Dynamics of Host Snail Species and Associated *Schistosoma/Fasciola* Infections: Testing the Citizen Science Model in Lake Albert Region, Uganda

BY

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Registration number at MUST (Host institution): 2019/PHD/032/PS

A Thesis Submitted to the Examination Office, Science Faculty, in Fulfilment of the Requirements Leading to the Award of a Degree of Doctor of Science (Doctor Rerum Naturalium) of Justus Liebig University Giessen

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May, 2024

ARTICLES PUBLISHED

First author:

1. **Tumusiime, J**., Rugunda, G. K., Tolo, C. U., Namirembe, D., Schols, R., Hammoud, C., Albrecht, C., & Huyse, T. (2023). An accident waiting to happen ? Exposing the potential of urogenital schistosomiasis transmission in the Lake Albert region , Uganda. *Parasites & Vectors*, 1–8. https://doi.org/10.1186/s13071-023-06017-3
2. **Tumusiime, J.**, Valderrama Bhraunxs, N., Kagoro-Rugunda, G., Namirembe, D., Albrecht, C., Twongyirwe, R., Tolo, C. U., Jacobs, L., & Huyse, T. (2024). Citizens can help to map putative transmission sites for snail-borne diseases. In *PLOS Neglected Tropical Diseases* (Vol. 18, Issue 4, p. e0012062). https://doi.org/10.1371/journal.pntd.0012062

Co-authored papers relevant to the study field:

1. Anyolitho, M. K., Poels, K., Huyse, T., **Tumusiime, J**., Mugabi, F., Tolo, C. U., Masquillier, C., & Nyakato, V. N. (2022). Knowledge, attitudes, and practices regarding schistosomiasis infection and prevention: A mixed-methods study among endemic communities of western Uganda. *PLoS Neglected Tropical Diseases*, *16*(2), 1–21. https://doi.org/10.1371/journal.pntd.0010190
2. Brees, J., Huyse, T., **Tumusiime, J**., Kagoro-Rugunda, G., Namirembe, D., Mugabi, F., Nyakato, V., Anyolitho, M. K., Tolo, C. U., & Jacobs, L. (2021). The potential of citizen-driven monitoring of freshwater snails in schistosomiasis research. *Citizen Science: Theory and Practice*, *6*(1), 1–13. https://doi.org/10.5334/cstp.388
3. Hammoud, C., Kayenbergh, A., **Tumusiime, J.**, Verschuren, D., Albrecht, C., Huyse, T., & Bocxlaer, B. Van. (2022). Parasites and Wildlife Trematode infection affects shell shape and size in *Bulinus tropicus*. *International Journal for Parasitology: Parasites and Wildlife*, *18*(April), 300–311. https://doi.org/10.1016/j.ijppaw.2022.07.003
4. Ssenkuba, F., Komujuni, C., & **Tumusiime, J**. (2022). In vitro anthelmintic activity of *Leonotis nepetifolia* ethanolic and aqueous leaf extracts on *Fasciola gigantica*. *Journal of Veterinary Medicine and Animal Health*, *14*(4), 82–90. https://doi.org/10.5897/JVMAH2022.1011

Manuscripts in preparation:

1. Tumusiime, J., Tolo, C. U., Rugunda, G. K., Namirembe, D., Hammoud, C., Albrecht, C., & Huyse, T. Diversity of *Biomphalaria* spp. and *Schistosoma* parasite prevalence along a spatial-temporal gradient in the Lake Albert Region, Uganda. [**In prep.]**
2. Chan, H.K., Huyse, T., Tumusiime, J., & Jacobs, L. Artificial Intelligence and the Future of Citizen Science: The Potential of Deep Learning Object Detection in Citizen-Driven Snail Host Monitoring to Map Putative Disease Transmission Sites. *Citizen Science: Theory and Practice* **[Accepted]**

DECLARATION (INSURANCE POLICY)

I declare that I have completed this dissertation single-handedly without the unauthorized help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and cited all text passages that are derived verbatim from or are based on the content of published work of others, and all information relating to verbal communications. I consent to the use of an anti-plagiarism software to check my thesis. I have abided by the principles of good scientific conduct laid down in the charter of the Justus Liebig University Giessen „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ in carrying out the investigations described in the dissertation.

Signature ……………………………….. Date……27.05.2024………

Julius Tumusiime

SUPERVISORS’ APPROVAL

We the undersigned, declare that the research work culminating into this thesis was conceived and conducted under our guidance and supervision:

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Signature……Grace………………………………….. Date ……16th May 2024……………

Prof. Grace Kagoro Rugunda

Signature… ... Date …13/05/2024……

Dr. Tine Huyse

Signature Date 13 May 2024....................

Assoc. Prof. Christian Albrecht

DEDICATION

I dedicate this work to my lovely family who had to bear with my absence for long periods when carrying out this research. To my wife Mrs. Esther Akello Ediu Tumusiime, daughter Jean Clara Asiimwe, and sons Kevin Businge, Seth Elisha Agaba and Jonan Basiima; I always derive encouragement to reach greater heights knowing that your love and prayers are worth every sacrifice. I hope that when you read this piece of work, you will be encouraged to pursue your dreams.

I also dedicate this piece of work to my parents: Mr. Edward Majara and Mrs. Paulina Majara. I owe my life and academic progress to you. Your sacrifice to nurture me into what I am today is greatly appreciated. Thank you for always praying for me and being my strongest cheerleaders.

ACKNOWLEDGEMENTS

I want to extend my hearty gratitude to a number of individuals and organisations who contributed significantly towards the completion of this research project. First, this research was financed by the Action Towards Reducing Aquatic Snail-borne Parasitic diseases (ATRAP) project of the Development Cooperation program of the Royal Museum for Central Africa, Tervuren, Belgium and the Mbarara University of Science and Technology, Uganda with support of the Directorate-General for Development Cooperation and Humanitarian Aid (DGD), Belgium. Therefore, my sincere appreciation goes to the above-listed institutions for the support rendered, without which this research project would have been impossible.

Secondly, I was privileged to work under the supervision of the following dedicated mentors: Dr. Tine Huyse, Dr. Casim Umba Tolo, Associate Professor Christian Albrecht and Professor Grace Kagoro-Rugunda. This supervisory team provided direction and guidance right from the conceptualisation of this research project to its completion. In the process, I have learnt a lot of lessons which will guide me in the academic journey which is only at inception. I specifically appreciate them for always looking out for me and ensuring that I was not deprived of opportunities for academic progress. They lifted me when I was down and used their experience to guide me to the last moments of the study.

Third, I would like to extend my appreciation to the entire ATRAP project team with whom we have worked throughout the project life. Ms. Daisy Namirembe worked with me through the thick and thin of fieldwork. I must admit, the fieldwork would have been tougher without Ms. Namirembe who demonstrated resilience and dedication to the field activities with the motivation to be revered. I thank her for being the catalyst of the otherwise cumbersome field trips. Even in my absence from laboratory work in Belgium, Ms. Namirembe ensured that data collection continued seamlessly. Similarly, I appreciate the contribution of Mr. Ben Lukubye who made logistical arrangements for the smooth running of the project. Mr. Lukubye also participated in the field sampling campaigns

during my absence for the lab training. In the same vein, I appreciate the contributions of Mr. Maxson Kenneth Anyolitho and Ms. Faith Mugabi, the PhD and Master's scholars on the social science strand of the ATRAP project. Alongside them, we selected the study sites and set up the citizen science network. In addition, we conducted concurrent fieldwork, training and refresher training for the citizen scientists, data dissemination workshops and awareness-raising campaigns. I am glad to have learnt from them different aspects of the outreach and community engagement approaches. Similarly, my gratitude extends to ATRAP students from Belgium: Mr. Jef Brees, Ms. Noelia Valderrama Bhraunxs and Ms. Mercy Gloria Ashepet. Mr. Brees was instrumental in the setting up of the citizen science network and designing the data collection tools used in the KoboCollect application by citizen scientists. I am grateful to have learnt this knowledge from him for later application in this study. Ms. Bhraunxs contributed significantly in the validation and collaborative analysis of the snail data collected by the citizen scientists. Her moral support in writing the sections we collaboratively worked on is greatly appreciated. Ms. Ashepet made our research stay in Belgium easier as she offered us important orientation in the new environment and especially shared significant insights on the best strategies to link up with our families back home.

Fourth, I extend my gratitude to the team at the RMCA during the months of research stay in the years 2021, 2022, and 2023. I start with Ms. Muriel Van Nuffel for making the logistical arrangements for the smooth travels and settling in Belgium. She also organised monthly cultural visits which exposed the culture and history of Belgium. Through these visits, we were able to break the routine of laboratory work with fun and enjoyment which I highly appreciate. Next, I would like to thank the personnel in the finance office of RMCA led by Ms. Eva November assisted by Ms. Ella Bert. They ensured that we did not lack when we were in Belgium and ensured a smooth flow of funds for the running of my research project. Next, I appreciate the support provided by the doctoral students under the supervision of Dr. Tine Huyse. First, Dr. Cyril Hammoud initiated and trained me at the Centre Accueil Personnel Africain (CAPA) molecular laboratory. DNA extraction, PCRs and sequence analysis were all done with his training. Dr. Hammoud was selfless and always created time for me amidst his very busy schedule as he was completing his doctoral programme. His mentorship and friendship are greatly valued. Next, Mr. Ruben Schols also contributed to the smooth laboratory work at RMCA. He shared his experiences and insights whenever needed. Mr. Tim Maes also shared from personal research, and his sense of humour is appreciated. Dr. Germain Kapour with whom we shared residence and laboratory for three years provided me company, more so during the COVID-19 period of 2021 when the laboratory was very lonely. I acknowledge the support provided by the laboratory personnel Ms. Nathalie Smitz and Mr. Kenny Meganck during the time spent at the CAPA molecular laboratory.

On a very special note, I extend my utmost appreciation Dr. Tine Huyse who held together and coordinated the entire ATRAP project and the team at RMCA. All the experiences were achieved under her leadership along with the team of local promotors in Uganda including Dr. Casim Umba Tolo, Dr. Viola Nyakato, Prof. Grace Kagoro-Rugunda and Dr. Ronald Twongyirwe.

I also greatly appreciate the great contribution of all the citizen scientists: Mr. Alinda Hassan, Mr. Chotum Friday, Mr. Atanasi Marisel, Mr. Opio Isingoma, Mr. Ategeka Augustine, Mr. Bahemuka Bob, Mr. Masereka Haruna, Mr. Sebakara Fobius, Ms. Nyamahunge Imelda, Mr. Businge Zabron, Ms. Tumusiime Janet, Mr. Nsenga David, Ms. Night Marygoret, Ms. Kyaligonza Noeline, Mr. Mwesige Robert, Mr. Barisigara Gard, Mr. Tusingwire Henry, Ms. Natabi Specioza, Ms. Nakingi Rose, Mr. Bamuturaki Charles, Mr. Kamukama Josias, Mr. Nuwagaba Emmanuel, Mr. Tweheyo Julius, Mr. Bahungirehe Crezestom, Mr. Ategeka Rogers, Ms. Unimu Hadijah and Mr. Fuwarinyo Richard.

Support from the Ugandan Ministry of Health Departments through the Vector Control Units of Kagadi and Ntoroko district local governments is also greatly acknowledged. Mr. Chris Twebaze, the Vector Control Officer of Kagadi District was instrumental in the entire ATRAP project setup and implementation.

Last but not least, I extend my appreciation to the Department of Biology at MUST including all my colleagues for the guidance during progress presentations and moral support extended to me. It has not been a lonely journey due to the team spirit exemplified by the colleagues. Specifically, I would like to single out colleagues who stepped in for me to take on my work load during my absence to the field and laboratory. Dr. Justine Nakintu, Ms. Ashaba Naome and Mr. Ssenkuba Francis helped in the teaching and practical sessions whenever I was not able to due to travels. I will forever be indebted to them. In the same vein, I extend my appreciation to the team at the Institute of Animal Ecology and Systematic, Justus Liebig University Giessen Germany led by my supervisor Assoc. Prof. Christian Albrecht. Thank you all for always hosting me (especially Ms. Marie Claire Dusabe and your dear husband Mr. Rwibutso) and for the guidance.

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LIST OF ACRONYMS/ABBREVIATIONS

ATRAP	Action towards reducing aquatic snail-borne parasitic diseases
BIC	Bayesian information criterion
CAPA	Centre Accueil Personnel Africain
COX1	Cytochrome c oxidase 1
CS	Citizen Scientist
DGD	Belgium Development Corporation
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
ddPCR	Droplet Digital Polymerase Chain reaction
DRC	Democratic Republic of Congo
EC	Electrical conductivity
FN	False negative
FP	False positive
FRET	Fluorescence Resonance Energy Transfer
GIS	Geographical Information Systems
HIV	Human Immunodeficiency Virus
ITS	Internal transcribed spacer
LAMP	Loop-mediated isothermal amplification
MDA	Mass Drug Administration
MEGA	Molecular Evolutionary Genetics Analysis
ML	Maximum likelihood
MoH	Ministry of Health
MUST	Mbarara University of Science and Technology
NNE	North-North-East
PCR	Polymerase Chain reaction

qPCR	quantitative polymerase chain reaction
RAPD	Random Amplified Polymorphic DNA
RD-	Rapid diagnostic
REC	Research Ethical Committee
RMCA	Royal Museum for Central Africa
RNA	Ribonucleic acid
SSA	sub-Saharan Africa
SSW	South-South-West
TCS	Temporal Compression of Sequences
TDS	Total dissolved solids
TN	True negative
TP	True positive
UBOS	Uganda Bureau of Statistics
VCD	Vector Control Division
VHT	Village Health Team
WHO	World Health Organisation

ABSTRACT (German)

Schistosomiasis ist eine durch Schnecken übertragene Krankheit, von der weltweit über 200 Millionen Menschen betroffen sind, 90 % davon leben in Afrika südlich der Sahara. Sie führt zu Problemen bei der Fortpflanzung und der körperlichen Gesundheit, Wachstumsstörungen bei Kindern, Schulabbruch und schwere Infektionen führen zu Morbidität und Tod. Die Krankheit ist in den großen Seen Ugandas weit verbreitet. Allerdings stellen unvollständige Kenntnisse über die Verteilungsmuster der Schneckenwirte und Krankheitsübertragungsorte aufgrund des Mangels an Malakologen eine Herausforderung bei der Umsetzung der Empfehlungen der Weltgesundheitsorganisation (WHO) zur Schneckenbekämpfung dar. Diese soll die medikamentöse Behandlung zur Beseitigung der Krankheit ergänzen. Daher untersuchte diese Studie die Variation in der Verteilung der Wirtsschnecken von *Schistosoma* und *Fasciola* spp. in Raum und Zeit innerhalb der endemischen Bilharziose-Region um den Albertsee in Uganda. Die Studie verfolgte auch einen Citizen-Science-Ansatz zur Schneckenüberwachung. Ab Juni 2020 beprobte der Doktorand 20 Monate lang monatlich Schnecken an 76 Standorten, während die *Citizen Scientists* (CSs) im gleichen Zeitraum wöchentlich dieselben Standorte beprobten. Es wurde ein zeitbasierter Schneckensammelansatz mit Sieben verfolgt, während die physikalisch-chemischen Wasserparameter in situ und einige in einem Feldlabor nach Standardprotokollen bestimmt wurden. Die Bestimmung der Schneckenart erfolgte nach der Analyse der Cytochrom-c-Oxidase-Untereinheit 1 (COX1) und des nukleären internen transkribierten Spacer-Marker (ITS). An den Schneckensequenzen wurde eine phylogenetische und Haplotyp-Netzwerkanalyse durchgeführt, um die Artidentität zu bestätigen und den Genfluss zwischen Populationen zu untersuchen. Es wurden logistische und verallgemeinerte lineare Modelle erstellt, um die vom Doktoranden und den CSs generierten Daten zu vergleichen. Es wurden Regressionsanalysen zwischen physikalisch-chemischen Parametern und der Schneckenhäufigkeit durchgeführt, während räumliche Unterschiede in der Schneckenhäufigkeit mithilfe der Chi-Quadrat-Tests analysiert wurden. In 1.382 Datenberichten (Besichtigungen vor Ort) wurden vom Doktoranden insgesamt 52.757 Schnecken der drei Zielgattungen (*Bulinus* (n = 6.123), *Biomphalaria* (n = 29.670) und *Radix* (n = 16.964)) gesammelt . Beeindruckend ist, dass die CSs Schneckengattungen mit einer Genauigkeit von über 99 % identifizierten (n = 4.034 Datenberichte). Darüber hinaus stimmten die vom CS gesammelten Schneckendaten mit den Daten des Doktoranden überein (70–90 %), was das Vorkommen von Schnecken betrifft, der Grad der Übereinstimmung variierte jedoch zwischen den Schneckengattungen. *Schistosoma mansoni* wurde nur bei *Biomphalaria* spp. nachgewiesen. an Seestandorten trotz höherer Schneckenzahlen an Standorten abseits des Sees, was die Existenz von Übertragungsbedingungen am See unterstreicht. Bei den *Biomphalaria*-Hochlandschnecken dominiert *B.* cf. *pfeifferi*, was in dieser Studie zum ersten Mal den Beweis liefert, dass es sich um eine Unterart von *B. sudanica* handelt, die dazu neigte, *Schistosoma rodhaini* zu beherbergen – ein Nagetier-Schistosom, das mit *S. mansoni* hybridisieren kann. Insbesondere wurden in der Studie zum ersten Mal in diesem Gebiet *Bulinus globosus* und *B. nasutus productus* gefunden, die Wirte von *Schistosoma haematobium*, das zur Harnwegsschistosomiasis führt. Allerdings ergaben Ausscheidungs- und PCR-Tests nur Infektionen mit dem Rinderparasiten *Schistosoma bovis*. Ein Anstieg des pH-Werts war signifikant mit der *Biomphalaria* spp. Häufigkeit verbunden, während der Standorttyp ein Prädiktor für die Abundanz der *Bulinus*- und *Radix*-Arten war. Somit unterstreicht diese Studie das tatsächliche und potenzielle Risiko von *S. mansoni* bzw. *S. haematobium* in der Albertine Region und die hohe Zuverlässigkeit der von Bürgern gesammelten Daten für die Schneckenüberwachung. Dennoch zeigt diese Studie auch die Mängel bei der Klassifizierung der *B. pfeifferi*-Gruppe und die Möglichkeiten für zukünftige Forschung auf. Andererseits unterstreicht das Vorkommen von *R. natalensis* das Potenzial einer hohen Fasziolose-Übertragung, über die in einer Parallelstudie in diesem Gebiet berichtet wurde.

ABSTRACT (English)

Schistosomiasis is a snail-borne disease that affects over 200 million people globally, 90% living in Sub-Saharan Africa. It leads to reproductive and physical health challenges, stunted growth in children, school dropouts, and severe infections lead to morbidity and death. The disease is highly prevalent among the great lakes in Uganda. However, incomplete knowledge of the distribution patterns of the snail hosts and disease transmission sites arising from a shortage of malacologists presents a challenge to implementing the World Health Organisation (WHO) recommendations on snail control to supplement drug treatment towards elimination of the disease. Therefore, this study assessed the variation in the distribution of host snails of *Schistosoma* and *Fasciola* spp. in space and time within the schistosomiasis endemic Lake Albert region of Uganda. The study also adopted a citizen science approach to snail monitoring. Snails were sampled from 76 sites monthly for 20 months by the PhD researcher starting from June 2020, while the citizen scientists (CSs) sampled the same sites weekly in the same period. A time-based snail scooping approach was followed while water physicochemical parameters were determined *in situ* and some in a field laboratory following standard protocols. Snail species determination followed analysis of the cytochrome c oxidase subunit 1 (*COX1*) and the nuclear internal transcribed spacer (ITS) markers. Phylogenetic and haplotype network analysis was conducted on the snail sequences to confirm species identity and study geneflow among populations. Logistical and generalised linear models were generated to compare the data generated by the PhD researcher and the CSs. Regression analyses were conducted between physicochemical parameters and snail abundance while spatial differences in snail abundance were analysed using the chi-square tests. In 1, 382 data reports (site visits), a total of 52,757 snails belonging to the three target genera (*Bulinus* (n = 6,123), *Biomphalaria* (n = 29,670) and *Radix* (n = 16,964)) were collected by the PhD researcher. Impressively, the CSs identified snail genera with over 99% accuracy (n = 4,034 data reports). In addition, the CS collected snail data agreed with the PhD researcher data (70-90%) in snail presence but the degree of agreement varied between snail genera. *Schistosoma mansoni* was only recorded among *Biomphalaria* spp. at lake sites despite superior snail numbers at sites away from the lake, thus highlighting the existence of transmission conditions at the lake. The upland *Biomphalaria* snails were dominated by *B.* cf. *pfeifferi*, which for the first time, this study provides evidence to be a subspecies of *B. sudanica* that tended to harbour *Schistosoma rodhaini* - a rodent schistosome that can hybridize with *S. mansoni.* Notably, the study found, for the first time in this area, *Bulinus globosus* and *B. nasutus productus,* the hosts of *Schistosoma haematobium,* which leads to urinary schistosomiasis. However, shedding and PCR tests only revealed infections with the bovine parasite *Schistosoma bovis*. Increasing pH was significantly associated with *Biomphalaria* spp. abundance while site type was a predictor of *Bulinus* and *Radix* spp. abundance. Thus, this study highlights the actual and potential risk of both *S. mansoni* and *S. haematobium* respectively in the Albertine region and the high reliability of citizen-collected data for snail monitoring. Nevertheless, this study also highlights the deficiencies in the classification of *B. pfeifferi* group and the opportunities for future research. On other hand, the presence of *R. natalensis* highlights the potential of high fasciolosis transmission reported by a parallel study in the area.

Keywords: *Biomphalaria*, *Bulinus*, Citizen Science, fasciolosis, Lake Albert, *Radix*, schistosomiasis, snail-host distribution, trematode parasites.

CHAPTER ONE

1.0 INTRODUCTION

This thesis developed from a bigger research project titled “*Action Towards Reducing Aquatic Snail-borne Parasitic diseases* (ATRAP)”. ATRAP is a multi-disciplinary academic project that incorporated actors from backgrounds of malacology, parasitology, sociology and geography to study different aspects of snail-borne disease transmission in Uganda’s Lake Albert region and parts of Eastern Democratic Republic of Congo (DRC) with funding from the Belgium Development Corporation. In this project, my research focused on studying the biology of intermediate snail hosts of *Schistosoma* spp. and *Fasciola* spp. Thus, this thesis elaborates on the molecular diversity of *Biomphalaria* spp. and *Bulinus* spp., the intermediate hosts of *S. mansoni* and *S. haematobium* parasites respectively and the implications of spatial in snail species distribution to snail-borne disease transmission are discussed. The prevalence of *S. mansoni* and *S. haematobium*, and the risk of hybridization with closely related *S. rodhaini* and *S. haematobium* respectively have been discussed in this study too. In addition, a selection of biotic and abiotic predictors of *Biomphalaria* spp., *Bulinus* spp. and *Radix* spp. presence at a given site were studied in this project.

Central to ATRAP is the ‘Citizen Science’ (CS) approach in which community members were recruited and trained as ‘citizen scientists’ (CSs) to participate in data collection and communicating to the wider community the behaviours and practices that predispose them to contracting snail-borne diseases. While this approach has proved effective in other fields like geography, astronomy and ecology among others, it is still a growing field which needs to be customised and tested for applicability in other fields of study, and in the Global South. In this study, therefore, we investigated the effectiveness of the novel Citizen Science approach to monitoring snail hosts of parasitic diseases.

1.1 Background

Snail-borne parasitic diseases form a major public and veterinary health burden, especially in Sub-Saharan Africa (SSA). The most prevalent diseases are schistosomiasis and fasciolosis, caused by *Schistosoma* and *Fasciola* worms, respectively. These parasites rely on freshwater snails of the genera *Biomphalaria* and *Bulinus* (for *Schistosoma*), and *Radix* (for *Fasciola*) to complete their life cycle (Mage et al., 2002; Morgan et al., 2001; Rollinson et al., 2001). In its chronic form, schistosomiasis can lead to severe health issues such as hepatic fibrosis, bladder cancer, infertility, stunted growth, and reduced learning ability in children (Gryseels et al., 2006). Schistosomiasis has a global burden of approximately 1.43 million disability-adjusted life years (Kyu et al., 2018). In Africa, fasciolosis (liver fluke disease) predominantly affects livestock and it has been estimated to cause annual losses worth billions of dollars. For instance,[2] in Uganda, bovine fasciolosis prevalence in Kampala abattoirs is as high as 84% causing an estimated annual loss of US$ 92.4 million due to condemned liver alone (Joan et al., 2015).

Currently, the primary approach to controlling schistosomiasis is mass drug administration (MDA) among school-aged children (Kokaliaris et al., 2022). Despite a recent modelling study using survey data from 44 countries in SSA indicating a considerable decrease in overall prevalence in the last decades (Kokaliaris et al., 2022; World Health Organization, 2023), schistosomiasis continues to (re-)emerge and scientists agree that MDA alone is not sufficient (King & Bertsch, 2015; Lo et al., 2018; Qian et al., 2018; Sokolow et al., 2017). Sokolow and colleagues evaluated control programs in 83 countries/territories and estimated that interventions for schistosomiasis that integrate snail control result in a 92% reduction in prevalence, compared to a 37% reduction in programs with no or limited snail control (Sokolow et al., 2016). Simulation studies also showed an additional reduction in total disability of 40% when snail control is added to MDA (Lo et al., 2018). In light of these findings, the World Health Organization (WHO) now advises complementing drug treatment with snail control and community involvement in the WHO roadmap for schistosomiasis elimination by 2030 (World Health Organization, 2019). Additionally, snail control will also impact animal diseases such as bovine schistosomiasis, amphistomiasis and fasciolosis, that are transmitted by the same or sympatric snail species, aligning with the One Health approach and steering away from a single-disease focus.

To minimize the environmental impact of snail control, a focal or targeted approach is necessary, whether it involves physical, chemical or biological methods (Allan et al., 2020; King & Bertsch, 2015; Sokolow et al., 2017). However, to design effective snail control interventions, we need detailed information on snail population dynamics at fine spatial and temporal scales, and a thorough understanding of the biotic and abiotic factors that influence these dynamics. Therefore, the ATRAP project set out to test the citizen science approach to meet the gap in snail data and in snail experts. More specifically, a two-year malacological survey on *Schistosoma* and *Fasciola* spp. snail host population dynamics was set up in the Lake Albert region by this study. There was a need to identify where *Biomphalaria* spp., *Bulinus* spp. and *Radix* spp. occur, the factors (biotic and abiotic) controlling their occurrence, and when they are likely to spread the flukes from the snail to the final hosts such as humans and livestock.

Nevertheless, there is currently a severe shortage of trained malacologists worldwide, particularly in SSA, where schistosomiasis is most prevalent (David Rollinson et al., 2013; Sokolow et al., 2017). To scale up data collection to complement the surveys by the few available malacologist, a citizen science-centred approach was instituted (Ashepet et al., 2020), where members of the general public are involved in scientific efforts within their communities (Fraisl et al., 2022; Vohland et al., 2021) especially in hard to reach areas. Citizen science involves collecting large volumes of research data or conducting scientific experiments that are often in response to the local community or societal needs. While citizen science is still in its early stages in Africa (Ashepet et al., 2020; Pocock et al., 2019), and has never been applied in schistosomiasis control, it has recently been proven to be a viable alternative or complement to monitor vectors of malaria, Lyme disease, yellow fever, and Zika (Eritja et al., 2019; Hamer et al., 2018; Murindahabi et al., 2021; Nieto et al., 2018; Palmer et al., 2017).

However, the quality and reliability of citizen-generated data is often questioned (Cox et al., 2016; Elbroch et al., 2011; Kosmala et al., 2016). Investigating data quality in citizen science projects is not straightforward since the nature and amount of incoming data are project-dependent, and so are the required protocols for data quality (Kosmala et al., 2016). Successful projects often deploy a combination of strategies including iterative designs, methods standardization, systematic capture, classification and data-entry procedures, refresher training for citizens, ongoing feedback, registration of metadata, statistical analysis to detect and correct potential bias, and PhD researcher validation (Fritz et al., 2019; Kosmala et al., 2016). However, implementing such tailored measures to monitor and evaluate data quality can be limited by associated costs in terms of human and material resources (Elbroch et al., 2011; Lukyanenko et al., 2016a; Van Eupen et al., 2021). Nonetheless, thorough validation processes are necessary to harness the potential of citizen-generated data for advancing scientific knowledge and guiding evidence-based policy (Hecker et al., 2018; Kennedy, 2016). This was therefore one of the aims of this research.

1.2 Problem statement

In Uganda, particularly along the shores of Lake Albert, the prevalence of schistosomiasis is the highest reported at approximately 90% despite previous efforts of MDA (PMA, 2020). The re-emergence of the disease after MDA could be attributed to several factors including the presence of key animal reservoirs such as cattle and sheep, intermediate snail hosts which are not affected by MDA (Sokolow et al, 2016; Tchuem Tchuenté et al., 2017), and the likely developed resistance to the treatment (Faust et al., 2019). The high prevalence of the disease in Africa is associated with risky behaviours such as washing clothes and fetching infected water, recreational swimming, fishing, and poor hygiene including open defecation (Adenowo et al., 2015; Clerinx & Van Gompel, 2011). Besides, cattle and sheep are known to host *Fasciola* flukes which also infest snails as intermediate hosts. Uganda is the second country most affected by fasciolosis in cattle in Africa with a prevalence of 70% after Sudan with a prevalence of 90% (Mehmood et al., 2017). The flukes cause great economic losses estimated in millions of dollars hence exacerbating the poverty burden in communities in Uganda, more so among the communities in the Lake Albert region where livestock is one of the most important sources of livelihood. Animals are grazed in the water-logged areas which are ideal habitats for freshwater snails that spread *Fasciola* parasites.

Identification of snails and *Schistosoma/Fasciola* trematodes is a key starting point in determining the occurrence, prevalence and distribution of both diseases. However, major challenges with the traditional morphology-based identification approaches like misclassification and underestimation of prevalence have been reported (Joan et al., 2015). This study, therefore, integrated the traditional morphological identification and shedding techniques with the more reliable and robust DNA-based analysis to ensure accurate parasite identification and prevalence estimation.

In addition, it has been established that schistosomiasis control through snail population control interventions receive about 60% more success than control programs without (Sokolow et al., 2016). However, snail population control requires a thorough understanding it’s ecology with a focus on aspects such as locations where the snails are most likely to be found, and critical times of the year when snail populations increase among others. Whereas Kazibwe et al. (2006) studied the distribution of *Biomphalaria* spp in Lake Albert, the determination of their association with the biotic factors like macrophytes that are potential predictors of snail habitats and hence schistosomiasis risk (Wood et al., 2019), was not done.

Further still, studies on snail-borne diseases have focused on the supposed disease transmission hotspots of the lakes and rivers with scanty information on small streams and spring wells (largely considered safe) yet these could be important habitats of the snail hosts, hence with implications in the disease cycle in upland human communities. This research, therefore, monitored the snail population from Lake Albert, connected river networks and smaller water sources on the Ugandan side of the lake. Additionally, whereas community involvement in research through the citizen science approach has been found to produce large volumes of quality data as well as streamlining communication with the wider community (Jacobs et al., 2019), this approach has not been used in snail-borne disease control. As a result, long-term high-resolution malacological data is lacking. Therefore, this study sought to train a critical mass of citizen scientists who were equipped with knowledge and practical skills for monitoring *Schistosoma/Fasciola* snail hosts, their distribution, and means of disease transmission. Consequently, this study did not only collect massive data on snail host occurrence, distribution and infestation but also contributed towards building awareness and eventual local community-facilitated interventions targeting *Schistosoma/Fasciola* parasitic disease control.

1.3 Objectives

1.3.1 Main objective

The overall objective of the study was to determine the distribution and population dynamics of snail species that act as an intermediate host for snail-borne parasites that affect humans and livestock; and assess the reliability of the citizen science approach for snail host population monitoring in the Lake Albert region of western Uganda in order to obtain reliable snail distribution data to guide targeted snail control.

1.3.2 Specific objectives

The specific objectives of the study were:

1. To describe the diversity and spatial-temporal distribution of freshwater snail intermediate hosts of *Schistosoma* (*Biomphalaria* spp. and *Bulinus* spp.) and *Fasciola* spp. (*Radix* spp.)
2. To examine the biotic (macrophyte) and abiotic (rainfall, pH, DO, EC, temperature, inorganic ions) factors that drive the distribution and population dynamics of these snail intermediate host species.
3. To determine the infection prevalence of the intermediate snails hosts by comparing traditional and molecular methods.
4. To test the potential of a citizen science approach towards snail monitoring in order to identify potential schistosomiasis transmission sites and guide targeted snail control.

1.4 Research Questions

Objective 1:

Is the reported higher prevalence of schistosomiasis at the lake shore communities than in neighbouring upland communities explained by intermediate host snail diversity (genetic differences) and abundance? How does *Schistosoma*/*Fasciola* host snail abundance change in space and time?

Objective 2:

Are there specific biotic or abiotic factors that predict the presence of snail intermediate hosts?

Objective 3:

Are the *Biomphalaria* spp. and *Bulinus* spp. south-east of Lake Albert infected with *Schistosoma* spp. and how does the prevalence vary in space and time?

Objective 4:

Can citizen scientists correctly sample and identify snail presence at a site, and if so, can they reliably monitor snail abundance?

1.5 Significance

This study provides very precise and novel information on the distribution of intermediate host snails of both *Schistosoma* and *Fasciola* along spatial and temporal gradients. Transmission hotspots of schistosomiasis were identified and communicated to the communities to adapt their behaviour: avoiding those hotspots, improving sanitation, amongst others. The transmission hotspots were also communicated to the Vector Control Division (VCD) of the Ministry of

Health (MoH) and other actors to plan targeted schistosomiasis/fasciolosis control intervention programs to eradicate/reduce the disease burden.

Notably, this study revealed for the first time the presence in the Lake Albert region of intermediate host snail species of *S. haematobium*, the causative agent of urogenital schistosomiasis. This data serves as an early warning of the viable risk of disease transmission in the area. In addition, the study provides insights into potential deficiencies in the classification of the *B. pfeifferi* species group, and hence an opportunity for future research as well as the implications for disease spread.

In addition, this study shows how local inhabitants of an endemic region, after training, can detect snail populations with acceptable accuracy, at a significantly lower cost than PhD researcher-led surveys. This citizen science approach could therefore generate unprecedented datasets in terms of spatiotemporal resolution and coverage. In addition, the citizen science approach empowers communities and increases knowledge of snail-borne diseases and their control and prevention. We therefore argue that this community-based approach presents a valuable and sustainable complement to classical surveillance programs, especially in remote areas, thereby generating the much-needed data and community support for targeted snail control.

1.6 Conceptual framework

In the conceptual framework (Figure 1.1), schistosomiasis and fasciolosis prevalence is directly dependent on the availability of the intermediate snail hosts in which the parasites multiply. The infectivity and quantity of trematode parasites produced by the host snails depend on the host-parasite compatibility that is determined by genetic and environmental factors (Douchet et al., 2023). For instance, lack of food resources due to inter- and intra-specific competition among snails could limit the establishment of schistosomes in the snails, hence reducing the *per capita* cercarial production that will consequently reduce the disease transmission risk (Civitello et al., 2022). Hybridization between related parasite species such as *S. mansoni* and *S. rodhaini*, and *S. haematobium* and *S. bovis* can increase the parasite virulence and range of hosts (Huyse et al., 2009; Léger & Webster, 2017; Savassi et al., 2020). The interaction between co-infecting trematode parasites within a single snail individual, either through competition or predation, will also affect the success of parasite transmission to the final host (Civitello et al., 2022).

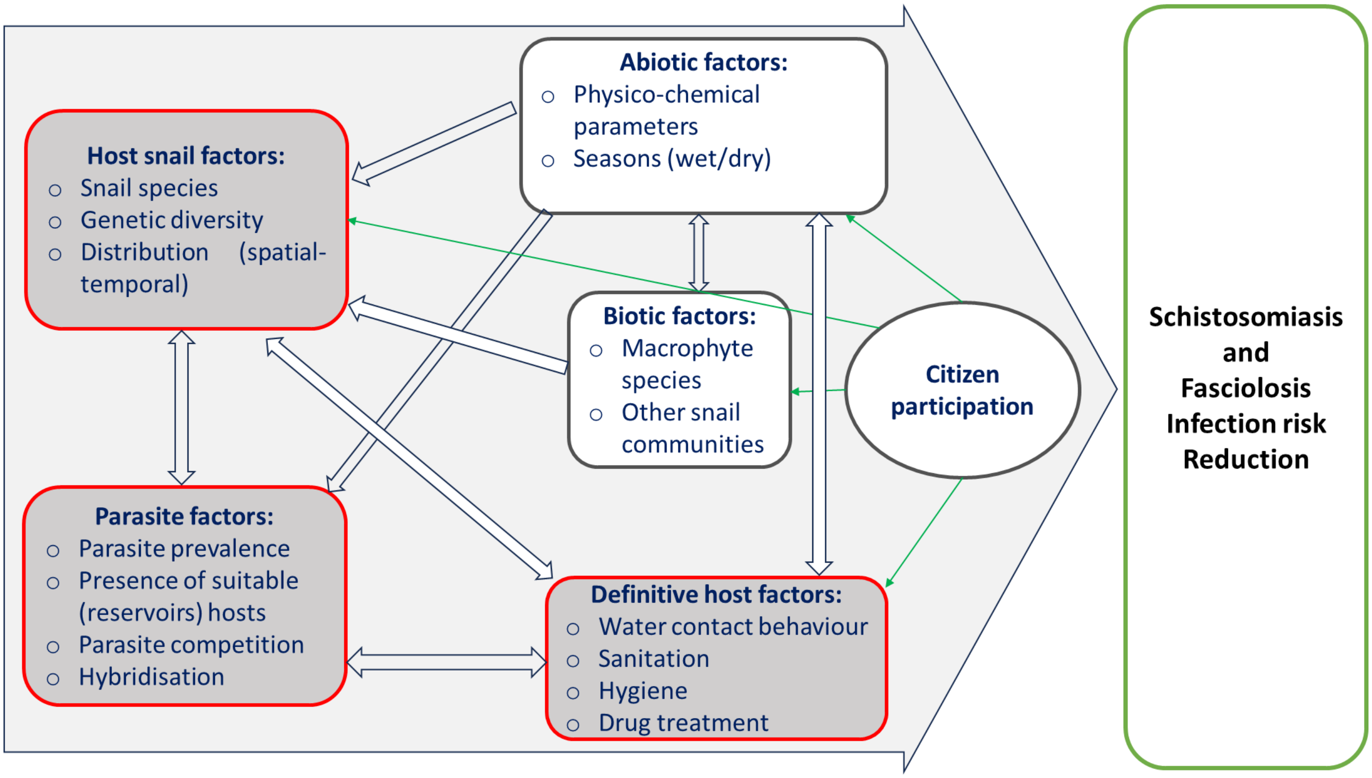

Figure 1. 1: The conceptual framework showing interactions of the dependent (red outlines) and the intervening (black outlines) factors that affect Fasciola/Schistosoma parasite infection risk to man and livestock, which make up the independent variable (green outline). The conceptual framework was developed by the PhD scholar.

The snail abundance varies in space and time in such a way that transmission hotspots and high-risk seasons can be identified. Freshwater snails depend on the availability of suitable habitats with the right combination of biotic and abiotic factors. Key among the predictors of snail presence are aquatic macrophyte species and density (Wood et al., 2019). The water chemistry (physicochemical) environment affects not only the survival of aquatic macrophyte but also the snail population as well. The mineral nutrients, dissolved oxygen, pH, electrical conductivity, etc affect the survival of all organisms that live in the water and these have specific tolerance ranges for survival. While presence of intermediate host snails represents the potential risk of schistosomiasis/fasciolosis outbreaks, the actual risk of the disease can be ascertained by the presence of infected snails. This requires accurate tools such as diagnostic PCR tests that can detect patent and prepatent infections as opposed to shedding techniques, which only detect patent infections (Schols et al., 2019). It is hypothesised in this study that shedding experiments underestimate the prevalence of *Fasciola/Schistosoma* parasites in the snails and thus the molecular assays are thought to give a better picture in estimating the fasciolosis/schistosomiasis risk in the communities (Allan et al., 2020).

Human water contact and hygiene practices play an important role in the transmission of trematode flukes in a given community. For instance, open defecation which is partly associated to myths and misconceptions (Anyolitho et al., 2022), is known to increase the risk of human schistosomiasis (Exum et al., 2019). Since high temperatures and water scarcity are common in the dry season, it expected that human water contact behaviour increases in the dry season while wet seasons will likely wash human faeces with the runoff from upstream rivers into water bodies, resulting in an increased risk of snail-borne disease transmission in an area.

However, interventions such as routine mass drug administration (MDA) with praziquantel reduce the prevalence of schistosomiasis among communities thus lowering the risk of diseases spread (Kokaliaris et al., 2022). Unfortunately, MDA does not affect non-human hosts such as the reservoir hosts, which perpetuate the cycle of the flukes (Duplantier & Sène, 2000; Oleaga et al., 2019; Savassi et al., 2020). Similarly, this study asserts that involvement of the local communities (e.g. citizen scientists) in the snail monitoring together with behavioural change campaigns increases the knowledge in the communities. Thus, it is anticipated that improved behavioural practices will reduce the risk of disease spread in endemic areas.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of schistosomiasis and fasciolosis prevalence

2.1.1 Schistosomiasis

Schistosomiasis is an important parasitic disease caused by trematodes of the genus *Schistosoma*. It afflicts over 250 million people worldwide, with more than 700 million at risk of infection (Loewenberg, 2014; World Health Organization (WHO), 2023), resulting in a substantial global disease burden of 1.43 million disability-adjusted life years (Kyu et al., 2018). Human infections are primarily attributed to six *Schistosoma* species: *S. mansoni*, *S. haematobium, Schistosoma intercalatum, Schistosoma guineensis, Schistosoma japonicum,* and *Schistosoma mekongi* (Adenowo et al., 2015; Clerinx & Van Gompel, 2011; Diego et al., 2021).

Infection of human hosts occurs through contact with water contaminated by cercariae (larvae) released from compatible snail species. The parasites then migrate to suitable body organs (*e.g.*, intestines for *S. mansoni* and bladder for *S. haematobium*), where sexual reproduction occurs, resulting in the production of eggs that partly remain in the body and partly released with stool or urine. Upon contact with water, the eggs hatch into miracidia (primary larval stage) which then seek a compatible snail host in which they develop and multiply asexually before they are released as cercariae (secondary larval stage) to continue the infection cycle. While the *Schistosoma* fluke infections are largely asymptomatic, pathological effects are due to the eggs getting trapped in the tissues of the host. In intestinal schistosomiasis, for example, migration of the eggs through the intestinal walls causes perforation and inflammation of the intestinal mucosa. Granulomas form followed by fibrosis around eggs that get stuck in the body, a multitude of signs and symptoms develop and may sometimes result in death (Betson et al., 2010). Urogenital schistosomiasis infection has severe consequences, including anaemia, stunted growth in children, kidney and urinary tract complications, reproductive system disorders, and an increased risk of HIV and human papilloma virus (HPV) infection (Aula et al., 2021).

Approximately 90% of the known schistosomiasis cases occur in impoverished communities in sub-Saharan Africa, where access to clean water and sanitation facilities is limited (French et al., 2018; Van Der Werf et al., 2003). Notably, *S. haematobium* and *S. mansoni* are the most important parasites causing the majority of the schistosomiasis cases in Africa. Urogenital schistosomiasis caused by *S. haematobium* is the predominant form, accounting for 75% of the known schistosomiasis cases (World Health Organisation, 2018). However, the distribution patterns of the different schistosome species vary geographically and can change over time. For example, in East Africa, intestinal schistosomiasis caused by *S. mansoni* predominates in the Great Lakes region (Aula et al., 2021). In Uganda, recent estimates indicate a national prevalence of intestinal schistosomiasis at 25%, with some communities residing near Lake Albert, Lake Victoria, and the River Nile reporting infection rates exceeding 50%, reaching 90% in certain areas (Exum et al., 2019; Loewenberg, 2014). Despite recent efforts to document schistosomiasis transmission in Uganda (Exum et al., 2019), prevalence data remains incomplete, and only outdated reports are present in some cases.

Schistosomiasis elimination campaigns have been successful in Japan and Tunisia while countries like China, Egypt, Brazil, Morocco and Caribbean Island countries are taking important steps towards the elimination of the disease (Adenowo et al., 2015). However, in poor rural agricultural and fishing communities of Sub-Saharan Africa, schistosomiasis remains very common resulting in an estimated 280,000 deaths per year (Exum et al., 2019). Nigeria, Tanzania, Ghana, the Democratic Republic of Congo and Mozambique are the most affected African countries by schistosomiasis with 29, 19, 15, 15 and 13 million infected people respectively (Adenowo et al., 2015; Stothard et al., 2013). In addition, national schistosomiasis control programmes have recently been introduced in SSA in 2002 compared to China and Brazil where it started more than 30 years ago. The control programmes majorly focused on the distribution of the antihelminthic praziquantel with little focus on the prevention of infections and were largely unsuccessful in reducing schistosomiasis by 2013 (Adenowo et al., 2015; Betson et al., 2010).

2.1.2 Fasciolosis

Fasciolosis (alternatively spelled as fascioliasis) is a parasitic disease caused by trematode flukes of the genus *Fasciola* that are spread by Lymnaeid snails. The most important fluke species is *F. hepatica* as it is widely distributed in Africa, Europe, the Americas, Oceania, and Asia while *F. gigantica* is restricted to the Old World (Europe, Asia and Africa) (Mas-Coma et al., 2009). Although *Fasciola* spp are characteristic of the great economic losses they cause to livestock, they are also zoonotic. The parasites affect a range of domestic animals including cattle, sheep, and wild animals. The multiplicity of reservoir hosts coupled with a complex lifecycle makes *Fasciola* spp. successful parasites that are hard to control (Webb & Cabada, 2018). It is therefore important to effectively identify the reservoir hosts of *Fasciola* spp. for targeted control of the parasites.

The global estimation of the prevalence of fasciolosis in livestock and therefore the losses incurred is difficult given that data from developing countries is scanty. However, data from developed countries indicates that 30-80% of the cattle is infected, causing significant losses in the quality and quantity of milk and beef (Mehmood et al., 2017). Nevertheless, a rough estimate of global economic loss was made at $3 billion per year but is thought to be significantly higher (Mehmood et al., 2017; Webb & Cabada, 2018). Given that livestock is a major source of food, income and cultural heritage, fasciolosis has a devastating impact on several households' income base and food security, more so in pastoral communities. Fasciolosis causes dullness, weakness, loss of appetite, pallor and oedema of mucosa and conjunctiva, and pain which in some cases results in the sudden death of sheep (Tsega et al., 2015). Fasciolosis has also been found to predispose infected cattle to tuberculosis (Kelly et al., 2018). Sharma et al. (2011) reported that up to 30% of Murrah buffalo yearlings died from acute disease associated with *F. gigantica* in India; Aliyu et al. (2014) reported that annual loss due to condemned liver in Nigeria was estimated at $5.8 million per year while Tsega et al. (2015) associated an annual loss of $23.4 million to ovine fasciolosis alone in Ethiopia.

Human fasciolosis was considered of secondary importance until 1990 when human *Fasciola* infection started to increase in Europe and America (Sah et al., 2018). Figures have since risen from below 3000 infections to between 2.4-17 million cases globally and an estimated 91 million at risk of infection. Infection occurs when one drinks water containing free-floating metacercarial cysts or by consuming uncooked/half-cooked vegetables either unwashed or washed in contaminated water (Aliyu et al., 2014; Keiser & Utzinger, 2009; Tolan Jr, 2011). The disease is mostly asymptomatic though acute fasciolosis has been linked with liver complications like cirrhosis and fibrosis (Machicado et al., 2016). In North Africa and the Middle East, 1.1 million people are infected with fasciolosis caused by *F. hepatica* with no recorded deaths worldwide (Nyindo & Lukambagire, 2015). Human fasciolosis has also been recorded in other African countries south of the Sahara including Burkina Faso, Senegal, Kenya, Zambia, and Mali (Mcdowell & Sima, 2014). There is scanty data on the existence of human fasciolosis in Uganda. Since the same *Fasciola* parasites affect both humans and other animals, it is likely many people have the disease but it is undetected. Tolan (2011) suggests that the prevalence of a veterinary fasciolosis in an endemic area may predict human disease.

Veterinary fasciolosis has been reported by Malatji et al. (2019) and Mehmood et al. (2017) ranging from 0.1% to 90.0% in cattle, 0.28% to 68.4% in goats, and 0.19% to 73.7% in sheep. The study further found varying prevalences of fasciolosis in wildlife across different territories where Kafue lechwe in Zambia had 52%, Kudu in South Africa had 12.5% and impala in Swaziland had 0.52% prevalence (Mehmood et al., 2017). The prevalence of *Fasciola* parasites is influenced by season, increasing mainly in the rainy season. Alemu (2019) reported fasciolosis to be the most important helminths infection infecting 30-90% of the cattle in Africa causing an annual loss estimated at $343 million in Ethiopia.

In Uganda, *F. gigantica* is prevalent in areas with altitudes between 1000 m and 1500 m above sea level and its prevalence is directly proportional to the population of *R. natalensis* intermediate host snails (Malatji et al., 2019, 2020). Studies have found that up to 84% of the liver in abattoirs in Kampala is condemned translating into an annual loss of US$ 92,474,620 every year (Joan et al., 2015; Mehmood et al., 2017; Webb & Cabada, 2018). It is therefore expected that economic losses due fasciolosis are even greater given that a lot of data from upcountry districts is missing.

2.2 The role of the snail intermediate hosts of *Schistosoma* and *Fasciola* species

2.2.1 *Biomphalaria* species and *Schistosoma mansoni*

The transmission dynamics of schistosomiasis are intricately tied to the presence and distribution of suitable snail intermediate hosts. Snails of the genus *Biomphalaria* are known to be compatible with *S. mansoni*, and thus, the presence of these snails plays a pivotal role in determining where infections are likely to occur (Perez-Saez et al., 2017). In Uganda, five *Biomphalaria* species have been identified: *Biomphalaria pfeifferi, B. sudanica, B. choanomphala*, *B. stanleyi* and *B. smithi* (Brown, 1994; Jorgensen et al., 2007). While *B. sudanica* and *B. pfeifferi* have a wide distribution pattern in both streams, wetlands and lakes, *B. stanleyi*, *B. choanomphala* and *B. smithi* are predominantly found in larger lacustrine water bodies, that is, Lake Albert, Lake Victoria and Lake Edward, respectively (Brown, 1994; Jorgensen et al., 2007).

The prevalence of intestinal schistosomiasis in Uganda is highest at locations near large water bodies, especially lakes, and reduces with distance away from a large water body (Exum et al., 2019; Kabatereine et al., 2004). This pattern suggests that lakes are either inhabited by the suitable snail hosts of the parasites, or have higher snail densities than smaller water bodies further away, or that the lake communities are more exposed and involved in risky behaviours like open defecation. Furthermore, variations in host-parasite compatibility and potency have revealed that certain *Biomphalaria* snail species, such as *B. stanleyi* and *B. pfeifferi*, support parasite multiplication more effectively than others (Adriko et al., 2013; Kazibwe et al., 2010). The role of non-lacustrine *Biomphalaria* spp. in schistosomiasis transmission remains, however, poorly understood. Therefore, investigating the composition of *Biomphalaria* communities in different types of waterbodies surrounding large lakes, as well as characterising their role in hosting *Schistosoma* parasites will potentially provide further insights into the transmission dynamics of schistosomiasis. The upland sites within the Lake Albert region represent an ideal area to address such a research question, due to its hydrological structure consisting of a mosaic of small streams, large rivers, artificial springs, and swamps (Brees et al., 2021). The hydrological setup presents suitable habitats for *Biomphalaria* spp. which are partially separated by the rift valley escarpment between the lake and upland sites. Moreover, some of the streams are isolated and form small catchments draining into the lake while others lie in the larger Nkusi River catchment. However, the frequent movement of humans between the upland and lake sites presents opportunities for transferring parasites between the snail subpopulations. In our study area, the most recent estimate (between 1998 and 2002) reports that over 80% of the population was infected in the present-day Ndaiga sub county (Kabatereine, et al., 2004). However, a more recent study indicated that the prevalence of intestinal schistosomiasis among communities at the shores of Lake Albert further north was over 50% (Exum et al., 2019).

In this context, our research aimed to: a) explore the genetic diversity and phylogeographical structure of *Biomphalaria* populations in the Lake Albert region across varying spatial scales and hydrological settings, b) determine the relative importance of *Biomphalaria* spp. in the transmission of *S. mansoni* at the lake and upland regions, and c) assess the seasonal variation in snail and parasite abundance.

2.2.2 *Bulinus* species and *Schistosoma haematobium*

Certain species of *Bulinus* snails, such as *B. globosus*, *Bulinus nasutus nasutus*, *B. nasutus productus*, *Bulinus africanus* and *Bulinus truncatus* act as intermediate hosts of *S. haematobium* (Brown, 1994; Zhang et al., 2022), and their distribution determines where *S. haematobium* infections can occur. Climate change is predicted to drastically alter the geographical distribution of snails and therewith that of their infecting parasites (Aula et al., 2021). In addition, snails of the genus *Bulinus* are implicated in the transmission of bovine schistosomiasis. *Schistosoma bovis* afflicts mainly cattle causing productivity losses, and hybridisation with *S. haematobium* results in offspring with increased host range, virulence and hybrid vigour (Huyse et al., 2009; Leger & Webster, 2017; Savassi et al., 2020). Therefore, a One Health approach is paramount for effective prevention and control of schistosomiasis.

In Uganda, intestinal schistosomiasis caused by *S. mansoni* is highly endemic and widespread, especially around the Great Lakes (Exum et al., 2019). On the other hand, urinary schistosomiasis is virtually absent except for a few cases in the central northern region, thus, not considered of public health importance (Adriko, Tinkitina, Tukahebwa, et al., 2018). Consequently, urogenital schistosomiasis remains largely neglected in Uganda and information about its prevalence is very scanty (French et al., 2015; Mbabazi et al., 2011; Tumwebaze et al., 2019). Nevertheless, the narrow geographical distribution of *S. haematobium* has been enigmatic but thought to be a result of the absence of suitable *Bulinus* snail hosts (Tumwebaze et al., 2019). Given the presence of *S. mansoni* in the Lake Albert region, open defecation and urination behaviour are present in these communities, thereby also favouring the potential transmission of urinary schistosomiasis if the respective *Bulinus* species were present. However, there are no reports on the latter, nor any reported *S. haematobium* cases.

2.2.3 Intermediate hosts of *Fasciola* species

Two species of liver flukes are known to cause fasciolosis in livestock, wildlife and human beings; *Fasciola hepatica* and *F. gigantica*. *F. hepatica* has a cosmopolitan distribution while *F. gigantica* is mostly tropical in distribution (Ashrafi & Mas-Coma, 2014; Elliott et al., 2015;

Jaja et al., 2017; S. Mas-Coma, 2005; Santiago Mas-Coma et al., 2009). The distribution of *Fasciola* spp is directly related to the distribution of the intermediate lymnaeid snails (Malatji et al., 2020). In Africa, *Fasciola* species are hosted by snail species *Radix natalensis*, *Radix auricularia*, *Radix rubiginosa*, *Galba truncatula* and *Pseudosuccinea columella* (Malatji et al., 2020). The most important species in the spread of *Fasciola* spp. are *R. natalensis* and *G. truncatula* and are the only species reported in East Africa (Jaja et al., 2017; Malatji et al., 2020).

Fasciola hepatica infest *Galba* snails while *F. gigantica* infest *Radix* snails as intermediate hosts (Mas-Coma et al., 2005; Walker et al., 2008). The snail intermediate hosts have different ecological requirements in that the intermediate hosts of *F. hepatica* prefer small and swallow water bodies while those of *F. gigantica* prefer deeper, permanent and vegetated waterbodies (Mahulu et al., 2019; Mas-Coma et al., 2005). In Uganda, *G. truncatula* has been reported at high altitudes in the Mt Elgon region (Mahulu et al., 2019; Santiago Mas-Coma et al., 2009; Stothard et al., 2004) while *R. natalensis* is more widely distributed. Despite the presence of intermediate hosts of *Fasciola* parasites, parasitological data remains scanty in Uganda (Howell et al., 2012; Malatji et al., 2020; Stensgaard et al., 2006). Moreover, up-to-date malacological data on the distribution of lymnaeid snails is lacking in the Lake Albert region. Howell et al. (2012) reported that the comprehensive surveys were done by Mandahl-Barth (1962) using morphological features to identify snails, an approach that is currently considered reasonably limited.

2.3 Detecting parasite transmission

Detection of active parasite transmission is vital for planning interruption of the transmission cycle. For this purpose, several techniques have been designed and these involve either detecting the presence of the trematode parasites in the host snails, or in the water inhabited by the snails.

While some of the techniques examine the morphology of the morphology of the larval stages of the parasites either in the water or the snail, more advanced techniques examine the presence of parasite DNA (Kamel et al., 2021).

2.3.1 Morphology-based techniques

2.3.1.1 Cercariae shedding

Snail intermediate host of helminths differ in their importance in the life cycle of *Schistosoma* parasites due to differences in susceptibility to miracidia and the number of cercariae shed thereafter. Using the cercariae shedding technique, Kazibwe et al. (2010) determined that *B. stanleyi* played a more important role in the transmission of *S. mansoni* in local communities of Lake Albert than did *B. sudanica.* The technique used was described in Webbe (1965); it involves exposing snails to 10 ml of water to a source of light for a minimum of two hours to induce cercariae emergence (Kazibwe et al., 2006, 2010; Levitz et al., 2013a). In the next step, the shed cercariae are identified morphologically under the magnifying power of the microscope. While this method is rapid and relatively cheap, there are high chances of wrongly considering infected snails in the pre-patent period as uninfected. The method does not detect immature parasite larval stages before the more developed cercarial stage, which takes between 20 to 35 days or longer in *Biomphalaria* and *Bulinus* snails (Allan et al., 2020). Wrong identification of the parasites is also likely given the lack of morphological diagnostic features in larval parasite stages.

Cercariae identification is based on: a) morphological traits such as number and position of suckers, shape and dimensions of the tail, and surface structures like the collar, stylets, etc, b) biological characteristics such as the host snail species, cercariae swimming and resting behaviour, formation of metacercariae, and host penetration behaviour (Frandsen & Christensen, 1984). Despite the presence of morphological differences between cercariae groups, it is almost impossible to identify the parasites to the species level basing on the structure and behaviour of the cercariae alone (Frandsen & Christensen, 1984; Kamel et al., 2021). Therefore, cercariae shedding and subsequent morphological identification are important steps in preliminary identification of areas where potential disease transmission occurs, pending verification with more sensitive techniques.

2.3.1.2 Snail crushing

This technique involves crushing and examining the snail tissues under a microscope to identify presence of trematode larval stages (usually cercariae or radiae) before they are released from the snail. It is a low-cost method capable of detecting patent and prepatent infections and may be applicable in field. On the other hand, this technique may not be useful for identifying the parasite species, may miss to detect light and early infections, and destroys the integrity of the snail complicating downstream morphological studies (Kamel et al., 2021). Since this study involved storing snail samples for additional analyses and photographing of the snail shells, this technique was not used.

2.3.2 Molecular PCR-based techniques for *Schistosoma* parasite detection

Molecular PCR-based diagnostic techniques have been developed to improve sensitivity in detection of pathogens in both humans and animals. Several PCR-based techniques such as the conventional PCR, PCR with restriction digestion, Random Amplified Polymorphic DNA (RAPD) PCR, Repeated Sequence PCR, Nested PCR, Multiplex PCR, quantitative PCR, Fluorescence Resonance Energy Transfer (FRET)-PCR, and Droplet Digital (dd)PCR have been developed and the pros and con of each techniques are elaborated by Kamel et al. (2021) in an extensive systematic review. In this study, we adopted the multiplex PCR technique due its ability to improve the detection of multiple infections at the same time (Schols et al., 2019). The diagnostic multiplex PCR allows the amplification of DNA fragments of different lengths enabling species-level identifications of parasites without the need for sequencing. Rapid diagnostic (RD) PCR techniques therefore significantly save the cost and time for parasite detection. It is now possible to identify the snail intermediate hosts and the parasites they harbour simultaneously by amplifying specific genes of the parasites of interest (Allan et al., 2020). However, lack of resources such as expensive equipment, biosafety cabinets, electricity supply and trained human resource makes the use of the sophisticated molecular techniques virtually impossible for regular use in the rural communities where schistosomiasis is prevalent (Qin et al., 2018). Therefore, additional research is needed to optimise the PCR techniques for use under field conditions.

In recent years, the loop-mediated isothermal amplification (LAMP) technology has been developed as a versatile and sensitive alternative to PCR potentially adaptable to field conditions (Qin et al., 2018). LAMP employs a single step amplification of target DNA or RNA under isothermal conditions and therefore does not require the thermocycler used in standard PCR reactions. It requires less reaction time and is adaptable for use is resource-limited areas (Ajibola et al., 2018). LAMP assays have been developed for detection of *S. mansoni, S. haematobium* and *S. japonicum* in the snail hosts both in the laboratory and under field conditions (Diego et al., 2021; Gandasegui et al., 2016; Hamburger et al., 2013; Qin et al., 2018). However, the use of LAMP technique in its current widely published protocols is not without limitation. DNA sample preparation and cleaning still employs complex procedures, making it less usable in resource constrained remote areas lacking electricity connectivity. In addition, it is not possible to target multiple species that might infect different snail species using the LAMP assay as in multiplex RD PCR that was used in this study.

2.4 Biotic and abiotic factors influencing *Schistosoma* and *Fasciola* host snail distribution

The geographical distribution of schistosomiasis is closely linked to that of intermediate snail hosts, with local ecological conditions determining the occurrence of particular snail species (Rollinson, 2009). Thus, snail control requires thorough knowledge of their habitat characteristics (Manyangadze et al., 2016). 'Easy-to-measure' environmental correlates have been determined for intermediate snail hosts of *S. haematobium* responsible for urogenital schistosomiasis in Senegal. Habitat characteristics such as the area and percentage cover of especially of non-emergent species like C*eratophllum* spp., *Ludwigia* spp. and *Potamogeton* spp. have been positively associated with persistence of *B. globosus* and *B. truncatus*, and thus the urogenital schistosomiasis risk (Wood et al., 2019). It is therefore important to determine environmental predictors of intermediate snail hosts of *S. mansoni* and *Fasciola* spp. and to check whether their findings also hold for *B. globosus* and *B. truncatus* in Uganda.

Water physico-chemical parameters such as temperature, salinity and pH have been linked to affect spatial and temporal dynamics in snail abundance (Douchet et al., 2023). While the general trend of snail abundance indicates increasing abundance in the wet season (Andrus et al., 2023; Douchet et al., 2023), some studies have reported opposite trends such as increasing snail abundance in the dry season in the Niger River valley (Rabone et al., 2019). It is therefore important to study the impact of physico-chemical and season variability considering the geographical differences in the Lake Albert region. In addition, water physicochemical parameters such as temperature affect the metabolic rates of snails as metabolism is favoured by warm temperatures within the tolerance range of the species. Additionally, warmer waters favour the development of cercariae within snails (De Leo et al., 2020; Mas-Coma et al., 2009). Similarly, the swimming behaviour of cercaria and their longevity after being shed are also affected by ambient water temperatures: cercariae swim faster in warmer water, but die faster (Douchet et al., 2023). Therefore, water physicochemical characteristics of a site affect not only snails but also free-living and hosted larval forms in snails.

The Lake Albert region of Uganda shows a gradient in the schistosomiasis prevalence with infections decreasing away from the lake (Chris Twebaze, 2019: personal communication) despite the presence of snail vector habitats along the gradient. There may be differences in the biotic (associated macrophyte) and abiotic (physico-chemical) environments that make some areas more susceptible to snail infestation than others. The biotic and abiotic factors may also explain the spatio-temporal distribution of *Schistosoma* and *Fasciola*-infested snail hosts.

Seasons have been linked to variations in populations of freshwater snails and the prevalence of *Fasciola* parasites in both snails and final hosts (Jaja et al., 2017; Malatji et al., 2020; Mas-Coma et al., 2009; Stensgaard et al., 2006). Rainfall, solar radiation and temperature are measurable parameters that change with seasons. Since temperature and rainfall change considerably daily, it is important to measure them with high enough resolution. Given the poikilothermic nature of snails and the parasitic helminths they host, their development is directly affected by environmental temperature (Pedersen et al., 2014).

2.5 Community-based interventions in snail-borne disease transmission control

2.5.1 Current status of community participation in snail-borne disease control

Community participation is very important for successful disease control and elimination interventions in a target population. This has been exemplified by the elimination of schistosomiasis in some parts of China, and the elimination of onchocerciasis and filariasis in Kenya (Macharia et al., 2016; Musuva et al., 2019). In many African settings, the community relies entirely on MDA where a few selected individuals merely supply medicines to the eligible communities. Considerable success in disease reduction has been attributed to MDA (Kokaliaris et al., 2022; World Health Organization, 2023). However, schistosomiasis continues to (re-) emerge despite decades of MDA leading to an inevitable conclusion that MDA alone is not sufficient (King & Bertsch, 2015; Lo et al., 2018; Qian et al., 2018; Sokolow et al., 2017). Evidence from previous studies indicates that incorporating snail control in the control programme results to significant snail-borne disease reduction (Lo et al., 2018; Sokolow et al., 2016), a recommendation adopted by the WHO in roadmap for schistosomiasis elimination by 2030 (WHO, 2019). Effective snail control will therefore require community involvement

In Uganda, the management of schistosomiasis is not very different from the rest of the Sub-Saharan African countries. A group of community medicine distributors (also known as village health teams, VHTs) distribute praziquantel (Chami et al., 2019). Each village has two community medicine distributors in the highly endemic area who actively distribute medicine to the eligible people. However, the drug distributors lack knowledge on the transmission of the disease which is important in disease prevention. Moreover, several myths and misconceptions such as witchcraft, defecating in the lake to increase fish catches, and farting as a cause of schistosomiasis have been recorded in the Lake Albert region (Anyolitho et al., 2022). Thus, there is a need to upscale the awareness, which calls for creative community empowerment for meaningful and efficient engagement approaches, but also collection of real-time and long-term snail host data to inform monitoring and snail control. Additionally, Macharia et al. (2016) highlighted the need for the direct involvement of local communities in prevention, control and treatment of schistosomiasis. This prompted the use the citizen science approach in this study.

2.5.2 The citizen science approach

The citizen science approach involves lay people in collecting and reporting data, usually by means of smartphone applications, about various topics. Citizen science involves collaborations between concerned citizens with various stakeholders such as government, academia, industry, etc. to monitor, track and respond to issues concerning the community's wellbeing (Conrad & Hilchey, 2011). This approach has grown in popularity in recent decades more so in the field of ecology with a focus on monitoring large-scale impacts of environmental change on ecological and social aspects (Dickinson et al., 2010; Lukyanenko et al., 2016b). The disciplines of astronomy and ornithology are among the leading users of the citizen science approach.

With the growth in technologies such as the development of mobile apps, the citizen science approach is expected to be an integral part of current and future research (Greg Newman et al., 2012). Since citizen scientists reside near the study sites, they provide a cheaper option for collecting malacological and associated environmental data weekly, a resolution that is practically impossible when expert researchers are involved to cover many sampling sites geographically far apart. Rather than being mere recipients of the research findings, community members need to be actively involved in the research process. This not only augments the research impact on the communities, but also enhances sustainability (Musuva et al., 2019).

Bonter and Cooper (2012) observed that "*Conducting research with assistance from the public, however, can be far more complex than a traditional scientist student-led approach. Although best practices are needed for all the steps in the citizen-science program .... improving data quality is critical to the success of such projects*". Therefore, data quality concerns should not be underestimated and regular validation should be done on citizen-collected data whenever possible. For instance, low motivation, disregard for proper sampling (e.g. sample size) and inaccurate data among others have been cited as challenges associated with data collected by citizen scientists (Conrad & Hilchey, 2011). Therefore, it is important to set up a robust data validation protocol to check reporting errors-inaccurate reporting of otherwise correctly collected data (Brees et al., 2021). In addition, observational errors, the difference between the sampled data by citizen scientists and the actual *in situ* situation, need to accounted for by trained collecting 'ground truth' data for comparison.

Nevertheless, Jacobs et al. (2019) collected a large volume of high-quality data in the Rwenzori region of western Uganda by involving citizen scientists, the so-called geo-observers. They also observed that geo-observers bridge the communication gap between professional scientists and the general public. This study sought to leverage high-resolution data collection in motoring snail-borne diseases and an efficient communication link of citizen scientists with their communities. Lukyanenko et al. (2016) suggested that differences in data collected by citizen scientists and professional scientists are likely to occur because they approach the same problem from different perspectives. However, experts researchers and trained lay people can work together in complementarity. This is also supported by the fact that data collected by citizen scientists has been greatly used in multiple studies in recent years (Follett & Strezov, 2015; Jacobs et al., 2019; Sekajugo et al., 2022).

In summary, this study built on existing works from several individual researchers, research teams and organisations. Entry points of this study have been highlighted to fill existing gaps. In the next chapter, the materials and methods employed in this study are elaborated.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

3.1.1 Location

The study was conducted in the districts of Ntoroko and Kagadi (Figure 3.1) in areas bordering Lake Albert western Uganda, a major hotspot of schistosomiasis. Lake Albert lies within the western rift valley at an altitude of 615 m above sea level. It is the northernmost lake in the western arm of the East African rift valley that extends from the Gulf of Aden to Mozambique covering more than 4500 km (Gagnevin et al., 2017). The lake was formed between 10-12 million years ago and covers 150 km in length and 35 km in width of which 46% is in Uganda and 54% is in the Democratic Republic of Congo, and has an average depth of 56 m (Kazibwe et al., 2006; Miriti, n.d.). The lake is alkaline with pH ranging between 8.5-9.0 and has a high salt content (Kazibwe et al., 2006).

The study site selection followed the Lake Albert water drainage system. The Lake Albert Basin forms part of a continuous series of NNE-SSW intracontinental rifts that began developing during the early Miocene. Three major evolutionary stages have been assigned for the evolution of the Albertine rift basin since the middle Miocene comprising (1) the Kisegi stage (12.5 to 7 Ma) with passive sediment infill from the proto-Nkusi River, (2) rift formation with major faulting episode and accelerated subsidence triggering formation of Paleolake Obweruka (from 7.5 to 2.5 Ma), and (3) splitting of Paleolake Obweruka into a southern and a northern basin during exhumation of the Rwenzori block (from 2.3 to 0.4 Ma). These events have exerted a strong influence on drainage evolution in the region” (Gagnevin et al., 2017). The drainage of the streams and rivers into Lake Albert is thought to influence the spread of snail intermediate hosts of parasitic helminths. For instance, the Rift Valley escarpment acts as a barrier to the upstream movement of aquatic biota from the lake. In addition, the watersheds in the study area constitute the large River Nkusi catchment and small streams that drain directly into Lake Albert. These small water systems are essentially ‘islands’ since they are disconnected from the large river catchment, and from the lake by the Rift Valley escarpment. It was important to study how this hydrological setting might affect snail and parasite dynamics and whether it influences molecular differentiations among snail hosts.

The study was conducted in the sub counties of Ndaiga, Kyatereka, Bwikara, Mpeefu, Ruteete, Muhorro and Kagadi Town council in Kagadi district, and Kanara town council in Ntoroko district (Figure 3.1). The sub counties were chosen based on their vicinity to major water bodies (L. Albert and river Nkusi), the presence of water contact points which harbour intermediate hosts of *Schistosoma* and *Fasciola* parasite species, the presence of human water contact, the prerequisite knowledge of the disease in the community to motivate citizen scientists’ selection.

