Tanzania Journal of Science 45(4): 686-696, 2019 ISSN 0856-1761, e-ISSN 2507-7961

Abundance and Distribution of *Afrosteles distans* an Indicator of Food Availability for the Kihansi Spray Toad (*Nectophrynoides asperginis)* in the Kihansi Gorge

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Abstract

Survivorship of the Kihansi Spray Toad in the wild depends on various factors such as environment, habitat, food availability, diseases and predation. This study assessed the abundance of *Afrosteles distans* Linnavuori 1959 as an indicator of food availability for the Kihansi Spray Toad. Samples were collected from sprinkled (A, B, C, D, E and F) and non-sprinkled (G and H) plots in the upper spray wetland in the Kihansi gorge during the dry period in 2007, 2008, 2015, 2016 and 2018. There was a significantly higher abundance of *A. distans* in sprinkled than non-sprinkled plots. ANOVA showed significant differences in the abundance of *A. distans* among plots in each year. ANOSIM (Global R = 0.756, $p = 0.001$) showed a significant difference between plots with an increasing trend (E, F and D) and plots with a decreasing trend (A, B and C). Cluster analysis resulted in 62% similarity of plots A, B and C and 68% similarity of plots E, F and D. Favourable conditions for the *A. distans* were not homogenous among the sprinkled plots and continued to vary over time. Abundance levels of *A. distans* and occurrence of other invertebrates indicated that food for the KST was sufficiently available in the gorge.

Keywords: *Afrosteles distans*, Abundance, Kihansi Spray Toad, KST, Kihansi gorge

Introduction

The Kihansi Spray Toad (KST), *Nectophrynoides asperginis* was discovered in the Kihansi Gorge, Tanzania in 1996 and formally described as a new species in 1998 (Poynton et al. 1998). In 2005 KST was listed as a critically endangered species by the IUCN because of its highly restricted geographic distribution, as it is only known to occur from four wetland meadows which were supported by mists from Kihansi River waterfalls within the Kihansi Gorge, Tanzania (Poynton et al. 1998). In 2009, KST was listed as extinct in the wild (EW) by the IUCN Red list of threatened species (IUCN 2015). The decline and extinction of the KST followed the cascades of environmental changes which emanated from the construction of the Lower Kihansi Hydroelectric Power (LKHP). Following the commissioning of the LKHP, the original wetland habitat was altered by approximately 95% (Doggart and Milledge 2001) including a significant decrease of the gorge's sprays which are critical for the survival of KST. There were significant and rapid changes in the wetland vegetation (Quinn et al. 2005), soil and the distribution of the toads was immediately noticeable. Vegetation from adjacent dry areas started to invade the former spray meadows, mosses declined almost by 95%, diversity of insects collapsed, and only 2000 toads were left alive (Krajick 2006). In 2000 the Government of Tanzania with support from the World Bank and other organizations initiated various mitigation measures such as the establishment of a captive breeding program for the KST in case the species became extinct in the gorge (NORPLAN 2002 and Lee et al. 2006). Besides, sprinkler systems were installed in the wetlands in order to mimic the spray once produced by the waterfall which offered the unique Kihansi gorge micro-climate particularly humidity, temperature and vegetation cover. Monitoring of several important parameters vital for re-establishment of KST in the wild such as microclimate, vegetation (habitat), food availability, diseases and predation was also recommended. Spray toads are generalist feeders; however, studies show that insects compose about 88.5% of the diet of KST including 18 orders of major groups of Diptera, Homoptera, Hymenoptera, Collembola, Coleoptera and Trichoptera (Channing et al. 2006). *Afrosteles distans* (Homoptera: Cicadellidae) and *Ortheziola sp*. were the commonest identifiable species in the gut contents of the KST (Channing et al. 2006), and therefore, were recommended as indicators of food availability in the gorge. Re-establishment guidelines of the KST in the wild further emphasized on the use of *A. distans* as an indicator of KST food availability because of its high abundance in the gorge is the principal diet constitute of the KST, and ease of identification. *A. distans* is closely associated with the spray wetlands, the habitat with significant biodiversity values which is also known to be changing in response to Kihansi hydropower project activities. This study assessed the abundance and trends of *Afrosteles distans* among the eight permanent plots established in the Kihansi USW in relation to the sprinkler system.

