Rufford Small Grants Foundation

Final report September 2024

Invasion and Management of the Red Swamp Crayfish (*Procambarus clarkii)* in Northern Rwanda

Introduction

The red swamp crayfish (*Procamabarus clarkii*) is a highly invasive species with significant negative impact on natural ecosystems and biodiversity worldwide (Oficialdegui et al., 2020). Native to the Mississippi River and Gulf of Mexico, commonly known as Louisiana crayfish and has been introduced to over 40 countries, excluding Antarctica (Adams & Jones, 2021, Putra et al., 2018). In Africa, introduction spans several countries including South Africa, Morocco, Egypt, Sudan, Uganda, Kenya, and recently Rwanda (Nunes et al., 2017, Madzivanziraa et al., 2020). In Rwanda, it has been identified in Mukugwa Valley where its introduction as biological control of water hyacinth is suspected (Madzivanziraa et al., 2020).

Due to its high adaptability, it colonizes all types of habitats including rivers, lakes, ponds, streams, canals, seasonally flooded swamps and marshes, and ditches with muddy or sandy bottoms and abundant organic debris (Loureiro et al., 2015). It exhibits considerable ecological plasticity and is tolerant of variation of salinities, PH, oxygen levels and temperatures (Bissattini et al., 2015), Huner & Barr, 1991). However, it is known that this species has a preference for habitats with a water temperature of 21 to 30°C (Peruzza et al., 2015). The species consumes a wide variety of plants, animals, detritus and sediments, making it the predominant violent animal in ecosystems (Grey &Jackson, 2012).

Despite its widespread distribution, detailed information on its occurrence and ecological impact in East Africa, particularly in Rwanda, remains limited. The current study worked on the twin lakes Ruhondo and Burera, also known as lava dammed lakes located in the northern part of Rwanda just near the board of Rwanda and Uganda, also close to the Volcanoas National park (the famous gorilla home) and where formed as a results of volcanism (Habimana & Nsabimana, 2020).

Supported by funding from the Rufford Small Grant Foundation, this study aimed to assess the occurrence of *Procambarus clarkii* Girard, 1852 in lakes Ruhondo and Burera. We conducted a comprehensive survey at varying depths. We used a combination of traditional morphological identification techniques and modern DNA-based methods to accurately identify and confirm the presence of the red swamp crayfish (*Procambarus clarkii*) in these lakes and the diversity of the associated macroinvertebrates.

In addition, we engaged local fishermen through an awareness campaign to highlight the importance of controlling *Procambarus clarkii* and preserving the lakes' habitats. The findings of this study inform effective management strategies to mitigate the spread of this invasive species and ensure the sustainability of freshwater ecosystems in Rwanda.

Methodology

Sampling

We conducted our sampling along the shores of Lake Ruhondo and Lake Burera in northern Rwanda, targeting different depths to collect different organisms (see Fig. 1). Our strategy was to sample the entire lake at 1 km intervals, with each interval lasting 45 minutes to maximize the chances of capture. The sites sampled included the entire Lake Ruhondo and accessible shores of Lake Burera (see Fig. 1).

Over a 12-day period, we collected *Procambarus clarkii* and other freshwater macroinvertebrates from both lakes. We also conducted campaigns with local fishermen to collect anecdotal information about *Procambarus clarkii* in the region. The field work and campaign took place in January 2024 and April 2024.

For sampling, we used a scoop net (diameter: 20 cm, mesh size: 1 mm) along the shore and manually collected samples from stones and rocky substrates. For sampling in deeper sections of the littoral zone, up to a depth of 20 meters, we used a dredge from the boat and we used traps for

Procambarus clarkii with different baits (cat food and Bacon). We collected detailed information, including geographic coordinates, site characteristics and physicochemical parameters (see Table S1). All collected specimens were labeled and preserved in 80% ethanol and are stored in the Laboratory of Animal Ecology and Systematics at Justus Liebig University (Giessen, Germany).

