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Genetic characterization of *Biomphalaria pfeifferi* snails in Mwea Irrigation Scheme, Kirinyaga County, Kenya

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ABSTRACT

Objective: To study the distribution and abundance of *Biomphalaria (B.) pfeifferi* species in the Mwea Irrigation Scheme, their role as intermediate hosts for *Schistosoma (S.) mansoni*, and the impact of water parameters.

Methods: A longitudinal field survey was conducted across eight administrative wards and five habitat types using a time-based metal scoop method. Physicochemical water quality parameters were measured both *in situ* and in the laboratory. The snails were examined for *S. mansoni* infections by shedding method, and cercariae were identified microscopically. Species identification was also performed. The data was analyzed using ANOVA, regression analysis, and Pearson correlation to identify the association between water physiochemical parameters and *Biomphalaria* snail abundance. Level of significance was determined at 5% ($P \leq 0.05$) with 95% confidence limit.

Results: A total of 2 013 *Biomphalaria* species were collected, mainly from Murinduko and Nyangati Wards. Streams (965, 47.93%) and tertiary canals (676, 33.58%) showed the highest abundance. *Biomphalaria* species populations were recorded in September 2022 (1 225), with declines noted in February and August 2023. Snail abundance was significantly and positively correlated with pH (β=27.22, P<0.001), turbidity (β=1.117, P=0.008), and total nitrate (β=71.4, P=0.04), and negatively correlated with conductivity, temperature, total dissolved solids, salinity, and dissolved oxygen. No *S. mansoni* infections were detected; however, 69.95% of snails were infected with echinostome cercariae. *B. pfeifferi* was the dominant species. Sequence analysis revealed genetic diversity, with five NCBI reference sequences forming a distinct clade from the study samples.

Conclusions: *B. pfeifferi* populations showed seasonal variation and strong environmental associations, emphasizing the need for ecological monitoring in schistosomiasis control.

KEYWORDS: *Biomphalaria pfeifferi*; *Schistosoma mansoni*; Mwea Irrigation Scheme; Physicochemical water parameters and *Pomacea canaliculata*

Summary

Question: What environmental factors influence *Biomphalaria pfeifferi* distribution and abundance in the Mwea Irrigation Scheme?

Findings: The longitudinal field survey showed a significant variation in *Biomphalaria pfeifferi* snails' population. The snails' abundance positively correlated with pH, turbidity, and nitrate levels, and negatively with salinity and conductivity. No *Schistosoma mansoni* infection were detected, but *Echinostome cercariae* were common.

Meaning: The understanding of ecological influences is important for effective vector management and schistosomiasis control in irrigation systems, emphasizing the need for continuous ecological monitoring to inform policies and research in affected areas.

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1. Introduction

Snails play a critical role in maintaining natural ecosystems by contributing to litter decomposition, nutrient cycling, community dynamics, and energy flow[1]. Despite these benefits, some snail species are known to cause significant economic and environmental damage or to transmit diseases of major public health significance[2]. These diseases are prevalent in 78 low- and middle-income countries particularly in Africa where they affect more than 251 million people resulting in an estimated global burden of 2.19 million disability-adjusted life years[3]. Therefore, knowledge of the distribution and abundance of snails, their parasite infection status, and the factors that influence the distribution and abundance has significant ecological and public health significance.

Snails inhabit diverse types of permanent and ephemeral aquatic habitats including ponds, ditches, drainage channels, rice fields, swamps, streams, rivers, lakes, underground aquifers and springs. The detrition and abundance of snails in these aquatic habitats is influenced by a variety of biotic and abiotic factors such as the physical factors of temperature, precipitation, aquatic macrophyte cover, hydrography and substrate composition chemical factors like pH, electrical conductivity, chemical oxygen demand, total nitrogen, total phosphorus and the biochemical oxygen demand and biological factors like competition, food and interactions between predators and prey[2]. The relative contribution of these factors to snail distribution and abundance varies considerably among geographic regions, highlighting the need for location-specific investigations to inform appropriate disease prevention and management strategies.

Schistosoma (*S.*) *mansoni* remains a major public health challenge in irrigated rice agroecosystems in Africa because rice require continuous flooding for their growth and optimum yield and this agroecosystem perfectly fits the ecological requirements of the *Biomphalaria* snails. In Mwea *S. mansoni* infections has remained a public health concern[4]. The infection is associated with intestinal schistosomiasis which should be addressed to alleviate its burden. This is may be attributed to the region's farming activities, environmental factors, and drainage patterns. The lack of toilet facilities in the rice paddies enhances open defecation in secondary water canals, therefore introduces parasite eggs in water that are later transported and spread to adjacent paddies through feeder canals[5]. According to the Compendium of WHO and other UN Guidance on Health and Environment: 2024 Update, the egregious behaviors and destructive actions of the local residents, including reckless disregard for the environment, flagrant pollution, and blatant disregard for laws and regulations, lead to catastrophic environmental degradation and devastating impacts on public health. All this contributes to the parasite's spread and survival[6]. Thus, the area has associated with stable transmission of the infection and the resulting burden especially among the farmers interacting with contaminated water. In addition, *Pomacea* (*P.*) *canaliculata* has recently invaded rice fields in East Africa where it has become a destructive pest of rice[7]. Recent studies show that *P. canaliculata* was first reported in the Mwea Irrigation Scheme in 2020. The introduction of *P. canaliculata* to East Africa, has raised concerns, including its potential environmental effects such as herbivory, competition, predation, disease spread, and bioaccumulation of pollutants, as well as its lack of a permit from Kenya's Standing Technical Committee on Imports and Exports[8]. This is likely to affect the distribution and abundance of the *Biomphalaria* species and *S. mansoni* transmission dynamics. Thus, this study sought to assess the *Biomphalaria* species' abundance, distribution, and infection status in the Mwea Irrigation Scheme, Kirinyaga County. Further, it sought to establish the effect of water physicochemical properties on the abundance of the *Biomphalaria* species.

2. Methods

2.1. Study area

The study was conducted within the Mwea Irrigation Scheme in Kirinyaga County, located between 0°36′S–0°48′S latitudes and longitudes 37°12′E–37°30′E (Figure 1). The region is classified as tropical with a semi-arid climate, having an annual range air temperature of 23 ℃-25 ℃ with about a 10 ℃ difference between the minimum temperatures in June and July and the maximum temperatures in October and March. The average annual rainfall is between 1 260 mm and 1 600 mm. The scheme has an area of approximately 122.82 km^2, of which 90 km^2 have been developed for paddy production. The scheme is served by two rivers, the Nyamindi and Thiba, and several water channels crisscross the area, providing irrigation water to the farms. The water is drawn from the rivers by gravity through weirs and is distributed using unlined open channels to the farms. Rice and horticulture canal irrigation is the main socio-economic activity.

2.2. Study design

A field survey to collect data on water physicochemical parameters, population abundance, and distribution of *Biomphalaria* spp. in their natural environment in the Mwea Irrigation System was conducted.

