COLLEGE OF SCIENCE AND TECHNOLOGY

BIOLOGY DEPARTMENT

MASTER'S THESIS

Bioacoustic assessment of anuran diversity in the Rugeramigozi wetland of Muhanga district, Rwanda

by

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Ref. No. 217298214

A research project submitted to the department of Biology in partial fulfilment for the award of a master's degree with honors in Biodiversity Conservation and Natural Resources Management

Supervisors:

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2019

Certification

This is to certify that **TUMUSHIMIRE Lambert** with reference number **217298214** carried out this research work titled "**Bioacoustic assessment of anuran diversity in the Rugeramigozi wetland of Muhanga district, Rwanda"** under my supervision and that this research work has not been previously submitted for the award of any degree in this or any other university.

TUMUSHIMIRE Lambert ______________________________

(Researcher) (Signature and Date)

Prof. Beth Ann Kaplin ______________________________

(Main supervisor) (Signature and Date

Declaration

I, the undersigned, declare that the research work entitled "**Bioacoustic assessment of anuran diversity in the Rugeramigozi wetland of Muhanga district, Rwanda"** is my original work and has not been presented for a degree in any university. Any reference in terms of books or any other written and electronic materials made concerning other people's works are indicated in the bibliography.

This dissertation has been submitted in partial fulfillment of the requirements for the Award of a Master's degree in Biodiversity Conservation and Natural Resources Management, University of Rwanda

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Signature……………………………… Date…………..…………

This dissertation is submitted with the supervision and approval of:

Prof. Beth Ann Kaplin

Signature ………………………………… Date………..……………

University of Rwanda-College of Sciences and technology

Department of Biology.

Dedication

My research project is dedicated to the Almighty God my creator and pillar. I also dedicate this work to my wife Mrs. NIYIGENA Myriam who has encouraged me all the way and whose encouragement has made sure that I give it all it takes to finish that which I have started. To my child ASHIMWE Juan who have been affected in every way possible during several days when I was out to the field. I cordially dedicate this thesis to my research partner Mr. Mapendo MINDJE for inspiring me with the research on anuran in Rwanda.

Thank you. My love for you all can never be quantified. God bless you.

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Abstract

Anuran (frogs and toads) are currently being studied for their ecological significance and spatial distribution influenced by both environmental changes and biotic processes. Anthropogenic alteration of ecological systems such as wetlands may be indicated by the presence or absence of anuran species as this group are considered biological indicators for wetland quality. In Rwanda, anuran diversity changes from one wetland to another and the use of this information in the management of wetlands are still inadequate. The present study informs about the diversity of the anuran community in Rugeramigozi wetland, a cultivated wetland of Rwanda. I estimated the abundance and richness of anuran species in the wetland and determined the variation in the calling activity of anuran species. Eight distinct microhabitats types were chosen for sampling in the Rugeramigozi wetland to assess the diversity of anuran species using eight bioacoustic night surveys coupled with visual detection. Sampling occurred on one night in October, three in November and four in December 2018. Results indicate a total of 13 anuran species *Hyperolius viridiflavus, H. rwandae, H. kivuensis, Afrixalus quadrivittatus, Kassina senegalensis, Amietia nutti, Sclerophrys regularis, Ptychadena anchietae, P. nilotica, P. porosissima, Phrynobatrachus natalensis, Phrynobatrachus kakamikro and Xenopus victorianus* detected in the wetland. Identified species presented significant variation in their calling patterns, dependent on the time of day and type of microhabitats. The detected anuran species are good representatives of an altered wetland following heavy human disturbance which confirms the current status of Rugeramigozi wetland in Muhanga district. The local α–diversity index presented a high species diversity at microhabitats with high vegetation and high water available and low diversity at microhabitats with poor or shallow vegetation despite presence of water. The β- Diversity index showed a high similarity of anuran species among microhabitats with similar characteristics. The presence of *Ptychadena spp* (*P. anchietae, P. porosissima* and *P. nilotica*) indicates a heavy disturbance of the Rugeramigozi wetland as Ptychadena ssp are distributed in wetlands with high human disturbances. Our study results show that the surveyed anurans are representative of heavily altered wetlands.

Key words: bioacoustics, wetlands, anuran, advertisement call activity, microhabitats.

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Abbreviations and Acronyms

asl: above sea level

ANCOVA: analysis of covariance

BCI: Bray-Curtis Index

FFT: Fast Fourier Transformation

Dr.: Doctor

GoR: Government of Rwanda

Hz: Hertz

IUCN: International Union for Conservation of Nature

MSc.: Masters of Science

ms: milliseconds

PCM: Pulse Code Modulation

SI: Sörensen similarity Index

%: Percentage

INTRODUCTION

Anuran (frogs and toads) are currently being studied for their ecological significance (Hocking & Babbitt, 2014) and spatial distribution (Hartel et al., 2010; Rittenhouse & Semlitsch, 2007) which is influenced by both environmental gradients and biotic processes (Sievers et al., 2019). The current status and trend in anuran diversity is being marked by new species discoveries despite the worldwide decrease in anuran diversity (Hanken, 1999). The decrease is due to climate change (Corn, 2005), anthropogenic alteration of ecosystems agriculture, development or other activities (Gardner, 2001) and diseases (Densmore & Green, 2007). Currently, Anuran make the largest group of the total amphibians' species worldwide with more than 7,061 species (88% of total amphibians' abundance) (Amphibiaweb.org, last accessed in October 2019). Anuran are known for their contribution to ecosystems services and considered to be biological indicators of wetland health (Saber, Tito, & Said, 2017; Hilty & Merenlender, 2000). Moreover, anuran act as biological control for pests that can cause diseases in human (Durant & Hopkins, 2008) and they are important sources of medical advances (Anyelet et al.,2013).