Materials and Methods

Study area and Experimental design

The Kihansi gorge is located along the eastern escarpment of the southern Udzungwa Mountains. The gorge is approximately 4 km long and 0.5 km wide (Lee et al. 2006) and had maintained the unusual microclimatic conditions by the presence of the Kihansi River and waterfalls. It is known to be the home for the Kihansi Spray Toads (*Nectophrynoides asperginis*) which occur in the unique habitat of four spray wetlands; the Upper Spray Wetland, Mhalala Spray Wetland, Mid-gorge Spray Wetland and Lower Spray Wetland. This study was conducted in the Upper Spray Wetland of about 6090 m^2 (S 08°35'0.03" E 035°51'0.05") located 900 masl (Figure 1). The upper spray wetland is fitted with a sprinkler system as one of the mitigation measures in efforts of wetland restoration. Under the sprinkler system, there are eight permanent plots (A-F) of 10 m x 10 m established where six of these plots A, B, C, D, E and F were fitted with sprinklers and two plots G and H were not fitted with sprinklers (control). Sprinkled plots A, B and C are closer to the waterfall than sprinkled plots E, F and G (NORPLAN 2002). The wetland substrate type is dominated by boulder and bedrock. Short grass and shrubs mainly cover the sprinkled areas, while the non sprinkled areas are mainly covered by shrubs and larger plants.

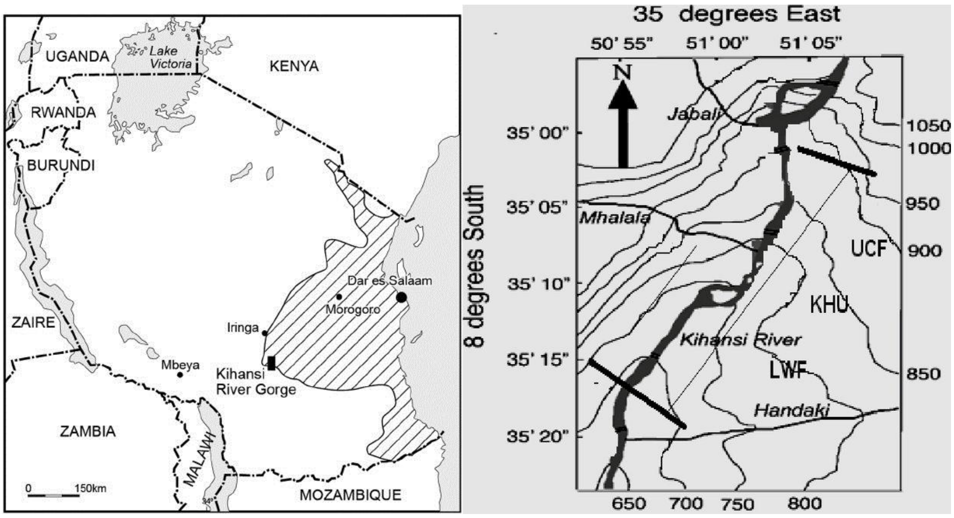

Figure 1: Map of the Kihansi gorge in southern Udzungwa Mountains, Tanzania showing the location of the study area (Adopted from Vandvik et al. 2014).

Sampling method
Samples were collected from vegetation using the beating vegetation method. A beating method is a suitable approach for estimating the numbers of insects that are easily dislodged when disturbed but do not fly away such as the cicadellids (leafhoppers). The method involves beating a vegetation a standard number of times and tapping the falling insects (Sutherland 1996, Southwood 1978). A 50 cm square frame was dropped on the vegetation to disturb the insects. Cicadellids jump when disturbed and the proportion of those that do so land back in the beating tray. The disturbed lodged insects were caught in a beneath plastic beating tray containing 50 ml of 70% ethanol. In each plot, 10 samples were collected from different vegetation spots where at each spot the square frame was dropped 3 times (triplicates). The contents of the dish were then strained and stored in a container containing 70% ethanol and transported to the University of Dar es Salaam Zoology laboratory for analysis. Samples were collected during the dry periods in 2007, 2008, 2015, 2016 and 2018.

Laboratory procedures
In the laboratory, samples were sorted and prepared for taxonomical analyses. Morphological identification under a binocular microscope was conducted using guides described in Ruppert and Barnes (1994), Gerber and Gabriel (2002) and Day et al. (2003). *A. distans* were identified and counted separately. Other invertebrates in the samples were identified to their major groups.