The distribution of *Biomphalaria* spp. was determined through morphological identification using standard taxonomic keys based on shell morphology characteristics, as described by Brown[9]. A longitudinal study design was employed. Observations and measurements were done as per the objectives. Water and *Biomphalaria* spp. samples were transported to the lab for additional examination. The water samples were tested in the lab for ammonia,

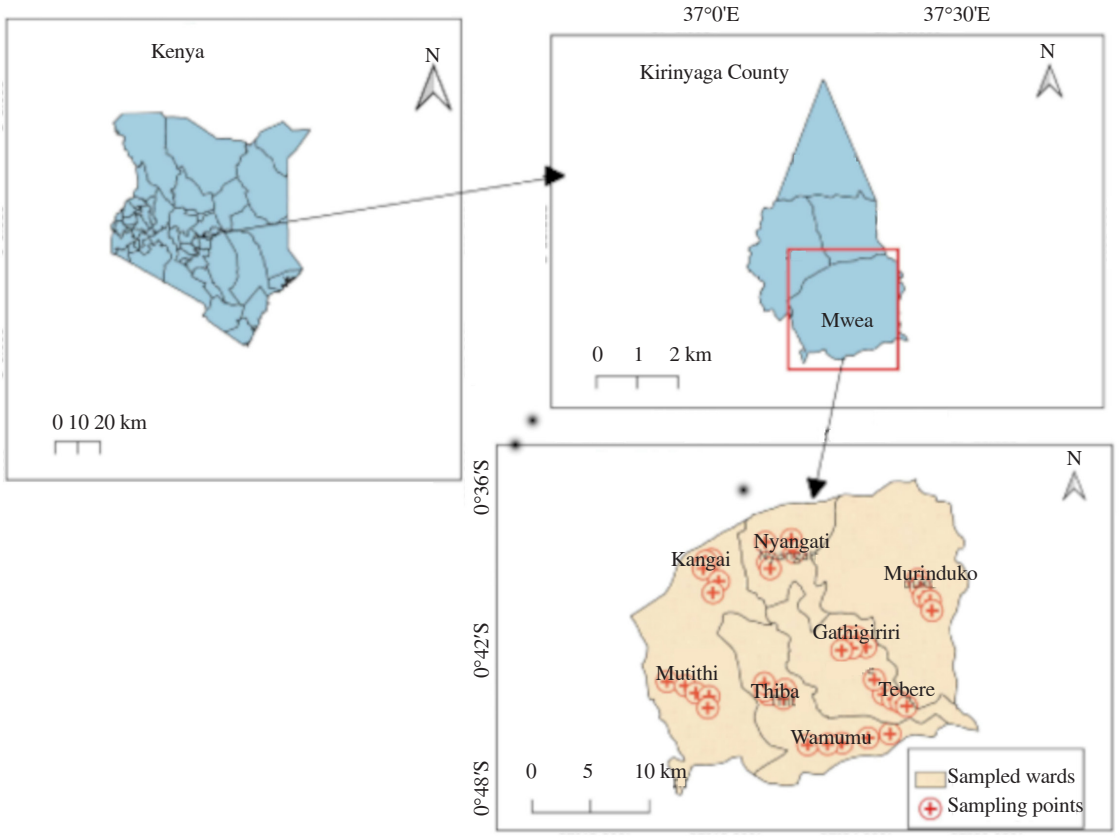

Figure 1. The map of Mwea Irrigation Scheme and the study sites.

total phosphate, and total nitrates using molecular absorption spectrophotometric analysis[9] and the *Biomphalaria* spp. samples were screened for *S. mansoni* infections using the shedding method and identified using molecular and morphological techniques.

2.3. Sampling design and sampling

Sampling was done in September 2022, February 2023, May 2023, June 2023, and August 2023. *Biomphalaria* spp. were sampled in eight administrative units (wards) of the Mwea Irrigation Scheme in Kirinyaga County: Murinduko, Nyangati, Kangai, Mutithi, Thiba, Gathigiriri, Tebere, and Wamumu. These wards were specifically chosen for the presence of active irrigation canals, streams, and human-water contact sites, all of which are possible habitats for *Biomphalaria* snails and areas with a high risk of schistosomiasis transmission.

A consistent procedure was used to examine water bodies for *Biomphalaria* spp.[10]. Five sampling habitats namely: secondary canals, tertiary canals, streams, swamps and rice paddies were sampled. Based on accessibility and the prior information that *Biomphalaria* spp. had a non-random distribution, sampling sites were selected. A time-based sample technique was employed, involving the collection of snails by passing a metal scoop through water, vegetation, and mud for a duration of 30 minutes. The *Biomphalaria* spp. were collected and stored in labelled, perforated plastic containers filled with canal water and vegetation from the irrigation system.

2.4. Physicochemical parameters analysis

Physico-chemical water quality assessments were conducted both in situ while sampling and in the laboratory. *In situ* measurements of water salinity, temperature (℃), conductivity (μS/cm), and total dissolved solids (TDS in ppm) were conducted using a combination pocket tester for salinity, conductivity, and TDS (Hanna HI98129; Hanna Instruments Ltd., UK). Dissolved oxygen was measured using a dissolved oxygen meter, turbidity using a turbidity meter, and depth using a graduated stick. The water samples were transported to the University of Embu chemistry laboratory for analysis of total nitrates, total phosphate, and ammonia using molecular absorption spectrophotometric analysis[11].

2.5. Parasitological test

For preservation and infection screening, the *Biomphalaria* spp. were taken to the laboratory of Kimbimbi Sub County Hospital.

The shedding method was used to examine for natural trematode infections in the *Biomphalaria* spp. Individual *Biomphalaria* spp. were put in shedding vials filled with deionized water and exposed to sunlight for one hour. The cercariae released by the *Biomphalaria* spp. were classified to the genus level based on their tail morphology using a dissecting microscope.

2.6. Morphological identification

Biomphalaria (B.) pfeifferi samples were identified to species level by specialists at the Kenya National Museum, utilizing established taxonomic criteria based on their physical characteristics.

2.7. Molecular identification

2.7.1. DNA extraction and preservation

Snail tissues were removed from the snail shell and processed by macerating them into fine fragments. Total genomic DNA was extracted from the tissues using a Qiagen kit (Qiagen Inc., MD, USA) following the manufacturer's protocol, with slight modifications. The incubation time was increased by 25 minutes, and the tissue-buffer mixture was homogenized with an applicator stick at 5-minute intervals to maximize DNA yield. Subsequently, 500 μL of cold absolute ethanol was added to precipitate the DNA. The lysate was centrifuged at 8 000 rpm for 30 seconds to pellet debris, and the supernatant was transferred into a silica matrix spin column. The DNA was then washed and eluted in 50 μL of molecular-grade water. The extracted DNA was quantified using a Qubit fluorimeter and stored at -20 ℃ for subsequent PCR amplification.