In Africa, the Albertine Rift is considered a biodiversity hotspot, with 119 amphibian species (Plumptre et al., 2007). Rwanda, located in east central Africa and within the Albertine Rift, is home to a diversity of anuran species including some newly described species such as *Hyperolius Jackie* (Dehling, 2012) and *Hyperolius rwandae* (Channing, Hillers, Schick, et al., 2013). Amphibian diversity in Rwanda have been studied in few wetlands of Rwanda including species of the Rweru-Mugesera, Rugezi, Akagera and Kamiranzovu complex wetlands (Fischer et al., 2011); Nyungwe National Park (Lehr et al., 2015; Maximilian Dehling, 2012); Volcano National Park (Roelke, 2010; Van der Hoek et al., 2019) and in Rwasave wetland of Huye (Sinsch et al., 2012), and in wetlands of the upper Nile (Dehling & Sinsch, 2013b).

The majority of Rwandan wetlands are now being used to grow rice and other crops, and this wetland transformation began in the 19^{th} century in order to cope with the populations' demand for food security. Wetlands are also currently exploited for clay to use in the fabrication of bricks. These activities have led to habitat fragmentation and alterations that affect wetland biodiversity including anurans (Nsengimana & Becker, 2017). Some species may be able to recover after habitat degradation (Lesbarrères et al., 2010) while others can become locally extinct due to failure in their (Nabahungu & Visser, 2016) reproduction success as a result of persistent disturbances within their respective microhabitats (Cushman, 2006). Anurans, like other organisms, have a niche breadth that reflects ecological requirements or needs such as calling in males during mating, access to adequate breeding grounds and predator escape (Toledo et al., 2014). Information about which anuran species persist in disturbed wetlands is crucial for wetlands conservation as the presence or absence of anuran species in a particular wetland provides information about the status of wetland degradation following anthropogenic activities (Lesbarrères et al., 2010).

Anuran vocalizations can be used in taxonomy studies, especially for species identification (Köhler et al., 2017). Call types have been used to study anuran bioacoustics, ecology, behavior, physiology and evolution and recent developments in information technology have supported this research (Toledo et al., 2014). The communication of amphibians and other vocalizing animals has attracted the attention of many scientists (Pijanowski et al., 2011), and bioacoustics is an effective technique to monitor and discover the presence of threatened species (Wijayathilaka et al., 2018). Based on the current information on acoustic niche partitioning in disturbed wetlands carried out in Rwanda (Sinsch et al., 2012) and on the lack of baseline information on anuran communities in relation to status of Rwandan wetlands (Dehling & Sinsch, 2013b), there is a need for additional data to substantiate what is known about spatial and temporal distribution of anuran communities inhabiting disturbed wetlands. This study presents (1) the anuran communities inhabiting the Rugeramigozi wetland, a cultivated wetland in Rwanda, and (2) the relationship between calling activity and the time of day and microhabitat type, (3) estimates of the diversity and richness of the anuran community in Rugeramigozi wetland.

METHODS

Study area

Species diversity and diel vocalization activity of the anuran community in a cultivated wetland called Rugeramigozi was studied. Rugeramigozi wetland is located in the southern province of Rwanda (2° 07' 40" S, 29° 45' 20" E, 1650 m a.s.l.), in Muhanga district with a size of 179.4 ha irrigated by the Rugeramigozi dam of 270,000 (Dusabimana, 2012; Nabahungu & Visser, 2016), Rugeramigozi is used under specific conditions as proposed by the Government of Rwanda (GoR, 2017) for the cultivation of rice crops since 2001 by the cooperative KIABR (Koperative Imparaniramusaruro y' Abahinzi Borozi ba Rugeramigozi), which is in charge of full operation and maintenance of the scheme (Dusabimana, 2012). The annual range of rainfall ranges between 1200-1400mm. The wetland was cultivated once a year (from June to September) before being officially transformed into full agriculture in 1999-2000 where soya beans, maize, sweet potatoes and vegetables were major crops (Dusabimana, 2012). Eight microhabitats were sampled based on distinction in their characteristics such as vegetation and water levels. These included a small lake (estimated at $<5m^2$) with shore vegetation without flowing water; mud holes with retained water but without vegetation; an irrigation channel with vegetation having a longitudinal narrow water body of 20 – 30 cm width without flowing water or very slowly running water located in the uncultivated area of Rugeramigozi wetland; a flooded uncultivated meadow with shallow water and vegetation comprised of sedges; a central ditch with flowing water body in the wetland, with > 1 m width, with less shore vegetation; partially flooded rice field with grown rice crops and shallow water; an irrigation channel without vegetation but with stagnant water and a potato field with surface water.

Table 1. GPS coordinates of sampling sites

Microhabitat	Elevation (m)	Latitude	Longitude
Mud hole	1798.55	-2.1030985	29.7592062
Small lake	1788.55	-2.1022627	29.7577492
Irrigation channel with vegetation	1796.55	-2.1016197	29.75757
Flooded uncultivated meadow	1787.55	-2.1013996	29.7578323
Central ditch	1794.55	-2.0998845	29.7585889
Potato field	1793.55	-2.0992161	29.7593119
Irrigation channel without vegetation	1787.55	-2.0985625	29.7603095
Partially flooded rice field	1792.55	-2.0984138	29.7606738