Photo ©: Rwibutso. Showing the removal of caught **Procambarus clarkii** *from traps using bacon bait by Kwizera.*

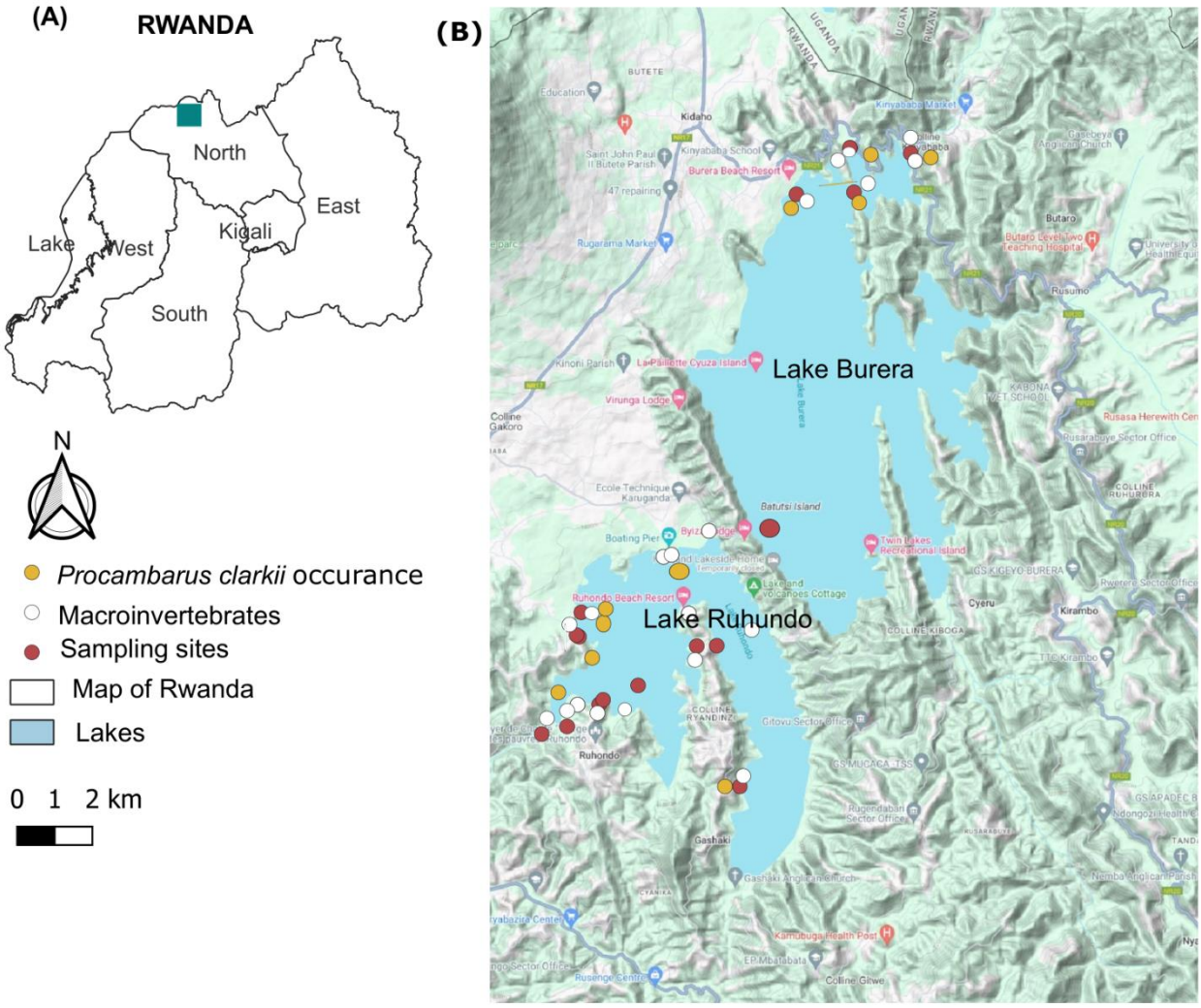

Figure 1. *Map of Lakes Ruhondo and Burera with sampling sites. A: Overview map of Rwanda indicating the location of twin lakes in northern Rwanda in green, with a green frame around the area where Lakes Ruhondo and Burera are located. B: Detailed map showing sites where samples were taken in both lakes (in red) sampling areas,* Procambarus clarkii *(in yellow) and Macroinvertebrates (in white).*

Physicochemical parameters

At each sampling site, we assessed physical chemical parameters (see Table S1) in parallel to red swamp crayfish and macroinvertebrate sampling. Collected physical-chemical parameters (temperature, total dissolved solids (TDS), conductivity, pH, and dissolved oxygen) were measured using the HANNA HI 9829 handheld multiparameter tool. The water samples were collected in plastic bottles for eDNA analysis of biodiversity from these lakes for further studies.

Photo ©: Habimana. Measuring physical chemical parameters by Rwibutso and Nzaramba

Morphological identification

The specimens of the genera *Procambarus* and other sampled specimen were identified to species level by MR on the basis of morphological features, with reference to the original literature for *Procambarus clarkii* and (de Moor and Day, 2002; de Moor et al., 2003 and Brown ,1994) for macroinvertebrate and Prior to DNA extraction, high-resolution images were taken with a digital microscope (Keyence VHX-600).