2.7.2. Polymerase Chain Reaction (PCR) and amplification

Biomphalaria spp. were amplified by PCR, using the primers ITS2F (5'-CGT CCG TCT GAG GGT CGG TTT GC-3') and ETTS1 (5'-TGC TTA AGT TCA GCG GGT-3')[12]. The primers target the conserved ITS2 region within the 5.8S and 28S ribosomal genes of *Biomphalaria* species, respectively. Primer sequences were synthesized by Macrogen EV, supplied in lyophilized form and reconstituted for PCR amplification. First optimized using OneTaq 2X Master Mix and *S. mansoni* laboratory isolate DNA obtained from BEI Resources through a gradient PCR approach to determine the optimal annealing temperature. Following optimization, PCR amplification was performed in a 25 μL reaction volume comprising 3 μL of template DNA, 0.2 μM of primer mix, 12.5 μL of OneTaq 2X Master Mix polymerase, and molecular-grade water to make up the remaining volume. The reactions were carried out using SimpliAMP thermal cycler (Applied Biosystems) under the following cycling conditions: an initial denaturation at 94 ℃ for 5 minutes, followed by 35 cycles of denaturation at 94 ℃ for 30 seconds, annealing at 55 ℃ for 30 seconds, and elongation at 72 ℃ for 30 seconds. This was followed by a final extension step at 72 ℃ for 5 minutes. Aliquots of the PCR products were electrophoretically resolved on 1.5% agarose gel before storage at 4 ℃[13].

2.7.3. PCR clean-up

For subsequent analyses of the PCR products, the amplified samples were cleaned using ExoSAP-IT kit. 2 μL of ExoSAP-IT was added to 10 μL of PCR amplicon, gently mixed and incubated at 37 ℃ for 15 minutes then at 80 ℃ for a further 15 minutes.

2.7.4. Sequencing

The purified PCR products were sequenced by the Sanger method on an ABI 3730xL automatic sequencer (Applied Biosystems) at The University of Nairobi-MMIDR Laboratory, using the same primers described above for amplification, sequences obtained were edited using Snap Gene Viewer software version 4.1.9.

2.8. Data analysis and phylogenetic analysis

Data was organized in Microsoft Excel and analyzed in SAS (r) proprietary software release 8.2 (TS2MO). A total of 2 013 *Biomphalaria* snails were collected from five habitat types across five study sites. For three of the four study sites (Murinduko, Kianjiru, and Togonye), all or nearly all snail samples were collected in September 2022. Thus, no additional statistics were conducted to evaluate how snail populations changed over time as it was clearly obvious. For Gathoge study site, where *Biomphalaria* spp. samples were distributed across all the five sampling periods, we created three models and used Akaike Information Criteria (AIC) to determine the best fit model for our data. These included two-way ANOVA with collection month and habitat types as fixed variables (model 1), linear mixed model (LMM) with habitat type as the fixed variable and collection month as the random variable (model 2), and LMM with collection month as the fixed variable and habitat type as the random variable (model 3). The variations in the physicochemical parameters were determined using the Analysis of Variance (ANOVA). Principal components analysis (PCA) was used to separate aquatic habitats based on the measured physical and chemical characteristics. The derived factors scores were assessed for multicollinearity using correlation analysis and found to have correlation coefficients of zero implying multicollinearity was not an issue. The association between the derived factor scores and *Biomphalaria* snail counts was determined using Person Correlation analysis. Snail counts were log transformed to normalize the data. All analyses were conducted using the R package "psych"). The level of significance was determined at $P \leq 0.05$.

A total of 23 samples were sequenced using the ITS2 primer. The resulting chromatograph sequencing data were processed using Chromas software (version 2.6.6, Technelysium Pty

Ltd, South Brisbane, Queensland, Australia, 2018) to obtain sequences in FASTA format. Sequence similarity analyses were performed using the BLASTn algorithm (National Center for BiotechnologyInformation[NCBI]; https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_L OC=blasthome#) with default settings. Given that all our sequences were less than 500 base pairs (bp) long, database entries with sequence lengths shorter than 1 000 bp were selected as close matches for comparison. Using close match sequences from NCBI the 23 sample sequences were aligned and trimmed using MEGA Version 11.0.13 to remove ambiguous nucleotides ("Noise") at the beginning and end of the sequences. All 23 samples and the five close match sequences were tested for model selection in MEGA, and the Kimura 2 parameter model, which had the lowest Bayesian Information Criterion (BIC=4 191.15), was selected. A maximum likelihood tree with 300 bootstrap replications was then generated to construct the phylogenetic tree.

3. Results

3.1. Spatial distribution and abundance of Biomphalaria pfeifferi

A total of 2 013 *B. pfeifferi* snails were collected from five habitat types across five study sites. For three of the four study sites (Murinduko, Kianjuri, and Tongonye), all or nearly all snail samples were collected in September 2022. Thus, no additional statistics were conducted to evaluate how snail populations changed over time as it was clearly obvious. For Gathoge study site, where *B. pfeifferi* samples were distributed across all the five sampling periods, we created three models and used AIC to determine the best fit model for our data. These included two-way ANOVA with collection month and habitat types as fixed variables (model 1), linear mixed model (LMM) with habitat type as the fixed variable and collection month as the random variable (model 2), and LMM with collection month as the fixed variable and habitat type as the random variable (model 3). The AIC values for models 1, 2, and 3 were 9.3, 1.42.1, and 28.0, respectively indicating that model 1 was the best fit for our data. This model revealed significant effects of habitat type and month of collection but not their interaction of the number of *B. pfeifferi* collected (Habitat type: F=28.46, P<0.001; Month of collection: F=25.34, P<0.001). No snails were collected in secondary canal and swamp habitats and these were excluded in the models. The number of snails collected in stream, tertiary canal, and rice paddies differed significantly from each other with the highest number of *B. pfeifferi* snails collected in stream habitats and the lowest number of *B. pfeifferi* snails collected in rice paddies. For the month effect, the number of *B. pfeifferi* snails collected in September 2022, May 2023, and June 2023 were significantly higher than those collected in February and August 2023.

For the site effect, ANOVA model with a fixed effect of study site [(F=7.184, P<0.001, AIC=390.4) was a better fit model than a model using study site as fixed variable and either habit type (AIC=1 032.0) or collection month (AIC=1 029.9) as a random variable. The number of snails collected in Gathoge were significantly higher than those collected in the other study sites.

A total of eight Wards, namely Murinduko, Nyangati, Kangai, Mutithi, Thiba, Gathigiriri, Tebere and Wamumu were sampled for *B. pfeifferi* snails: for the period September 2022 to August 2023. *Biomphalaria* snails were found in only two wards, that is Murinduko Ward (n=1 552) and Nyangati Ward (n=461). In Murinduko Ward, *Biomphalaria* snails were found in Murinduko locality (n=534) and Gathoge locality (n=1 018). Also, in Nyangati Ward, *B. pfeifferi* snails were found in Kianjiru locality (n=408) and Togonye locality (n=53) (Table 1).

According to Table 1 five habitats were sampled, namely, secondary canals, tertiary canals, rice paddies, streams, and swamp. Most *B. pfeifferi* snails were recorded in streams (n=965) (Table 1). In Murinduko Ward, Gathoge locality recorded the highest snail abundance with 1 018 individuals, mainly found in streams (n=560), tertiary canals (n=312) and rice paddies (n=146). Murinduko locality had 534 snails, found in streams (n=200), tertiary canals (n=224) and rice paddies (n=110). In Nyangati Ward, Kianjiru locality recorded a total of 408 snails, primarily found in streams (n=205), tertiary canals (n=120), and rice paddies (n=83). Togonye had the lowest counts of 53 *B. pfeifferi* snails, distributed among the tertiary canals (20), rice paddies (17), and swamps (16). Notably, no *B. pfeifferi* snails were found in secondary canals across all localities, and swamps generally had very low snail numbers, except for Togonye. Streams and tertiary canals proved to be the most productive habitats, supporting the majority of *B. pfeifferi* snails populations in the sampled areas.