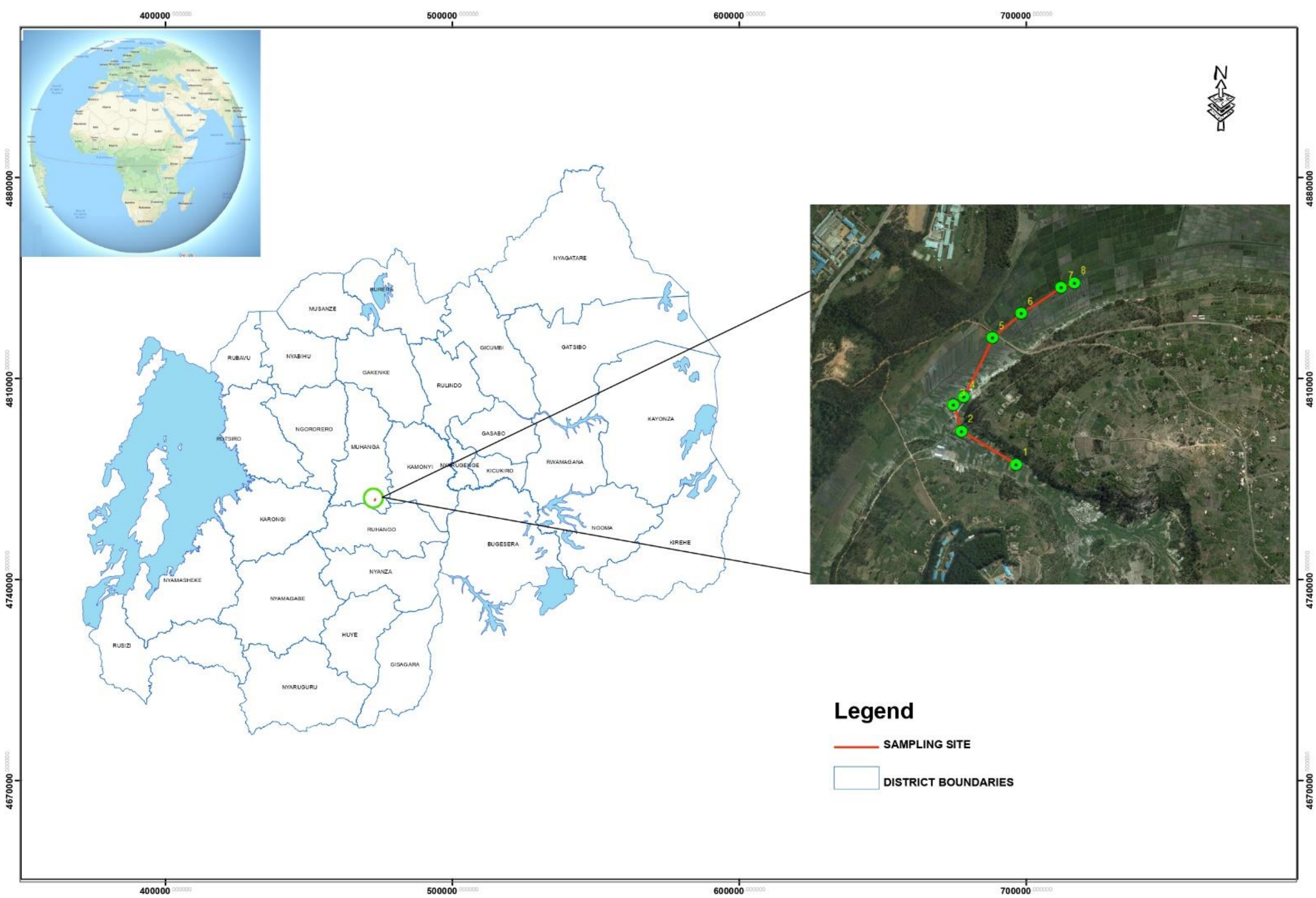

Figure 1. Study area location

Sampling design

To cover all calling species, a motionless position was maintained for 2 minutes before recording. Air temperature was measured and recorded at each selected microhabitat. Sampling was done in a total of 192 recording sessions, 24 per microhabitat during eight bioacoustics night survey in October (29th), November (12^{th}, 18^{th} and 25^{th}) and December (2^{nd}, 9^{th}, 16^{th} and 23rd). Each night recording was done shortly after sunset at 7pm, 8pm and 9pm inclusive. The survey did not record calling species after 9pm due to insecurity and safety uncertainty. The recorder was rotated slowly 360 degrees at each microhabitat sampled with an estimated distance of 5 meter maintained from the recording point. The main objective was to record the frogs or toads calling from the microhabitats. After two minutes, I noted the details of the sampling location into the recorder, including microhabitat type, date and time of day, air temperature and any important features found in the microhabitat. Advertisement calls were recorded with a Sony PCM–D50 Linear PCM Recorder with stereo microphones and a few records have been taken by the cell phone when the recorder was not available. Species that were recorded included both visually as well as acoustically detected. A visual survey was done following no transect sampling but when moving from one microhabitat to another or when anuran is moving and detected during recording.

Species identification

Species identification was done based on external morphological features (mainly color pattern, shape and size), and on advertisement call structure (Köhler et al., 2017). For visual identification, a homing-in approach was used to single specimens calling with LED torches to recover calling frogs for specimen identification. Collected specimens were kept in a well secured plastic box for further identification. Photographs of the calling or caught specimen were kept for morphological corroboration during species identification. Species known to call under water such as *Xenopus spp* were not recorded; a hydrophone is needed to recover advertisement calls done underwater. However, *Xenopus spp* was recorded present when observed visually with a LED torch during the visual inspection of each mirohabitat. Sonograms and frequency analyses (oscillograms) were obtained applying Blackman–Harris Fast Fourier transformation with a FFT size of 1024 Hz to be used for identification based on the call structure by measuring its call duration [ms], pulses per call, pulse rate [Hz], pulse duration [ms], interpulse interval [ms] and dominant frequency

[Hz](Köhler et al., 2017; Sinsch et al., 2012). Species nomenclature was done following Frost (2019).

Statistical analysis

Alpha diversity and Beta diversity measures were computed using the statistical package EstimateS version 9.1.0 (Colwell & Elsensohn, 2014) – for each microhabitat. Alpha diversity was quantified as local number of species and calculated using the Shannon-Wiener index (H') and Simpson diversity Index considering species abundance. To measure changes in species among microhabitats, a Beta diversity index was calculated using Bray-Curtis Dissimilarity Index and Sörensen Similarity Index. ANCOVAs (analysis of covariance) were used to estimate the influence of microhabitat type and time of day (categorically fixed factors) on the local number of species and individuals per species calling within a 2 min record. Analysis was done using the raw data because distributions did not deviate significantly from normality. All statistical procedures including average number of species per microhabitat were performed using the program package Statgraphics Centurion version 18.1.01 (64-bit).