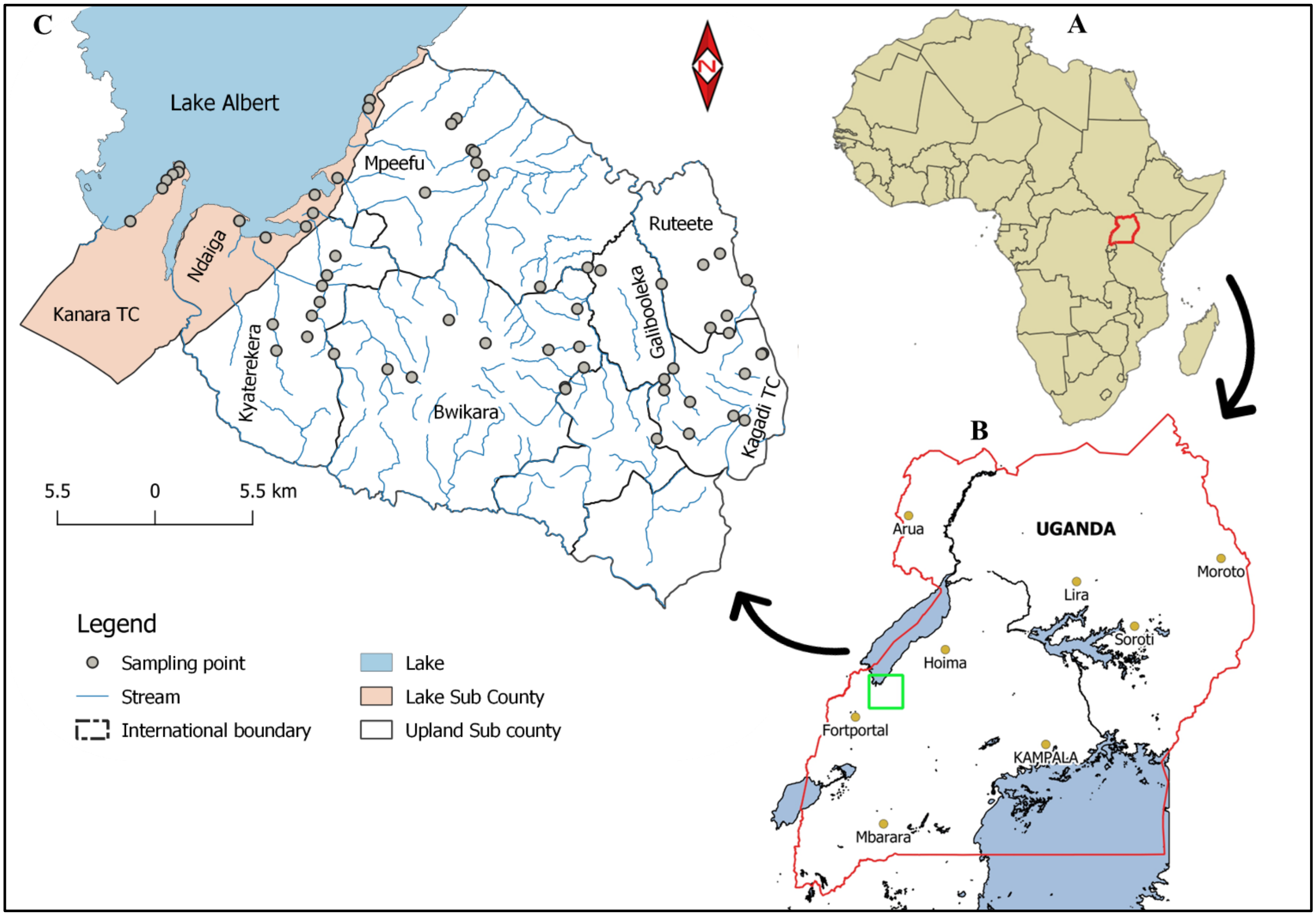

Figure 3. 1: A map of the study sub counties and water contact points therein (C) expanded from the map of Uganda showing district boundaries (B) and the location of Uganda in Africa (A) (Developed by Tumusiime, 2024 using QGIS 3.4.13)

3.1.2 Schistosomiasis prevalence in the study districts/sub counties

Ntoroko and Kagadi districts are located along the shores of Lake Albert (Figure 3.1), which is a major and persistent hotspot of schistosomiasis transmission. Recent estimates indicate that the prevalence of intestinal schistosomiasis along the shores of Lake Albert is over 50% (Exum et al., 2019). However, in the comprehensive survey by Exum et al. (2019), Ntoroko and Kagadi districts were not sampled, resulting in a gap of up-to-date data figures, possibly due to the hard-to-reach nature of our study area. For instance, the most recent data on the prevalence of intestinal schistosomiasis in the present-day Ndaiga sub-county indicated that over 90% of the population was infected (Kabatereine et al., 1996). Nevertheless, five out of eight sub-counties in this study including Kanara TC, Ndaiga, Mpeefu, Kyaterekera and Bwikara are mapped for routine MDA since they are considered to be of high endemicity of *S. mansoni*. However, in the pilot study, efforts to retrieve hospital records about the extent of schistosomiasis in the districts were not successful. It was revealed that the schistosomiasis test is not usually sanctioned by doctors despite the existing knowledge of the disease in the community. In simple terms, the disease is neglected and not usually treated in the primary health care system but relying on donor funding for MDA with praziquantel. Therefore, this study chose the highly endemic sub-counties that are often neglected by previous studies.

3.1.3 Human population socio-economic characteristics

The most recent records from the population census of 2014 indicate that Kagadi district had a population of 351,033 people while Ntoroko district has a population of 67,005 people, 58% being below the age of 12. However the 2020 population projections indicate a population of 76,000 and 430,200 for Ntoroko and Kagadi districts respectively (*Kagadi (District, Uganda) - Population Statistics, Charts, Map and Location*, n.d.). Given the dynamic nature of fisher communities coupled with the recent oil exploration activities in the region, it is likely that populations in the districts bordering Lake Albert (Hoima, Kikuube, Buliisa) mix frequently. Livestock farming is the main source of livelihood for the community in Ntoroko district. Some households practice crop farming-beans, maize, banana, coffee, sweet potato, and millet. Beans and maize are the main crops grown here (Uganda Bureau of Statistics, 2016). According to Koojo et al. (2018), agriculture accounts for 70% of the economic activities of the people of

Kagadi. Similarly, the major crops cultivated include maize, banana, beans, rice, coffee, tea, groundnuts, and cassava. Livestock kept include cattle, goats, poultry, pigs, and sheep. Other activities include trade, especially in agricultural produce and finished products, small cottages, leisure and hospitality and schools and health facilities.

Access to safe water is important in the prevention of schistosomiasis. According to the annual performance and accountability report for the Financial Year 2017/2018, Kagadi district (vote Code 613) performed poorly in the water, sanitation and hygiene sector with water coverage being at only 49%, which is less than the national figure. Some of the sub counties in the study sites have water coverage below the district figures i.e. Muhorro (42%), Bwikara (47%), Kyatereka (12%) and Mpeefu (10%). In the study area however, alarming levels of lack of access to safe water have been documented in the sub counties of Ndaiga (83.2%), Mpeefu (67.5%), Kyaterekera (50%) and Bwikara (40%). The lack of safe water results in people and livestock getting water directly from ponds, streams, rivers and the lake, which put them at the risk of parasite infection (Plate 3.1).

Proper disposal of human faeces and urine are primary in the control of schistosomiasis. In Kagadi district, a significant number of households lack toilet facilities. The worst affected sub county is Ndaiga (33% lack toilet facilities) with the rest of the sub counties having less than 10% of the households lacking facilities (Uganda Bureau of Statistics, 2016). The situation of lack of safe water and toilet facilities is not very different for Kanara town council in Ntoroko district standing at 81.3% and 34.2% respectively.

Plate 3. 1: People and cattle obtaining water directly from Lake Albert that is known to be highly infested with *Schistosoma* and *Fasciola* parasites (photo by Tumusiime, 2019).

3.2 Research design

The study majorly adopted ecological survey techniques, where monthly field sampling campaigns were conducted by the PhD researcher and weekly sampling by the citizen scientists (referred to as CSs henceforth) to collect malacological data and associated water physicochemical environmental parameters. Field campaigns were followed by laboratory analysis and data interpretation majorly focusing on parasitological and molecular data.

Each of the study sites was monitored and sampled on a weekly basis by trained CSs. Each CS monitored a maximum of four sites with an equal sampling effort of 30 minutes to ensure effectiveness. Monthly field data sampling surveys were conducted by the PhD researcher following the same procedure as the CSs.

3.3 Sampling methods

3.3.1 Sampling sites for snail collection

Before the selection of the study area, pilot visits were carried out in a stretch beginning from Mbarara to Hoima districts between February and May 2019. This pilot study was facilitated by a multistakeholder grant from KU Leuven titled: “**SWAMP: S**chistosomiasis in **W**est-Uganda: **A**nalysis and **M**apping of **P**revalence”. Along the way, we mapped health facilities present and major waterbodies in the vicinity of the facilities. Central to this project was the citizen science approach, in which the local citizens voluntarily participate in scientific research to solve an existing problem in their community. Therefore, during the reconnaissance visits, we interacted with different members of the public including ordinary members of the public, teachers, and health workers at the nearest health facility (from Health Centre II to Regional Hospital). We asked them if they ever heard of or knew about schistosomiasis. At the health facilities, we interacted with laboratory staff and asked whether they tested patients who presented with signs of schistosomiasis. In addition, we sampled all potential water contact points along the main road for the presence of intermediate host snails of schistosomes and liver flukes. In each of the districts visited, we held key-informant interviews with the district vector control officer to gain insights into the extent of schistosomiasis in their district. To further understand the volunteering function of the community, in collaboration with the Uganda Red Cross, we rolled out a survey to the community volunteers known as the village health teams (VHTs). This survey sought to understand the motivations for volunteering in the healthcare provision in their communities. The insights from this survey informed the selection of citizen scientists as described below in section 3.3.5. The results of the pilot study are included in the field report (Annexes 1-4).

A total of seventy-three sites were purposively chosen to represent a diverse range of hydrological settings, encompassing slow-flowing seasonal streams and large rivers (the “stream” site type), wetlands, spring wells, and lake sites (Plate 3.1). Springs are underground water sources trapped by concrete walls to direct water through a metallic pipe with continuously flowing water, accessed by the communities. The pool of water created on the ground here the spring water pours and the draining channel were the snail habitats that constituted the spring site type. Stream sites are water contact sites with flowing surface water. Lake sites on Lake Albert have highly used water contact sites within fishing villages in Ndaiga Sub County and Ntoroko Town Council, on the South-Eastern side of the lake. Wetland sites consist of flooded areas for most of the year mostly characterised by rooted aquatic vegetation. The selection of sites also considered their proximity to Lake Albert, with the furthest sampling point located approximately 40 km away from the lake. The 73 sampling points that were monitored regularly throughout the study are indicated in Figure 3.1.

Plate 3. 2: Site types used in the grouping of data collected by this study.

3.3.2 Monitoring of snail community structure

At each sampling point, all the snails belonging to the three genera of interest were collected using a timed counts sampling approach (Stothard et al., 2017; Utzinger & Tanner, 2000) by both the PhD researcher and the citizen scientist. However, only the snails collected by the PhD research were used for downstream laboratory analyses to determine the population molecular diversity. However, the snails collected by the CSs were recorded, photographed and returned to the sites of collection to minimise artificial manipulation of snail abundance, which was under investigation.

3.3.3 Sampling for biotic and abiotic factors

All biotic and abiotic factors were determined *in situ* at the same sites for snail sampling. Physicochemical parameters were measured at each site of snail collection by randomly dipping the appropriate meter in water (see below). Aquatic vegetation was determined at each site of snail collection using quadrats that were placed by simple random sampling.

3.3.4 Selection of snail hosts for shedding

Schistosoma and *Fasciola* intermediate snails from each site were first sorted to morphospecies using the morphology of the shells using appropriate identification keys (see section 3.4.1). A representative sample of each of the species were selected using the Krejcie and Morgan (1970) sample size table per site of collection. The snails to be included in the shedding were selected randomly from a pool of each species for each site. After shedding, all the snails were preserved in ethanol for additional molecular assays as described below.

3.3.5 Selection of snail hosts for molecular diagnosis of infection and barcoding

Only snail hosts of *Schistosoma* spp. (*Bulinus* and *Biomphalaria*) were considered for the subsequent steps of DNA extraction and analysis due to the limitation of time available at the laboratory in Belgium. Thus, *Radix* snails were identified morphologically and parasite prevalence only estimated using the shedding technique described in section 3.4.2 below. Before DNA extraction, all snails were grouped according to morphospecies per site per sampling month. The extraction of DNA was done to answer three primary objectives: 1) to confirm the snail species identity, 2) to estimate the extent of trematode parasite infection, especially by *Schistosoma* spp., and 3) for phylogenetic and phylogeographic studies. To estimate the parasite prevalence in space and time, ten snails from each site per sampling time point (per month) were randomly selected for DNA extraction. This number was considered to balance between the sample size and the cost of extraction (cost of the laboratory supplies and the limited time available). For phylogeography studies, five samples were selected per morphospecies per site. For this step, the samples selected had to be collected within the same sampling period to minimise potential confounding effects of temporal variation. Therefore, the maximum time difference between sampling periods considered was six months within the same growing season (August to February), for cases where it was impossible to obtain snails at all sites within the same sampling period.

3.3.6 Selection and training of citizen scientists

Citizen scientists were selected following a bottom-up approach to ensure effective participation of all the stakeholders and ownership of the interventions by the community. A similar approach was used by Jacobs et al. (2019) in selection of local community members as “geo-observers” of disasters in the Rwenzori region of Uganda. In summary, our team held community entry meetings with the district political and technical leadership, including the District Health officer, Chairman LC V, Chief Administrative Officer, the District Health Educator, the Vector Control officer, Disease surveillance officer, the Residence District Commissioner, and the District Speaker. In these meetings, the leadership was informed about the objectives of the study and our intentions to work with the local communities in their jurisdictions. In the next step, in each sub county, community meetings were held with the health officer, sub county chief, chairperson Local Council (LC) III, and opinion leaders, religious leaders and community representatives at the parish and LC I levels for the selected areas. Involvement of the community at all levels was done to ensure inclusiveness in the selected CSs and avoid homogeneity (Lukyanenko et al., 2016b). After introducing the project and its objectives, the local leaders were allowed a week to collaboratively mobilize and preselect potential candidates from within their sub-county based on a set of criteria described below. For every one CS needed, three candidates were nominated and final selection was done by the MUST research team based on the same set of criteria communicated to all stakeholders in advance (Brees et al., 2021).

Inclusion criteria for citizen scientists

Below were minimum requirements for an individual to qualify as citizen scientist (who preferred to be called 'citizen researcher' after selection):

a) Be in possession of national ID. Only Ugandan nationals were considered since local capacity building is an important aspect of the study.
b) Being of consenting age of 18 years and above
c) Should have had some experience of or exposure in utilizing smartphones, or willing to adopt the technology.
d) Being a resident of the area (preferably permanent or at least for the last five years). The CS had to be a resident in the parish where water contact sites were mapped to minimise cost of moving between sites.
e) Having necessary sanitary facilities such as toilet, bathroom/showers, utensil drying racks, mosquito nets, etc. This to ensure that the selected participant was exemplary in the sanitation and hygiene areas for effective sensitisation of other community members.
f) Having a track record of voluntarily participating in community development programs such as campaigns on Ebola prevention, malaria, safe male circumcision, cholera, schistosomiasis, Red Cross, etc.

g) Having a good ability to read, write and speak English. Basic literacy was important for the CS to effectively operate the data collection tool in the smartphone.

h) Being respected and trusted by the community. This criterion was based on the findings of the pilot study conducted prior to the commencement of the project.

i) Having the ability to communicate to wider communities in local language.

j) Having a good knowledge of local community dynamics i.e. how things are done in the community.

Exclusion criteria for citizen scientists

Not all the nominated candidates were considered to volunteer as citizen scientists. The following criteria were used in exclusion:

a) Lack of motivation to volunteer in the activities explained to them. Having too high monetary expectations in case facilitation is given.

b) Being none Ugandan national.

c) Having less than 18 years or being physically unable to perform the rigorous tasks due to very advanced aged or chronic illness.

d) Having plans to move from the respective area in the foreseeable future, for example a student in S.6 vacation, who would soon leave the project for further studies.

e) Lacking necessary sanitary facilities such as toilet, bathroom/showers, drying rags, mosquito nets, etc. to be considered a hygiene role model.

f) Being very engaged with other work such that the research activities would be a great inconvenience.

g) Inability to read, write and/or speak English.

h) Inability to communicate to wider communities in local language.

i) Lack of knowledge of local community dynamics i.e. how things are done in the community. This was ascertained through asking relevant questions during the interviews.

3.4 Methods of data collection

3.4.1 Snail collection

Snail sampling by the PhD researcher and citizen scientists was conducted in the period spanning from June 2020 to April 2022. However, the months of December 2020, and February and March 2021 were not sampled by the PhD scholar due to logistical challenges. At each site, monthly snail collection involved a 30-minute search by one person using a handheld scoop net, equipped with a stainless-steel sieve, securely attached to a metallic handle (Kazibwe et al., 2006). For sites where water levels were too low to allow the use of scoop nets, the hand-picking method for 30 minutes was used. The time-based sampling approach was used since it provides good estimates for snail abundance to study temporal changes in snail abundance in a single snail population (Perez-Saez et al., 2019). Nevertheless, some scholars have raised concerns about the use of time-based sampling when comparing absolute abundances between sites due to varying sampling efficiency and different habitat typology (Perez-Saez et al., 2019). Area-specific techniques for snail surveys using quadrats (Wood et al., 2019) have been proposed for comparing absolute snail abundance between sites. In this study, time-based sampling was preferred to the more accurate yet cumbersome quadrat sampling (Perez-Saez et al., 2019), as the latter would be time-consuming for volunteers, difficult to validate remotely compared to time stamps in the former, and the increased time investment would potentially lower the motivation of the CSs (Sprinks et al., 2017).

The collected snails were placed in plastic containers, pooled per sampling site, and transferred in ambient water to the laboratory where they were identified morphologically to species level using identification keys by Brown (1994) and Mandahal-barth (1967). The snails were then sorted per species and prepared for cercariae shedding.

3.4.2 Cercariae shedding

The Emergent Method (Webbe, 1965) was used to shed cercariae from snails. The intermediate hosts of *Schistosoma* species were put individually in culture plates containing clean water and exposed to light from 60W lamps for 2-4 hour to simulate natural light and induce shedding. The plates were viewed under a dissecting microscope and any cercaria shed identified according to morphotype (Frandsen & Christensen, 1984; Kamel et al., 2021). Infected snails were individually placed in 2ml tubes together with a subset of the emerged cercariae and fixed with 90% ethanol. The remaining snails were pooled per species/morphotype, per site, fixed in 90% ethanol and stored for later DNA extraction.

3.4.3 Molecular Assays

Whenever possible, two to five specimens were selected per morphotype of *Bulinus* and *Biomphalaria* and photographed (each specimen individually) on a macro photography stand in such a way that a vertical tangent could be fit to its most peripheral margin and ensuring the deepest point was clear enough (Hammoud et al., 2022). The ventral, dorsal and lateral views were photographed for *Biomphalaria* snails, while only the dorsal and ventral views were photographed for *Bulinus* specimens. Depending on the snail size and the view, several high-resolution pictures per specimen were taken between the lowest and highest point of the specimen with a Canon EOS 1200D camera. The photos were then stacked with Zerene®

software (T2019-10-07-1410) to obtain a composite structure of the snail specimen. After photography, the entire snail tissue was separated from the shell, homogenised using a sterile scalpel, and excess ethanol was removed with absorbed paper. The DNA was extracted using the E.Z.N.A. Mollusc DNA Kit protocol (OMEGA Bio-tek, Norcross, GA, USA), diluted with 1/10 ultrapure water, and stored in the freezer at -20°C.

The detection of *Schistosoma* spp. in the snail DNA extracts was conducted using a two-step approach described by Schols et al. (2019). In the first step, termed infection rapid diagnostic PCR (RD-PCR), three markers were used to detect the presence of respectively snail, trematode, and *Schistosoma* spp. DNA. The performance of the PCR reactions was compared with positive controls (DNA extract of adult worms) and a negative control (water). The second PCR, called *Schistosoma* RD-PCR, discriminates among *Schistosoma* species and was only applied to samples that tested positive in the infection RD-PCR. A universal forward primer, Asmit1, was combined with species-specific reverse primers (Sh.R for *S. haematobium*, Sman.R for *S. mansoni*, Smat.R for *S. mattheei* and Sb.R for *S. bovis*/*S. curassoni*/*S. guineensis*) resulting in the amplification of cytochrome *c* oxidase subunit (*COX1*) gene fragments of different lengths depending on the parasite species in a ThermoFisher Platinum PCR thermocycler (Biometra). Both diagnostic PCR steps were conducted under the following conditions (Schols et al., 2019): initial denaturation for 3 min at 95°C, followed by 39 cycles each of 30 s at 94°C, 45 s at 62 °C, 45 s at 72°C, and then a final elongation step of 10 min at 72°C. PCR products were visually checked under UV light exposure after electrophoresis for 2 h 30 min at 120 V in a 3% agarose gel with Midori Green Direct®.

Partial *COX1* gene fragments for *Bulinus* snails were amplified using the universal barcoding primers (LCO1490-HCO2198) (Folmer et al., 1994). In cases where the PCR amplification with the universal *COX1* primers was not successful, BulCox6-BulCox12, and Asmit1-Asmit2 were used as alternatives (Kane et al., 2008). Additionally, the entire internal transcribed spacer (ITS) region was amplified using the primers ETTS1 and ETTS2 (Kane & Rollinson, 1994). The PCR reaction was performed with the Qiagen^{TM} Taq DNA polymerase kit (Qiagen, Venlo, Netherlands) and the conditions were as described by Kane et al. (2008): 2.5µL of DNA solution was added to 22.5 µL of the PCR master mix (2.5µL PCR buffer X10, 0.75µL dNTP, 0.75µL $MgCl_2$, 1µL of 10µM primer1, 1µL of 10µM primer2, 0.15µL TAQ, and 16.35µL dH_2O).

In addition, snail infections that were not well resolved in the second step of the RD-PCR described above were amplified using the primers COIdigF and COIdigR. The cycling conditions were: 94°C for 5 min, 45 cycles of 94°C for 15 sec, 40°C for 30 sec, 72°C for 45 sec, and 72°C for 7 min. Cycling conditions for this and the ITS PCR were as follows: 94°C for 5 min, 45 cycles of 94°C for 15 sec, 40°C for 30 sec and 72°C for 45 sec and 72°C for 7 min.

PCR fragments were separated on a 1% agarose gel. The PCR products were purified for sequencing using the ExoSAP-IT™ PCR Product Cleanup procedure and sequenced in both directions using the BigDye Terminator protocol V3 (Qiagen, Venlo, Netherlands) by Macrogen. The DNA sequences were edited and assembled using Geneious Prime® version 2023.1.1 (Biomatters Ltd). We then checked and removed *COX1* sequences with stop codons and/or indels, as they are probably nuclear mitochondrial DNA sequences (NUMTs), which are not functional anymore as this results in reading frame shifts. In this study, we employed BLAST (Basic Local Alignment Search Tool) search to identify snail species by determining their closest resemblance to the genetic sequences of the snail specimens in the GenBank reference database. In addition, the species delineation was inferred from the phylogenetic analysis (see section 3.5.1.1). For the case of the *B. pfeifferi* group, haplotype network analysis using PopART software (© 2023 University of Otago), was further used to separate *B. pfeifferi* proper and *B.* cf. *pfeifferi* since the latter did not form highly supported clades during phylogenetic analysis (see section 3.5.1.2 below).

In addition, the following samples within the sub-Saharan region were retrieved from GenBank for comparison purposes: AM921839, AM921808, FN546814, OP233113, AM286294, ON117872, ON117894, AM921970 and MK414454 for *B. globosus*; AM286299 and AM921811 for *B. nasutus nasutus*; AM921815, OP233133, AM921989, AM921988 and AM286302 for *B. nasutus productus;* AM286295 and AM286296 for *B. africanus*; and OP233141, ON117874, ON117882 and AM286308 for *Bulinus forskalii*; DQ084842 and DQ084841 for *Biomphalaria* cf. *pfeifferi*; OQ0849820 and OQ0849823 for *B. stanleyi*; DQ084840 and DQ084843 for *B.sudanica*; HM768906, HM768946 and DQ084828 for *B. choanomphala*; DQ084836 for *B. smithi*; and MG780186 for *B. pfeifferi*. See section 3.5.1 below for the details on model selection, p-distance computation and phylogenetic analysis.

3.4.4 Determination of biotic and abiotic factors

The biotic factor determined in this study was the impact of aquatic macrophyte species density on the abundance of *Schistosoma*/*Fasciola* host snails and other snail populations while the abiotic factors measured include dissolved oxygen, electrical conductivity, pH, water hardness, salinity, total dissolved solids, nitrate concentration, phosphates and chloride ion concentration.

To determine the macrophytes species richness and abundance, a wooden quadrat (30 cm x 30 cm) was laid on the ground at the wells, small streams and slow-flowing rivers while an equivalent area was demarcated using four sticks fixed into the substrate for the lake shores and fast-moving water (Plate 3.3). The quadrats were laid at the same points where snail collection was done. For each macrophytes species, the number of independent stems (whole plants) were counted per quadrat. Runners and stolon forming plants, which did not originate from the quadrat were included in the quadrat as long as part of the plant was in the designated quadrat. Initial identification of the macrophytes in the field was done by assigning them tentative field names or codes.

Plate 3. 3: The research lays a quadrat at a site for macrophyte enumeration in the Lake Albert region. Photo credit Namirembe (2021).

A pair of secateurs was used to cut the stem, while rooted macrophytes were uprooted and pressed individually between newspapers with the leaves and flowers laid flat. The macrophyte species were collected in triplets and tagged with a label containing information about the collection site, name/code of the plant, locality, date of collection, habitat type, plant description (submerged, emergent, rooted or floating) and GPS location. The macrophyte specimens were prepared and dried for identification at the Makerere University herbarium (Bowles, 2004).

Other abiotic factors such as pH, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS), salinity, turbidity, resistivity and temperature were also recorded *in situ* at every site of snail collection using a multiprobe water quality meter (HANNA HI9829). The meter was calibrated once every two months using the quick calibration solution following the manufacturer's procedures. These parameters were measured *in situ* by immersing the probes into the water to be tested. Stable readings were then recorded. In cases when it was not possible to obtain a stable reading for example due to continuous wave action, or in flowing waters, a water sample was drawn using a plastic container rinsed in the water being tested. The probes were then immediately immersed and the stable readings were taken.

On the other hand, chemical parameters such as nitrates, ammonium, chlorides, and phosphates were measured in a field laboratory by spectrophotometry. For this, water samples were drawn into plastic 50 ml containers rinsed with the site water. The samples were well-labelled and stored in a cool box for transportation at ambient temperatures. The selected parameters were then measured using a HANNA Multiparameter Benchtop Photometer (HI83300) following the manufacturer’s guidelines. This instrument uses the test sample as the control and measures the colour changes when the chemical species being determined reacts with the standard reagents provided. The concentration of the chemical parameter is proportional to the intensity of the colour change following a specified reaction time.

3.4.5 Citizen scientists and snail monitoring

Twenty-five CSs were selected and assigned to 2-4 fixed sites which they sampled weekly. They collected data using Android OS Huawei Y7 smartphones. A good battery capacity of 3500 mAh, a rear camera of 8 MP, and GPS functionality at a reasonably low price influenced the choice of the type of phone. Apart from a mobile phone, a backpack and a t-shirt with the ATRAP project logo, the CSs also received monthly financial compensation of 20 USD to cover transportation costs and data bundles.

The KoboCollect application was chosen as it allows offline data collection and online data submission to a central server that can be accessed by researchers. The KoboToolbox platform, on which the application works, was designed for data collection in hard-to-reach areas with connectivity challenges (KoBoToolBox, 2014). Moreover, the platform allows researchers to modify the data collection tools to meet the objectives of the study, and multiple submissions by different data collectors are accumulated as single records in a single data file retrievable for validation and analysis.

Before the commencement of the data collection campaigns, the selected CSs were invited for a residential two-day training. On the first day, the CSs were taken through the theoretical background including what schistosomiasis and fasciolosis are, the disease transmission cycles, prevention and control measures, and how citizen involvement can bolster the intermediate host monitoring in their area. In addition, the CSs were guided through the smartphone technology and how the KoboCollect application works. This was done by filling out a dummy questionnaire developed for the training. This was an exciting day, given that the majority of the CSs acquired and used a smartphone for the very first time. On the second day of the training, snail identification was emphasised. Ethanol-preserved specimens were used to illustrate the peculiar characteristic traits of the three genera including *Biomphalaria*, *Bulinus* and *Radix* snails that host *Schistosoma* spp. and *Fasciola* spp., and how to distinguish them from other freshwater and terrestrial snail genera. A simplified identification guide adopted from Brown (1994) and Mandahal-barth (1965) was printed and laminated for waterproofing to guide the CSs in their subsequent snail identification (see Appendix 6). To concretise their snail identification skills, each citizen was individually asked to identify the preserved snails presented to them and was evaluated. This was followed by two field training sessions where the CSs were taught how to sample snails in different habitat types and segregate the different species. In these sessions, all the CSs had the opportunity to practice all they had learned by filling out data collection forms similar to what they would eventually do independently in their communities. This allowed real-time validation of the submissions and feedback was given when it was needed. This step also allowed the CSs to learn from the experiences of their peers, especially about the potential challenges they might encounter while they were back in their communities. The final step consisted of field visits to operationalise the network and allocation of study sites to be monitored from then onwards. Each CS was accompanied to their mapped site and allowed to fill out one data collection form at a site. This step was important to provide additional context-specific feedback and additional training to each CS. In addition, annual refresher trainings were organised, in which the CSs shared their experiences from the previous year for mutual learning. Refresher trainings were an avenue for ironing out the challenges faced and reporting errors committed in the data collection process. Social networking was a bonus from these gatherings.

The established network operated from March 2020 until February 2023. The reporting protocol for CSs consisted of multiple-choice and open questions about the presence, identity, and quantity of snails, GPS location, sampling duration, and time. At each mapped water contact site, sampling occurred within a diameter of 10 m. The CSs, wearing appropriate protective gear (latex gloves and gumboots), actively searched for snails for ca. 30 minutes using a hand-held scoop net attached to a two-meter-long metallic handle. The collected snails were sorted into groups (genus level) based on their morphological features as learned during the CSs' training. *Biomphalaria*, *Bulinus* and *Radix* snails were counted and recorded in the questionnaire, while snails from other genera were classified as 'pool'. Subsequently, all the snail groups were placed on a graph paper and photographed at close range. The picture was then uploaded to a central server for later verification. All the collected snails were returned to the site after recording the information to minimise environmental impact.

The verification protocol was designed to minimise *reporting errors*, i.e. the difference between sampled data by the CSs and the data submitted in the questionnaire (Brees et al., 2021). Reporting errors minimization was done by using skip logic and favouring multiple-choice questions, setting up a robust semi-automatic validation protocol that flags faulty entries, providing regular feedback, and organising an annual refresher training for the CSs. The data validation code developed in Python is available on GitHub at https://github.com/CiSciUganda/data_validation/blob/main/Automatic_KOBO. The semi-automatic validation is described in Brees et al. (2021). A portion of the validation process was automated, relying solely on the (meta)data within each report, while another portion required input from a researcher. This preliminary validation did not ensure completely error-free reports but rather identified obvious errors.

The automated validation began by cross-referencing an ID to confirm if the designated smartphone was utilized for reporting at a particular site, ensuring that citizen scientists only reported on their assigned sites. Following this, the reported GPS location was compared to the actual location documented during field exploration. If the reported location deviated by more than 200 meters in latitude and/or longitude, it was flagged. This threshold accommodated potential site shifts due to changing water levels throughout the year while still serving as a reliable indicator for potential inaccuracies in sampled locations. Additionally, the timestamps recorded before and after the sampling session were scrutinized to verify the actual sampling duration. Any reported sampling duration of less than 20 minutes or exceeding 40 minutes was flagged. Reports indicating temperatures outside the expected range of 10-35°C were also flagged as errors and not considered for subsequent data processing.

Next to the automatic verifications, manual verifications were done based on the submitted pictures. The pictures of the remaining snail species labelled as ‘pool’ were used to verify any false absence-cases where the CS reported absence of *Bulinus*, *Biomphalaria* or *Radix* when they were actually collected by the CS. Each of the snail genera (*Biomphalaria*, *Bulinus* and *Radix*) was independently verified, meaning that reporting errors for one genus do not affect the validity of correct reports for another genus. All reports with reporting errors were filtered out (<2.5% of total reports) before further analysis.

On the other hand, *observational errors* relate to the difference between the snail presence and abundance reported by the citizens, and the (actual) *in situ* situations. This bias could not be tested due to a lack of ‘ground-truth’ data. Therefore, a trained malacologist (hereafter referred to as the ‘PhD researcher’) visited the same sites that were monitored by the CSs weekly on a monthly basis, following the same standardised snail collection protocol (see section 3.4.1 above). The ‘PhD researcher’ dataset was used as a proxy for the ‘ground truth’, allowing a thorough comparison with the CSs data. Each CS sampled two to four water contact sites, but three of these sites were excluded from the analysis as the PhD researcher was unable to regularly access these remote sites due to logistical and safety. As a result, 73 out of 76 sites monitored by 24 of 25 CSs on a weekly basis were considered for data analysis. We included data from June 2020 to April 2022, when the PhD researcher stopped the monthly sampling campaigns. Due to the flooding of Lake Albert at the end of 2019 through 2021, the initially selected sampling locations (14 lake sites) shifted in space. Therefore, the CSs and PhD researcher agreed on new locations to monitor each time the water levels changed. Interaction with the local community members revealed that flooding of the lake was a rare event that had previously happened more than 50 years ago.

In the last four months of snail sampling, all snails collected by the PhD researcher were returned to the site after enumeration to assess the bias potentially introduced by snail removal on agreement/disagreement between the CSs and the PhD student (the so-called raters). The slight deviation of the PhD researcher from the CS protocol and the possible consequences for data comparison are discussed in the sections below.

3.5 Data Analysis Methods

3.5.1 Diversity and distribution of freshwater snails

Site and monthly differences in the abundancies of snail species was compared using χ^2 test. To get more reliable estimates, the snail counts were converted into abundance rate (counts per 30 minutes) since in some months some sites were not sampled due to inaccessibility of the sites. Therefore, means of snail counts per 30 minutes were compared between site types using repeated measurements ANOVA while the dry and wet seasons were compared using paired sample student t-test.

3.5.1.1 Phylogenetic analysis

A comprehensive analysis of *Biomphalaria* spp. and *Bulinus* spp. haplotypes was conducted using Geneious Prime® version 2023.1.1 (Biomatters Ltd). After removing duplicates, the unique haplotype sequences were aligned using the MUSCLE algorithm (Edgar, 2004) in MEGA X (Kumar et al., 2018). The genetic divergence was then estimated using correlated p-distance and modelled rate variation among sites with a gamma distribution (shape parameter = 0.19). In the next step, additional sequences from regional populations were retrieved from NCBI GenBank and aligned with the collected haplotypes from this study. The best-fitting nucleotide substitution model for the *COX1* gene was selected in MEGA X based on the Bayesian Inference Criterion (BIC). The Hasegawa-Kishino-Yano model (HKY+G+I) was the best fit for *Biomphalaria* spp., while the *Bulinus* dataset was best fit by the HKY model without a gamma distribution or invariable sites. Finally, a Maximum Likelihood (ML) phylogenetic tree was constructed for using 10,000 bootstrap replicates in MEGA X to infer phylogenetic relationships. *Biomphalaria sudanica* (OL423117) was used as an outgroup for the *Bulinus* spp. phylogenetic tree while *Helisoma anceps* (MF544974) was used for the *Biomphalaria* spp. tree. *Helisoma anceps* (MF544974) was used as an outgroup for the *Biomphalaria* spp. phylogeny.

3.5.1.2 Phylogeographic analysis

The TCS (Temporal Compression of Sequences) algorithm (Clement et al., 2002) was used to construct a statistical parsimony network in PopART software (Leigh & Bryant, 2015) (© 2023 University of Otago) for *Biomphalaria* spp. and *Bulinus* spp. in this study. Further, genetic diversity indices between and within groups were analysed using the Analysis of Molecular Variance (AMOVA) test in Arlequin software v3.5 (Excoffier & Lischer, 2010). A geographical distance matrix between the sampling sites was computed using a Geographic Distance

Generator (Version 1.23) and used in Arlequin software to carry out the Mantel test for assessing genetic isolation by distance. The correlation between the geographic distance and genetic distance (the p-distance) was computed. For this purpose, the *Biomphalaria* spp. DNA samples were divided into two subgroups based on the geographical location in proximity to Lake Albert. The lake subgroup constituted samples collected from the lake (sheltered bays, and open shoreline) and streams flowing into the lake at points less than 1km from the lake. The upland group constituted streams, wetlands (ephemeral vegetated pools, and swamps), streams, and artificial spring wells. The lake and upland subpopulations are separated by a rapid altitudinal change from over 1,120 m to an average 620m as one descends the Albertine rift valley escarpment, which was hypothesised to constitute a barrier to gene flow between lake and upland sub populations.

3.5.2 Cercariae shedding *versus* molecular determination of prevalence

The snail infection prevalence was descriptively analysed as percentages of the infected snails from shedding and diagnostic PCR data separately. Monthly trends in the prevalence of infections in the snails were presented graphically in Microsoft excel software. For the case of *Biomphalaria* spp., sufficient numbers of infected snails were present (89 snails) and mean prevalence of *S. mansoni* between *B. sudanica* and *B. pfeifferi* were compared using the Mann-Whitney U test since the assumption of normality of the distribution of the data was violated.

3.5.3 Biotic and abiotic predictors of snail abundance

All the measured physicochemical parameters were checked for normality using the Shapiro-Wilk test in R and were not normally distributed. Consequently, a generalized linear multiple linear regression analysis (GLM) with mixed effects was considered. The GLM is free of the distribution and homoscedasticity (equivalence of variance) assumption (Schweinberger, 2022).

Since the data constituted of repeated measurements, the measured site was used as a random effect (1|Site). A manual stepwise step-up forward inclusion procedure was used to include the various physicochemical parameters to examine their explanatory power for snail abundance. The Restricted Maximum Likelihood (REML) estimation method was used in the context of linear mixed-effects models to estimate the variance components. The REML method is preferred to the Maximum Likelihood (ML) method in that it removes the fixed effects from the likelihood function, resulting in a more unbiased estimate of the variance components. The inclusion of independent variables was based on a minimization of the Akaike information criterion (AIC) to balance model complexity and explanatory power (Schweinberger, 2022). The regression model was explored for collinearity through the calculation of the generalised variance inflation factor (VIF) values. The VIF of three was considered the threshold for collinearity (Zuur et al., 2010).

For the biotic predictors of snail species presence and abundance, the data was grouped into categories as either from Lake or other sites types. This grouping was based on observed peculiarities where by the lake macrophyte structure was different from the other upland sites (streams, wetland and springs). For each macrophyte morphospecies, plant density was summing the total stem count and dividing by the number of quadrats at a site. The macrophyte density was then matched with the snail count at the site and used for further analysis. Exploratory analysis included correlation of the macrophyte density with snail species abundance.

In the next step, multiple linear regression models were used to predict snail abundance rates (count per 30 minutes) by the density of each of the plant species. A stepwise forward inclusion method was used to building the best fitting model of macrophyte that predict snail species abundance. For this step, each variable was included if it significantly improved model fit ($p \leq 0.05$), while the exclusion threshold for plant species to eliminate from model was the p-value > 0.1. In addition, the reduction in the residual sum of squares for the models was used as a proxy for model improvement. For this analysis, the nested structure of the sampling design was not considered due to model complexity. Similarly, the presence of other snail species was used as a predictor for the density of the snails per site using the same approach above.

3.5.4 Traditional malacological surveys *versus* citizen scientist collections

3.5.4.1 Snail presence/absence data

The data points collected by the CSs and the PhD researcher were matched in space and time, considering the closest observation within a period of seven days. The closest time points were selected for the pairwise comparison to minimise natural differences due to mortality, birth, and passive or active dispersal of snails between both time points (Rabone et al., 2019).

Subsequently, the reported snail abundance was simplified to occupancy records (presence/absence) and compared in terms of binary agreement or disagreement. The extent of agreement between the CSs and PhD researcher data was expressed as a percentage of all paired data across the site types. Site type was used as the grouping variable as the categories (lake, spring, stream or wetland) have distinctive characteristics. Agreement/disagreement was split into four components of the confusion matrix. Agreement consisted of true positives (TP) when both the PhD researcher and CSs independently recorded snail presence at a site and true negatives (TN) when both reported snail absence at a site. Disagreement was split into false negatives (FN) when the PhD researcher recorded snail presence at a site while the CS did not, and false positives (FP) when the CS recorded snail presence at a site, but the PhD researcher did not. False positives and false negatives in this case are thus considered relative to the PhD researcher data (the so-called ground truth). As such, 'false' observations in our case can thus either be caused by observation errors by CSs, the PhD researcher, or actual differences in occurrence due to changes in the local conditions (paired samples can be as much as 7 days apart).

Sensitivity describes how well an observer detects the presence of objects/phenomena when they are actually present (Trevethan, 2017). It is calculated by dividing the TP by the sum of TP and FN and expressed as a percentage. PhD researcher-collected snail data was used as the reference for the computation of sensitivity of CSs in detecting snail presence at a site. A statistical analysis was then performed to further investigate why the PhD researcher and CS data might differ. The variables considered for this are summarised in Table 3.1 below.

Table 3.1: Variables used in the comparison of citizen scientist and PhD researcher collected snail data

Variable	**Type**	**Levels**
Citizen scientist’s ID	Nominal	1-25 (ID 11 excluded)
Site name	Nominal	73 unique names
Site type	Nominal	Stream, wetland, lake, or spring
Sampling date difference	Numerical	0 – 7
Snail species	Nominal	*Biomphalaria, Bulinus* & *Radix* species
Number of snails	Numerical	0 – 282 per 30 minutes of sampling

A generalised binomial logistic mixed-effects regression (GLMER) was used to analyse the binary agreement of the PhD researcher with the CS snail presence/absence data. GLMER treats data points as grouped (hierarchical) considering the nested nature of data based on a random effect such as repeated measurements of the same subject (Schweinberger, 2022). Our dataset consisted of repeated observations of sampling locations that are nested at the CS level (Figure 3.1). Site type, CS ID, the difference in sampling dates between CS and PhD researcher, and snail abundance were tested as predictors (fixed effects) of binary agreement between the PhD researcher and CS snail presence/absence data. Sampling location (site) nested between CS ID was considered as a random effect and included as a random intercept to avoid autocorrelation (repeated observations at the same site and/or by the same CS). The snail abundance reported by the PhD researcher was transformed to a count per 30 minutes and used as an independent predictor of binary agreement in presence/absence of snails at a site.

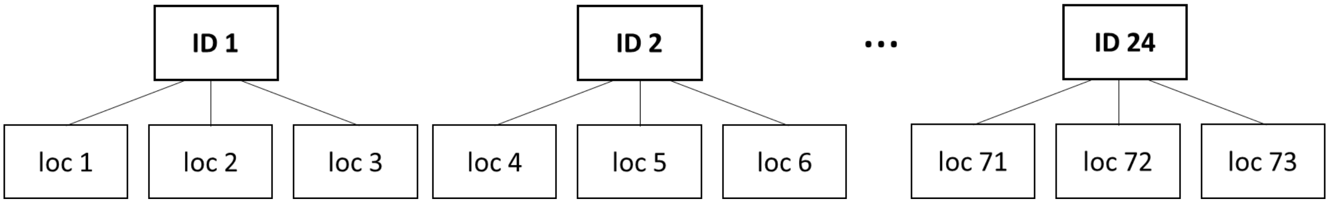

Figure 3. 2: Structure of the nested random effect implemented in the linear mixed-effect model. ID is the CS ID, loc is the sampling site/location.

A generalised linear mixed model framework of the GLMER is described following Ugwu and Zewotir (Ugwu & Zewotir, 2018). Here Y_{ij} is the binary response (agree/disagree) in the presence of snails between the CS at the *j*th location and the PhD researcher, with $P(Y_{ij} = 1)$ denoting the probability that at an *i*th time and the *j*th site, the CS agrees in snail occupancy with the PhD researcher. The GLMER then takes the following form:

$$P(Y_{ij} = 1) = \frac{exp\ (\eta_{ij})}{1 + exp\ (\eta_{ij})}$$

Eq. 1

With

$$\eta_{ij} \ = \ \beta_0 + \beta_1 . ID_j + \ \beta_2 . ST_j + \beta_3 . DD_{ij} + \beta_4 . NS_{ij} + \gamma_{\square} . (1|ID_j/Site_j)$$

Eq. 2

Where, ST_j is the site type, DD_{ij} is the sampling date difference, NS_{ij} is number of snails for each genus, ID_j is the citizen scientist identification number at $Site_j$ and $Site_j$ is the sampling location. β denotes the fixed effects coefficients while γ denotes random effect coefficients.

A simplified model for each of the genera was developed by a manual stepwise step-up forward inclusion procedure. The inclusion of independent variables was based on a minimization of the Akaike information criterion (AIC) to balance model complexity and explanatory power (Schweinberger, 2022). The model was explored for collinearity through the calculation of the generalised variance inflation factor (VIF) values. The VIF of three was considered the threshold for collinearity (Zuur et al., 2010). The reduction in model deviance (chi-square) was used to evaluate improvement in model fit after inclusion of independent variables compared to the null model. The function *somers2()* was used to compute Somers' D_{xy} and the C index of concordance (Baayen, 2008). Somers' D_{xy} is a rank correlation between predicted probabilities and observed responses and takes values ranging from 0 (randomness) and 1 (perfect prediction), with values greater than 0.5 considered meaningful. On the other hand, C is an index of concordance between the predicted probability and observed response. Values of C of 0.5 indicate randomness, 0.8 indicate the model has real predictive capacity while one (1) indicates perfect prediction (Baayen, 2008). All the analyses were done in R statistical software (v4.1.0; R Core Team 2021).

3.5.4.2 Snail abundance data

An exploratory analysis was performed to visualize the concordance between the snail abundance reported by the citizens and the one reported by the PhD researcher through time; as well as relative to the site types. After visualisation, the quantitative similarity- *raters' reliability*- between reported snail abundance was measured. For this analysis, only paired observations (previously adjusted to a scoop time of 30 minutes) where both the CSs and the PhD researcher reported snail presence (i.e. the TP pairs) were considered. We assess how well snail abundance is reflected by the CSs when both PhD researcher and CS detect the respective snail genus. For a detailed measure of these similarities, the data were analysed in subsets considering the site type, and the concept of reliability subdivided into consistency and numerical agreement.

Consistency is here defined as the resemblance between rankings of abundance by both rater types (CS and the PhD researcher). To compare ordinal association between two ranks, Kendall's Tau-b correlation coefficient was used (Agresti, 2010; Kendall, 1945). Kendall's Tau-b allows assessing how both rater types agree in the determination of abundance ranking of the sites. Particularly, Kendall's Tau-b coefficient is used when the analysed data presents ties within a group (observations when a certain group has two or more instances with the same quantity of snails in their sampling). Kendall's Tau-b coefficient was calculated from the following formula (Agresti, 2010; Kendall, 1945):

$$\hat{t}_b = \frac{C - D}{\sqrt{\left[\frac{n(n-1)}{2} - T_x\right]\left[\frac{n(n-1)}{2} - T_y\right]}}$$

Eq. 3

$$T_x = \sum_{p=1}^{ex} \frac{n_p\left(n_p - 1\right)}{2} \qquad\qquad T_y = \sum_{q=1}^{cs} \frac{n_q\left(n_q - 1\right)}{2}$$

Eq. 4

where C represents the concordant pairs, defined as those where the sign of ($X_p – X_q$) equals the sign of (Y_p-Y_q) whereby X_p and X_q are ranks of two observations by the PhD researcher and Y_p and Y_q are the paired observations made by the CSs and D the discordant ones where the sign of ($X_p – X_q$) is opposite to the sign of (Y_p-Y_q) (Agresti, 2010); n represents the number of paired observations, T_x and T_y represent the number of tied observations as defined above for either the CS data or the PhD researcher data, respectively; and p, q represents observations of snail abundance at sites where PhD researcher and CS sampled within less than one week of each other. Kendall’s Tau-b coefficient range from -1 to 1, with 0 indicating no correlation and (+ or -) 1 indicating a perfect correlation. For this analysis, each TP paired observation was used to assess the overall agreement in rank, without considering the nested structure of the data.

In contrast to the binary agreement defined above, the numerical agreement was defined in terms of the algebraic differences between the values reported by the raters relative to an absolute zero point; and calculated through Krippendorff’s alpha coefficient. Krippendorff’s alpha measures numerical agreement while correcting for chance agreement and is calculated from the following formula (Krippendorff, 2011):

$$\alpha = 1 - \frac{D_o}{D_e}$$

Eq. 5

whereby D_o represents the observed numerical disagreement and D_e represents the numerical disagreement that is attributable to chance. Both measures rely on values specific to frequencies reported in coincidence matrices, for which the construction is specific to the data type, which in our case is ratio metric differences. For a full description of the computation of α, we refer to Krippendorff (Krippendorff, 2011). Algebraically, when observers have a perfect match (no disagreement) α is equal to 1; but Krippendorff's α coefficient usually ranges from -1 to 1, with -1 being opposite numerical agreement, 0 no numerical agreement or numerical agreement due to chance and 1 perfect numerical agreement (Krippendorff, 2011).

Additionally, the factors that could influence differences between the snail abundance reported by CSs and the PhD researcher were analysed. For this purpose, a linear mixed-effect model (GLMMs) was computed considering a nested structure due to the multiple observations from the same locations made by each CS (Figure 3. 2). The linear mixed-effects regression was used to explain the difference of the snail abundance reported between CSs and PhD researcher as follows:

$$\Delta A_{ij} = \beta_0 + \beta_1 . DD_{ij} + \ \beta_2 . ST_j + \beta_3 . date_{ij} + \gamma_2 . (1|ID_j/site_j) \qquad \text{Eq.6}$$

The counting difference between reported snail abundance (TP: true positives, cases when the CSs and the PhD researcher reported snails) for observation *i* per location j, ΔA_{ij}, has a normal distribution with mean μ_{ij}, which is modeled as a linear function of the predictors (DD_{ij}: time difference in days between sampling made by the PhD researcher and CSs; ST_j: site type; and $date_{ij}$: scaled date of sampling) as well as a random intercept term for each ID_j: CS ID and $site_j$: sampling location. The scaled data represents the number of days since the first day of sampling. The collinearity of the predictors was evaluated through VIF values with a threshold of three (Zuur et al., 2010). The data of each snail genus was analysed per group: (a) data whereby snail abundance reported by PhD researcher is larger than snail abundance reported by CSs, and (b) data with lower abundance reported by the PhD researcher compared to the CSs, as we aimed to explore which factors could underly both situations.

3.6 Ethical considerations

There was due adherence to ethical code of conducting research. A full proposal with associated protocols was presented to Mbarara University ethical review committee (REC) for scrutiny, and was approved with reference number MUREC 1/7 (Appendix 9). Thereafter, a proposal was also sent to Uganda National Council of Science and Technology (UNCST) for approval and to ensure due adherence to Nagoya protocol before issuing material transfer agreement (MTA) to enable exportation of study samples for laboratory processing outside Uganda. The UNCST approval reference number is NS148ES (see appendix 10) and the approved signed MTA is in Appendix 11. Since the study was extractive for snail vectors, the benefit in terms of knowledge of effective control of snail-borne parasitic diseases, which affect health of millions of people and cripple economies in developing countries like Uganda outweighs any decreases in vector populations. Moreover, all snail samples collected weekly by CSs were returned in water after sampling to avoid excessive removal of snails since they play important roles in the ecological balance of freshwater ecosystems. Nevertheless, sampling was only done at the water contact points, which constitute only a small section of the waterbodies, thus not significantly affecting ecosystem balance.

Written informed consent was sought from the CSs and other community members that participated in this study (Appendix 11). The extent of disclosure of the information they provide and their facilitation were fully discussed and formalised in a memorandum of understanding (Appendix 12) before the commencement of the data collection process. The memorandum of understanding was renewed annually following a refresher training to allow the CSs to take into considerations their experiences. In cases where some citizen scientists dropped out, we followed the procedures described in section 3.3.2 to make replacements. In one case, we followed up the family for counselling sessions when a female participant disclosed that she was having family problems affecting her involvement in the research project. In addition, confidentiality was maintained in that each CS was only identified by an identification number assigned to him/her and the same was used in data analysis and reporting but not their names. In case the pictures of the CSs and other community members or stakeholders were needed or shared on social media, informed written or verbal consent was sought first.

CHAPTER FOUR

4.0 RESULTS

This chapter presents the results from data collected about snail species abundance and *COX1* variation, changes in *Schistosoma* spp. prevalence among the competent snail species, biotic and abiotic factors that influence snail distribution, and finally the reliability of the citizen science model in snail sampling and identification. In addition, it is important to note that this study emphasises schistosomiasis more than fasciolosis as described in the methods section. Therefore, more detailed information will be provided about the *Bulinus* and *Biomphalaria* snails compared to *Radix natalensis* and its associated parasites (*Fasciola* spp.), which were also not being studied at a molecular level.

4.1 Diversity of the target freshwater snail species

This section contains data collected by the PhD researcher while the data collected by citizen scientists can be found in section 4.2. A total of 52,757 snails belonging to the three target genera (*Bulinus*, *Biomphalaria* and *Radix*) were collected by the PhD researcher over the 20 months sampled. *Biomphalaria* spp. constituted the majority followed by *Radix* sp. and finally *Bulinus* spp. having total counts of 29,670; 16,964 and 6,123 respectively ($\chi^2 = 15798$, df = 2, p-value < 0.001). Thus, there is a viable risk of transmission of intestinal schistosomiasis, fasciolosis and urogenital schistosomiasis respectively, provided other conducive factors such as open defecation/urination are present. The data was collected from a total of 1,355 sampling reports, of which 691 reports were made at streams, 241 at lake sites, 217 at wetland sites, and 206 at springs from the 76 study sites surveyed monthly. The detailed results for each snail genus are in the sections 4.1.1 to 4.1.3 below.

4.1.1 *Biomphalaria* species diversity, abundance and distribution

A total of 29,670 *Biomphalaria* snails were collected from 70 out of 73 sites for 20 months. Surprisingly, spring sites had the highest mean *Biomphalaria* snail abundance with a mean count of 38 ± 68 snails sampled per 30 minutes (n = 7,516). On the other hand, stream sites had a mean count of 25 ± 32 *Biomphalaria* snails (n = 16,749), Lake Albert sites had 15 ± 31 (n = 3,279), and wetlands had 11 ± 19 snails sampled per 30 minutes (n = 2,126; Figure 4.1).

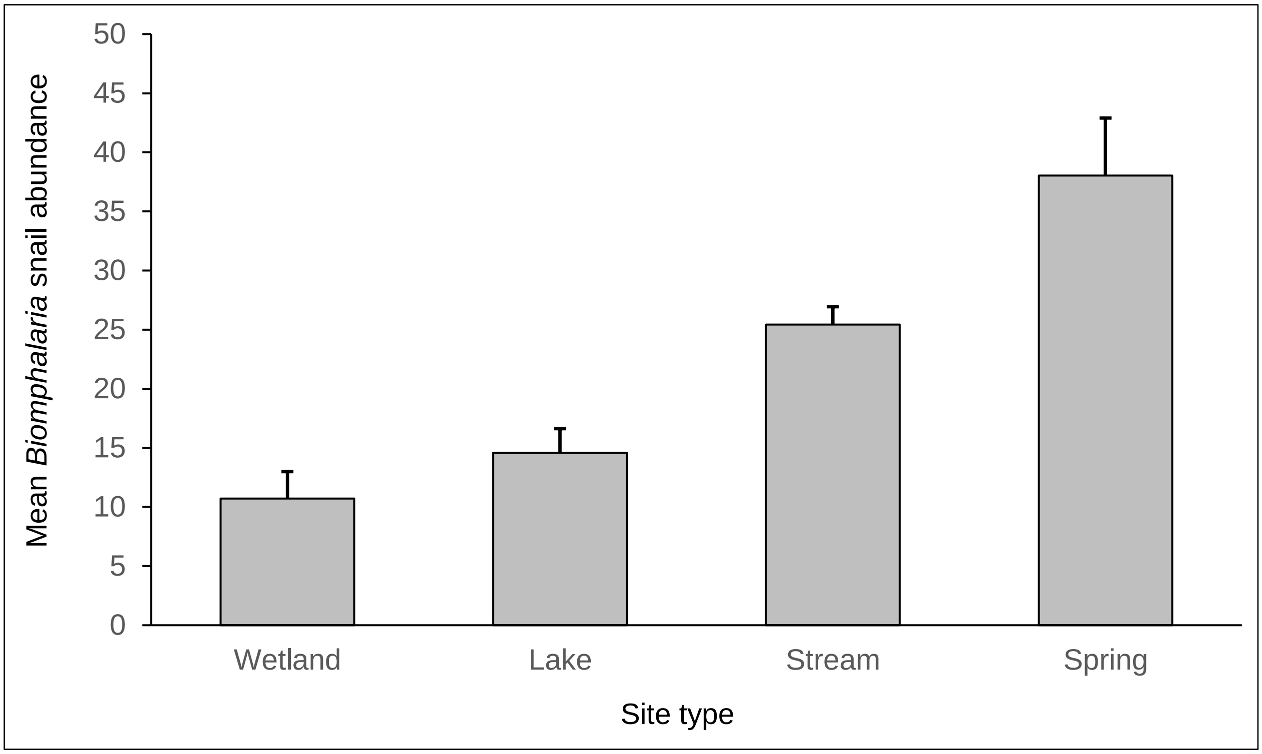

Figure 4. 1: The distribution of *Biomphalaria* spp. across habitat types in the Lake Albert region, Western Uganda. The error bar represents the standard error of the mean at 95% confidence level.

Generally, the *Biomphalaria* snail abundance peaked at the end of the rainy season and beginning of the dry season months (May-June and December-January), gradually increasing during the rainy season and decreasing in the dry seasons (Figure 4.2). Therefore, the wet season is the breeding season for *Biomphalaria* snails, which could result in a potentially higher risk of schistosomiasis transmission given other factors are favourable for parasite transmission.

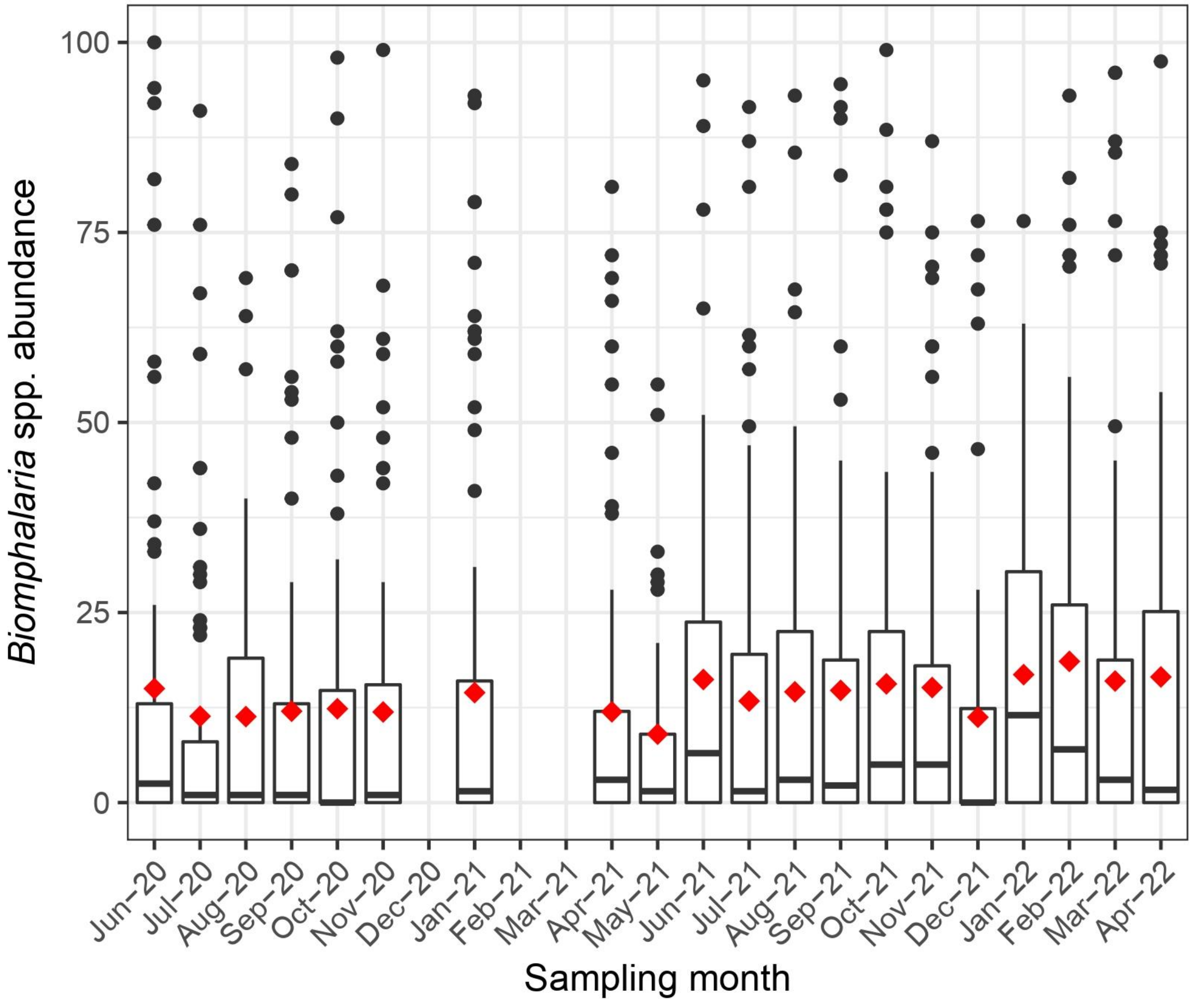

Figure 4. 2: The distribution of *Biomphalaria* spp. Abundance with all site counts aggregated per month for 20 months within the Lake Albert region.

The central line within each box represents the median value, while the box covers the interquartile range, which contains the middle 50% of the data. The whiskers extend to the minimum and maximum non-outlier data points, and outliers are plotted individually. Mean monthly snail abundances is indicated by the red diamond points.

Three morphospecies including *B. sudanica*, *B. stanleyi*, and *B. pfeifferi* were identified based on conchological (shell morphological) characteristics. However, considerable morphological variability between snail shells was observed among the *B. pfeifferi* group with some non-lacustrine *B. pfeifferi* snails appearing as morphological intermediates between *B. pfeifferi* proper and *B. sudanica* indicated in Table 4.1 below.

Table 4. 1 showing the morphotypes of *Biomphalaria pfeifferi* group and *Biomphalaria sudanica* collected in the Lake Albert region of Uganda.

Snail code	Photographed view
Non-lacastrine *Biomphalaria pfeifferi*	
RU1_02	
Lacastrine *Biompalaria pfeifferi*	
KOG02	
Biomphalaria cf. *pfeifferi* (non-lacastrine)	

2BAG02	
KAF03	
E18_02	
Biomphalaria sudanica	
2NT6_01	

The *Biomphalaria* snail morphospecies were validated using the BLAST search of partial *COX1* gene sequences to be *Biomphalaria pfeifferi, B. sudanica, B. stanleyi,* and *B. smithi.* We refer to the latter species as *Biomphalaria* sp. collected from Lake Albert due to deviation in shell morphology from *B. smithi* proper and failure to cluster with other GenBank sequences (Fig. 3), despite the 99.7 similarity with the sequence with the GenBank accession number DQ084836. Similarly, snails morphologically identified as belonging to the *B. pfeifferi* group constituted *B. pfeifferi* proper, and *B.* cf. *pfeifferi*. *Biomphalaria stanleyi* and *Biomphalaria* sp. were found only in Lake Albert while *B. pfeifferi*, *B.* cf. *pfeifferi*, and *B. sudanica* were found in both lacustrine and non-lacustrine environments. Overall, the *B. pfeifferi* group (including snails identified as *Biomphalaria* cf. *pfeifferi* in GenBank) constituted 19,054 (64.2%) specimens, *B. sudanica* 10,600 (35.7%), and *B. stanleyi* 13 (0.004%) of the total *Biomphalaria* snails collected from the Lake Albert region. The *Biomphalaria* spp. from Lake Albert clustered with the same species identified in the region downloaded from GenBank as shown in the phylogenetic tree in Figure 4.3.

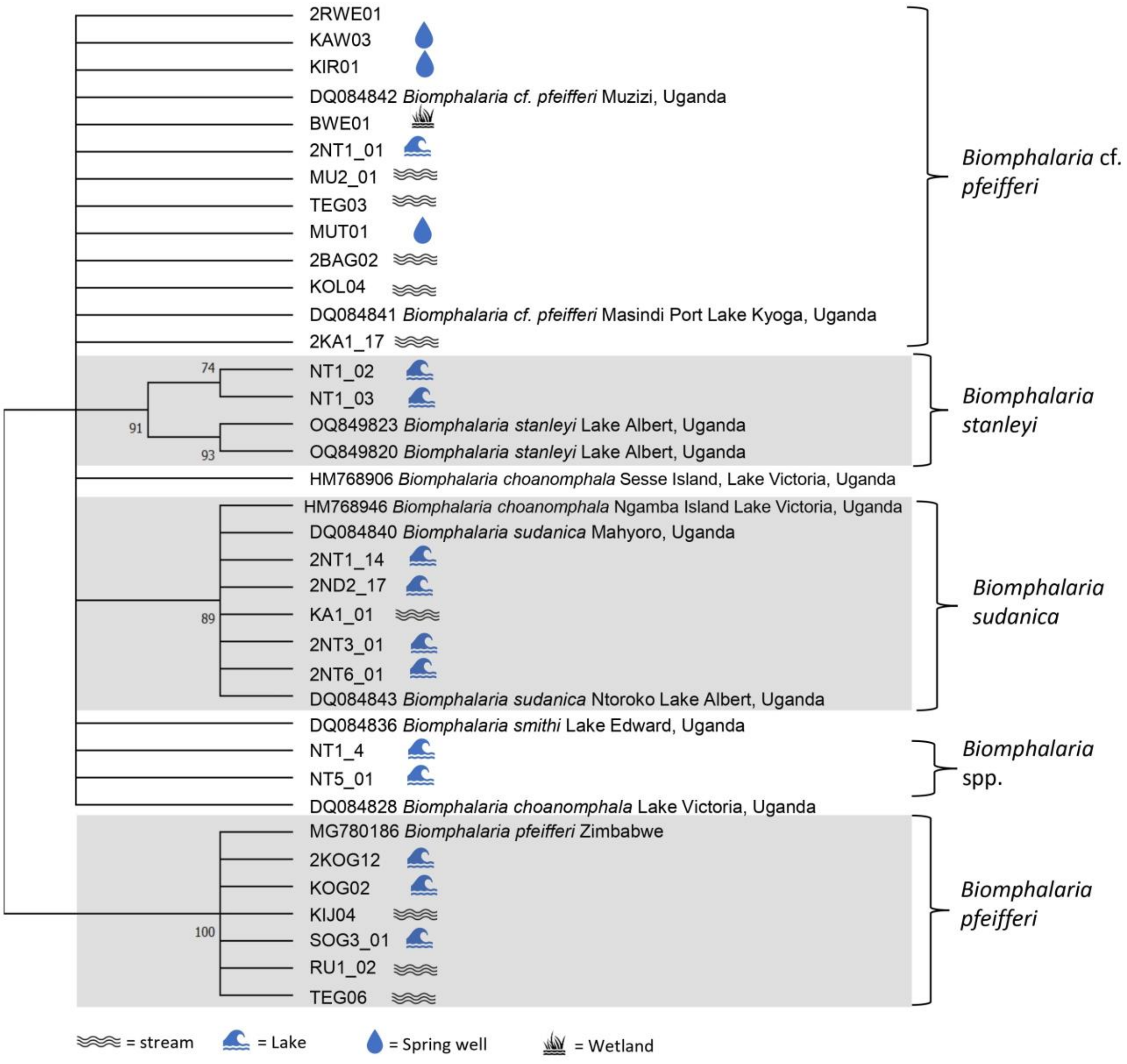

Figure 4. 3: Phylogenetic relationships of *Biomphalaria* spp. from the Lake Albert region obtained in this study and from GenBank

Sequences obtained from this are indicates with no accession numbers while the GenBank sequences are indicated with accession numbers, place and countries of origin where available. The phylogenetic tree was inferred using 567 bp of the cytochrome c oxidase subunit I and Maximum Likelihood with the Hasegawa-Kishino-Yano model + gamma (0.05) (Hasegawa et al., 1985). The tree was constructed using 10,000 bootstrap replicates and the clades that are supported by more than 70 bootstraps are highlighted in grey. The site types (stream, lake, wetland, or spring well), from which snails sequenced in this study were collected, are indicated by the symbol after the specific ID of the snail sample.

Considering the *COX1* gene, 43 haplotypes of the *B. pfeifferi* complex were obtained. Six of the haplotypes were found at Lake Albert sites, while 37 haplotypes were found in the upland sites (streams, springs, and wetlands) and only two haplotypes were shared between the lake and upland sites.

One major haplotype was found at various locations up the Rift Valley escarpment and thus constituted mainly the none-lacustrine *Biomphalaria* cf. *pfeifferi* group (Figure 4.4). Several haplotypes were linked to the haplotype H1 with a few mutations ranging from one to 12 single nucleotide polymorphisms (SNPs). However, there was a subgroup of haplotypes distantly related to the first haplotype subgroup with between 26 and 29 SNPs separating the subgroups. This second subgroup constituted the snails identified as *B. pfeifferi* from the GenBank references and was found mainly in Lake Albert and a few streams upland. Considering the distinct clustering of the *B. pfeifferi* proper supported by high nodal support (Figure 4.3) and clear separation of the haplotype network from *B.* cf. *pfeifferi* (Figure 4.4), the two groups can be considered distinct species or subspecies. Thus, *B. pfeifferi* proper constituted nine haplotypes while *B.* cf. *pfeifferi* constituted 33 haplotypes (Figure 4.4).

Generally, the intraspecific genetic diversity of the *B. pfeifferi* population (including the *B.* cf. *pfeifferi* and *B. pfeifferi* proper) from the Lake Albert region was 0.7453 ± 0.0404, and when the populations were split into two groups: the low-altitude (~620 m) lake sites (with lacustrine and non-lacustrine *B. pfeifferi* proper), and the high-altitude sites (1,120 to 1,221 m) which constituted main *B.* cf. *pfeifferi* and non-lacustrine *B. pfeifferi* proper, the highest genetic variation (56.3%) was between the groups (fixation index = 0.5095, $p < 0.001$) while 22.3% of the variation was within groups (fixation index = 0.7857, $p < 0.001$) and 21.4% of the variation was between the individual populations per site (fixation index = 0.5630, $p < 0.001$). Therefore, these results suggest that the rift valley escarpment between the lake and upland communities acts as a barrier to bidirectional gene flow between the two populations. However, more research is needed to ascertain whether this variation is due to geographic isolation alone, or whether you the *B. pfeifferi* group in the study area is made up of two different species that have a non-overlapping distribution, which are adapted to two different environments.

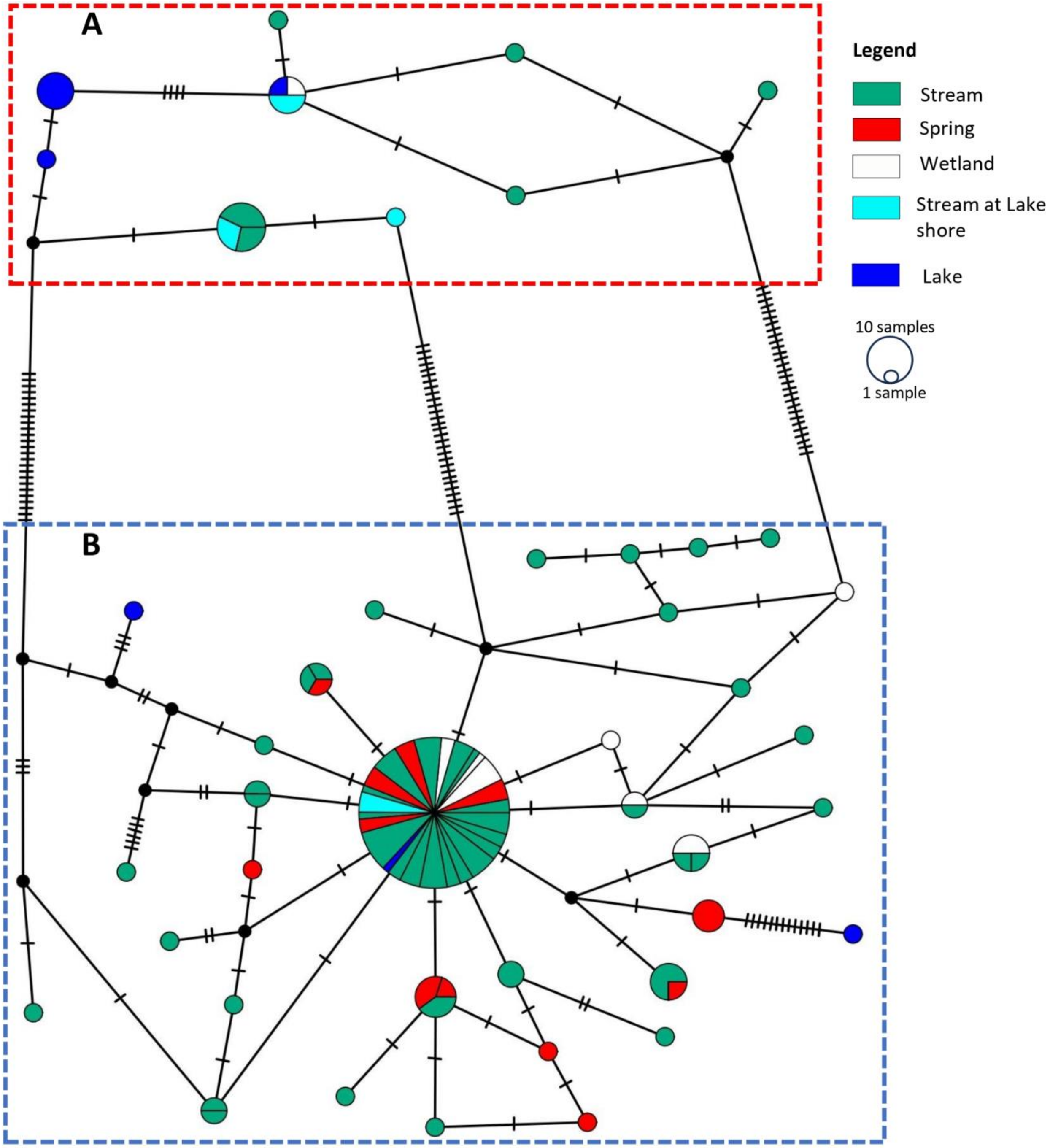

Figure 4.4: The maximum parsimony haplotype network for the 43 haplotypes of the *Biomphalaria pfeifferi* group collected from the Lake Albert region.

Note the distance between *B. pfeifferi* proper enclosed in in a red dashed rectangle A and *B. cf. pfeifferi* enclosed in the blue dashed rectangle B. The two groups are connected by three links, one of 25 mutations while the other two have 29 mutations each, resulting in p-distance ranging from 5.47 to 6.35% respectively. The colour codes represent the site types, from which the snails were sampled. The network was computed using the TCS algorithm in PopArt software.

When the p-distance of all the *Biomphalaria* species in the study area were further analysed, evidence indicates that *B.* cf. *pfeifferi* is distantly related to *B.pfeifferi* proper (p-distance = 5.81±0.25) and more closely related to *B.sudanica* (p-distance = 2.02±0.38) as indicated in Table 4.2 below. Therefore, there is need to revisit the classification of the *Biomphalaria* spp. Complex in the Lake Albert region. The intra- and inter-specific varation observed across the species reflects the expected. *Biomphalaria pfeifferi* proper was the most distantly related species to the other species with p-distance values greater than 5%. This implies that *B. pfeifferi* is ancestral to the other species in the region.