Data analysis
Mean abundance and standard deviation of *A. distans* were calculated for each plot in the five years of sampling. Higher and lower mean values among plots and years were also recorded. Simple bar graphs were used to show abundance across plots and years. Analysis of Variance (ANOVA) was used to compare the abundance of *A. distans* among the plots. Trend analysis was done for individual plots along the five years of study using linear analysis. Multivariate analysis was done to assess the degree of similarity among plots using Analysis of Similarity (ANOSIM) followed by pairwise testing to show the levels of similarly among the groups based on the observed trends. Cluster analysis

based on Bray Curtis similarity was used to visualize the groupings. A constrained multivariate analysis using canonical analysis of principal coordinates (CAP) was used to uncover patterns that are masked in unconstrained ordinations.

Results

The total numbers of *A. distans* in all plots were recorded as 567, 955, 707, 745 and 2075 for years 2007, 2008, 2015, 2016 and 2018, respectively. Other invertebrates collected in the samples were only identified to their major taxa group. Other invertebrates included groups of Araneae, Blattodea, Coleoptera, Collembola, Copepoda, Diptera, Heteroptera, Homoptera, Hymenoptera, Lepidoptera Lymnaeidae, Oligochaeta, Orthoptera, Phasmatodea and Succineidae.

Abundance of *Afrosteles distans* among plots

Mean abundance of *A. distans* was lower in the non-sprinkled plots with the highest value of (1 ± 1.41) and (0.2 ± 0.63) in plots G and H, respectively. Abundance was generally higher in the sprinkled plots however with variations among plots. Highest recorded abundance was in plot D (88.5 ± 38.68), E (52.5 ± 30.33) and F (38.88 ± 32.29) in the year 2018. Lowest abundance in sprinkled plots was recorded in the year 2015 in plots A (3.3 ± 3.68) and B (3.1 ± 1.79).

There were variations in the abundance of *A.distans* among plots which varied across different years (Figure 2). In 2007, higher abundance were recorded in plots A, B and C, while plots D, E and F had a lower abundance. In the following year, 2008, plot B recorded a higher abundance of the same levels as plots A, B and C. In years 2015 and 2016, the abundance of *A. distans* increased in plots D, E and F, while the abundance in plots A, B and C did not show any increase. In 2018, the abundance was higher in plots D, E and F and lower in plots A, B and C.

Single factor analysis of variance (ANOVA) showed a significant difference among all plots in 2007 ($F = 11.58451$, $p = 9.17E^{-10}$), 2008 ($F = 22.74806$, $p = 6.12E^{-16}$), 2016 ($F = 5.23383$, $p = 7.37E^{-05}$) and 2018 ($F = 19.5196$, $p = 2.2E^{-14}$). When only sprinkled plots were analysed, ANOVA resulted in significant differences among plots in 2007 ($F = 9.9535$, $p = 8.71E^{-07}$), 2008 ($F = 17.0114$, $p = 4.53E^{-10}$), and 2018 ($F = 14.86293$, $p = 3.67E^{-09}$). Plots did not show significant difference in 2015 among all plots ($F = 2.065513$, $p = 0.05848$), sprinkled plots ($F = 3.726512$, $p = 0.005703$) and in sprinkled plots in 2016 ($F = 1.257023$, $p = 0.295912$).