RESULTS

Anuran communities of Rugeramigozi wetland

During this study, 13 anuran species were detected in Rugeramigozi wetland, among which ten were detected by both visual and acoustic survey (Figure 2) and three by only visual survey detection (Figure 3). The sonogram and oscillograms of advertisement call features for anuran species detected visually and acoustically are shown in Figure 4. Generally, all detected species fell into one of six families. Hyperoliidae had the highest number of individuals (five individuals), followed by Ptychadenidae (three individuals), Phrynobatrachidae (two individuals) and the remining families were represented by one individual only (Pipidae, Bufonidae and Pixycephalidae. Except for *Phrynobatrachus kakamikro*, which is listed as data deficient on the International Union for Conservation of Nature (IUCN) Red List (IUCN, 2019, www.iucnredlist.org), the remaining species are listed as least concern (Table 2). Sampling was done in the eight microhabitats to obtain the species counts and assessment of the anuran communityof Rugeramigozi wetland (Table 3).

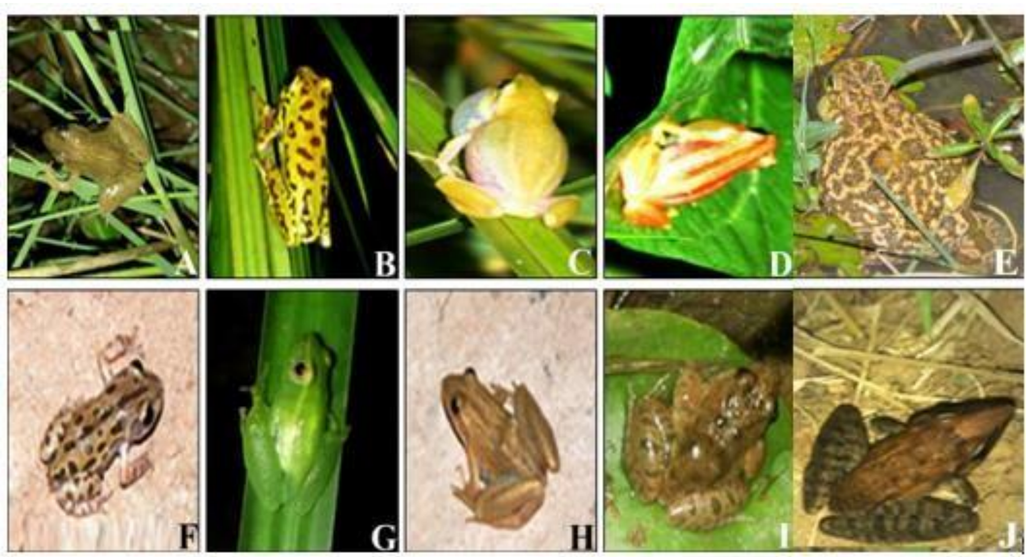

Figure 2. Visually and acoustically detected anuran of Rugeramigozi wetland

A. *Phrynobatrachus kakamikro*, **B.** *Hyperolius viridiflavus*, **C.** *Hyperolius kivuensis*, ***D.*** *Afrixalus quadrivittatus*, **E.** *Sclerophrys regularis*, **F.** *Kassina seneglensis*, **G.** *Hyperolius rwandae*, **H.** *Amietia nutti*, **I.** *Phrynobatrachus natalensis*, **J.** *Ptychadena anchietae.*

Figure 3. Visually detected anuran species

K. *Ptychadena nilotica,* **L.** *Ptychadena porosissima,* **M.** *Xenopus victorianus.*

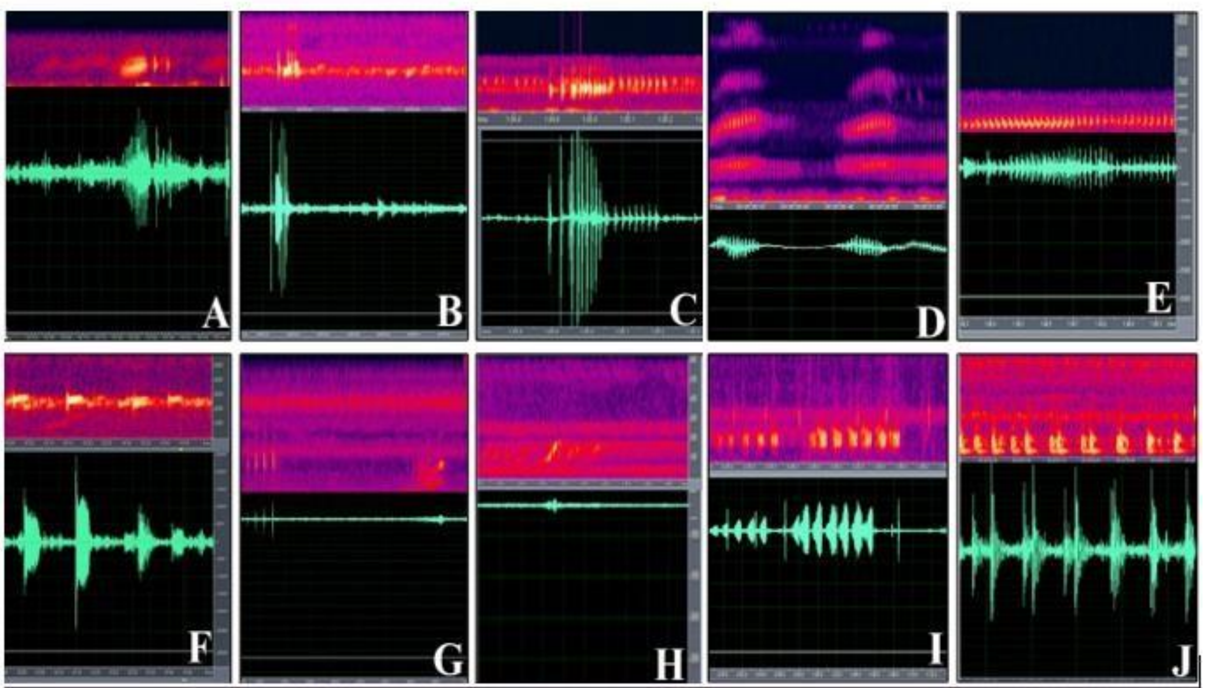

Figure 4. Sonagrams and oscillograms of the advertisement call for acoustically and visually detected species

