded to have preyed on the smaller forms when they both happened to be caught in the same trap. At least two toads were found partly eaten by crabs (one with a leg amputated) and a third one was rescued after been dragged away on fence by a small crab (Plate 3). The crabs were controlled by removal from acclimatizing cages by opportunistic searching and trapping. The traps proved to be effective in luring crabs.

Plate 3: Observation of predators and events of predation (a) KST been dragged away by a small crab, (b) KST hind limb amputated possibly by a crab, (c) Baited crab trap set in cage 1, and (d) Fresh water crab caught in one of the traps.

KST diseases and parasites load

Some of the toads in the cages were found to be physically weak despite the abundance of prey items observed in the cages. Faecal tests conducted on 16th and 19th Dec 2016 in cage 2 and 1, respectively revealed relatively high gut parasites load (Plate 4) which were 4+ Strongyloides per field of vision through Bresser LCD microscope. All the toads that could be observed in cage 1 and 2 were collected and treated by bathing in 0.001% of 1% ivermectine for 6 minutes. Toads tested after 2 weeks following treatment had a low level of parasites indicating that the treatment was effective. The health of the toads subsequently improved in both cages and the rate of population decline was lowered.

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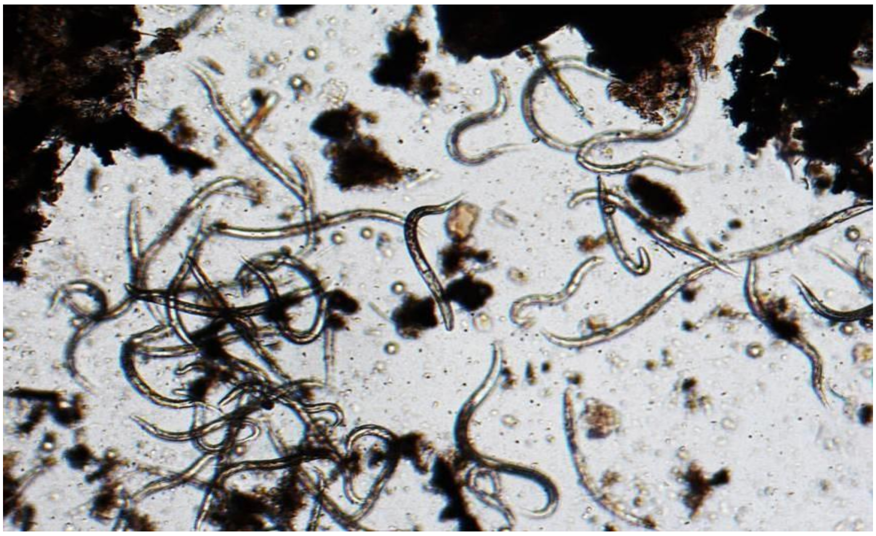

Plate 4: Strongyloides observed under microscope during fecal test.

Discussion

Cage design and performance

The Kihansi spray wetlands are really wet throughout the year receiving water spray from natural fall as well as artificial spraying system. Acclimatizing cages constructed from rust proof metal was important to minimize introduction of polluting material in the wetlands. Galvanized water pipes and aluminium fencing material used have so far maintained for eight months without showing signs of deterioration. However, the fence needs to be cleaned regularly to remove scum formed by spray water to ensure free movements of invertebrates foods for Kihansi Spray Toads across the mesh. The toads were retained in enclosed environment well isolated from predators outside the enclosures. Most of the invertebrates foods for KST, *Afrosteles distans*, *Afralebra* sp, *Ortheziola sp* and others documented before (NORPLAN 2002, Tamatamah and Suya 2007) were moving across the cage freely.

The fine aluminium mesh (1/16 inch) was rust proof in mud and was effective in preventing small babies of toads from escaping the enclosure while maintaining free movements of micro-invertebrates including collembolans and other known foods of the KST juveniles. Young KST once born occupy the lower strata of vegetation matrix and ground level where food of their size is abundant. This age group is hardly found on open vegetation, and therefore is hardly encountered during most surveys. Juveniles emerge to upper vegetation strata when they approach late juvenile stage and colour pattern of adult KST has started to appear and sub adult stage is reached.

The larger wire mesh screen (1/8 inch) size was effective in preventing adult toads from leaving the cages and preventing other animals including frogs, snakes, large spiders and crabs from entering. After one month of vegetation recovery, there was mingling of some plants from both sides of the cages across the 1/8 inch mesh easing small invertebrates' movements across, and therefore, replenishing and assurance of food supply to the reintroduced KST. Leaf hoppers, *Afrosteles distans* were found to be moving freely across the mesh when flushed from vegetation surrounding the cages.