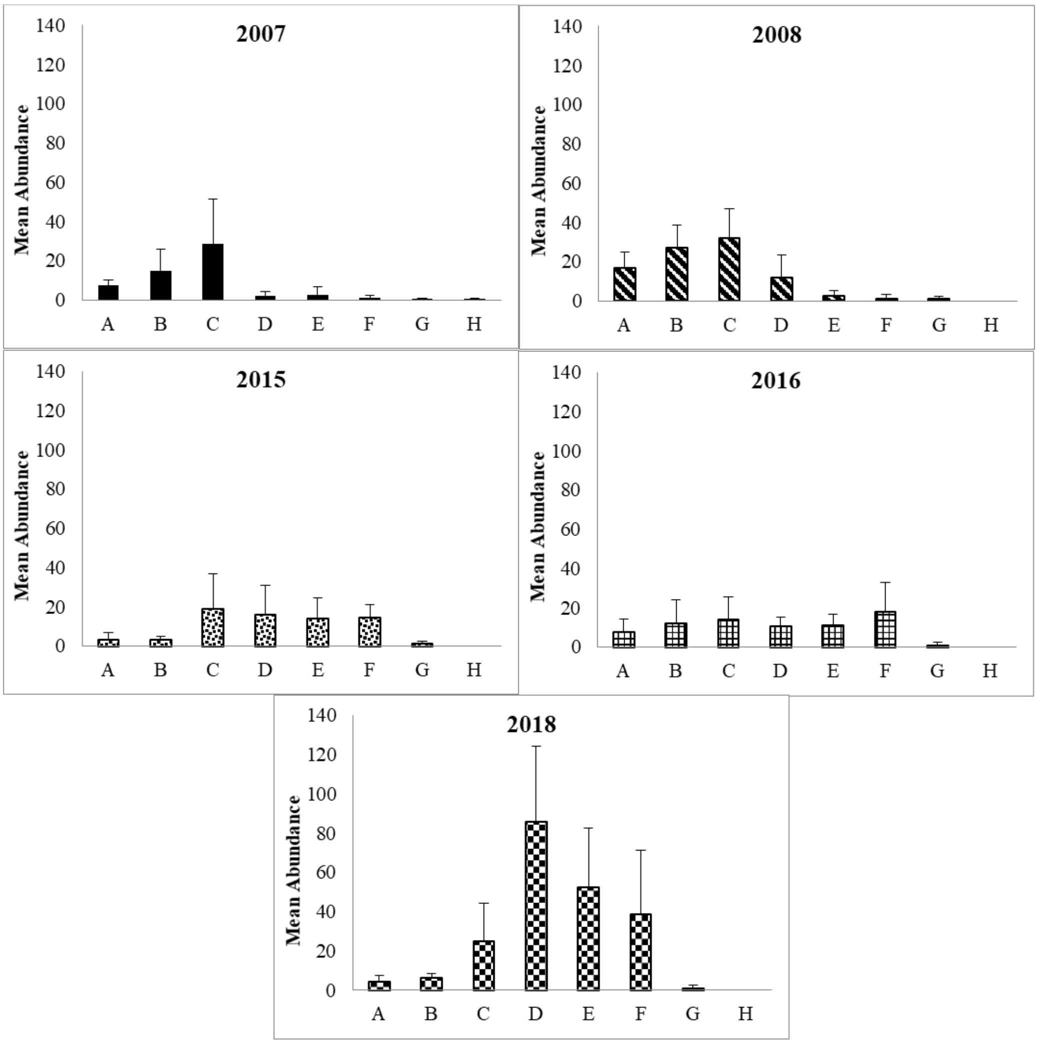

Figure 2: Mean abundance of *Afrosteles distans* in the sprinkled and non sprinkled plots in the Upper Spray Wetland of the Kihansi gorge as sampled in the dry period of 2007, 2008, 2015, 2016 and 2018. Error bars represent standard deviation.

Trends of abundance of *Afrosteles distans*

The change over time in the mean abundance of *A. distans* showed variations among sprinkled plots. Plots A, B and C showed a decreasing trend, however not significant (R^2 = 0.2081, 0.2994 and 0.3076, respectively). The mean abundance in these plots remained below 40 with low standard deviations. Plots D, E and F exhibited a reverse increasing trend with time. Linear regression resulted in R^2 = 0.5866 in plot D, R^2 = 0.6862 in plot E and R^2 = 0.8865 in plot F. Mean abundance spiked in 2018 in all the three plots (Figure 3).