A. *Hyperolius rwandae,* **B.** *Hyperolius kivuensis,* **C.** *Afrixalus quadrivittatus,* **D.** *Ptychadena anchietae,* **E.** *Phrynobatrachus kakamikro,* **F.** *Hyperolius viridiflavus,* **G.** *Amietia nutti,* **H.** *Kassina senegalensis,* **I.** *Phrynobatrachus natalensis,* **J.** *Sclerophrys regularis.*

Table 2. Detected anuran species from Rugeramigozi wetland, Rwanda and IUCN status

Anuran Taxa	**IUCN status**	**Families**
Afrixalus quadrivittatus	Least concern	Hyperoliidae
Amietia nutti	Least concern	Pyxicephalidae
Hyperolius kivuensis	Least concern	Hyperoliidae
Hyperolius rwandae	Least concern	Hyperoliidae
Hyperolius viridiflavus	Least concern	Hyperoliidae
Kassina senegalensis	Least concern	Hyperoliidae
Phrynobatrachus kakamikro	Data Deficient	Phrynobatrachidae
Phrynobatrachus natalensis	Least concern	Phrynobatrachidae
Ptychadena anchietae	Least concern	Ptychadenidae
Ptychadena porosissima	Least concern	Ptychadenidae
Ptychadena nilotica	Least concern	Ptychadenidae
Sclerophrys regularis	Least concern	Bufonidae
Xenopus victorianus	Least concern	Pipidae

Table 3. Detected anuran species and corresponding microhabitats in Rugeramigozi wetland, Rwanda

Species/taxa	**Microhabitats**							
	Mud hole	**Lake**	**Irrigation channel with vegetation**	**Flooded uncultivated meadow**	**Central Ditch**	**Partially flooded rice field**	**Irrigation channel without**	**Potato field**
Afixalus quadrivittatus		+	+	+				+
Amitia nutti					+			
Hyperolius kivuensis	+	+	+	+	+	+	+	+
Hyperolius rwandae		+	+	+				+
Hyperolius viridiflavus		+	+	+	+	+	+	+
Kassina senegalensis	+	+	+	+		+	+	
Phrynobatrachus kakamikro		+	+	+		+		+
Phrynobatrachus natalensis	+			+		+	+	
Ptychadena anchietae	+							
Ptychadena porosissima								
Ptychadena nilotica								
Sclerophrys regularis							+	
Xenopus victorianous	+			+				

+: Present

Correlation between anuran calling activity (abundance) and the time of the day and type of microhabitats in Rugeramigozi wetland

Among the 13 detected species, six were considered common species as they were recorded in most microhabitats sampled. The remaining species were recorded rarely or were restricted to single microhabitats. The species restricted to single microhabitats included *Amietia nutti, Phrynobatrachus natalensis, Ptychadena porosissima, P. anchietae, P. nilotica, Sclerophrys regularis* and *Xenopus victorianus.*

Table 4 indicates that the call activity of *Hyperolius kivuensis* was independent of microhabitat type (p-value=0.137) but depended on time of day (p-value=0.0208) (Table 3).

Table 4. Calling activity dependence factors in *Hyperolius kivuensis*

Source	**Square sums**	**Degrees of freedom**	**Mean squares**	**F-Quotient**	**p-value**
Main effects					
A: Time of day	3.37	2	1.68	4.03	0.02
B: Microhabitat types	4.76	7	0.68	1.63	0.13
Residuals	41.03	98	0.41		
TOTAL (CORRELATION)	49.16	107			

Hyperolius kivuensis showed variation in the calling activity with time of day, with call frequency low shortly after sunset but increasing with the night (Figure 4).

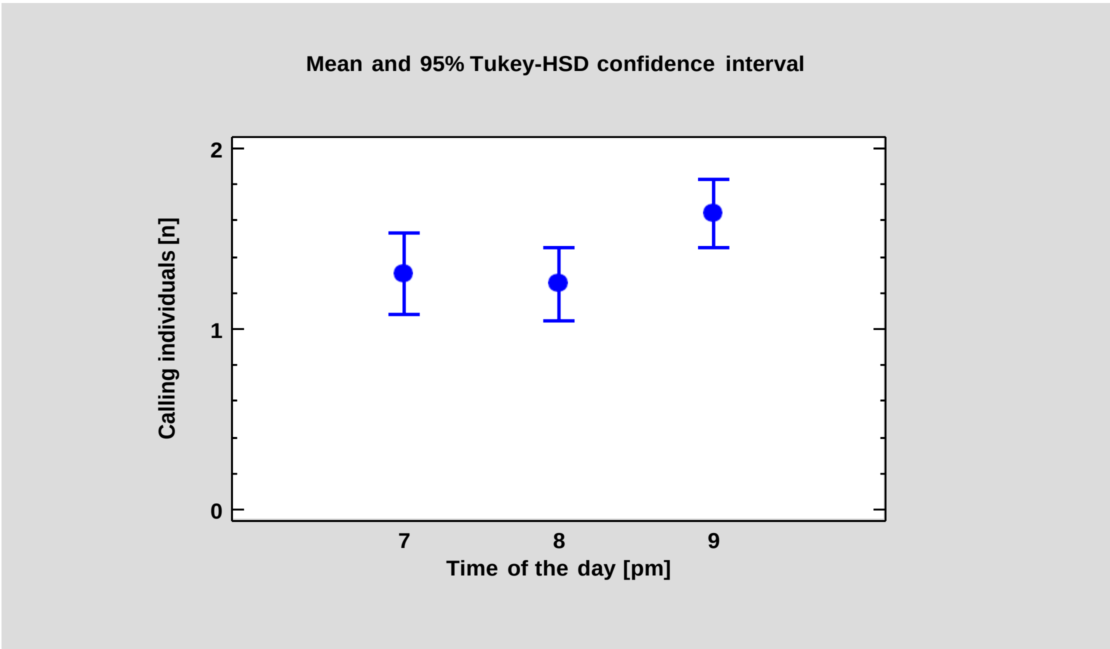

Figure 5. Time dependence of the calling activity in *Hyperolius kivuensis*

The calling activity in *Hyperolius viridiflavus* was independent of hour of day (p-value=0.09) but significantly dependent on microhabitat type (p-value<0.00001) (Table 4).