Status of the released Kihansi Spray Toads

There was a general decline in number of toads found in both cages 1 and 2 due to several reasons. One of the reasons is that, if the recruitment of juveniles is not yet to take place, the population will obviously decrease with time due to deaths caused by environmental factors such as predation, diseases and ageing of individuals. The life span of the KST in captivity which may as well apply in the wild is four years. Some of the released toads were gravid and had given birth to youngs which were always difficult to spot in the vegetation matrix. At least few newborns were spotted but could not help to balance the number of declining adults. Both experimental cages indicated the KST population decline of up to 50% after two and half months following their release. Similar trends had been observed to toads released in soft and hard release experiments conducted in July 2012 and October 2012 respectively (Tuberville and Buhlmann 2014). Factors such as adaptation to new environment, predation and diseases were responsible for the declines. In this study, reintroduced toads were observed closely by using acclimatizing cages instead of letting them wonder freely in the entire wetland. We are convinced that predation and gut parasite overloads are the two major factors that are responsible for the decline of the toads both in cages and the entire wetlands at Kihansi Gorge. Treatment of the KST for gut parasite overloads by bathing in ivermectine minimized the declining trends and proved to be important for the survival of the toads in the cages. Treatment of toads is necessary at the period of acclimatization before they can acquire natural immunity.

Detection of the Kihansi Spray Toads was primarily influenced by the activity patterns which varied over the wet and dry seasons and over day and night. Our findings which are limited to the period between October 2016 and April 2017 covered a period of extreme dryness and very wet conditions. Kihansi Gorge environment was the driest of all the years of our field survey experience in the area. The long rains season in the region ended in mid-May 2016 and there were no short rains which normally occur between October and December. The long spell of dry season affected activity patterns and breeding of other amphibians in the Kihansi Gorge. Occupiers of the spray wetlands including the KSTs were not affected by the variations in humidity in their microhabitat, but rather by the temperature rise especially during the day. The spray toads were responding to this by staying deep in vegetation matrix and ascended to the top in the night when the ambient temperature was low. Day ambient temperature in the wetland was rising far above optimum level for KST (20 °C) and when combined with the risk of been exposed to predators, the toads remained deep in vegetation all day and started to come out immediately after sunset. Other studies have shown that, in the USW and LSW there was variation in temperature and relative humidity depending on the location and time of the day (Mutagwaba and John 2014), but these parameters remained within the range that can be tolerated by the KST for its survival. Relative humidity ranges between 60 and 100% is common. Temperature rise above 30 °C can readily kill the toads as experienced in the captive breeding facilities in Tanzania (Msuya, per comm). Occasionally, ambient temperature in the USW rose to above 30 °C (data from data loggers) during the day for few hours but that in deep vegetation was usually 1 to 2 °C lower. This partly explains why the behaviour of the KST is to hide deep in vegetation during high temperatures and cannot readily be detected in day light. The number of toads detected during night hours was higher than during the day. Such pattern is consistent to all other amphibians in Afro-tropical environment, where activities are restricted to the periods of high humidity or rainfall to avoid desiccation (Vitt and Caldwell 2009). The Kihansi Gorge spray wetlands receive high amounts of spray, thus temperature is usually lower and humidity is always high, and therefore, the risks of desiccation is minimal. In this study, the majority of the KSTs were hiding under vegetation during the day as a strategy to avoid predators and strong sunlight, which was also observed in other surveys (Ngalason et al. 2015).

Feeding of the toads prior to the release helped to minimize deaths associated with starvation due to inexperience in hunting wild food in their first and second days after release. This period of acclimatization is associated with a lack of knowledge on location of new food items, refuge and predators (Bertolero et al. 2007). This naivety to the environment was expected to lower the survivorship before the toads adapt to the environment. In the previous releases, the KST were fed in captivity and transported to the Gorge; and once they arrived, they were released in the wetland without being fed again. The protocol developed to feed the captive bred KST before release worked well and stressed animals had time to relax and were ensured to have enough energy and food reserve to start them off while exploring their new environment.

Controlling population decline of the Spray Toads in acclimatizing cages

It was possible to control the predators inside acclimatizing cages than in the entire wetland. Crabs are able to dig tunnels and access the toads inside cages if they do not meet obstacles, i.e., fence wire mesh dug in the soil, although properly constructed cages should constantly be checked for emerging leakages and unwanted species entry. Although all these were considered during the construction, both acclimatizing cages had small crabs and few large ones trapped inside either as adults or juveniles and laid eggs. Crab funnel traps were effective in catching both small and large cabs when appropriate baits were used.

Acclimatizing cages proved to control entry of other amphibians and reptiles commonly encountered in the USW, with exception of those trapped inside the cages during construction. One *Afrixalus fornasini* was found and removed from the cage. More than 10 *Hyperolius mitchelli* were heard calling from inside cage 1 and a clutch of eggs was found and removed from the cage. Tree frogs found in the cage could have been trapped inside as juveniles or as in their earlier stages of development. However, *H. mitchelli* may not be considered as potential predators to the adult KST due to relatively smaller size (*H. mitchelli* up to 32 mm of snout-vent length and *N. asperginis* up to 30 mm). However, if left to breed inside the wetland, they may prey on small KST youngs and compete for food and space. Torrent frog (*Arthroleptides yakusini*) due to its relatively large size is considered a potential predator of the Kihansi Spray Toad (Channing et al. 2006), but has successfully been controlled from reaching the acclimatizing toads inside cages.