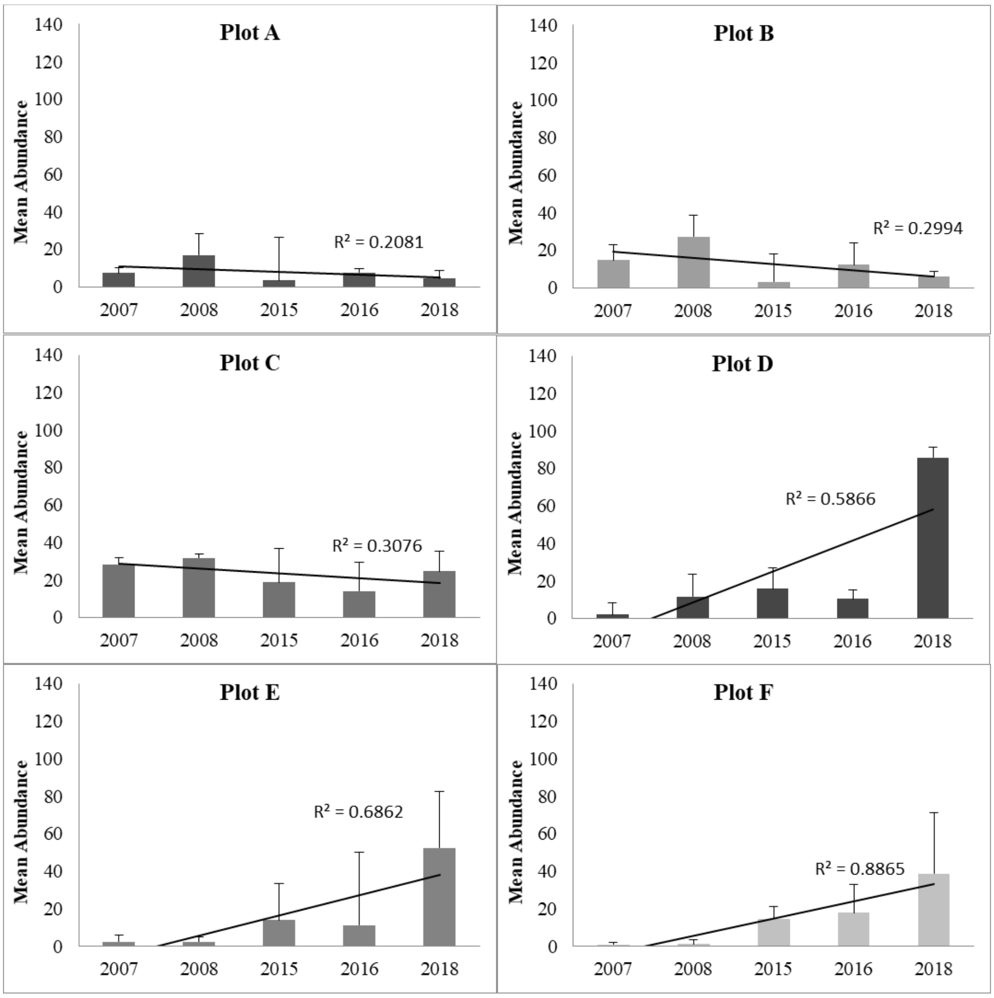

Figure 3: Linear trends in mean abundance of *Afrosteles distans* as recorded in the sprinkled plots in the Upper Spray Wetland of the Kihansi gorge as sampled in the dry period of 2007, 2008, 2015, 2016 and 2018. Error bars represent standard deviation.

Clustering of plots based on the abundance of *A. distans*

Statistical analysis of similarities (ANOSIM) using One Way Analysis with trend characteristic as a factor, showed a significant difference in mean abundance of *A. distans* among the plots with Global R = 0.756, p= 0.001. Pairwise testing indicated a significant difference in all three groups of trends in mean abundance (Table 1).

Table 1: Pairwise tests among groups of plots defined by the trend of the mean abundance of *Afrosteles distans* in the Upper Spray Wetland of the Kihansi gorge as sampled in the dry period of 2007, 2008, 2015, 2016 and 2018 (Increasing (D, E and F), Decreasing (A, B & C) and Non (G & H)).

Pairwise test				
Groups (Trends of mean abundance)	R Statistic	Significance Level %	Actual Permutations	Number >= Observed
Increasing, Decreasing	0.713	0.1	999	0
Increasing, Non	0.857	0.1	999	0
Decreasing, Non	0.891	0.1	999	0

Hierarchical cluster analysis resulted in significant different clusters with Cophenetic correlation R = 0.88048 (Figure 4). One cluster with 62% similarity includes samples from sprinkled plots E, F, and D which exhibited an increasing trend in mean abundance of *A. distans*. Another cluster with 68% similarity includes samples from sprinkled plots A, B, and C which exhibited a decreasing trend in mean abundance of *A. distans*. Two samples from plots E and D characterized with a decreasing trend clustered with samples of plots which characterized with an increasing trend.