Table 5. Calling activity dependence factors in *Hyperolius viridiflavus*

Source	**Square sums**	**Degrees of freedom**	**Mean squares**	**F-Quotient**	**p-value**
Main effects					
A:time of day	6.68	2	3.34	2.41	0.09
B: Microhabitat types	263.6	6	43.93	31.62	<0.00001
Residuals	126.43	91	1.38		
TOTAL (CORRELATION)	396.71	99			

Calling activity of *H. viridiflavus* was the highest at small lake microhabitat and lowest at the potato field (Figure 6).

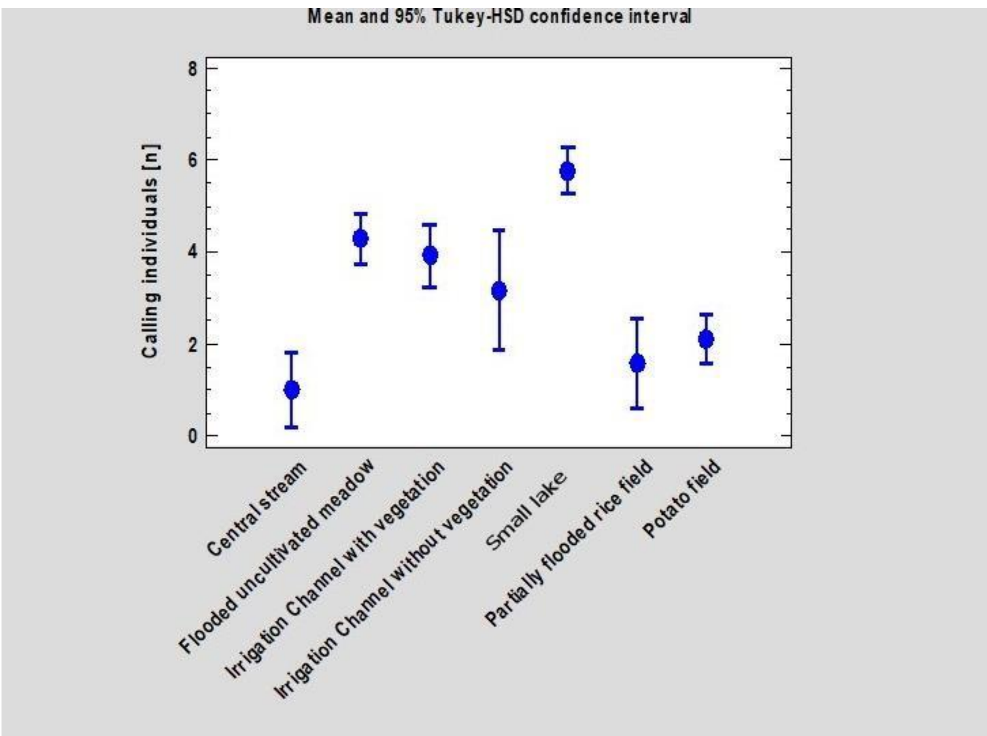

Figure 6. Calling activity dependence on microhabitat types in *Hyperolius viridiflavus.*

In *Hyperolius rwandae,* calling activity was independent of hour of day (p-value=0. 38) and of habitat type (p-value=0. 39) (Table 5).

Table 6. Calling activity dependence in *Hyperolius rwandae*

Source	**Square sums**	**Degrees of freedom**	**Mean squares**	**F-Quotient**	**p-value**
Main effects					
A:time of day	2.80	2	1.40	1.01	0.38
B:Microhabitat types	4.34	3	1.44	1.04	0.39
Residuals	24.96	18	1.38		
TOTAL (CORRELATION)	32.1	23			

The call activity in *Kassina senegalensis* was independent of microhabitat type (p-value=0.4865), but dependent on time of day (p-value=0.0393) (Table 7).

Table 7. Calling activity dependence in *Kassina senegalensis*

Source	Square sums	Degrees of freedom	Mean squares	F-Quotient	p-value
Main effects					
A:time of day	12.68	2	6.34	3.72	0.03
B:Microhabitat types	6.06	4	1.51	0.89	0.48
Residuals	40.97	24	1.70		
TOTAL (CORRELATION)	59.71	30			

Calling activity for *Kassina senegalensis* decreased at the last sampling time of the night (Figure 7).