Photo ©: Dusabe. Morphological identification.

DNA extraction, PCR and Sequencing

DNA was extracted from the abdominal tissue using the CTAB protocol as described by Wilke et al. (2006). Fragments of two mitochondrial markers, COI (cytochrome *c* oxidase subunit I) and 16S rRNA, were amplified using the primers COF14 (Folmer et al. 1994) and COR722b (Wilke and Davis 2000) and 16Sar and 16 Sbr (Palumbi et al. 1991) respectively. PCR conditions followed those described by Albrecht et al. (2004). Sanger sequencing was performed on an ABI 3730xl DNA analyser using the Big Dye Terminator Kit (Life Technologies, LGC Genomics GmbH, Berlin, and Germany). All DNA sequence errors at the beginning and end of the sequences were manually edited and the alignment was performed using BioEdit v7.2 (Hall, 1999).Vouchers are deposited in the University of Giessen Systematics and Biodiversity collection. BLAST was used to taxonomically ascertain the specimen by comparing the sequences to those in the database of NCBI. The COI sequences were supplemented with some previously published relevant sequences on GenBank (Table 2).

Photo ©:Dusabe. DNA isolation by Muyumbana.

Campaign

Throughout the campaign, we involved fishermen from most of the cooperatives in the vicinity of the two lakes in the awareness campaign. We exchanged ideas and gathered some information about the first appearance of *Procambarus clarkii* and information about native species in the lakes.

Results

Morphological identification of the targeted species (Procambarus clarkii): Presence/Absence

Photo©: Kwizera. The traps of Procambarus clarkii

Through morphological identification, we have confirmed the presence of *Procambarus clarkii* and macroinvertebrates. Although *Procambarus clarkii* was observed in both Lake Ruhundo and Lake Burera at 11 (34.3%) of 32 (100%) sampling sites, its distribution and abundance differed between the two lakes.

In Lake Ruhundo, *P. clarkii* occurred at 6 (18.7%) of 23 (71.8%) sites and in Lake Burera at 5 (15.6%) of 9 (28.1%) sites (Table 1).

Table 1. Overview of the sampling sites at Lake Ruhondo (LR) and Lake Burera(LB): distribution of species among sampling sites, latitude and longitude coordinates, voucher number, occurrence of Procambarus clarkii, sequenced specimens and associated macroinvertebrate.

Localities	Coordinates		Voucher No	Presence	Sequenced	Associated Macroinvertebrates
	Latitude(°N)	Longitude (°E)				
LB(Kagogo)	-1.39406	29.7685	UGSB 30109	presence	yes	
LB(Kagogo)	-1.39359	29.78225	UGSB 30110	presence	yes	*Melanoides*
LB(Kagogo)	-1.38302	29.78134	UGSB 29722	presence	no	*Physa, Radix*
LB(Kinyababa)	-1.38428	29.7958		absence		*Radix*
LB(Bugamba)	-1.38611	29.79686		absence		*Radix*
LB(Kagogo)	-1.38295	29.78129		absence		*Radix*
LB(Burera)	-1.38295	29.78129		absence		*Physa*
LB(kagogo/ Rukenke)	-1.38295	29.78129	UGSB 28463	presence	no	
LB(Kinyababa /Rukore)	-1.38611	29.79686	UGSB 28464	presence	no	
LR(Gacaca)	-1.52304	29.70763		absence		*Melanoides, Sphaerium,Radix*
LR(Remera)	-1.51595	29.72138		absence		*Radix*
LR(Remera)	-1.51485	29.72231		absence		*Bulinus, Biomphalaria, Melanoides, Radix*
LR(Gashaki/Birwa)	-1.51142	29.73067		absence		
LR(Remera/mumana)	-1.51601	29.71621	UGSB 30111	presence	yes	*Bimphalaria, Melanoides,Radix, Sphaerium*
LR(Remera/Mukiriba)	-1.52122	29.71371		absemce		*Melanoides,Sphaerium*
LR(Gashaki)	-1.49424	29.74278		absemce		*Melanoides,Radix*
LR(Gashaki)	-1.50192	29.74947		absemce		*Bulinus,Biompalaria,Lentobis, melanoides,Radix*
LR(Gashaki)	-1.502	29.74469		absemce		*Bulinus,Biompalaria,Lentobis, Melanoides,Radix*
LR(Gashaki)	-1.53555	29.75498	UGSB 29778	presence	no	*Bulinus,Biompalaria,Melanoides, Radix*