3.2. Temporal distribution and abundance of Biomphalaria pfeifferi

According to Table 1 most *B. pfeifferi* snails were recorded in September 2022 (n=1 225) dropping in February 2023 (n=114), increasing in May 2023 (n=300) and June 2023 (n=320) and an eventual drop again in August 2023 (n=54). In September 2022, *B. pfeifferi* snails were found in all four localities, *i.e.*, Murinduko, Gathoge, Togonye and Kianjiru, (Table 1). In February 2023, *B. pfeifferi* snails were found in Gathoge (n=84) and Kianjiru (n=30). In May, June and August 2023, *B. pfeifferi* were found in Gathoge.

There were variations in abundance of *B. pfeifferi* snails in the different habitats over time (Table 1). There were no *B. pfeifferi* snails throughout the sampling period in secondary canals. In swamps, snails were recorded only in September 2022 (n=16). In

Table 1. Summary of *Biomphalaria pfeifferi* snail collections categorized by locality, habitat type, and collection period, including results from ANOVA models.

Ward	Locality	Habitat type	September 2022	February 2023	May 2023	June 2023	August 2023	Total snails	% of total (n=2013)
Murinduko	Gathoge	Secondary canals	0	0	0	0	0	0	0.00
		Tertiary canals	90	29	60	94	18	291	14.46
		Rice paddies	54	9	40	36	8	147	7.30
		Streams	116	46	200	190	28	580	28.81
		Swamps	0	0	0	0	0	0	0.00
		Total	260	84	300	320	54	1018	50.57
	Murinduko	Secondary canals	0	0	0	0	0	0	0.00
		Tertiary canals	224	0	0	0	0	224	11.13
		Rice paddies	110	0	0	0	0	110	5.46
		Streams	200	0	0	0	0	200	9.94
		Swamps	0	0	0	0	0	0	0.00
		Total	534	0	0	0	0	534	26.53
Nyangati	Kianjiru	Secondary canals	0	0	0	0	0	0	0.00
		Tertiary canals	130	11	0	0	0	141	7.00
		Rice paddies	79	3	0	0	0	82	4.07
		Streams	169	16	0	0	0	185	9.19
		Swamps	0	0	0	0	0	0	0.00
		Total	378	30	0	0	0	408	20.27
	Togonye	Secondary canals	0	0	0	0	0	0	0.00
		Tertiary canals	20	0	0	0	0	20	0.99
		Rice paddies	17	0	0	0	0	17	0.84
		Swamps	16	0	0	0	0	16	0.79
		Total	53	0	0	0	0	53	2.63
		Overall total	1225	114	300	320	54	2013	100.00

ANOVA – Effect of habitat type: F=28.46, df= 2,8, P=0.0002 ; ANOVA – Effect of month of collection: F=25.34, df=2,8, P=0.0001; ANOVA – Fixed effect of study site: F=7.184, df=3, 96, P=0.0002; The model incorporating study site as a fixed effect (AIC=390.4) demonstrated superior fit relative to models utilizing habitat type (AIC=1032.0) or collection month (AIC=1029.9) as random variables. The population of snails in Gathoge was markedly greater than in other locations.

September 2022, most *B. pfeifferi* snails were recorded in Streams (n=485), tertiary canal (n=464) and Rice Paddies (n=260). There was a decline in number of *B. pfeifferi* snails from September 2022 to February 2023, and again from May to August 2023.

The invasive freshwater *P. canaliculata* were found in seven of the sampled wards (Table 2) in June 2023. The highest counts of *P. canaliculata* were recorded in Wamumu Ward (n=299) and the lowest in Kangai Ward (n=135). Interestingly, no *B. pfeifferi* collected in areas where *P. canaliculata* was present.

Table 2. Abundance of *Pomacea canaliculata* and *Biomphalaria* sp. in June 2023.

Ward	*Pomacea canaliculata*	*Biomphalaria* sp.
Murinduko	0	320
Kangai	135	0
Nyangati	144	0
Mutithi	228	0
Thiba	251	0
Gathigiriri	263	0
Tebere	277	0
Wamumu	299	0
Total	1597	320

3.3. Water physicochemical parameters and Biomphalaria pfeifferi

The data from the Mwea Irrigation Scheme reveals differences in physicochemical parameters between two wards—Gathoge locality (Murinduko Ward) and Kianjiru locality (Nyangati Ward)—where *B. pfeifferi* were found. In contrast, other wards were primarily inhabited by *P. canaliculata*, a species that serves as a biological control agent, helping to prevent the establishment of *B. pfeifferi* in those regions. Unfortunately, physicochemical data for September 2021 could not be gathered due to the unavailability of suitable measuring equipment.

Table 3 presents February data revealing moderate dissolved oxygen (DO) levels, with Gathoge at 4.92 mg/L and Kianjiru at 3.9 mg/L. Gathoge exhibited a notable snail abundance of (10.8±1.3), alongside increased conductivity levels of 625.8 μS/cm, a temperature of 28.4 ℃, and turbidity measured at 20.61 NTU. Kianjiru exhibited a marginally elevated water temperature of 30.18 ℃ and conductivity of 908.2 μS/cm, while demonstrating a reduced snail abundance of (6.00 ± 1.22).

In May, the abundance of *B. pfeifferi* in Gathoge increased significantly (64±5.43), corresponding with decreased conductivity (11.48 μS/cm), minimal salinity (0.01), and stable pH (8.12). Turbidity was measured at 19.15 NTU, and dissolved oxygen (DO) levels rose to 5.64 mg/L. The conditions may have fostered a more conducive aquatic environment for the survival and reproduction of *Biomphalaria* spp. snails.

Snail abundance peaked in June at (77.20±15.74), coinciding with

Table 3. Variability of standard water physicochemical parameters measured across five study sites and *P. canaculata* in relation to the abundance of *Biomphalaria pfeifferi*.