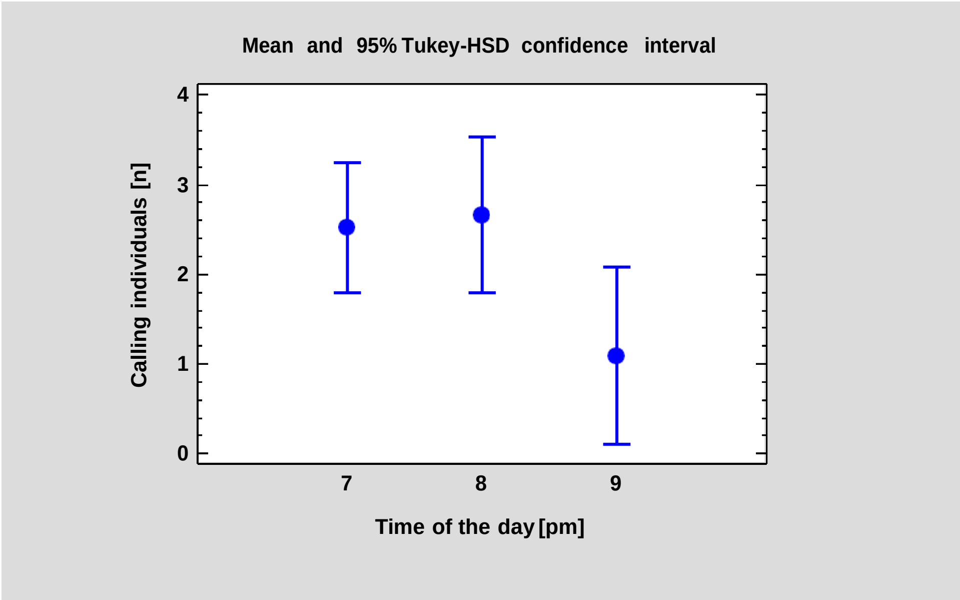

Figure 7. Calling activity dependence with time of day in Kassina senegalensis

In *Afrixalus quadrivittatus*, the calling activity was found to be independent of time of day (p-value=0.2166) and independent of habitat type (p-value=0. 0801) (Table 8).

Table 8. Calling activity dependence in *Afrixalus quadrivittatus*

Source	**Square sums**	**Degrees of freedom**	**Mean squares**	**F-Quotient**	**p-value**
Main effects					
A:time of day	5.68	2	2.84	1.63	0.21
B:Microhabitat types	13.27	3	4.42	2.54	0.08
Residuals	41.78	24	1.74		
TOTAL (CORRELATION)	60.73	29			

The calling activity of *Phrynobatrachus kakamikro* was also found to be independent of time of day (p-value=0,26) and independent of the microhabitat types (p-value=0.46) (Table 9).

Table 9. Calling activity dependence in *Phrynobatrachus kakamikro*

Source	**Square sums**	**Degrees of freedom**	**Mean squares**	**F-Quotient**	**p-value**
Main effects					
A: time of day	5.84	2	2.92	1.41	0.26
B:Microhabitat types	7.73	4	1.93	0.93	0.46
Residuals	49.89	24	2.07		
TOTAL (CORRELATION)	63.46	30			

Anuran species diversity and richness in Rugeramigozi wetland

Alpha diversity was computed to estimate the average number of species detected at each microhabitat during the period of the bioacoustics survey. Results indicate a high species diversity at the flooded uncultivated meadow (H'=1.68, D=0.2), and lowest diversity at the central ditch (H'=0.64, D=0.56) (Table 10).

Table 10. α –diversity of anurans in sampled microhabitats in Rugeramigozi wetland

Microhabitats	**Shannon Mean (H')**	**Simpson Inverse Mean**	**Simpson value (D)**
Mud hole	1.08	2.58	0.39
Small Lake	1.17	2.18	0.46
Irrigation channel with vegetation	1.49	3.79	0.26
Flooded uncultivated meadow	1.68	4.46	0.22
Central ditch	0.64	1.8	0.56
Partially flooded rice field	1.26	2.71	0.37
Irrigation channel without vegetation	1.16	2.78	0.36
Potato field	1.24	3	0.33

To measure the β –diversity, a measurement of the changes in species diversity from one microhabitat to another, the Bray-Curtis dissimilarity index and Sörensen similarity index were calculated.

Table 11. Measurement of β –diversity index

	Mud hole	Small Lake	Irrigation channel with vegetation	Flooded uncultivated meadow	Central ditch	Partially rice field	Irrigation channel without vegetation	Potato field
Mud hole		**0.4**	**0.4**	**0.545**	**0.333**	**0.6**	**0.5**	**0.22**
Small Lake	0.24		**1**	**0.92**	**0.5**	**0.66**	**0.4**	**0.90**
Irrigation channel with vegetation	0.26	0.62		**0.92**	**0.5**	**0.66**	**0.4**	**0.90**
Flooded uncultivated meadow	0.31	0.73	0.72		**0.44**	**0.76**	**0.54**	**0.83**
Central ditch	0.19	0.16	0.24	0.16		**0.5**	**0.66**	**0.57**
Partially rice field	0.55	0.28	0.41	0.30	0.56		**0.6**	**0.54**
Irrigation channel without vegetation	0.17	0.12	0.17	0.13	0.54	0.42		**0.44**
Potato field	0.23	0.55	0.75	0.64	0.3	0.45	0.21	

The Bray-Curtis similarity Index (BCI) showed a high dissimilarity of species between small Lake Small Lake and irrigation channel without vegetation (BCI= 0.12). The Sörensen similarity index (SI) indicated the highest similarity between small Lake and irrigation channel with vegetation (SI=1) (Table 11).

Results indicated a high species richness at the flooded uncultivated meadow (eight species) and lowest at the central ditch (three species).

Table 12. Species richness in Rugeramigozi wetland

Species/taxa	Microhabitats							
	Mud hole	**Lake**	**Irrigation channel with vegetation**	**Flooded uncultivated meadow**	**Central Ditch**	**Partially flooded rice field**	**Irrigation channel without vegetation**	**Potato field**
Afixalus quadrivittatus		+	+	+				+
Amitia nutti					+			
Hyperolius kivuensis	+	+	+	+	+	+	+	+
Hyperolius rwandae		+	+	+				+
Hyperolius viridiflavus		+	+	+	+	+	+	+
Kassina senegalensis	+	+	+	+		+	+	
Phrynobatrachus kakamikro		+	+	+		+		+
Phrynobatrachus natalensis	+			+		+	+	
Ptychadena anchietae	+							
Ptychadena porosissima								
Ptychadena nilotica								
Sclerophrys regularis							+	
Xenopus victorianous	+			+				
Species richness	**5**	**6**	**6**	**8**	**3**	**5**	**5**	**5**

DISCUSSION

Using visual and acoustic surveys, a total of 13 anuran species were found in the Rugeramigozi wetland including 12 frogs and one toad, belonging to six families with Hyperoliidae having the largest number of species.