LR(Gacaca)	-1.49978	29.71656	UGSB 30112	presence	yes	*Biomphalaria,,Melanoides, Ictinogompus,Sphaerium*
LR(Gacaca)	-1.49396	29.7171	UGSB 29788	presence	yes	*Melanoides,Radix*
LR(Kanoni)	-1.48021	29.73865	UGSB 30113	presence	yes	*Bulinus,Bellamya,Melanoides, Radix*
LR(Gitovu)	-1.47638	29.75647		absence		
LR(Gacaca/Gasenyi)	-1.49939	29.71595		absence		*Biompalaria*
LR(Kinoni/Ntaruka)	-1.47449	29.7476		absence		*Biompalaria*
LR(Gacaca/Gasenyi)	-1.49939	29.71595		absence		*Radix*
LR(Kinoni/Ntaruka)	-1.47449	29.7476		absence		*Radix*
LR(Kinoni/Gikoro)	-1.4807	29.73674		absence		*Radix*
LR(Musanze)	-1.49939	29.71595		absence		*melanoides*
LR(Burera)	-1.4807	29.73674		absence		*melanoides*
LR(Musanze)	-1.49939	29.71595		absence		*Physa*
LR(Gacaca/Gasenyi)	-1.49939	29.71595	UGSB 28465	presence	no	

Identification outcomes using DNA barcoding

Our molecular identification focused on 5 sequences from new specimens performed with COI and 16S. The BLAST search confirmed our taxonomic assignment based on morphology. Both the COI and 16S sequences had a percentage identity (similarity) of >98% with the closest sequences in GenBank (see Table 2).

Table 2: Overview of specimens from both lakes in DNA barcoding: species prep number, morphology description, similarity with Genbank sequences, source of identified specimens and locality.

Species No	**Morphology ID**	**Blast ID CO1**	**Blast ID 16S**	**GenBank accession**	**source**	**Location**
	Site description	**Identity %**	**Identity %**			
29,699	*Procambarus clarkii*		100	KJ645816.1	*Procambarus clarkii* isolate TW4 16S ribosomal RNA gene, partial sequence; mitochondrial.	USA: Chicarita Creek, San Diego, CA
29698	*Procambarus clarkii*		97.31	KJ645829.1	*Procambarus clarkii* isolate LV9 16S ribosomal RNA gene, partialsequence; mitochondrial.	USA: Las Virgenes Creek, Los Angeles, CA
29,700	*Procambarus clarkii*	100		PP409480.1	*Procambarus clarkii* voucher ANMB96 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial.	Kenya: Nyeri

29695	*Procambarus clarkii*	100		OQ870906.1	*Procambarus clarkii* isolate Muscle cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial.	France
29640	*Procambarus clarkii*	99.8		OQ979159.1	*Procambarus clarkii* voucher SMNH-AR30275 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial.	Israel
29695	*Procambarus clarkii*		100	KJ645816.1	*Procambarus clarkii* isolate TW4 16S ribosomal RNA gene, partial sequence; mitochondrial.	USA: Chicarita Creek, San Diego, CA
29694	*Procambarus clarkii*		100	KJ645816.1	*Procambarus clarkii* isolate TW4 16S ribosomal RNA gene, partial sequence; mitochondrial.	USA: Chicarita Creek, San Diego, CA
29694	*Procambarus clarkii*	100		PP409480.1	*Procambarus clarkii* voucher ANMB96 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial.	Kenya: Nyeri

With 16S (580 bp length) and COI (658 BP length) sequences represented a single Network (see Figure 2, Table S2) with the maximum of 6 nucleotides mutations. Three haplotypes for the partial COI fragment of *P. clarkii* were identified from 6 countries (Table 2). Three haplotypes from Genbank (OQ979159, PP409480 and OQ870906) from Israel, Kenya and France were clustered together with our new haplotype sequences from Rwanda and two from Uganda (29689 and 29693) and two additional haplotypes from Genbank (KX417114, KT959364) from the USA were clustered separately with our new haplotype sequences (Figure 2) with six maximum mutations of and minimum of two mutations.