Table 4. 2: The p-distance matrix for the unique haplotypes of *Biomphalaria* species collected in the Lake Albert region of Uganda.

	B. pfeifferi	*B. sudanica*	*B.* cf. *pfeifferi*	*B. stanleyi*	*Biomphalaria* sp.
B. pfeifferi	0.62±0.39				
B. sudanica	6.11±0.18	0.54±0.66			
B. cf. *pfeifferi*	5.81±0.25	2.02±0.38	0.63±0.22		
B. stanleyi	5.75±0.23	2.21±0.43	1.21±0.24	0.35±0.35	
Biomphalaria sp.	5.53±0.08	3.14±0.14	2.57±0.65	3.19±0.09	0

On the other hand, eight haplotypes of *B. sudanica* were obtained from the study sites, dominated by two main haplotypes. Four of the haplotypes were shared between upland and lake sites, while two were found at the lake sites only, and two were at upland sites only (see Figure 4.5). The haplotypes were only separated by a single SNP between them, except the haplotype at Tegeta, which was separated from its closest haplotype by nine SNPs. The overall haplotype diversity was 0.5452, and 88.86% of the variation was contributed by genetic diversity within each population while only 11.14% of the variation was observed between populations.

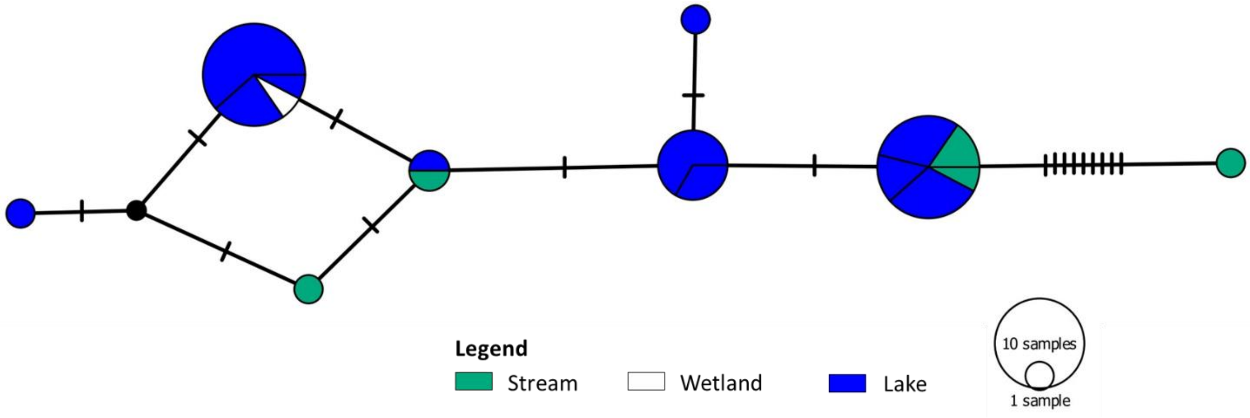

Figure 4. 5: The maximum parsimony haplotype network for the eight haplotypes of *Biomphalaria sudanica* collected in the Lake Albert region.

Haplotypes collected from the lake sites are filled with shades of blue while other colour shades indicate haplotypes collected from other site types (streams or springs). The network was created with PopArt software.

4.1.2 *Bulinus* species diversity, abundance and distribution

In the Lake Albert region, a comprehensive survey resulted in the collection of 6,123 snails belonging to the genus *Bulinus*. An interesting pattern emerged in the distribution of snail species, diverging from the observed distribution of *Biomphalaria* spp. While *Biomphalaria* spp. exhibited greater abundance in spring wells, *Bulinus* spp. were notably more prolific in wetland sites.

The mean snail abundance per sampling period of 30 minutes was calculated, revealing distinct variations across different habitats. Notably, the wetland sites demonstrated the highest mean snail abundance, with a value of 12 ± 30. Following were stream sites, exhibiting a mean snail abundance of 4 ± 20, and lake sites with a mean of 4 ± 12. In contrast, spring wells registered the lowest snail abundance, recording a mean of 2 ± 4, as visually represented in Figure 4.6 below. This nuanced non-random distribution underscores the ecological heterogeneity within the surveyed area, shedding light on the preferential habitats of *Bulinus* spp.

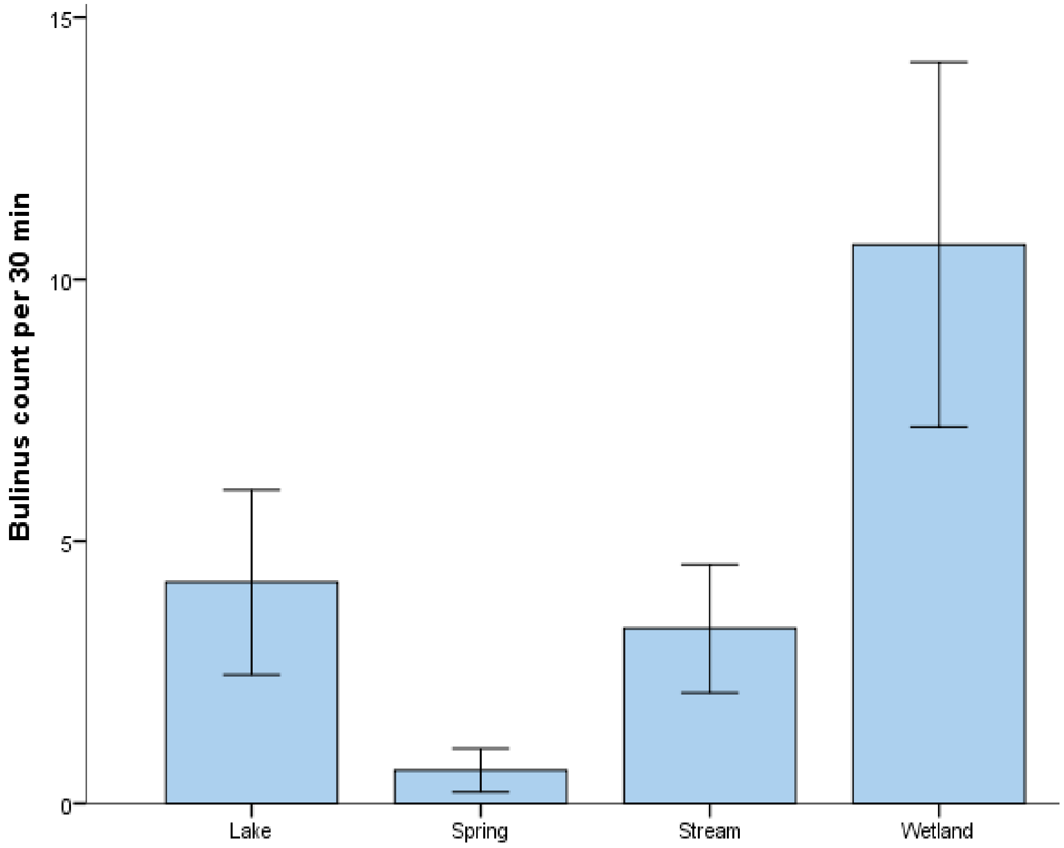

Figure 4. 6: The variation in the mean *Bulinus* species abundance across the site types in the Lake Albert region sampled over 20 months between 2020 and 2022. The error bars represent the standard error of mean at 95% confidence level. The difference in the means is significant with p-value for the ANOVA test < 0.001.

Similar to the trends observed for *Biomphalaria* snails, the *Bulinus* snail species increased as the wet season progressed and reduced gradually to the minimum at the peak of the dry months. The highest abundance was recorded in the months of September and October in 2020, and May, June and December in 2021, corresponding to the climax of the wet seasons in both years

(Figure 4.7). Some of the typical habitats of the *Bulinus* snails were observed to completely dry out in the dry season and snails would reappear at the sites when the rains returned.

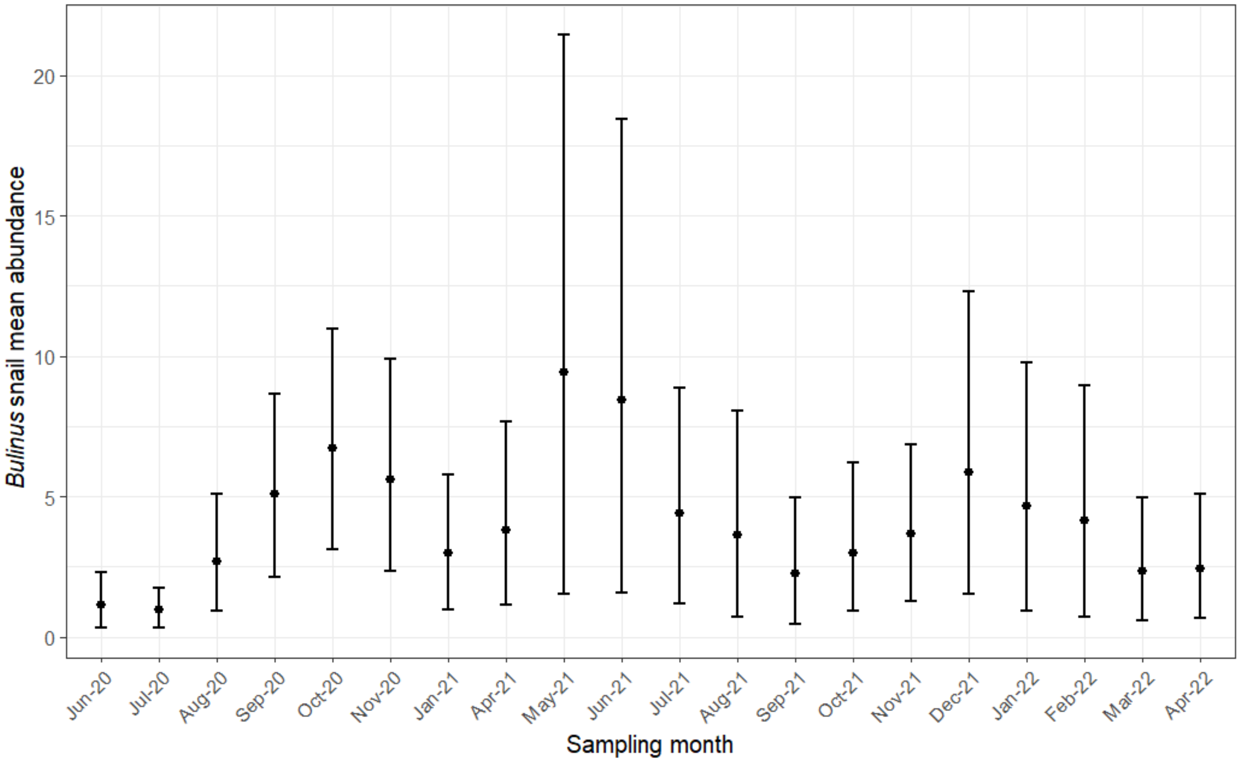

Figure 4. 7: The trend of mean snail abundance for *Bulinus* spp. collected across different months in the Lake Albert region of Uganda. The bars represent the standard error in computing the mean values, thus indicating the spread of the data values.

Bulinus spp. hosts of *Schistosoma haematobium* were found in the study area for the first time. It is important to highlight this result from the general results for the genus *Bulinus* since it has major implications in the understanding of the schistosomiasis landscape of Uganda. The presence of these hosts is the first elaborate demonstration of the potential of the outbreak of urogenital schistosomiasis spread caused by *S. haematobium* in the Lake Albert region of Uganda. This result dispels the long-held belief that absence of *S. haematobium* is because of absence of suitable intermediate hosts. Therefore, this section presents the data from the first two months (October and November 2020) when the host snails were first found abundant in the study area.

A total of 267 individuals of *Bulinus globosus* (n = 188) and *B. nasutus productus* (n = 79) morphotypes were collected from five out of the 73 sampled sites (Figure 4.8). The identity of *B. globosus* and *B. nasutus productus* collected at three and one sampling site(s), respectively, was confirmed by molecular barcoding (Figure 4.9). At Bulenge2 site, the *B. globosus* was identified morphologically due to failure of DNA amplification.

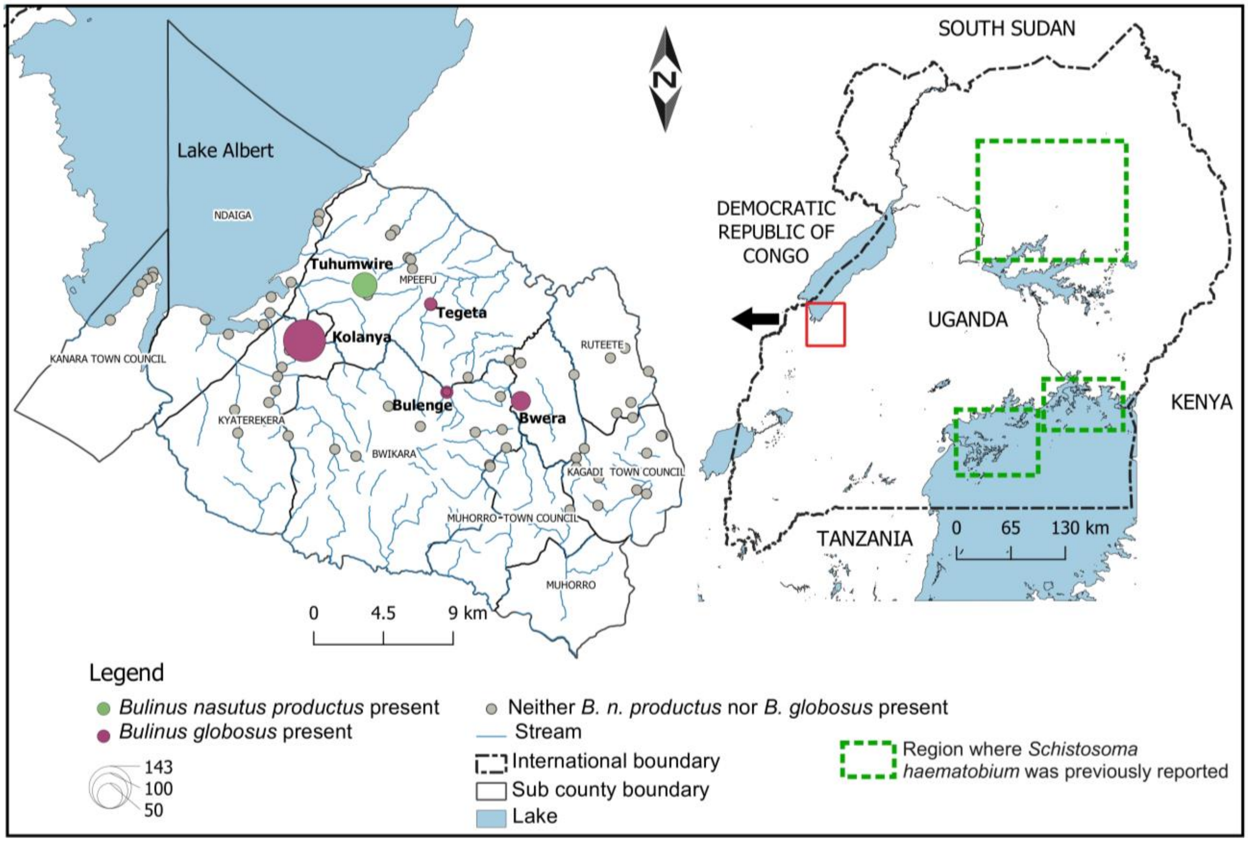

Figure 4. 8: The distribution of *Bulinus globosus* and *Bulinus nasutus productus* across 73 sites south-east of Lake Albert in Uganda (area in red rectangle) sampled in October and November 2020.

The maroon filled points indicate locations where either *B. globosus* was found, the green filled point represents the location of Tuhumwire site where *B. nasutus productus* was found, and the size of the circle represents the total number of snails collected. The locations where *Schistosoma* haematobium was previously reported (London

Applied & Spatial Epidemiology Reseach Group, n.d.) are indicated by green rectangles with dashed outlines. *Schistosoma* haematobium has also been reported in neighbouring countries including the Democratic Republic of Congo, South Sudan, Kenya, and Tanzania.

Bulinus globosus was more abundant and was collected from permanent stream points frequently used by humans and animals while *B. nasutus productus* was collected at a seasonal pond from a communal livestock grazing field in Tuhumwire village (N1.05143 E30.66487). Additionally, more *B. globosus* snails were collected in November (110) than in October (78; chi-square = 5.45, p = 0.02) while *B. nasutus productus* abundance remained constant (39 and 40, resp.; chi-square = 0.013, p = 0.91) for the two sampling time points (Table 4.”).

None of the *B. globosus* shed cercariae and the RD-PCR results were negative for *Schistosoma* spp. infection. On the other hand, two of the 32 (6.25%) *B. nasutus productus* shed *Schistosoma* sp. cercariae and the prevalence increased to 16% (four out of 25) when checked by infection RD-PCR. The infecting *Schistosoma* species was identified as *Schistosoma bovis* through the *Schistosoma* RD-PCR. Details on the infection trends across all the *Bulinus* spp. along with infection among *Biomphalaria* spp. during the entire study period are in section 4.2.1 below.

Table 4. 3: Observed human activities and abundance of snails at locations in the Lake Albert region of western Uganda where *Bulinus globosus* and *Bulinus nasutus productus* were sampled in October and November 2020. The accession numbers of the sequenced samples for the partial COX1 and ITS rDNA regions are appended. For the 'Folmer' region, each species had only one haplotype while three haplotypes, one at each site, were obtained among *B. globosus* considering the 'Asmit' region

Site	GPS Coordinates	Observed activities	*B. globosus*		*B. nasutus productus*		GenBank no. *COX1*		GenBank no. *ITS* rDNA
			Oct.	Nov.	Oct.	Nov.	'Folmer' region	'Asmit' region	
Bulenge	N0.98913 E30.71254	Bathing, cattle drinking	0	2	0	0	_	_	_
Bwera	N0.98388 E30.75554	Water collection, cattle drinking	20	10	0	0	OR553989; OR553992	OR553988	_
Kolanya	N1.01909 E30.63013	Washing, water collection	54	89	0	0	OR553990; OR553991	OR553985; OR553986; OR553987	OR553999; OR554002
Tegeta	N1.04047 E30.70325	Washing, water collection, swimming	4	9	0	0	_	OR553983; OR553984	_
Tuhumwire	N1.05143 E30.66487	livestock grazing	0	0	40	39[a]	OR553993; OR553994; OR553995; OR553996	_	OR554000; OR554001; OR554003
		TOTAL	**78**	**110**	**40**	**39**			

[a] *B. nasutus productus* at Tuhumwire had a *Schistosoma bovis* prevalence of 6.25% (n = 32) and 16% (n = 25) as determined by shedding and diagnostic PCR techniques, respectively.

A total of 11 *COX1* sequences were generated for *B. globosus* across all sites. However, *B. globosus* DNA was challenging to amplify using the Folmer primers with only four successful sequences, of which three were from the same site (Kolanya). Primers targeting the Asmit region (Asmit1-Asmit2; 443bp) were successfully used to amplify *B. globosus* from three of the four sites (Kolanya, n= 4; Tegeta, n=2 and Bwera, n=1). Therefore, *B. globosus* at Bulenge site was only identified morphologically. Based on the partial *COX1* sequences (631 bp from the Folmer region), one haplotype of *B. globosus* was identified, which was highly similar (uncorrected p-distance = 0.16%) to the sequences from the Albert Nile (about 300 km away from our study area, AM9286291) in Moyo, Uganda, differing by three substitutions. This close relationship is also reflected in the phylogenetic tree (Figure 4.9). Lower similarity was found with *B. globosus* haplotypes from Kenya (3.65% and 4.13% p-distance with reference sequences MK414454 and OP233113, respectively), Zanzibar Island (p-distance = 4.60%), Nigeria (p-distance = 4.92%), and Senegal (p-distance = 5.56%). However, higher variability was observed in the Asmit region. The intraspecific p-distances between the three obtained haplotypes ranged from 0.28% to 0.83% between the populations at Bwera and Tegeta, and Kolanya and Bwera, respectively. The haplotype at Bwera was identical to the haplotype from Kyaninga Crater Lake (ON112320). A similar phylogenetic clustering as in Figure 4.9 was observed where the Ugandan population clustered with the East African populations (Figure 4.10), while *B. nasutus productus, B. africanus* and *B. globosus* from West Africa formed a second clade. However, the bootstrap support values were low indicating low resolution in the Asmit alignment.

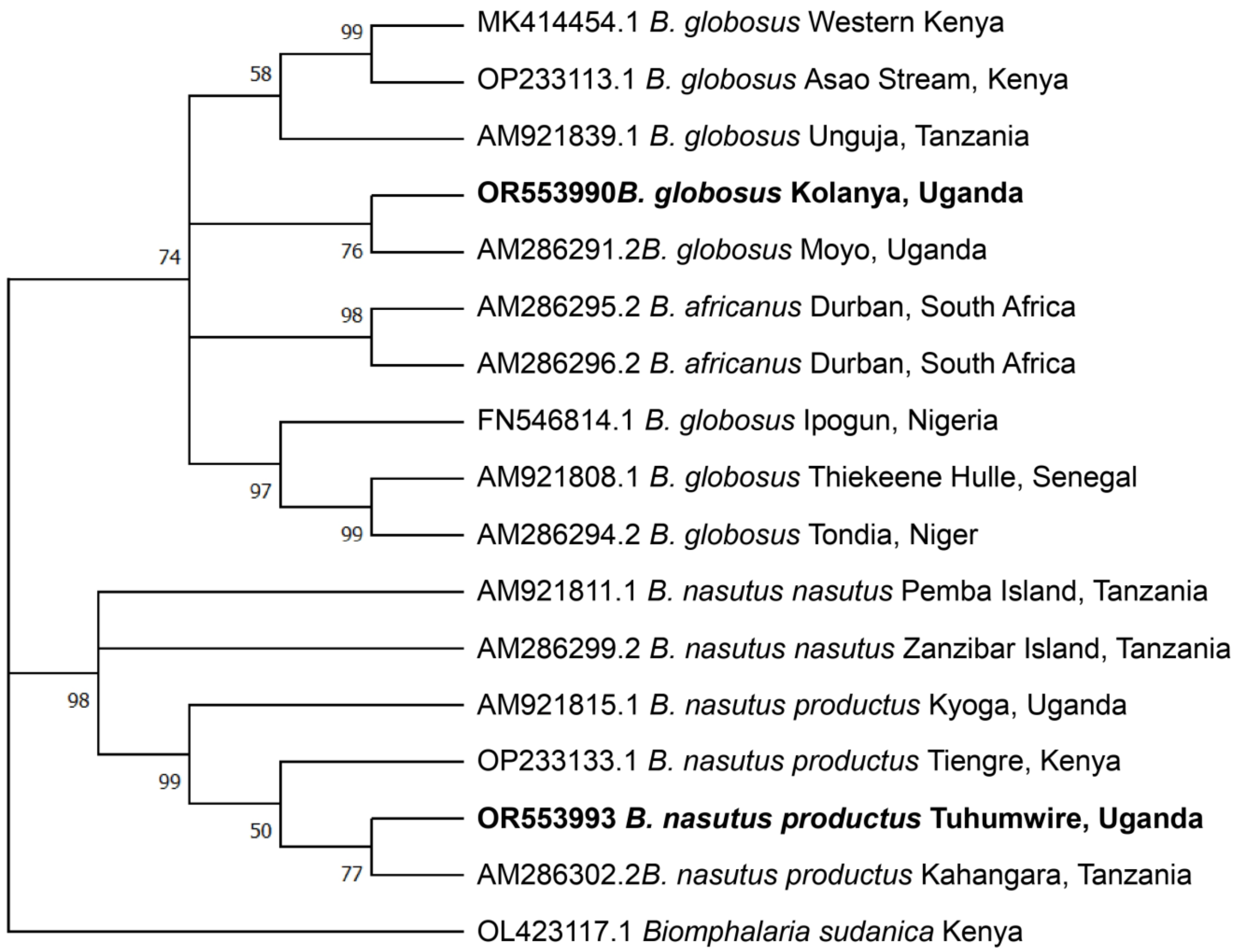

Figure 4. 9: Phylogenetic relationships of *Bulinus globosus* and *Bulinus nasutus productus* from Lake Albert region and regional populations based on the Folmer region of cytochrome c oxidase subunit I.

Sequences obtained from snails in this study are in bold and GenBank reference sequences with accession numbers, place and countries of origin where available are in regular font. The tree was inferred using 631 bp and Maximum Likelihood with the Hasegawa-Kishino-Yano model (Hasegawa et al., 1985). Only bootstrap values (10,000 replicates) above 50 are shown.

Seven identical *COX1* sequences were obtained from *B. nasutus productus* from site Tuhumwire using the Folmer *COX1* primers. The intraspecific differences with GenBank sequences ranged from 0.31% from the Tanzanian population, 0.63% with the Cawente (Lake Kyoga-Ugandan) population, to 1.27% with the Kenyan populations. Generally, the *COX1* phylogenetic analysis positioned both *B. globosus* and *B. nasutus productus* firmly within their respective species (Figure 4.9). The *ITS*2 phylogenetic tree follows the same topology as the *COX1* tree (Figure 4.11).

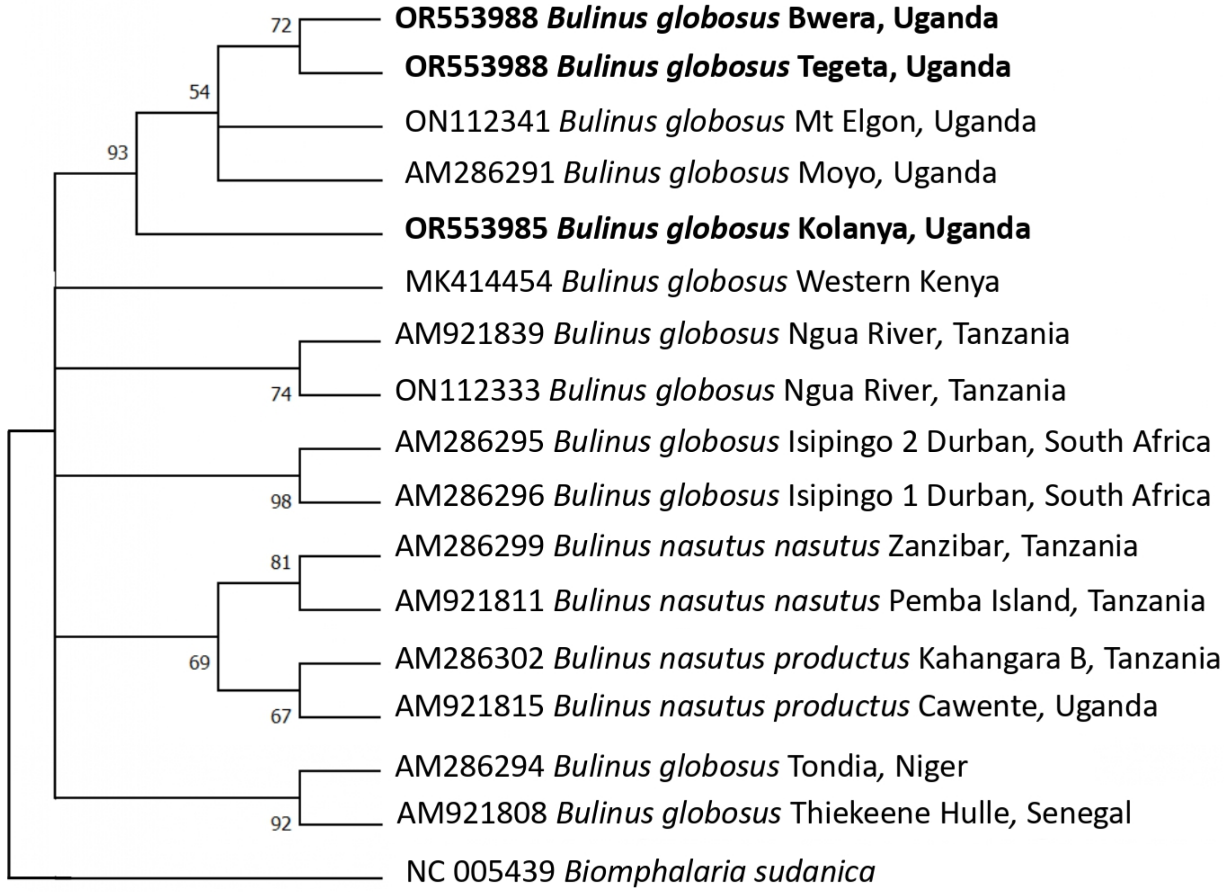

Figure 4. 10: Phylogenetic relationships of the *Bulinus globosus* and *Bulinus nasutus productus* from Lake Albert region and regional populations based on the Asmit region of cytochrome c oxidase subunit I.

Sequences obtained from snails in this study are in bold and GenBank reference sequences with accession numbers, place and countries of origin where available are in regular font. The tree was inferred using 360 bp and Maximum Likelihood with the Hasegawa-Kishino-Yano + Gamma model (Hasegawa et al., 1985). Only bootstrap values (10,000 replicates) above 50 are shown.

Figure 4. 11: Phylogenetic relationships of *Bulinus globosus* and *Bulinus nasutus productus* from Lake Albert region and regional populations based on the nuclear internal transcribed spacer 2 (ITS2) .

Sequences obtained from snails in this study are in bold and GenBank reference sequences with accession numbers, place and countries of origin where available are in regular font. The tree was inferred using 439 bp and Maximum Likelihood with the Hasegawa-Kishino-Yano (HKY + I) model (Hasegawa et al., 1985). Only bootstrap values (n= 10,000) above 70% are shown.

Other ***Bulinus* spp.** of veterinary importance were also found. In addition to the *B. globosus* and *B. nasutus productus* described above, this study found *B. forskalii* and *B. truncatus*.

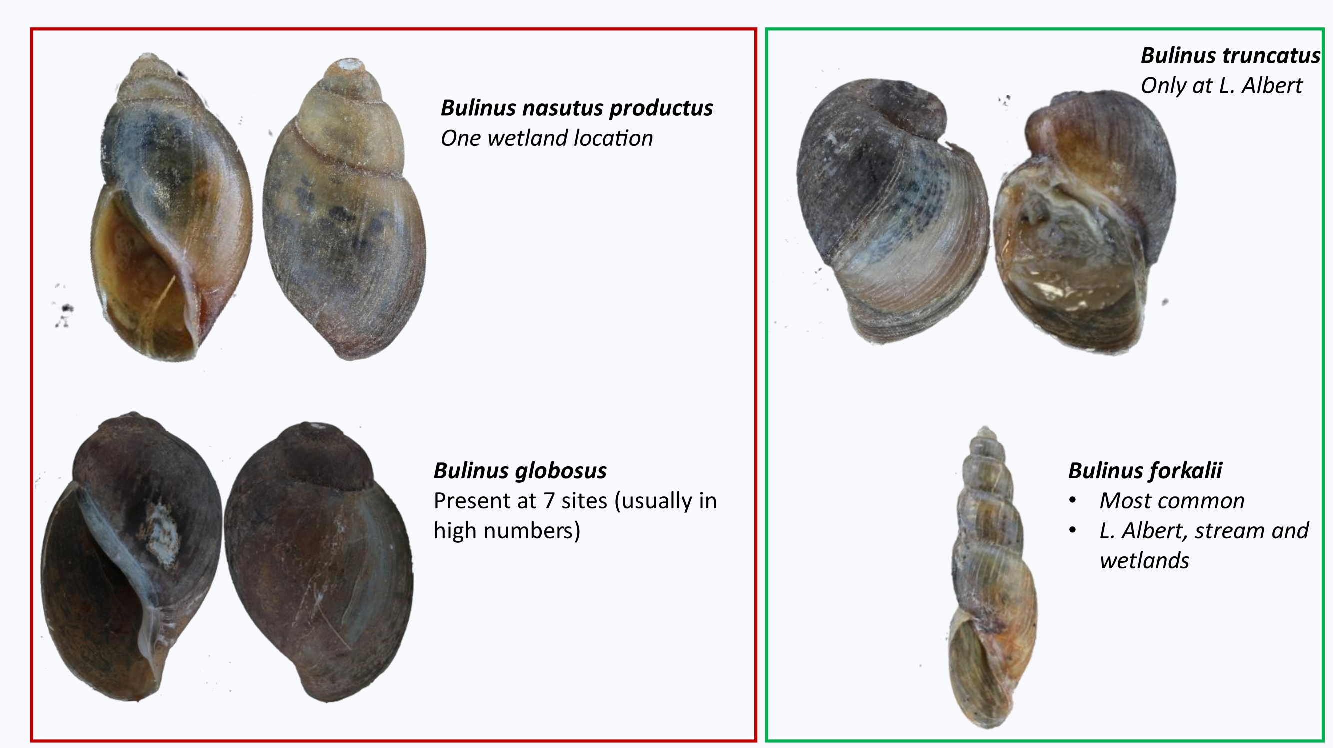

Plate 4. 1: The *Bulinus* species collected from the different site types in the Lake Albert region of Uganda. Snails in the red frame are species known to host *Schistosoma haematobium* in the East African region while snails in the green frame are natural hosts of *Schistosoma bovis*.

Moreover, *B. forskalii* was the most abundant and widespread *Bulinus* species occurring in all habitat types while *B. truncatus* occurred only in the Lake with the lowest abundance over the 20 months sampled. Therefore, the risk of bovine schistosomiasis transmission is high both at the Lake Albert shores and upland sites since the *B. truncatus* and *B. forskalii* are not known to naturally host *S. haematobium* but rather host *S. bovis*. While *B. globosus* was equally abundant (see Figure 4.12), it was not collected at lake sites. A chi-square test indicated no significant difference between the *B. forskalii* and *B. globosus* abundance ($\chi^2 = 0.55188$, df = 1, $p = 0.4576$). However, significantly lower counts of *B. nasutus productus* and *B. truncatus* were recorded ($p < 0.05$) as indicated in Figure 4.12 below. The low numbers might reflect the less favourable conditions for proliferation of the species and this could be a subject for further investigation. Such unfavourable conditions could be applicable in other areas where the snail species pose a significant health burden.

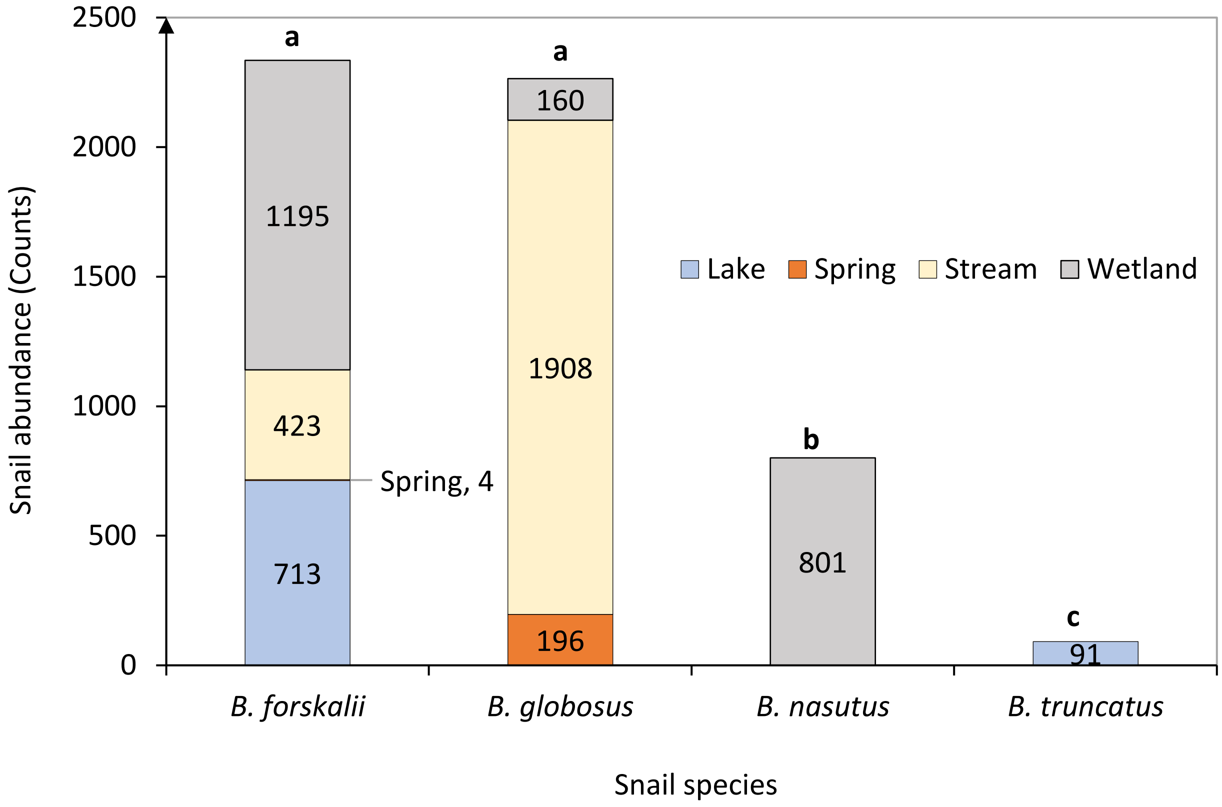

Figure 4. 12: The distribution of *Bulinus* species collected from the Lake Albert region of Uganda.

The letters “a-c” above the bars are used to symbolise statistical differences in abundance following a chi-square goodness of fit test, where by similar letter implies no statistical difference while a different letter indicates statistical significance at 5% level of significance.

The temporal distribution patterns showed that *B. globosus* and *B. forskalii* had similar dynamics tending to increase in the wet months and decreasing gradually in the dry season (June-July and January-February). However, there was no clear pattern observed for *B. nasutus productus* and *B. truncatus* (Figure 4.13). Notably, the populations of *B. nasutus productus* and *B. forskalii* were observed to completely disappear when the sites dried out completely, but would reappear at the beginning of the rain season. When independent samples t-tests were carried on the *Bulinus* species abundance rate (counts per 30 minutes) between the dry and wet seasons, only *B. forskalii* showed significant reduction int he abundance in the dry season (t =

-2.0645, df = 1127.6, p = 0.0392). Therefore, putative transmission risk for schistosomes carried by *B. forskalii* is higher in the wet season and if control measures such as snail control should consider seasonality of snail abundance changes. The differences in abundance for other species between the wet and the dry seasons were not significant (p > 0.05).

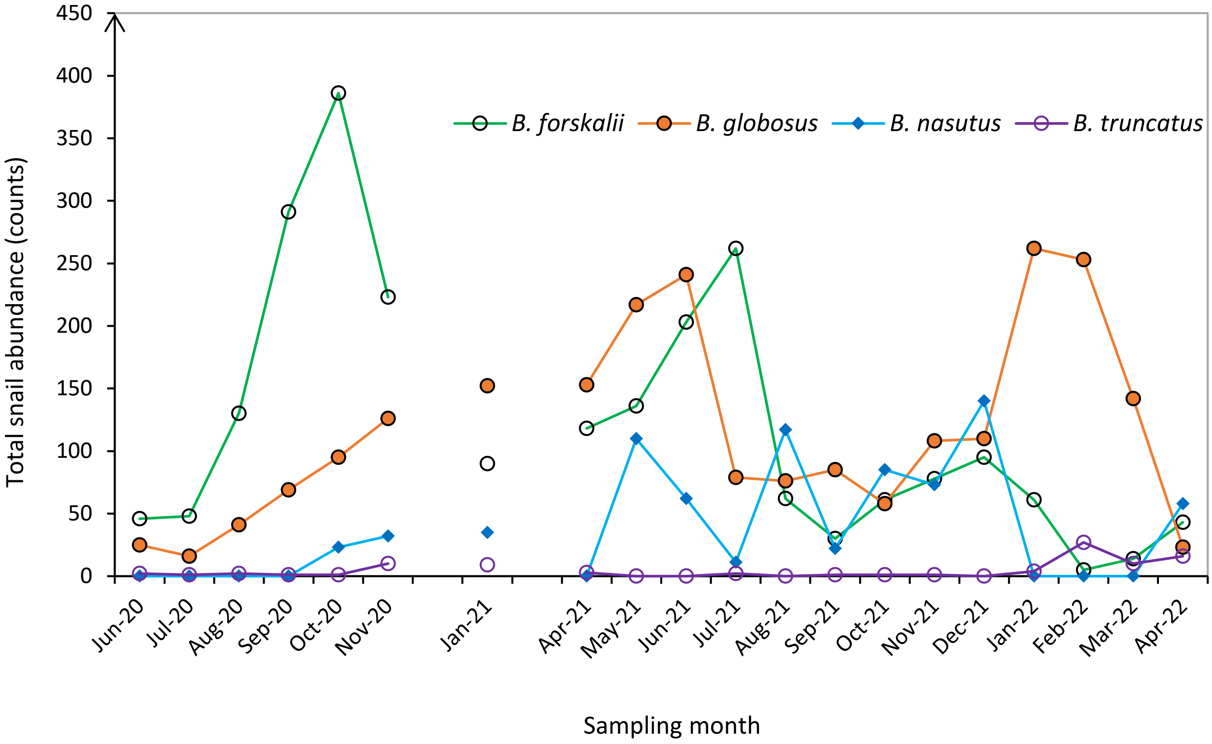

Figure 4. 13: Temporal changes in the *Bulinus* snail abundance in the Lake Albert region between the period of June 2020 and April 2022.

4.1.2.3 Bulinus forskalii and B. truncatus genetic diversity

A total of 12 haplotypes of *B. forskalii*, and two haplotypes *B. truncatus* were sequenced from this study. For *B. forskalii*, there was one major haplotype that was found in all site types while six haplotypes were exclusively in the Lake, two haplotypes each were exclusive to wetlands and the streams, while others were shared between some site types as indicated in Figure 4.14 below.

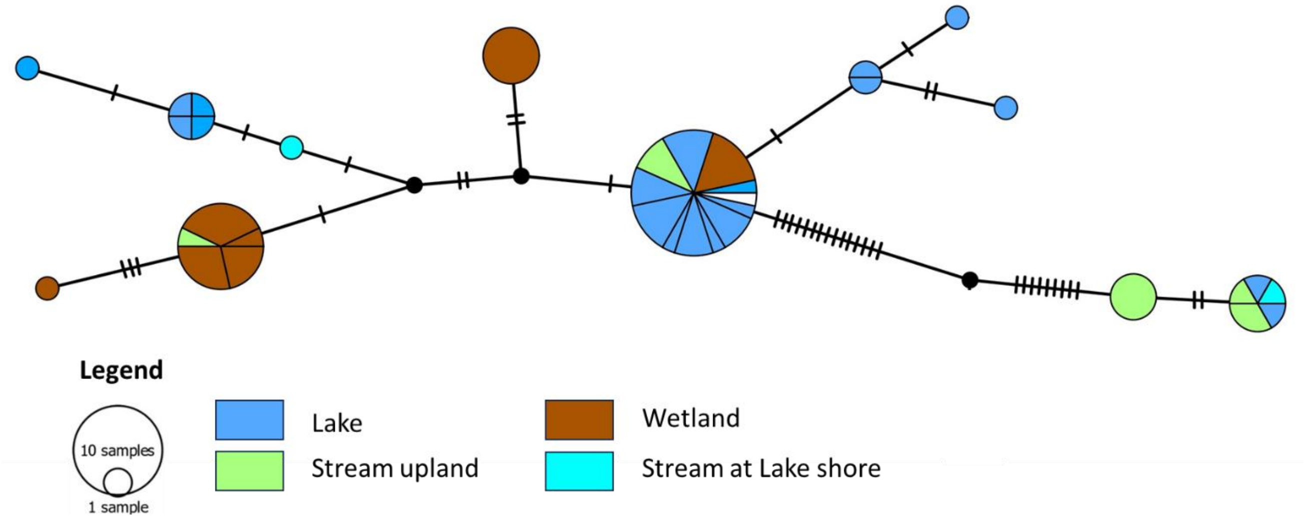

Figure 4. 14: A parsimony haplotype network for *Bulinus forskalii* snails collected in the Lake Albert region of Uganda.

The colour shades represent the different habitats from which the snails were sampled while the size of the circle is proportional to the number of individuals sequenced for a particular haplotype. All the computations were done in PopArt software using the TCS algorithm.

Generally, the intraspecific p-distance between the *B. forskalii* haplotypes ranged from 0.0023 to 0.0703 as indicated in Table 4.4 below. The low values indicate closely interacting populations with regular gene flow between them.

Table 4. 4: the p-distance matrix for the unique haplotypes of *Bulinus forskalii* collected in the Lake Albert region of Uganda. The bolded values indicate haplotypes which have p-distance values greater than 0.05.

	22ND2_07	22TU2_30	KAT1	22KIJ17	RU2_4	22ND2_08	KAM1	TUH8	22KIJ18	NT3_4	SOG1_7	NT6_8
22ND2_07												
22TU2_30	0.0094											
KAT1	0.0070	0.0117										
22KIJ17	**0.0609**	**0.0656**	**0.0632**									
RU2_4	**0.0562**	**0.0609**	**0.0585**	0.0047								
22ND2_08	0.0117	0.0070	0.0141	**0.0656**	**0.0609**							
KAM1	0.0023	0.0117	0.0094	**0.0632**	**0.0585**	0.0141						
TUH8	0.0164	0.0070	0.0187	**0.0703**	**0.0656**	0.0141	0.0141					
22KIJ18	0.0094	0.0047	0.0117	**0.0632**	**0.0585**	0.0023	0.0117	0.0117				
NT3_4	0.0141	0.0094	0.0164	**0.0656**	**0.0609**	0.0023	0.0164	0.0117	0.0047			
SOG1_7	0.0070	0.0164	0.0141	**0.0656**	**0.0609**	0.0187	0.0047	0.0141	0.0164	0.0164		
NT6_8	0.0047	0.0141	0.0117	**0.0656**	**0.0609**	0.0164	0.0023	0.0117	0.0141	0.0187	0.0070	

When a phylogenetic analysis was conducted, it was observed that two highly supported sub clusters of *B. forskalii* existed in the Lake Albert region (Figure 4.15). The two subgroups appear to be genetically distant as indicated by the distances greater than 5% in the p-distance matrix for the *B. forskalii* haplotypes (see table 4.3). Similarly, in the haplotype network up to 24 mutations separated two haplotypes (extreme right) from the rest of the haplotypes that constituted the majority (Figure 4.14). Surprisingly, these seemingly distant haplotypes were sympatric at Kijanibarora stream down the escarpment in Ndaiga sub county (i.e. sample 22KIJ17 and 22KIJ18), which might suggest that we have a case of recent or cryptic speciation.

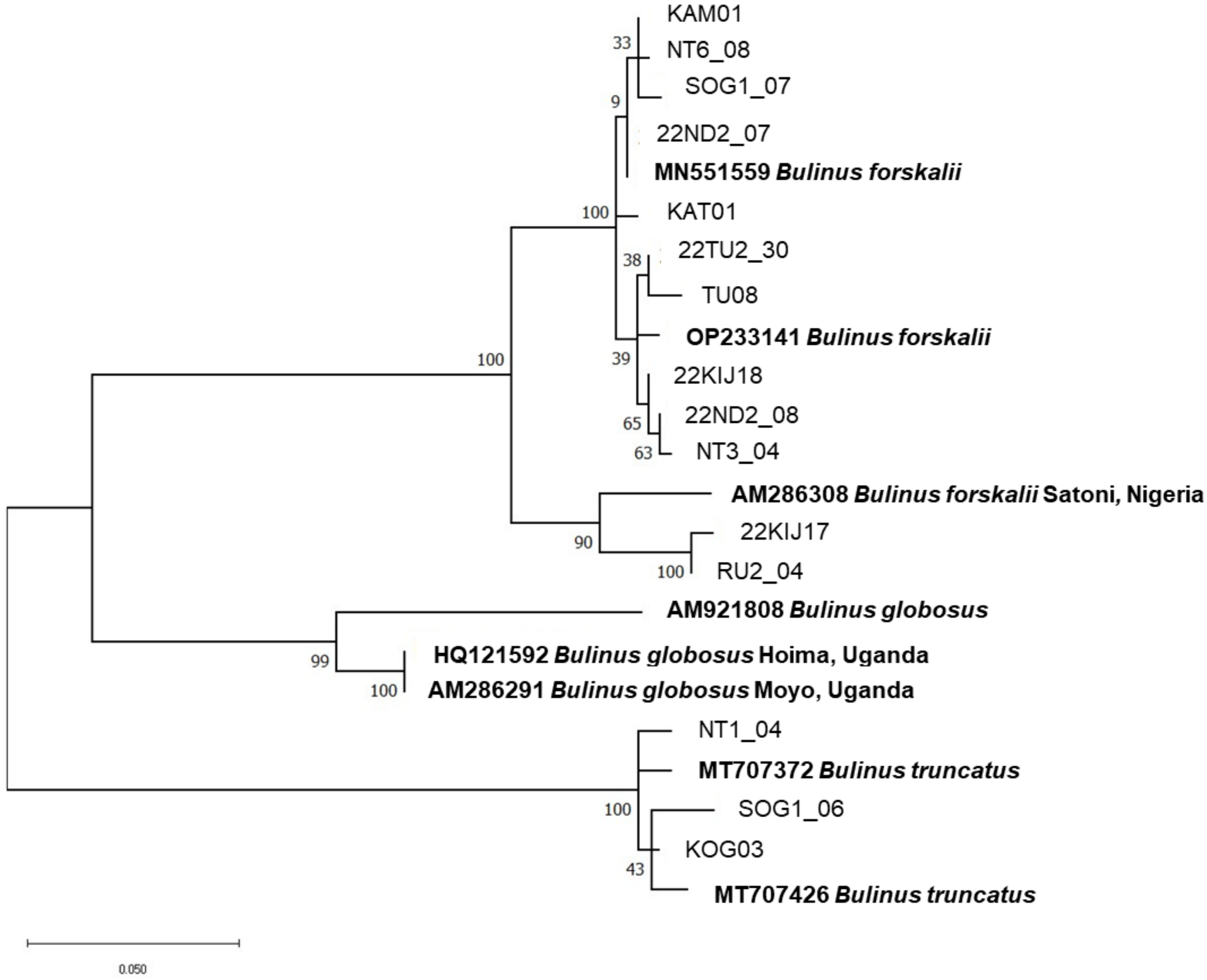

Figure 4. 15: Phylogenetic relationships of the *Bulinus forskalii* and *Bulinus truncatus* from Lake Albert region and the GenBank reference sequences based on the cytochrome c oxidase sub unit I.

The GenBank sequences are bolded with accession numbers, place and countries of origin whenever available. The tree is inferred using 425 bp and Maximum Likelihood with the Tamura-Nei (T93 + I) model (Hasegawa et al., 1985). 10,000 bootstrap values replicates were used in the construction of the phylogenetic tree in MEGA-X software.

4.1.3 *Radix natalensis* distribution and abundance

Radix natalensis (determined morphologically) was most prevalent in wetland sites with a mean abundance of 25 ± 3 snails per 30 minutes of sampling, followed by stream sites (15 ± 02), spring wells (10 ± 02) and was least prevalent at the lake sites (02 ± 01), see Figure 4.16 below. The differences were statically significant following an ANOVA test (F = 18.02, df = 3, p < 0.01). In addition, a post hoc Tukey HSD test indicated a significant difference between site types ($p < 0.05$) except between spring and stream site types ($p = 0.23$) as shown in figure 4.16. Therefore, wetlands are the main sites where fasciolosis transmission is likely to occur. Moreover, in the dry seasons, these low-lying areas are mostly the sources of fresh pasture and water, which further increases the risk of transmission of *Fasciola* flukes. Similarly, streams are important putative transmission sites, especially if they contain vegetation along their courses. Since the *R. natalensis* is present in both upland and lake (lowland) areas, the fluke transmission potential appears not to be geographically biased. However, if the transmission potential of the parasites is associated with the number of snails, upland sites where most wetland sites and streams are located would have a higher likelihood of disease transmission. Nevertheless, the higher abundance of free-ranging livestock at the lake shores may increase the risk of fasciolosis transmission at the lake more than the uplands.

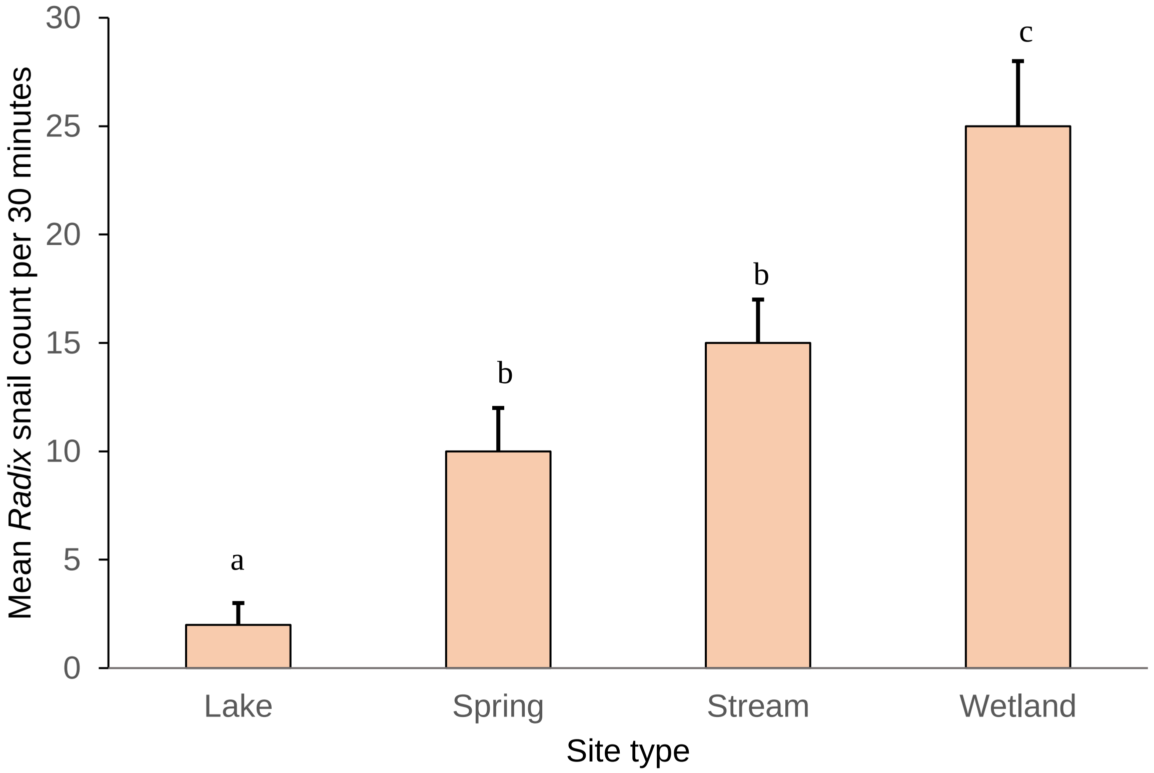

Figure 4. 16: The mean abundance of *Radix natalensis* across the different site types in the Lake Albert region of Uganda.

The overall mean abundance between sites was significant, the letters ‘a-c’ above each bar shows statistical difference between site types when the letters are different. The error bar denotes the standard error of mean.

Unlike the *Biomphalaria* and *Bulinus* snails, which tended to peak in the rainy months, the peak months for *R. natalensis* were in the dry months of June-August 2021, and January-February 2022 (see Figure 4.17). As described above, given the preference of *R. natalensis* for wetlands, which are the major source of fresh pastures in the dry season, high numbers of snail hosts could imply higher disease transmission of *Fasciola* flukes. However, the differences in mean snail counts per 30 minutes in the dry and wet seasons were not significant (t = 0.79933, df = 855.1, p = 0.4243). Notwithstanding, the persistent presence/survival of the *R. nataensis* snails throughout the season demonstrates a potentially continuous role in parasite transmission and hence need for continuous vigilance among livestock farmers.

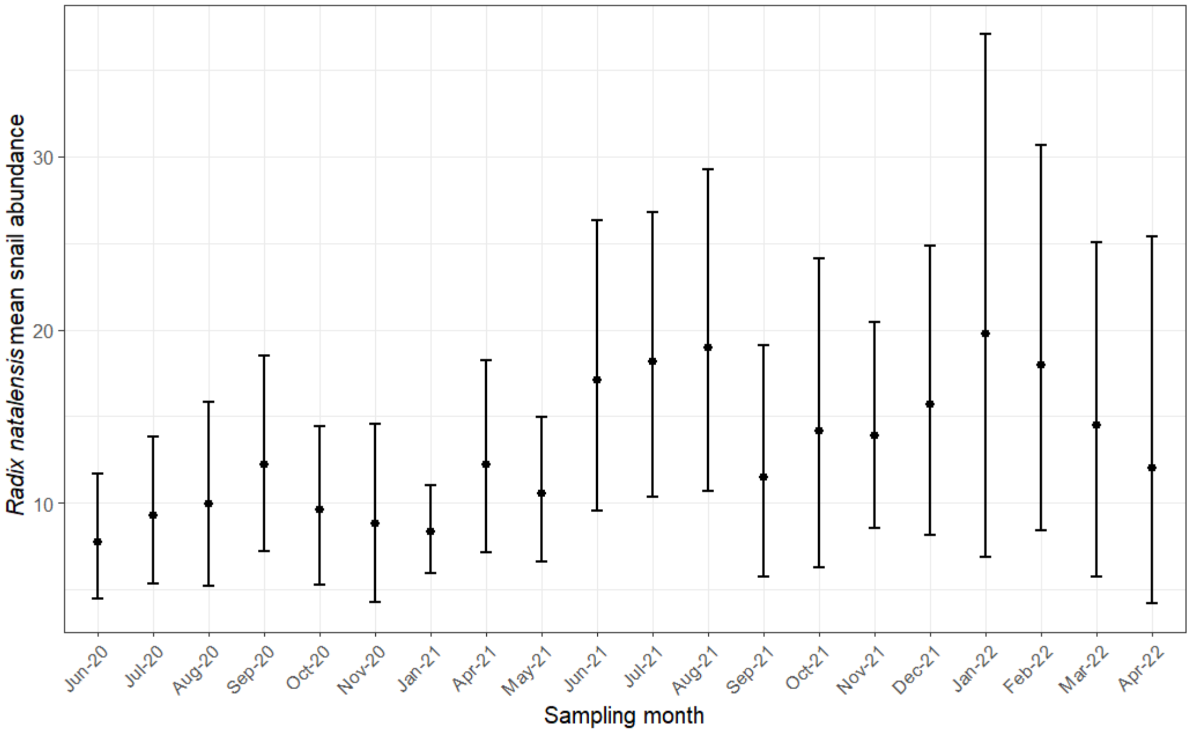

Figure 4. 17: Variation in *Radix* snail abundance with the month of the year

4.2 Prevalence of *Schistosoma* and *Fasciola* species parasites

4.2.1 Schistosoma parasite prevalence

Patent parasite prevalence was established from cercariae (larvae) shedding snails (see section 3.4.2). Of the 6,706 *Biomphalaria* snails screened, 45 (0.67%) shed schistosome cercariae (Table 4.5). The highest number of shedding snails was in the wet season in October 2021 with a prevalence of 3.9% (n = 511). When only sites with shedding snails were considered, the prevalence of schistosome cercariae ranged from 2.1% (n = 48) to 15.5% (n = 127), with a mean cercariae shedding prevalence of 7.7% (n = 584).

Table 4. 5: The prevalence of *Schistosoma* species among *Biomphalaria* when using the shedding technique

Month	No. induced to shed	No. of shedding *Biomphalaria* (%)	No. of *Biomphalaria* at positive sites	% infection at positive sites
Jun-20				
Jul-20				
Aug-20	720	11(1.5)	171	6.4
Sep-20				
Oct-20				
Nov-20	868	1(0.1)	37	2.7
Dec-20				
Jan-21	817	3(0.4)	41	7.3
Feb-21				
Mar-21				
Apr-21				
May-21	669	1(0.1)	48	2.1
Jun-21	934	0(0)	0	0
Jul-21	736	3(0.4)	31	9.7
Aug-21	757	0(0)	0	0
Sep-21	69	1(1.4)	47	2.1
Oct-21	511	20(3.9)	129	15.5
Nov-21	571	4(0.7)	56	7.1
Dec-21	54	1(1.9)	24	4
Overall	**6,706**	**45(0.67)**	**584**	**7.71**

Next, we determined the prepatent infections and identified the *Schistosoma* spp. infecting snails using molecular techniques. Two *Schistosoma* species were found infecting the *Biomphalaria* snails in the Lake Albert region: *Schistosoma mansoni* (confirmed using diagnostic PCR) and *S. rodhaini* (confirmed through genotyping). The two species showed heterogeneous spatial distribution: *S. mansoni* was present at the lake and two stream sites close to the lake whereas *S. rodhaini* was distributed widely in the upland sites (Figure 4.18). *Biomphalaria sudanica*, which was dominant at the Lake site, had a higher overall infection rate of 9.02% (n = 255) than *B. pfeifferi* with a rate of 1.91% (n = 1,364), while *B. stanleyi* (n

= 13) and *Biomphalaria* sp. (n = 03) snails screened in this study were not infected with *Schistosoma* spp. In addition, *S. mansoni* infection among *B. pfeifferi* at positive sites ranged from 1.8% to 14.3% with a mean infection rate of 5.11% (n = 147). On the other hand, higher *S. mansoni* infection was detected among *B. sudanica* snails, ranging between 10% (n = 10.0) to 33.3% (n = 48), and a mean infection rate of 24.72% (n = 89). Nevertheless, the mean difference in percentage prevalence between the *S. mansoni* among *B. pfeifferi* and *B. sudanica* was not statistically significant following a Mann-Whitney U test (p = 0.367). Therefore, *S. mansoni* transmission at Lake Albert is contributed by almost equally by the *Biomphalaria* snails present. Since no *S. mansoni* snails were detected in the upland sites, it is likely that there are spatial differences in other favouring factors for disease transmission.

In contrast, there was a higher prevalence of *S. rodhaini* among *B. pfeifferi* (especially *B.* cf. *pfeifferi*) snails with an overall rate of 12.24% (n = 147) than in *B. sudanica* where the overall prevalence was 1.12% (n = 89). Since almost all the *S. rodhaini* infections were recorded in the upland sites (Figure 4.18), it is likely that the rodents, the definitive hosts, are more distributed in the upland than at the lake shores. Incompatibility of *S. sudanica* in the study area with *S. rodhaini* is also a possibility. Table 4.6 indicates the detailed temporal distribution of *Schistosoma* spp.

Table 4. 6: The percentage infection rate of *Biomphalaria pfeifferi* and *B. sudanica* snails with *Schistosoma mansoni* and *Schistosoma rodhaini* as determined by rapid diagnostic multiplex PCR reactions on the snails collected in the Lake Albert region of Uganda.

	Biomphalaria pfeifferi group			
Month	Total tested	No. infected by *Schistosoma* spp. (%)	No. with *S. mansoni* at positive sites (%)	No. with *S. rodhaini* at positive sites (%)
Jun-20	43	0(0)		
Jul-20	23	0(0)		
Aug-20	68	0(0)		
Sep-20	14	0(0)		
Oct-20	9	1(11.1)	0(0)	1(11.1)
Nov-20	76	1(1.3)	0(0)	1(1.3)
Dec-20	-	-	-	-
Jan-21	90	4(4.4)	0(0)	4(4.4)
Feb-21	-	-	-	-
Mar-21	-	-	-	-
Apr-21	106	1(0.9)	1(0.9)	0(0)
May-21	197	1(0.5)	1(0.5)	0(0)
Jun-21	194	1(0.5)	0(0)	1(10.0)
Jul-21	112	1(0.9)	1(0.9)	0(0)
Aug-21	208	9(4.3)	1(0.48)	8(3.85)
Sep-21	19	0(0)		
Oct-21	124	3(2.4)	1(0.8)	2(0.16)
Nov-21	43	4(9.3)	3(7.0)	1(2.33)
Dec-21	38	0(0)		
Overall	**1,364**	**26(1.91)**	**8(0.59)**	**18(1.32)**
	Biomphalaria sudanica			
Jun-20	1	0(0)		
Jul-20	1	0(0)		
Aug-20	6	0(0)		
Sep-20	2	0(0)		
Oct-20	4	0(0)		
Nov-20	8	0(0)		
Dec-20	-	-	-	-
Jan-21	0			
Feb-21	-	-	-	-
Mar-21	-	-	-	-
Apr-21	30	1(3.33)	1(3.33)	0(0)
May-21	10	0(0)		
Jun-21	21	2(9.5)	2(9.5)	0(0)
Jul-21	24	1(4.2)	1(4.2)	0(0)
Aug-21	72	0(0)		
Sep-21	10	0(0)		
Oct-21	16	3(18.8)	2(12.5)	1(6.3)
Nov-21	50	16(32.0)	16(33.3)	0(0)
Dec-21	0			
Overall	**255**	**23(9.02)**	**22(24.72)**	**1(1.12)**

In summary, the distribution of *Schistosoma* species appeared to follow a spatial pattern (Figure 4.18) with the upland site, dominated by *B.* cf. *pfeifferi* having high *S. rodhaini* prevalence while the lake sites, dominated by *B. sudanica* were infected by *S. mansoni.*

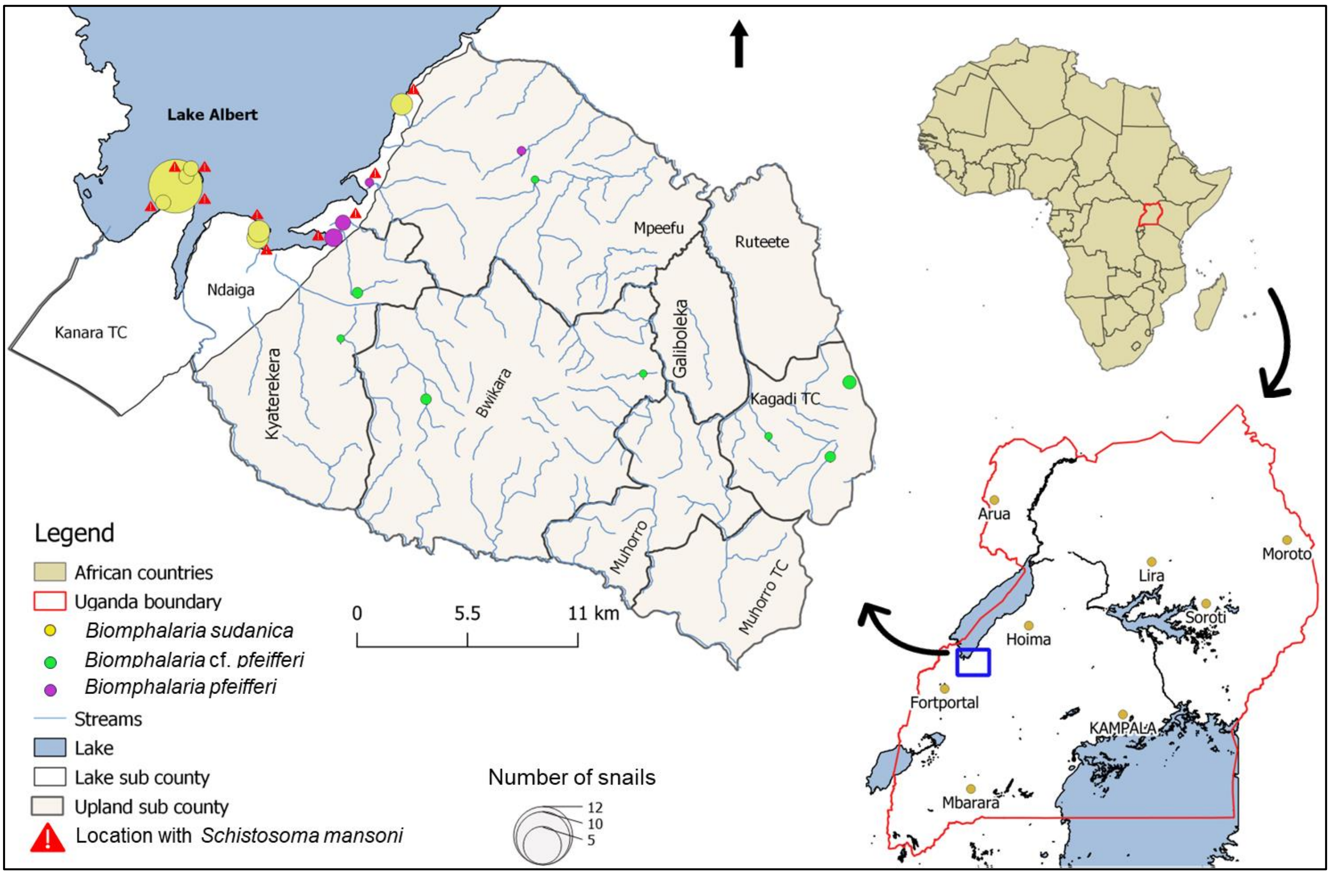

Figure 4. 18: A map of the occurrence of *Biomphalaria* snails infected with *Schistosoma mansoni* and *Schistosoma rodhaini* in the Lake Albert region of Uganda.

Locations that are not indicated to have *S. mansoni* had *S. rodhaini*. Infection status was determined using diagnostic PCR (S. mansoni) and genotyping (for *S. rodhaini*).

For the case of the *Bulinus* snails, of the 1,315 snails exposed to shed cercariae, 67(5.10%) shed cercariae, of which only 23(1.75%) snails shed schistosome cercariae. The highest prevalence of *Schistosoma* cercariae was reported in *B. nasutus productus* (17.53%, n = 97), followed by *B. forskalii* (0.82%, n = 709) while *B. globosus* (n = 416) and *B. truncatus* (n = 4) did not shed any schistosome cercariae. This underscores the importance of *Schistosoma* spp. transmission in the study area. Given that *B. nasutus productus* was present at one site (Tuhumwire) - a community grazing ground, there is urgent need to instate disease control strategies such as regular deworming of the livestock that utilise this ground. The spread of *B. nasutus productus* needs to be closely monitored since it appears to be an important schistosome host. In addition, the snails also hosted other types of trematodes at varying prevalences among *Bulinus* spp. as in indicated in Figure 4.19 A below. In addition, the shedding prevalence was highest in May 2021 as shown in Figure 4.19 B.

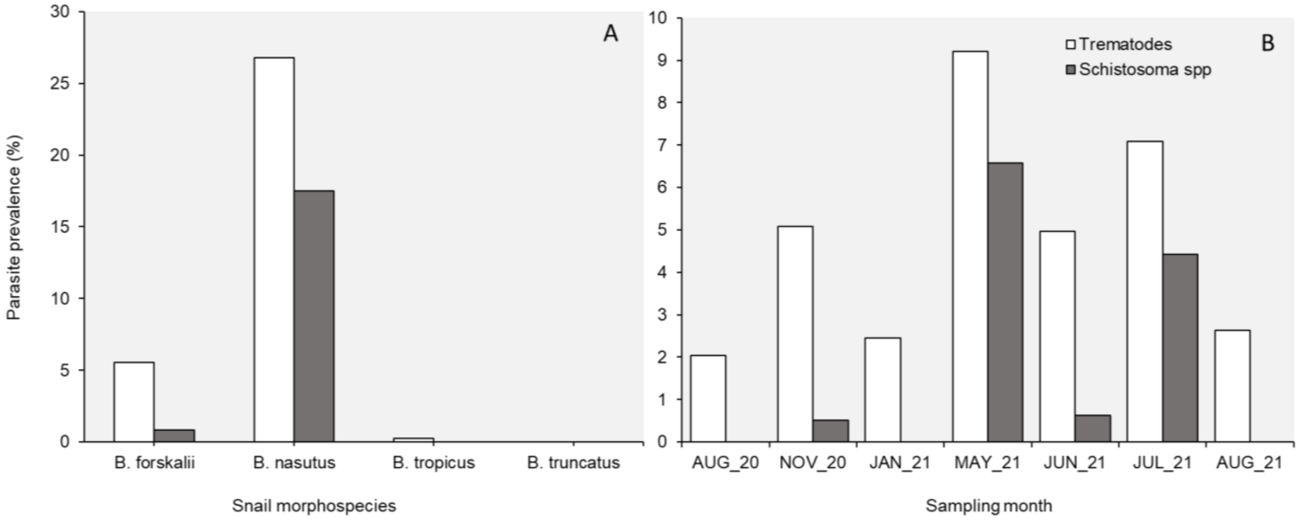

Figure 4. 19: The percentage prevalence of *Schistosoma* and other trematode cercariae among *Bulinus* spp. (A), and the prevalence variance with sampling month (B).

When the prepatent *Bulinus* snails were checked for parasite infection using the RD-PCR technique described above, a similar trend in *Schistosoma* parasite prevalence was obtained, the highest infection with *S. bovis* observed among the *B. nasutus productus* (35.87%, n = 92), followed by *B. forskalii* (09.00%, n = 311), while one snail of *B. globosus* (0.81%, n = 124) was detected to be infected with *S. bovis* as indicated in Table 4.7 below. The proportion of infected snails was significantly higher when detected by diagnostic PCR than the shedding technique ($p < 0.01$). Generally, the *S. bovis* prevalence in the *B. nasutus productus* increased from 0% in April 2021 to 80% in September of the same year, except in August where the prevalence was only 9% (Table 4.7). Increasing parasite prevalence coincides with the rainy season in which the habitat for *B. nasutus productus* floods and possibly increasing contact of the parasite eggs with water inhabited by snails. There was no clear pattern of parasite prevalence among the *B. forskalii* snails. Nevertheless, the highest prevalence was reported in June 2020 (55.56%) and September 2021 (41.67%).

Table 4. 7: The temporal variation in *S. bovis* prevalence among the *Bulinus* spp. collected in the Lake Albert region of Uganda between June 2020 and December 2021. The infection status was determined using the diagnostic PCR approach.