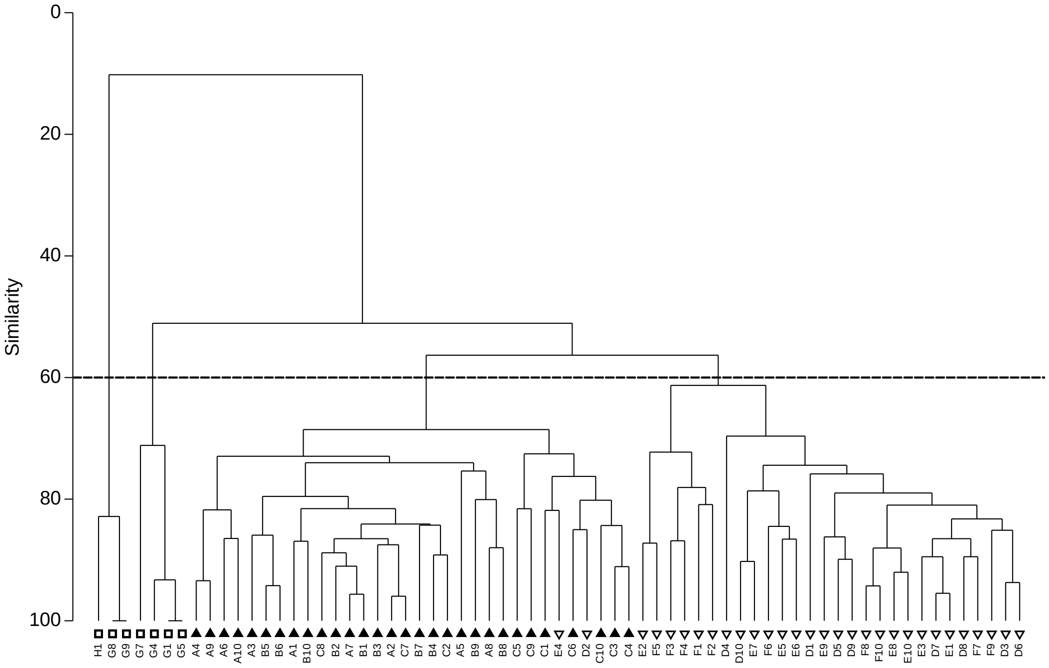

Figure 4: Cluster analysis of similarity of plots based on the mean abundance of *Afrosteles distans* in the Upper Spray Wetland of the Kihansi gorge as sampled in 2007, 2008, 2015, 2016 and 2018 (□ = no trend, ▽ = increasing trend, and ▲= decreasing trend).

Canonical analysis of principal coordinates was used to uncover patterns of distribution of *A. distans* that are masked in an unconstrained MDS ordination. CAP showed significant ordination of three groups of plots. The CAP correlation were 0.8929 and 0.8133 with diagnostics m = 4. Total correctness in the grouping was 95.52% and the misclassification was 4.48%. Misclassified samples were D4, D9 and E4 (Figure 5).