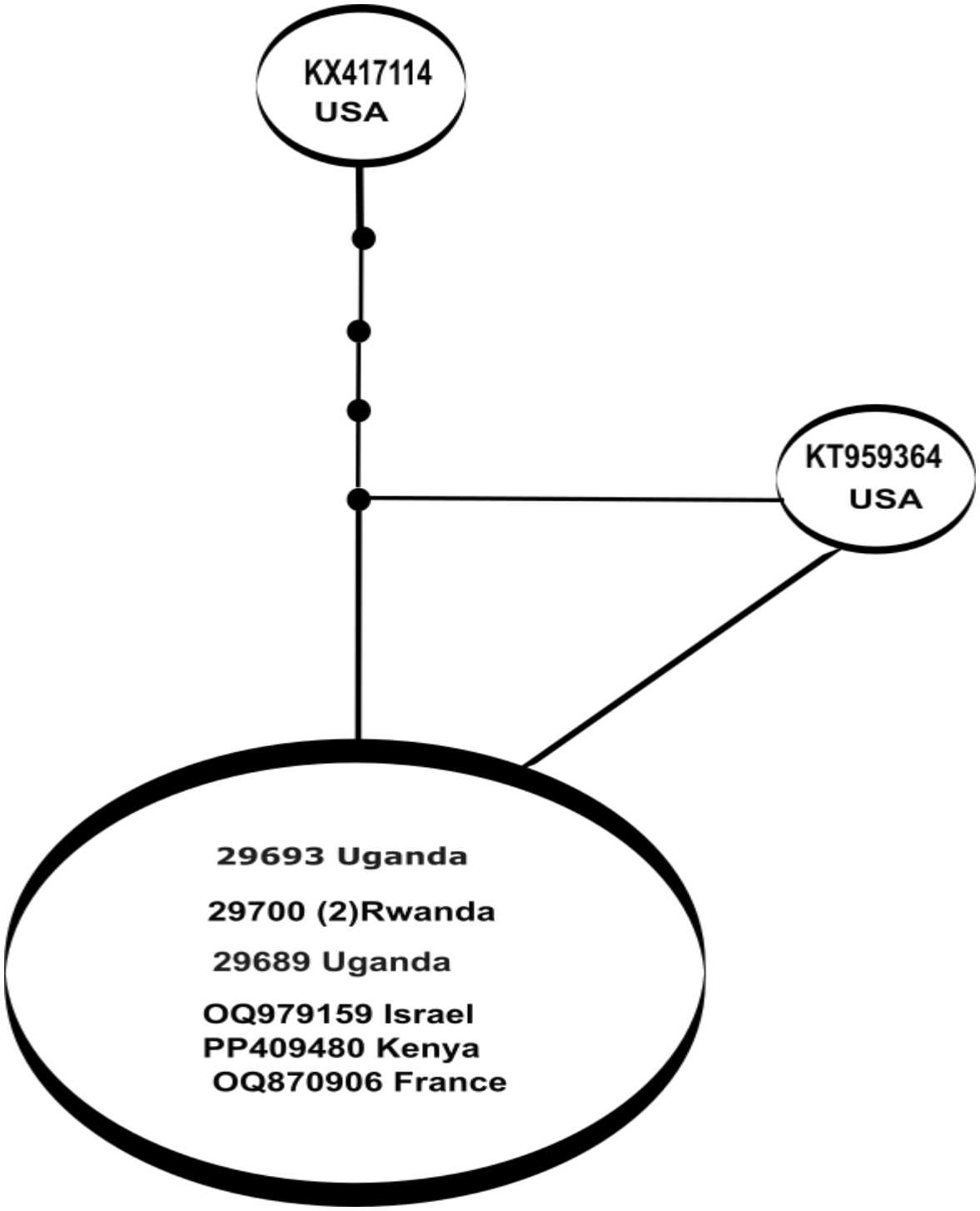

Figure 2: Maximum parsimony network of Procambarus clarkii *using COI (658 base pairs length). A network inferred by the TCS method using 10 COI sequences from 6 countries. The big circle in a network represents 1haplotypes presented by 8 sequences (two from Uganda, three from Rwanda, one from France, Israel, and Kenya), and the small cycle represents 1 haplotype from the USA each and dots represent mutations.*

Physicochemical parameters and environmental variables

The water parameters at the sampling sites where *Procambarus clarkii* was sampled had the following average values: pH of 8.2 and 7.9, dissolved oxygen (DO) of 6.2 and 6.8 mg/L, total dissolved solids (TDS) of 205.7 and116.5 mg/L, temperature of 23.2 and 22.9°C, electrical conductivity (EC) of 411.5 and 232.8µS/cm, Ruhondo and Burera, respectively. Table S1 contains a detailed summary of these parameters. The following figure summarizes the relationship between the water parameters and the measured values at the different sites.