Month	Locality	DO (mg/L)	pH	Cond (µS/cm)	Temp (C)	TDS (mg/L)	Sal	Turb (NTU)	Depth (cm)	PO_4 (mg/L)	NO_3 (mg/L)	NH_4 (mg/L)	*P. canaculata*	*B. pfeifferi*
Feb	Gathoge	4.92±0.66 (4.10-5.74)	7.24±0.17* (7.03-7.50)	625.8±2.28* (622.97-628.63)	28.40±0.42* (27.87-28.93)	310.84±0.86* (309.78-311.90)	0.30*	20.61±2.18* (17.90-23.32)	15.40±0.55 (14.72-16.08)	2.57±0.73 (1.66-3.48)	0.35±0.11* (0.23-0.48)	2.29±0.89 (1.18-3.40)	0	10.80±1.30 (9.18-12.42)
	Kianjiru	3.90±0.29 (3.54-4.26)	7.16±0.18* (6.95-7.37)	908.2±1.3* (906.58-909.82)	30.18±0.18* (29.96-30.40)	646.4±11.28* (632.39-660.41)	0.63*	3.85±0.05* (3.79-3.91)	10.60±0.80 (9.49-11.71)	3.00±0.32 (2.60-3.40)	0.27±0.12* (0.12-0.42)	2.57±0.29 (0.97-4.17)	0	6.00±1.22 (4.48-7.00)
May	Gathoge	5.64±0.15 (5.45-5.80)	8.12±0.11* (7.98-8.26)	11.48±0.18* (11.26-11.70)	29.34±0.25 *(29.03-29.65)	5.64±0.17* (5.43-5.85)	0.01*	19.15±0.03* (19.11-19.19)	14.70±2.30 (14.54-20.26)	3.20 (3.21-3.21)	0.48* (0.48-0.48)	2.24 (2.24-2.24)	0	64.00±5.43 (57.26-70.74)
Jun	Gathoge	3.82±0.18 (3.6-4.04)	9.64±0.05* (9.57-9.71)	10.84±0.65* (10.03-11.65)	24.64±0.05* (24.57-24.71)	5.64 ±0.38* (4.99-5.93)	0.01*	38.88±22.03* (11.52-66.24)	22.20±2.28 (19.37-25.03)	3.43±0.41 (2.92-3.93)	0.60±0.09* (0.49-0.72)	3.43±1.60 (1.44-5.41)	0	77.20 ±15.74 (57.66-96.74)
Aug	Gathoge	6.00±0.60 (5.26-6.74)	8.22±0.11* (8.08-8.36)	80.8±5.65* (73.78-87.82)	31.32±1.37* (29.62-33.02)	47.20 ±1.47* (45.38-49.02)	0.04*	25.67±1.29* (24.07-27.27)	34.20±3.19 (30.23-38.17)	2.36±0.54 (1.68-3.03)	0.62±0.23* (0.34-0.90)	3.14±1.36 (1.46-4.83)	0	10.80±0.84 (9.76-11.84)

*Represent physical and chemical parameters that showed significant differences ($P<0.05$) among sampling months and locations in relation to the abundance of *Biomphalaria pfeifferi*, as determined by one-way ANOVA analysis. Expressed by mean±SD and the minimum–maximum range.

cool temperatures of 24.64 ℃, elevated pH levels of 9.64, and significantly high turbidity measured at 38.88 NTU. The observed conditions may indicate elevated organic content or suspended particles, potentially enhancing food availability for snails or diminishing predator visibility. In August, a significant decrease in snail populations was recorded (10.8 ± 0.84), despite dissolved oxygen levels peaking at 6.00 mg/L and temperatures increasing to 31.32 ℃. Turbidity and nutrient concentrations, such as ammonia and nitrate, were moderately elevated; however, the reduction in snail populations may indicate seasonal variations or stress due to increased temperatures. The results indicate that the abundance of *B. pfeifferi* is likely affected by a complex interplay of environmental parameters, with moderate dissolved oxygen, alkaline pH, low salinity, and increased turbidity identified as potential favorable factors.

In February, *B. pfeifferi* snails were observed in both Gathoge and Kianjiru localities, however *P. canaliculata* was not detected at any of the sampling sites. In the following months, *B. pfeifferi* was mainly found in Gathoge, while Kianjiru was only dominated by *P. canaliculata*. The current study concentrated on *B. pfeifferi*, leading to data collection in subsequent months that prioritized Gathoge, where this species persisted, whereas no *B. pfeifferi* individuals were observed in Kianjiru or other locations. The lack of *B. pfeifferi* in Kianjiru corresponds with an increased occurrence of *P. canaliculata*, indicating potential habitat alterations or interspecific competition between the two species.

Regression analysis was performed to determine how water physicochemical parameters influenced the abundance of *B. pfeifferi* across the study sites. The model revealed that several parameters significantly affected snail abundance as shown in Table 4. A significant positive association was observed between *B. pfeifferi* abundance and pH (β=27.22, $P<0.001$), turbidity (β=1.117, $P= 0.008$), and total nitrate (β=71.400, $P=0.040$), indicating that slightly alkaline conditions, higher turbidity, and elevated nitrate levels favored the presence of *B. pfeifferi*. Conversely, significant negative associations were found with conductivity (β=–0.059, $P=0.001$), temperature (β=–9.021, $P<0.001$), total dissolved solids (β=–0.083, $P<0.001$), and salinity (β=–83.910, $P<0.001$), suggesting that higher levels of these parameters may inhibit *B. pfeifferi* populations. Dissolved oxygen (β=–4.831, $P=0.475$), ammonia (β= 2.051, $P=0.719$), and total phosphate (β=20.920, $P=0.087$) showed no significant relationship with *Biomphalaria* snails abundance.

Table 4. Regression analysis statistics for the relationships between abundance of *Biomphalaria* snails and water physiochemical characteristics.

Parameters	Regression coefficient (β)	Standard error	*t*	*P*-value
Intercept	57.220	32.912	1.738	0.096
Dissolved oxygen	-4.831	6.649	-0.727	0.475
PH	27.22	4.424	6.153	<0.001
Conductivity	-0.059	0.012	-4.748	0.001
Temperature	-9.021	2.017	-4.472	<0.001
Total dissolved solids	-0.083	0.019	-4.254	<0.001
Salinity	-83.910	20.360	-4.121	<0.001
Turbidity	1.117	0.388	2.880	0.008
Depth	0.166	0.787	0.211	0.834
Total phosphate	20.920	11.500	1.788	0.087
Total nitrate	71.400	32.750	2.180	0.040
Ammonia	2.051	5.638	0.364	0.719

According to Supplementary Table 1 two principal components (PC) explained 98% of the total variance based on the 9 environmental variables that were measured with PC1 accounting for 80% of the total variance. PC 1 was a linear combination of high loads on turbidity separating clean water from turbid water. PC2 was a linear combination of high loads on habitat depth, separating shallow habitats from deeper habitats.

3.4. Infection status of Biomphalaria pfeifferi species

According to Table 5, 69.95%, that is 1408 out of the 2013 *B. pfeifferi* snails collected, were found to be infected with echinostomes cercariae. None of the samples was infected with *S. mansoni* cercariae. In September 2022, samples from the four localities were infected (range 55.56%-74.91%; mean 66.29%). In February 2023, Kianjiru recorded had 66.67% infection rate while Gathoge had 53.57%. In Gathoge, infection rates decreased from 86.67% in May 2023 to 29.63% in August 2023.

Table 5. Infection rates of *Biomphalaria* snails with *Echinostomes cercariae*.

Month	Locality	Total number of snails	Snails infected *n* (%)
September 2022	Gathoge	260	172 (66.15)
	Kianjiru	378	210 (55.56)
	Murinduko	534	400 (74.91)
	Togonye	53	30 (56.60)
February 2023	Gathoge	84	45 (53.57)
	Kianjiru	30	20 (66.67)
May 2023	Gathoge	300	260 (86.67)
June 2023	Gathoge	320	255 (79.69)
August 2023	Gathoge	54	16 (29.63)
Total		2013	1408 (69.95)

3.4.1. Morphological and molecular Identification of Biomphalaria species

Morphometric standard taxonomic keys determined *Biomphalaria* species dominating the Mwea irrigation scheme as *B. pfeifferi*. PCR was used to amplify the snails' ITS2 region, yielding 500 bp products. Positive amplification is observed for pools 1-37, while pools 38-46 yielded a negative result (Supplementary Figure 1).