Diseases and parasite loads

It has been observed in captive breeding facilities that toads have relatively high gut parasites loads after 3 months without treatments. The high gut parasite loads have been associated with mortality in captivity; therefore captive bred toads are regularly been treated to control gut parasites. Treatment protocols established in the USA zoos captivity (Lee et al. 2006) by dusting prey items with crushed fenbendazole (Intervet panacur), proved to be successful. After six weeks of monitoring in cage 1 and one week in cage 2, it was revealed some of the toads were physically weak despite the abundance of preys observed in the cages. Faecal tests conducted confirmed high accumulations of gut parasites and therefore treatment was necessary. It was not possible to treat the toads with medicine dusted on prey items due to continuous spray in the wetland and the chance of the toads to feed on medicated prey was not certain. Therefore, it was decided to bath them in 0.001% of 1% ivermectin solution. This method has proven to be successful in Bronx and Toledo zoos (Borek 2007). The health of the treated toads improved and other faecal tests conducted 2 weeks later revealed low levels of gut parasite loads. However, the KSTs are reported to be prone to relapses of the infestations and treatment is required regularly (Lee et al. 2006). It is also important to diagnose and find the effects of other internal parasites in addition to the gut parasites.

The acclimatizing cage provides a useful arena for the treatment of the reintroduced toads which would be very difficult if the toads are released in the entire wetland. It is important to examine the health and treat the toads reintroduced in cages at least on monthly basis. The treatments of toads in the gorge were done with caution while bearing in mind about effects on breeding success and intention to expose the released toads to new pathogens so that they may acquire self-immunity.

Changes in wetland ecology

The Upper Spray Wetland and Mhalala Wetland have been invaded by tree frogs, mainly *Hyperolius mitchelli and Afrixalus fornasini*. Both species normally visit the nearby wetlands with standing water bodies to breed over the long rainy season between March and May. They gather in vegetation surrounding common breeding ponds, and once there is assurance of water to support their tadpoles' developments, males move first to breeding microhabitats and calls are intensified and are accompanied by females (Msuya 2001). *H. mitchelli* was recorded in the USW for the first time in 2015 (Ngalason 2015) and observations were estimated to be one frog per man hour searching effort. Our observation which coincided with the peak of 'tree frogs' breeding season recorded 66 calls and 5 animals, equivalent to 5.6 calling males per man hour in the USW. This number suggests a rapid increase of *H. michelli* populations in the wetland over a period of two years. The calls were spread in almost the entire wetland and one clutch of fertilized eggs collected in acclimatizing cage 1 confirmed that the wetland is capable to support breeding and propagation of the species. This species is known to occur in lowland forest and bushland in Tanzania and Mozambique (Schiøtz 1999) and use pond water for tadpoles' developments. Such microhabitats were not originally supported by the Kihansi Gorge wetlands system. It can be argued that; (1) ecological surveys and sprinkler maintenance activities in the wetland cause trampling which create small water pools capable of supporting tadpoles' developments, otherwise much of sprinkled water drain away readily due to steep terrain nature of the wetlands; (2) the species is in adaptation to having their tadpoles develop in constantly sprayed wetland environment. The success of *H. mitchelli* and other tree frogs breeding in the wetland is a new threat to the KST, a native species in the Kihansi Gorge spray wetlands. However, calls of tree frogs in wetlands ended in mid-June and there is no proof that the frogs had gone back to surrounding unsprayed vegetation. It is common for juveniles to stay behind and grow to sub-adult stage before dispersing away (Msuya 2001). The invasion of Kihansi Gorge wetlands by tree frogs is threatening the future of the KST through food and space resources competition in the near future.

Conclusion and recommendations

Acclimatizing cages constructed for use at the Kihansi Gorge Spray wetlands have met the proposed objectives and the idea can be employed in similar situations elsewhere. The materials used in the construction of the cages have lasted for eight months and are still in good conditions; however, regular maintenance of the cages by checking leakages around doors and cleaning of scum formed on fence is essential.

We are strongly convinced that pathogens overloads and diseases are the main factors responsible for the KST not reestablishing readily in the wild. Therefore, it is proposed that reintroduced toads in the cages should be treated regularly whenever gut parasite overloads are high to control diseases while they acquire immunity and survive to reproduce. Meanwhile, there is need to improve existing captive breeding populations as a conservation measure and source of experimental animals and for successful releases in the wild.

The invasion and colonization of other species of amphibians, especially *Hyperolius mitchelli* in the wetlands has turned out to be another challenge to the reestablishment of the Kihansi Spray Toads in the Kihansi Gorge. Predictable challenges include predation, competition for food and space and transfer of diseases. Understanding the changing wetland ecology is therefore critical for the survival of the KST during reintroduction and for its future conservation. Research activities in the Kihansi Gorge wetlands may change the ecology of the spray wetlands, all activities need to be assessed.

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