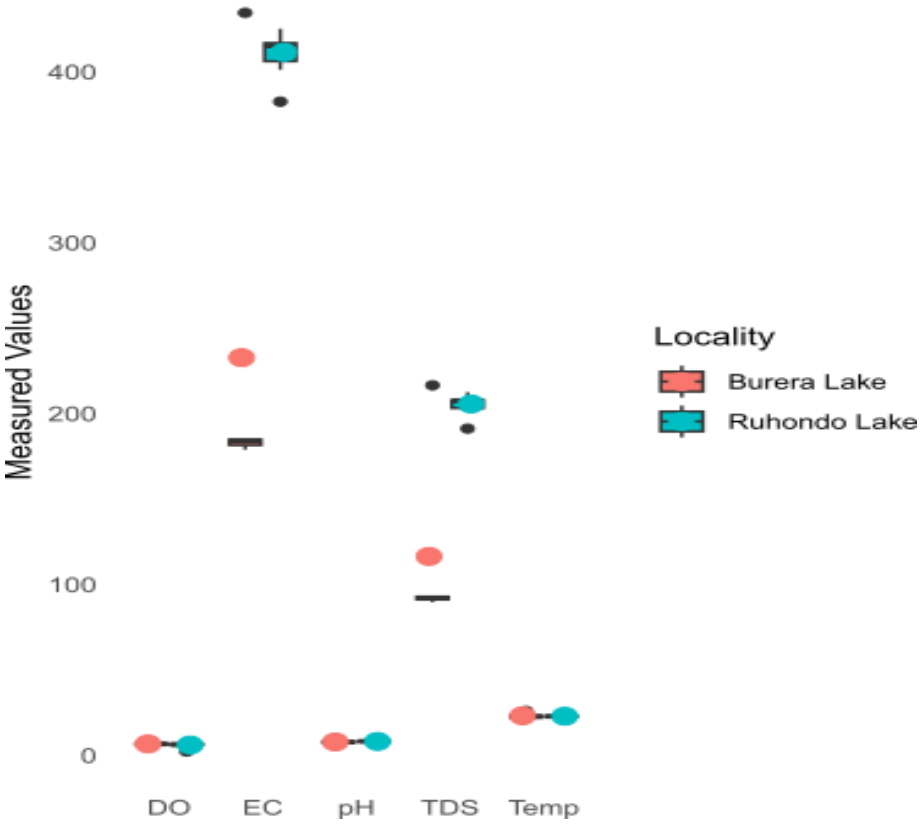

Figure 3: water parameters comparison between Ruhondo and Burera Lakes. Red color present parameters of Burera and Ruhondo (blue).

The comparison of the most important water quality parameters between Lake Ruhondo and Lake Burera shows some important differences. Dissolved oxygen (DO) arrange from 2.3 up to 8.2 mg/L these values some are low and show a narrow range in both lakes, indicating limited oxygen

availability in the water, which could affect aquatic organisms. Electrical conductivity (EC) varies significantly between the two lakes from179 up to 425.338µS/cm, with Lake Ruhondo having much higher conductivity values compared to Lake Burera. This suggests that Ruhondo has a higher concentration of dissolved ions, likely due to mineral content or external sources of pollution. Remarkably, Ruhondo also showed several outliers in the EC measurements, indicating occasional spikes in conductivity.

The pH in both lakes is in a relatively constant and neutral range, indicating the range between 7.5 up to 8.7. However, total dissolved solids (TDS) varied more, with Lake Ruhondo having higher concentrations than Lake Burera varies from91.3 up to216.67 mg/L, suggesting a greater amount of dissolved minerals or pollutants in the Ruhondo water. Finally, the temperature values of the two lakes were quite similar and showed little variation 22.14 up to 26.09°C, suggesting that both lakes have comparable thermal conditions (Table S1).

These observations highlight the possible environmental or anthropogenic factors influencing the higher EC and TDS values of Lake Ruhondo, while the low dissolved oxygen values in both lakes may require further investigation into possible ecological impacts.