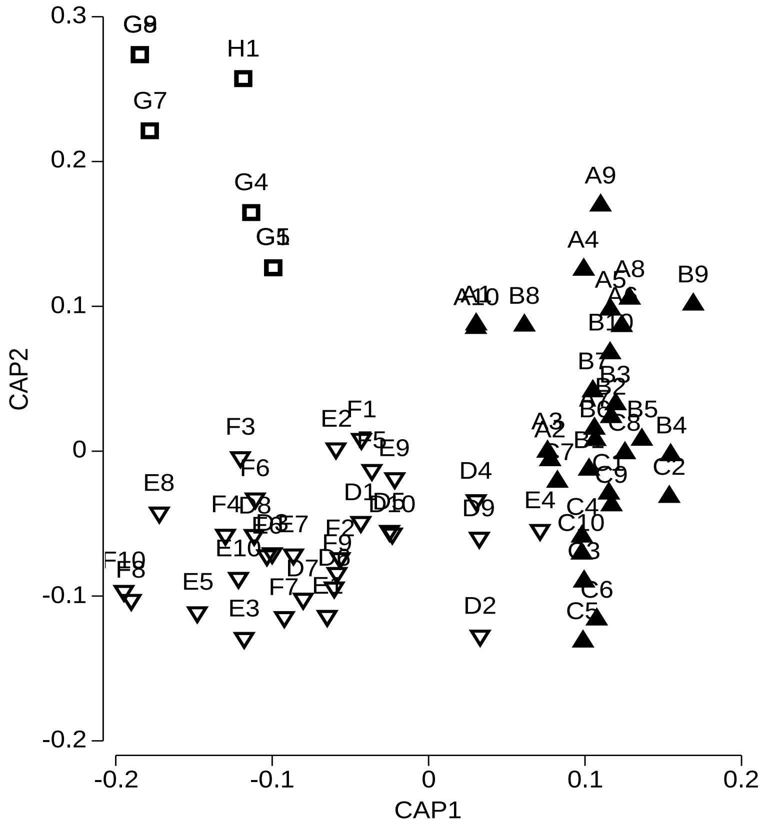

Figure 5: Canonical analysis of principal coordinates (CAP) of *Afrosteles distans* among 8 plots in the Upper Spray Wetland of the Kihansi gorge as sampled in 2007, 2008, 2015, 2016 and 2018 (□ = no trend, ▽ = increasing trend, and ▲ = decreasing trend).

Discussion

To maintain a viable population of the Kihansi Spray Toad (KST) in the wild, the wetlands habitat must provide shelter, high humidity and abundant food (Vandvik et al. 2014). Therefore, understanding the occurrence of *Afrosteles distans* in the Kihansi gorge as an indicator of food availability for KST is an important aspect in the efforts of restoration of the KST in the wild. The observed abundance of *A. distans* in the upper spray wetland was mostly an effect of the existing favourable ecological conditions during the study period. Studies conducted in the pre-diversion wetland had reported *A. distans* as one of the most abundant and visually dominant insects in vegetated areas close to where KST were found (Channing et al. 2006). Even with lack of baseline abundance levels, the occurrence of *A. distans* post diversion of the river indicates that the existing ecological conditions in the USW such as habitat, temperature and moisture to a larger extent provide either pre-existing or adaptive suitable. Water diversion reduced water input, lowered humidity, and caused larger temperature fluctuations in the wetland resulting to reduction of vegetation species composition and abundance (Quinn et al. 2005, Channing et al. 2006, Vandvik et al. 2014). Field observations during this study indicated habitat preference by *A. distans* to mostly vegetation species of *Crydon sp* and *Microstagium vegans* and *A. distans* were more abundant in areas dominated by these species. *Crydon sp* and *M. vegans* were known to be the dominant vegetation species along the entire upper and lower gorge area of the Kihansi River before construction of the Kihansi Hydropower Station (Channing et al. 2006). However, assessment conducted just after the installation of the sprinkler system showed absence of *Crydon sp* and *M. vegans* in the wetland (Quinn et al. 2005). This explains that the vegetation cover in the wetlands was changing with time and influencing the habitat for *A. distans*.

Variations in the abundance of *A. distans* between sprinkled and non sprinkled plots are also associated with the differences in vegetation cover. During all sampling periods, non sprinkled plots were characterized mostly by larger plants of *Aframomum mala*, *Costus afer* and *Senacio manii* which are not among preferred species by *A. distans*. These species were known to occur at the edges of the wetlands during the pre-diversion of the river (Channing et al 2006). Vegetation species of *Brillantasia madagasceriensis* and *M. vegans* were noted to occur only in sprinkled plots. *B. madagasceriensis* was among the dominant vegetation species in the gorge prior to the hydropower project (Poynton et al. 1998, Quinn et al. 2005). The difference in the plots emphasizes on the critical role of the sprinkler system in the efforts of the ecosystem and KST restoration because, without sustenance of the wetland, the suitable habitat for biota and KST will collapse.