According to Supplementary Table 2, the sequence lengths of the 23 samples ranged from 344 bp (>9-ETTS1_F) to 442 bp (>14-ETTS1_F and >16-ETTS1_F). The percentage identity of the sequences, as determined by BLASTn analysis, ranged from 93.44% (>3-ETTS1_F) to 98.34% (>2-ETTS1_F). Close matches to GenBank accessions were identified for all samples. Specifically, two samples (>8-ETTS1_F and >10-ETTS1_F) closely matched accession KX644865.1, while three samples (>3-ETTS1_F, >19-ETTS1_F, and >21-ETTS1_F) had close matches to accession KX644869.1. Ten samples were identified as closely matching accession KX644872.1. Two samples (>9-ETTS1_F and >18-ETTS1_F) closely matched accession PQ783664.1, and six samples closely matched accession PQ783665.1.

Figure 2 presents the results of a maximum Likelihood phylogenetic analysis based on 23 *B. pfeifferi* ITS2 sequences from Mwea and five reference sequences retrieved from GenBank (PQ783664.1, PQ783665.1, KX644865.1, KX644869.1, and KX644872.1). The analysis revealed a significant genetic divergence between the study samples and the GenBank reference sequences, with all five references clustering together in a distinct clade. Notably, one sample from the study, 9-ETTS1_F1, showed closer genetic proximity to the reference sequences than the other samples, indicating a degree of genetic variability within the Mwea *B. pfeifferi* population.

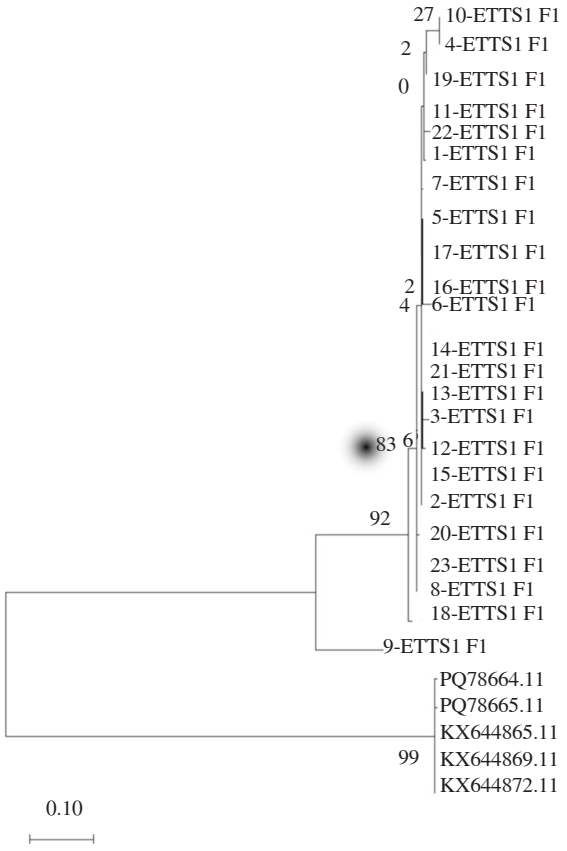

Figure 2. The maximum likelihood tree of 23 snail samples and an additional five close match sequences from NCBI.

4. Discussion

The present study aimed to characterize *Biomphalaria* spp., an intermediate host of *S. mansoni* parasites, their location, distribution, abundance, ecology and infectivity in the Mwea Irrigation Scheme in Kirinyaga County. The available literature on the *Biomphalaria* spp. in the Mwea Irrigation Scheme has been narrowing in terms of scope and method. A few studies solely based on morphological

identification may cause a misidentification, because the plasticity of the phenotype in snails is often coupled with a resemblance of shell morphology in closely associated species[14]. Based on these results, all 23 samples were assigned as *B. pfeifferi*. This assignment aligns with previously published ITS2-based identification studies of *Biomphalaria* species[15]. The sequence variation observed within the dataset reflects the natural intraspecific variability of *B. pfeifferi* populations, as reported in prior studies done by Palasio *et al.*[16]. The phylogenetic analysis revealed that the five NCBI sequences formed a distinct clade separate from the 23 *B. pfeifferi* samples suggesting genetic divergence between the sampled populations.

In the Mwea samples obtained from select locations in Murinduko and Nyangati Wards, a minimum of three clades were detected, signifying substantial population genetic structure and restricted gene flow among these areas, possibly driven by ecological and environmental factors[17]. Additionally, the presence of invasive *P. canaliculata* snails, known for their role as biological control agents against *Biomphalaria* spp., may have contributed to the observed genetic structure by exerting selective pressure or reducing population sizes in certain areas, thereby limiting gene exchange among snail populations[18].

Identification keys and molecular DNA barcoding confirmed that *B. pfeifferi* is the dominant species in this rice-growing region. This is consistent with results in[19], which also found *B. pfeifferi* to be common in this area. DNA barcoding also identified all sequences as belonging to *B. pfeifferi*. Nevertheless, inconsistent results have been described by Palasio[16] who, in different regions using molecular analysis, observed high interspecific genetic differentiation among *Biomphalaria* species, even when morphometric features indicated phenotypic constancy. This highlights the need to integrate molecular and classical morphological analyses in order to build a complete picture of *Biomphalaria* snails taxonomy[16]. This study presents a novel approach by integrating morphometric, molecular and ecological analyses to create a robust and multidimensional framework for identifying and studying *Biomphalaria* spp. populations. DNA barcoding effectively overcomes the limitations of traditional morphological methods. These results confirm the identification of the snails as *B. pfeifferi* and emphasize the reliability of molecular identification techniques in verifying species identity.

In earlier work within the Mwea Irrigation Scheme, Bulinus and other species have been found adapted to the same environment with *B. pfeifferi* snails[20]. Nevertheless, the invasive *P. canaliculata* has been recently reported in the scheme and the surrounding areas. The presence of *P. canaliculata* is notable because it poses a significant ecological threat due to its high reproductive capacity, competitive advantages, and ability to outcompete native snail species like *B. pfeifferi*[8]. This study found *P. canaliculata* snails in seven Wards and most localities of Murinduko Ward was also invaded. These findings suggest that the presence of *P. canaliculata* snails in Mwea Irrigation Scheme could be the reason for the diminished population of *B. pfeifferi* in Murinduku Ward and their absence in seven wards, which were not detected at all despite the extensive search indicating a possible exclusion or displacement effect. This observation highlights the ecological impact of *P. canaliculata* and its potential influence on the distribution of *Biomphalaria* spp. in the sampled areas. They are able to outcompete other snail species because of their comparatively bigger size, high trophic flexibility, high feeding rates, and reproductive potential[21]. Their ability to withstand abiotic stressors like hypoxia, high salinity, desiccation, extreme temperatures, adaptability in life cycles, and resistance to starvation, further enhances their competitive advantage[22]. These snails provide a crucial medicinal and ecological role by eating aquatic vegetation and diminishing *Biomphalaria* spp. by preying on their egg masses and juveniles, hence reducing the prevalence of schistosomiasis[23]. No attention has been given to the relationship between *Biomphalaria* spp. and invasive species, such as *P. canaliculata*, in the Mwea Irrigation Scheme. This sheds light on how invasive species can disrupt native snail ecology and potentially alter the dynamics of schistosomiasis transmission within the region. Rising temperatures and changing rainfall patterns in East Africa are predicted to increase suitable *P. canaliculata* snail habitats by the 2050s, increasing the risk of colonization in previously unsuitable areas[21]. Surveys and modeling reveal the snail's spread across Eastern Africa's water-connected rice ecosystems, a trend exacerbated by climate-induced flooding events[24]. Uncertainty surrounds *P. canaliculata* introduction in Kenya, possibly for research or biocontrol, but lack of formal documentation or import permits from Kenyan regulatory bodies raises concerns[25].