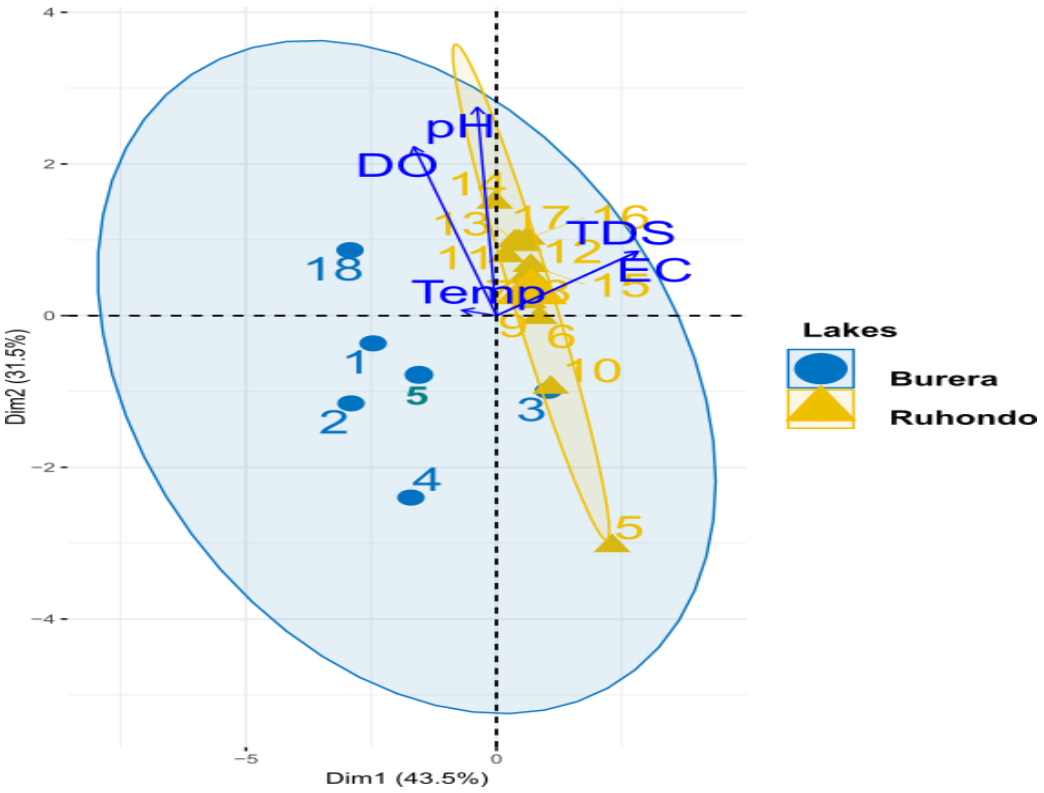

Figure 4: PCA Biplot analysis Illustrates how the different water quality parameters (Temp, pH, EC, TDS, DO) contribute to the overall variance in our dataset and how Procambarus clarkii and macroinvertebrates from different locations (Burera Lake, Ruhondo Lake) are grouped based on these parameters. Dim1 and Dim2 are likely referring to the first two principal components (or axes) from a Principal Component Analysis (PCA) or a similar dimensionality reduction technique. The percentages given (43.5% and 31.5%) indicate how much of the total variance in the data is captured

The PCA biplot illustrates how the water samples from Lakes Burera and Ruhondo are affected by different physicochemical parameters. Samples located near the origin are less influenced by the measured parameters, while those aligned with the direction of a vector are more influenced by the corresponding factor. In Lake Burera, sample 1 is strongly influenced by temperature and electrical conductivity (EC), sample 2 shows a moderate influence of dissolved oxygen (DO), and sample 3 is slightly influenced by pH. In Lake Ruhondo, sample 14 is strongly influenced by total dissolved solids (TDS), sample 5 is moderately influenced by pH and DO, and sample 6 shows a slight influence of temperature. This analysis clearly shows how specific environmental factors influence the water properties in each lake (Table S1).

Discussion

The average of water parameters of Ruhondo and Burera lakes showed pH of 8.2 and 7.9 indicates an alkaline environment, as previously described in a study on physicochemical parameters of

Lakes Burera and Ruhondo (Habimana&Nsabimana, 2020). *Procambarus clarkii* is known to thrive in a wide pH range, but prefers slightly alkaline conditions, as described by Loureiro et al. (2015). An average DO value of 6.2 and 6.8 mg/L indicating limited oxygen availability in the water, which could affect aquatic organisms as discussed by Ultsch & Nordlie (2019), a TDS value of 205.7 and 116.5 mg/L indicates a moderate mineral content, a temperature of 23.2 and 22.9°C is an optimal range for *Procambarus clarkii* and an EC value of of 411.5 and 232.8µS/cm provides an ion concentration in the water. All variable parameters are favorable for the survival and reproduction of this species (Bissattini et al., 2015, Huner & Barr, 1991).

The phylogenetic network shows that the samples from these lakes were closely clustered with other *Procambarus clarkii* specimens from four different countries (Uganda, Kenya, France and Israel). This clustering indicates a100% genetic similarity between our specimens and those from these countries, suggesting either a common origin or recent gene flow between the populations. The presence of *Procambarus clarkii* in these lakes could have a significant ecological impact given the known invasive potential of this species. It is important to monitor these populations closely as they could affect native biodiversity, water quality and ecosystem functioning. Given the species' adaptability to a range of environmental conditions and its potential for rapid dispersal, it is necessary to implement management strategies to control the population and mitigate its impact on the local ecosystem.