Month	No. of *B. forskalii*	No. with *S. bovis* (%)	No. of *B. n. productus*	No. with *S. bovis* (%)	No. of *B. globosus*	No. with *S. bovis* (%)	No. of *B. truncatus*	No. with *S. bovis* (%)
Jun-20							1	0(0.00)
Jul-20	9	5(55.56)						
Aug-20	4	0(0.00)					1	0(0.00)
Sep-20	13	0(0.00)						
Oct-20	35	0(0.00)	3	0(0.00)				
Nov-20	38	0(0.00)	3	0(0.00)	5	0(0.00)	1	0(0.00)
Dec-20	-	-	-	-	-	-	-	-
Jan-21	6	6(100)			10	0(0.00)		
Feb-21	-	-	-	-	-	-	-	-
Mar-21	-	-	-	-	-	-	-	-
Apr-21	16	0(0.00)	10	0(0.00)	22	0(0.00)	-	-
May-21	48	5(10.42)	27	11(40.74)	19	0(0.00)	6	0(0.00)
Jun-21	17	0(0.00)	10	5(50.00)	11	0(0.00)		
Jul-21	38	1(2.63)	8	6(75.00)	14	0(0.00)		
Aug-21	38	0(0.00)	11	1(9.01)	11	0(0.00)		
Sep-21	12	5(41.67)	10	8(80.00)			1	0(0.00)
Oct-21	27	4(14.81)			12	1(8.33)		
Nov-21	10	2(20.00)	10	2(20.00)	20	0(0.00)		
Dec-21								
TOTAL	**311**	**28(09.00)**	**92**	**33(35.87)**	**124**	**1(0.81)**	**10**	**0(0.00)**

4.2.2 Fasciola parasite prevalence

In this section, the *Fasciola* spp. data presented is based on the shedding technique and may not therefore indicate the true identity of the *Fasciola* spp. since their identification was based on parasite morphology and behaviour (formation of metacercariae). Nevertheless, 2,719 snails were exposed to shed cercariae of which 139(5.11%) shed trematode cercaria. Only eight snails (0.29%) shed encysting cercariae with morphological characteristics of *Fasciola* spp. The

Fasciola shedding snails were sampled in the months of November 2020 and July 2021 as shown in Figure 4.20 below.

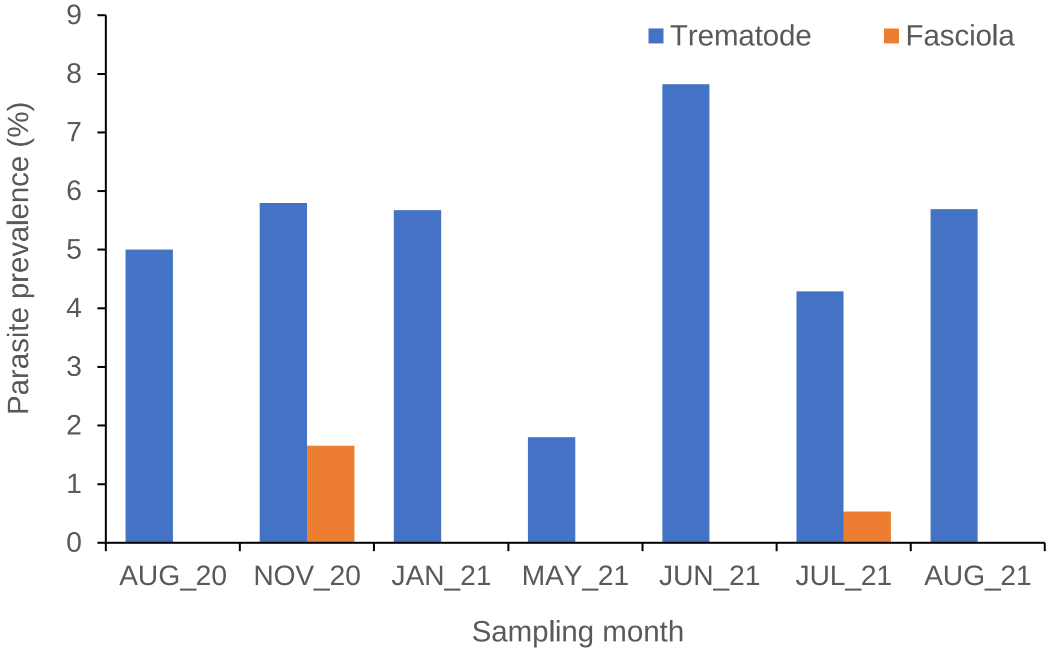

Figure 4. 20: The prevalence of *Fasciola* and other trematode cercaria in *Radix natalensis* collected in the Lake Albert region of Uganda as determined by a one-time shedding approach.

4.3 Physico-chemical parameters and aquatic macrophytes influence on snail distribution

4.3.1 Physico-chemical parameters

The following physicochemical parameters that were measured routinely to constitute abiotic factors in this study include pH, water temperature, conductivity (µS/cm), dissolved oxygen (DO, ppm), total dissolved solids (TDS, ppm), percentage saturation of water with oxygen (%), salinity (PSU), turbidity (FNU) Phosphates (PO_4^{3-}, ppm), and the ammonium ion (NH_4^-, ppm), water resistivity (Ωm). As indicated in Table 4.8 below, the results of the one-way ANOVA test indicated that the parameters varied significantly between site types except for the phosphates ($p<0.05$). For instance, the lake had a slightly alkaline pH (7.810±0.052) while the other site types were slightly acidic with pH ranging from 6.4 to 6.9 ($F = 26.58$, $df = 3$, $p < 0.01$). similarly, the lake had higher temperatures (29.402±0.139 °C, $F = 466.43$, $p < 0.01$), electrical conductivity (626.416±33.36 µS/cm, F = 197.34, p < 0.01), oxygen saturation in the water (43.638±1.784 %, F = 7.379, p < 0.01), TDS (290.592±4.434 ppm, F = 552.207, p < 0.01), and salinity (0.278±0.004 PSU, F = 542.402, p < 0.01). The dispersion indicated is the standard error in the computation of means at a 95% confidence level. On the other hand, the wetlands had higher concentrations of ammonium ions (0.640±0.084 ppm, F = 5.18.858, p < 0.01).

Table 4. 8: The means of the measured physicochemical parameters for the different site types in the Lake Albert region. significant differences between the site types for a parameter are bolded.

	Lake	Spring	Stream	Wetland	P-value
pH	7.810±0.052^{a}	6.431±0.060^{b}	6.873±0.030^{c}	6.819±0.049^{c}	**<0.01**
Oxidative reductive potential, ORP	39.195±4.118^{a}	76.694±3.884^{b}	32.815±2.176^{a,c}	22.281±5.154^{c}	**<0.01**
Temperature (°C)	29.402±0.139^{a}	23.537±0.112^{b}	22.728±0.094^{c}	24.147±0.207^{b,d}	**<0.01**
Conductivity (µS/cm)	626.416±33.360^{a}	162.213±6.495^{b}	208.702±4.493^{b,c}	279.426±11.212^{d}	**<0.01**
Dissolved oxygen (ppm)	3.233±0.111^{a}	3.059±0.107^{a,b}	3.075±0.111^{a}	2.565±0.119^{b}	**0.025**
Dissolved oxygen (%)	43.638±1.784^{a}	36.111±1.571^{b}	36.204±0.930^{b,c}	33.147±1.813^{b,d}	**<0.01**
Total dissolved solids (ppm)	290.592±4.434^{a}	82.975±3.187^{b}	104.402±2.387^{c}	140.564±5.610^{d}	**<0.01**
Phosphates (ppm)	0.323±0.071^{a}	0.684±0.462^{a}	0.559±0.109^{a}	0.417±0.200^{a}	0.697
Ammonium (ppm)	0.375±0.070^{a}	0.114±0.013^{b}	0.286±0.015^{a}	0.640±0.084^{c}	**<0.01**
Salinity (PSU)	0.278±0.004^{a}	0.077±0.003^{b}	0.099±0.002^{c}	0.133±0.005^{d}	**<0.01**
Turbidity (FNU)	67.904±9.886^{a}	44.817±7.770^{a,b}	35.163±3.393^{b}	82.941±8.015^{a,c}	**<0.01**
Resistivity (Ωm)	0.002±0.0000^{a}	0.007±0.0002^{b}	0.006±0.0002^{c}	0.006±0.0006^{c,d}	**<0.01**

For each parameter, the superscript indicates pairwise post hoc comparison whereby the same later indicates no significant difference between the site types while a different letter indicates a significant difference at a 05% level of significance.

When the parameters were correlated with the snail abundance for the three genera, only resistivity had a low positive correlation with the abundance of *Biomphalaria* spp ($r = 0.258$, $p < 0.01$, $n = 1282$). In addition, pH ($\rho = -0.187$, $p < 0.01$), ORP ($\rho = -0.059$, $p = 0.03$), water temperature ($\rho = -0.252$, $p < 0.01$), conductivity ($\rho = -0.265$, $p < 0.01$), DO ($\rho = -0.139$, $p < 0.01$), TDS ($\rho = -0.264$, $p < 0.01$), salinity ($\rho = -0.276$, $p < 0.01$), and turbidity ($\rho = -0.209$, $p < 0.01$) correlated negatively with the abundance of *Biomphalaria* spp., which implies than the *Biomphalaria* spp. abundance reduces when these parameters are increased. Phosphates and ammonium ions had no significant correlation with *Biomphalaria* spp. abundance. At the species level, the trends of the correlation coefficients for *B. pfeifferi* were similar to the general genus level. However, for *B. sudanica*, only pH ($\rho = -0.16$, $p < 0.01$), DO ($\rho = -0.089$, $p < 0.01$), NH_4^+ ($\rho = -0.082$, $p = 0.02$) and turbidity ($\rho = -0.141$, $p < 0.01$) had a significant negative correlation with snail abundance. Therefore, increase in these water physicochemical parameters is associated with a reduction in snail abundance reduction. See Table 4.9 below.

On the other hand, the *Bulinus* spp. snail abundance was positively correlated with pH ($\rho = 0.22$, $p < 0.01$), water temperature ($\rho = 0.31$, $p < 0.01$), conductivity ($\rho = 0.286$, $p < 0.01$), DO saturation ($\rho = 0.105$, $p < 0.01$), salinity ($\rho = 0.284$, $p < 0.01$), and turbidity ($\rho = 0.159$, $p < 0.01$), and negatively correlated with ORP ($\rho = -0.102$, $p < 0.01$) and water resistivity ($\rho = -0.277$, $p < 0.01$). The species level correlation coefficients for *Bulinus* snails are indicated in Table 4.9. Generally, the *Bulinus* spp. displayed site type preferences as described in Figure 4.12 above and the site types displayed variation in the physicochemical parameters. For instance, the lacustrine *B. truncatus* was positively correlated with parameters that are higher in lakes than other stream sites. Similarly, *B. forskalii*, which predominated the wetland site, was correlated with parameters associated with wetland sites. This means that snails prefer to inhabit habitats that provide the best environment for their survival and reproduction. The characteristics of the habitat thus determine the snail populations that inhabit the habitat.

On the other hand, *Radix natalensis* had a low positive correlation with pH ($\rho = 0.073$, $p < 0.01$) and DO ($\rho = 0.114$, $p < 0.01$), but negatively correlated with NH_4^+ ($\rho = -0.14$, $p < 0.01$). All the other measured parameters did not correlate significantly with *Radix natalensis* abundance.

Table 4. 9: correlation of the *Biomphalaria*, *Bulinus* and *Radix* species with measured physicochemical parameters. *Biomphalaria* spp. and *Bulinus* spp. represents the total abundance of the species under each of the genera while individual species abundances are correlated separately

	Biomphalaria spp.	*B. sudanica*	*B. pfeifferi*	*R. natalensis*	*Buinus* spp.	*B. forskalii*	*B. globosus*	*B. truncatus*	*B. nasutus*
pH	-.187**	-.160**	-.041	.072**	.220**	.220**	.053	.090**	-.009
ORP	-.059*	-.013	-.081**	.013	-.102**	-.086**	-.023	-.079**	-.019
Temperature	-.252**	.008	-.294**	-.035	.310**	.277**	.048	.233**	.045
Conductivity	-.265**	-.014	-.281**	-.018	.286**	.282**	.014	.215**	-.047
DO	-.139**	-.089**	-.079**	.114**	.045	.007	.034	.075**	.041
DO saturation	-.136**	-.029	-.135**	.083**	.105**	.044	.069*	.097**	.058*
TDS	-.264**	-.016	-.282**	-.016	.290**	.284**	.017	.219**	-.046
PO4	.059	.065	.003	.046	-.023	-.064	.059	-.086*	-.030
NH4	.026	-.082*	.070*	-.140**	.016	.092**	-.046	-.057	.116**
Salinity	-.276**	-.021	-.288**	-.018	.284**	.284**	.011	.215**	-.040
Turbidity	-.209**	-.141**	-.076**	-.008	.159**	.183**	.020	.020	.138**
Resistivity	.258**	.009	.277**	.015	-.277**	-.274**	-.013	-.214**	.042

**. Correlation is significant at the 0.01 level; *. Correlation is significant at the 0.05 level.

However, when a generalised mixed effects regression analysis was conducted, only pH significantly explained 38.8% of the *Biomphalaria* spp. abundance ($R^2 = 0.388$, $p = 0.007$). The model's intercept, corresponding to pH = 0, is at 57.34 (95% CI [32.03, 82.66], t(735) = 4.45, $p < .001$). Within this model: The effect of pH is statistically significant and negative (beta = -4.88, 95% CI [-8.42, -1.34], t(735) = -2.71, p = 0.007; Std. beta = -0.10, 95% CI [-0.17, -0.03]). The model estimates of -4.88 indicates that a unit increase in pH results in a reduction in the *Biomphalaria* spp. abundance by about five snails as illustrated in Figure 4.21 below. All the other measured physicochemical parameters did not significantly predict changes in the *Biomphalaria* spp. abundance (see appendix 14-16).

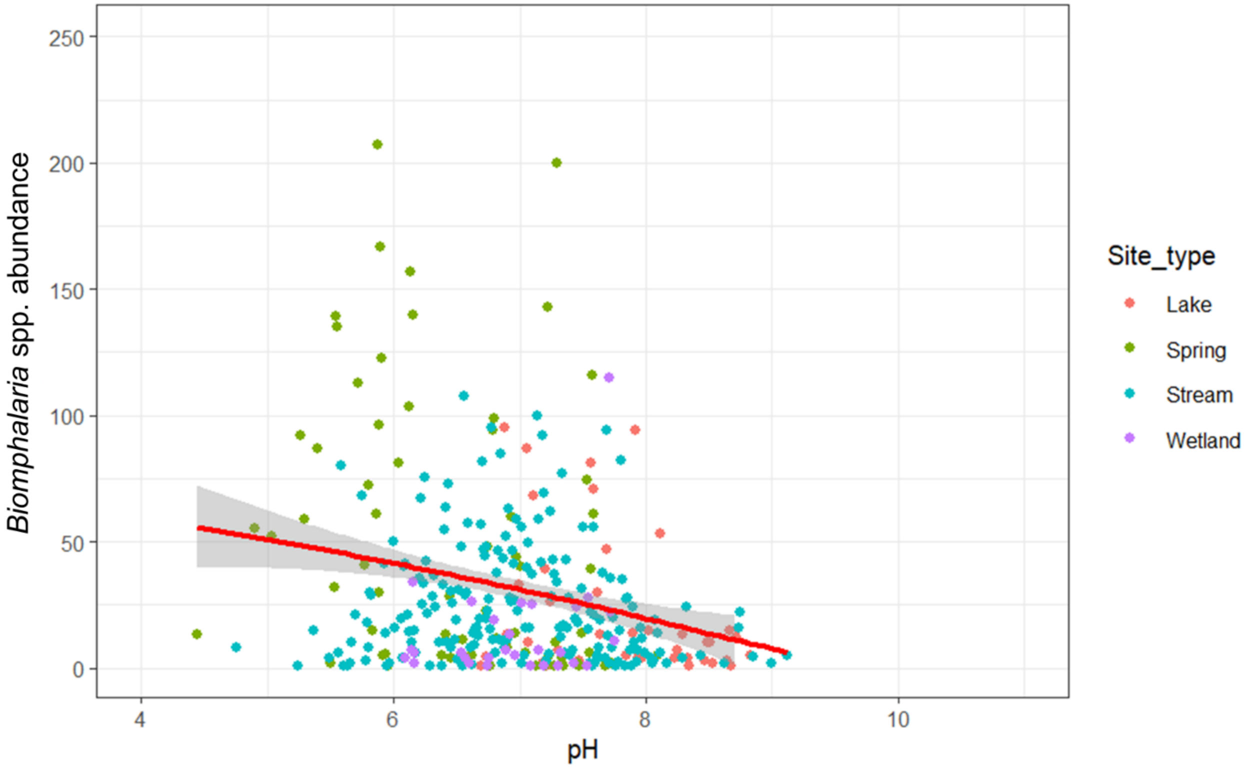

Figure 4. 21: Variation in *Biomphalaria* spp. abundance with pH across the site types

However, unlike *Biomphalaria* spp. and *Radix natalensis*, *Bulinus* spp. abundance was significantly predicted by site type. From the generalised linear mixed effects model summarised in Table 4.10 below, a unit increase in *Bulinus* snail abundance at the lake results in abundance increase of about eight and nine snails increase when site type changes to stream and wetland respectively. In other words, stream sites were eight times more likely to have *Bulinus* snails (p = 0.029) while wetland sites were about 10 times likely to have *Bulinus* snails (p = 0.031) compared to lake sites. Spring sites were likely to have less snails than lake sites but the difference was not significant (p = 0.400). Overall, site type explained about 49.7% of the variability in the snail abundance at any given site as indicated by the conditional R^2 value of 0.497. The marginal R^2 value represents the proportion of the variability that is explained by the random variable (site name), which explains the structure of data, i.e., repeated counts of snails at the same location along a temporal gradient.

Table 4. 10: The mixed effect model estimates indicating the that site type significantly predicts *Bulinus* spp. abundance

		***Bulinus* spp. abundance**		
Predictors		*Estimates*	*CI*	*p*
(Intercept)		0.20	-5.78 – 6.18	0.949
Site type (Ref. Lake)				
	Spring	-3.40	-11.33 – 4.53	0.400
	Stream	7.48	0.77 – 14.18	**0.029**
	Wetland	9.91	0.93 – 18.89	**0.031**
Random Effects σ^2		198.79		
τ_{00} Site name		174.71		
ICC*		0.47		
Observations		755		
Marginal R^2 / Conditional R^2		0.055 / 0.497		

* ICC denotes the Intraclass Correlation Coefficient (ICC) - the proportion of total variability in abundance that is a result of variability between sites (τ_{00}). The ICC is derived from $\frac{\tau_{00}}{\tau_{00}+\sigma^2}$

4.3.2 Aquatic macrophytes and snail abundance

A total of 40 macrophyte species were collected at the snail sampling site in the Lake Albert region. A full list of the macrophytes is attached in Appendix 23

The results of the multiple linear regression indicated that at Lake Albert, the predictors of *Biomphalaria* species density were the plant species *Ipomea aquatica* ($\beta = 0.542$, $p < 0.001$), *Potamogeton schweinfurthii* ($\beta = 0.416$, $p = 0.004$), *Pistia stratiotes* ($\beta = 0.346$, $p = 0.014$) and *Vallisneria spiralis* ($\beta = 0.271$, $p = 0.048$). The best fitting model indicated that the selected plant species explained 56% of the variation in *Biomphalaria* spp. abundance at the lake sites (adjusted $R^2 = 0.56$; $p < 0.01$). This implies that other factors than macrophyte species contribute to the presence of snails at a site. When the model was not adjusted for overfitting resulting from model complexity, the coefficient of determination, i.e., the explanatory power (R^2) was 62.5%. When simpler models were considered by removing a plant species at a time from the best fitting, the explanatory power reduced to 49.8% (adjusted R^2) without *V. spiralis*, to 40.8% without *V. spiralis* and *P. stratiotes*, and to 26.1% with only *I. aquatica* (i.e., without *V. spiralis*, *P. stratiotes* and *P. schweinfurthii*). From this analysis, the most important macrophyte predictors of *Biomphamaria* snail abundance at Lake Albert are: *Ipomea aquatoca* > *Potamogeton. schweinfurthii* > *Pistia stratiotes* > *Vallisneria spiralis.* The regression equation of the best model, which follows the form $y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + C$, is displayed below. The constant is the standard error of the model estimate.

Biomphalaria abundance = 0.542**Ipomea aquatica* + 0.416**Potamogeton schweinfurthii* +
0.346**Pistia stratiotes* + 0.271**Vallisneria spiralis* + 20.346

On the other hand, when the macrophyte data was considered for other site types, there was no plant species that predicted the presence of *Biomphalaria* spp ($p > 0.05$). In other words, macrophyte species was not an important factor for prediction of snail presence.

For *Bulinus* snails, *B. globosus* was significantly explained by presence of *Cynodon dactylon* with 64.1% increase in snail abundance by increase in *C. dactylon* density ($R^2 = 0.641$, $p < 0.01$). It was not possible to analyse the *B. nasutus productus* since it occurred at only one site and could not offer enough data for replication. Similarly, the very few *B. truncatus* snails could not easily be associated to macrophyte diversity.

On the other hand, *B. forskalii* abundance increased with increase in *Azolla filiculoides* ($\beta = 0.837$, $p < 0.001$), *P. stratiotes* ($\beta = 0.424$, $p < 0.001$) and *Leersia hexandra* ($\beta = 0.404$, $p < 0.001$), but decreased with increase in *Cyperus digitatus* ($\beta = -0.032$, $p = 0.03$) despite the effect size being small. Overall, the best model indicated that a notable 99% of the variability in the *B. forskalii* abundance was explained by the macrophyte species listed above (adjusted $R^2 = 0.991$, $p = 0.03$, $F = 1216.921$). This is contributed by 65.7% explanation by *A. filiculoides* (adjusted $R^2 = 0.657$, $p < 0.01$), and increases by 16.5% when *P. stratiotes* (adjusted $R^2 = 0.822$, $p < 0.01$), further increases by additional 16.1% when *L. Hexandra* is added (adjusted $R^2 = 0.990$, $p < 0.01$), and finally increased marginally by 0.1% when *C. digitatus* was added (adjusted $R^2 = 0.991$, $p < 0.01$).

Therefore, the regression equation of the best model for abundance of *B. forskalii* in the form $y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + C$ is displayed below where x represents the plant species, β is the regression coefficient and C is the standard error of the model estimate.

Bulinus forskalii abundance = 0.837**Azolla filiculoides* + 0.424* *Pistia stratiotes* +
0.404**Leersia hexandra* - 0.032**Cyperus digitatus* + 0.34

Finally, *Radix natalensis* abundance increase was also significantly explained by in increase in macrophyte density of the following plants: *P. stratiotes* ($\beta = 0.658$, $p < 0.01$), *L. Hexandra* ($\beta = 0.392$, $p < 0.01$), *Utricularia stellaris* ($\beta = 0.245$, $p = 0.014$) and *C. dactylon* ($\beta = 0.197$, $p = 0.044$). With *R. natalensis*, *P. stratiotes* was the most important predictor explaining 38.2% of the variability in the abundance of the snails, next *L. hexandra* contributed an additional 13.4% explanation to the variability, while *U. stellaris* and *C. dactylon* contribute 6.4% and 3.9% respectively ($p < 0.05$) and indicated by the change in the adjusted coefficients of determination. Similarly, the best fitting model for _R. natalensis_ is displayed below:

Radix natalensis abundance = 0.658* _P. stratiotes_ + 0.392* _L. hexandra_ + 0.245*_U. stellaris_ +
0.032*_C. digitatus_ + 0.197*_C. dactylon_ + 16.54

4.4 Citizen science and snail monitoring

In this section, data collected by the PhD researcher is referred to as PhD researcher data while the data collected by trained community members is referred to as citizen scientists (CSs) collected data. The PhD researcher collected a total of 1,382 data reports, while the CSs collected 4,034 reports over the same period of 20 months. After filtering for differences in sampling dates, errors in snail identification, and sampling time, the datasets from the PhD researcher malacologist and CSs were merged. This resulted in a total of 907 paired reports on _Biomphalaria_ spp., 892 on _Bulinus_ spp. and 898 on _Radix_ sp. It is worth noting that out of the 73 sites observed, 72 sites (98.6%) contained at least one of the three snail genera during the observed period.

4.4.1 Snail presence and absence

Similar to the PhD researcher data, _Biomphalaria_ snails were the most abundant and widely distributed among the three genera, being recorded at 68 (93%) sites (Figure 4.22).

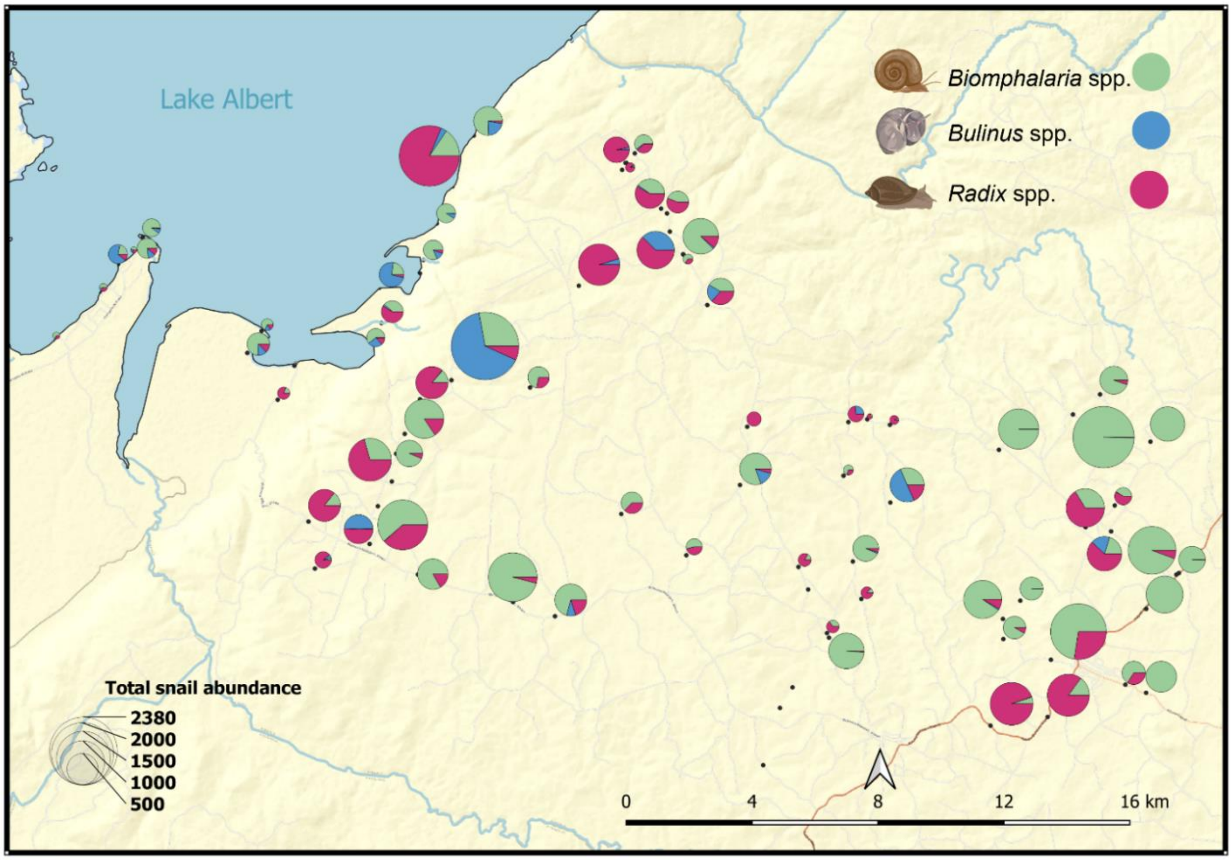

Figure 4. 22: Map of the study area showing total abundance (PhD researcher data 06/2020 – 04/2022) of the studied snail genera in each water contact site, black points indicate locations without snails.

In approximately 76% of the paired reports on *Biomphalaria*, there was a binary agreement in snail occupancy at a site (presence/absence) between the PhD researcher and CSs. However, the frequency of agreement varied across different site types, as depicted in Figure 4.23A. The highest agreement was observed at wetland sites (86%), followed by streams (78%), and spring wells (72%), while lake sites had the lowest agreement (60%; N = 907). Overall, the true positive rate (sensitivity) was determined to be 74.6%.

Bulinus snails exhibit lower abundance and a patchy distribution within the study area. Approximately 86% of the paired reports on *Bulinus* showed binary agreement between the CSs and the PhD researcher. However, the sensitivity of *Bulinus* snail detection by the CSs was found to be low (43.4%; Figure 4.23B). Conversely, the *Radix* group displayed the lowest binary agreement (70%) but with a moderate sensitivity (59.5%; see Figure 4.23C).

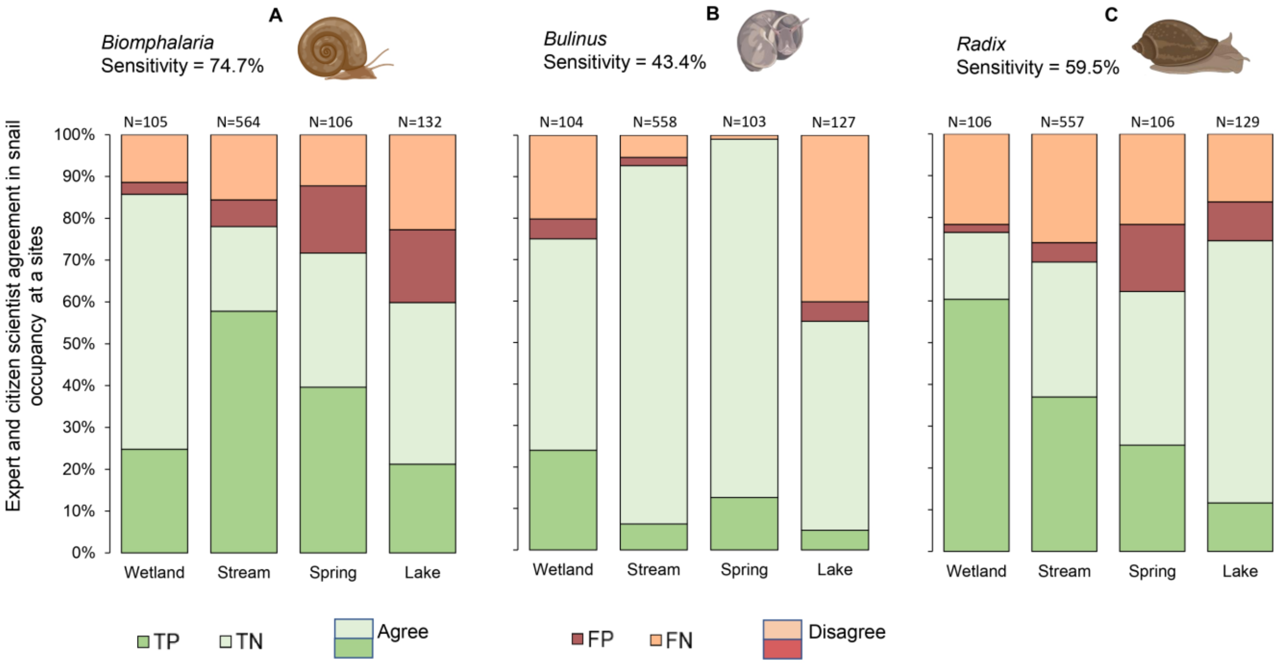

Figure 4. 23: Agreement between the PhD researcher and citizen scientists collected data on the presence/absence of *Biomphalaria* (A), *Bulinus* (B) and *Radix* (C) snails at different site types.

The GLMER model for *Biomphalaria* was defined as $P(Y = 1) \sim SN_{i,j} + ST_j + DD_{i,j} + 1 \mid ID_j/Site_j$ (refer to Figure 4.24A-C), with a probability of binary agreement, P(Y = 1), equal to 0.76. The fitted model indicated a significant prediction of agreement by the independent variables (C = 0.795, Somers' D_{xy} = 0.590; N = 907), as evidenced by the reduction in deviance compared to the random effects model (chi-square (5) = 35.5; $p < 0.01$).

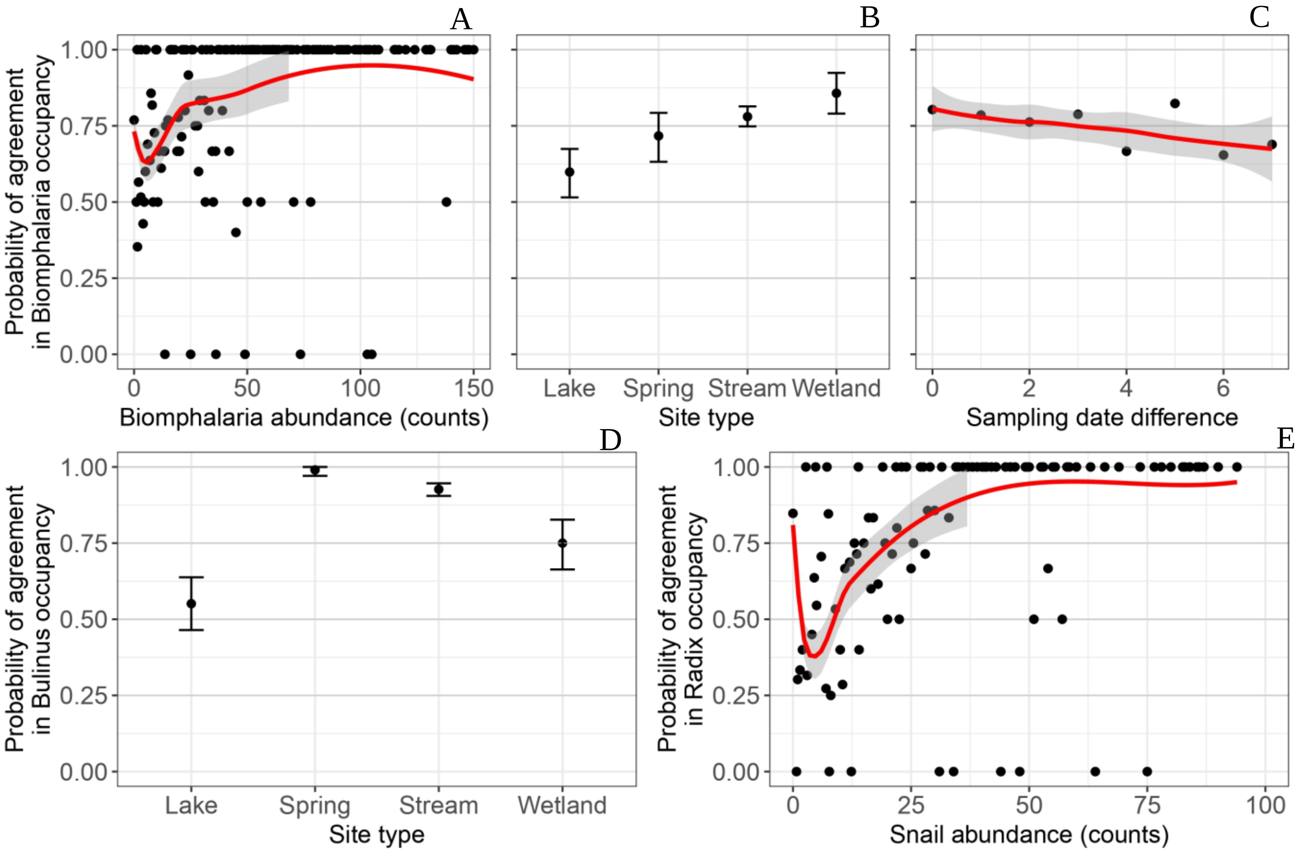

Figure 4. 24: Variables influencing observed mean probabilities of agreement in snail presence/absence

Biomphalaria spp. presence/absence by snail abundance (A), site type (B) and sampling date difference (C); in *Bulinus* spp. presence/absence by site type (D); in *Radix* presence/absence by snail abundance (E). The 95% confidence level is indicated by the grey limits on the fitted red line for continuous data and by the error bars for site type.

In general, the likelihood of binary agreement showed a significant increase as the abundance of *Biomphalaria* snails increased (odds ratio [OR] = 1.02; $p < 0.001$), particularly in wetland sites (OR = 4.30; $p = 0.002$) or stream sites (OR = 2.33, p = 0.008) (see Figure 4.24A and B). However, the binary agreement significantly decreased with an increase in the difference in sampling dates between the PhD researcher and the CSs (OR = 0.91; $p = 0.02$; Figure 4.24C), indicating that disagreement is at least partially driven by differences in sampling date, rather than errors committed by the sampler(s). The model diagnostics are indicated in Table 4.11 below.

When fitting a GLMER model for agreement in *Bulinus* spp. presence/absence, only site type significantly explained the agreement (Figure 2.20D above), with a probability, P(Y = 1), equal to 0.86. Spring sites (OR = 104.7; p <0.001), stream sites (OR = 11.8; p <0.001) and wetland sites (OR = 3.4; p = 0.032) exhibit a higher likelihood of achieving binary agreement compared to lake sites (Figure 2.24D above). The fitted model demonstrated a significant improvement over the reference random effects model when site type was included as a fixed variable (chi-square (3) = 42.3; $p < 0.01$; C = 0.889; D_{xy} = 0.778; N = 892), see Table 4.11 below.

For *Radix*, the agreement in presence/absence was determined by the formula: $P(Y=1) \sim SN_{i,j} + 1 \mid ID_j/Site_j$, with a probability of binary agreement, P(Y=1), equal to 0.70. The probability of agreement between the CSs and the PhD researcher increased significantly with an increase in *Radix* sp. abundance (Figure 2.24D above; OR = 1.01; p = 0.031). Despite the simplicity of the model with only one fixed variable, the explanatory power of snail abundance was relatively high (C = 0.780; D_{xy} = 0.560; N = 867), see Table 4.11 below for model diagnostics.

Table 4. 11: *Biomphalaria*, *Bulinus* and *Radix* predictors of agreement in snail presence/absence between the citizen scientists and the PhD researcher, and their metrics. Ref. stands for reference, CI for confidence interval, and significant values are in bold.

		Agreement				
Snail genus	**Predictors, x_{ij}**	Effect size, β (95% CI)	Odds ratios (OR)	95% CI		*p*
				Lower	upper	
Biomphalaria	Intercept	0.57 (-0.04, 1.18)	1.76	0.96	3.25	0.068
	Snail abundance	0.02 (0.008, 0.02)	1.02	1.01	1.02	**<0.001**
	Sampling date difference	-0.10 (-0.18, -0.02)	0.91	0.83	0.98	**0.020**
	Site type (Ref. Lake)					
	Spring	0.49 (-0.38, 1.36)	1.63	0.68	3.89	0.272
	Stream	0.84 (0.22, 1.47)	2.33	1.24	4.35	**0.008**
	Wetland	1.46 (0.54, 2.38)	4.30	1.72	10.76	**0.002**
Bulinus	Intercept	0.39 (-0.33, 1.12)	1.48	0.72	3.05	0.284
	Site type (Ref. Lake)					
	Spring	4.65 (2.37, 6.93)	104.66	10.72	1021.46	**<0.001**
	Stream	2.47 (1.59, 3.35)	11.83	4.93	28.39	**<0.001**
	Wetland	1.21 (0.11, 2.32)	3.36	1.11	10.17	**0.032**
Radix	Intercept	0.85 (0.49, 1.20)	2.33	1.63	3.33	**<0.001**
	Snail abundance	0.0085 (0.00078, 0.02)	1.01	1.00	1.02	**0.031**

Other factors such as site type and the difference in sampling dates between the PhD researcher and the CSs did not significantly explain the binary agreement in detecting *Radix* snail presence/absence at a site. The order in which the PhD researcher and CSs conducted the sampling (i.e., whether the PhD researcher sampled before, after, or on the same day as the CS) was not a significant predictor of binary agreement for all three genera ($p > 0.05$), suggesting that the PhD researcher's snail removal had minimal impact on snail presence/absence (Figure 4.25). It was hypothesised that if snail removal impacted the extent of agreement between the PhD researcher and CS data, agreement would be higher if the CS sampled before the PhD researcher, more so on same date. Similarly, the effect of snail removal by the PhD researcher was expected to decrease with time due to migration. Thus, “Same” stands for the PhD researcher and CS having sampled a site on the same day, “Before” when the PhD researcher sampled before the CS and “After” when the PhD researcher sampled after the CS. If the sampling date difference was between one and three, it was considered “Close” (grey error bars) and if the difference was between four and six days, it was considered “Apart” (black error bars). Except for *Biomphalaria*, the difference in sampling days did not explain significant agreement/disagreement in snail presence/absence. Across all the genera and site types, the effect of snail removal by the PhD researcher was not significant (X^2 (2) = (2.3, 2.1 & 2.5) $p > 0.05$, for *Biomphalaria, Bulinus* and *Radix* respectively) when the PhD researcher sampled before the CS. Moreover, the reduction in model deviance (chi-square) when including sampling order was small, ranging from 2.1 to 2.7 and statistically insignificant.

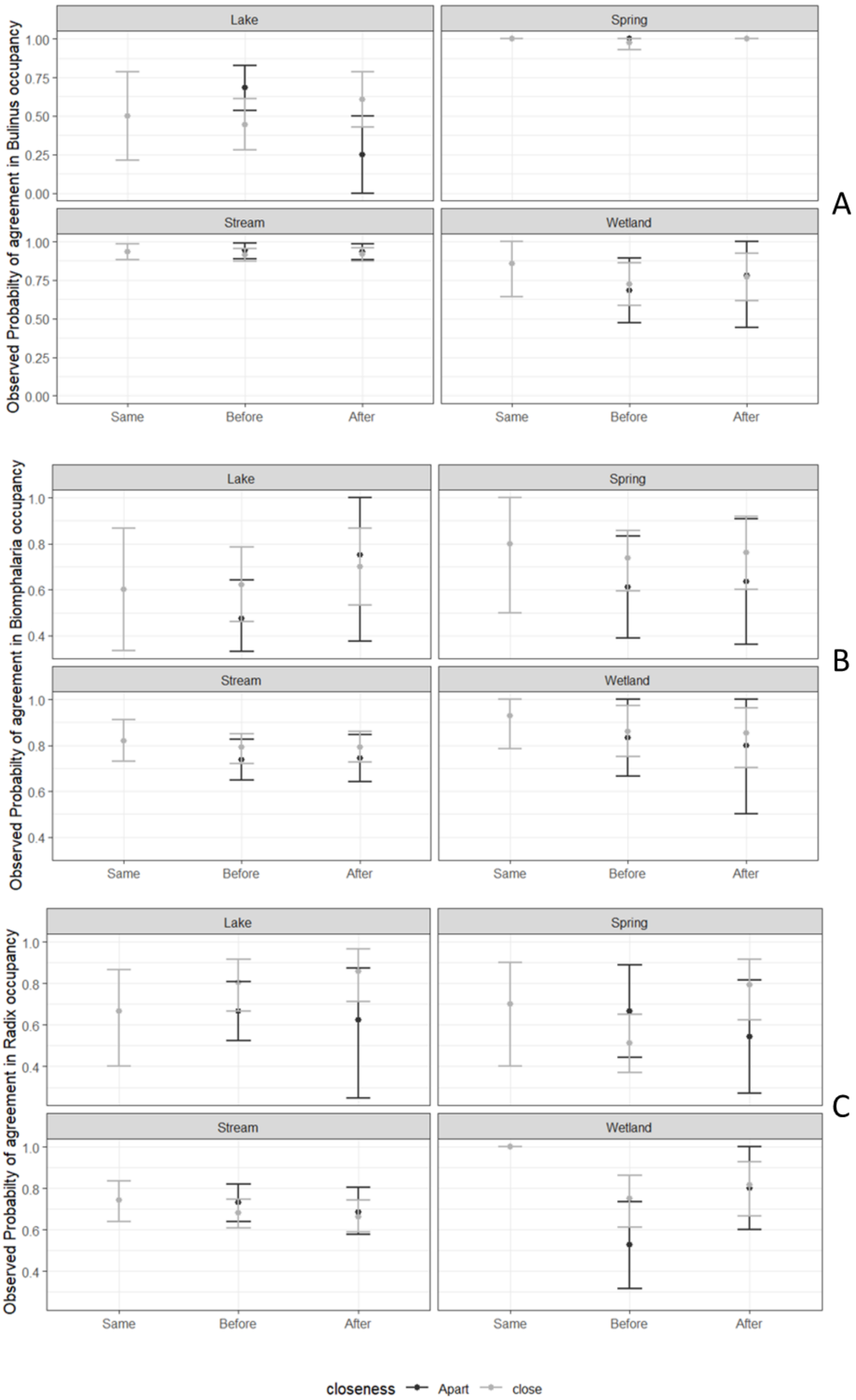
Observed Probabilty of agreement in Bulinus occupancy
Lake
Spring
Stream
Wetland
1.00
0.75
0.50
0.25
0.00
Same
Before
After
A
Observed Probabilty of agreement in Biomphalaria occupancy
1.0
0.8
0.6
0.4
B
Observed Probabilty of agreement in Radix occupancy
C
closeness
Apart
close

Figure 4. 25: Variation in the degree of agreement between the citizen scientists and the PhD researcher if the PhD researcher sampled before, on the same date or after the citizens (5A)-*Biomphalaria*, (B)-*Bulinus* and (C)- *Radix*

Furthermore, the effect of snail replacement by the PhD researcher in the last four months of snail sampling on the binary agreement between CS and PhD researcher data could not conclusively be determined. The agreement decreased for *Biomphalaria*, remained the same for *Bulinus* and increased for *Radix* (Figure 4.26). For the three genera, the probabilities of agreement are statistically different with $p<0.05$. If snail removal changes snail population dynamics, the probability of agreement was expected to be lower when there was snail removal. However, except for *Radix* snails, the probability of was higher in the period when there was snail removal, contrary to our expectation. There was no clear pattern in agreement as a result of snail removal implying no clear influence of the snail removal by the PhD researcher on agreement in snail presence/absence.

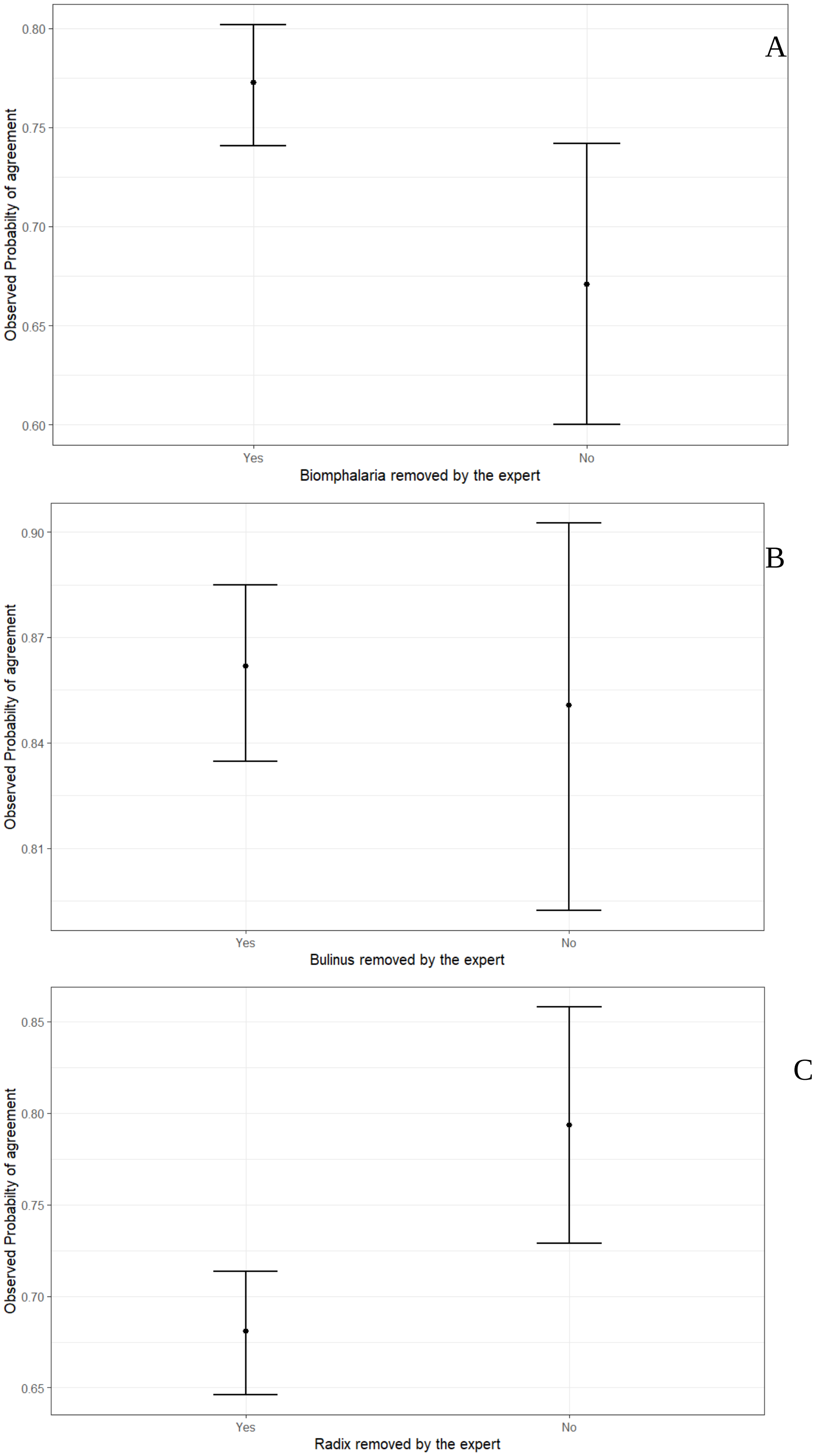

Figure 4. 26: Observed probability of binary agreement (presence/absence) of *Biomphalaria* (A), *Bulinus* (B) and *Radix* (C) compared in the period when the PhD researcher removed snails from a site without replacement (16 months) with when the PhD researcher replaced the snails (4 months).

4.4.2 Snail Abundance

The total number of true positives cases (TP) were 422 for *Biomphalaria* spp., 79 for *Bulinus* spp., and 313 for *Radix*. *Biomphalaria* snails exhibited the highest accumulated abundance (Figure 4.27A), while *Bulinus* snails had the lowest abundance. Furthermore, the PhD researcher consistently reported tended higher abundance compared to the CSs for all snail genera. However, there appears to be a general agreement between the CSs and the PhD researcher regarding temporal trends, with minor variations observed (Figure 4.27A). Additionally, when comparing the snail abundance data between CSs and the PhD researcher, the relative dominance of each genus by site type remained consistent (Figure 4.27B).

To visually assess the agreement in rankings across time and space, Figure 4.27C illustrates the overlap in the top 15 sites (ranked by abundance) for *Biomphalaria* spp. in all comparisons of PhD researcher and CS data (TP, TN, FP, FN). All months but one exhibit more than 75% of concordance, with an average concordance of 84.1%. Additionally, the refresher training conducted in January 2021 (Figure 4.27D) resulted in significantly higher agreement in the subsequent months (t = -2.59; p = 0.023; df = 12.739).

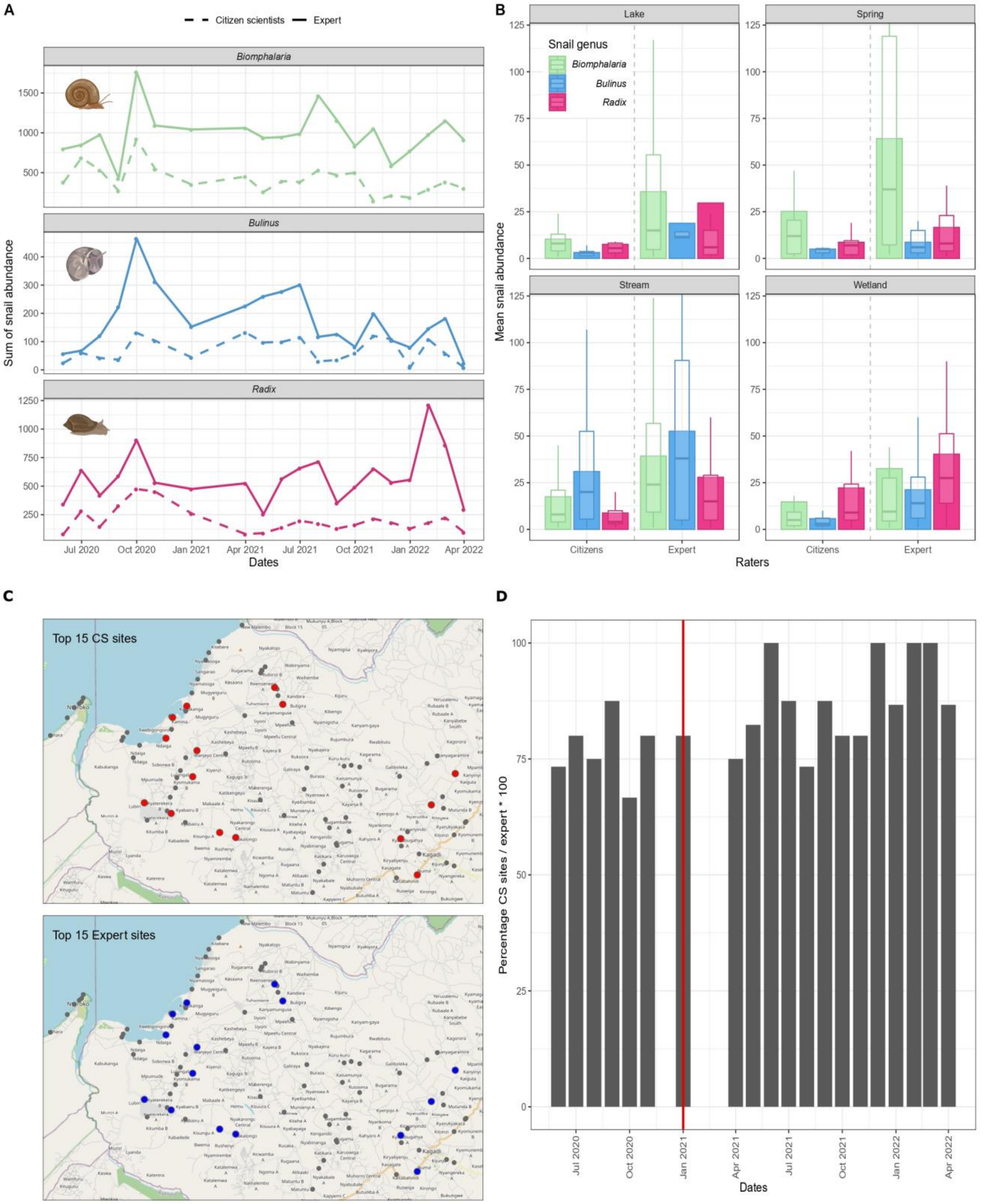

Figure 4. 27: Spatial and temporal trends comparison of snail abundance reported by citizen scientists and PhD researcher.

Sum of snail abundance over time per type of rater (A). Mean snail abundance per site type and type of rater (limit of y-axis has been set to 120) (B). For those instances where paired reports are available for more than 40 sites, agreement was considered in the top 15 locations in terms of abundance. Top 15 locations with the highest abundance of *Biomphalaria* spp. in February 2022 (citizen reports on the top, PhD researcher on the bottom, results from the other months showed similar trends) (C). Percentage of concordance between top 15 sites of *Biomphalaria* spp. abundance reported by raters over time, the red line indicates the refresher.

Regarding the assessment of raters' reliability (Table 4.12), the consistency results (Kendall's Tau-b) displayed a constantly significant correlation coefficient for stream and wetland sites across all three genera. Among the snail genera, *Bulinus* presented the highest values of Kendall's Tau-b, implying strong relationships between the ranking of its abundance reported by the PhD researcher and the CSs at each site type, particularly at lake and stream sites. However, it should be noted that there were limited TP observations around the lake (n=6). *Biomphalaria* and *Radix* snail abundance had the highest values of consistency among raters at wetland sites (0.541 and 0.381, respectively). For *Bulinus*, the lake site type had the highest Kendall's Tau-b but was not considered due to the small sample size (n=6). The second-highest consistency for *Bulinus* corresponded to the stream site type where snails from this genus are more abundant (0.637).

In terms of numerical agreement (Krippendorff's α), positive coefficients were observed for spring, stream and wetland site types, indicating a positive relationship between the snail abundance reported by CSs and the PhD researcher. The stream site type exhibited the highest numerical agreement for *Bulinus* spp. (0.612), while the wetland site type showed the highest numerical agreement for *Biomphalaria* spp. (0.457). Although the values of the numerical agreement are lower than the ones of consistency, this outcome was expected since the numerical agreement metric is stricter than the one of consistency.

Table 4. 12**:** Raters' reliability assessment: consistency and agreement of snail abundance reported by citizens scientists and PhD researcher

	Biomphalaria spp.				*Bulinus* spp.				*Radix* sp.			
		Cons		Agree		Cons		Agree		Cons		Agree
Site	N	K. Tau-b	*p*	K. α	N	K. Tau-b	*p*	K. α	N	K. Tau-b	*p*	K. α
Lake	28	-0.15	0.27	-0.10	6	0.74	0.05	-0.12	16	0.23	0.28	0.18
Spring	42	0.41	<.01	0.27	13	0.34	0.12	0.27	27	0.11	0.45	0.06
Stream	326	0.38	<.01	0.19	35	0.64	<.01	0.61	206	0.30	<.01	0.08
Wetland	26	0.54	<.01	0.46	25	0.49	<.01	0.06	64	0.38	<.01	0.25

N is the sample size; Cons is consistency; K. Tau-b is Kendall's Tau-b; Agree is agreement and K. α is Krippendorff's alpha. Grey cells were discarded due to a small sample size.

In addition, the factors influencing the disparity between snail abundance reported by the PhD researcher and the CSs were analysed, and none of the variables reached statistical significance ($p<0.05$). See Table 4.13-14 below. In this analysis, two scenarios were considered: case (a) when snail abundance reported by PhD researcher > snail abundance reported by CS, and it was observed that for *Biomphalaria* and *Radix* snails the predictor: scaled date is statistically significant ($p<0.05$) with coefficients with values close to zero (Table 4.13). In the case of *Bulinus* spp., the result of the GLMM was a singular fit related to overfitting due to the complex random effect structure. Since the random effect structure is a characteristic of the sampling design, this configuration could not be altered, hence the results of *Bulinus* spp. were not included.

Table 4. 13: Generalized linear mixed model output – case A: snail abundance reported by PhD researcher > snail abundance reported by CS

	Biomphalaria **spp.**			*Radix* **sp.**		
Predictors	***Estimates***	***CI***	***p***	***Estimates***	***CI***	***p***
(Intercept)	2.59	2.01 – 3.18	**<0.001**	1.52	0.97 – 2.07	**<0.001**
Sampling date difference	0.01	-0.04 – 0.06	0.667	-0.01	-0.05 – 0.04	0.788
Date	0.00	0.00 – 0.00	**0.001**	0.00	0.00 – 0.00	**0.003**
Site type (ref. Lake)						
Spring	0.07	-0.66 – 0.81	0.841	0.41	-0.26 – 1.08	0.231
Stream	-0.38	-0.93 – 0.18	0.182	0.41	-0.11 – 0.94	0.121
Wetland	-0.79	-1.53 – -0.05	**0.036**	0.95	0.33 – 1.57	**0.003**
Observations	470			440		
Marginal R^2	0.051			0.055		
Conditional R^2	0.343			0.378		

For case (b), when snail abundance reported PhD researcher < CS snails, none of the variable was statistically significance ($p<0.05$) (Table 4.14). In addition, the results for *Radix* sp., indicated a singular fit related to overfitting due to the complex random effect structure. Since the random effect structure is a characteristic of the sampling design, this configuration could not be altered, hence the results of *Radix* sp. are not included in Table 4.14.

Table 4. 14: Generalized linear mixed model outputs – case B: snail abundance reported by PhD researcher < CS

	***Biomphalaria* spp.**			***Bulinus* spp.**		
Predictors	**Estimates**	**CI**	***p***	**Estimates**	**CI**	***p***
(Intercept)	1.84	1.24 – 2.43	**<0.001**	0.29	-0.84 – 1.41	0.61
Sampling date difference	-0.02	-0.08 – 0.04	0.568	0.12	-0.04 – 0.28	0.13
Date	0	-0.00 – 0.00	0.74	0	-0.00 – 0.00	0.39
Site type (ref. Lake)						
Spring	0.44	-0.33 – 1.20	0.261	0.95	-0.63 – 2.53	0.23
Stream	-0.01	-0.58 – 0.56	0.975	0.72	-0.25 – 1.69	0.14
Wetland	-0.25	-1.13 – 0.63	0.57	0.23	-0.98 – 1.43	0.7
Observations			165			35
Marginal R^2	0.032			0.141		
Conditional R^2	0.403			0.512		

CHAPTER FIVE

5.0 DISCUSSION OF RESULTS

5.1 Diversity, abundance and temporal distribution of snail species

This study aimed at understanding the diversity of snail that are intermediate hosts of *Schistosoma* spp. and *Fasciola* spp. with their distribution in space and time put into perspective. In this section, the distribution of snails in the genera *Biomphalaria, Bulinus* and *Radix* were collected comparing two geographical contexts: 1) the lowland lake and its immediate surroundings, 2) the upload streams, wetland, and artificial spring wells.

5.1.1 *Biomphalaria* species Abundance, diversity and Distribution

Morphological, molecular and phylogenetic analysis indicated that indicated that three well-resolved species, including *B. sudanica, B. pfeifferi,* and *B. stanleyi,* and two unresolved species, i.e., *B.* cf. *pfeifferi* and *Biomphalaria* sp. were present in the Lake Albert region. The single snail specimen collected from Lake Albert in this study was identified as *Biomphalaria* sp. Despite being 99.7% identical to the GenBank sequence DQ084836 identified as *B. smithi* (Jorgensen et al., 2007) and 99.3% to the *B. alexandrina* GenBank sequence KF412766 (Grabner et al., 2014). The snail shell morphology did not conform to either *B. smithi* or *B. alexandrina* thus precluding conclusive species identification.

Similarly, the *B.* cf. *pfeifferi* reported in this study is similar to that recorded by Jorgensen et al. (2007) as '*Biomphalaria pfeifferi*' due to *B. pfeifferi*-like morphology. It was observed that, from the haplotype network analysis of the partial *COX1* gene, there were up to 29 mutations between the *B. pfeifferi* proper and *B.* cf. *pfeifferi* resulting in p-distance ranging from 5.47 to 6.35% (Fig. 4). Interestingly, the p-distance between *B. pfeifferi* and *B.* cf. *pfeifferi* was larger than the p-distance between *B. sudanica* and *B.* cf *pfeifferi* (ranging between 1.2-2.5%). Therefore, it is likely that *B.* cf.

pfeifferi may be a different species altogether or a subspecies closely related to *B. sudanica*. Considering a threshold of a p-distance of approximately 1% mean variability within populations of a species (Kartavtsev, 2011), *B.* cf. *pfeifferi* is neither a morphotype of *B. sudanica* nor *B. pfeifferi* but may be classified as a subspecies recently evolved from *B. sudanica*. The p-distances greater than 5% indicate a more distant relationship with *B. pfeifferi* proper (Kartavtsev, 2011). Nevertheless, *B. sudanica* and *B.* cf. *pfeifferi* were further grouped along with *B. stanleyi*, *B. smithi* and *B. choanomphala* as the Nilotic species complex with interspecific p-distances ranging between 3.7 and 7.3% compared to *B. pfeifferi* proper (Jorgensen et al., 2007). Therefore, there is need for further studies to untangle the *Biomphalaria* species classification in the Lake Albert region.

In this study, *B. pfeifferi* and *B. sudanica* were present in both lake and upland water bodies, while *B.* cf. *pfeifferi* was predominantly non-lacustrine. A recent study by Andrus et al. (2023) reported the presence of the same species (*B. pfeifferi*, *B. sudanica*, *B. stanleyi*) in Lake Albert, except for *Biomphalaria* sp. and *B.* cf. *pfeifferi*, which were only found in this study. In this study, we did not find *B. choanomphala*, which is in agreement with recent studies by Adriko et al. (2013), Andrus et al. (2023) and Levitz et al. (2013), although Plam et al. (2008) did report this species in the lake. Overall, the snail species composition of Lake Albert provides an opportunity for further research into their genetic and ecological dynamics as previously noted by (Jorgensen et al., 2007). In addition, presence of *Biomphalaria* sp. reported for the first time in Lake Albert by this study confirms the need for regular update of the snail database in the region. The role of *Biomphalaria* sp. in trematode transmission could not be ascertained due to very low numbers (only one specimen). In addition, there is a need for extensive deep-water studies on Lake Albert to ascertain the true extent of the biodiversity in the lake.

Surprisingly, artificial spring wells had the highest mean abundance of the *Biomphalaria* snails per 30 min followed by streams, the lake, and lastly wetland systems. The artificial spring wells were characterised by very slow-flowing water derived from underground sources and directed through pipes. They also tended to have large quantities of detritus deposited by people as they collected water for domestic use, which could act as organic food sources for the snails. *Biomphalaria* (and *Bulinus*) snails have been previously reported to have a high preference for human modified habitats with high pollution and low numbers of molluscivorous fish (Stothard et al., 2017). Similarly, Perez-Saez et al. (2016) reported that *Bulinus* spp. and *B. pfefferi* demographics depend on habitat type and hydrological characteristics such as change in water level, conductivity and temperature. Similarly, Civitello et al. (2022) noted the high dependence of snails on periphyton and detritus. Moreover, *Biomphalaria* snails have been reported to survive less in habitats that are prone to desiccation (Perez-Saez et al., 2017) or fast-flowing water bodies (Woolhouse, 1992). The presence of high numbers of *B.* cf. *pfeifferi* at artificial spring wells, which are largely considered to be safe sources of groundwater for domestic use, calls for improved vigilance and avoidance of faecal contamination of the springs to prevent the spread of schistosomiasis. The water may be free from bacterial contamination compared to open water sources but at the points of water collection, if infected snails are present, schistosomiasis transmission is possible. Open defecation was observed at some of the spring sites during the study.

High molecular diversity in *Biomphalaria* species in upland areas

Generally, there was a high molecular diversity among the *B. pfeifferi* group consisting of 43 haplotypes: nine haplotypes of *B. pfeifferi* and 32 of *B.* cf. *pfeifferi* (Fig. 4). The number of haplotypes in our study is higher than that reported by Andrus et al. (2023) who recorded only four haplotypes for *B. pfeifferi* from three sites at Lake Albert, farther north of our study area. In this study, three haplotypes were recorded from the lake sites while the remaining six were from other site types both upland and close to the lake. The high diversity in our study confirms the presence of diverse populations of *B. pfeifferi* snails in the streams and wetlands upland, which are often neglected in preference to Lake Albert. The presence of population bottlenecks such as the rift valley escarpment limits efficient gene flow between the populations. Moreover, temporary water bodies such as ephemeral streams in our area are more likely to experience population bottlenecks such as desiccation, thus influencing upland populations to adapt differently from the more stable lake populations. Similar findings were obtained by Maes et al. (2022) who observed higher genetic diversity among *B. truncatus* from temporary water bodies in Senegal and Egypt. Similarly, habitat diversity and small-scale isolation of the upland subpopulations could be responsible for upland diversity in *B.* cf. *pfeifferi*. Even when unidirectional flow of genes down the escarpment is possible, studies have indicated that self-fertilization frequency and genetic drift can a have strong effect in determining the population structure of snails (Maes et al., 2022). The study area lies between two main rivers: Muzizi and Semliki. However, the majority of the small streams studied have small water sheds draining directly to the lake through a steep escarpment. Therefore, some of the upland streams and wetland systems act as ‘islands’ geographically isolated from neighbouring systems (Lake Albert and larger rivers). This hydrological arrangement creates a diverse range of habitats, supporting distinct ecological niches that are inhabited by different *Biomphalaria* species. Notably, the phylogenetic relationships among these species remain incompletely resolved, highlighting the need for further research to clarify their evolutionary history. Thus, *B.* cf. *pfeifferi* is proposed by this study to be a subspecies of *B. sudanica* with a high affinity for *S. rodhaini* but unknown compatibility with *S. mansoni*. It is therefore possible that *B.* cf. *pfeifferi* in the upland is not compatible with *S. mansoni.* Nevertheless, studies into the compatibility of *B.* cf. *pfeifferi* with *S. mansoni* will provide insights into the role of the (sub)species in the disease distribution patterns in the region.

On the other hand, eight haplotypes of *B. sudanica* were recorded in this study, three of which were shared between the lake and upland sites, two were found at upland sites only, and three exclusively at lake sites (Fig 5). In addition, two of the shared haplotypes between upland and lake sites were also the most frequent and widespread haplotypes across the study area. Therefore, these haplotypes have persisted across multiple generations in the study area and potentially gave rise to the less frequent haplotypes and could be ancestral to the other haplotypes. Only five upland sites were inhabited by *B. sudanica*, thus having a much lower geographical range of distribution than *B.* cf. *pfeifferi*. Overall, the haplotype diversity for *B. sudanica* reported in this study (0.545) is consistent with 0.553 found by Andrus et al. (Andrus et al., 2023) at Lake Albert. Moreover, 88.86% of the genetic variation among *B. sudanica* was recorded within populations and only 11.14% between populations. This further confirms that the upland and lake populations of *B. sudanica* are genetically similar and, in principle, present an equal likelihood to transmit *S. mansoni* to people in both communities that appear geographically separated by the rift valley escarpment. In the next section, we discuss where actual *S. mansoni* transmission was detected and the likely reasons.

5.1.2 Abundance, diversity and distribution of *Bulinus* species

This study aimed to understand the enigmatic absence of *S. haematobium* in the Lake Albert region of Uganda and specifically to investigate whether this absence results from the lack of suitable intermediate snail hosts. Our results indicate that *B. globosus* and *B. nasutus productus* are present in the study area, the former being more widespread in our study area and more abundant than the latter. Similarly, the geographical distribution of *B. globosus* is known to be wider in sub-Saharan Africa compared to *B. nasutus productus* and even other hosts of *S. haematobium* (Brown, 1994), making it an important host of the disease. Notably, the *B. nasutus productus* recorded from this study is the western-most case report in the known range of the species. Both species were present in relatively high numbers, suggesting that these are viable populations sustained in time. This assertion is supported by the presence of three haplotypes of *B. globosus* collected at three locations, indicating the presence of distinct sub populations with possibly different colonisation origins in the East African region. Nevertheless, the isolates cluster closely with *B. globosus* populations from the East African region (Babbitt et al., 2023; Kane et al., 2008; Tumwebaze et al., 2022), confirming their close relationship and possible (recent) introduction from neighbouring endemic regions of *S. haematobium*. However, our study recorded high genetic distance in the *B. globosus* partial *COX1* gene from the West African snail populations similar to other studies when compared with the East African populations (Babbitt et al., 2023; Kane et al., 2008; Zhang et al., 2022), indicating the complexity of *B. globosus* group (Mccullough et al., 1968). Relatedly, Tumwebaze et al. (2022) reported that the name "*Bulinus globosus*" was being used to group different species basing on morphological similarity of the shell. For instance, the GenBank sequence from Senegal with accession number AM921808 (Kane et al., 2008) used for comparison in this study was considered a different species (Tumwebaze et al., 2022). Our study, therefore, provides additional evidence for considering the re-evaluation of the taxonomy of *B. globosus* species complex.

Bulinus globosus is by far the most important snail host of *S. haematobium* in Africa, occurring in most areas with active disease transmission (Babbitt et al., 2023; Pennance et al., 2022). The absence of *S. haematobium* in *B. globosus* snails in this study suggests the absence of infective larvae in the study area, despite the regular water contact and open urination that were observed at the snail infested sites. Nevertheless, urinary schistosomiasis was previously reported in Lango region of Uganda north of Lake Kyoga (about 300 km away from our study area) as early as 1951 with a mean prevalence of 56.8% (Schwetz, 1951), and more recently in 2011 with a mean prevalence of 3.74% in 2011(Adriko et al., 2018). However, Adriko et al. (2018) did not find any *B. globosus* or *B. nasutus productus* specimens during their malacological surveys. They only identified, morphologically, *B. tropicus* and *B. forskalii*, which are not known to naturally transmit *S. haematobium* (Babbitt et al., 2023; Brown, 1994). None of these snails were shedding *S. haematobium* cercariae, despite urinary schistosomiasis cases detected in the community. Nevertheless, Kane et al. (2008) previously identified *B. globosus* previously collected in the Moyo district, and confirmed this through molecular barcoding, which could be responsible for *S. haematobium* transmission in the region. The rapid reduction of urogenital schistosomiasis is a subject of discussion, and partly attributed to mass drug administration (MDA). There is some evidence indicating a reduction in infection pre-MDA from 28.2% to 12.3% (Adriko et al., 2018), potentially attributed to the reduction in the presence of the snail intermediate hosts.

Our current findings show that all conditions are ideal to support the transmission of urogenital schistosomiasis in Lake Albert hinterlands. The suitable snail hosts are present, as well as risky water practices like bathing, and washing open defecation/urination (Table 4.3 and Exum et al. (2019), and the parasite is present in neighbouring regions and countries. It is a very dynamic area in terms of human mobility (partly due to cross-border trade, the recent oil exploration activities and refugee influx) from neighbouring endemic regions and countries such as the Democratic Republic of Congo, thus introduction from these areas is possible. There are many cases of outbreaks of urinary and intestinal schistosomiasis following ecological changes and subsequent snail introduction, e.g. dam constructions in Senegal (Talla et al., 1990) or the recent emergence of *S. mansoni* in Lake Malawi as a result of colonisation by *Biomphalaria pfeifferi* snails (Alharbi et al., 2019). *Schistosoma mansoni* emergence quickly transitioned to an intestinal schistosomiasis outbreak in an area that was only known to be endemic for *S. haematobium* (Kayuni et al., 2020). It is therefore only a matter of time before urinary schistosomiasis could break out, which call for efficient monitoring and vigilance.

The partial *COX1* sequences of *B. nasutus productus* obtained from this study were highly similar to the GenBank samples from Tanzania and Kenya. Natural transmission of *S. haematobium* by *B. nasutus productus* was recently reported in neighbouring Kenya (Babbitt et al., 2023) and previously in Tanzania (Mccullough et al., 1968), indicating that the Ugandan population could also support the transmission of urogenital schistosomiasis. However, we so far only found the presence of *S. bovis* cercariae in *B. nasutus productus* and no infections with *S. haematobium* as confirmed by RD-PCR tests. The prevalence of *S. bovis* was high (16% based on the infection PCR). This indicates ongoing transmission of bovine schistosomiasis in cattle from the communal grazing fields where the snails were sampled. Recent studies indicate that significant economic losses are incurred when livestock schistosomiasis is not diagnosed and treated (Díaz et al., 2023). It is therefore important to investigate the extent of *S. bovis* distribution among livestock in the surrounding communities and the associated economic damage caused to the livestock industry in the area. This information should be shared with the respective communities together with the possible treatment and prevention strategies.