Our study did not detect *Hyperolius cinnamomeoventris* and *H. lateralis* which are species restricted to few habitats such as semi-natural reeds, shrubs or even trees (Bell et al., 2015; Chick et al., 2005). This study did detect *H. rwandae* which also co-occurs with *H. lateralis and H. cinnamomeoventris* (Sinsch et al., 2012). Detected species of Rugeramigozi wetlands that were similar to the species detected in the cultivated wetland in the Sinch et al. (2012) study were found in nearly the same microhabitats and hence, this study suggests the Rugeramigozi wetland as an altered wetland from its natural state. A study by Sakané et al. (2011) showed that natural wetlands are usually dominated by natural vegetation such as Cyperaceae and Typhaceae families. Rugeramigozi as a cultivated wetland that may have lost its natural state (personal observation) but still has small remnant patches of *Cyperus spp* especially at the flooded uncultivated meadow, irrigation channel with vegetation and at a distant site away from the potato field. *Hyperolius rwandae* was recorded in these sites, which is a species indicating natural vegetation (Channing et al., 2013). However, the presence of *Ptychadena spp* such as *P. nilotica, P. porosissima* and *P. anchietae* indicate the Rugeramigozi wetland as a heavily disturbed wetland (Dehling & Sinsch, 2013a).

Anuran species detected in the Rugeramigozi wetland were found to have varied calling activities in relation to time of the day and microhabitat type. Time of day was an important factor for the calling activity in *Hyperolius kivuensis* and *Kassina senegalensis*. A study by Oseen & Wassersug (2002) and Hsu, Kam, & Fellers (2006) indicated that time of day influences calling activity of anuran species where some species are active shortly after sunset. This behavior is stated to be used by anurans in locations with low ambient light (i.e. sunset) decreases the risk of anurans to be perceived by diurnal predators such as snakes and birds (Oseen & Wassersug, 2002). In our study, the calling activity in *Hyperolius kivuensis* was highest at 9pm and in *Kassina senegalensis* it was lowest at 9pm, the last time period sampled each sampling evening. In addition to the time of day microhabitat type also influenced calling activity in some species detected. *Hyperolius viridiflavus* was mainly calling in a habitat with open water and shore vegetation. The calling activity of *H.*

viridiflavus was high at the small lake microhabitat and the species called at all sampled microhabitats compared to other species which used few habitats for calling. Habitat characteristics such as vegetation structure and water levels influence calling patterns in anuran species (Vasconcelos & Rossa-Feres, 2008). Vegetation and water levels have been positively correlated to anuran breeding patterns as they are important for egg laying sites (Gottsberger & Gruber, 2004; Guilherme et al., 2019). However, the calling activity of *Hyperolius rwandae* and *Afrixalus quadrivittatus* were not found to correlate with the two investigated factors (time of day and microhabitat type).

The diversity of anuran species in Rugeramigozi wetland varied with the type of microhabitats where highest alpha diversity was observed at the flooded uncultivated meadow and lowest at the central ditch. The diversity of anuran species is associated with type of microhabitats where heterogeneous microhabitats usually harbor a high number of species at a particular site (Guilherme et al., 2019). Heterogeneity is important in understanding species composition in anuran community since these microhabitats provide calling sites for anuran species. Not all males call on same substrate but use a variety of substrate types such as open water with floating vegetation, tall grasses and dry land (Sinsch et al., 2012). This was the case of our study where the microhabitat with varied substrates was occupied by different greater number of species. The flooded uncultivated meadow consisted of reed vegetation, ground, open waters with floating water hyacinth and shallow grasses which therefore contained the highest diversity. However, microhabitats which had more or less similar characteristics in vegetation and water shared similar species than those with distinctive features. We observed similarity to be high between small lake and irrigation channel with vegetation. These microhabitats had in common shallow vegetation without flowing water which was obvious to have similar species in common. According to studies by Guilherme et al. (2019) and Ramalho et al. (2019) habitats that share common geographic features are most likely to have common species. Concerning the richness, our study found that microhabitats with high diversity also had high species richness. This was the case of the flooded uncultivated meadow whose Shannon index was high and the number of species higher than in the other microhabitats. Species richness is related to environmental heterogeneity where species occurrence in a particular microhabitat is associated with water availability (Vasconcelos & Rossa-Feres, 2008) and type of vegetation (Guilherme et al., 2019).

CONCLUSION AND RECOMMENDATION

A total account of 13 anuran species was detected by both bioacoustics and visual night surveys in the Rugeramigozi wetland from eight different microhabitats. These last were characteristically distinct in terms of the vegetation abundance and availability of amount of water which influenced diversity of anuran species, richness and changes among species at each microhabitat. The study indicates that the type of microhabitats and time of day were important factors influencing the variation in the calling activity for surveyed anurans where some species calling activity was high after sunset and decreases with night and in other species, the calling activity started low but increases with the night. Currently, the Rugeramigozi wetland in Muhanga district has a high number of species commonly found in heavily disturbed wetlands of Rwanda such as species studied in the Rwasave wetland of Huye disctrict. It was surprising to detect *Hyperolius rwandae*, a species that is typical of uncultivated swamp. Its presence in Rugeramigozi wetland suggests that this wetland still possesses some patches of natural vegetation that can be a stepping-stone of regeneration for the wetland once the decision to protect this wetland is taken. The high level of loss of natural vegetation in the wetland is due to anthropogenic activities. The results show that heavy human disturbance is impacting Rugeramigozi wetland although some remnant patches of natural vegetation are still observed. Our prediction is that with no action to conserve the wetland, there will be no chance for recovery of the natural wetland and respective local anuran species. In conclusion, the present study confirms that the Rugeramigozi wetland is a disturbed wetland due to human disturbances such as agriculture for rice field growing and clay exploitation for bricks making. The anuran community of the area represents species of a heavily disturbed wetland.

Based on the strength of our results, we recommend the institutions in charge of wetlands management and conservation in Rwanda to take into consideration cultivated wetlands while developing policies for wetlands management by identifying which activities should be carried out in cultivated wetlands to limit excessive exploitation by human. Despite agriculture allowed in cultivated wetlands, policies should stipulate ways to protect these wetland types even if they are left for human use. This is due to the idea that wetlands support biological diversity and hence have to be conserved.

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