The AIC model comparison provides a significant statistical support for Model 1 as the most suitable for examining *B. pfeifferi* snail distribution. Model 1, with an AIC of 9.3 significantly lower than Models 2 (42.1) and 3 (28.0) demonstrates an effective equilibrium between the complexity and fitness. This aligns with previous studies that promote the use of AIC in ecological modelling to prevent overfitting while maintaining sufficient explanatory power[26].

The ecological distinctiveness of *Biomphalaria* spp. is significantly influenced by their habitat type and collection month. Comparable results have been documented in research conducted throughout sub-Saharan Africa where *B. pfeifferi* populations are found to be more abundant in permanent, flowing water bodies like streams and canals, unlike temporary or stagnant habitats like swamps and rice paddies that may experience drying or possess lower oxygen levels[27]. The total absence of snails in secondary canals and swamps environments corresponds with the findings of Olkeba *et al.* and Cando *et al.*[2,3], who highlighted that the quality of water, vegetation presence, and substrate type are essential for snail colonization. Streams provide

constant biological settings as well as an abundance of debris or algae, which increases snail productivity.

The seasonal fluctuations in snail population, peaking in September 2022, May 2023, and June 2023, likely indicate favorable climatic conditions, including enhanced precipitation, mild temperatures, and elevated water levels, which facilitate snail reproduction. The observed patterns align with previous findings by Kazibwe *et al.* and Nwoko *et al.*[28] which indicated increased freshwater snails abundance during or following the rainy seasons. The temporal peaks correspond to periods when people are more susceptible to schistosomiasis transmission if they come into touch with infested waters. Prior to these high-risk periods, targeted snail control or public awareness campaigns could be implemented.

Prior studies, which lacked temporal sampling, could not adequately document seasonal fluctuations in *B. pfeifferi* snail populations and their associations with environmental factors and largely overlooked the significance of physicochemical data in influencing snail distribution and abundance[20]. In contrast, year-round sampling across multiple localities in this study provided detailed insights into both temporal and spatial variations within *Biomphalaria* populations. These variations were analyzed in relation to key environmental factors such as salinity, conductivity, pH, and temperature that have rarely been studied comprehensively in the region. The findings underscore the importance of specific physicochemical parameters in determining the habitat suitability for *B. pfeifferi* and provide critical insights for the management of environments to control the spread of snails, which are key intermediate hosts in the schistosomiasis lifecycle. The regression analysis emphasizes the important role that physicochemical water parameters play in determining the distribution of *B. pfeifferi* across the study sites. The analysis showed both positive and negative correlations between the abundance of *B. pfeifferi* and different water parameters.

The abundance of *B. pfeifferi* snails was recorded from Murinduko and Nyangati Wards, *i.e.*, two out of eight Wards sampled. Two localities in each of this two Wards were infested, *i.e.*, Murinduko and Gathoge in Murinduko Ward, and Togonye and Kianjiru in Nyangati Ward. Within Wards and localities, snails were most abundant in streams followed by tertiary canals, rice paddies, and swamps. No *B. pfeifferi* snails were found in the secondary canals. Snail abundance is known to vary from region to region depending on a variety of environmental and biotic factors[29]. A lack of snails in secondary canals could be attributed to the cemented structure of most secondary canals that enhances the speed of the flowing water. Cement-lining ditches are the most effective snail management method because they keep them free of silt and vegetation where snails could breed[30]. The overall variation in *B. pfeifferi* snails abundance can be related to water properties, aquatic plant types, and aquatic substrate properties[29].

This study found marked temporal variations in snail abundance, although patterns differ among localities and habitats. *B. pfeifferi* snails' abundance varied considerably from September 2022 to August 2023, within the study sites. This was consistent with earlier findings reported by Oso & Odaibo[31]. *Biomphalaria* snails were more abundant in September 2022 after a long rainy season. The pools close to the streams and swamps provide appropriate environments for *B. pfeifferi* snails. Also, most of the study sites had not been invaded by *P. canaliculata* snails. The distribution and abundance of *B. pfeifferi* snails declined in February. This occurred during the hot, dry season when the majority of streams and swamps were desiccated, resulting in a limited number of *B. pfeifferi* snails being discovered. *B. pfeifferi* snails may have perished, or some may have been aestivating. The long rainy season occurred from March to June and the abundance of *B. pfeifferi* snails was high during this period. This could be attributed to the vegetation along the stream that provided a favorable environment for *B. pfeifferi* snail growth, proliferation, and protection[32]. *B. pfeifferi* snails' abundance and spread declined in August 2023. This was the rice-planting period when streams and irrigation canals had been cleared. The number of *B. pfeifferi* snails was low during this rainy season due to flooding by the water bodies. A similar observation was reported in the study done by Oso & Odaibo[31]. DO levels fluctuated across months, with Gathoge showing a range from (3.82±0.21) mg/L (range: 3.51-4.13) in June to (6.00±0.34) mg/L (range: 5.54-6.46) in August. Kianjiru in February recorded a lower DO level of (3.90± 0.17) mg/L (range: 3.67-4.13), possibly reflecting localized environmental conditions or higher organic matter decomposition rates. The low DO levels in both wards could stress aquatic organisms, especially in June when Gathoge recorded its lowest DO level.

Kianjiru recorded the highest conductivity [(908.2±1.3) μS/cm, (range: 906.58-909.82) and TDS (646.4±2.3) mg/L (range: 643.88-648.92)] in February, indicating a higher ionic content. This might be due to agricultural runoff or localized soil characteristics contributing to salinity. Gathoge had significantly lower values, with conductivity and TDS decreasing dramatically in May and June, reflecting either dilution from precipitation or lower solute input during these months. A negative correlation existed between water conductivity and *B. pfeifferi* populations. This was evident in February, when conductivity values were highest, and low numbers of *B. pfeifferi* snails. This corresponds with the findings of the study done by Rowel *et al.*[33]. An elevation in water conductivity was noted during the dry season, which diminished during the rainy season. The rise in conductivity during the dry season was likely attributable to elevated evaporation rates. The total dissolved solids were also shown to influence the *B. pfeifferi* populations and showed a statistically significant negative relationship with *B. pfeifferi*

populations. This result means that high TDS values correspond to low population values in *B. pfeifferi* populations. Total dissolved solids are important in determining the total concentration of substances dissolved in water (inorganic salts and organic materials). The TDS mean ranges from (5.64±0.38) mg/L (range: 4.99-5.93) to (646.4±2.3) mg/L (range: 643.88-648.92). The TDS demonstrated a trend similar to salinity and conductivity, in line with the results reported by Oso and Odaibo[31].