In this discussion, environmental parameters, morphological and DNA identification results, and phylogenetic analyses are effectively linked to confirm the presence of *Procambarus clarkii* and consider its broader ecological implications. However, it is of great interest to understand the dynamics of this species in East Africa.

Fishermen Feedback Summary on the Red Swamp Crayfish Campaign

Overview of the campaign: The campaign, which was led by Mr. Marcellin Rwibutso, started in the afternoon with 94 local fishermen from different cooperatives and more than 20 local people. The aim of the event was to introduce and inform the fishermen about the research study on *Procambarus clarkii*, especially its occurrence in the local lakes and its impact on biodiversity.

Mr. Rwibutso, with the support of the Animal Resource Officer, explained the objective of the study: to investigate the occurrence of *Procambarus clarkii* in the local lakes, to understand its introduction, its benefits and its negative impact on biodiversity. He provided information on the origins of *Procambarus clarkii* and explained how it has spread to different countries and its proven impact on native species. He discussed both the potential benefits and negative impacts on local ecosystems.

Insights from fishermen:

Participant 1: Was unaware of the invasive nature of crayfish, but noted its negative impact on native species in the lakes. “Causing a sharp decline” since its appearance.

Participant 2: Knew the crayfish as "Magurukotone or Masokoza" local name and mentioned its use in the community: known as pig feed, noting the increased market price due to the introduction of consumption by the community.

Leopard: He recalled seeing crayfish in a lake while fishing in 1995.

Babyariyehe: Gave a historical overview and noted that *Procambarus clarkii* was introduced in 2003. Initially it was shunned due to its aggressiveness and cultural barriers, resulting in high reproduction rates. By 2014, consumption increased and the crayfish were used as pig feed, driving up their market price. Since their appearance, a significant decline in amphibian larvae and juveniles has been observed, probably related to the introduction of the crayfish, possibly by stocking fish introduced by PAIGELAC (projet d’appui `a la gestion des lacs interieurs). The origins of the seeds were traced back to Uganda and Burundi (reported by fishermen during

Campaign (2024). All participants agreed that *Procambarus clarkii* is an invasive species that disturbs native biodiversity in its habitats and negatively affects the reproduction of native species.

Photo ©: Richard Habimana. Fishermen, local people and the project team members through campaign activities

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Supplementary data

Table S1: Physicochemical parameters of Ruhondo and Burera Lakes

Locality	DO	pH	TDS	Temp	EC	Substrate	Utization
Burera Lake	7.11	8.23	89.67	22.14	179	stones	Boating,fishing,Tourism
Burera Lake	6.79	7.85	91.33	26.09	181.67	detritus	fishing,toursim,water sport
Burera Lake	6.38	7.47	216.67	23.36	434.67	Sand	water sport,boating,fishing,
Burera Lake	5.55	7.56	92	21.78	184	detritus	boating,fishing,Tourism
Ruhondo Lake	2.31	7.48	210.67	22.22	421.33	sand	fishing,toursim,water sport
Ruhondo Lake	6.07	8.1	207.67	22.71	416.33	sapropel	fishing,toursim,water sport
Ruhondo Lake	6.4	8.3	206	23.2	412	Sand	fishing,toursim,water sport
Ruhondo Lake	6.03	8.4	205.33	23.02	410	Sand	fishing, boating ,tourism
Ruhondo Lake	6.13	8.18	208	23.81	416.67	sapropel	fishing, boating ,tourism
Ruhondo Lake	5.12	7.84	210	23.8	420.33	sapropel	fishing, boating ,tourism
Ruhondo Lake	6.97	8.32	201	23.62	401.33	sand	fishing,toursim
Ruhondo Lake	6.23	8.58	203.33	22.95	406.33	Sand	fishing,toursim,water sport
Ruhondo Lake	6.97	8.42	203	22.78	406	Sand	fishing,toursim,water sport
Ruhondo Lake	6.8	8.78	191.33	23.2	382.67	Sand	fishing,toursim,water sport
Ruhondo Lake	6.59	8.33	207.67	22.44	415.33	Sand	fishing,toursim,water sport
Ruhondo Lake	7.23	8.33	212.67	22.45	425.33	sapropel	fishing,toursim,water sport
Ruhondo Lake	7.23	8.33	208	22.78	417	Sand	Fishing, boating
Burera Lake	8.2	8.57	93	22.91	185	Sand	fishing,boating ,tourism