Although the sprinkler system was expected to provide a constant homogenous unique microclimate in the wetland, the shift in abundance of *A. distans* from plot-to-plot and year-to-year indicate a rather non-uniform effect. The difference in the abundance of *A. distans* among sprinkled plots was mostly caused by variations in microclimate (humidity and temperature) as a regulated by both the sprinkler system and the waterfall sprays, and continuous changes in the dominance of vegetation species among the sprinkled plots (personal communication Dr. H. Ndangalasi 2018). The differences among sprinkled plots resulted in mainly two groups; one with a significant increasing trend in abundance of *A. distans* (similarity = 62%) and another with a decreasing trend (similarity = 68%) (Figure 4). Plots with a decreasing trend A, B and C are closer to the Kihansi gorge site, thus in addition to the artificial sprinklers, these plots were also inundated by the natural spray coming from the gorge. This fact would possibly tend to separate the three plots from other sprinkled plots D, E and F. Higher abundance occurred first in plots which are closer to the waterfall and considered to be wetter (A, B and C) and was delayed in plots D, E and F which are further from the gorge. A combination of the effects of variations in moisture availability and vegetation cover composition is largely the driver of the observed group patterns as shown with CAP analysis (Figure 5). Plots G and H; A, B and C; and D, E and F are characteristically different (CAP correlations = 0.8929 and 0.8133).

Furthermore, the dynamics of the *A. distans* abundane could also be attributed to annual rainfall variations and their effects on microclimate or the trend in changes in vegetation cover and dominance (Quinn et al. 2005). The pre-diversion condition offered precipitation of about 300 mm per day to the gorge (Channing et al. 2006) which supported the required moisture content and habitat for the *A. distans* as well as for KST. The design of the sprinkler system, when efficiently functioning provides precipitation 70 mm per day to the wetland (Quinn et al. 2005, Channing et al. 2006), which is only 23.3% of the pre-diversion precipitation indicating that the response to mitigation by biota is bound to a low percentage of success.

This study lacked comparative seasonal information which could have provided an in-depth understanding of the trends during the wet period. Information on microclimate characteristics (temperature and relative humidity) was not collected for the individual plots. Studies by Mutagwaba and John (2014) showed that temperature in the spray wetlands was as low as 12.71 °C and relative humidity of 99.88%, however, they were not recorded for the individual plots. Such information could have supported the analysis of moisture and temperature gradients among plots which could have supported the variations in abundance of *A. distans* and give the suitable requirements in terms of temperature and relative humidity.

The abundance of *A. distans* observed in this study indicates sufficient availability of food for the KST in the wetland. Occurrences of other invertebrates support the assurance of food availability for the KST which is a generalist feeder. However, the survivorship of KST in the wetland depends on the continued presence of its quality habitat as well as sufficient and accessible food. The abundance of food for the once in the wild KST does not necessarily transform to the availability and accessibility of the food to the re-introduced KST that has been kept in captivity. Further investigation on whether the feeding behaviour of KST has remained the same over the ecological and possibly genetically dynamics of the species is important. Questions to whether the KST which has been raised in captivity exhibit the same feeding behaviour (i.e., hunting efficiency) and food preferences with the KST that has been re-introduced in the wild, and a study on the feeding behaviour of the captive-bred and the re-introduced KST in the wild will be useful for re-defining further monitoring of food availability for the KST in the gorge.

Conclusions

The existing ecological conditions in the upper spray wetland such as habitat, temperature and moisture to a larger extent provide either pre-existing or adaptive suitable conditions for maintaining a population of *Afrosteles distans*.

There were significant differences in the abundance of *A. distans* between the sprinkled and non sprinkled plots. The difference was attributed to the difference in vegetation cover where for example the sprinkled plots supported the occurrence of vegetations which are preferred by *A. distans*. Variations in the abundance of *A. distans* among plots between different years are mostly influenced by the changes in the dominance of vegetation species among plots. Variations in the abundance of *A. distans* among plots in different years resulted in increasing and decreasing patterns. Plots A, B and C showed a decreasing trend, while plots D, E and F showed an increasing trend. The clustering could be an effect of moisture gradient among plots.

Variation in abundance of *A. distans* among the plots in the wetland shows that the wetland is still stabilizing and the operation of the sprinkler system is critical. The abundance of *A. distans* indicated sufficient availability of food for KST in the wetland. The occurrence of other invertebrates in the wetland also supports the sufficiency of food availability for the KST in the wild.

Acknowledgements

Authors acknowledge the Lower Kihansi Catchment Conservation Management Project for financial support, the National Environment Management Council (NEMC) for coordination support and the Zoology and Wildlife Department of the University of Dar es Salaam for provision of laboratory facilities.

Conflicts of interest

Authors declare that there are no any conflicts of interest.

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