This study found a negative regression between salinity and *B. pfeifferi* populations. This result implies high water salinity values correspond to low population values in *B. pfeifferi* populations. The salinity mean was (0.30±0.01) (range: 0.29-0.31) at Gathoge and (0.63±0.02) (range: 0.60-0.66) at Kianjiru in February. It declined to (0.01±0.00) (range: 0.01-0.01) at Gathoge in May and June. It then rose to (0.04±0.01) (range: 0.03-0.05) in August. During the dry month of February, the salinity was high. This was during the hot dry season when in most of the streams and swamps, the water level had dropped. Salinity levels decreased during May and June and then increased a little bit in August. This fluctuation can be explained by the fact that water salinity is controlled by dilutions; for instance, direct precipitation lowers the salinity. A decline in salinity favors the proliferation of aquatic plants that prevent the penetration of solar rays hence depleting dissolved oxygen and this lower *B. pfeifferi* populations[34].

The pH values across both locations remained slightly alkaline, ranging between (7.16±0.18) (range: 6.95-7.37) at Kianjiru in February and (9.64±0.05) (range: 9.57-9.71) at Gathoge in June. The more alkaline conditions in June at Gathoge could result from reduced precipitation or increased evaporation, concentrating bicarbonates in the water[31]. The slightly alkaline pH favors the existence of freshwater snails. The notable positive link between pH and *B. pfeifferi* populations in this study corresponds with the research conducted by Oso & Odaibo[31]. The temperature was also shown to influence the *B. pfeifferi* populations and showed a statistically significant negative relationship with *B. pfeifferi* populations. Water temperatures ranged between (24.64±0.42) ℃ (range: 24.10-25.18 ℃) at Gathoge in June to (31.32±1.37) ℃ (range: 29.62-33.02 ℃) at Gathoge in August. Higher temperatures in August correspond with seasonal warming, potentially influencing metabolic rates and reproduction of *B. snails*[35]. The turbidity has also been shown to influence the *B. pfeifferi* populations and showed a statistically significant positive relationship with *Biomphalaria* spp. populations. Turbidity showed significant variation, peaking at (38.88±22.03) NTU (11.52-66.24) in Gathoge in June.

Elevated turbidity could reduce light penetration, affecting primary productivity and aquatic vegetation growth[36]. The total nitrate has also been shown to influence the *B. pfeifferi* and showed a statistically significant positive relationship with *B. pfeifferi*. The notable positive association between total nitrate and *B. pfeifferi* populations in this study corresponds with the research conducted by Marie *et al.*[37]. Nitrate increases organic matter and promotes the growth of aquatic vegetation such as algae, which are the snails' main food source.

Previous studies in the Mwea Irrigation Scheme have documented *B. pfeifferi* snails infected with *S. mansoni* parasites[15]. However, the study uniquely investigated *Biomphalaria* spp. infectivity, revealing no schistosome infections; instead, it found a high prevalence of *E. cercariae* (66.95%). The results indicate that *E. cercariae* infection was widespread in all locations, showing a significant persistence in Gathoge over time. The lack of schistosome cercariae suggests a possible disruption in their lifecycle, which could be attributed to competition from *Echinostoma* species or other ecological influences[38]. The antagonistic relationship between *E. cercaria* and *S. mansoni* cercariae could be the cause of the total lack of *Schistosoma* larval stages at all examined locations[39]. Antagonism is a complicated interaction between species of larval trematodes that can affect abundance patterns among larval trematode species.

Inter-species competition for habitat and resources imposes significant selection pressure on trematodes, frequently resulting in echinostomes being the predominant species when various digenetic trematodes inhabit the same snail host[40]. *E. cercariae*, which exhibit strong competitiveness against human schistosomes by using the same intermediate snail hosts, pose a threat to *Schistosoma* species establishment, and some *Echinostoma* species have even been proposed for inclusion in management programs due to their potential dominance over human schistosomes[41]. This observation underscores the potential of E. cercariae as a biological control agent for schistosomiasis.

4.1. Conclusions

In conclusion, the abundance and distribution of *B. pfeifferi* snails were positively correlated with water temperature, depth, pH, total nitrate, dissolved oxygen, and turbidity, indicating that these physicochemical parameters influence the presence of snail intermediate hosts of human schistosomes. Conversely, snail density decreased with increased salinity, conductivity, and total dissolved solids, emphasizing the importance of water quality in the growth, development of *B. pfeifferi* and *S. mansoni* they transmit.

This study revealed that *B. pfeifferi* snails, the intermediate hosts of *S. mansoni*, dominate the Mwea Irrigation Scheme. The invasive snail *P. canaliculata* was found in seven of the eight Wards sampled but did not co-occur with *B. pfeifferi*, suggesting that *P. canaliculata* may be a highly successful invader, competitor, and predator of other snails. The study also found that *B. pfeifferi* snails were infected with echinostome cercariae, a parasite that causes human echinostomiasis, with infection rates below 100%, suggesting variability in host susceptibility, environmental conditions, or parasite distribution,

which may have implications for biological control strategies. Additionally, the absence of *S. mansoni* cercariae in *B. pfeifferi* snails suggests a low or possibly absent transmission of schistosomiasis in the area, potentially influenced by environmental factors or competition with other parasites such as *E. cercariae*. Understanding genetic variation and ecological interactions between the *E. cercariae* parasites and *B. pfeifferi* snail hosts is crucial for effective biological strategies. Therefore, integrating molecular tools with ecological investigations improves targeted, sustainable, and environmentally responsible interventions for controlling schistosomiasis.

This study has limitations, *P. canaliculate* was found to invade and reduce *B. pfeifferi* snail populations, and this invasion modified the natural population dynamics of the *B. pfeifferi* snails, resulting in their diminished numbers and a curtailed distribution within the Mwea Irrigation Scheme. The *B. pfeifferi* snails were restricted to a small number of locations and were growing scarcer with time.

4.2. Recommendations

This study successfully established the distribution, abundance, and species diversity of *Biomphalaria* species in the Mwea Irrigation Scheme and examined the influence of physicochemical water parameters on their distribution. However, given the invasion of *P. canaliculata*, further studies should assess its long-term effects on *Biomphalaria* species and its implications for schistosomiasis transmission. Additionally, this study focused on the prevalence and intensity of *S. mansoni* infections in *B. pfeifferi*, but future research should extend to human definitive hosts. Assessing *S. mansoni* prevalence in humans would provide a more comprehensive epidemiological picture and evaluate whether the integrated control measures in Mwea, including snail control and environmental modifications, are effectively reducing schistosomiasis transmission. Moreover, this study highlights the competitive interactions between echinostome parasites and *Schistosoma* species within *Biomphalaria* spp. snails. Given the potential of echinostomes as a natural competitor that could suppress *Schistosoma* infections, further investigations should explore their role in biological control programs. Research should focus on the ecological interactions, safety, and long-term effectiveness of using echinostomes as a sustainable, eco-friendly intervention for reducing *Schistosoma* parasites transmission.

Conflict of interest statement

There is no conflict of interest regarding the publication of this article.

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Authors' contributions

S. W. C. conceived and designed the study, defined the intellectual content, conducted the literature search, participated in data acquisition and analysis, and prepared and edited the manuscript. L. O. contributed to the study concept and design, participated in data analysis, and assisted in manuscript editing and review. F. N. N. participated in data analysis, statistical analysis, and manuscript review. C. M. contributed to data acquisition, analysis, and manuscript editing and review. J.W. N. participated in study design, data acquisition, statistical analysis, and manuscript editing and review. All authors read and approved the final version of the manuscript.

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