On the other hand, high molecular diversity was reported among the *B. forskalii* snails with two distinct haplotype clades. This diversity is attributed to the ability of *B. forskalii* to colonise small temporary water pools allowing the establishment of subpopulations from various geographical origins. Similarly, population bottlenecks and genetic drift could explain the molecular diversity within the *B. forskalii* snails in our study area (Maes et al., 2022). From this study, it was observed that *B. forskalii* inhabited all habitat types from small isolated wetland pools to the large Lake Albert. In addition, the ability to survive in multiple habitats and disconnected pools could provide an avenue for adapting to the localised environmental conditions and accumulating mutations in the *B. forskalii* snails. Since *B. forskalii* is implicated in the spread of *S. bovis* in the East African region (Brown, 1994) and also in Mpeefu sub county for this study, it is important to monitor the grazing grounds of livestock in the region to limit the spread of bovine schistosomiasis. Regular treatment of cattle and interventions such as snail control in the grazing grounds are highly encouraged. Notably, *B. forskalii* snails have recently been implicated in the transmission of *S. haematobium* in Senegal (Gaye et al., 2023). Therefore, the potential for transmission of *S. haematobium* by *B. forskalii* in Uganda remains a subject of further research. However, a recent study in Kenya reported incompatibility of the *B. forskalii* with the *S. haematobium* strain in the East African region (Babbitt et al., 2023). Nevertheless, given the interconnectedness of the continent and human mobility, it is possible that the compatible *S. haematobium* strain from Senegal could spread to other geographical regions (Díaz et al., 2023).

5.1.3 Abundance and distribution of *Radix natalensis*

Whereas DNA barcoding was not done for the *R. natalensis* reported in this study, the shell morphological traits were used to confirm the species. In addition, only two lymnaeid species are known to be present in Uganda: *R. natalensis* and *Galba trancatula*, the latter only reported at high (>3000 m) altitudes on Mt Elgon (Howell et al., 2012; Malatji et al., 2019). This study was carried out in the altitude ranges below 1500 m, which are within the tolerance range of *R. natalensis*. In addition, *R. natalensis* snails were more abundant in wetlands in the upland than other water bodies with the lake having the lowest abundance. Streams draining to the lake down the escarpment were important habitats for *R. natalensis*, thus, indicating the putative fasciolosis risk both in the upland and lake sites. Wetlands are eutrophic systems supporting growth of autotrophs such as filamentous algae, which act as food for the *Radix* snails. Anthropogenic disturbance such as agriculture is known to cause eutrophication creating suitable habitats for lymnaeid snails (Olkeba et al., 2020). In this study, the abundance of *R. natalensis* was positively correlated with pH and dissolved oxygen. Similarly, Howell et al. (2012) recorded a positive correlation between pH and *R. natalensis* abundance. Nevertheless, physicochemical parameters were not found to significantly predict occurrence of *R. natalensis* in Ethiopia (Olkeba et al., 2020). Therefore, the importance of physicochemical parameters in snail distribution may not follow a similar pattern and may require context-specific analysis for different studies.

Given that *R. natalensis* is prevalent in the Lake Albert region, there is a high likelihood of fasciolosis transmission among livestock in the region. A recent parallel study reported high prevalence of fasciolosis among livestock faecal sample(Namire et al: in prep). Our one-time shedding approach may not provide a complete picture of the extent of *Fasciola* spp. transmission by the *R. natalensis* snails in this study. As evidenced from this study, a one-time cercariae shedding grossly underestimate the parasite prevalence as prepatent infections are missed. Thus, it is important to further assess actual contribution of the *R. natalensis* snails in fasciolosis epidemiology in the Lake Albert region using effective techniques such as diagnostic PCRs (Schols et al., 2019).

5.2 *Schistosoma* species prevalence: comparison of traditional and molecular methods

In this study, the shedding prevalence of *Schistosoma* spp. among *Biomphalaria* snails was relatively low at 0.67% (n = 6,706). However, when the non-shedding snails were screened for prepatent *Schistosoma* infections, the prevalence ranged from 1.9% among the *B. pfeifferi* group (n = 1,364) to 24.0% among *B. sudanica* (n = 255). Clearly, a single timepoint shedding approach greatly underestimates the parasite prevalence in snails, thus underscoring the advantage of molecular diagnostic techniques (Schols et al., 2019). Moreover, identification of the *Schistosoma* species based on the morphology of the cercariae is not possible. Here, 12.7% (30 snails out of 236) of the *Biomphalaria* snails (*B. sudanica* and *B. pfeifferi*) screened from L. Albert were PCR positive with *S. mansoni*. This result is similar to the 12.5% infection rate found further north of Lake Albert in Buliisa district (about 150 km away) in 2010 (Andrus et al., 2023). This implies that the risk of acquiring schistosomiasis infection from Lake Albert remains high over time and relatively uniform regardless of the geographical location. However, in this study, we found a much higher prevalence of *S. mansoni* in *B. sudanica* (24.7%, n = 89) and a lower prevalence in *B. pfeifferi* (5.11%, n = 147) than reported by Andrus et al. (2023). Based on the findings of this study, *B. sudanica* was the main species contributing to the schistosomiasis burden in Ndaiga and Ntoroko at the shores of Lake Albert.

However, we found infected *B. pfeifferi* (identified through shedding and PCR) at Kijanibarora (1.03533°N, 30.61033°E)-a small stream at the foot of the escarpment (approximately 1km from the lake) preferred by nearby community members as a source of water (personal observations) due to relatively cold waters and lower salinity than the lake water. At this stream, the infection was consistently detected in the *B. pfeifferi* snail in the months between August and January for the years 2020, 2021 and 2022, implying that this site is a persistent infection hotspot. This finding confirms that schistosomiasis transmission can occur at other sites, which are not necessarily the lake provided the right conditions such as open defecation and presence of compatible snails. In this study *Schistosoma* spp. distribution showed a spatial pattern whereby *S. mansoni* was only recorded down the rift valley escarpment and *S. rodhaini* only recorded at the upland sites. However, our approach had some limiting factors. For example, we only barcoded *Schistosoma* parasites in the snail specimens that showed the *Schistosoma* spp. infection signal in the first step of RD-PCR but did not amplify *S. mansoni* in the second step. Consequently, it was not possible to identify co-infections of *S. mansoni* and *S. rodhaini,* in case they were present. Nevertheless, if the status quo as determined form the data of this study remains, the chances of hybridisation between the two parasite species are minimal, however, passive transport of the parasite eggs or infected snails to the lake potential hybridisation of the parasites in the vertebrate hist.

The probability of occurrence of schistosomiasis is dependent on the presence of suitable snail intermediate hosts (Perez-Saez et al., 2017). However, in this study, *S. mansoni* was only recorded at either lake sites or streams close to the lake more prevalent among *B. sudanica* than in *B. pfeifferi* snails. At all the upland sites, which had relatively higher *Biomphalaria* spp. abundance (mainly *B.* cf. *pfeifferi*), none of the snails were infected with *S. mansoni.* Snail abundance alone is not sufficient to predict a schistosomiasis risk as very high abundances are associated with resource competition, which results in low production of infective cercariae due to competition among snails (Civitello et al., 2022). In addition, a single infected snail is sufficient to maintain transmission, provided other conditions are provided. As discussed above, similar *B. sudanica* haplotypes were shared between the upland and lake sites, thus snail genetic factors cannot explain the absence of *S. mansoni* upland as this snail species played the most important role in disease transmission at the lake sites in this study. Therefore, we suggest that other factors such as socio-cultural factors could be the main drivers behind the presence of human schistosomiasis at Lake Albert and its absence in the upland areas. For example, behaviours like open defecation, myths, and misconceptions have been reported to be more pronounced among the lake-dwelling communities than the upland communities (Anyolitho et al., 2022). In addition, the lake communities have much lower latrine coverage (~60%) and access to clean and safe water (<20%) than the upland communities (Uganda Bureau of Statistics, 2016), thereby predisposing the lake communities to a higher risk of contracting schistosomiasis. However, caution is still warranted given the presence of *B. sudanica* in many upland sites to avoid a possible expansion of disease beyond its current range.

A combination of approaches could be explored to support the local communities with the highest schistosomiasis burden to alleviate the transmission challenge, by increasing latrine coverage and by targeted snail control (World Health Organization, 2023). If targeted snail control is to be implemented against intestinal schistosomiasis in the Lake Albert region, this study suggests focusing the efforts on the *Biomphalaria* snails mostly at the lake shores and streams in the valley where active disease transmission has been recorded. In addition, there is a need to do regular monitoring of changes and shifts in transmission hotspots (David Rollinson et al., 2013; Sokolow et al., 2017). Our study demonstrates that snail abundance increases in the wet season and decreases as the dry season advances. If snail control is to be implemented, it is critical to initiate it before the onset of the wet season, which is the breeding season of the snails to interrupt snail abundance at transmission hotspots to levels where disease transmission is significantly reduced (Sokolow et al., 2017).

This study found a wide distribution of *S. rodhaini* among *Biomphalaria* spp. in the upland aquatic systems. *Biomphalaria* cf. *pfeifferi* had an infection prevalence of 12.24% (18 out of 147) snails infected with *S. rodhaini* while *B. sudanica* had an infection rate of 1.12% (1 out of 89 snails). The widespread distribution of *S. rodhaini* in our study area reflects the presence of infected rodents at many sites in the upland regions of Lake Albert. Given that *S. rodhaini* and *S. mansoni* are not sympatric in the study area, it appears that hybridization of the two species is not probable in the current situation. However, this situation could change with the changing distribution of (infected) snails or rodents. Given that the natural hybridisation of *S. rodhaini* and *S. mansoni* has been recorded in the Lake Victoria region (Morgan et al., 2003), spreading *S. rodhaini* at the lake shores could potentially result in hybrids, which would complicate the schistosomiasis control strategies

5.3 Biotic and abiotic factors influencing snail distribution

5.3.1 Physico-chemical parameters and snail distribution

This study found that *Biomphalaria* spp. preferred certain site types due to the associated physico-chemical parameters. Generally, the lake had a higher pH, temperature, conductivity, Dissolved Oxygen (DO) and Total Dissolved Solids (TDS) than other site types. Being at a relatively lower altitude than other sites, the lake is characterised by higher water temperature, which increases the productivity of the phytoplankton and macrophyte in the lake thereby increasing the amount of DO in the water. Similarly, high atmospheric pressure and wave action at the lake increase the DO levels. The high productivity of the lake supports fish fauna including molluscivorous species which are known to decrease the abundance of snails (Stothard et al., 2017). The upland sites which are smaller in size and less productive are less likely to support a large number of molluscivorous species thus supporting relatively larger numbers of *Biomphalaria* snails. Nevertheless, *B. stanleyi* snails are exclusively lacustrine and endemic to Lake Albert (Brown, 1994) owing to the possession of special adaptions such as relatively small and hard shell, the ability to live in deep waters and strongly attach to macrophyte mats to survive in the lake environment. *Biomphalaria* spp. abundance significantly decreased with an increase in pH ($R^2 = 0.388$, $p = 0.007$) possibly indicating that reproduction in the snails reduces at high pH. Nevertheless, the pH values recorded in this study fall within the tolerance range for *Biomphalaria* spp., which implies that all the study sites can support the multiplication of *Biomphalaria* snails.

For the case of *Bulinus* spp. and *Radix* spp., only site type was explanatory of the changes in the snail abundance. This means that the physicochemical parameters at the different site types collectively contribute to snail abundance than when the individual parameters are considered independently. For instance, high pH, water temperature, conductivity, DO saturation, salinity and turbidity are associated with the lotic lake and wetland sites, which tended to favour increase in the *Bulinus* spp. abundance. While the physico-chemical parameters are important, it appears that other factors such as the physical microhabitat characteristics (Utzinger & Tanner, 2000) and macrophytes communities (Haggerty et al., 2020; Wood et al., 2019) play a more important role in explaining snail presence and abundance differences.

5.3.2 Aquatic macrophyte and snail distribution

In this study, *Biomphalaria* spp. abundance was not significantly predicted by aquatic macrophyte density in upland sites away from the lake. This is likely due to the adaptability of *Biomphalaria* spp., especially *B.* cf. *pfeifferi* to multiple food sources. In addition, *Biomphalaria* spp. in the upland sites preferred spring wells, which are often littered with organic matter especially as people collect water from these sources. The availability of diverse food sources in the form of decaying organic matter provides a richer alternative source of food than macrophytes. Indeed, previous studies indicated that freshwater snail diet constitutes 50-90% detritus and <1% living macrophyte (Brönmark, 1989). However, in the Lake, higher *Biomphalaria* spp. abundance was associated with *Ipomea aquatica*, *Potamogeton schweinfurthii*, *Pistia stratiotes* and *Vallisneria spiralis*. These plants provide surfaces for the growth of algae and periphyton, which are food sources for snails (Haggerty et al., 2020). The rooted *V. spiralis* with dense mats submerged in the water provides a surface for attachment to the snails. The flattened leaves provide a large surface area for snail attachment and growth of periphyton and algae. Given that the lake environment is characterised by strong wave action, the above plant species provide a good refuge for lacustrine *Biomphalaria* snails. Similar results were recorded when *B. pfeifferi* was recorded to invade Lake Malawi, particularly among *Vallisneria* spp. beds (Alharbi et al., 2019).

On the other hand, *Bulinus globosus* mainly inhabited river sites only associated with *C. dactylon*. This macrophyte species is mainly terrestrial but the grass stem extends in the water. Therefore, the tough stem of *C. dactylon* might provide a good surface for attachment of *B. globosus*, which was observed to mainly attach to the bottom substrate. *C. dactylon* stems and leaves could probably also provide good spawning surfaces for the snail species.

Bulinus forskalii snails were positively associated with *Azolla filiculoides*, *Pistia stratiotes* and *Leersia hexandra*. These macrophyte species are characteristic of wetland sites, which were preferred by *B. forskalii*. These plants thrive in nutrient rich waters, an environment suitable for periphyton which is a rich food source for snails. Similar trends were observed for *R. natalensis*, which also dominated wetland sites.

Generally, the feeding habits of freshwater snails characterised by high dietary intake of detritus (Brönmark, 1989) imply that the observed association of snails to specific macrophytes in this study may not be for direct feeding benefits. In the unstable lake environment, macrophytes offer both shelter from wave action and predators and act as substrates for growth of the sought out periphyton. Therefore, the identified macrophytes only act as a proxy for the potential presence of snails at a site.

Removal of such vegetation from snail habitats may have a significant impact on the snail numbers in the affected habitats. However, extensive studies to test this hypothesis need to be done to establish the contribution of macrophytes on snail distribution patterns.

5.4 Reliability of citizen scientists’ malacological survey and snail identification

5.4.1 Citizen science and snail monitoring

This study aimed to assess the suitability of a citizen science approach for monitoring snail intermediate hosts, with the goal of guiding targeted snail control efforts and to support species distribution modelling. To evaluate the quality of the collected data in relation to these objectives, the study employed the concept of ‘fitness for use’ as suggested by Parrish et al. (2018). To ensure the generation of relevant and high-quality data, the participants were purposively selected and a rigorous sampling protocol with fixed sampling times and sites was developed (Brees et al., 2021). This deliberate approach helps to mitigate issues related to sampling bias, which are commonly encountered in many citizen science projects that rely on opportunistic participation and self-selection by volunteers, as seen in projects like *Mosquito alert* and *Spider in da house* (Hart et al., 2018; Palmer et al., 2017). However, to assess the reliability of citizen-collected data, this study chose to compare it with temporally matched data collected by a PhD researcher at the same locations, considering the PhD researcher's data as the ‘ground truth’. This choice and the snail sampling method have some repercussions, which are discussed before going into the results. ‘Ground truth’ is difficult to establish because freshwater snail species are difficult to sample exhaustively for several reasons. Firstly, *Biomphalaria*, *Bulinus* and *Radix* snails are relatively small, with juvenile stages measuring only a few millimetres, usually hidden in the vegetation or sediment (Woolhouse, 1992). Secondly, their dispersal is not spatially continuous but rather stratified, involving a combination of passive long-range dispersal through water currents, waterbirds, and humans, as well as active short-range movements in search of food, mates or shelter from predators, heat and strong currents (Dewitt et al., 1999). Additionally, circadian vertical migration has also been observed (Morgan & Last, 1982). Consequently, observational inconsistencies are likely to occur, even when the same PhD researcher samples the same site at different times of the day. It is therefore worth noting that our quality control measures might be overly stringent, as some of the observation errors like false positives/negatives could be attributed to factors unrelated to the CSs' performance. In the next paragraphs, two fundamental questions for prospects involving citizen scientists in snail monitoring are discussed; 1) can CSs determine snail presence at a site? And if so, 2) can they reliably monitor snail abundance?

5.4.2 Can citizens correctly identify snail presence and absence?

This study demonstrates a substantial level of agreement between CS-collected and PhD researcher-collected data regarding the presence or absence of snails at a given site. The binary agreement rates were highest for *Bulinus* (86%), followed by *Biomphalaria* (76%) and *Radix* (70%). These agreement values are relatively high compared to the 50-80% reported among marine divers as CSs compared to PhD researchers in recording the presence of biological taxa and litter (Goffredo et al., 2010), but similar to the 79% reported in the *Mosquito alert* project (Palmer et al., 2017). However, our binary agreement was influenced by factors such as snail abundance and site type. For example, presence/absence of *Biomphalaria* spp. was more likely to match between the PhD researcher and CSs at sites with higher snail abundance (see Fig 4.20A) such as wetland sites (86%), while it was lowest at lake sites (60%). These findings could be explained by the fact that snail population dynamics are influenced by habitat type and hydrological conditions (Perez-Saez et al., 2016). Wetland sites in the study area are relatively stable systems with minimal chances of snail migration due to their shallow lentic nature, small size, and clearly delineated boundaries. Therefore, the chances that two independent observers will find snails at such a site are higher compared to a larger and deeper lake system. Wave action in lakes can also alter the shoreline vegetation and thus snail communities within a few hours in response to heavy winds (Grabas et al., 2019). Disagreement was also relatively high in spring wells (see Fig 4.20B), which are regularly unblocked by removing mud and debris, which are substrates that support snails. Spring wells are also usually located in valleys where they are prone to runoffs from heavy rains. The probability of agreement for *Biomphalaria* occupancy decreased when the difference in the number of days between sampling by the PhD researcher and CS increased (Fig 4.20C). This could be explained by an increasing chance for changing weather events with time, affecting the sampling sites as described above. Previous studies have shown how heavy rains or wave action can lead to passive snail dispersal (Woolhouse, 1988) and subsequently low recapture values as low as 20% after one week (Woolhouse, 1992). As none of the interaction effects for site type and sampling date difference was significant, none of the site types seem to attenuate the effect of short-term fluctuations significantly, thus corroborating our statement earlier that using PhD researcher data as ground truth might be too strict.

Bulinus spp. presence/absence data had the highest binary agreement between the PhD researcher and CS data. However, the detection sensitivity was the lowest (43.4%) of the three snail genera studied. Lower values of sensitivity and higher values of specificity are likely when the frequency of occurrence of the event is low (Gallas et al., 2019; Leeflang et al., 2013; Trevethan, 2017). In our study area, *Bulinus* spp. snails were rarely encountered and when present, in relatively low numbers. For most of the sampling months (15/20), the monthly total abundance of *Bulinus* reported by all raters was less than 250 snails across the 73 sites (see Fig 4.23A). Consequently, the class with true negatives (TN) was very high (77%). The CSs were, therefore, able to correctly identify sites where *Bulinus* snails did not occur, increasing the chances of binary agreement with the PhD researcher (86%). Similar trends were found in *Mosquito alert* for the comparison of mosquito trapping by PhD researchers and mosquito reporting by CSs in areas with low mosquito abundance (Palmer et al., 2017).

Radix snail presence/absence agreement between the CSs and the PhD researcher was the lowest of the three genera and the agreement was significantly predicted by snail abundance. *Radix* snails tend to attach firmly to the substrate and are often found on substrate suspended in fast-flowing waters, unlike *Bulinus* and *Biomphalaria* snails. The large shell aperture relative to the size of the snails (Falade & Otarigho, 2015; Pretorius, 1963) could provide a large surface area for the foot to attach to the substrate firmly. We observed that sampling *Radix* snails from the water using a scoop net requires extra effort in agitating the substrate, making it more difficult compared to sampling *Biomphalaria* and *Bulinus*. This could explain why there were more false negatives for *Radix* paired reports (23.6%, N = 898). However, the degree of binary agreement in *Radix* presence/absence increased from 70% to over 80% from September 2021 to February 2022 (Appendix 17), corresponding to a period of higher *Radix* abundance reported by the PhD researcher as shown in Figure 4.27A. Generally, as the abundance of *Radix* snails increased, the CSs were more likely to agree with the PhD researcher, similar to *Biomphalaria* snail data. However, the decline in snail abundance from the peak in February to April 2022 was contrasted by increasing binary agreement in the same period (Appendix 17). We attribute this to the hands-on refresher training organised in January 2021, which emphasised the snail sampling technique. This underpins the importance of training and feedback in improving CSs' performance (Brees et al., 2021), especially when high quality data is desired, and when sophisticated sampling procedures and fine-scale morphological identification of organisms are involved (Bancks et al., 2018; Engel & Voshell, 2002; Galloway et al., 2006; Parrish et al., 2018; Roman et al., 2017).

The effect of CS ID on presence/absence could not be conclusively analysed due to an increase in model complexity. There is however an apparent association between CS ID and binary agreement in the presence/absence of snails. This association could be reflected by the majority of them performing well (over 50% agreement), and a few performing excellently (>80% agreement), or poorly (<50% agreement) (see Appendix 18). Future research should investigate the factors behind this inter-individual variation, by studying the link between motivation and data quality for example.

The agreement in presence/absence is more straightforward when both the CS and the PhD researcher do (not) find snails at a site (true positives or negatives). However, the consequences of and reasons behind disagreement (false positives or negatives) are more complex. High numbers of false negatives would result in CSs underestimating the risk of schistosomiasis infection at a site. In this study, this risk is partly offset by having frequent monitoring (weekly sampling by CSs). By aggregating weekly reports by CSs and calculating the cumulative number of snails collected per month, the number of false negatives significantly decreased (chi-square (1) = 33.9; $p < 0.01$) for all genera (Appendices 20 and 21). False positives on the other hand, represent cases where snail pictures uploaded by the CSs are carefully validated for correct snail identification, so the reported snail was actually present. This again illustrates the limitation of our 'ground truth', as discussed above.

5.4.3 Can citizens reliably monitor snail abundance in given site/habitat types?

For all snail genera, snail abundance reported by the CSs were positively correlated with those obtained by the PhD researcher. However, the correlation values were lower than those reported in other studies about intercoder reliability (Camilleri et al., 2020; Schilling et al., 2021; Terzis et al., 2021). Nevertheless, most of these studies focus on medical diagnosis, which involves fewer categories of classification, and therefore fewer opportunities for disagreement. In our study, we used Kendall's Tau-b coefficient to assess the level of consistency for a discrete variable (snail counts), with values from 0 to 282 (Appendix 21). The high number of reports included in our study (422, 79 and 313 for *Biomphalaria*, *Bulinus* and *Radix* snails, respectively) can significantly impact the estimated marginal distribution of the snail counts. This, in turn, leads to an increased number of possible ranks, resulting in a greater occurrence of discordant pairs, as discussed by Agresti (Agresti, 2010). Consequently, these factors could possibly contribute to lower Kendall-tau values.

Similarly, the results of numerical agreement, as indicated by Krippendorff's alpha, revealed a lower agreement between CSs and the PhD researcher compared to other studies evaluating intercoder reliability in a citizen science framework (Berland et al., 2019; Crall et al., 2013; G. Newman et al., 2017; Palacin et al., 2020). Again, the majority of these studies were focused on the agreement between a limited number of categories (from 2 to 6) whereas we worked with ratio data that considered the algebraic differences between the values reported by the raters. Although using abundance intervals could potentially yield higher alpha values, we opted to retain the highest level of detail in our data. Nonetheless, Berland et al. (2019) reported higher values of alpha (>0.8) when assessing intercoder agreement among novices, intermediate analysts and PhD researcher analyst counting trees. In our study, the lower values of snail abundance agreement are most likely also partly attributed to the nature of our study subjects, freshwater snails, which, unlike trees, are not immobile (see discussion above).

In line with the reported results of the presence/absence analyses, snails from the genera *Biomphalaria* and *Radix* presented higher values of consistency and numerical agreement in wetlands (Table 4.11), probably due to the shallow and lentic nature of these water bodies. In contrast, the highest agreement for *Bulinus* snail abundance was observed in stream sites (Table 4.11), which may be attributed to specific locations where *Bulinus* snails were found, such as Kolanya and Nyamasoga. These sites are characterized by small, slow-flowing streams with confined access points, which support relatively stable populations of *Bulinus*. It is important to note that both Krippendorff's α and Kendall Tau-b are calculated with all paired observations for each genus, and thus the resulting values do not account for the temporal structure of the data. When calculating these metrics for each timestep, we observe less variations (especially for *Bulinus* snails) in Krippendorff's α than in Kendall Tau-b, which is more likely to be affected by sample size (Appendix 19).

A plausible explanation for the differences in snail abundance between CSs and the PhD researcher is the task difficulty. Determining the presence or absence of a particular snail genus requires relatively low skill, which CSs would already acquire after the initial malacological training. However, obtaining reliable abundance data comparable to the ground-truth data (PhD researcher data) requires that, in addition to identifying the potential microhabitats of the snails at the sampling sites, a vigorous and continuous sampling for 30 minutes should follow, which demands considerable physical effort and dedication. In the latter, individual factors such as motivation or skill, could contribute to the observed variations in performance among CSs (see Appendix 18). The observed increase in agreement in the ranking of high abundance sites over time supports the hypothesis of task difficulty, indicating that CSs improve their performance with more practice and experience, but also after the refresher training that stressed the importance of correct sampling (Figure 4.27D).

For all evaluated snail genera, consistency values were higher than numerical agreement, suggesting that using proportional abundance (Fig 4.23B) can be a more effective approach than exact numerical agreement, although both metrics remain relatively low and are strongly influenced by the site type.

5.4.4 Can citizens help to map putative schistosomiasis transmission sites to guide targeted snail control?

Our study provides evidence of high agreement in presence/absence and spatial congruence between the CSs and the PhD researcher in monitoring snail populations. The CSs successfully identified the top 15 sites with the highest abundance of *Biomphalaria* spp. in over 84% of the matched reports. In addition, this study reported high values of the binary agreement for *Biomphalaria* spp. (76%) across all the site types, and a low number of false negatives (15.4%) (see above). The latter is important since false negatives would lead to refraining from snail control in potential transmission sites. These findings, together with the similar trend in relative snail abundance over time generated from PhD researcher and CS data, suggest that involvement of CSs has the potential to bridge the gap in snail data availability and the lack of snail PhD researchers (Rollinson et al., 2013; Shiff, 2017; Sokolow et al., 2017). It could generate the data needed by the national Vector Control Division for planning targeted snail control interventions, and meet the increasing need for high-resolution data in precision mapping as the efforts shift from control to elimination of schistosomiasis (Rollinson et al., 2013; Sokolow et al., 2017). Involving local citizens could increase the spatial coverage, facilitate access to remote areas and decrease the monitoring costs (see below). A notable example to illustrate the potential is the *Mosquito alert* project in Spain (Palmer et al., 2017), which utilizes a mobile app allowing citizens to report sightings of the Asian tiger mosquito. This project demonstrated collaboration between citizens and the traditional monitoring surveys improves monitoring effectiveness (>60% new information) than when either party works in isolation (<40% new information) (Palmer et al., 2017). In our study, CSs collected high-resolution data both in terms of spatial coverage, encompassing 73 sites across an area of approximately 750km², and temporal coverage, sampling on a weekly basis for 20 months. Notably, ten of these sites were located in remote areas along the shores of Lake Albert in the highly endemic and hard to access Ndaiga sub-county, making the resident CS the ideal person to regularly monitor them. Therefore, we argue that citizen-generated snail data can guide targeted snail control.

However, it is important to note that in this study many locations without active schistosomiasis transmission but with high *Biomphalaria* spp. densities were found (e.g. the sites further away from the lake). Indeed, in order for schistosomiasis transmission to occur, other requirements like human water contact and open defecation need to be met (Exum et al., 2019; Utzinger et al., 2011). Therefore, CSs also record the type and frequency of water-related activities they encounter when sampling. However, to prove actual schistosomiasis transmission, human or snail infection data is needed. Since this is outside the scope of our current citizen science approach, we recommend that citizen-driven snail monitoring should only be implemented in areas with known endemicity for schistosomiasis. In the future, one could envision the involvement of citizens in eDNA monitoring of schistosome DNA in waterbodies (Sengupta et al., 2022), as already done for the eDNA monitoring of birds and marine species (Biggs et al., 2015; Tøttrup et al., 2021). One could even foresee the involvement of citizens in village-based mollusciciding as done in Cameroon (Greer et al., 1996), after snail or schistosome detection in the respective water contact sites. In absence of molluscicides, citizen scientists can assist in alternative snail control measures like manual aquatic vegetation removal, which has been proven in effective snail reduction in Senegal (Rohr et al., 2023). In this case, citizen scientists instead of satellite imagery-assisted researchers identify the sites for intervention, based on the data they collect on snail abundance and human water contact patterns.

5.4.5 Added value of citizen science beyond data quality

Citizen scientists offer a unique perspective on scientific and social issues that can complement or enrich those of professional scientists (Lukyanenko et al., 2016a). This fosters mutual learning between CSs and PhD researchers, allowing for subsequent exchange of knowledge and skills within their communities. Within the ATRAP project, the same CSs are also involved in community mobilisation for contextualised awareness campaigns. We have gained valuable insights from this citizen-led awareness campaigns where the CSs designed tailored messages on schistosomiasis prevention and control upon community input (Anyolitho et al., n.d.). This led to diverse, contextualised communication approaches specific to each community, stimulating the knowledge uptake and moving away from a one-size-fits-all approach. Furthermore, Anyolitho et al. (2022) reported existing myths and misconceptions regarding schistosomiasis in our study area, suggesting that bottom-up participatory approaches led by CSs can help address and reduce these misconceptions. Additionally, the process of data collection itself, which involves identifying snails associated with schistosome transmission, raises awareness about snail-borne diseases in the area (Anyolitho et al., 2023). We observed that the support and demand for snail control by the CSs and their fellow community members increased during the project, and witnessed a multiplication effect by having CSs safely training their family members and friends in snail sampling and identification, schistosomiasis transmission, and practices that influence it.

From a cost-benefit perspective, there are significant advantages to engaging CSs. It would require one PhD researcher at least 80 months to collect the data collected by the 24 CSs in 20 months. Given the size of our study area, weekly monitoring is simply not feasible for one person. For instance, monitoring the 73 sites on a weekly basis would require at least four PhD researchers, amounting to €178,640, which is about 7.6 times more expensive than working with CSs. This cost is based on the involvement of a PhD student as an expert, so the total price would increase if governmental experts were involved. The current cost for our sampling campaign was €23,475 including the initial non-recurring cost of buying equipment such as the smartphone, protective gear, and scoop net (for details, see Appendix 22).

In summary, we agree with Parrish et. (Parrish et al., 2018) that the citizen science approach leads to increased ownership and sustainability of interventions while providing opportunities for citizen involvement in scientific endeavours cost-effectively.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

This study sought to determine the diversity and distribution patterns of snail intermediate hosts of *Schistosoma* and *Fasciola* species in the south-eastern region of Lake Albert in Uganda. In addition, there was the need to understand whether the absence of urogenital schistosomiasis in the region, and intestinal schistosomiasis away from the shores Lake Albert was due to a lack of suitable snail intermediate snail hosts. Equally important, the study also determined where actual disease transmission was occurring using traditional shedding and modern PCR-based diagnosis. As an innovation, this study thought to test the citizen science model in snail monitoring to alleviate the data scarcity and knowledge gap among the affected communities.

In this chapter, therefore, the study summarises the key conclusions and recommendations based on the findings that have been discussed. In addition, the limitations of the study and potential areas for further research are highlighted.

6.1 Conclusions

This study notes that *Biomphalaria* spp. in the Lake Albert region are present at both the lower altitude lake and streams and at the higher altitude sites with some haplotypes of *B. pfeifferi* and *B. sudanica* shared between the upland and lake sites, despite the rift valley escarpment barrier to the bidirectional movement of snails. Since endemic regions were only described around the Great Lakes, we unexpectedly found that upland sites are also putative intestinal schistosomiasis transmission sites. However, *S. mansoni* infections in both *B. sudanica* and *B. pfeifferi* were only recorded at the lowland sites (the lake and streams) highlighting the importance of human behaviour and water contact practices in the transmission of the schistosomes.

For the first, this study provides evidence, based on the p-distance in *COX1* gene fragment, for re-classification of the non-lacustrine *Biomphalaria* cf. *pfeifferi* as a subspecies of *B. sudanica* given its distant relationship with *B. pfeifferi* proper and closer relationship with *B. sudanica*. Based on our molecular analyses, *Biomphalaria* cf. *pfeifferi* appears to play a major role in the transmission of a rodent schistosome, *S. rodhaini*, in the upland sites but its role in the transmission of *S. mansoni* is hypothesised to be minimal. This study did not detect *S. mansoni* in *B.* cf. *pfeifferi*. However, given that *S. mansoni* showed a spatial bias to the lake shore *B. sudanica* and *B. pfeifferi* snail communities despite shared haplotypes in the upland snails, we cannot rule out the possibility of compatibility of *B.* cf. *pfeifferi* with *S. mansoni*. This calls for vigilance and avoidance of risky behaviours such as open defecation among the upland communities to expansion of the current range of intestinal schistosomiasis.

Contrary to our expectations, in the upland sites of the Lake Albert region, *Bulinus globosus* and *B. nasutus productus* are present, the former being more prevalent than the latter. The two species are known to naturally transmit *S. haematobium* in the East African region and beyond. Therefore, there is a viable risk of the outbreak of urogenital schistosomiasis in the area if infected people introduce it in the snail habitats. This study reports the westernmost record of *B. nasutus productus* in sub-Saharan Africa. This finding demonstrates the need for extensive and regular malacological surveys to better describe the snail host species' range of distribution which is predicted to change with the changing climate (Aula et al., 2021). In addition, the presence of *B. forskalii* and *B. truncatus* with former actively transmitting *S. bovis* demonstrates an existing challenge to livestock production in the region.

In this study, the presence of non-human schistosomes, *S. rodhaini* and *S. bovis,* which are respectively known to hybridise with the human schistosomes *S. mansoni* and *S. haematobium* presents a risk if the compatible species were sympatric, which is currently not the case. Potentially, the emergence of hybrids could have far-reaching implications such as the replacement of the well-studied parasite species and strains, the extension of the range of the intermediate and final hosts, and/or the increase in the infectivity and virulence of the schistosomes. Therefore, there need to regularly study the *Schistosoma* spp. distribution range in the Lake Albert region to quickly detect the emergence of hybrids, especially down the rift valley escarpment where the passive movement of *S. rodhaini* with water currents is most likely to occur.

Among the physicochemical parameters of the water, pH is a significant predictor for *Biomphalaria* spp. abundance whereby abundance decreases with increasing pH. Therefore, human activities such as agriculture and waste disposal could provide suitable conditions for the proliferation of parasite-transmitting snails. Similarly, wetlands were the most important habitats for the *Bulinus forskalii* and *Radix natalensis* snails. Since the two snail species are implicated in the transmission of bovine schistosomiasis and fasciolosis respectively, continuous communal grazing of livestock in wetlands exposes the livestock to these diseases.

Macrophyte species predict the abundance of snail species differently according to habitat characteristics in line with the needs of the snail species. For instance, macrophyte species were associated with *Biomphalaria* spp. abundance at the lake but not in the upland sites with the decreasing order of importance as: *Ipomea aquatoca* > *Potamogeton schweinfurthii* > *Pistia stratiotes* > *Vallisneria spiralis*. In the turbulent lake, unlike relatively stable streams and wetlands, macrophytes provide shelter, breeding grounds and attachments for algae and periphyton (food for snails). Human activities that increase nutrient enrichment in the snail habitats (e.g. pollution with organic waste) will likely increase the abundance of macrophytes and the associated snails. Thus, given that macrophyte communities only mediate their influence on snail distribution, macrophyte abundance may only be used as a proxy for potential snail presence.

This study demonstrates that citizen scientist-collected data on freshwater snail intermediate hosts of *Schistosoma* and *Fasciola* species can be used to map putative snail-borne disease transmission sites with up to 86% agreement of the citizen-collected data with PhD researcher data on snail presence/absence. Citizen scientists can effectively detect snail occupancy where snails are abundant, but their effectiveness decreases in sites with low snail abundance. The approach of this project is fit for the purpose of guiding targeted snail control and also increasing community awareness. By transferring knowledge about snail-borne diseases among local community members, awareness and ownership increase, which is vital for the success of control and prevention strategies. We therefore argue that this inclusive, powerful and cost-effective approach can be more sustainable than top-down monitoring and intervention campaigns.

6.2 Study Limitations

It was not possible to resolve the *Biomphalaria cf. pfeifferi* snail species identity. Species delineation to resolve this challenge would require analysis of multiple mitochondrial and nuclear markers, which was outside the scope of this study. In addition, a study of the compatibility of *B.* cf. *pfeifferi* was out of the scope of this study. Therefore, it is recommended that infection experiments are conducted to confirm the hypothesis developed after this study that possibly, the snail subspecies is incompatible with *S. mansoni.*

It was not possible to ascertain whether the absence of urinary schistosomiasis was due to the natural incompatibility of the snails with the *S. haematobium* strains in the region, or rather a lack of chance to infect the snails from the community.

In this study, time and resource constraints could not allow for a detailed analysis of the contributions of *Radix natalensis* in the spread of *Fasciola* spp. Assessments were done using the limited shedding technique and conchological classification.

The time-based sampling of snails was preferred in this study to the more accurate yet cumbersome quadrat sampling (Perez-Saez et al., 2019), as the latter would be time-consuming for volunteers, difficult to validate remotely compared to time stamps in the former, and the increased time investment would potentially lower the motivation of the CSs (Sprinks et al., 2017). In addition, with respect to *Bulinus* spp., the snail intermediate hosts of urogenital schistosomiasis, there is need to further assess the applicability of the citizen science approach to map their distribution. Whereas the CSs correctly identified the sites where these snails occur, the relatively low spatial distribution of *Bulinus* snails. Therefore, only a few CSs monitored the sites where they occurred, which could not allow an in-depth comparison between CSs and the PhD researcher.

6.3 Recommendations

Species delineation of *Biomphalaria* cf. *pfeifferi* would require analysis of the morphology, and multiple mitochondrial and nuclear markers, which was outside the scope of this study. Therefore, there is a need to revisit the classification of the *Biomphalaria pfeifferi* and *B. sudanica* snails in Uganda to improve the resolution in species delineation for a better understanding of the role played by each species in sustaining the *Schistosoma* spp. lifecycle.

In addition, this study found natural intermediate hosts of *S. haematobium* in the Lake Albert region. Therefore, there is a need for increased snail monitoring and *S. haematobium* surveillance among the surrounding communities to rapidly detect a potential outbreak. Additionally, controlled snail infection experiments are needed to study the susceptibility of both snail species towards Ugandan *S. haematobium* isolates and those of neighbouring countries.

The presence of active shedding snails and snails infected with *S. mansoni* is a clear indication of active disease transmission among the communities at the shorelines of Lake Albert. There is therefore need to enhance the disease control strategies in the area. From our observations, hygiene challenges like open defecation and lack of access to clean safe water for the communities are still major. Given that myths and misconceptions regarding schistosomiasis have been recorded by a colleague in the same study area (Anyolitho et al., 2022), more effort need to be put towards mindset change if a significant reduction in the disease situation is to be realised.

In addition, a one-health approach that considers both human and environmental health should be considered in the prevention and control of schistosomiasis to avoid a potential exchange of parasites between man and wild animals. This inclusive approach will also reduce the prevalence of bovine schistosomiasis and fasciolosis in livestock, thereby improving productivity.

This project studied *Radix natalensis* and associated *Fasciola* spp. using the limited conchological classification and shedding technique. Therefore, this study recommends an assessment of the prevalence of *Fasciola* spp. in the *Radix* snails using molecular techniques. In addition, molecular diversity studies could be carried out on the *R. natalensis* snails in the region to answer specific research questions as required on phylogeography and evolutionary trends of both the snails and parasites they contain.

This study was conducted in a confined geographical area with only 25 CSs actively involved. This approach can be scaled up to other areas that are endemic for schistosomiasis. Considerations like the complexity of the tasks and the ability of the CSs to learn through feedback and experience should be carefully planned in the project design. Parrish at al. (2018) suggested that for complex assignments continuous expert participation is recommended to improve the data quality, which was also shown by our own study (Brees et al., 2021). Finally, identifying and understanding the motivations of citizen scientists will further improve the design and execution of citizen science projects to ensure mutual benefits for all involved parties. Within the ATRAP project, the same CSs are also involved in community mobilisation for contextualised awareness campaigns (Anyolitho et al., 2023). IN addition, there is a need to test the citizen science model in areas endemic for urogenital schistosomiasis to allow a significant number of CSs to monitor *Bulinus* snail species. In this study, the patchy distribution of *Bulinus* spp., especially in the upland sites, could not permit this detailed study.

6. 4 Areas for further research

There is need to investigate the compatibility of *S. mansoni* and *S. haematobium* with *B.* cf. *pfeifferi* and *B. globosus* respectively in the Lake Albert region. In the former case, *B.* cf. *pfeifferi* were present in the upland sites but none was infected with *S. mansoni*. Similarly, *B. globosus* is present in the study area but no infections were detected during this study. Thus, extra research will reveal the actual possibility of spreading schistosomiasis in the Lake Albert region.

In this study, we used rapid diagnostic PCR approaches but these are not yet field-friendly and are costly, necessitating the need for point-of-care diagnostics like LAMP and eDNA detection methods (Sengupta et al, 2022). Thus, modern techniques for xenomonitoring using environmental DNA (eDNA), which do not require sampling for snails to detect the presence of the parasites in the aquatic environment can be explored.

In addition, studies on targeted snail control at confirmed transmission sites and how this would affect the disease epidemiology in the Lake Albert region are encouraged. More research is needed to identify the appropriate control strategy, especially for the large and very dynamic lake. As evidenced by this study, citizen-led interventions are highly encouraged.

The design of this study did not allow to detect the presence of *S. mansoni/S. rodhaini* hybrids since only snails with a positive schistosome and negative *S. mansoni* signal were sequenced. Similarly, co-infections of *S. mansoni* and *S. rodhaini* in snails were not detected. Therefore, future surveillance programmes should monitor this situation more closely, by sequencing snails with *S. mansoni* infections, especially along the lake where *S. mansoni* infection among snails was detected.

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APPENDICES

Appendix 1: Pilot study field report for Rubirizi, Kazinga Channel and Bunyaruguru crater lakes

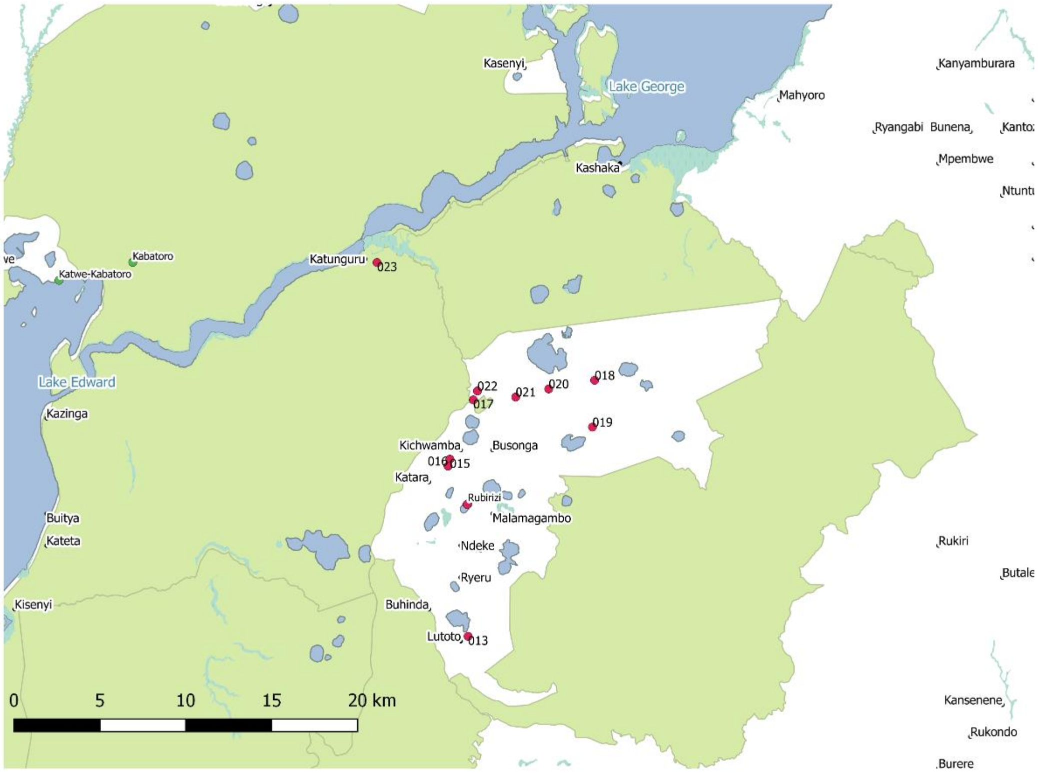
FIELD WORK 10-11-12TH FEBRUARY 2019 19-22H

1. 10TH FEBRUARI : KAZINGA CHANNEL, RUBIRIZI, CRATER LAKES (S)

Overview GPS points and observations (for location see map below)

GPS point	Name	Comment
13	Makondo village near Nyanjakufu river	Flooded area in the wet season (currently dry). No Bilharzia cases known to locals. Cattle keeping is the major source of livelihood.
14	Nyanjakufu river at Makondo	Very large river bed but completely dry. Cattle keepers collect water from dug deep wells in dry season.
15	Swamp in Rwebisengo	Swamp contains water. Snail of *Pila* species found. Could potentially support other snails.
16	Rwebisengo HC III	Acting In-charge Mujanama Vicent (mujanamavicent@gmail.com; 0772403366) • MDA in community by VHTs • HC rarely gets cases of schisto • In march, they tested and confirmed a case of intestinal schisto. The patient had been staying in Rwangara (at L. Albert). • They have a good lab with a technician (Muhindo Jockus: muhindojocks@gmail.com; 0775196818) • Jockus is interested in schisto and did research on it in Buliisa district (Northwards) during his training. • Vicent much willing to take part in SWAMP and coordinate VHTs
17	Swamp after Rwebisengo	Cattle grazing ground. Dirty smelly water. Snails found were *Bulinus foskali* and *Ceratophalus* sp.
18-19	Seasonal swamps	No snails in the water.
20	Karongo village (swampy)	Contacts know about Bilharzia. Say its not in this area because there are no permanent waterbodies. The surroundings are also salty they say. That schisto is common at the lake.
21	Private clinic in Butungama	No much info on schisto. Attendant not that knowledgeable as the main "doctor?" was away.
22-24	Swamps	Temporary/seasonal swamps

25	Rwangara HC III	• Contact person Kabagonza Gertrude (peer mother). Works mainly with HIV patients to ensure compliance with treatment. • People get MDA in time and comply. • People seek MDA when there are delays. They are concerned that their environment floods so much. • Contacted on phone the coordinator for VHTs who doubles as the lab technician at Rwangara HCIII (Ruhweza Steven; 0779480535) o Gave contradictory info e.g. schisto is tested in the lab (could not tell what analysis is done!) o No cases of schisto recorded. • VHTs give MDA annually.
26	Songa Ochaki (landing site)	• Large floodplain with a lot of mud. • Access to the main water isn't easy (without a boat) because of mud • Fish cleaning, intestines dropped in the water. • Cattle keeping still key activity. • Snail shells seen in the water including *Biomphalaria*. • Locals say need schisto check-up since it has not been done. They fear they might have it. Have seen colleagues with signs.
27	Rwangara fishing village	• Contact person Lukia Ahabwe (0772364138). She is the coordinator for VHTs at village and parish level. • VHTs are trained on dosage during MDA. Distribute drugs from house-to-house. • They do not know whether people have schisto or not but follow directions from VCD. No check-ups are done. • People get side effects from the drugs. • There is no way of knowing whether people get cured. • That volunteering is very hectic with too little facilitation. Some people opt out of being VHTs. • She was very positive about the prospective of SWAMP project and would be much willing to participate.
28	Nyanjakufu river	River bed completely dry.
29	River in the Park	Apparently, no snails. No sampling done because I had no permit.

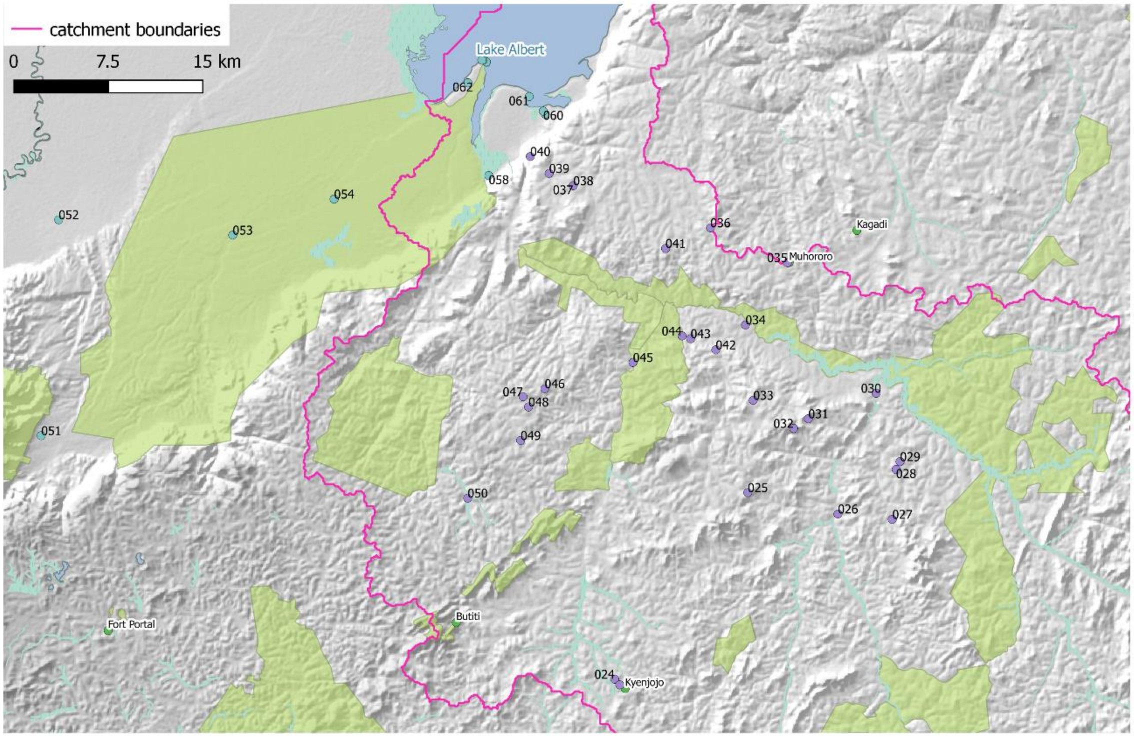


1. 13TH AND 14TH APRIL: KAGADI (OLD NAME KIBAALE) AND KYENJOJO DISTRICTS, TRAVELLING BACK TO MBARARA

Overview GPS points and observations (for location see map below)

GPS point	Name	Comment
30	Nyamalembo	Small stream arising from the hill. Local contact says schisto is not a problem here, it is a problem in Mpefu sub county. MDA occurs.
31	Nyakitukuma river	People washing moto cycles and bathing in the water. Local says no known cases of schisto. MDA is carried out by VHTs. Snails of Lymnaea species (intermediates of *Fasciola*) found. Cattle access the point for water.
32	Muzizi river	People showering and fishing from the water. People know about schisto but from the lake. Snails found include: Pila, Melanoides-like?, Lymnaea and bivalves.
33	Buhirira	Contact didn't know whether people have schisto or not. Biomphalaria (human schisto intermediate) and Lymnaea snails found

34	Siisa	Vehicle washing occurs in the river. Lymnaea snails found.
35	Kasaba medical centre (private clinic)	The nurse, Twinamatsiko Medard (0782081279) said they have a functional lab but they have not registered any cases of schisto.
36	Waswa	Site in the forest with no snails found. We previous accessed this point.
40	Nturagye	Small stream. *Pila* and *Segmentobis* snails found
41	Nyabukoni	Cattle access point. Shells of *Biomphalaria.* Contact doesn't know about schisto.
42	Muzizi river	• A lot of human activity: swimming, grazing animal, water pumping, road construction, fishing, etc. • Snails of *Biomphalaria* found. • Scoop net broke down before completion of sampling at this site.



Impressions after field work:

- Kazinga channel: lower prevalence
- Near lake albert: high prevalence, but decreasing. System is still unclear (who is keeping which record, why are people not going to HC, are diagnoses ever done?).
- Strong geographical contrast: at lake, on rift shoulder. But still signs of snails and disease at higher areas: gradient in snail population? Migration of people? Also west of Ntoroko there must be a high prevalence: currently unvisited but potentially very relevant, and perhaps more water bodies and lower elevation gradient than the subcounties visited now (in pink watershed).

Appendix 2: Pilot study meeting with the national VCD Officer in Kampala

MEETING NATIONAL VCD OFFICER MOSES ADRIKO KAMPALA 13TH FEBRUARY 2019 12-13H

- Rubirizi (crater lakes near Kazinga channel): last prevalence measurements are 2013: 24% prevalence
- Ntoroko: last prevalence measurement in 2015: 75%
- Crater lakes near Kamwenge: 16% prevalence. Moses says that crater lakes are infected by people from Ntoroko area migrating.
- Districts for MDA application are based upon these studies: these are done every 3-5y but heavily depend on funding availability. Ideally they also do an impact study after 5 Y.
- There diagnosis and impact studies (what VCD refers to as mapping data) are not available until they are published: they depend on their funding agencies/academic partners and cannot release any data.
- Makere university also did a country-wide study, but not with VCD.
- Most HC will not have diagnostic tools to detect schisto, and early stages are difficult to detect.
- BUT VCD is in favour of a pilot study where health facilities are trained to report and sensitize the community. VCD are in favour of more structured monitoring. In addition to this they would welcome an initiative training the VHT to report via sms/app on their drug availability, interventions and difficulties.
- VHT submit reports to parish, parish supervisor sends it to SC and district combines this into a report which is then send to VCD national office but because of compiling, spatial detail is missing (also not known if reports are complete etc.);
- Drugs are not optimally distributed: sometimes drugs are available at the HC2 (village/parish level) and not at the district, while the district reports shortages.
- For setting up SWAMP I can contact Dr. Edrida to get the SC prevalence data for Ntoroko district.

2. 06TH APRIL 2019: NTOROKO DISTRICT TO LAKE ALBERT VIA RWEBISENGO

Overview GPS points and observations (for location see map below)

GPS point	Name	Comment
13	Makondo village near Nyanjakufu river	Flooded area in the wet season (currently dry). No Bilharzia cases known to locals. Cattle keeping is the major source of livelihood.
14	Nyanjakufu river at Makondo	Very large river bed but completely dry. Cattle keepers collect water from dug deep wells in dry season.
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22-24	Swamps	Temporary/seasonal swamps
25	Rwangara HC III	• Contact person Kabagonza Gertrude (peer mother). Works mainly with HIV patients to ensure compliance with treatment. • People get MDA in time and comply. • People seek MDA when there are delays. They are concerned that their environment floods so much.

		• Contacted on phone the coordinator for VHTs who doubles as the lab technician at Rwangara HCIII (Ruhweza Steven; 0779480535) o Gave contradictory info e.g. schisto is tested in the lab (could not tell what analysis is done!) o No cases of schisto recorded. • VHTs give MDA annually.
26	Songa Ochaki (landing site)	• Large floodplain with a lot of mud. • Access to the main water isn't easy (without a boat) because of mud • Fish cleaning, intestines dropped in the water. • Cattle keeping still key activity. • Snail shells seen in the water including *Biomphalaria*. • Locals say need schisto check-up since it has not been done. They fear they might have it. Have seen colleagues with signs.
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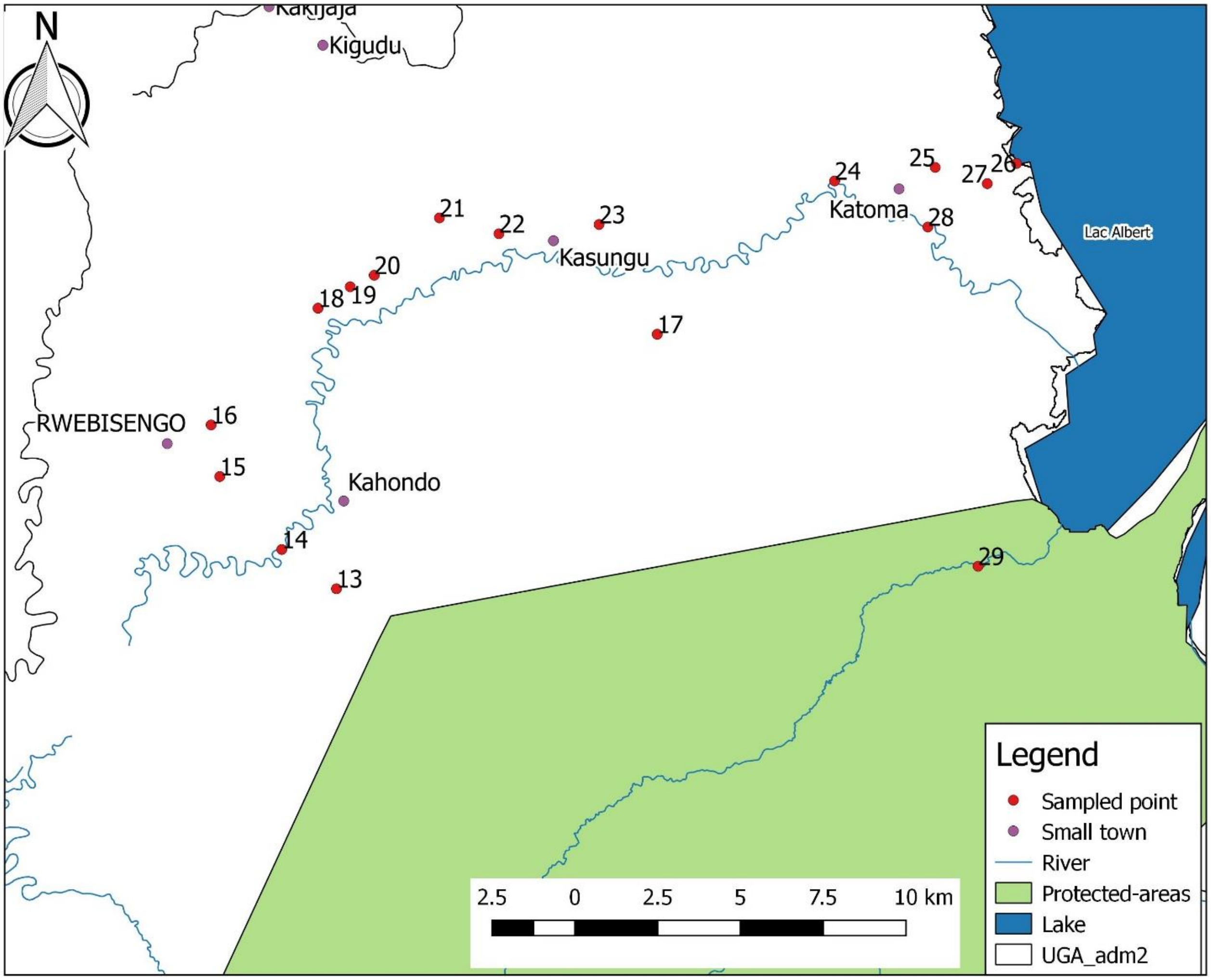
29	River in the Park	Apparently, no snails. No sampling done because I had no permit.

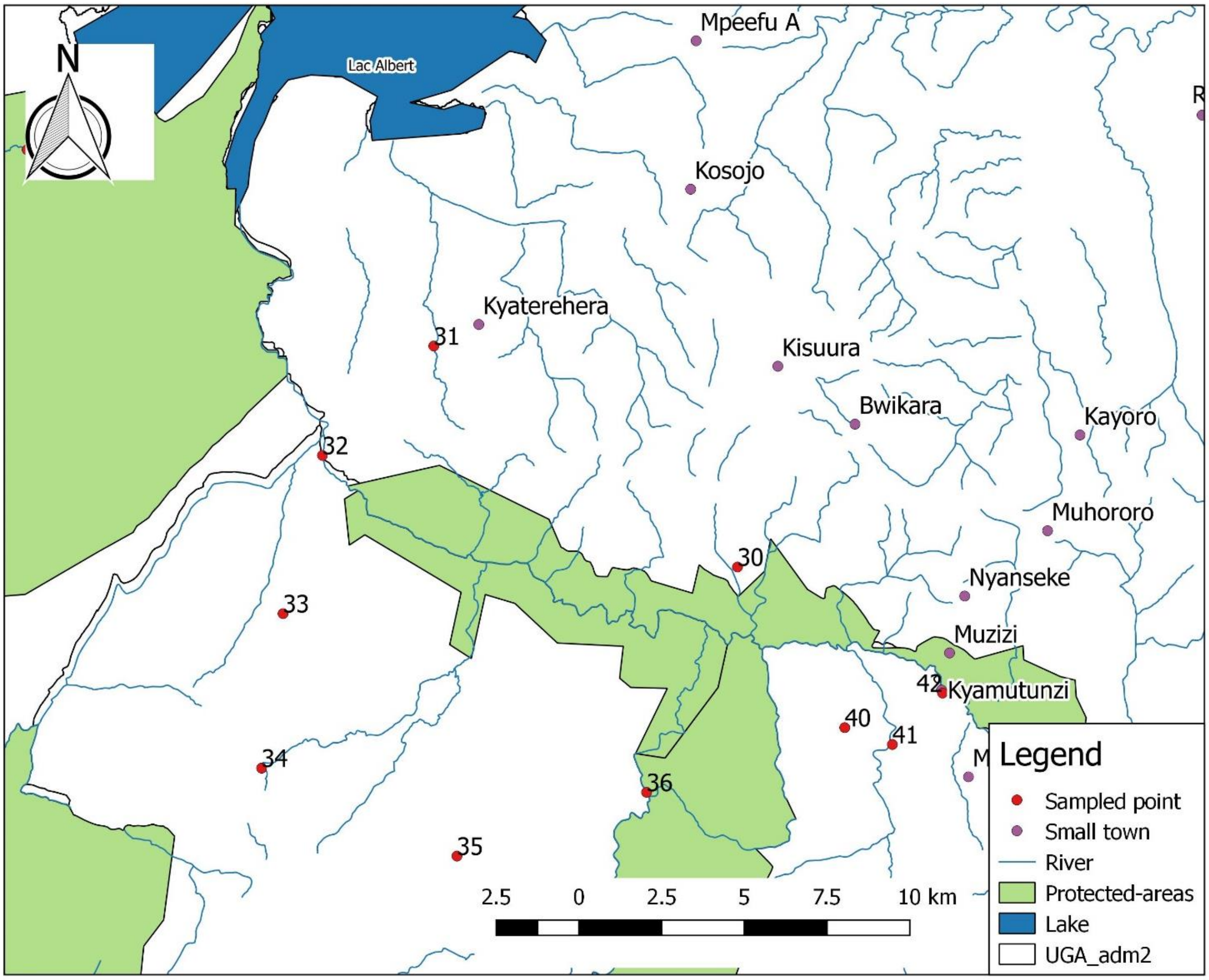


3. 07TH AND 08TH APRIL: KAGADI (OLD NAME KIBAALE) AND KYENJOJO DISTRICTS, TRAVELLING BACK TO MBARARA

Overview GPS points and observations (for location see map below)

GPS point	Name	Comment
30	Nyamalembo	Small stream arising from the hill. Local contact says schisto is not a problem here, it is a problem in Mpefu sub county. MDA occurs.
31	Nyakitukuma river	People washing moto cycles and bathing in the water. Local says no known cases of schisto. MDA is carried out by VHTs. Snails of Lymnaea species (intermediates of *Fasciola*) found. Cattle access the point for water.
32	Muzizi river	People showering and fishing from the water. People know about schisto but from the lake.

		Snails found include: Pila, Melanoides-like?, Lymnaea and bivalves.
33	Buhirira	Contact didn't know whether people have schisto or not. Biomphalaria (human schisto intermediate) and Lymnaea snails found.
34	Siisa	Vehicle washing occurs in the river. Lymnaea snails found.
35	Kasaba medical centre (private clinic)	The nurse, Twinamatsiko Medard (0782081279) said they have a functional lab but they have not registered any cases of schisto.
36	Waswa	Site in the forest with no snails found. We previous accessed this point.
40	Nturagye	Small stream. *Pila* and *Segmentobis* snails found
41	Nyabukoni	Cattle access point. Shells of *Biomphalaria.* Contact doesn't know about schisto.
42	Muzizi river	• A lot of human activity: swimming, grazing animal, water pumping, road construction, fishing, etc. • Snails of *Biomphalaria* found. • Scoop net broke down before completion of sampling at this site.



** Some of the rivers (close to Muzizi river) in this map don’t seem to cross any access road. They could not be seen in the field with even a local guide on boda boda.

Key observations from the field:

- Rwebisengo area (up to the lake) is seasonally flooded. There are many swamps with stagnant water which dries in dry season leaving few isolated ‘permanent?’ swamps. Some of these have snails and could thus be included the study.
- River Nyanjakufu is seasonal and may not be appropriate for this project.
- The many small streams that drain into Muzizi river are not very accessible even by motorcycle (boda boda).
- Sub counties in Kyenjojo and Kagadi district have relatively more (and more stable) water bodies and could be given priority for the study on rivers/stream. Some of the streams do not flow into Muzizi river but flow to the lake (from map of day 2). Schisto in these subcounties is also known to have high prevalence even from reports of the community.
- Regarding following Muzizi river till there are no more snail, this does not seem logical. There is no explanation why snails would not inhabit some sections of the river. The river continues upstream through forests and dense swamps but snails could still be there.

- Absence of snails at a point doesn’t suggest that they are completely absent in the river. From previous studies, the distribution of water snails can be quite patchy. As long as the waterbody is relatively permanent (or connected), snails could reside somewhere (sometimes not seen with rapid explorations).
- We could probably designate the study area within a certain radius from L. Albert (as suggested by Lies) and sample key villages for thorough systematic studies in the framework of ATRAP and SWAMP.
- The people in the areas close to lake Albert Know about schisto and regularly receive MDA.
- Despite the capacity to test for schisto, many labs at health centres do not do it. Perhaps the technicians are not very familiar or are not interested in the procedure. We could find out more within SWAMP.
- The lab technician at Rwebisengo HCIII is highly motivated and loves to study and even test for schisto.
- Generally, VHTs and hospital administrators have great enthusiasm to participate in SWAMP (later on ATRAP) when asked.

Appendix 4: Pilot study planning meeting for volunteer motivation survey in Ntoroko and Kagadi districts

10th MAY 2019: Ntoroko district

Stop	Name	Comment
1	Ntoroko district	• Contact person: Musana Patrick, the Community Development Officer (CDO). Mobile: 0782806585, Email: pmusana2015@yahoo.com. • Most officers had gone to Karugutu for a conference. • A total of 17 people could potentially come for the meeting on the 22nd May. They include assistant Chief Administrative Officer(ACAO) in charge of health, district biostatistician, District Health Educator (DHE), District Health Officer (DHO), Health assistants (4 from the entire district), Health in-charges (2, each from a HC III in the target sub counties, e.g. Vincent Mujanama from Rwebisengo HC III), VCD officer, 2 Sub county chiefs and the District Community Development Officer (DCDO). • The venue will be the district council hall and would not be paid for.
2	Rwebisengo Town council	• We got a contact from the In-charge of Rwebisengo HC III (Vincent Mujanama) by phone. Contact person was Birungi Edward (0782896581). Edward is the VHT coordinator for present-day Rwebisengo SC. • Rwebisengo SC is different Rwebisengo TC. Rwebisengo SC, as we know it, was divided into Rwebisengo TC, Rwebisengo Sc and Butungama SC. • The present-day Rwebisengo SC has five parishes, four villages and each village has two VHTs making a total of 40 VHTs. • If a meeting were to be organised, it would be better at Rwebisengo HC III. Previous meetings were held there.
3	Butungama SC headquarters.	• Contact person: Asiimwe Robert Bamwenda (0774883293/0777306125). He is the VHT coordinator for the sub county. He has 23 years of experience as a VHT and has faced a number of challenges but his motivation to help the community supersedes the challenges. • He rides a moto cycle ambulance to help especially pregnant mothers and other emergencies cases to go to the hospital. Ambulance donated by Save the Children NGO. They praise the NGO for the much help and that all members of community recognise it. • MDA is given though he says he hasn’t encountered cases of schisto in his community. • The SC was created in 2017 and has six parishes, 27 villages and 54 VHTs (two per village).

		• If a meeting were to be organised, it would be in the SC hall. A few seats would need to be hired since they do not have enough furniture. • Another VHT found was Mugabe Keneth (0783370861) who is a Parish coordinator for Kyabukunguru parish. He says he gets renewed hope when he sees people coming to help the community given previous support from NGOs like Save the Children has phased out. He is looking forward for the workshop to share more of their experiences as VHTs.
4	Rwangara HC III	Received by Mr. Rude Wilson, a nurse at the HC (Contact: 0777295129). • Kanara SC has a total of 42 VHTs. Some come from quite far e.g. Katolingo parish which is an island in the lake and Mulango village in Kamoga parish. Previous engagements with these VHTs included transport refund of 50,000 shillings for those from far places. • The VHTs are attached to Save the Children since it does outreach programmes. • The Health Inspector (HI) deals with the VHTs directly and would be the lead in coordinating them. The HI is called Mumbere Nason (0775555727). He stays in Bundibugyo and we were unable to meet him. However, he could coordinate activities by phone. • That the Health In-charge (Nyakahuma Simon, 0784414599) should be informed of the activities that are to be conducted. He was however away for a workshop in Karugutu. • The workshop would be organised at Kanara primary school. Agreement with the chairman of the school needs to be reached with a small facilitation (they said maybe 30000). The nurse called Katusiime Jessy (0782037125) would help us in organisations.
4	Kanara TC at Kanara HC III (Ntoroko town)	• No direct connection between Kanara TC and Kanara SC by car. • We were received by the nurse Kato James (0782972901). • The TC has 32 VTHs from 16 cells and four parishes. • All the VHTs are within the town and can easily be mobilised. • The VHT coordinator is Kato James (0782972901) but could not be reached by phone or even physically. • More coordination needed for this place.

4. 11TH MAY: KAGADI (OLD NAME KIBAALE) AND KYENJOJO DISTRICTS

Stop	Name	Comment
1	Kyenjojo town.	• Meeting with the district VCD officer (Edwin) following an appointment. We could not meet the officials since it was a Saturday. • The meeting with the district health team can be organised in the council hall.

		• There is need for an introductory letter (official communication) to the district officials before formal arrangements are done. • The DHO is Tumusiime Charles (0772358115). • Edwin advised that we foresee transport refund for all participants and breakfast, even those that work at the district. • As for Kyarusozi SC, the VCD officers said the sub county was also divided into Kyarusozo TC, Kyarusozi sub county and Kyembogo SC. More details to be obtained before the dates are confirmed. • Edwin is willing to help in coordination of the activities in his district.
2	Kagadi TC, Kagadi district headquarters and Kagadi hospital	• We were unable to get the official because of the weekend. • Through Joshua, we contacted the Red Cross manager for Kagadi district (Pascal Kasaija, 0775989466, pkasaija@redcrossug.org). • Pascal informed us that some of the VHTs are also RC volunteers. • That Kyaterekera SC (formally part of Muhorro SC) was recognised as the best in the country in handling schisto cases. • That in case a combined meeting is foreseen, Kyaterekera SC would be the central place for Ndaiga and Mpeefu SCs. • Pascal is willing to help in coordinating the meetings especially with the district officials given his office is close by and he knows them individually. • **Pascal asked whether it is possible for Red Cross Kagadi branch to work with us (MUST; KUL) to in the forthcoming projects. He is enthusiastic** • Contacts of some district officials ○ DHO: Dr. Olowo Samuel (0772574279) ○ DHI: Mr. Setuma Peter (0782794275)
3	Mpeefu, Kasojo trading centre	• We contacted (through Pascal) the VHT coordinator for Mpeefu SC. Teopista Muhindo is also a nurse at Mpeefu HC II. • Mpeefu SC has four parishe, 42 villages and 84 VHTs. • Two training centres were used by VHTs previously (Mpeefu church of Uganda and Mpeefu SC headquarters) but the SC headquarters are preferred. • Parish coordinators can be contacted by the SC coordinator to mobilise the VHTs when needed. • Transport refund should be foreseen for those travelling from far. We also met the health inspecto for Mpeefu SC (Mr. Thembo Silvano, 0772364320) who is also the husband to Teopista Humindo. He has been working on schisto since the year 2000. • He said L. Albert is the breeding ground for schisto. • Schisto is also recorded in SC neighbouring but a little far from the lake. He thinks its because people from a distance visit the

		lake to trade with the residents and end up contacting the water. He thinks children are exposed especially in the holidays. • He is not sure whether locals also get schisto from nearby streams, ponds and rivers and wondered whether our study would address that gap.
4	Mpeefu ya Sande	• We met Julius Tweheyo. He is a very active VHT and RC volunteer. • Informed us that Mpeefu SC and Mpeefu TC are still operating together (administratively and otherwise) though they were separated in 2018. • MDA was last given in 2018.
5	Kyaterekera SC.	• We contacted the VHT coordinator Ms. Allen Kansiime on phone since she was not available physically. She is very much willing to work with us in coordinating the workshop. • Kyaterekera SC has 46 VHTs. The possible venue is at the HC II in Kyaterekera where previous meetings were held. • After contacting the coordinator in Ndaiga SC, she confirmed that the VHTs in Ndaiga are 16 in number. • That both Ndaiga and Kyaterekera can be combined in case a workshop is organised though it would cost more to transport the VHTs from Ndaiga Sc up the escarpment.

Key observations from the field:

- There is a good connection from Ntoroko district headquarters to Rwebisengo TC. If activity schedule is to be revised, the option putting the activities in both places on the same day is feasible.
- The sub counties as in the map we had earlier have been split into town councils (TCs) and other sub counties. This implies that we need to revise our map or to consider a sub count in either the ‘old’ or ‘new’ sense. We need to decide on the sub counties to have workshops in.
- Kanara SC and Kanara TC are the ones that border the lake.
- There are two VHTs per village and from 32-84 VHTs per sub county. This means that we can potentially have a workshop each of the chosen sub counties. Alternatively, we may need to sample the VHTs.
- The possible venues are mostly public places (we may not necessarily have to pay for them) where VHTs have had meetings before.
- We need to foresee a budget for coordination by the local people (phone calls etc) who will contact the stakeholders of interest. From this report, we have identified a few key people but these will definitely need some support as they will not sacrifice their own money.
- The stakeholders are willing and looking forward to the engagements in the forthcoming projects.
- There is a lot of information we can gather regarding the motivation for VHTs to do what they do. They tend to open up about their enthusiasm and challenges.

- There are different groups of VHTs attached to different outreach programmes by NGOs. E.g. Baylor Uganda and Save the Children NGOs have different VHTs. However, when you explain what the study is about, the set of VHTs related to the study are quickly identified. The VHTs are in pairs per villages meaning each village could actually have up to 4 or more VHTs if all were to be called upon.

Appendix 5: Data collection quaestion about their motivation for volunteering

This is a questionnaire that can be applied to all citizen scientists that will be active within the D-Sire, ATRAP or HARISSA projects. This Questionnaire intends to poll for socio-economic characteristics of the citizen scientists as well as identify explanatory factors for their degree of activity within the project. Therefore this questionnaire consists of 4 parts:

(1) Socio-economic questions
(2) Questions related to their work and role in their communities
(3) Questions based upon the Volunteering Functions Inventory
(4) Questions based upon the Theory of Planned behaviour

Text-box indicate additional information for the enumerators. This version of the questionnaire is tailored to Village Health Team volunteers (VHTs) who are potential citizen science for ATRAP.

Instructions to the interviewer: It is important that you have a short discussion with the respondent before actually starting to ask questions. During this discussion you should at least go through the following points. Please make sure to go discuss all these elements in a similar way with all respondents in order to make sure that they are in the same understanding before starting the interview.

First discuss what it means to be a Village Health Team (VHT). What are **the typical tasks of a VHT**? **Are there many VHT's** in the village? **Do VHT's get a compensation** for their work?

Secondly, elaborate on the word "volunteering". What do they **understand under "volunteering"**? What is **the difference with a classical job**? And what is the **difference with just helping out friends**?

Third, discuss the link between VHT and "volunteering". **Are VHT always volunteers? Why yes/no?**

If the respondents answer that VHT are not always volunteers, then it is important to stress that at least some tasks of VHT are largely uncompensated and can therefore be considered as volunteering. It is important to make sure that the understanding of the word volunteering is clear before starting the questions. All questions are about the volunteering aspects of VHT's and not about other, possibly compensated, activities related to being a VHT.

Module 1: Socio-economic characteristics of the volunteer.

First Name	....					
Last name	....					
Tel. Number	....					
Gender:	M/F					
Age	.....Y					
Economic occupation (several answers possible)	o	(small) business owner	estimated net income from this activity (optional)	....	UGX/	day/week/month
	o	caretaker/taking care of the household				
	o	farmer/pastoralist	estimated net income from this activity (optional):	....	UGX/	day/week/month
	o	fisher/fish trader	estimated net income from this activity (optional):	....	UGX/	day/week/month

o	teacher	estimated net income from this activity (optional):	....	UGX/	day/week/month
o	tailor	estimated net income from this activity (optional):	....	UGX/	day/week/month
o	religious leader if so: which function:	estimated net income from this activity (optional):	....	UGX/	day/week/month
o	Politician If so: which function:	estimated net income from this activity (optional):	....	UGX/	day/week/month
o	public servant: If so: which function:	estimated net income from this activity (optional):	....	UGX/	day/week/month
o	No income generating activity				

Numbers of days in the week working on the above occupation(s)		.... Days		
Religion	....			codes: RELIGION
Highest Education	....			Codes: EDUC
Relationship status	o single		o separated	
	o married		o widowed	
	o Other; please specify:			

Number of children below 18Y
(if several households (for men), only consider the household where you spend most time and money) ....Children

Number of other people living in the household	.............Persons	
estimated collective income of other adults in the household (excluding own income)		................................UGX/ day/week/month

Address
district: ....
sub-county: ....
parish: ....
village: ....

Are you originally from this village/parish?		Y/N
Is your extended family present in your village/parish?		Y/N
Number of friends in village (people you could ask for money in case of need)?	....................persons	
Do you think people in this village trust one another in matters of lending and borrowing?	Y/N	
Ethnicity	....	
Do you own your own house?		Y/N
Number of plots you own	.... Plots	
Total area of land you own	....	acre/ha
Total area of land you cultivate for agriculture	....	acre/ha

Do you own cattle? Y/N	if Yes: how many/which?	....	cows
		....	goats

	……….	Others Specify:………. …………………….
Did you ever take up a pu lic function (chief, LOC, NGO, vice ch …)?	Y/N	
if yes: from when to when?	………………….	
if yes: which function?	………………….	

APPENDIX: CODES RELIGION AND EDUCATION

RELIGION	1	Catholic	RELIGION	5	Born again
RELIGION	2	Muslim	RELIGION	6	Traditional religion
RELIGION	3	Protestant/anglican	RELIGION	7	Other
RELIGION	4	Seventh Day Adventist			
EDUC	0	No education	EDUC	21	University - 1 year (without degree/diploma)
EDUC	1	Primary school - P1	EDUC	22	University - 2 years (without degree/diploma)
EDUC	2	Primary school - P2	EDUC	23	University - 3 years (without degree/diploma)
EDUC	3	Primary school - P3	EDUC	24	University - 4 years (without degree/diploma)
EDUC	4	Primary school - P4	EDUC	25	University - 5 years (without degree/diploma)
EDUC	5	Primary school - P5	EDUC	26	University with Bachelor degree/diploma
EDUC	6	Primary school - P6	EDUC	27	University with master degree/diploma
EDUC	7	Primary school - P7, without certificate	EDUC	28	Higher Institute for Education (HIE) - 1 year
EDUC	8	PLE certificate	EDUC	29	Higher Institute for Education (HIE) - 2 year
EDUC	9	Secondary school - S1	EDUC	30	Higher Institute for Education (HIE) - 3 years with diploma
EDUC	10	Secondary school - S2	EDUC	31	Vocational Training Institute (VTI) - 1 year (without diploma)
EDUC	11	Secondary school - S3	EDUC	32	Vocational Training Institute (VTI) - 2 years (without diploma)
EDUC	12	Secondary school - S4, without UCE certificate	EDUC	33	Vocational Training Institute (VTI) - 3 years (without diploma)
EDUC	13	Secondary school - S4, with UCE certificate	EDUC	34	Vocational Training Institute with certificate
EDUC	14	Advanced level - S5	EDUC	35	Adult literacy training
EDUC	15	Advanced level - S6, without UACE certificate			
EDUC	16	Advanced level - S6, with UACE certificate	EDUC	39	Other

Module 2: VHT Characteristics

How long are you in a VHT?	………… months/years
Are you paid as VHT?	Y/N
Were you promised money?	Y/N if yes: how much, when? and have you received this? ………………………………………………………………………………
how many households are you responsible for?	……….. HH
Area of operation as VHT:	VILLAGE level / PARISH level/SC level + name of Village/parish/SC :……………………………………………………………………….
Other members of your village VHT Team:	
How were you selected as VHT?	○ people voted ○ the local chief/chairman suggested me ○ i don't know ○ i put my own candidacy forward ○ Other, please specify…………….
Before you were a VHT were you a drug distributor?	Y/N
Before you were a VHT were you a condom distributor?	Y/N
Before you were a VHT were you a traditional birth attendant?	Y/N
Before you were a VHT were you a traditional healer/herbalist?	Y/N
Before you were a VHT were you a parish health mobilizer?	Y/N
Before you were a VHT were you a community reproductive health worker?	Y/N
Before you were a VHT were you a community vaccinator?	Y/N
Before you were a VHT were you a community counsellors?	Y/N
Before you were a VHT were you a traditional birth recorder?	Y/N
Before you were a VHT were you a local council leader?	Y/N
As a VHT, do you perform house visits?	Y/N
As a VHT, do people visit your house for medical advice/assistance?	Y/N
As a VHT, do you perform mobilization actions?	Y/N
As a VHT, do you perform health promotion and education?	Y/N
As a VHT, do you manage common ill health conditions?	Y/N
As a VHT, do you follow up pregnancies and post-natal mothers?	Y/N
As a VHT, do you perform neonatal follow-up?	Y/N
As a VHT, do you follow up discharged cases from health facilities?	Y/N
As a VHT, do you follow up people on long term treatment?	Y/N
As a VHT, do you distribute health commodities?	Y/N
As a VHT, do you do community information management?	Y/N
As a VHT, do you perform disease surveillance?	Y/N
As a VHT, do you identify health problems?	Y/N
As a VHT, do you perform immunization activities?	Y/N
As a VHT, do you provide basic health care messages for behavioural change?	Y/N

As a VHT, do you monitor children growth?	Y/N		
As a VHT, do you perform community work (eg. school cleaning) ?	Y/N	if Yes: which:	…………………
As a VHT, do you link the community with health centers?		Y/N	

What is your main mode of transport as a VHT?

- ○ foot
- ○ bicycle
- ○ motor cycle
- ○ Car
- ○ rent boda boda
- ○ rent car
- ○ Other, please specify:…….

How often were you trained as a VHT

(select one answer only)

- ○ not at all
- ○ one time at the start
- ○ Once a year on average
- ○ twice a year on average
- ○ three times a year on average
- ○ between three and five times a year
- ○ more than 5 times a year

As a VHT, my relationship with public administration and politicians is …	very bad	Bad	somewhat bad	neutral	somewhat good	good	very good

Additional comments of respondent on this question:

……

As a VHT, my relationship to health workers in health centers is …	very bad	bad	somewhat bad	neutral	somewhat good	good	very good

Additional comments of respondent on this question:

……

I'm respected by the community as a VHT	Completely disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Completely agree
People in my community know I'm a VHT	Completely disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Completely agree

As a VHT, I talk about my role as VHT to members of my community	Y/N
i only talk about my role as VHT if people ask me	Y/N
i never talk about my role as VHT	Y/N

As a VHT, I experience hostility from people as they don't know me	Y/N
As a VHT, I experience hostility from people as they believe i'm paid	Y/N

As a VHT, I receive appreciation from the community	Y/N	if yes, how often:	once every two weeks	once a week	twice a week	daily

As a VHT, I consider myself to have received enough training	completely disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	completely agree

As a VHT, i'm disturbed by

➢ drugs and diagnostic tools that are frequently out of stock	completely disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	completely agree
➢ the fact that my job as VHT is difficult to combine with my regular job	completely disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	completely agree

➢ there is too much work as VHT for me, i feel overburned	completely disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	completely agree
➢ lack of supervision	completely disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	completely agree
➢ hostility from the community	completely disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	completely agree
○	O						
How much volunteering as a VHT did you do over the past month	not at all	only sporadically	once in the month	once every two weeks	once a week	twice a week	daily

Module 3: VFI: volunteering function inventory.

	Not at all important/accurate for you	Not important/not accurate	Somewhat unimportant/inaccurate	Neutral	Somewhat important/accurate	Important/accurate	Extremely important/accurate for you
Volunteering allows me to gain a new perspective on things.	1	2	3	4	5	6	7
Volunteering makes me feel needed.	1	2	3	4	5	6	7
Volunteering is a good escape from my own troubles.	1	2	3	4	5	6	7
Volunteering will help me succeed in my chosen profession	1	2	3	4	5	6	7
People I'm close to want me to volunteer.	1	2	3	4	5	6	7
My friends volunteer.	1	2	3	4	5	6	7
Volunteering experience will look good on my resume (CV)	1	2	3	4	5	6	7
I feel it is important to help others.	1	2	3	4	5	6	7
Others with whom I am close place a high value on community service.	1	2	3	4	5	6	7
Volunteering makes me feel better about myself	1	2	3	4	5	6	7
I am concerned about those less fortunate than myself.	1	2	3	4	5	6	7
Volunteering makes me feel important	1	2	3	4	5	6	7
I feel compassion toward people in need.	1	2	3	4	5	6	7
People I know share an interest in community service.	1	2	3	4	5	6	7
I can do something for a cause that is important to me.	1	2	3	4	5	6	7
I can learn more about the cause for which I am working.	1	2	3	4	5	6	7
Volunteering can help me get my foot in the door at a place where I'd like to work	1	2	3	4	5	6	7
No matter how bad I've been feeling, volunteering helps me to forget about it.	1	2	3	4	5	6	7
I can learn how to deal with a variety of people.	1	2	3	4	5	6	7

I can explore my own strengths.	1	2	3	4	5	6	7
I am genuinely concerned about the particular group I am serving.	1	2	3	4	5	6	7
Volunteering increases my self-esteem	1	2	3	4	5	6	7
I can make new contacts that might help my business OR career.	1	2	3	4	5	6	7
Volunteering is an important activity to the people I know best.	1	2	3	4	5	6	7
Volunteering is a way to make new friends.	1	2	3	4	5	6	7
Doing volunteer work relieves me of some of the guilt over being more fortunate	1	2	3	4	5	6	7
Volunteering lets me learn through direct “hands on” experience.	1	2	3	4	5	6	7
By volunteering, I feel less lonely.	1	2	3	4	5	6	7
Volunteering helps me work through my own personal problems.	1	2	3	4	5	6	7
Volunteering allows me to explore different career options.	1	2	3	4	5	6	7
Do you want to continue your activities as VHT	o Yes o No o I'm not sure						

Module 4: TPB: Theory of Planned Behaviour

I think treating each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month **is** **[good or bad]**	very bad	bad	somewhat bad	neutral	somewhat good	very good	excellent
[foolish or wise]	very foolish	foolish	somewhat foolish	neutral	somewhat wise	wise	very wise
[harmful or beneficial]	very harmful	harmful	somewhat harmful	neutral	somewhat beneficial	beneficial	very beneficial
[pleasant or unpleasant]	very unpleasant	unpleasant	somewhat unpleasant	neutral	somewhat pleasant	pleasant	very pleasant
[satisfying or unsatisfying]	very unsatisfying	unsatisfying	somewhat unsatisfying	neutral	somewhat satisfying	satisfying	very satisfying
[valuable or worthless]	completely worthless	worthless	somewhat worthless	neutral	somewhat valuable	valuable	very valuable
Those people most important to me think treating each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month **is [desirable or undesirable]**	completely undesirable	undesirable	somewhat undesirable	have no opinion	somewhat desirable	desirable	completely desirable
I wish to treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
Those people who are important to me think that I should treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	completely disagree	disagree	somewhat disagree	no opinion	somewhat agree	agree	completely agree
I think of myself as someone who would treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
Most of the VHT members that are important to me treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month :	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
If I wanted to, I could easily treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree

I am not the type of person that would treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home whenever they would present	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
Events or conditions outside my control will stop me from treating each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
I am determined to treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
It would go against my principles not to treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
If I wanted to, I could easily treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
How much control do you have over whether you can treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	very little control	little control	somewhat little control	neutral	somewhat control	considerable control	a great deal of control
How likely do you think it is that your fellow VHT members that are important to you, treat each child <5Y for Pneumonia, diarrhoea or Malaria at their home (home of VHT), whenever such a sick child presents during the coming month	very unlikely	unlikely	somewhat unlikely	neutral	somewhat likely	likely	very likely
It is mostly up to me whether I treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
I would feel guilty if I didn't treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree

It would be very difficult for me to treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
I will feel happy if I manage to treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
I do not intend to treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
Most People who are important to me treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
Being someone who would treat each child <5Y for Pneumonia, diarrhoea or Malaria whenever such a sick child presents **is an important part of who I am**	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
Those people who are important to me regularly tell me that I should treat each child <5Y for Pneumonia, diarrhoea or Malaria at my home (home of VHT), whenever such a sick child presents during the coming month	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
General attitudes							
Access to health care is a basic right of all children <5y	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
we would take care of children <5Y who are sick or have health issues	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree
Health care for children <5Y should be the top priority when making policy decisions	strongly disagree	disagree	somewhat disagree	neutral	somewhat agree	agree	strongly agree

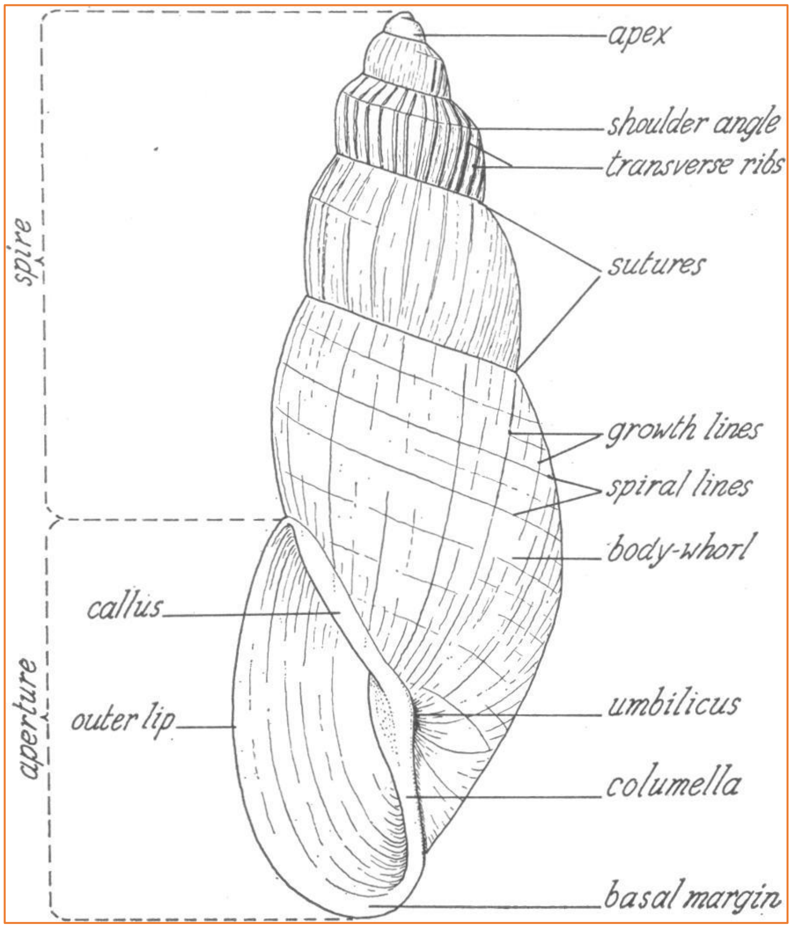
Appendix 6*: Snail Identification Key for Citizen Researchers*

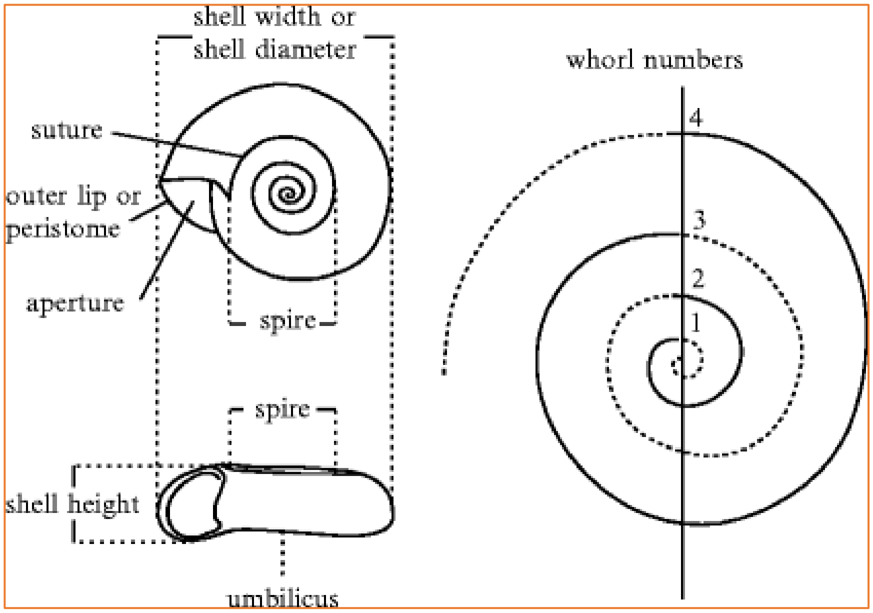
SNAIL IDENTIFICATION KEY FOR CITIZEN SCIENTISTS

Strategy:

- **Use physical specimens of the snails during training to aid in learning the names of the snails.**
- **Take picture of the snails so far collected from the field and associate them to their genus names.**
- **Emphasise identifying *Biomphalaria*, *Bulinus* and *Lymnaea* species**
- **Teach them easily confused genera of snails yet they are different**

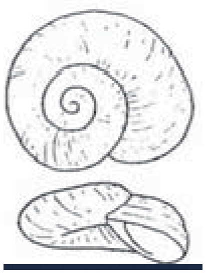
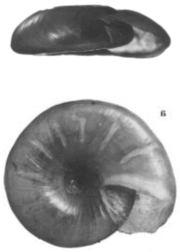
Structure of a typical water snail

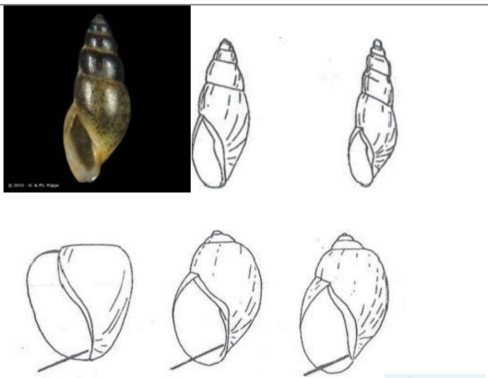
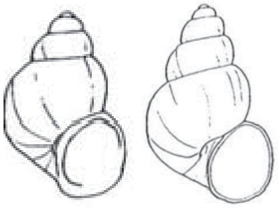




Mandahl-barth (1962)

Common Freshwater snail Genera of L. Albert and Surroundings

Genus	Image	Description (characteristics)
Biomphalaria	 Ponder et al. (2016)	• Medium size (shell height more than 2 mm but less than 6 mm). Diameter rarely reaches 20 mm • Rings (whorls) rounded (convex) on both sides. •
Ceratophallus	 Alè (1987)	• small size (shell height less than 2 mm). • Diameter less than 2½ times the height. • Smooth shell with no angle at the edge
Segmentorbis	 Brown (1994)	• small size (shell height less than 2 mm). • Shell is curved on the upper side (convex) but flat on the under side. • Shell has divisions (septa) on the under side
Lymnaea **(Radix)**		• Thin shell opening on the right (dextral) • Large, short, triangular antennae • Large opening higher than spire. • Large body whorl usually spotted

Bulinus	 Alè (1987)	• Thin shell opening on the **left** (sinistral) • May have very short or high spire
Gabbiella		• small size (shell height not more than 10mm). • Cone shaped • Colourless to pale brown shell. • Operculum (opening) central just below body wall
Pila		• Medium to large size (up to 40 mm) • Thick shell opening on the right (dextral) •
Mellanoides		
Bellamaya		• Shell apex sharply pointed • Medium size; dextral • Shell may have sculpture (markings)

Appendix 7*: Additional information collected by Citizen Researchers weekly*

ATRAP KOBO QUESTIONNAIRE SUGGESTIONS (To be uploaded on the mobile phone application)

1. Background information

Date ---------------------------- Time ----------------------------

Site Name ---------------------------- River ☐ Lake ☐ Pond ☐

Latitude ---------------------------- Longitude ---------------------------------

Other site characteristics with 50 m radius (tick where applicable)

Washing of clothes ☐

Washing Vehicles ☐

Swimming ☐

Bathing ☐

Fetching water ☐

Fishing ☐

Grazing animals ☐ Please specify -------------------------------------

Animal drinking place ☐ Please specify -------------------------------------

Crop growing ☐

Settlement (home) ☐

Garbage disposal point ☐

Flooded area ☐

Presence of human faeces ☐

Presence of fresh animal dung/urine ☐

Snail shell collection ☐

Site picture(s)

2. Snails collected at the site

Name	**Number**	**Pictures**
Biomphalaria		
Bulinus		
Lymnaea (Radix)		
All others		

3. Water parameters at each site

Temperature ------------------------

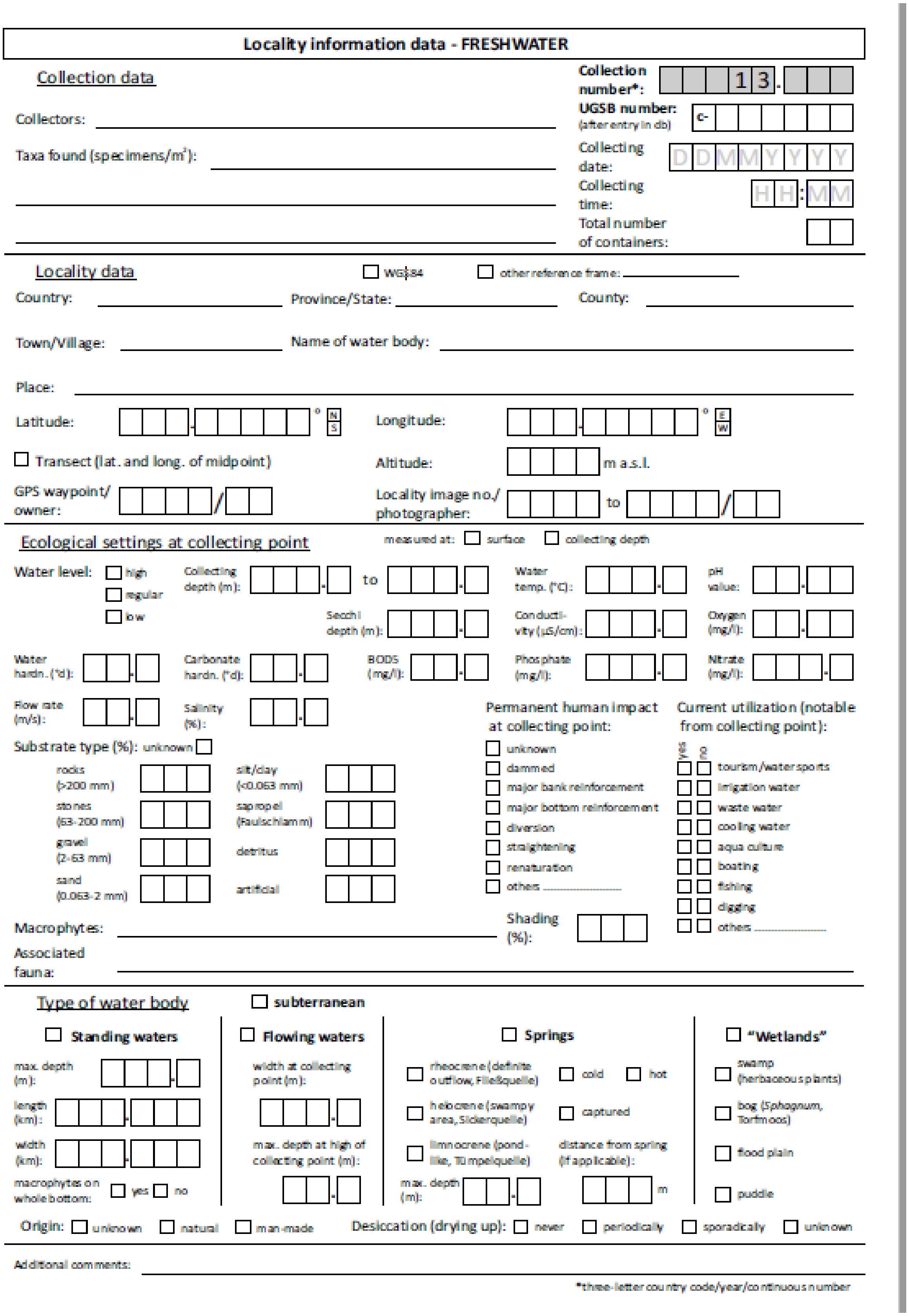
pH ------------------------

Conductivity ------------------------

TDS ------------------------

ETC

***Appendix 8**: Locality sheet for data recording by the PhD researcher*



Locality information data - FRESHWATER

Collection data

Collection number*: 13.

UGSB number: (after entry in db) C-

Collectors:

Taxa found (specimens/m²):

Collecting date: DDMMYYYY

Collecting time: HH:MM

Total number of containers:

Locality data ☐ WGS84 ☐ other reference frame:

Country:

Province/State:

County:

Town/Village:

Name of water body:

Place:

Latitude: ° N S

Longitude: ° E W

☐ Transect (lat. and long. of midpoint)

Altitude: m a.s.l.

GPS waypoint/ owner: /

Locality image no./ photographer: to /

Ecological settings at collecting point

measured at: ☐ surface ☐ collecting depth

Water level: ☐ high ☐ regular ☐ low

Collecting depth (m): . to .

Water temp. (°C): .

pH value: .

Secchi depth (m): .

Conductivity (µS/cm): .

Oxygen (mg/l): .

Water hardn. (°d): .

Carbonate hardn. (°d): .

BOD5 (mg/l): .

Phosphate (mg/l): .

Nitrate (mg/l): .

Flow rate (m/s): .

Salinity (%): .

Substrate type (%): unknown ☐

rocks (>200 mm)

silt/clay (<0.063 mm)

stones (63-200 mm)

sapropel (Faulschlamm)

gravel (2-63 mm)

detritus

sand (0.063-2 mm)

artificial

Permanent human impact at collecting point:

- ☐ unknown
- ☐ dammed
- ☐ major bank reinforcement
- ☐ major bottom reinforcement
- ☐ diversion
- ☐ straightening
- ☐ renaturation
- ☐ others ....

Current utilization (notable from collecting point):

yes	no	
☐	☐	tourism/water sports
☐	☐	irrigation water
☐	☐	waste water
☐	☐	cooling water
☐	☐	aqua culture
☐	☐	boating
☐	☐	fishing
☐	☐	digging
☐	☐	others ....

Macrophytes:

Shading (%):

Associated fauna:

Type of water body ☐ **subterranean**

☐ **Standing waters**

max. depth (m): .

length (km): .

width (km): .

macrophytes on whole bottom: ☐ yes ☐ no

☐ **Flowing waters**

width at collecting point (m): .

max. depth at high of collecting point (m): .

☐ **Springs**

☐ rheocrene (definite outflow, Fließquelle) ☐ cold ☐ hot

☐ helocrene (swampy area, Sickerquelle) ☐ captured

☐ limnocrene (pond-like, Tümpelquelle)

distance from spring (if applicable): m

max. depth (m): .

☐ **"Wetlands"**

☐ swamp (herbaceous plants)

☐ bog (*Sphagnum*, Torfmoos)

☐ flood plain

☐ puddle

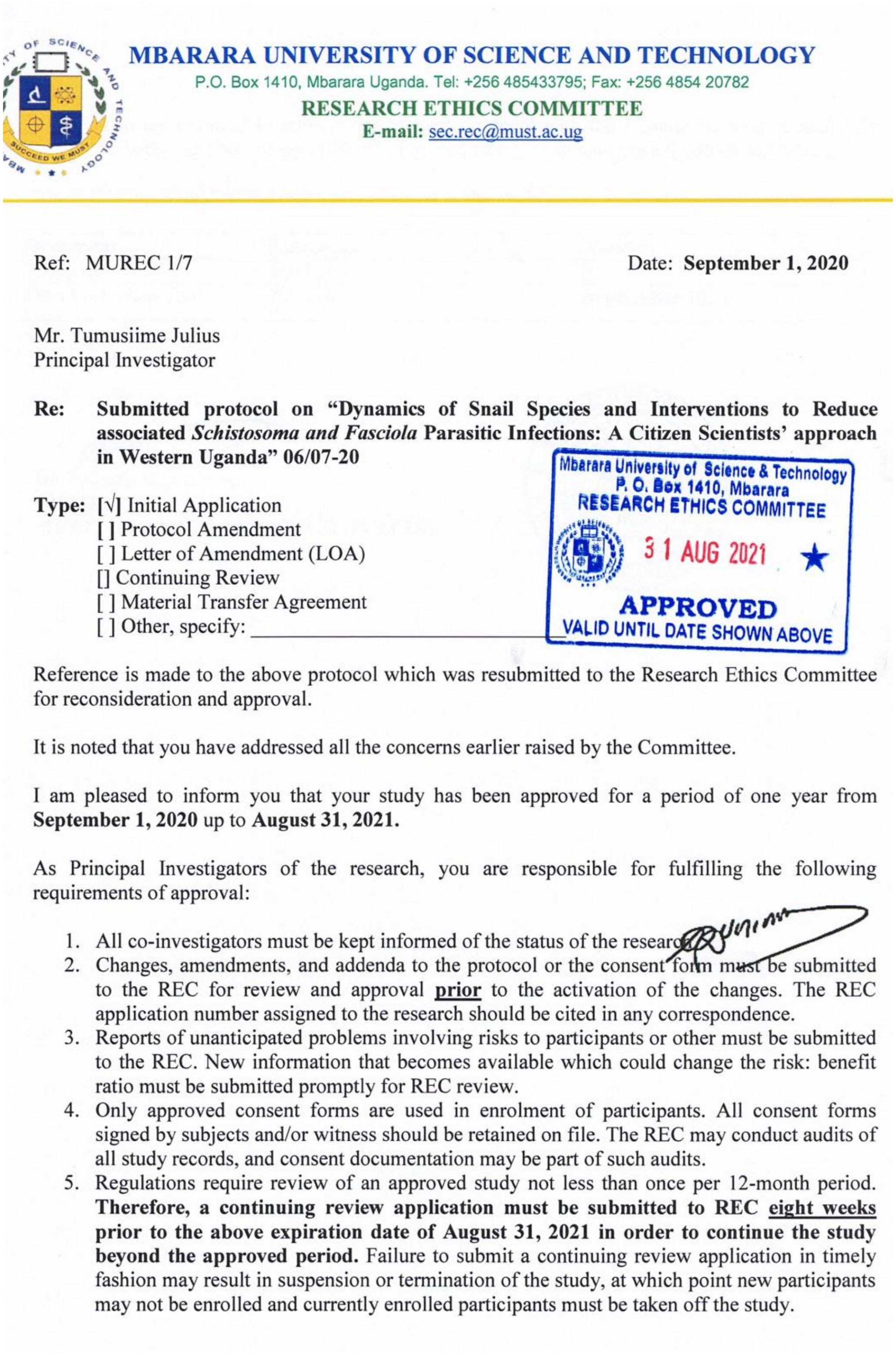
Origin: ☐ unknown ☐ natural ☐ man-made

Desiccation (drying up): ☐ never ☐ periodically ☐ sporadically ☐ unknown

Additional comments:

*three-letter country code/year/continuous number

Appendix 9: Consent form for citizen Researcher



MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY
P.O. Box 1410, Mbarara Uganda. Tel: +256 485433795; Fax: +256 4854 20782
RESEARCH ETHICS COMMITTEE
E-mail: sec.rec@must.ac.ug

Ref: MUREC 1/7 Date: **September 1, 2020**

Mr. Tumusiime Julius
Principal Investigator

Re: Submitted protocol on "Dynamics of Snail Species and Interventions to Reduce associated *Schistosoma and Fasciola* Parasitic Infections: A Citizen Scientists' approach in Western Uganda" 06/07-20

Type: [√] Initial Application
[ ] Protocol Amendment
[ ] Letter of Amendment (LOA)
[] Continuing Review
[ ] Material Transfer Agreement
[ ] Other, specify: ______________________________

Mbarara University of Science & Technology
P. O. Box 1410, Mbarara
RESEARCH ETHICS COMMITTEE
31 AUG 2021 ★
APPROVED
VALID UNTIL DATE SHOWN ABOVE

Reference is made to the above protocol which was resubmitted to the Research Ethics Committee for reconsideration and approval.

It is noted that you have addressed all the concerns earlier raised by the Committee.

I am pleased to inform you that your study has been approved for a period of one year from **September 1, 2020** up to **August 31, 2021.**

As Principal Investigators of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for review and approval **prior** to the activation of the changes. The REC application number assigned to the research should be cited in any correspondence.
3. Reports of unanticipated problems involving risks to participants or other must be submitted to the REC. New information that becomes available which could change the risk: benefit ratio must be submitted promptly for REC review.
4. Only approved consent forms are used in enrolment of participants. All consent forms signed by subjects and/or witness should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Regulations require review of an approved study not less than once per 12-month period. **Therefore, a continuing review application must be submitted to REC eight weeks prior to the above expiration date of August 31, 2021 in order to continue the study beyond the approved period.** Failure to submit a continuing review application in timely fashion may result in suspension or termination of the study, at which point new participants may not be enrolled and currently enrolled participants must be taken off the study.

6. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

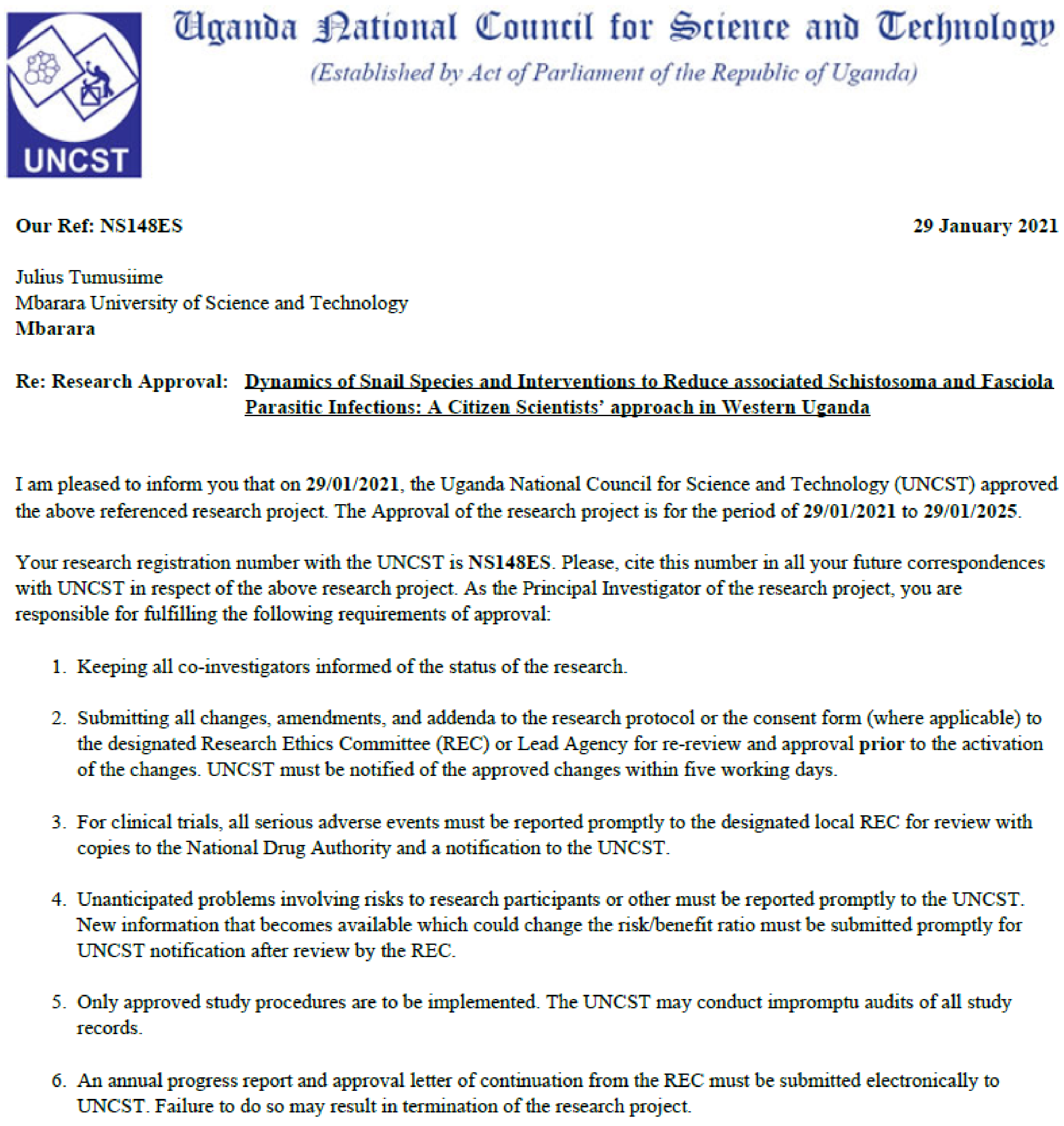
The following is the list of documents approved in the application:

Document	**Language**	**Version**
Proposal	English	**2**
Data Collection Tool	English	**September 2020**

I wish you all the best.

Dr. Francis Bajunirwe
CHAIR,
MUST RESEARCH ETHICS COMMITTEE

Appendix 10: The Uganda National Council for Science and Technology Approval for this study



UNCST

Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: NS148ES **29 January 2021**

Julius Tumusiime
Mbarara University of Science and Technology
Mbarara

Re: Research Approval: **<u>Dynamics of Snail Species and Interventions to Reduce associated Schistosoma and Fasciola Parasitic Infections: A Citizen Scientists' approach in Western Uganda</u>**

I am pleased to inform you that on **29/01/2021**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **29/01/2021** to **29/01/2025**.

Your research registration number with the UNCST is **NS148ES**. Please, cite this number in all your future correspondences with UNCST in respect of the above research project. As the Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. Keeping all co-investigators informed of the status of the research.
2. Submitting all changes, amendments, and addenda to the research protocol or the consent form (where applicable) to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local REC for review with copies to the National Drug Authority and a notification to the UNCST.
4. Unanticipated problems involving risks to research participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST notification after review by the REC.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. An annual progress report and approval letter of continuation from the REC must be submitted electronically to UNCST. Failure to do so may result in termination of the research project.

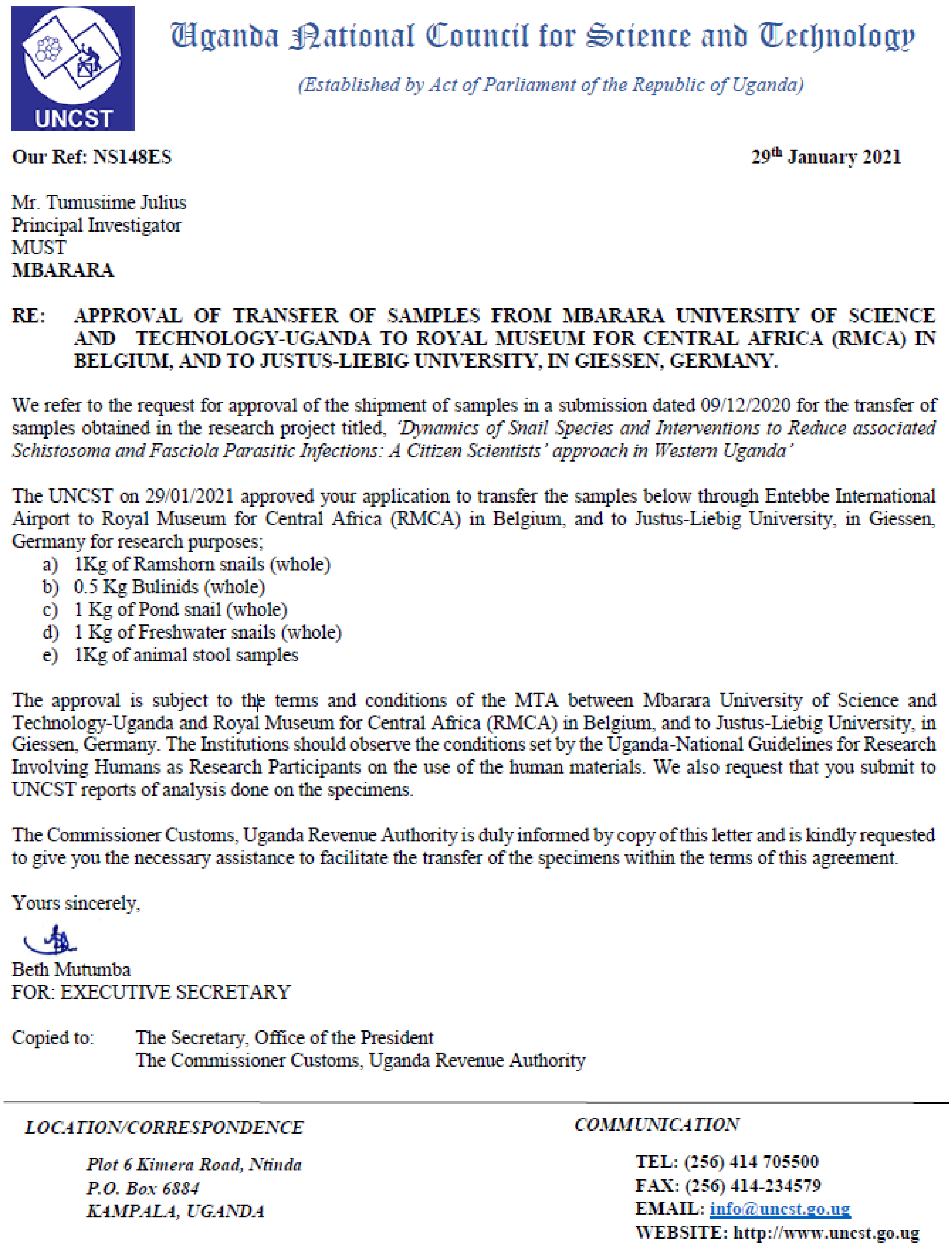
Please note that this approval includes all study related tools submitted as part of the application as shown below:

No.	Document Title	Language	Version Number	Version Date
1	Informed concent form for citizen researchers	English	APPROVED BY MUST REC	10 July 2020
2	MoU with citizen researchers	English	APPROVED BY MUST REC	10 July 2020
3	Project Proposal	English	MUST REC APPROVED PROPOSAL	
4	Approval Letter	English	MUST REC APPROVED PROPOSAL	2020-07-10

Yours sincerely,

Hellen Opolot
For: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Appendix 11: The material transfer agreement for transportation of the samples out of the country for molecular analysis as approved by the Uganda National Council for Science and Technology



Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

UNCST

Our Ref: NS148ES **29th January 2021**

Mr. Tumusiime Julius
Principal Investigator
MUST
MBARARA

RE: APPROVAL OF TRANSFER OF SAMPLES FROM MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY-UGANDA TO ROYAL MUSEUM FOR CENTRAL AFRICA (RMCA) IN BELGIUM, AND TO JUSTUS-LIEBIG UNIVERSITY, IN GIESSEN, GERMANY.

We refer to the request for approval of the shipment of samples in a submission dated 09/12/2020 for the transfer of samples obtained in the research project titled, *'Dynamics of Snail Species and Interventions to Reduce associated Schistosoma and Fasciola Parasitic Infections: A Citizen Scientists' approach in Western Uganda'*

The UNCST on 29/01/2021 approved your application to transfer the samples below through Entebbe International Airport to Royal Museum for Central Africa (RMCA) in Belgium, and to Justus-Liebig University, in Giessen, Germany for research purposes;

a) 1Kg of Ramshorn snails (whole)
b) 0.5 Kg Bulinids (whole)
c) 1 Kg of Pond snail (whole)
d) 1 Kg of Freshwater snails (whole)
e) 1Kg of animal stool samples

The approval is subject to the terms and conditions of the MTA between Mbarara University of Science and Technology-Uganda and Royal Museum for Central Africa (RMCA) in Belgium, and to Justus-Liebig University, in Giessen, Germany. The Institutions should observe the conditions set by the Uganda-National Guidelines for Research Involving Humans as Research Participants on the use of the human materials. We also request that you submit to UNCST reports of analysis done on the specimens.

The Commissioner Customs, Uganda Revenue Authority is duly informed by copy of this letter and is kindly requested to give you the necessary assistance to facilitate the transfer of the specimens within the terms of this agreement.

Yours sincerely,

Beth Mutumba
FOR: EXECUTIVE SECRETARY

Copied to: The Secretary, Office of the President
The Commissioner Customs, Uganda Revenue Authority

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
P.O. Box 6884
KAMPALA, UGANDA

COMMUNICATION

TEL: (256) 414 705500
FAX: (256) 414-234579
EMAIL: info@uncst.go.ug
WEBSITE: http://www.uncst.go.ug

MATERIALS TRANSFER AGREEMENT (MTA) FOR NONHUMAN BIOLOGICAL MATERIAL

Signature Date (Effective date of the MTA) ---------------------- ***a. Parties***

This MTA is by and between the following parties:

The principal investigator

Name: Tumusiime Julius

Student registration number: 2019/PHD/032/PS

Tel: +256(0)785886136

Email: jtumusiime90@must.ac.ug

Research Institution: Mbarara University of Science and Technology

Permanent Address: Mbarara University of Science and Technology,
P.O. Box 1410, Mbarara-Uganda

Signature:

Date: December 03, 2020

(Hereof referred to as "the researcher")

And

The representatives of the principal investigator (Supervisors)

1. Name: Dr. Tine Huyse Promoter "Action Towards Reducing Aquatic snail-borne Parasitic diseases (ATRAP)" project

Institution: Royal Museum for Central Africa
Leuvensesteenweg 13
3080 Tervuren, Belgium

Email: tine.huyse@kuleuven.be Tel: +32497299748

Signature:

Date: 13/11/2020

2. Name: Assoc. Prof. Dr. Christian Albrecht

Institution: Institute of Animal Ecology and Systematics, Justus Liebig University Giessen Heinrich-Buff-Ring 26-32 (IFZ), D-35392 Giessen, Germany

Phone: (+49) 641 9935722, fax: (+49) 641 9935709, email: christian.albrecht@allzool.bio.uni-giessen.de URL: www.uni-giessen.de/wilke

Signature:

Date: 16/11/2020

3. Name: Dr. Casim Umba Tolo

Local Promoter “Action Towards Reducing Aquatic snail-borne Parasitic diseases (ATRAP)” project.

Institution: Mbarara University of Science and Technology, Mbarara-Uganda

Tel: +256 772 2837055

Email: tolocas@must.ac.ug

Signature:

Date: 04/12/2020

4. Name: Assoc. Prof. Dr. Grace Kagoro Rugunda

Institution: Mbarara University of Science and Technology, Mbarara-Uganda

Tel: +256 772 576622

Email: kgraceug2002@must.ac.ug

Signature:

Date: 4th December 2020

This material transfer agreement (MTA) has been organised to enable authorisation, by Uganda National council of Science and Technology (UNCST), for the transfer of research material from Uganda to Royal Museum for Central Africa (RMCA) in Belgium, and to Justus-Liebig University, in Giessen, Germany.

The materials/samples are to be transferred solely for a PhD research project titled "Dynamics of Snail Species and Interventions to Reduce associated *Schistosoma* and *Fasciola* Parasitic Infections: A Citizen Scientists' approach in Western Uganda". The PhD project is part of a bigger research project "Action Towards Reducing Aquatic snail-borne Parasitic diseases (ATRAP)". ATRAP is an interdisciplinary project cutting across Biology, Geography and Social Sciences in contributing towards reduction of the Neglected Tropical Diseases of schistosomiasis (bilharzia) and fasciolosis (liver fluke infections) that are transmitted by freshwater snails. The ATRAP project under which research materials are to be transferred, has already been cleared by UNCST and the reference number is **NS128ES**. The PhD research project has also been cleared by institutional ethical review board of Mbarara University of Science and Technology under reference number MUREC1/7.

b. Description of materials being transferred

The materials being transferred/exchanged are described below:

Species Name		Specimen specifications	Quantity
Scientific Name	Common Name		
Biomphalaria spp	Ramshorn snails	Whole animals	1 kg
Bulinus spp	Bulinids	Whole animals	0.5 kg
Radix spp	Pond snail	Whole animals	1 kg
Gastropoda	Freshwater snails	Whole animals	1 kg
Animal stool	Stool samples	Faecal samples	1 kg

c. Purpose and usage

The samples shall be taken to the molecular laboratory of the Royal Museum for Central Africa in Belgium and Justus Liebig University, Giessen-Germany for analysis. The laboratories have been identified because they have specialised equipment in advanced molecular analysis like PCR and next generation sequencing to concurrently extract DNA for snail vectors and the parasites they host. Animal stool samples will also be analysed for presence of *Schistosoma* and *Fasciola* parasite eggs with the aim of profiling animals acting as reservoir hosts of the parasites. The research will benefit from the availability of specialised equipment lacking in Uganda, and technical support provided by the supervisors.

All the studies on the transferred materials/samples have no commercial background and are purely basic science. The principle investigator (Mr. Tumusiime Julius), who is a Ugandan citizen, will be author of all the scientific papers related to the material studied.

The following are some of the potential benefits of the research project to the community:

- The project is expected to contribute and fill significant knowledge gaps in the spread of *Schistosoma* and *Fasciola* parasites that cripple the health and incomes of communities.
- The molecular analyses will allow to estimate the risk for infection with *Schistosoma* and *Fasciola* parasites in the southern Lake Albert region
- Based on this risk maps will be constructed that can guide targeted snail control
- The project will produce academic manuscripts that will be published in peer reviewed international journals and shared with the wider scientific community and health practitioners.
- In addition, the outcome will also be communicated to the respective communities through awareness campaigns in order to inform them on disease risks for them and their livestock, and about preventive measures that can be taken.

d. User(s)

Authorized users of the materials include the parties mentioned above from Royal Museum for

Central Africa-Belgium, Justus Liebig University Giessen-Germany and Mbarara University of Science and Technology-Uganda in the postgraduate study groups. The users cited agree to abide by the terms and conditions of the MTA. Transfer to third parties not mentioned in the MTA is prohibited without written consent of the provider organization or their assignees. The use of the materials transferred shall adhere to the principles of fairness and equity as stipulated in Article 5 of the Nagoya Protocol.

e. Location (The place where material is to be transferred, used and or stored).

Institution	Royal Museum for Central Africa	Justus Liebig University
Department	Biology	Animal Ecology & Systematics
Street	Leuvensesteenweg 13	Heinrich-Buff-Ring 26-32, D-35392
City	Tervuren	Giessen
Country	Belgium	Germany

f. Period of use of material

The material is to be used during the period of the PhD study course (4 to 6 years). Material transfer is planned for a period between January 2021 to December 2024. This date may be extended by written mutual consent of the parties. At the termination date, the unused samples will be kept at the Biology lab of Mbarara University of Science and Technology, Royal Museum for Central Africa, and Justus Liebig University for future reference.

g. Disposal of material

Remaining snail shells and the sequencing products will be disposed off following proper and safe laboratory procedures in the bounds of the laws of the Kingdom of Belgium and the Federal Republic of Germany.

h. Restrictions

There are particularly no specific restrictions for the recipient organization. The material will be used for a PhD research project and follow-up studies, and the laboratory procedures will be conducted under the laws of the research ethics committee in the host institutions of the principle investigator.

i. Ownership of derivatives

For all the future major research findings with the transferred material, the Principal investigator will be an author with co-authors such as the supervisors and some other colleagues.

j. Ownership of the product

There are no intended/ anticipated new products from the use of the transferred material apart from the research thesis, which is automatically owned by the principal investigator.

k. Commercialisation rights

The research project whose materials are to be transferred is purely academic with no commercial value whatsoever.

l. Technology transfer

Possible forms of technology both in soft and hardware to be transferred to Uganda shall include:

- Expertise in bioinformatics and molecular analyses
- Snail sampling and parasite study equipment like scoop nets, microscopes and other scientific equipment
- Water quality measuring equipment like multi-probe meters, spectrophotometers, etc.

m. Publication

Publication of research findings is one of the requirements for progress and completion of the PhD project in the awarding institution. Therefore, publications in peer review journals will be done by the principal investigator during the course of the study.

n. Citation requirements

The organizations of affiliation as well as all permit references will be acknowledged during the publications of research findings. The research permits provided by UNCST for the ATRAP project will be cited/acknowledged in the published articles. The project seeks due compliance with the Nagoya Protocol guidelines.

o. Governing law

This MTA puts into consideration the governing laws of the provider's and recipient's countries. Major considerations of the Nagoya protocol are respected in this MTA.

p. Responsibilities

The Principal investigator is responsible for the proper handling and use of the material.

q. Liability

Both the provider and recipient are accountable for any misuse or consequences of use of the material. Parties agree on liability.

r. Warranty

Provider makes no representations and extends no warranties of any kind, either expressed or implied.

s. Amendment

The MTA may be amended at any time by written mutual consent of the parties.

t. Termination of MTA

The MTA may be terminated by either party providing a written notice in an agreed time frame.

In the event that the project terminates, for reasons such as project completion or withdrawal of funding by the scholarship providers or any other plausible reason, the principal investigator will provide the UNCST with a Termination Report.

Appendix 12: Informed consent form for the citizen scientists to participate in the study

MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY

RESEARCH ETHICS COMMITTEE

P.O. Box 1410 Mbarara, Tel: +256-48-543-3795, Fax: +256-48-542-0782

E-mail : irc@must.ac.ug, mustirb@gmail.com

INFORMED CONSENT FORM

Dynamics of Snail Species and Interventions to Reduce associated *Schistosoma and Fasciola* Parasitic Infections: A Citizen Scientists' approach in Western Uganda

Principal Investigator(s)

Tumusiime Julius

Introduction

Hello, I am a student of Mbarara University of Science and Technology conducting an academic study aimed at understanding factors favouring the spread snail-borne infections among rural communities and livestock of Western Uganda.

Brief background to the study

The research project for which you are being asked to participate is focusing on understanding Schistosomiasis (Bilharzia) and Fasciolosis (Liver fluke) infections around the Lake Albert region of western Uganda. These diseases currently present serious public health and socio-economic problems not only in Uganda but the world at large. It is hoped that the outcome of the study will greatly help in the prevention of the said diseases which can later be replicated to other areas with same or similar problems.

Purpose of the research project

This study aims at understanding the contribution of snail vectors in the spread of schistosomiasis and fasciolosis risk in your community and the factors favouring the snail survival and reproduction. You, as a citizen researcher, have been selected to participate in this project to participate in monitoring the snail populations and changes in the water environment in which they live. You will be trained on how to carry out your duties in a safe way without putting your life at risk of the said diseases. You will be required to participate in the project for one year initially but the engagement is renewable on mutual agreement.

Why you are being asked to participate?

You have been asked to participate in the study because the community leadership and the project team have selected you as a person capable to use the tools that will be provided to you and effectively collect the required data. Additionally, your parish has been identified as a high-risk area for contracting schistosomiasis. you will be instrumental in later community sensitization.

Procedures

If you agree to participate in the study, you will receive special training on how to collect data. You will be provided data collection equipment including a scoop net, water testing strips, a thermometer, a smart phone, protective gear (gum boots and gloves) and a field notebook. You will follow the guidelines in an application in the phone to fill the questionnaire and submit the filled questionnaire online. You will be assigned up to a maximum of four water contact points in your parish and facilitated to move from one water with a modest transport refund and internet data to submit the filled questionnaire.

Risks or discomforts

The main risk of participating is exposure to contaminated water that could cause you to contract schistosomiasis. The project however values your safety above anything else. You will therefore be trained and equipped to avoid this risk. Because you will move between different sites in your community through out the year, you may face elements of weather like rainfall and sunshine. We encourage you to collect data only when it is safe to do so. Additionally, you will be required to dedicate only one day in a week so the risk is expected to be minimal.

Benefits

The most important benefit of your participation in this project is gaining knowledge on how schistosomiasis and fasciolosis spread, and the favourable conditions for multiplication of the vector snails. This will help in sensitizing to control the spread of schistosomiasis and fasciolosis in your community.

Incentives or rewards for participating

The project team will provide a modest incentive of 75,000 monthly to facilitate you to do the work. You will also be provided with a branded t-shirt, a smart phone, gum boots, and materials for your safety. The phone is project property but you may use it for personal communication as long as you protect its safety.

Protecting data confidentiality

Any information shared with the research team will be treated as confidential. When processing and publishing data, anonymity is guaranteed, as all data will be coded. This means that data that can identify a citizen researcher will be systematically coded such that your identity can be protected.

Protecting subject privacy during data collection

Your privacy is important to us as indeed it is your right. In case any private data like pictures of you are needed, you will be requested to permit that the picture is taken. In case you do not agree, there will not be any consequences whatsoever.

Right to refuse or withdraw

Your participation in this study is voluntary, you have the right not to participate in the study if you so wish or to leave at any moment. The terms of termination of your engagement with us are outlined in a detailed Memorandum of Understanding, a copy of which you will be provided for your approval.

Who do I ask/call if I have questions or a problem?

In case of further information, do not hesitate to contact me, or my supervisor or the MUST-REC on the telephone contacts below:

1. Mr. Tumusiime Julius, (Researcher) Tel. 0785886136
2. Assoc. Prof. Grace Kagoro Rugunda (Supervisor) Tel. 0772576622
3. Dr. Casim Umba Tolo (Supervisor) Tel. 0772837055
4. Dr. Francis Bajunirwe (Chairman, MUST-REC) Tel. 0485433795

What does your signature or thumbprint on this consent form mean?

Your signature on this form means that you have been informed about this study’s purpose, procedures, possible benefits and risks and that you have been given the chance to ask questions before you sign. You have voluntarily agreed to participate in this study.

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _
Name of adult participant	Signature of participant or Legally authorized representative	Date
_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _
Name of person obtaining consent	Signature	Date
_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _
Print Name of witness	Signature or thumbprint or mark	Date

Appendix 13: Memorandum of understanding signed between Citizen Scientists and MSUT ATRAP project before undertaking the activities

MEMORUNDUM OF UNDERSTANDING BETWEEN MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY (MUST) TOGETHER WITH THE ATRAP CONSORTIUM AND THE CITIZEN RESEARCHERS

INTRODUCTION

Aware that Mbarara University of Science and Technology (MUST) represented by Dr. Casim Umba Tolo in partnership with the ATRAP project partners intend to collect data about the population dynamics of freshwater snails, water parameters, and the behaviours that expose communities to infections transmitted by freshwater snail-borne diseases like bilharzia, in selected sites of Lake Albert and surrounding areas in collaboration with Citizen Researchers. Aware of the above, MUST/ATRAP project and .... (Here after referred to as Citizen Researcher) of .... (village/affiliation) have agreed on the following:

OPERATION AREA: .... sub county...Parish**.**
Number of water contact sites to monitor: ....

ARTICLE: **1- Project title:** Action Towards Reducing Aquatic snail-borne Parasitic diseases (ATRAP).

ARTICLE: **2- DURATION**: The engagement is foreseen for an initial period of one year with possibilities of renewal based on positive evaluation (with a maximum of 3 years in total). The process officially starts on March ...., 2020 and shall end on March ...., 2021.

ARTICLE: 3- OVERALL OBJECTIVE

To monitor changes in the number of freshwater snails in relation to the changes in the waters they inhabit, as well as to report on behaviour and practices of the communities that expose them and their livestock to snail-borne parasitic diseases in the area of operation (water contact sites). These aspects of the study are recorded through weekly visits to selected water contact sites. The observations are recorded as written notes in a diary and through the submission of reports via the Kobo application on a smartphone provided by the project. These reports are submitted digitally on a weekly basis to the MUST supervisor. In addition, the Citizen Researcher disseminates information on snail-borne diseases received from the ATRAP project team to his/her community as need arises.

ARTICLE: 4- OVERALL OUTPUT:

The Citizen Researcher will provide his/her diary to MUST supervisor at each Citizen Researcher’s meeting upon request. The Citizen Researcher will submit correctly filled in report protocols regarding snail identity and number, as well as supporting non-snail data as required by the ATRAP team supervisor. S/he will monitor the snail population without disrupting the natural recovery or decline of snail populations.

ARTICLE: 5- OWNERSHIP AND USE OF EQUIPMENT: The Citizen Researcher will be provided with a smartphone, gumboots, gloves, a thermometer, scoop net, water testing strips, a diary, t-shirt(s) and additional equipment if deemed necessary for the job and when available. The Citizen Researcher is allowed to use the smartphone for personal purposes but is responsible for the maintenance and security of the device (**if proven destroyed or lost beyond doubt, it will not be replaced, and the contract will be discontinued**). Excessive downloading and using applications can harm the phone and should thus be avoided.

Project equipment remains the property of the MUST/ATRAP project and shall be maintained by the Citizen Researcher during the project period and will be retuned in sound state at the end of the project. If, for any reason, the contract or collaboration with the Citizen Researcher is stopped, the Citizen Researcher has to return all equipment back to MUST within four (4) weeks. Only the t-shirt and gumboots may become the property of the Citizen Researcher after a period of twelve (12) months of continuous service as a Citizen Researcher in the ATRAP project.

ARTICLE: 6 DATA SHARING AND GPDR

All data reported by the Citizen Researchers will be the property of MUST and the ATRAP consortium, and can be used for scientific and communication purposes without further permission. All third parties that want to make use of the data are referred to the website https://www.citizenscienceuganda.info/datarequest.html

Data on snail distribution can be shown online, with the exception of photo material where individuals can be recognized. Privacy will be respected at all times and the Citizen Researchers will not take pictures of people in the public space without their permission (conform the new European legislation GDPR (General Data Protection Regulation)).

ARTICLE: 7 PERSONAL SAFETY

All Citizen Researchers will receive adequate training on the symptoms, prevention and treatment of snailborne diseases and the risks of the field sampling before the start of the study. They will always wear protected gear (gumboots and gloves) when sampling at the water contact sites. They will not put themselves at risks; in unsafe weather conditions or any other suspect circumstances, all activities will be stopped immediately.

ARTICLE: 8- TERMS AND CONDITIONS OF THE PARTIES TO THIS MOU a) MUST/ATRAP project

- Shall ensure that all activities are implemented in the manner such that the Citizen Researcher takes lead and manages the collaborative process with the communities with the support of the local leadership.
- Shall meet the financial requirements (related to the project's data collection) of the Citizen Researcher for the period of engagement as per the section on terms of facilitation.
- Shall provide technical assistance- Trainings, Monitoring and Assessment of the Citizen Researcher.
- Shall provide information and Training Guides where necessary to the Citizen Researcher.

b) Citizen Researcher:

- Timely documentation and reporting of all snail and non-snail data within your area of deployment according to articles 3 and 4. (Note: You will need permission from your MUST supervisor in order to operate outside the geographical area you have been allocated). To the very best of your ability (taking safety issues into account, see article 7) each water contact point should be visited once every week and a report submitted thereafter.
- Provide any extra information on your report as requested by the MUST supervisor.
- Participate in meetings/trainings organised by MUST/ATRAP project.
- Ensure participation of local leadership in your area of deployment.
- Maintain project equipment under your custody in a safe and sound state.
- Perform any other duties assigned to you by your immediate supervisor
- Take up the role of a devoted Citizen Researcher and act as a contact point for researchers within the MUST/ATRAP project.
- Disseminate information received from the project within the community according to established strategies discussed with MUST/ATRAP project members.

ARTICLE: 9- MONITORING/ EVALUATION:

- The Research Assistant from MUST, (Ben Lukubye, 0759668325, lukubyeben@gmail.com) shall be the MUST supervisor and contact person for concerns on project activities. He will be working alongside PhD students Jef Brees (KU Leuven), Julius Tumusiime (MUST), Mercy-Gloria Ashepet (KU Leuven) and Maxson Anyolitho (MUST).
- Following the reports submitted, your MUST supervisor shall make the necessary follow-ups (announced/unannounced) to verify the data.
- The report from the participatory evaluation (conducted by both community leaders and MUSTATRAP project team) shall contribute to the contract renewal whenever necessary.

ARTICLE: 10a- Terms of facilitation:

- You shall receive UGX 55, 000/= fixed facilitation per month plus 10,000/= per month for internet data and additional 10,000/= to facilitate your transport to your study sites. You shall therefore receive a total sum of **75,000/=** which shall be paid into your bank account or phone account after approval of the reports (by your MUST supervisor) made at the end of every month.

- **10b-** Here below you indicate your preferred mode of payment and details

Account name: ....

Number: ....

Bank/Mobile service provider: ....

ARTICLE: 11- TERMINATION:

- Any party has the right to terminate this contract at any time.
- Written communication indicating reasons for discontinuation shall be made and, in this case, provide for four weeks in advance for the termination to be effected.
- In case of contract termination, all equipment and data collected so far by the Citizen Researchers will be returned to MUST before the effective termination of the collaboration.

ARTICLE: 12- MODIFICATIONS:

Modification of the terms of this Memorandum, including any modification of the scope of the services (with exception to changes in the protocols) and responsibilities, may only be done by written and signed agreement between both parties indicating any changes that will take course four weeks after the signing of amended copies.

ARTICLE: 13- DISCLAIMER

The Citizen Researcher willingly accepts to participate in the ATRAP project for the good of the community at large. MUST or any of its partners in the ATRAP project will not take responsibility of any eventualities that may arise during the project activities. ATRAP therefore does not guarantee any form of insurance to the Citizen Researchers. The ATRAP team will however endeavour to train the Citizen Researchers to ensure appropriate safety measures are taken whenever carrying out ATRAP project activities.

In witness hereof both parties have set their hands hereunto this day……........of March 2020 at Mbarara University of Science and Technology, Mbarara.

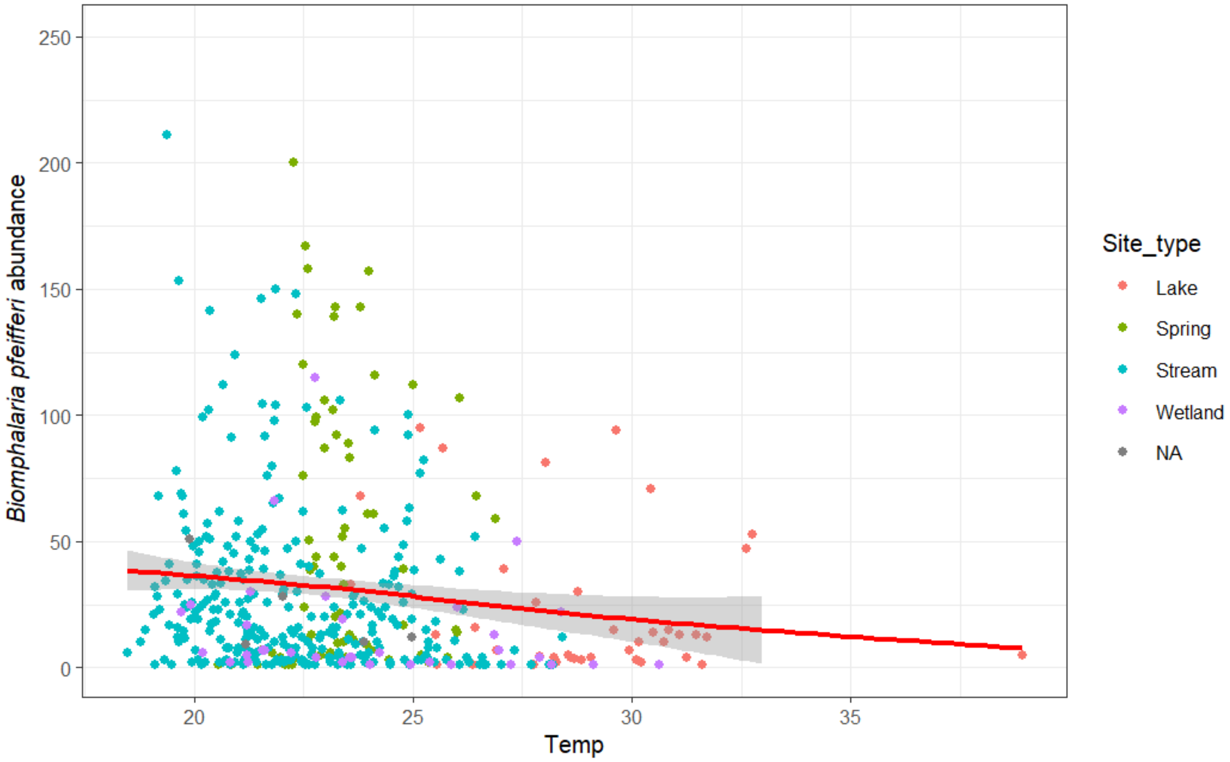
Name………………………………………………………Sign…..…………………………………

MUST/ATRAP Local Promoter

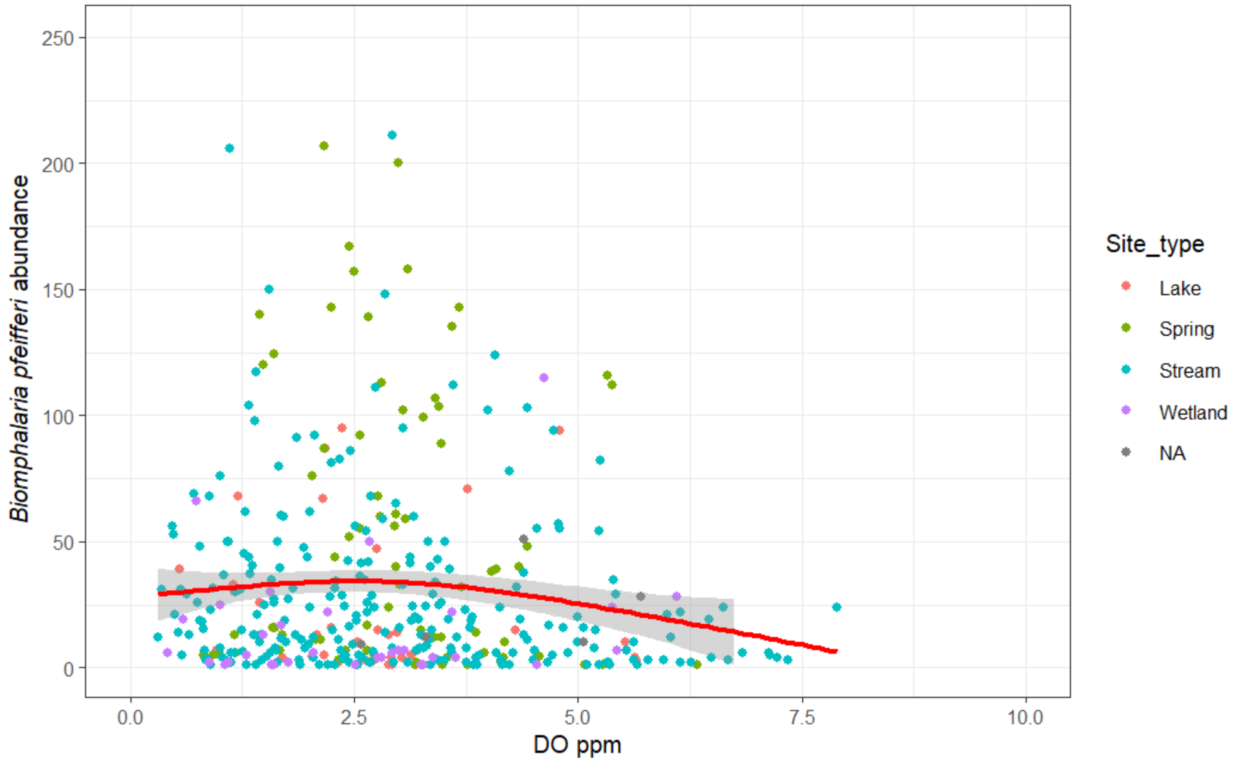
Name ……………………………………………………….Sign…………………………………

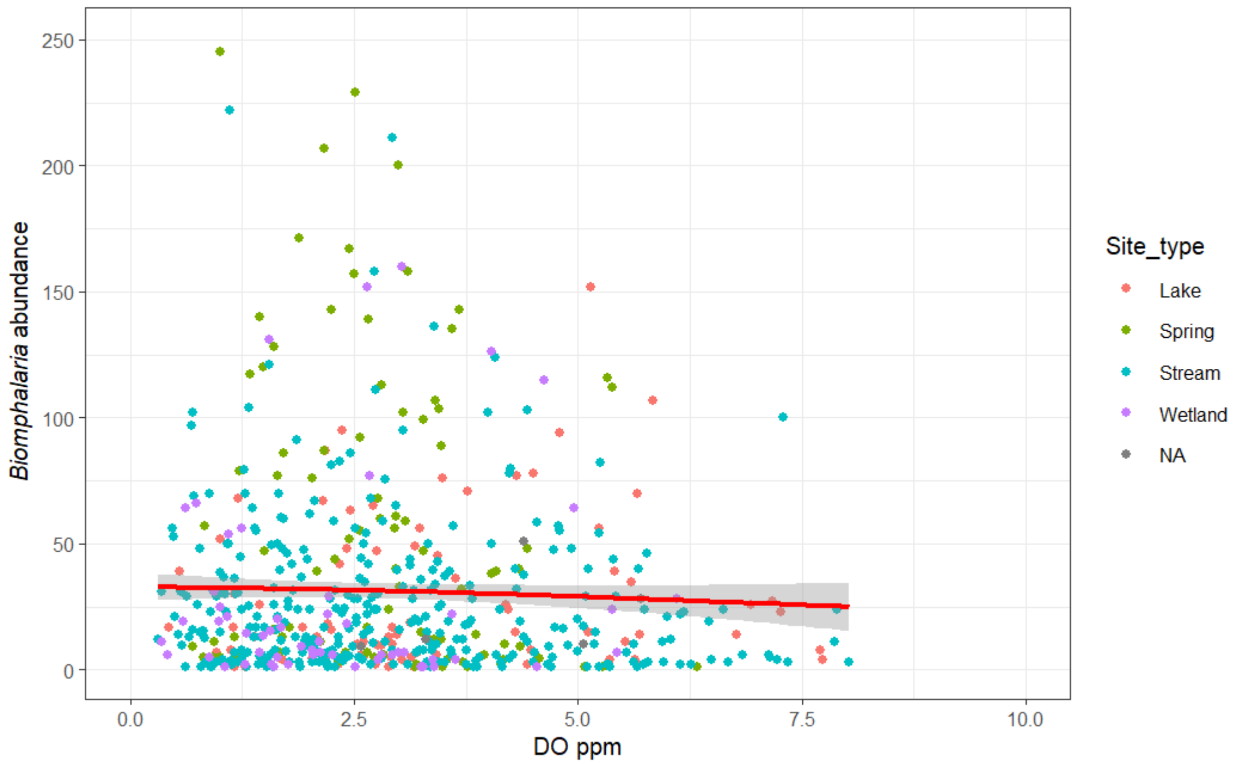
Citizen Researcher

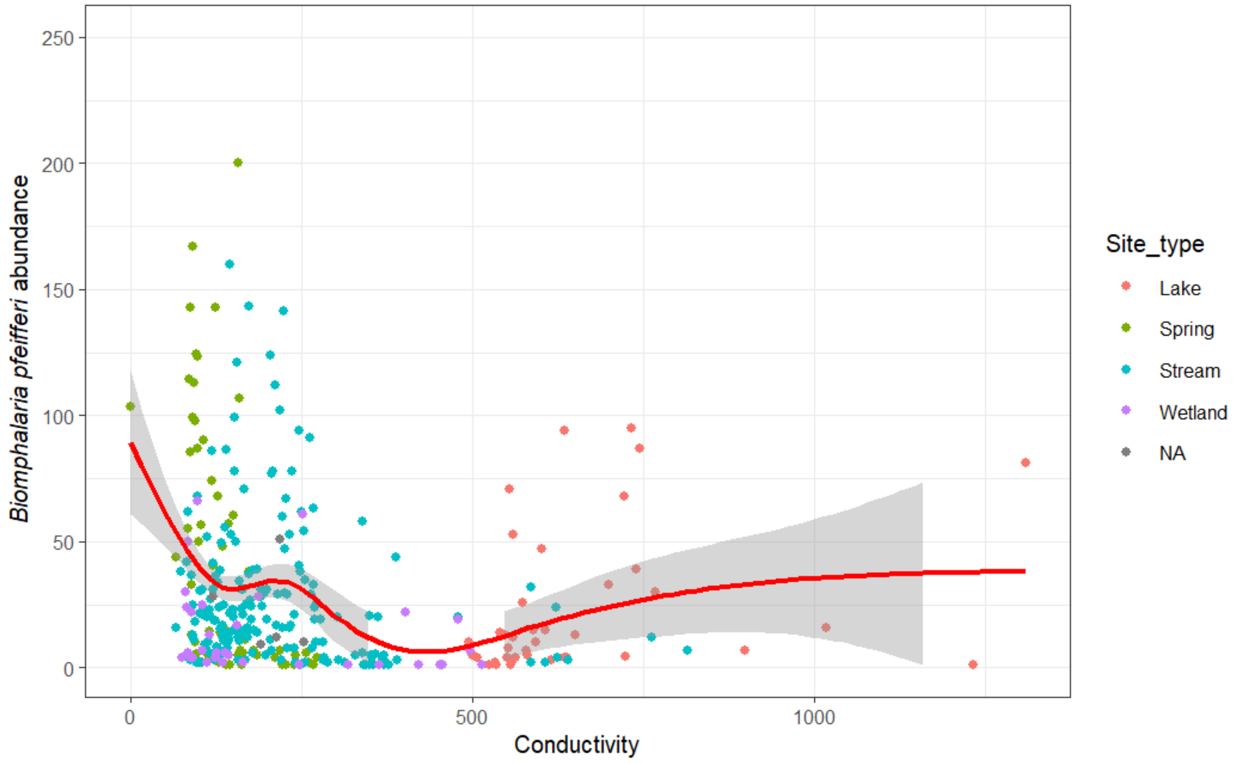
Appendix 14: Variation of Biomphalaria spp. abundance with selected physicochemical parameters in the Lake Albert region. The site types are indicated by different colour codes of the points. For each parameter, B. pfeifferi was singled out as it constituted the greatest majority of the snails in the genus in the upland sites. A similar plot was made for the genus level.

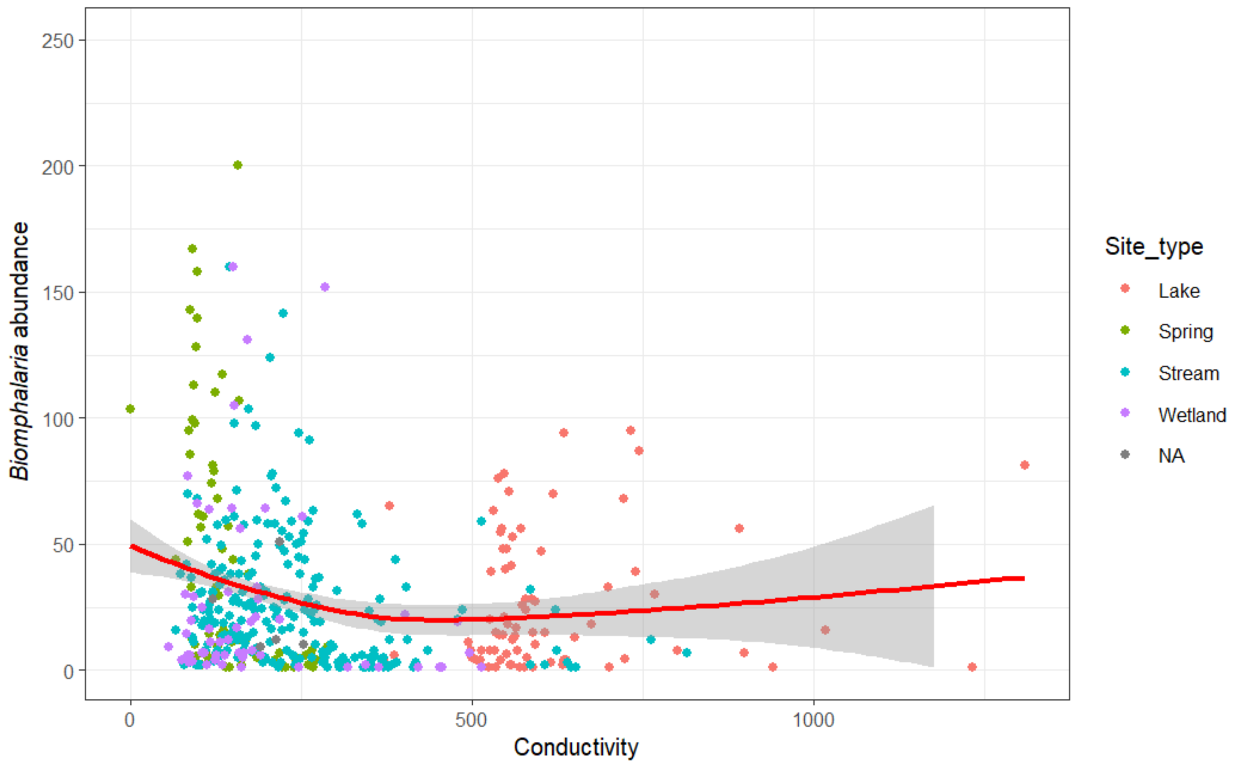


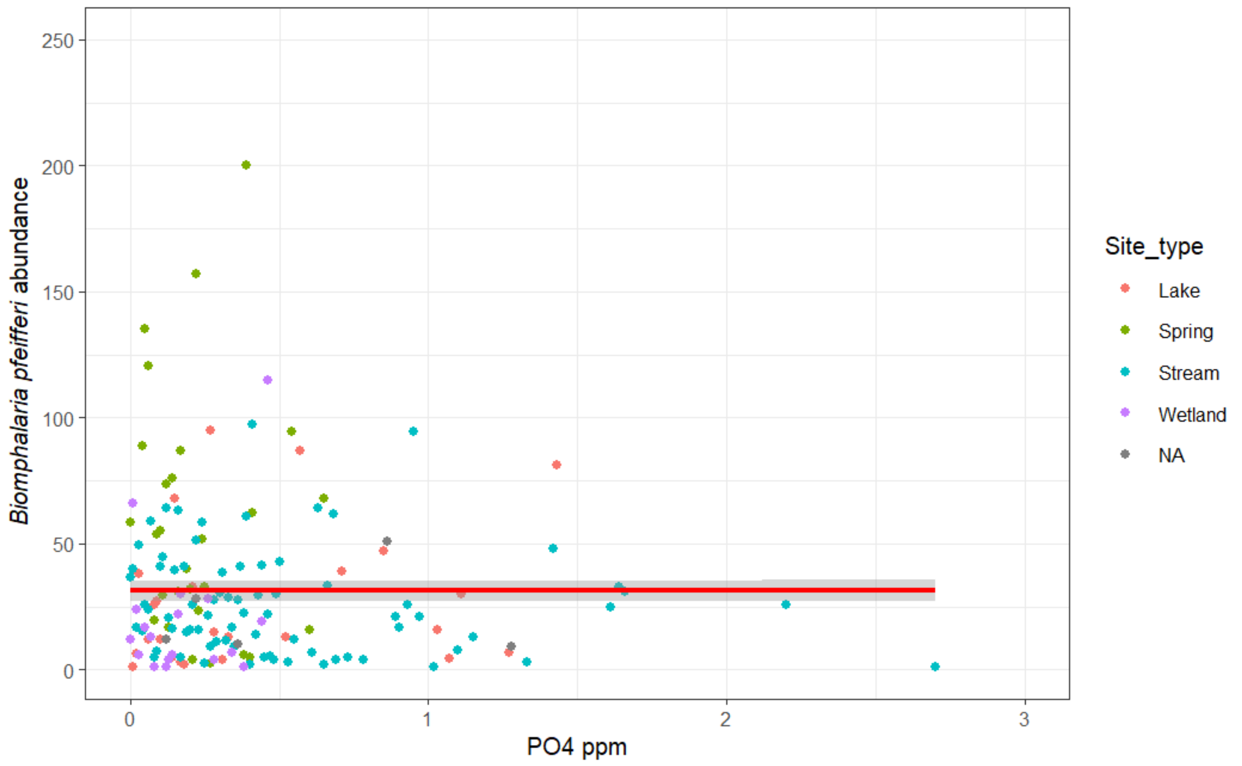

Biomphalaria abundance
250
200
150
100
50
0
20
25
30
35
Temp
Site_type
Lake
Spring
Stream
Wetland
NA

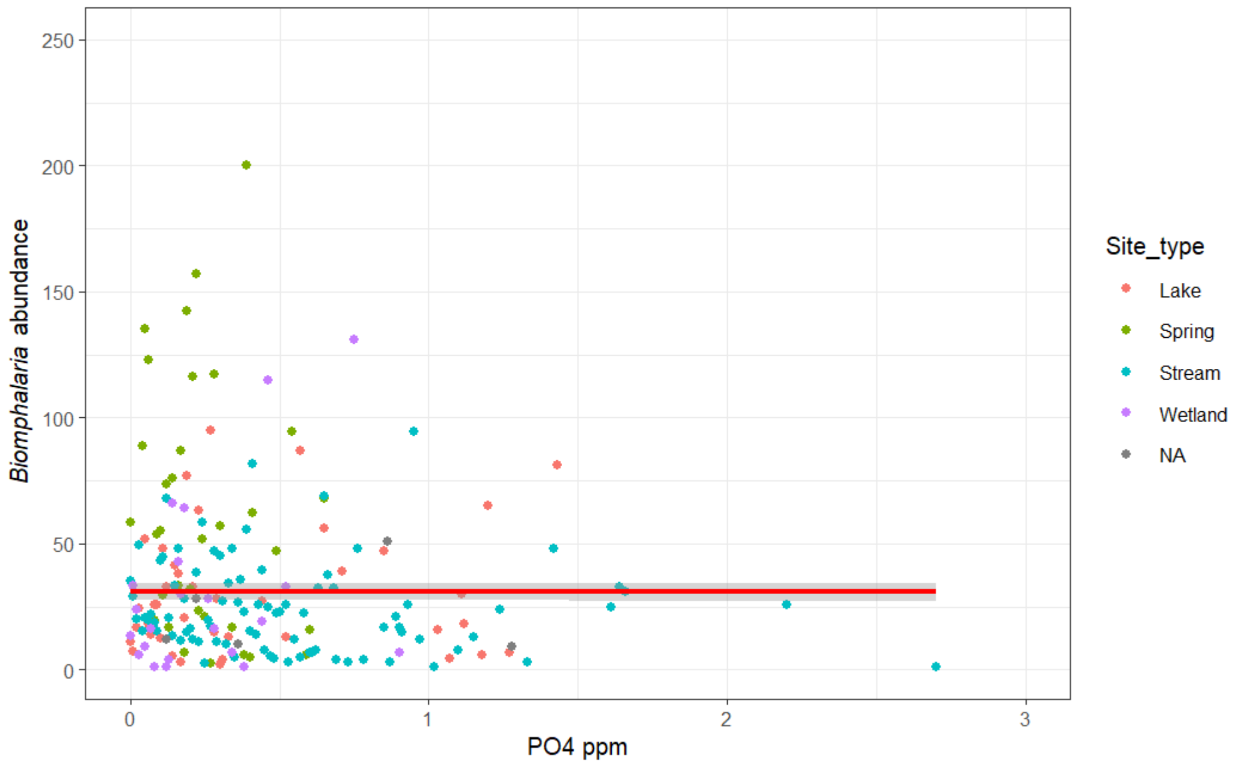

Biomphalaria pfeifferi abundance
250
200
150
100
50
0
0.0
2.5
5.0
7.5
10.0
DO ppm
Site_type
Lake
Spring
Stream
Wetland
NA


Biomphalaria abundance
250
200
150
100
50
0
0.0
2.5
5.0
7.5
10.0
DO ppm
Site_type
Lake
Spring
Stream
Wetland
NA

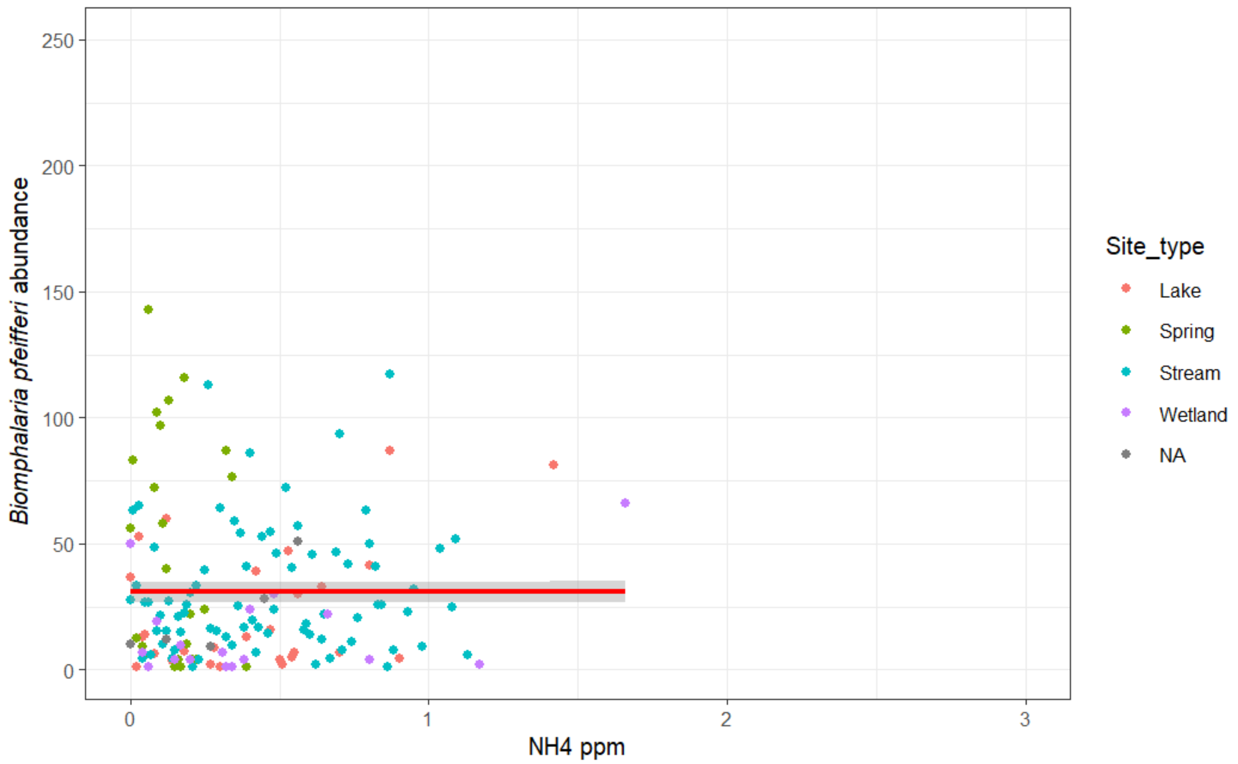

Biomphalaria pfeifferi abundance
250
200
150
100
50
0
0
500
1000
Conductivity
Site_type
Lake
Spring
Stream
Wetland
NA


250
200
150
100
50
0
Biomphalaria abundance
0
500
1000
Conductivity
Site_type
● Lake
● Spring
● Stream
● Wetland
● NA

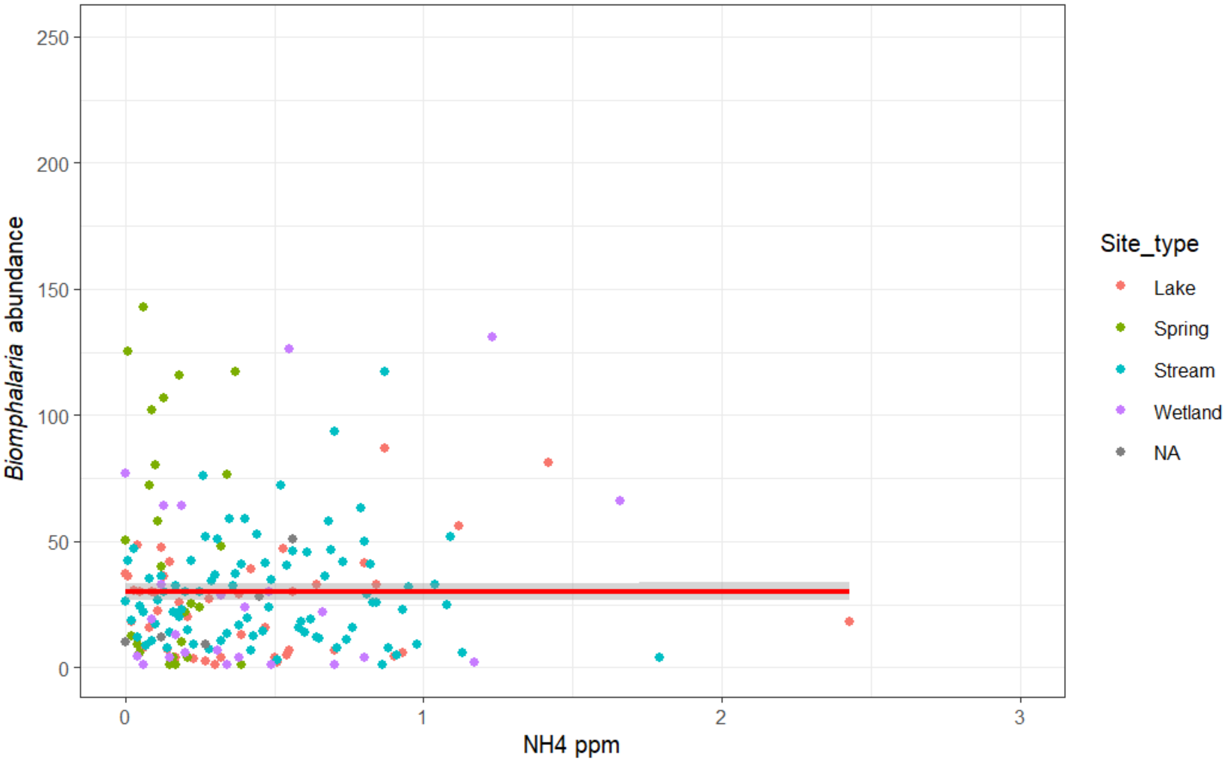

250
200
150
100
50
0
Biomphalaria pfeifferi abundance
0
1
2
3
PO4 ppm
Site_type
● Lake
● Spring
● Stream
● Wetland
● NA



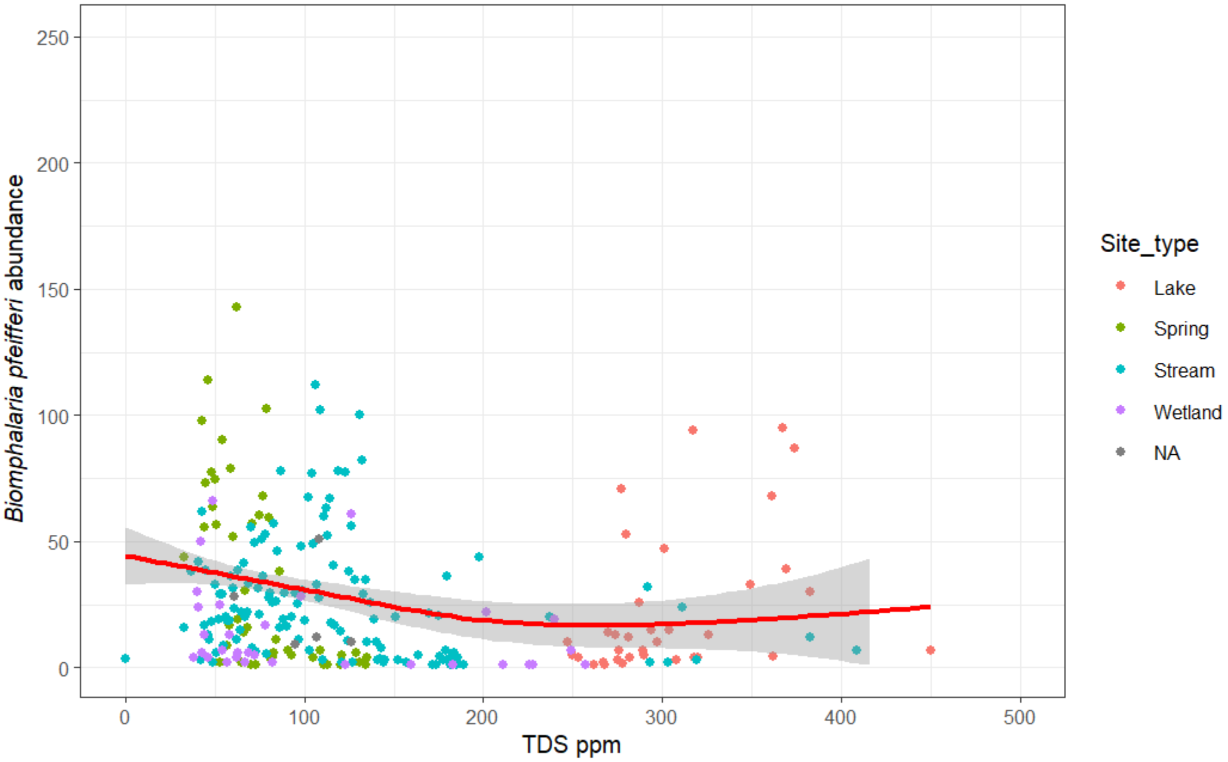
250
200
150
100
50
0
Biomphalaria abundance
0
1
2
3
PO4 ppm
Site_type
Lake
Spring
Stream
Wetland
NA



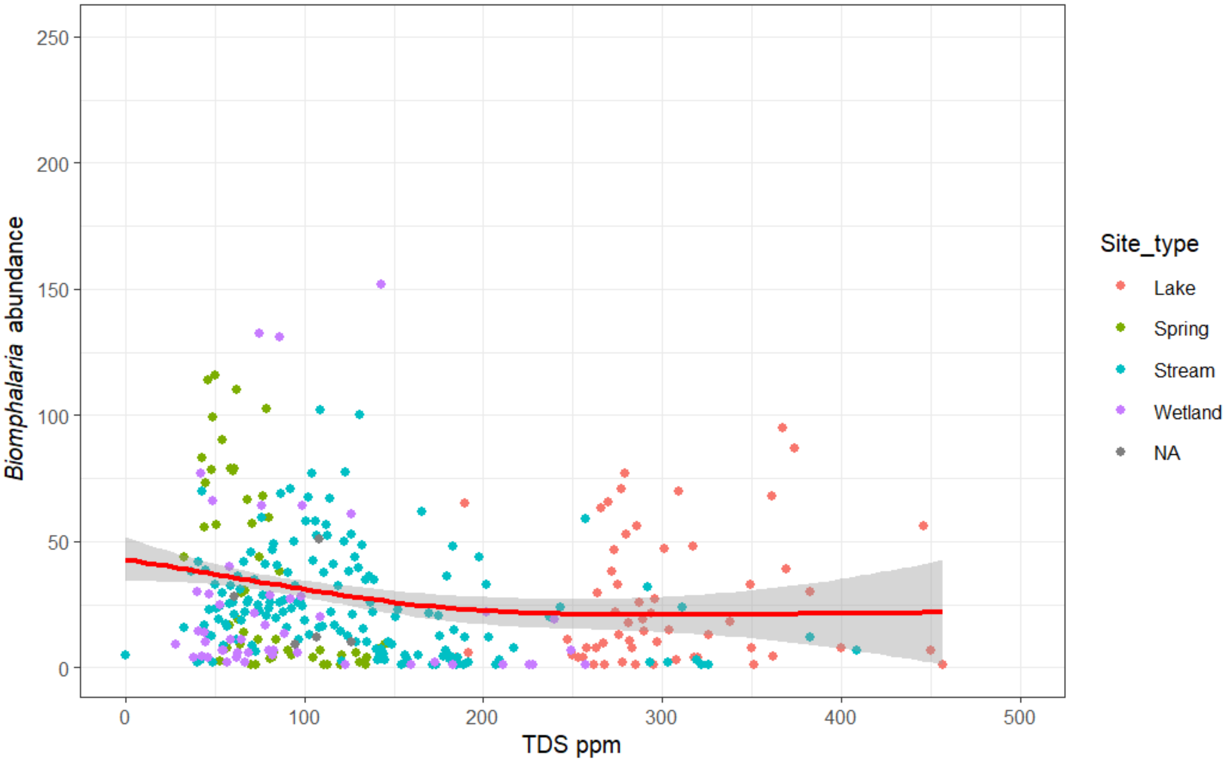
250
200
150
100
50
0
Biomphalaria pfeifferi abundance
0
1
2
3
NH4 ppm
Site_type
Lake
Spring
Stream
Wetland
NA

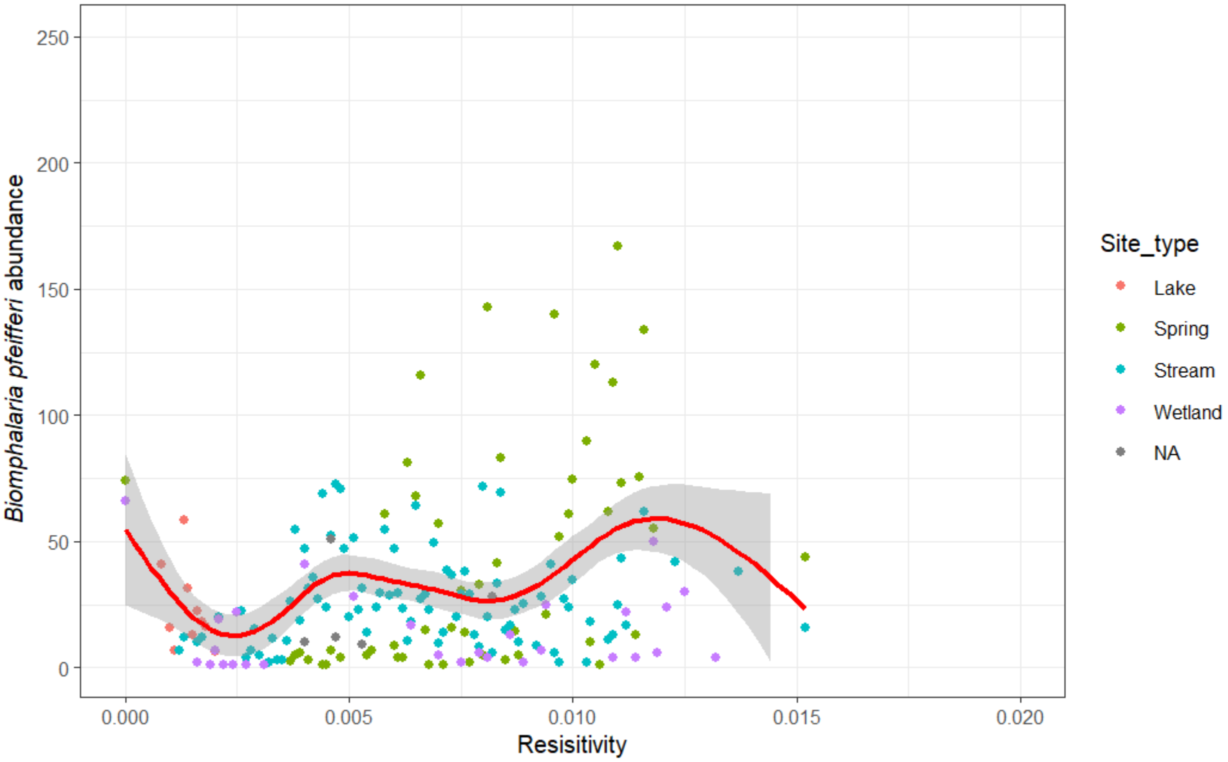


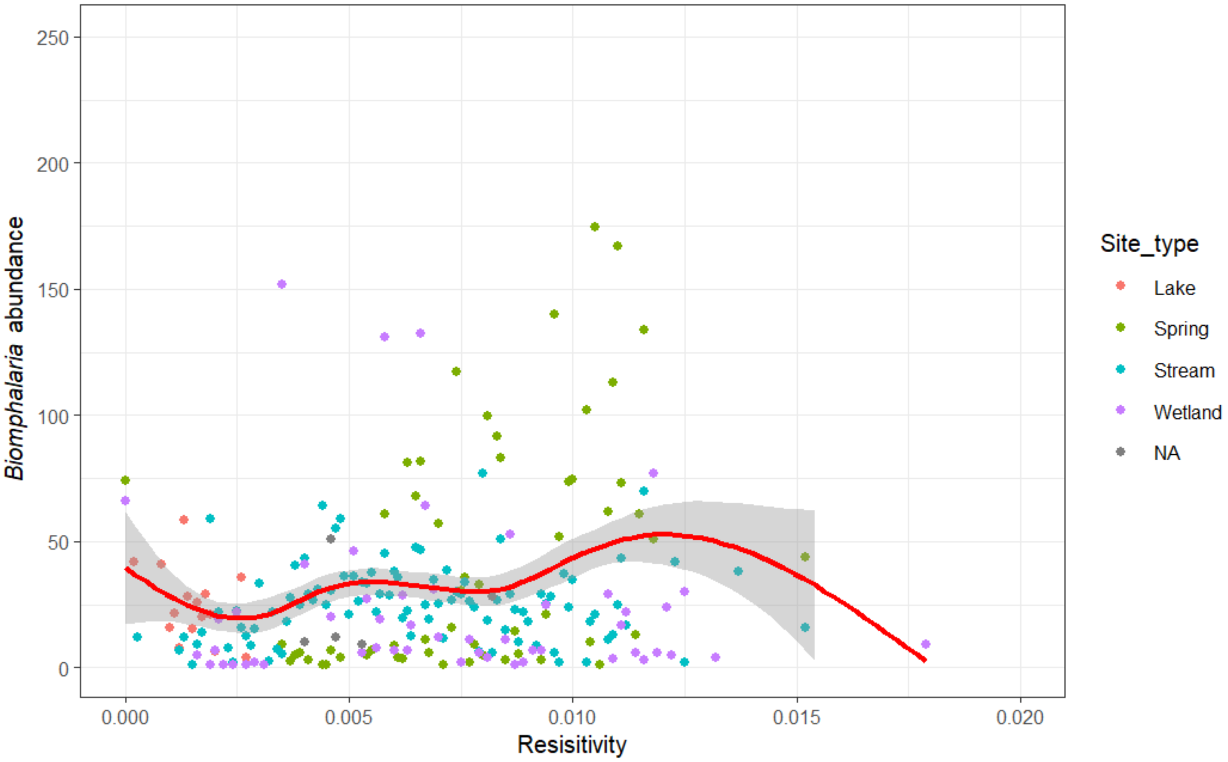
Biomphalaria abundance
250
200
150
100
50
0
0
1
2
3
NH4 ppm
Site_type
Lake
Spring
Stream
Wetland
NA



Biomphalaria pfeifferi abundance
250
200
150
100
50
0
0
100
200
300
400
500
TDS ppm
Site_type
Lake
Spring
Stream
Wetland
NA

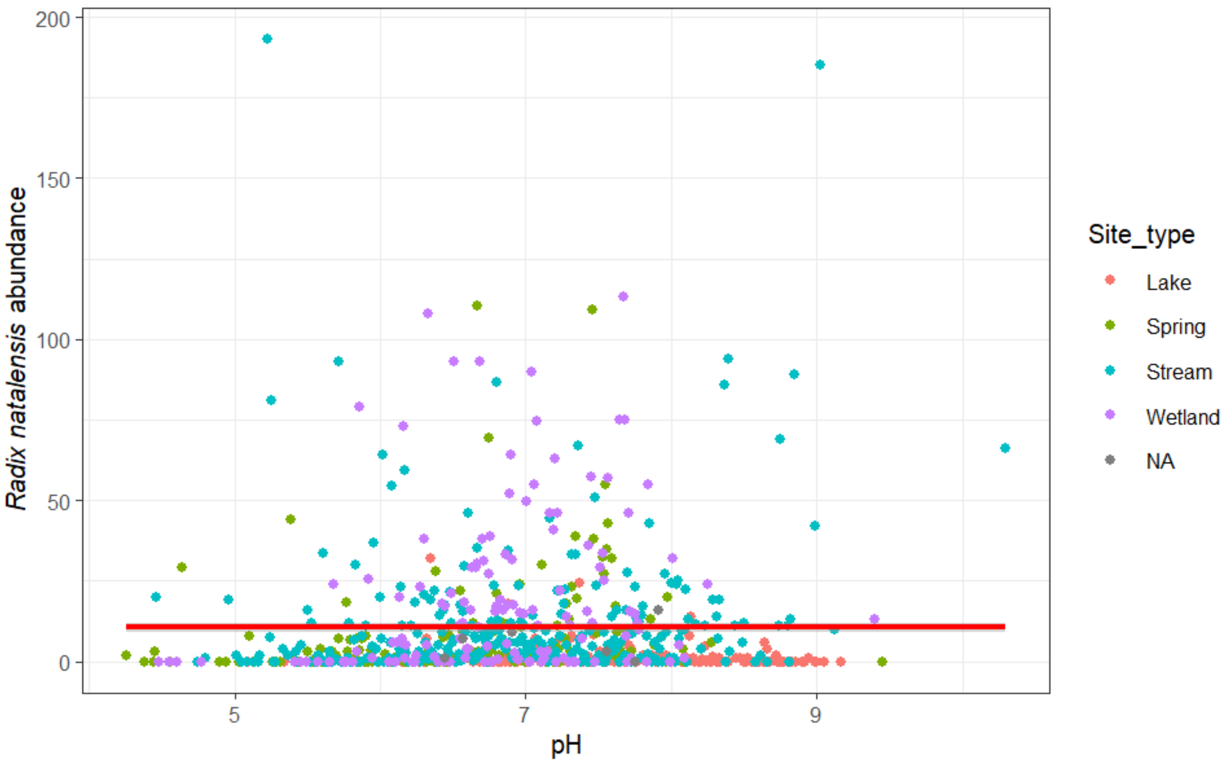

Biomphalaria abundance
250
200
150
100
50
0
0
100
200
300
400
500
TDS ppm
Site_type
Lake
Spring
Stream
Wetland
NA

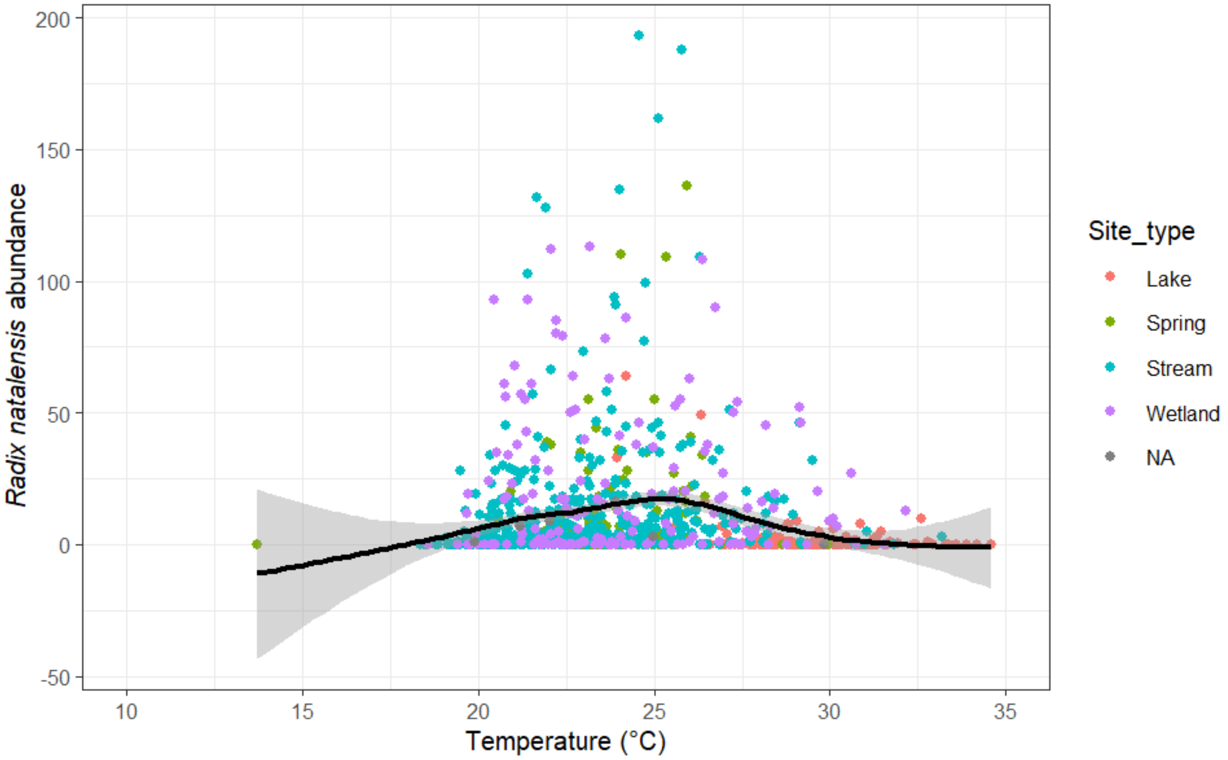

Biomphalaria pfeifferi abundance
250
200
150
100
50
0
0.000
0.005
0.010
0.015
0.020
Resisitivity
Site_type
Lake
Spring
Stream
Wetland
NA

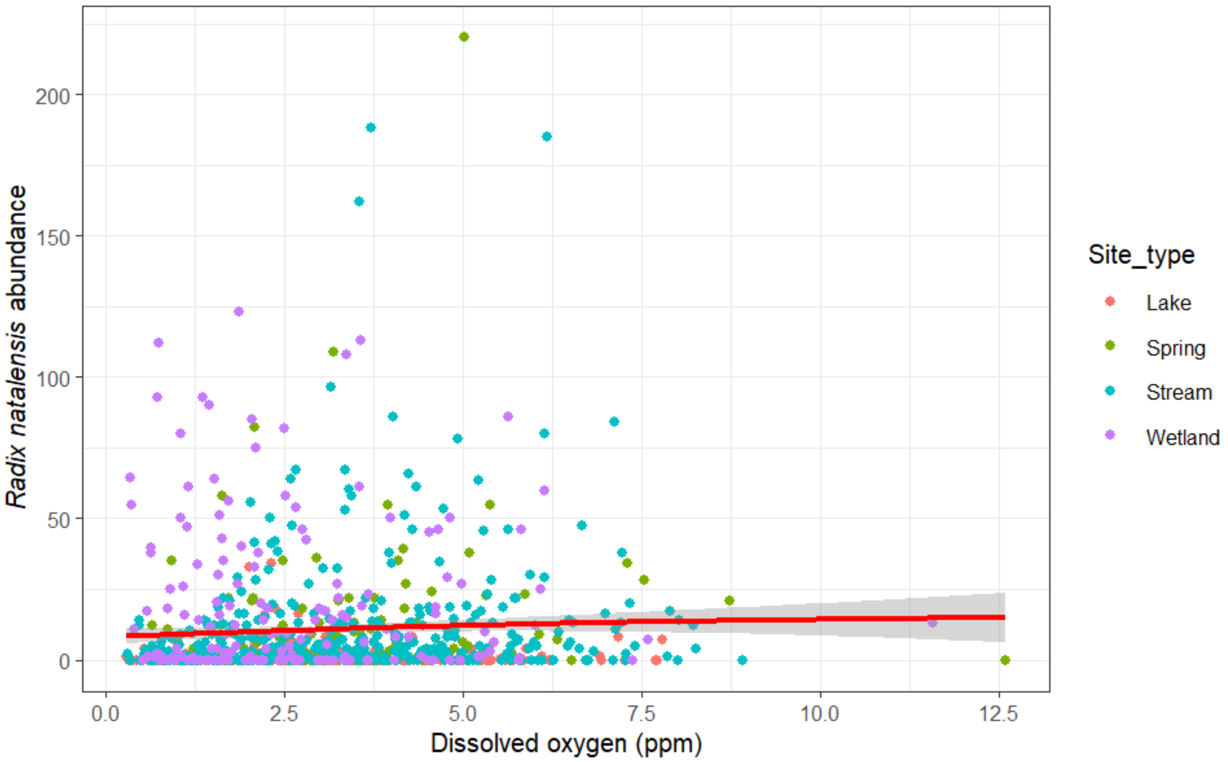


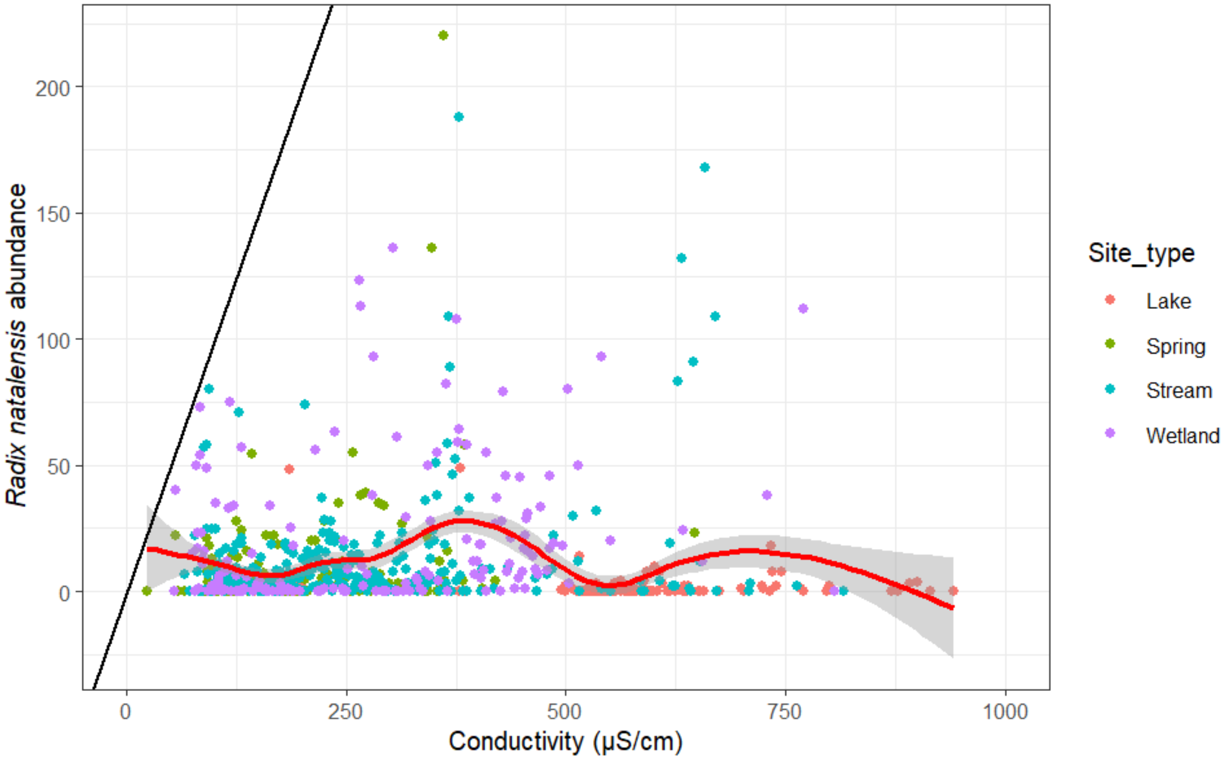
250
200
150
100
50
0
Biomphalaria abundance
0.000
0.005
0.010
0.015
0.020
Resisitivity
Site_type
Lake
Spring
Stream
Wetland
NA

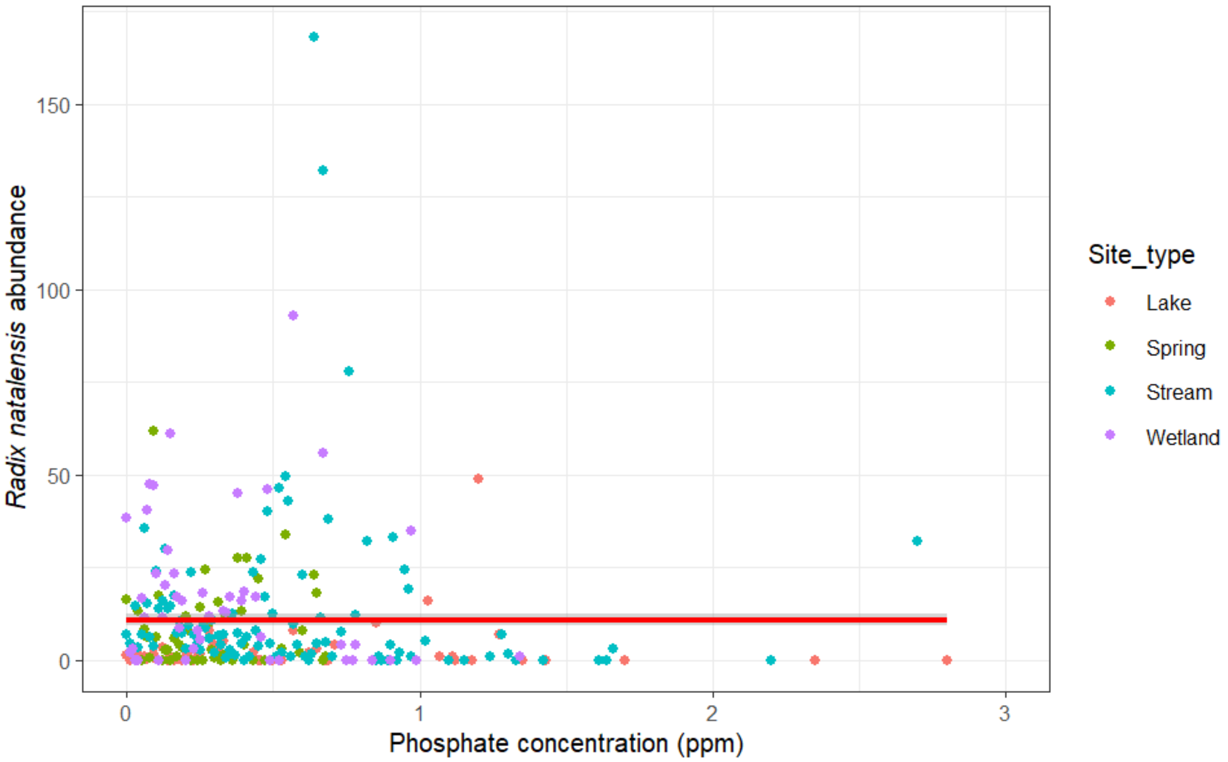
Appendix 15: Variation of Radix natalensis abundance with selected physicochemical parameters in the Lake Albert region. The site types are indicated by different colour codes of the points.

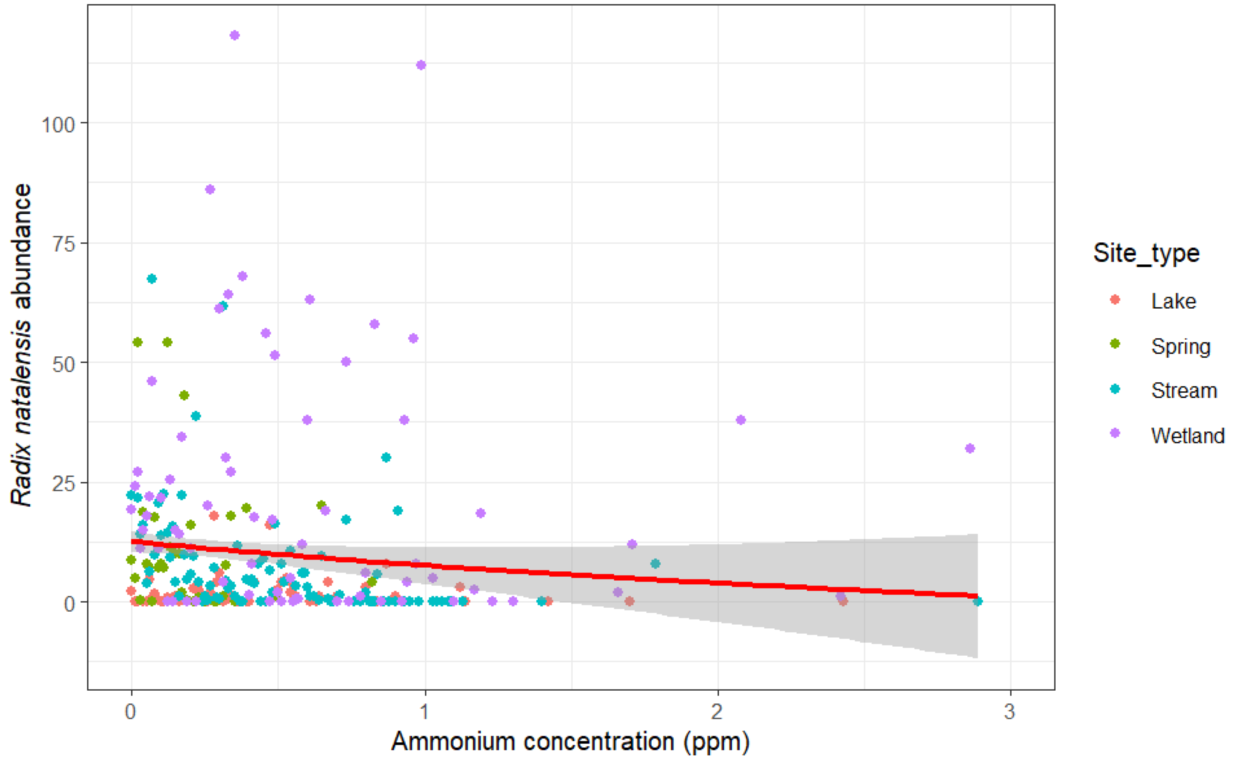


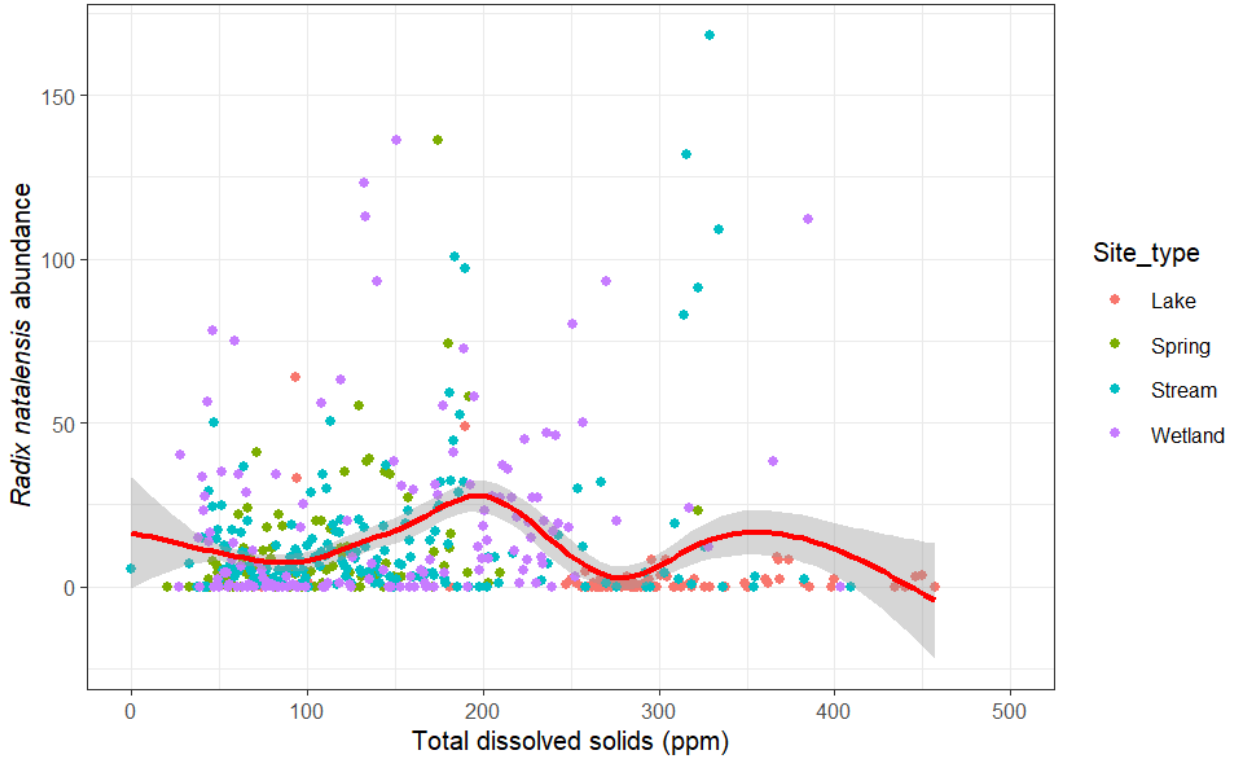

Radix natalensis abundance
200
150
100
50
0
-50
10
15
20
25
30
35
Temperature (°C)
Site_type
Lake
Spring
Stream
Wetland
NA

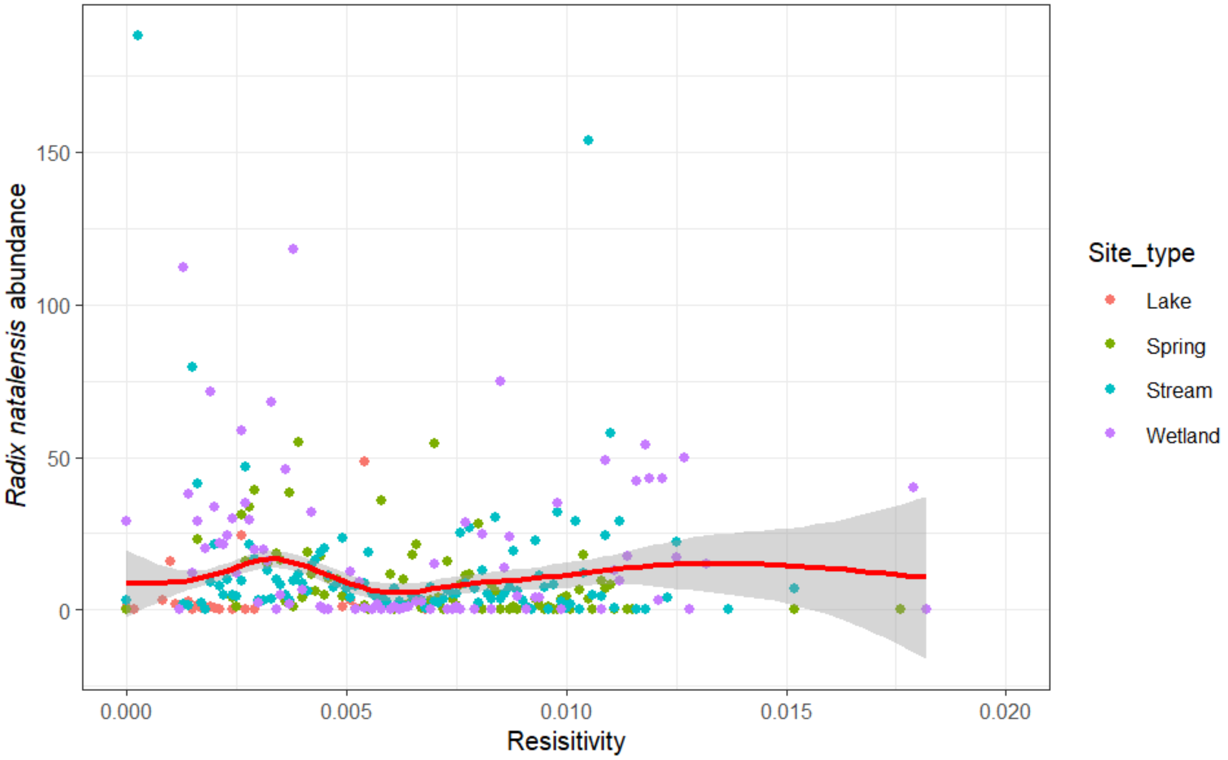

Radix natalensis abundance
200
150
100
50
0
0.0
2.5
5.0
7.5
10.0
12.5
Dissolved oxygen (ppm)
Site_type
Lake
Spring
Stream
Wetland


Radix natalensis abundance
200
150
100
50
0
0
250
500
750
1000
Conductivity (µS/cm)
Site_type
Lake
Spring
Stream
Wetland

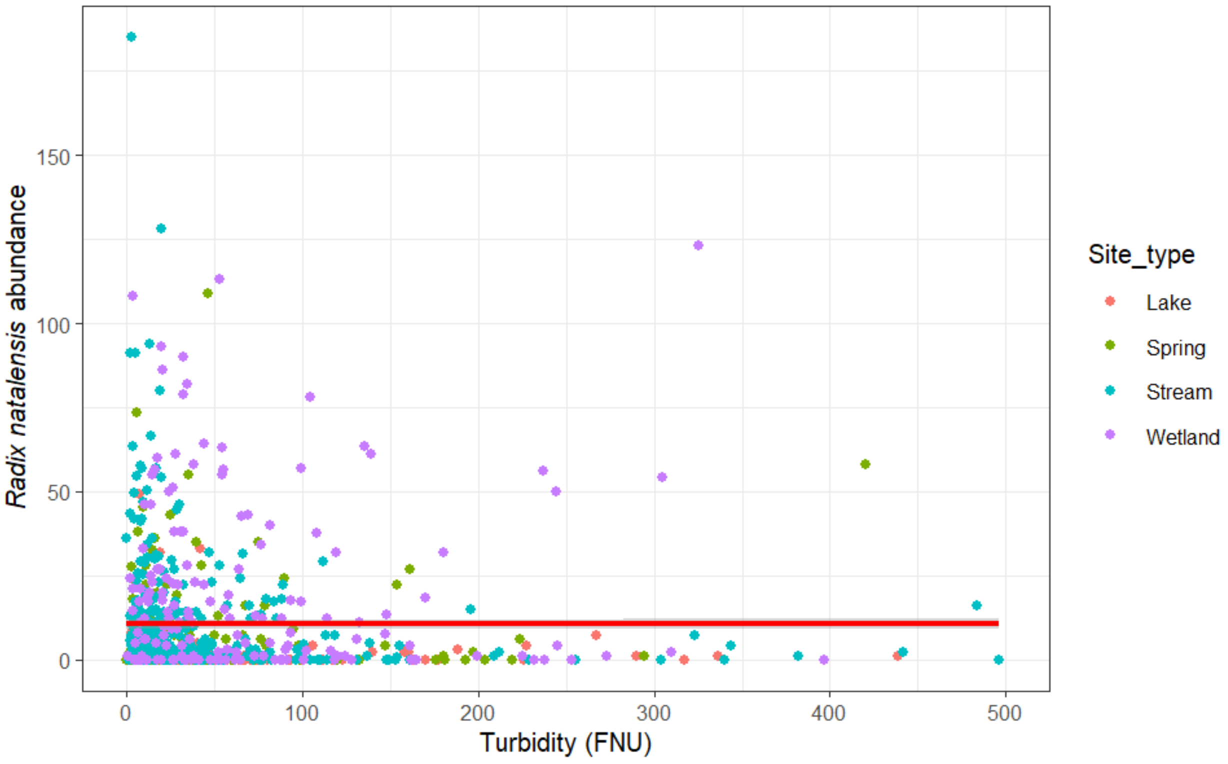

Radix natalensis abundance
150
100
50
0
0
1
2
3
Phosphate concentration (ppm)
Site_type
Lake
Spring
Stream
Wetland


Radix natalensis abundance
100
75
50
25
0
0
1
2
3
Ammonium concentration (ppm)
Site_type
Lake
Spring
Stream
Wetland


Radix natalensis abundance
150
100
50
0
0
100
200
300
400
500
Total dissolved solids (ppm)
Site_type
Lake
Spring
Stream
Wetland

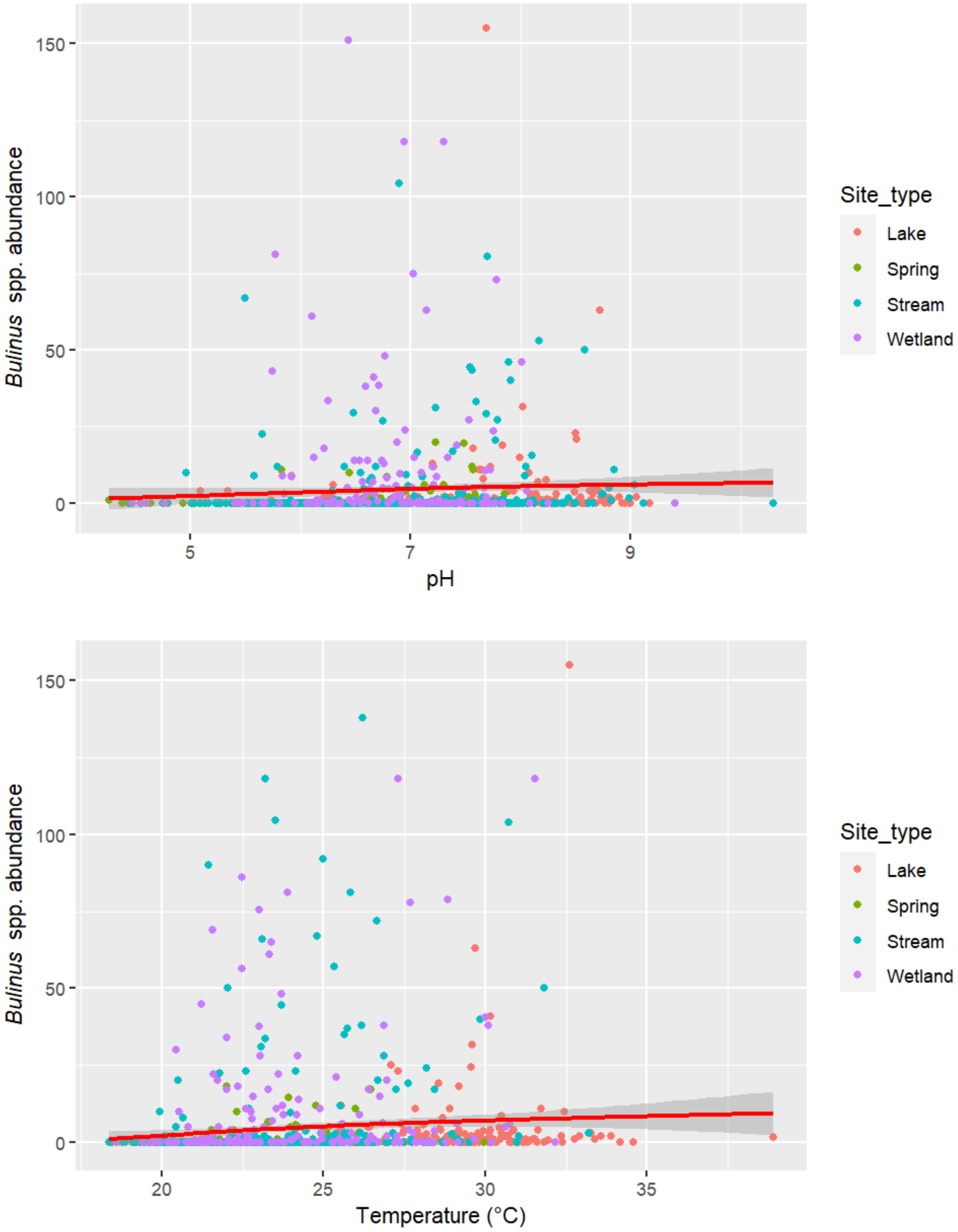


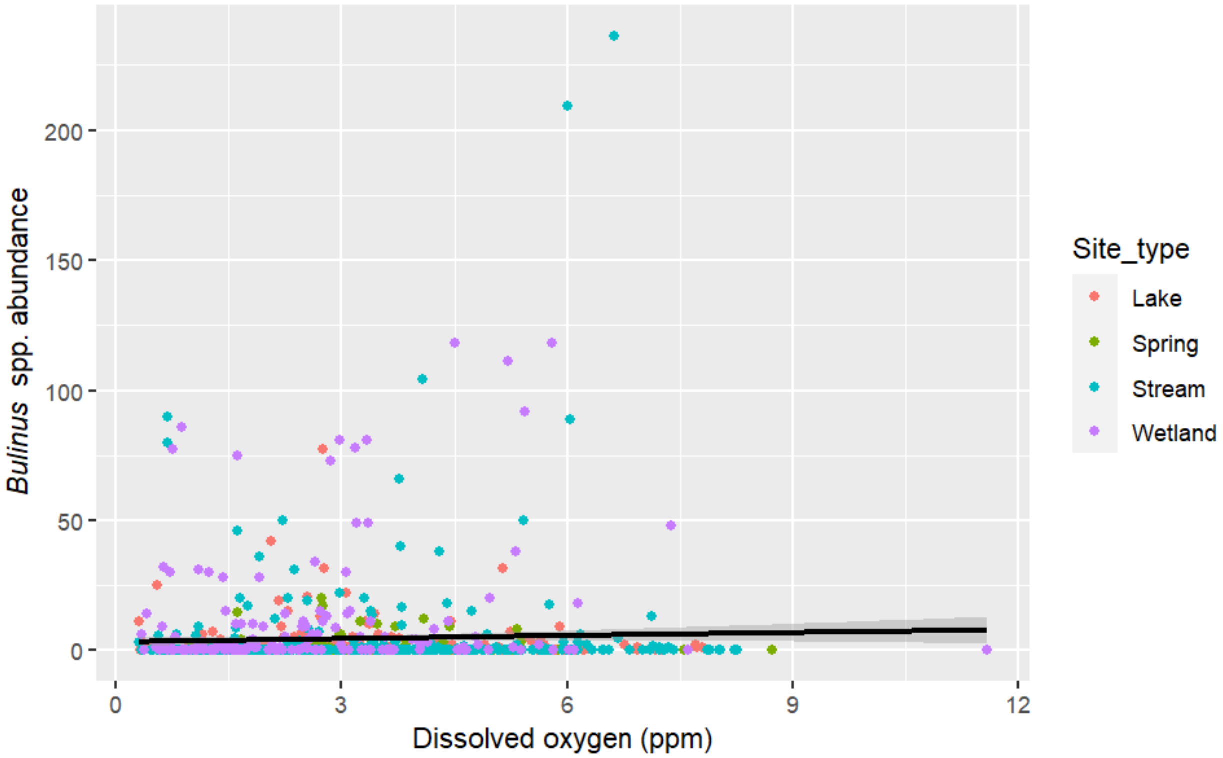
Radix natalensis abundance
150
100
50
0
0.000
0.005
0.010
0.015
0.020
Resisitivity
Site_type
● Lake
● Spring
● Stream
● Wetland

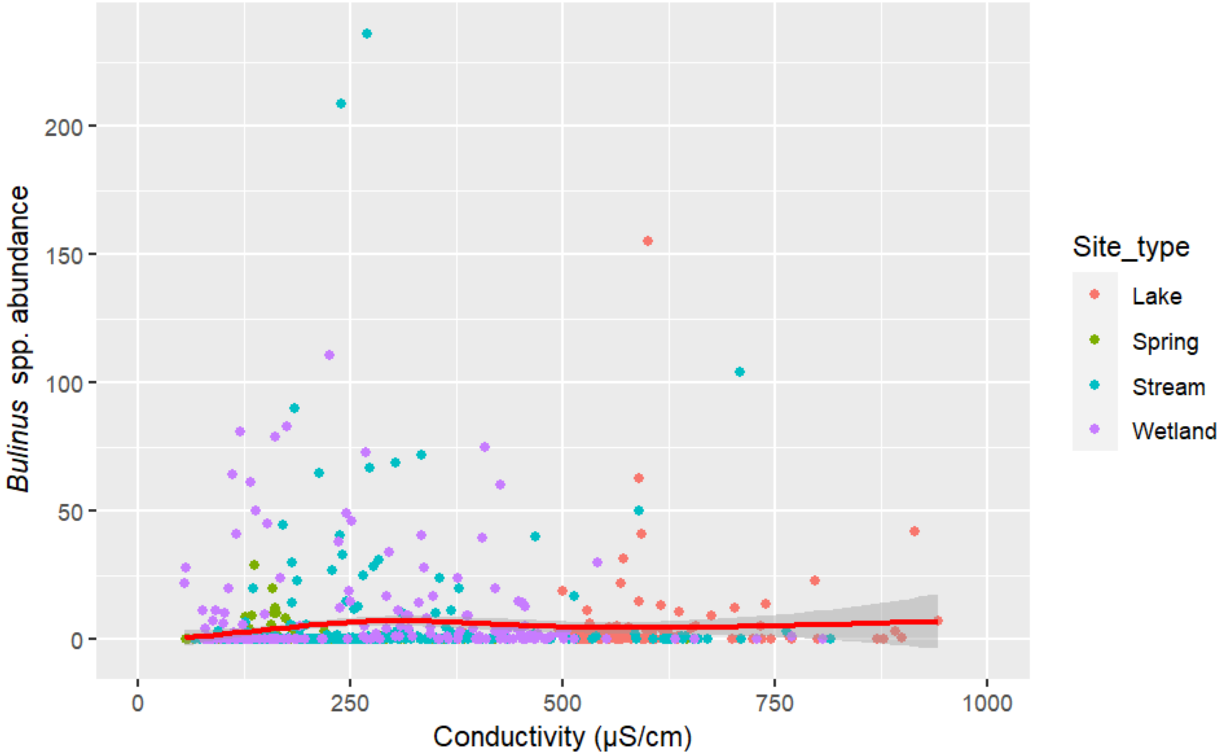


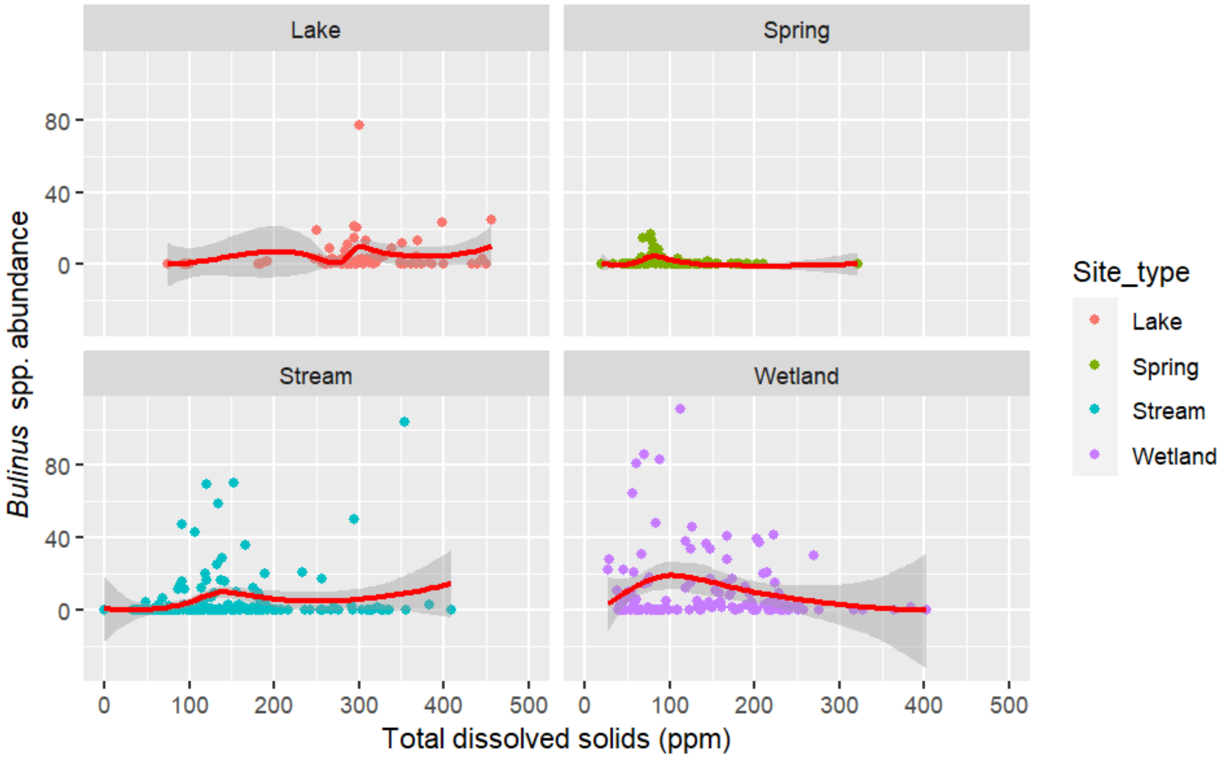
Radix natalensis abundance
150
100
50
0
0
100
200
300
400
500
Turbidity (FNU)
Site_type
● Lake
● Spring
● Stream
● Wetland

Appendix 16: Variation of Bulinus spp. abundance with selected physicochemical parameters in the Lake Albert region. The site types are indicated by different colour codes of the points.

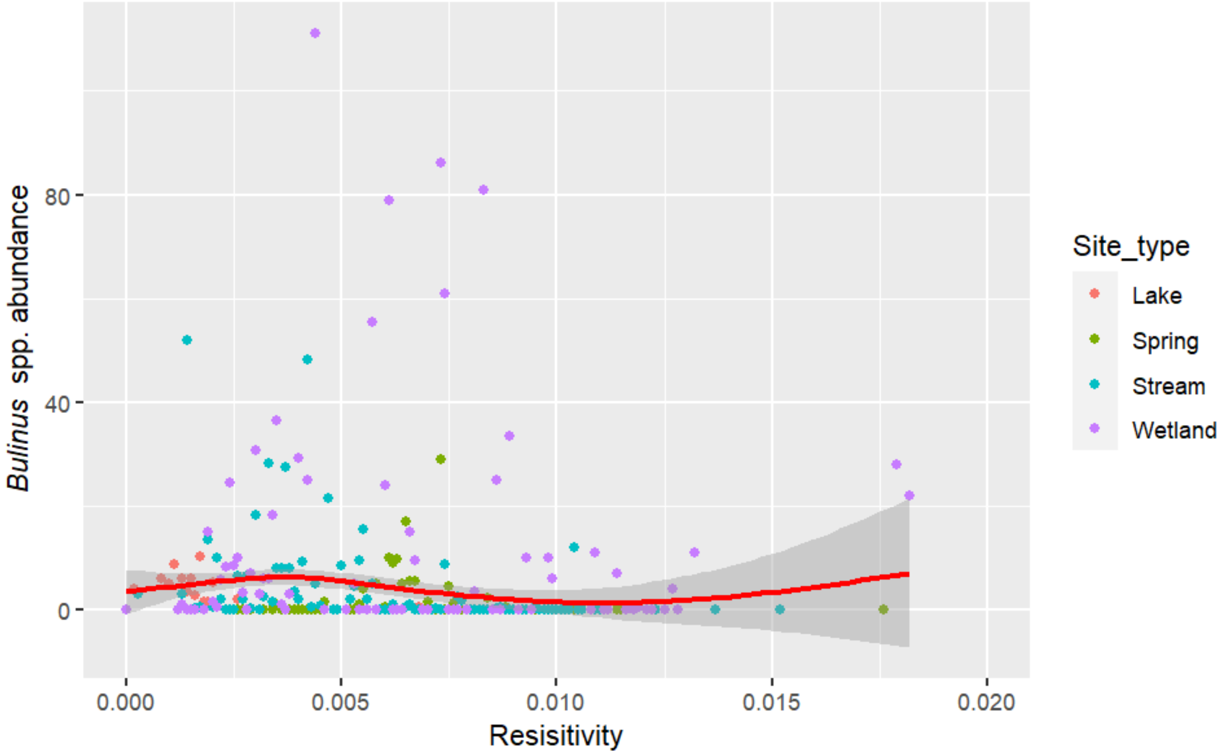



Bulinus spp. abundance
0
50
100
150
200
0
3
6
9
12
Dissolved oxygen (ppm)
Site_type
Lake
Spring
Stream
Wetland


Bulinus spp. abundance
0
50
100
150
200
0
250
500
750
1000
Conductivity (µS/cm)
Site_type
Lake
Spring
Stream
Wetland

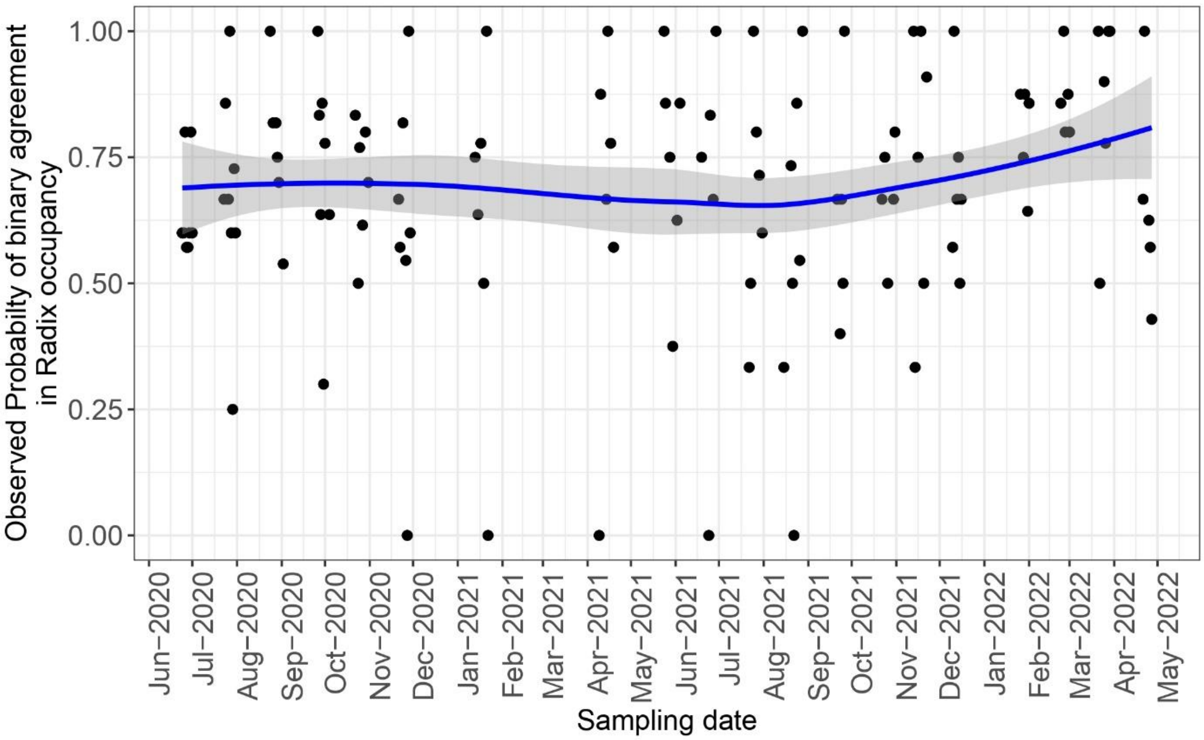


Lake
Spring
Stream
Wetland
80
40
0
Bulinus spp. abundance
0
100
200
300
400
500
Total dissolved solids (ppm)
Site_type
Lake
Spring
Stream
Wetland

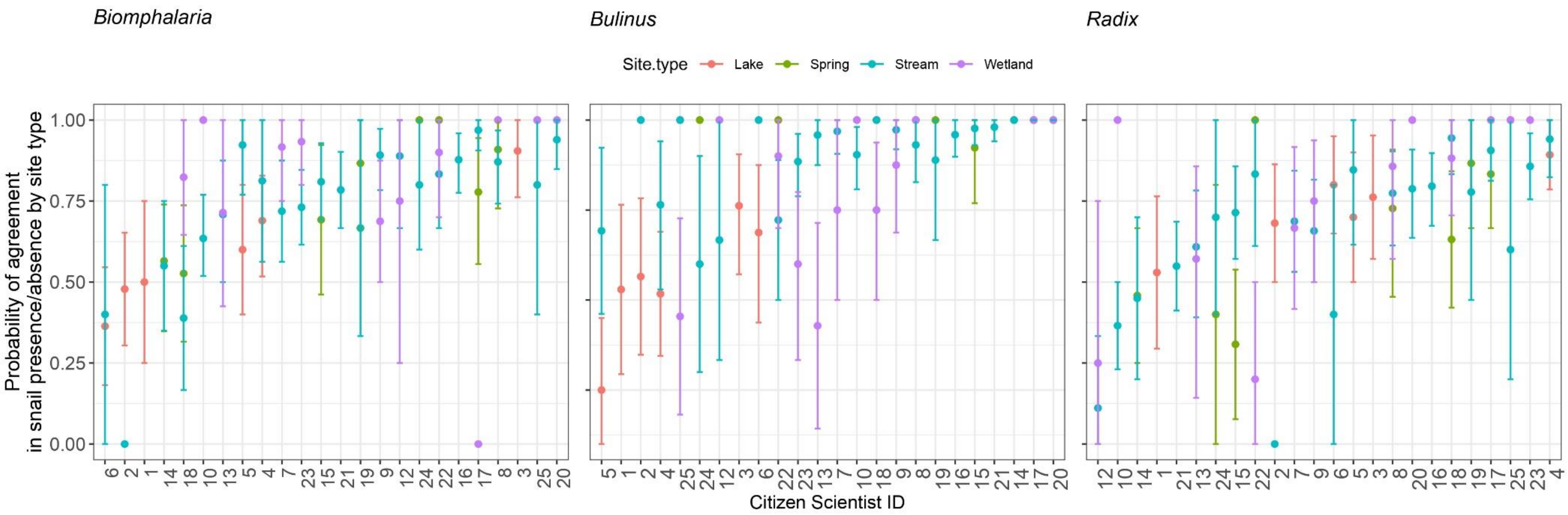


80
40
0
Bulinus spp. abundance
0.000
0.005
0.010
0.015
0.020
Resisitivity
Site_type
Lake
Spring
Stream
Wetland

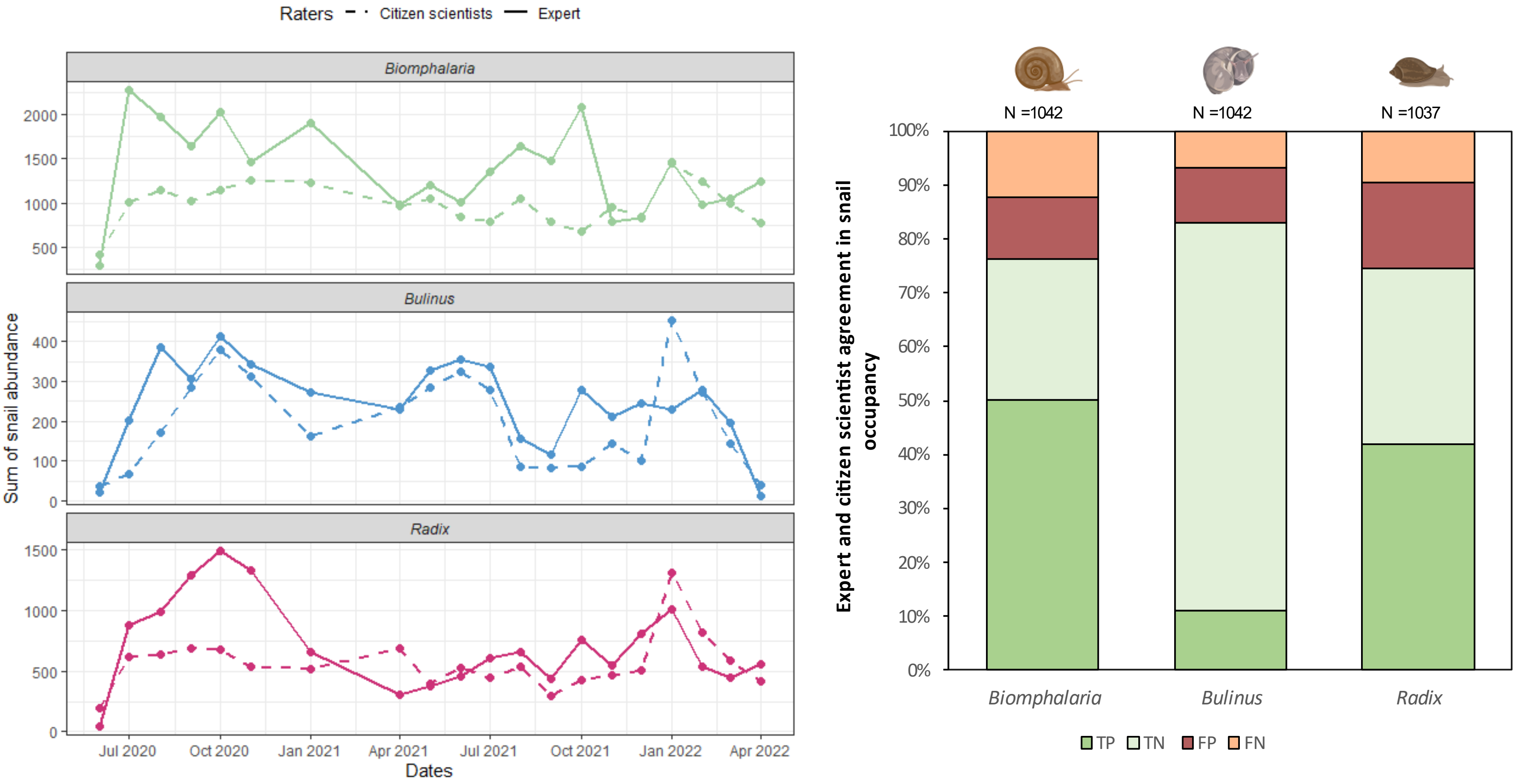
Appendix 17: Observed probability of binary agreement with time between the citizen scientists and the expert in Radix sp. presence/absence with time.



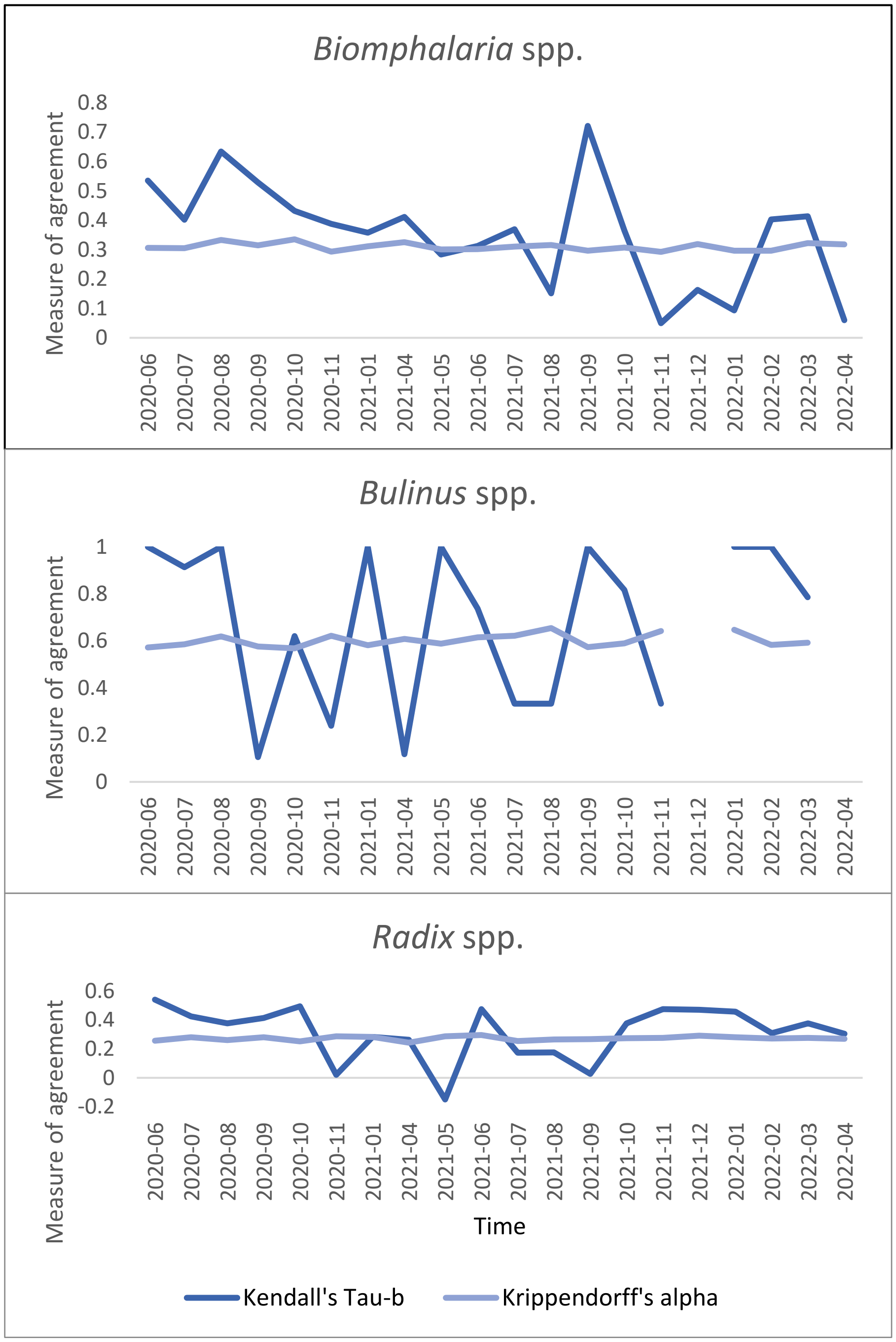
Appendix 18: Observed variation in the degree of agreement of each citizen scientists with the expert in detection of presence/absence of Biomphalaria, Bulinus and Radix snails. We observed variations in agreement for the same citizen scientist when sampling different site types. The citizen scientist IDs were sorted from lowest to highest mean agreement with the expert for each snail genus.



Appendix 19: Analysis including cumulative abundance reported by the citizen scientists (dotted line) and the expert (full lines) per month (left panel). The differences between the graphs (Figure 4.27A – main text) are due to the number of points that are being considered (1037 points for each snail genus, in contrast with 911 paired reports on Biomphalaria spp., 900 on Bulinus spp. and 905 on Radix sp. from the original analysis) because originally, only points that were apart +/- 7 days were considered. The false positives increased, but also the agreement (TP + TN) increased for Radix by around 10%.



Appendix 20: Values of consistency and numerical per time step (month) for all the snail genera studied.



Appendix 21: Consistency (Kendall Tau-b) and numerical agreement (Krippendorff’s alpha) for analysis including cumulative abundance per month (CS) / Abundance

Site	*Biomphalaria* spp.				*Bulinus* spp.				*Radix* sp.			
		Cons.		Agree.		Cons.		Agree.		Cons.		Agree.
	N	K. Tau-b	*p*	K. α	N	K. Tau-b	*p*	K. α	N	K. Tau-b	*p*	K. α
Lake	37	0.10	0.39	0.14	16	0.24	0.24	0.11	25	0.22	0.15	0.16
Spring	52	**0.37**	**<0.01**	0.39	14	**0.54**	**0.01**	0.35	38	**0.17**	0.15	0.11
Stream	401	**0.35**	**0.01**	0.32	50	**0.45**	**<0.01**	0.45	287	**0.37**	**<0.01**	0.36
Wetland	33	**0.34**	**<0.01**	0.45	36	**0.52**	**<0.01**	0.47	85	**0.29**	**<0.01**	0.25

Only the statistically significant values are in bold (Kendall Tau-b) In general, the consistency is lower with the new arrangement, but the agreement increases (this is something expected since always the expert was reporting more than the citizens and now the values are closer).

Appendix 22: Summary of cost of data collection by the citizen scientist compared to the expert.

	Item	Units	Unit cost (UGX)	20 months (UGX)	20 months (1€ = 4000 UGX)
Expert	Fuel	167	6,000	20,040,000	5,010
	Vehicle hire	12	300,000	72,000,000	18,000
	Per diem for the expert	12	150,000	36,000,000	9,000
	Per diem for the driver	12	150,000	36,000,000	9,000
	Scoop net	1	100,000	100,000	25
	Monthly facilitation lumpsum[‡]	1	720,000	14,400,000	3600
	Gum boots	2	50,000	100,000	25
	Disposable gloves	2	30,000	1,200,000	300
	TOTAL*1 sampling time per site per month			178,640,000	44,660
	TOTAL*4 sampling times per site per month			714,560,000	178,640
Citizen scientists	Monthly compensation	24	75,000	36,000,000	9,000
	Smartphones	24	800,000	19,200,000	4,800
	Scoop nets	24	100,000	2,400,000	600
	Gum boots	24	50,000	1,200,000	300
	Disposable gloves	24	30,000	14,400,000	1,200
	Data validation cost	-	-	-	210
	Annual trainings on data collection and safety	3	-	29,460,000	7,365
	TOTAL*4 sampling times per site per month			102,660,000	23,475

[‡] Note that the cost for the monthly facilitation of the expert is based on conservative estimates of half of the stipend of a PhD student dedicating 14 days a month. The cost would be much higher if a hired expert with a salary is considered.

For the expert, two scenarios are considered: when one expert samples each site once a month as in our approach, and if the expert were to sample each site four times a month like the citizen scientists in this study.

Appendix 23: The distribution of macrophytes across sampling points in the Lake Albert region of Uganda.

Site name	Date	Plant species	Stem count	Area (m²)	Density (stems/m²)
ntoroko1	22/07/2021	*Cynodon dactylon*	7	2	4
	22/07/2021	*Lemna spp.*	125	2	63
	22/07/2021	*Pontederia crassipes*	34	2	17
ntoroko3	22/07/2021	*C. papyrus*	5	4	1
	22/07/2021	*Echinochloa pyramidalis*	3	1	3
	22/07/2021	*Lemna spp.*	4	1	4
	22/07/2021	*Pontederia crassipes*	28	4	7
	22/07/2021	*Typha spp.*	8	4	2
	22/07/2021	*Utricularia reflexa*	2	1	2
ntoroko4	22/07/2021	*Echinochloa pyramidalis*	5	1	5
	22/07/2021	*Leersia hexandra*	315	2	158
	22/07/2021	*Lemna spp.*	19	2	10
	22/07/2021	*Pontederia crassipes*	26	2	13
	22/07/2021	*Typha spp.*	12	2	6
	22/07/2021	*Utricularia reflexa*	14	2	7
	22/07/2021	*Utricularia stellaris*	8	2	4
ntoroko5	22/07/2021	*Alternanthera nodiflora*	29	3	10
	22/07/2021	*Commelina benghalensis*	3	3	1
	22/07/2021	*Echinochloa colona*	12	3	4
	22/07/2021	*Ipomea aquatica*	2	3	1
	22/07/2021	*Leersia hexandra*	4	1	4
	22/07/2021	*Utricularia reflexa*	142	3	47
	22/07/2021	*Utricularia stellaris*	42	3	14
	22/07/2021	*Water lettuce*	3	3	1
hemu2	23/07/2021	*Coix lacryma*	7	1	7
	23/07/2021	*Persicaria spp.*	7	1	7
	23/07/2021	*Scleria melanomphala*	20	1	20
kanyogoga	23/07/2021	*Brillantaisia owariensis*	15	2	8
	23/07/2021	*Commelina benghalensis*	15	2	8
	23/07/2021	*Leersia hexandra*	16	2	8
	23/07/2021	*Phragmites mauritianus*	5	2	3
	23/07/2021	*Scleria melanomphala*	4	2	2
kolanya	23/07/2021	*Commelina benghalensis*	7	2	4
	23/07/2021	*Cynodon dactylon*	49	3	16
	23/07/2021	*Cyperus digitatus*	1	2	1
	23/07/2021	*Leersia hexandra*	23	1	23
	23/07/2021	*Persicaria spp.*	8	2	4
mukondo	23/07/2021	*Commelina benghalensis*	65	2	33
	23/07/2021	*Persicaria spp.*	1	2	1
nyakitukuma1	23/07/2021	*Commelina benghalensis*	14	2	7

	23/07/2021	*Cynodon dactylon*	5	1	5
	23/07/2021	*Cyperus digitatus*	45	2	23
	23/07/2021	*Jussiaea abyssinica*	3	2	2
	23/07/2021	*Leersia hexandra*	4	2	2
	23/07/2021	*Utricularia stellaris*	3	2	2
nyakitukuma2	23/07/2021	*Commelina benghalensis*	12	4	3
	23/07/2021	*Cynodon dactylon*	6	3	2
	23/07/2021	*Cyperus digitatus*	3	1	3
	23/07/2021	*Jussiaea abyssinica*	5	2	3
	23/07/2021	*Leersia hexandra*	34	4	9
	23/07/2021	*Persicaria spp.*	2	1	2
nyinawiri	23/07/2021	*Commelina benghalensis*	3	2	2
	23/07/2021	*Cyperus digitatus*	8	2	4
	23/07/2021	*Jussiaea abyssinica*	1	1	1
	23/07/2021	*Leersia hexandra*	21	3	7
	23/07/2021	*Persicaria spp.*	15	3	5
	23/07/2021	*Typha spp.*	1	2	1
rwebigongoro	23/07/2021	*Azolla filiculoides*	2473	3	824
	23/07/2021	*Commelina benghalensis*	15	2	8
	23/07/2021	*Cyperus digitatus*	12	3	4
	23/07/2021	*Jussiaea abyssinica*	11	2	6
	23/07/2021	*Jussiaea abyssinica*	1	1	1
	23/07/2021	*Leersia hexandra*	21	3	7
kijanibarora	25/07/2021	*Cyperus digitatus*	4	2	2
	25/07/2021	*Echinochloa colona*	66	2	33
	25/07/2021	*Jussiaea abyssinica*	4	2	2
	25/07/2021	*Leersia hexandra*	11	2	6
kitebere2	25/07/2021	*Cyperus mundtii*	2	1	2
	25/07/2021	*Echinochloa pyramidalis*	4	1	4
	25/07/2021	*Pontederia crassipes*	30	1	30
	25/07/2021	*Typha spp.*	1	1	1
kooga	25/07/2021	*Cynodon dactylon*	8	3	3
	25/07/2021	*Echinochloa colona*	6	3	2
	25/07/2021	*Pontederia crassipes*	18	3	6
	25/07/2021	*Utricularia reflexa*	8	3	3
	25/07/2021	*Utricularia stellaris*	73	3	24
ndaiga2	25/07/2021	*Azolla filiculoides*	7	1	7
	25/07/2021	*Cynodon dactylon*	10	1	10
	25/07/2021	*Lemna spp.*	22	1	22
	25/07/2021	*Pontederia crassipes*	22	1	22
	25/07/2021	*Salvinia molesta*	310	1	310
ndaiga3	25/07/2021	*Cynodon dactylon*	5	2	3
	25/07/2021	*Lemna spp.*	120	2	60
	25/07/2021	*Panicum spp.*	6	2	3
	25/07/2021	*Pontederia crassipes*	52	2	26

	25/07/2021	*Utricularia reflexa*	4	2	2
nyamasoga3	25/07/2021	*Commelina benghalensis*	59	2	30
bulenge2	27/07/2021	*Commelina benghalensis*	23	3	8
	27/07/2021	*Cyperus digitatus*	17	3	6
	27/07/2021	*Leersia hexandra*	70	3	23
kitengeta	27/07/2021	*Commelina benghalensis*	12	1	12
	27/07/2021	*Cynodon dactylon*	3	1	3
kobushera	27/07/2021	*Azolla filiculoides*	2	1	2
	27/07/2021	*Commelina benghalensis*	7	1	7
	27/07/2021	*Cynodon dactylon*	9	1	9
	27/07/2021	*Leersia hexandra*	63	1	63
nyakatojo1	27/07/2021	*Commelina benghalensis*	15	2	8
	27/07/2021	*Commelina benghalensis*	2	2	1
	27/07/2021	*Cyperus digitatus*	68	2	34
	27/07/2021	*Leersia hexandra*	4	2	2
	27/07/2021	*Phragmites australis*	12	2	6
nyakatojo3	27/07/2021	*Commelina benghalensis*	14	2	7
	27/07/2021	*Cynodon dactylon*	2	2	1
	27/07/2021	*Cyperus digitatus*	57	2	29
	27/07/2021	*Leersia hexandra*	3	2	2
	27/07/2021	*Water lettuce*	12	2	6
nyakatojo5	27/07/2021	*Commelina benghalensis*	80	1	80
	27/07/2021	*Cynodon dactylon*	50	1	50
	27/07/2021	*Leersia hexandra*	21	1	21
	27/07/2021	*Persicaria spp.*	3	1	3
	27/07/2021	*Water lettuce*	23	1	23
rubirizi1	27/07/2021	*Commelina benghalensis*	6	2	3
	27/07/2021	*Cynodon dactylon*	14	2	7
	27/07/2021	*Cyperus digitatus*	6	2	3
	27/07/2021	*Leersia hexandra*	50	2	25
	27/07/2021	*Persicaria spp.*	7	2	4
rubirizi2	27/07/2021	*Azolla filiculoides*	15	2	8
	27/07/2021	*Commelina benghalensis*	31	2	16
	27/07/2021	*Cyperus digitatus*	14	2	7
	27/07/2021	*Leersia hexandra*	295	2	148
	27/07/2021	*Lemna spp.*	3800	2	1900
tegeta	27/07/2021	*Commelina benghalensis*	72	3	24
	27/07/2021	*Cynodon dactylon*	2	3	1
	27/07/2021	*Jussiaea abyssinica*	17	3	6
	27/07/2021	*Leersia hexandra*	87	2	44
tuhumwire1	27/07/2021	*Cynodon dactylon*	1	3	0
	27/07/2021	*Cyperus digitatus*	4	3	1
	27/07/2021	*Hygrophila schulli*	4	3	1
	27/07/2021	*Leersia hexandra*	100	3	33
tuhumwire2	27/07/2021	*Leersia hexandra*	37	2	19

	27/07/2021	*Scleria melanomphala*	101	2	51
	27/07/2021	*Utricularia reflexa*	27	2	14
bulenge2	29/07/2021	*Commelina benghalensis*	1	2	1
	29/07/2021	*Cyperus digitatus*	5	2	3
	29/07/2021	*C. papyrus*	24	2	12
	29/07/2021	*Desmodium salicifolium*	3	2	2
	29/07/2021	*Leersia hexandra*	24	2	12
butahulira	29/07/2021	*Commelina benghalensis*	57	1	57
	29/07/2021	*Cyperus mundtii*	33	1	33
	29/07/2021	*Leersia hexandra*	7	1	7
bwera	29/07/2021	*Brillantaisia owariensis*	38	2	19
	29/07/2021	*Commelina benghalensis*	9	2	5
	29/07/2021	*Cyperus digitatus*	2	2	1
	29/07/2021	*Leersia hexandra*	59	2	30
	29/07/2021	*Persicaria spp.*	36	2	18
kajuma	29/07/2021	*Aframomum mildbraedii*	1	2	1
	29/07/2021	*Brillantaisia owariensis*	1	2	1
	29/07/2021	*Commelina benghalensis*	26	2	13
	29/07/2021	*Jussiaea abyssinica*	1	2	1
	29/07/2021	*Leersia hexandra*	74	2	37
kawamara	29/07/2021	*Brillantaisia owariensis*	93	3	31
	29/07/2021	*Commelina benghalensis*	162	1	162
	29/07/2021	*Commelina benghalensis*	15	2	8
	29/07/2021	*Cynodon dactylon*	1	3	0
	29/07/2021	*Jussiaea abyssinica*	2	1	2
	29/07/2021	*Leersia hexandra*	75	1	75
	29/07/2021	*Leersia hexandra*	75	3	25
	29/07/2021	*Persicaria spp.*	30	3	10
mukikunyu	29/07/2021	*Commelina benghalensis*	3	1	3
	29/07/2021	*Cynodon dactylon*	2	1	2
nyanjategera	29/07/2021	*Commelina benghalensis*	2	2	1
	29/07/2021	*Cynodon dactylon*	2	2	1
	29/07/2021	*Leersia hexandra*	72	2	36
	29/07/2021	*Persicaria spp.*	19	2	10
	29/07/2021	*Commelina benghalensis*	8	1	8
	29/07/2021	*Leersia hexandra*	270	1	270
	29/07/2021	*Persicaria spp.*	3	1	3
rwabitujul	29/07/2021	*Commelina benghalensis*	3	3	1
	29/07/2021	*Desmodium salicifolium*	1	3	0
	29/07/2021	*Jussiaea abyssinica*	24	3	8
	29/07/2021	*Leersia hexandra*	4	3	1
	29/07/2021	*Persicaria spp.*	26	3	9
rwabituju2	29/07/2021	*Coix lacryma*	23	3	8
	29/07/2021	*Commelina benghalensis*	18	3	6
	29/07/2021	*Cynodon dactylon*	13	3	4

	29/07/2021	*Desmodium salicifolium*	3	3	1
	29/07/2021	*Leersia hexandra*	27	3	9
	29/07/2021	*Persicaria spp.*	7	3	2
rwabitujju3	29/07/2021	*Commelina benghalensis*	11	3	4
	29/07/2021	*Cynodon dactylon*	77	3	26
	29/07/2021	*Persicaria spp.*	2	3	1
extra17	31/07/2021	*C. papyrus*	66	2	33
	31/07/2021	*Leersia hexandra*	2	2	1
	31/07/2021	*Persicaria spp.*	2	2	1
kamiranjojo1	31/07/2021	*Commelina benghalensis*	8	1	8
	31/07/2021	*Cyperus digitatus*	2	1	2
	31/07/2021	*Desmodium salicifolium*	2	1	2
	31/07/2021	*Jussiaea abyssinica*	7	1	7
	31/07/2021	*Leersia hexandra*	35	1	35
kamiranjojo2	31/07/2021	*Brillantaisia owariensis*	8	2	4
	31/07/2021	*Commelina benghalensis*	2	2	1
	31/07/2021	*Jussiaea abyssinica*	1	2	1
	31/07/2021	*Leersia hexandra*	23	2	12
	31/07/2021	*Persicaria spp.*	11	2	6
kiryani	31/07/2021	*Commelina benghalensis*	8	1	8
	31/07/2021	*Cynodon dactylon*	4	1	4
	31/07/2021	*Jussiaea abyssinica*	7	1	7
	31/07/2021	*Leersia hexandra*	6	1	6
musandika1	31/07/2021	*Commelina benghalensis*	2	3	1
	31/07/2021	*Desmodium salicifolium*	1	3	0
	31/07/2021	*Leersia hexandra*	21	3	7
	31/07/2021	*Scleria melanomphala*	16	3	5
musandika2	31/07/2021	*Brillantaisia owariensis*	3	2	2
	31/07/2021	*C. papyrus*	1	2	1
	31/07/2021	*Desmodium salicifolium*	1	2	1
	31/07/2021	*Jussiaea abyssinica*	10	2	5
	31/07/2021	*Leersia hexandra*	13	2	7
	31/07/2021	*Persicaria spp.*	9	2	5
musandika3	31/07/2021	*Azolla rubra*	1900	1	1900
	31/07/2021	*C. papyrus*	3	1	3
	31/07/2021	*Leersia hexandra*	32	1	32
nyabwegereka	31/07/2021	*Hydrocotyle leucocephala*	2	2	1
	31/07/2021	*Jussiaea abyssinica*	1	2	1
	31/07/2021	*Leersia hexandra*	600	2	300
	31/07/2021	*Persicaria spp.*	5	2	3
nyakabungo	31/07/2021	*Brillantaisia owariensis*	121	3	40
	31/07/2021	*Coix lacryma*	8	3	3
	31/07/2021	*Commelina benghalensis*	22	3	7
omukyeeya	31/07/2021	*Cyperus digitatus*	42	2	21
	31/07/2021	*Leersia hexandra*	201	2	101

ntoroko1	15/08/2021	*Cyperus mundtii*	33	2	17
	15/08/2021	*Leersia hexandra*	1	2	1
	15/08/2021	*Lemna spp.*	190	2	95
	15/08/2021	*Ludwigia stolonfera*	6	2	3
	15/08/2021	*Phragmites mauritianus*	1	2	1
	15/08/2021	*Pontederia crassipes*	42	2	21
	15/08/2021	*Scleria melanomphala*	13	2	7
	15/08/2021	*Typha spp.*	12	2	6
	15/08/2021	*Water lettuce*	1	2	1
ntoroko2	15/08/2021	*Cynodon dactylon*	24	1	24
	15/08/2021	*Echinochloa colona*	1	1	1
	15/08/2021	*Ipomea aquatica*	1	1	1
	15/08/2021	*Leersia hexandra*	20	1	20
	15/08/2021	*Lemna spp.*	100	1	100
	15/08/2021	*Pontederia crassipes*	40	1	40
	15/08/2021	*Typha spp.*	1	1	1
ntoroko3	15/08/2021	*C. papyrus*	3	3	1
	15/08/2021	*Echinochloa colona*	4	3	1
	15/08/2021	*Lemna spp.*	5	3	2
	15/08/2021	*Pontederia crassipes*	33	3	11
	15/08/2021	*Salvinia molesta*	2	3	1
	15/08/2021	*Typha spp.*	15	3	5
	15/08/2021	*Utricularia reflexa*	4	3	1
	15/08/2021	*Utricularia stellaris*	4	3	1
ntoroko6	15/08/2021	*Azolla filiculoides*	45	2	23
	15/08/2021	*Commelina benghalensis*	1	2	1
	15/08/2021	*Ipomea aquatica*	7	2	4
	15/08/2021	*Leersia hexandra*	3	2	2
	15/08/2021	*Ludwigia stolonfera*	33	2	17
	15/08/2021	*Pontederia crassipes*	41	2	21
	15/08/2021	*Utricularia reflexa*	292	2	146
	15/08/2021	*Utricularia stellaris*	20	2	10
extra17	20/08/2021	*Brillantaisia owariensis*	2	2	1
	20/08/2021	*C. papyrus*	25	2	13
	20/08/2021	*Cyperus digitatus*	1	2	1
extra18	20/08/2021	*Coix lacryma*	14	2	7
	20/08/2021	*Commelina benghalensis*	16	2	8
	20/08/2021	*Jussiaea abyssinica*	4	2	2
kafrasiko	20/08/2021	*Commelina benghalensis*	16	1	16
kamiranjojo2	20/08/2021	*Brillantaisia owariensis*	8	3	3
	20/08/2021	*Jussiaea abyssinica*	1	3	0
	20/08/2021	*Leersia hexandra*	40	3	13
	20/08/2021	*Persicaria spp.*	11	3	4
musandika2	20/08/2021	*Brillantaisia owariensis*	2	3	1
	20/08/2021	*Commelina benghalensis*	2	3	1

	20/08/2021	*Jussiaea abyssinica*	17	3	6
	20/08/2021	*Leersia hexandra*	142	3	47
	20/08/2021	*Persicaria spp.*	16	3	5
musandika3	20/08/2021	*Azolla rubra*	11000	2	5500
	20/08/2021	*Commelina benghalensis*	5	2	3
	20/08/2021	*Jussiaea abyssinica*	2	2	1
	20/08/2021	*Leersia hexandra*	179	2	90
	20/08/2021	*Persicaria spp.*	1	1	1
nyabwegereka	20/08/2021	*Hydrocotyle leucocephala*	1	2	1
	20/08/2021	*Leersia hexandra*	550	2	275
	20/08/2021	*Persicaria spp.*	3	2	2
nyakabungo	20/08/2021	*Brillantaisia owariensis*	226	2	113
	20/08/2021	*Commelina benghalensis*	42	2	21
	20/08/2021	*Leersia hexandra*	66	2	33
omukyeeya	20/08/2021	*Cyperus digitatus*	61	2	31
	20/08/2021	*Leersia hexandra*	24	2	12
ruhangiire	20/08/2021	*Commelina benghalensis*	20	2	10
	20/08/2021	*Cyperus digitatus*	17	2	9
	20/08/2021	*Cyperus mundtii*	115	2	58
	20/08/2021	*Jussiaea abyssinica*	1	2	1
	20/08/2021	*Leersia hexandra*	83	2	42
	20/08/2021	*Persicaria spp.*	3	2	2
bulenge2	22/08/2021	*C. papyrus*	11	2	6
	22/08/2021	*Commelina benghalensis*	1	2	1
	22/08/2021	*Cyperus digitatus*	2	2	1
	22/08/2021	*Desmodium salicifolium*	9	2	5
	22/08/2021	*Jussiaea abyssinica*	2	2	1
	22/08/2021	*Leersia hexandra*	6	2	3
	22/08/2021	*Scleria melanomphala*	20	2	10
bwera	22/08/2021	*Brillantaisia owariensis*	6	2	3
	22/08/2021	*Commelina benghalensis*	8	2	4
	22/08/2021	*Jussiaea abyssinica*	1	2	1
kajuma	22/08/2021	*Brillantaisia owariensis*	1	1	1
	22/08/2021	*Commelina benghalensis*	8	1	8
	22/08/2021	*Leersia hexandra*	76	1	76
kawamara	22/08/2021	*Commelina benghalensis*	100	1	100
	22/08/2021	*Leersia hexandra*	218	1	218
	22/08/2021	*Persicaria spp.*	3	1	3
kayanja	22/08/2021	*Brillantaisia owariensis*	138	2	69
	22/08/2021	*Commelina benghalensis*	8	2	4
	22/08/2021	*Cyperus digitatus*	1	2	1
	22/08/2021	*Leersia hexandra*	140	2	70
	22/08/2021	*Persicaria spp.*	18	2	9
mukikunyu	22/08/2021	*Commelina benghalensis*	14	1	14
musandika1	22/08/2021	*Commelina benghalensis*	6	1	6

	22/08/2021	*Jussiaea abyssinica*	3	1	3
	22/08/2021	*Scleria melanomphala*	2	1	2
nyanjategera	22/08/2021	*Commelina benghalensis*	3	2	2
	22/08/2021	*Jussiaea abyssinica*	2	2	1
	22/08/2021	*Leersia hexandra*	38	2	19
	22/08/2021	*Persicaria spp.*	6	2	3
omukatenge	22/08/2021	*Azolla filiculoides*	53	1	53
	22/08/2021	*Commelina benghalensis*	1	1	1
	22/08/2021	*Commelina benghalensis*	2	2	1
	22/08/2021	*Desmodium salicifolium*	1	2	1
	22/08/2021	*Jussiaea abyssinica*	6	2	3
	22/08/2021	*Leersia hexandra*	500	1	500
	22/08/2021	*Leersia hexandra*	4	2	2
	22/08/2021	*Persicaria spp.*	6	1	6
	22/08/2021	*Persicaria spp.*	17	2	9
rwabituju2	22/08/2021	*Brillantaisia owariensis*	1	2	1
	22/08/2021	*Coix lacryma*	1	2	1
	22/08/2021	*Commelina benghalensis*	12	2	6
	22/08/2021	*Desmodium salicifolium*	3	2	2
	22/08/2021	*Leersia hexandra*	7	2	4
	22/08/2021	*Persicaria spp.*	10	2	5
rwabituju3	22/08/2021	*Commelina benghalensis*	7	1	7
	22/08/2021	*Cynodon dactylon*	50	1	50
hemu1	24/08/2021	*Cynodon dactylon*	36	2	18
	24/08/2021	*Cyperus digitatus*	14	2	7
	24/08/2021	*Persicaria spp.*	1	2	1
kamina	24/08/2021	*Commelina benghalensis*	4	2	2
	24/08/2021	*Cyperus mundtii*	1	1	1
	24/08/2021	*Echinochloa colona*	74	2	37
	24/08/2021	*Leersia hexandra*	90	1	90
	24/08/2021	*Leersia hexandra*	41	2	21
	24/08/2021	*Pontederia crassipes*	35	1	35
kitebere2	24/08/2021	*Lemna spp.*	260	1	260
	24/08/2021	*Pontederia crassipes*	31	1	31
kooga	24/08/2021	*Cynodon dactylon*	6	2	3
	24/08/2021	*Echinochloa colona*	10	2	5
	24/08/2021	*Pontederia crassipes*	22	2	11
	24/08/2021	*Utricularia stellaris*	58	2	29
ndaiga1	24/08/2021	*C. papyrus*	4	1	4
	24/08/2021	*Echinochloa colona*	2	1	2
	24/08/2021	*Leersia hexandra*	56	1	56
	24/08/2021	*Phragmites mauritianus*	4	1	4
	24/08/2021	*Pontederia crassipes*	9	1	9
ndaiga2	24/08/2021	*Cynodon dactylon*	41	2	21
	24/08/2021	*Cyperus mundtii*	2	2	1

	24/08/2021	*Echinochloa colona*	4	2	2
	24/08/2021	*Pontederia crassipes*	51	2	26
ndaiga3	24/08/2021	*Azolla filiculoides*	260	3	87
	24/08/2021	*Cynodon dactylon*	133	3	44
	24/08/2021	*Lemna spp.*	120	3	40
	24/08/2021	*Ludwigia stolonfera*	3	3	1
	24/08/2021	*Pontederia crassipes*	20	3	7
	24/08/2021	*Salvinia molesta*	397	3	132
nyamasoga1	24/08/2021	*Azolla filiculoides*	416	1	416
	24/08/2021	*Cynodon dactylon*	22	1	22
	24/08/2021	*Lemna spp.*	816	1	816
	24/08/2021	*Ludwigia stolonfera*	25	1	25
	24/08/2021	*Phragmites australis*	1	1	1
	24/08/2021	*Pontederia crassipes*	13	1	13
nyamasoga3	24/08/2021	*Commelina benghalensis*	119	2	60
extra10	26/08/2021	*Desmodium salicifolium*	3	2	2
	26/08/2021	*Jussiaea abyssinica*	2	2	1
	26/08/2021	*Leersia hexandra*	13	2	7
kanyogoga	26/08/2021	*Aframomum mildbraedii*	10	3	3
	26/08/2021	*Brillantaisia owariensis*	22	3	7
	26/08/2021	*Brillantaisia owariensis*	8	3	3
	26/08/2021	*Commelina benghalensis*	25	3	8
	26/08/2021	*Desmodium salicifolium*	2	3	1
	26/08/2021	*Leersia hexandra*	16	3	5
	26/08/2021	*Persicaria spp.*	10	3	3
	26/08/2021	*Phragmites mauritianus*	1	3	0
kolanya	26/08/2021	*Commelina benghalensis*	23	3	8
	26/08/2021	*Cynodon dactylon*	75	3	25
	26/08/2021	*Cyperus digitatus*	3	3	1
	26/08/2021	*Jussiaea abyssinica*	18	3	6
	26/08/2021	*Leersia hexandra*	45	3	15
mukondo	26/08/2021	*Commelina benghalensis*	86	3	29
	26/08/2021	*Jussiaea abyssinica*	12	3	4
	26/08/2021	*Leersia hexandra*	56	3	19
nyakitukuma1	26/08/2021	*Brillantaisia owariensis*	6	1	6
	26/08/2021	*Commelina benghalensis*	2	1	2
	26/08/2021	*Leersia hexandra*	32	1	32
nyakitukuma2	26/08/2021	*Brillantaisia owariensis*	5	2	3
	26/08/2021	*Commelina benghalensis*	9	2	5
	26/08/2021	*Cynodon dactylon*	22	2	11
	26/08/2021	*Cyperus digitatus*	46	2	23
	26/08/2021	*Jussiaea abyssinica*	16	2	8
	26/08/2021	*Leersia hexandra*	25	2	13
	26/08/2021	*Persicaria spp.*	5	2	3
nyinawiri	26/08/2021	*Commelina benghalensis*	6	2	3

	26/08/2021	*Cyperus digitatus*	1	2	1
	26/08/2021	*Jussiaea abyssinica*	5	2	3
	26/08/2021	*Leersia hexandra*	335	2	168
	26/08/2021	*Persicaria spp.*	7	2	4
rwebigongoro	26/08/2021	*Azolla filiculoides*	450	2	225
	26/08/2021	*Commelina benghalensis*	3	2	2
	26/08/2021	*Cynodon dactylon*	1	1	1
	26/08/2021	*Cyperus digitatus*	19	2	10
	26/08/2021	*Jussiaea abyssinica*	31	2	16
	26/08/2021	*Leersia hexandra*	140	2	70
bulenge1	28/08/2021	*Commelina benghalensis*	55	2	28
	28/08/2021	*Cyperus digitatus*	19	2	10
	28/08/2021	*Leersia hexandra*	37	2	19
eumu	28/08/2021	*Cyperus digitatus*	7	2	4
	28/08/2021	*Jussiaea abyssinica*	2	2	1
	28/08/2021	*Leersia hexandra*	46	2	23
kamiranjojo1	28/08/2021	*Commelina benghalensis*	17	2	9
	28/08/2021	*Cyperus digitatus*	2	2	1
	28/08/2021	*Jussiaea abyssinica*	9	2	5
	28/08/2021	*Leersia hexandra*	98	2	49
	28/08/2021	*Persicaria spp.*	1	2	1
kobushera	28/08/2021	*Commelina benghalensis*	3	1	3
	28/08/2021	*Cynodon dactylon*	13	1	13
	28/08/2021	*Leersia hexandra*	27	1	27
muhumbu	28/08/2021	*Commelina benghalensis*	6	1	6
	28/08/2021	*Jussiaea abyssinica*	1	1	1
	28/08/2021	*Leersia hexandra*	13	1	13
	28/08/2021	*Scleria melanomphala*	70	1	70
nyakatojo5	28/08/2021	*Commelina benghalensis*	43	2	22
	28/08/2021	*Cynodon dactylon*	72	2	36
	28/08/2021	*Cyperus digitatus*	2	2	1
	28/08/2021	*Persicaria spp.*	10	2	5
	28/08/2021	*Water lettuce*	39	2	20
rubirizi2	28/08/2021	*Commelina benghalensis*	7	1	7
	28/08/2021	*Leersia hexandra*	164	1	164
tegeta	28/08/2021	*Brillantaisia owariensis*	1	3	0
	28/08/2021	*Commelina benghalensis*	1	3	0
	28/08/2021	*Cynodon dactylon*	19	3	6
	28/08/2021	*Leersia hexandra*	68	2	34
tuhumwire1	28/08/2021	*Leersia hexandra*	62	2	31
tuhumwire2	28/08/2021	*Leersia hexandra*	27	2	14
	28/08/2021	*Scleria melanomphala*	185	2	93
kitebere2	21/09/2021	*C. papyrus*	2	2	1
	21/09/2021	*Cyperus digitatus*	14	2	7
	21/09/2021	*Leersia hexandra*	14	2	7

	21/09/2021	*Lemna spp.*	57	2	29
	21/09/2021	*Pontederia crassipes*	78	2	39
	21/09/2021	*Salvinia molesta*	8	2	4
	21/09/2021	*Typha spp.*	3	2	2
	21/09/2021	*Water lettuce*	1	2	1
kooga	21/09/2021	*Echinochloa colona*	72	2	36
	21/09/2021	*Pontederia crassipes*	3	2	2
	21/09/2021	*Utricularia reflexa*	5	2	3
	21/09/2021	*Utricularia stellaris*	17	2	9
ndaiga1	21/09/2021	*Cynodon dactylon*	23	1	23
	21/09/2021	*Echinochloa colona*	9	1	9
	21/09/2021	*Leersia hexandra*	13	1	13
	21/09/2021	*Pontederia crassipes*	1	1	1
ndaiga2	21/09/2021	*Cyperus mundtii*	11	1	11
	21/09/2021	*Pontederia crassipes*	8	1	8
ndaiga3	21/09/2021	*Azolla filiculoides*	260	2	130
	21/09/2021	*Cynodon dactylon*	133	2	67
	21/09/2021	*Lemna spp.*	120	2	60
	21/09/2021	*Ludwigia stolonfera*	3	2	2
	21/09/2021	*Pontederia crassipes*	20	2	10
	21/09/2021	*Salvinia molesta*	397	2	199
nyamasoga1	21/09/2021	*Azolla filiculoides*	416	1	416
	21/09/2021	*Commelina benghalensis*	119	2	60
	21/09/2021	*Cynodon dactylon*	22	1	22
	21/09/2021	*Lemna spp.*	816	1	816
	21/09/2021	*Ludwigia stolonfera*	25	1	25
	21/09/2021	*Phragmites australis*	1	1	1
	21/09/2021	*Pontederia crassipes*	13	1	13
nyamasoga3	21/09/2021	*Commelina benghalensis*	30	1	30
	21/09/2021	*Cynodon dactylon*	16	2	8
eumu	23/09/2021	*Cyperus latifolia*	59	2	30
	23/09/2021	*Leersia hexandra*	53	2	27
	23/09/2021	*Ludwigia stolonfera*	1	2	1
extra10	23/09/2021	*Desmodium salicifolium*	3	2	2
	23/09/2021	*Jussiaea abyssinica*	2	2	1
	23/09/2021	*Leersia hexandra*	13	2	7
extra8	23/09/2021	*Commelina benghalensis*	2	1	2
	23/09/2021	*Leersia hexandra*	33	1	33
kanyogoga	23/09/2021	*Aframomum mildbraedii*	10	3	3
	23/09/2021	*Brillantaisia owariensis*	22	3	7
	23/09/2021	*Brillantaisia owariensis*	8	3	3
	23/09/2021	*Commelina benghalensis*	25	3	8
	23/09/2021	*Desmodium salicifolium*	2	3	1
	23/09/2021	*Leersia hexandra*	16	3	5
	23/09/2021	*Persicaria spp.*	10	3	3

	23/09/2021	*Phragmites mauritianus*	1	3	0
kolanya	23/09/2021	*Commelina benghalensis*	23	3	8
	23/09/2021	*Cynodon dactylon*	75	3	25
	23/09/2021	*Cyperus digitatus*	3	3	1
	23/09/2021	*Jussiaea abyssinica*	18	3	6
mukondo	23/09/2021	*Commelina benghalensis*	86	3	29
	23/09/2021	*Jussiaea abyssinica*	12	3	4
	23/09/2021	*Leersia hexandra*	56	3	19
nyakitukuma1	23/09/2021	*Brillantaisia owariensis*	6	1	6
	23/09/2021	*Commelina benghalensis*	2	1	2
	23/09/2021	*Leersia hexandra*	32	1	32
nyakitukuma2	23/09/2021	*Commelina benghalensis*	9	2	5
	23/09/2021	*Cyperus digitatus*	13	2	7
	23/09/2021	*Jussiaea abyssinica*	3	2	2
	23/09/2021	*Leersia hexandra*	34	2	17
nyinawiri	23/09/2021	*Commelina benghalensis*	6	2	3
	23/09/2021	*Cyperus digitatus*	1	2	1
	23/09/2021	*Jussiaea abyssinica*	5	2	3
	23/09/2021	*Leersia hexandra*	335	2	168
	23/09/2021	*Persicaria spp.*	7	2	4
rwebigongoro	23/09/2021	*Azolla filiculoides*	450	2	225
	23/09/2021	*Commelina benghalensis*	3	2	2
	23/09/2021	*Cynodon dactylon*	1	2	1
	23/09/2021	*Cyperus digitatus*	19	2	10
	23/09/2021	*Jussiaea abyssinica*	31	2	16
	23/09/2021	*Leersia hexandra*	140	2	70
bulenge1	24/09/2021	*Commelina benghalensis*	55	2	28
	24/09/2021	*Cyperus digitatus*	19	2	10
	24/09/2021	*Leersia hexandra*	37	2	19
kobushera	24/09/2021	*Azolla filiculoides*	3	1	3
	24/09/2021	*Commelina benghalensis*	7	1	7
	24/09/2021	*Leersia hexandra*	700	1	700
nyakatojo3	24/09/2021	*Cynodon dactylon*	3	1	3
	24/09/2021	*Cyperus digitatus*	25	1	25
	24/09/2021	*Leersia hexandra*	23	1	23
	24/09/2021	*Water lettuce*	7	1	7
nyakatojo5	24/09/2021	*Commelina benghalensis*	60	1	60
	24/09/2021	*Leersia hexandra*	30	1	30
	24/09/2021	*Persicaria spp.*	31	1	31
rubirizi2	24/09/2021	*Azolla filiculoides*	15	2	8
	24/09/2021	*Commelina benghalensis*	31	2	16
	24/09/2021	*Cyperus digitatus*	14	2	7
	24/09/2021	*Leersia hexandra*	295	2	148
tuhumwire2	24/09/2021	*Scleria melanomphala*	92	2	46
bulenge2	25/09/2021	*C. papyrus*	2	1	2

	25/09/2021	*Cyperus digitatus*	5	1	5
	25/09/2021	*Desmodium salicifolium*	4	1	4
	25/09/2021	*Jussiaea abyssinica*	7	1	7
	25/09/2021	*Panicum spp.*	40	1	40
	25/09/2021	*Phragmites mauritianus*	2	1	2
butahulira	25/09/2021	*Commelina benghalensis*	57	1	57
	25/09/2021	*Cyperus mundtii*	33	1	33
	25/09/2021	*Leersia hexandra*	7	1	7
extra17	25/09/2021	*C. papyrus*	17	2	9
	25/09/2021	*Leersia hexandra*	20	2	10
	25/09/2021	*Phragmites mauritianus*	6	2	3
kajuma	25/09/2021	*Brillantaisia owariensis*	1	1	1
	25/09/2021	*Commelina benghalensis*	8	1	8
	25/09/2021	*Leersia hexandra*	76	1	76
musandika1	25/09/2021	*Commelina benghalensis*	6	1	6
	25/09/2021	*Jussiaea abyssinica*	3	1	3
	25/09/2021	*Scleria melanomphala*	2	1	2
musandika3	25/09/2021	*Azolla filiculoides*	6	1	6
	25/09/2021	*C. papyrus*	1	1	1
	25/09/2021	*Leersia hexandra*	43	1	43
	25/09/2021	*Persicaria spp.*	4	1	4
nyabwegereka	25/09/2021	*Hydrocotyle leucocephala*	1	2	1
	25/09/2021	*Leersia hexandra*	550	2	275
	25/09/2021	*Persicaria spp.*	3	2	2
nyanjategera	25/09/2021	*Commelina benghalensis*	3	2	2
	25/09/2021	*Jussiaea abyssinica*	2	2	1
	25/09/2021	*Leersia hexandra*	38	2	19
	25/09/2021	*Persicaria spp.*	6	2	3
rwabituju2	25/09/2021	*Coix lacryma*	23	3	8
	25/09/2021	*Commelina benghalensis*	18	3	6
	25/09/2021	*Cynodon dactylon*	13	3	4
	25/09/2021	*Desmodium salicifolium*	3	3	1
	25/09/2021	*Leersia hexandra*	27	3	9
	25/09/2021	*Persicaria spp.*	7	3	2
rwabituju3	25/09/2021	*Commelina benghalensis*	15	1	15
	25/09/2021	*Cynodon dactylon*	60	1	60
	25/09/2021	*Persicaria spp.*	3	1	3
ntoroko1	27/09/2021	*Cynodon dactylon*	6	1	6
	27/09/2021	*Lemna spp.*	1000	1	1000
	27/09/2021	*Ludwigia stolonfera*	14	1	14
	27/09/2021	*Pontederia crassipes*	54	1	54
	27/09/2021	*Scleria melanomphala*	4	1	4
ntoroko2	27/09/2021	*Lemna spp.*	230	1	230
	27/09/2021	*Pontederia crassipes*	29	1	29
ntoroko3	27/09/2021	*Alternanthera nodiflora*	20	2	10

	27/09/2021	*C. papyrus*	13	2	7
	27/09/2021	*Cynodon dactylon*	1	2	1
	27/09/2021	*Cyperus digitatus*	3	2	2
	27/09/2021	*Echinochloa pyramidalis*	13	2	7
	27/09/2021	*Ipomea aquatica*	1	2	1
	27/09/2021	*Ludwigia stolonfera*	11	2	6
	27/09/2021	*Pontederia crassipes*	40	2	20
	27/09/2021	*Salvinia molesta*	28	2	14
	27/09/2021	*Typha spp.*	10	2	5
ntoroko6	27/09/2021	*Alternanthera nodiflora*	112	1	112
	27/09/2021	*Commelina benghalensis*	1	1	1
	27/09/2021	*Ipomea aquatica*	1	1	1
	27/09/2021	*Leersia hexandra*	2	1	2
	27/09/2021	*Ludwigia stolonfera*	12	1	12
	27/09/2021	*Pontederia crassipes*	4	1	4
bulenge2	22/10/2021	*Commelina benghalensis*	6	2	3
	22/10/2021	*Desmodium salicifolium*	2	2	1
	22/10/2021	*Leersia hexandra*	11	2	6
nyabwegereka	22/10/2021	*Desmodium salicifolium*	3	2	2
	22/10/2021	*Leersia hexandra*	115	2	58
eumu	24/10/2021	*Cyperus digitatus*	40	2	20
	24/10/2021	*Leersia hexandra*	158	2	79
nyakatojo2	24/10/2021	*Commelina benghalensis*	21	1	21
	24/10/2021	*Cyperus digitatus*	26	1	26
	24/10/2021	*Leersia hexandra*	19	1	19
nyakatojo5	24/10/2021	*Commelina benghalensis*	48	1	48
	24/10/2021	*Cynodon dactylon*	17	1	17
	24/10/2021	*Leersia hexandra*	35	1	35
	24/10/2021	*Persicaria spp.*	33	1	33
rubirizi1	24/10/2021	*Brillantaisia owariensis*	8	2	4
	24/10/2021	*Commelina benghalensis*	42	2	21
	24/10/2021	*Cynodon dactylon*	12	2	6
	24/10/2021	*Cyperus digitatus*	3	2	2
	24/10/2021	*Leersia hexandra*	16	2	8
	24/10/2021	*Persicaria spp.*	10	2	5
rubirizi2	24/10/2021	*Azolla filiculoides*	1700	1	1700
	24/10/2021	*Commelina benghalensis*	17	1	17
	24/10/2021	*Commelina benghalensis*	234	1	234
extra8	26/10/2021	*Commelina benghalensis*	4	1	4
	26/10/2021	*Leersia hexandra*	120	1	120
mukondo	26/10/2021	*Commelina benghalensis*	32	2	16
	26/10/2021	*Leersia hexandra*	36	2	18
nyakitukuma2	26/10/2021	*Commelina benghalensis*	30	2	15
	26/10/2021	*Cynodon dactylon*	2	2	1
	26/10/2021	*Cyperus digitatus*	5	2	3

	26/10/2021	*Jussiaea abyssinica*	3	2	2
	26/10/2021	*Leersia hexandra*	170	2	85
rwebigongoro	26/10/2021	*Cynodon dactylon*	9	2	5
	26/10/2021	*Cyperus digitatus*	16	2	8
	26/10/2021	*Jussiaea abyssinica*	16	2	8
	26/10/2021	*Leersia hexandra*	145	2	73
ntoroko1	28/10/2021	*Cynodon dactylon*	3	2	2
	28/10/2021	*Lemna spp.*	25	2	13
	28/10/2021	*Ludwigia stolonfera*	19	2	10
	28/10/2021	*Pontederia crassipes*	52	2	26
	28/10/2021	*Scleria melanomphala*	3	2	2
ntoroko2	28/10/2021	*Pontederia crassipes*	30	2	15
ntoroko3	28/10/2021	*Alternanthera nodiflora*	20	2	10
	28/10/2021	*C. papyrus*	13	2	7
	28/10/2021	*Cynodon dactylon*	1	2	1
	28/10/2021	*Cyperus digitatus*	3	2	2
	28/10/2021	*Echinochloa pyramidalis*	13	2	7
	28/10/2021	*Ipomea aquatica*	1	2	1
	28/10/2021	*Ludwigia stolonfera*	11	2	6
	28/10/2021	*Pontederia crassipes*	40	2	20
	28/10/2021	*Salvinia molesta*	28	2	14
	28/10/2021	*Typha spp.*	10	2	5
ntoroko4	28/10/2021	*Pontederia crassipes*	15	2	8
	28/10/2021	*Typha spp.*	2	2	1
kijanibarora	30/10/2021	*Commelina benghalensis*	4	2	2
	30/10/2021	*Echinochloa colona*	74	2	37
	30/10/2021	*Leersia hexandra*	41	2	21
kitebere2	30/10/2021	*Cyperus mundtii*	4	1	4
	30/10/2021	*G6*	37	1	37
	30/10/2021	*Leersia hexandra*	14	1	14
	30/10/2021	*Lemna spp.*	30	1	30
	30/10/2021	*Ludwigia stolonfera*	1	1	1
	30/10/2021	*Pontederia crassipes*	43	1	43
	30/10/2021	*Vallisneria spiralis*	10	1	10
kooga	30/10/2021	*Echinochloa colona*	72	2	36
	30/10/2021	*Pontederia crassipes*	3	2	2
	30/10/2021	*Utricularia reflexa*	5	2	3
	30/10/2021	*Utricularia stellaris*	17	2	9
ndaiga2	30/10/2021	*Azolla filiculoides*	493	2	247
	30/10/2021	*Cynodon dactylon*	17	1	17
	30/10/2021	*Cyperus mundtii*	49	2	25
	30/10/2021	*Pontederia crassipes*	98	2	49
ndaiga3	30/10/2021	*Azolla filiculoides*	260	2	130
	30/10/2021	*Cynodon dactylon*	133	2	67
	30/10/2021	*Lemna spp.*	120	2	60

	30/10/2021	*Ludwigia stolonfera*	3	2	2
	30/10/2021	*Pontederia crassipes*	20	2	10
	30/10/2021	*Salvinia molesta*	397	2	199
nyamasoga3	30/10/2021	*Commelina benghalensis*	26	1	26
ndaiga2	13/11/2021	*Alternanthera nodiflora*	27	2	14
	13/11/2021	*Azolla filiculoides*	160	3	53
	13/11/2021	*Cyperus mundtii*	2	3	1
	13/11/2021	*Ipomea aquatica*	2	3	1
	13/11/2021	*Ludwigia stolonfera*	2	2	1
	13/11/2021	*Pontederia crassipes*	25	3	8
nyakitukuma1	13/11/2021	*Commelina benghalensis*	23	2	12
	13/11/2021	*Cynodon dactylon*	64	2	32
	13/11/2021	*Cyperus digitatus*	22	2	11
	13/11/2021	*Jussiaea abyssinica*	7	2	4
	13/11/2021	*Leersia hexandra*	17	2	9
rwebigongoro	13/11/2021	*Azolla filiculoides*	6	2	3
	13/11/2021	*Commelina benghalensis*	3	2	2
	13/11/2021	*Cynodon dactylon*	42	2	21
	13/11/2021	*Cyperus digitatus*	13	2	7
	13/11/2021	*Jussiaea abyssinica*	7	2	4
	13/11/2021	*Leersia hexandra*	57	2	29
ntoroko5	25/01/2022	*Vallisneria spiralis*	1328	3	443
kitebere2	26/01/2022	*Utricularia reflexa*	370	1	370
kooga	26/01/2022	*Utricularia reflexa*	1300	3	433
	26/01/2022	*Utricularia stellaris*	7	3	2
ndaiga1	26/01/2022	*Potamogeton schweinfurthii*	8	4	2
	26/01/2022	*Utricularia reflexa*	692	4	173
	26/01/2022	*Vallisneria spiralis*	1205	3	402
ndaiga3	26/01/2022	*Azolla filiculoides*	1	1	1
	26/01/2022	*Utricularia reflexa*	10	3	3
	26/01/2022	*Vallisneria spiralis*	2112	4	528
nyamasoga1	26/01/2022	*Utricularia reflexa*	563	3	188
	26/01/2022	*Vallisneria spiralis*	20	3	7
nyamasoga2	26/01/2022	*Utricularia reflexa*	1100	3	367
hemu2	28/01/2022	*Persicaria spp.*	7	1	7
ntoroko3	22/02/2022	*C. papyrus*	5	3	2
	22/02/2022	*Lemna spp.*	130	3	43
	22/02/2022	*Potamogeton schweinfurthii*	30	3	10
	22/02/2022	*Utricularia reflexa*	13	3	4
	22/02/2022	*Vallisneria spiralis*	290	3	97
ntoroko5	22/02/2022	*Utricularia reflexa*	6	3	2
	22/02/2022	*Vallisneria spiralis*	1028	3	343
ntoroko6	22/02/2022	*Vallisneria spiralis*	1678	3	559

kamina	23/02/2022	*Utricularia reflexa*	52	3	17
	23/02/2022	*Vallisneria spiralis*	1240	3	413
kitebere2	23/02/2022	*Azolla filiculoides*	172	3	57
	23/02/2022	*Cyperus mundtii*	83	3	28
	23/02/2022	*Pontederia crassipes*	2	3	1
	23/02/2022	*Salvinia molesta*	7	3	2
	23/02/2022	*Utricularia reflexa*	520	3	173
	23/02/2022	*Vallisneria spiralis*	54	3	18
kooga	23/02/2022	*Cynodon dactylon*	8	3	3
	23/02/2022	*Echinochloa colona*	6	3	2
	23/02/2022	*Utricularia reflexa*	8	3	3
	23/02/2022	*Utricularia stellaris*	73	3	24
ndaiga1	23/02/2022	*Potamogeton schweinfurthii*	8	4	2
	23/02/2022	*Utricularia reflexa*	692	4	173
	23/02/2022	*Vallisneria spiralis*	1205	3	402
ndaiga2	23/02/2022	*Potamogeton schweinfurthii*	5	3	2
	23/02/2022	*Utricularia reflexa*	143	3	48
	23/02/2022	*Vallisneria spiralis*	246	3	82
ndaiga3	23/02/2022	*Potamogeton schweinfurthii*	13	3	4
	23/02/2022	*Utricularia reflexa*	68	3	23
	23/02/2022	*Vallisneria spiralis*	439	3	146
nyamasoga1	23/02/2022	*Potamogeton schweinfurthii*	2	3	1
	23/02/2022	*Utricularia reflexa*	368	3	123
	23/02/2022	*Utricularia stellaris*	24	3	8
	23/02/2022	*Vallisneria spiralis*	505	3	168
nyamasoga2	23/02/2022	*Potamogeton schweinfurthii*	5	3	2
	23/02/2022	*Utricularia reflexa*	449	3	150
	23/02/2022	*Vallisneria spiralis*	380	3	127
nyamasoga3	23/02/2022	*Commelina benghalensis*	28	3	9
ntoroko1	21/03/2022	*Pontederia crassipes*	2	1	2
	21/03/2022	*Typha spp.*	4	1	4
ntoroko2	21/03/2022	*Cyperus digitatus*	37	3	12
ntoroko3	21/03/2022	*Potamogeton schweinfurthii*	1	2	1
	21/03/2022	*Vallisneria spiralis*	585	2	293
ntoroko4	21/04/2022	*Commelina benghalensis*	2	3	1
	21/04/2022	*Cyperus mundtii*	9	3	3
	21/04/2022	*Echinochloa pyramidalis*	66	3	22
	21/04/2022	*Phragmites mauritianus*	2	3	1
	21/04/2022	*Pontederia crassipes*	14	3	5

	21/04/2022	*Utricularia stellaris*	9	3	3
	21/04/2022	*Vallisneria spiralis*	1	3	0
ndaiga2	22/04/2022	*Utricularia stellaris*	51	2	26
	22/04/2022	*Vallisneria spiralis*	1065	2	533