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# Freshwater malacological fauna of the Ruzizi plain (South Kivu, Democratic Republic of the Congo): biodiversity, infestation rates and outbreaks of infection of schistosomiasis

**By**

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# DEDICATION

To my parents Asende Ekyamba and Julie Mwaliasha who gave me the opportunity to study natural sciences.

To my fiancée Jeannine Safi who strengthened me to fulfil these studies.

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**SUMMARY**

Located in the Albertine Rift, the Ruzizi plain is a natural area of high freshwater molluscan Biodiversity. Unfortunately, there is very little information about the freshwater gastropods of the Ruzizi Congolese plain. In the Ruzizi plain, intestinal schistosomiasis due to *Schistosoma mansoni* is one of the most important public health problems and its intermediate host mollusc is *Biomphalaria pfeifferi*.

This study provides information about molluscan biodiversity in the freshwaters of Ruzizi Congolese plain, infestation rates and the prevalence of schistosomiasis.

Sampling was carried out in three representative sites, including Luvungi, Sange and Kiliba. The samples were collected from 2$^{nd}$ May 2015 to 2$^{nd}$ June 2015. Each of the sites included 2 ponds, 2 irrigation canals and 2 rivers. In total, 18 collecting points have been visited twice each one. The medical statistics data of outbreaks of infection of schistosomiasis were recorded from hospitals in these sites. Statistical analyses were performed using ANOVA and Kruskal Wallis tests in R software. We also used Shannon, Simpson and Fisher-alpha diversity indices in PAST software.

A total of 3763 snails were collected belonging to 11 gastropod species such as *Biomphalaria pfeifferi* (30.75%), *Melanoides tuberculata* (25.64%), *Melanoides sp* (18.89%), *Pila ovata* (17.06%), *Lymnaea natalensis* (3.19%), *Bulinus tropicus* (2.84%), *Bulinus globosus* (1.44%), *Bulinus forskalii* (0.05%), *Lymnaea sp* (0.05%), *Biomphalaria sp* (0.05%) and *Bellamya sp* (0.03%). Of them, only *B. pfeifferi* was found in the Ruzizi plain as the highest abundant species. The ponds and irrigation canals dug for the agricultural needs provide a higher abundance of molluscs than rivers. There was no significant difference in the total numbers of gastropods collected these three freshwater habitats ($p= 0.4819$).

The findings showed schistosomiasis is reported in all Ruzizi Congolese plain, but Luvungi is a locality where the prevalence of schistosomiasis becomes greatest increased ($P < 0.001$). Only *B. pfeifferi* was shedding *S. mansoni* cercariae in the Ruzizi Congolese plain, but *B. globosus* and *B. forskalii* were never found shedding human schistosome cercariae during our survey. The infestation rates of *B. pfeifferi* at Luvungi, Sange and Kiliba were 24.5%, 18.3% and 15.4% respectively.

**Key words:** Freshwater molluscs, infestation rates, outbreaks of schistosomiasis, Ruzizi plain.

**RESUME**

Située dans le Rift Albertin, la plaine de la Ruzizi est une zone naturelle à haute biodiversité malacologique. Malheureusement, il y a très peu d'informations sur les gastéropodes des eaux douces de la plaine de la Ruzizi. Dans la région de la Ruzizi, la schistosomiase intestinale à *Schistosoma mansoni* est l'un de plus grands problèmes de la santé publique et son hôte intermédiaire est le mollusque *Biomphalaria pfeifferi*.

Ce travail fournit une information sur la biodiversité des mollusques dans les eaux douces de la plaine de la Ruzizi, le taux d'infestation et la prévalence de la schistosomiase. La collecte des échantillons a été effectuée dans trois sites, comprenant Luvungi, Sange et Kiliba. Les échantillons étaient collectés du 02 Mai 2015 au 02 Juin 2015. Chaque site comprenait 2 étangs, 2 canaux d'irrigation et 2 rivières. Au total, 18 stations ont été visitées deux fois chacune. Les données statistiques médicales des foyers d'infection de la schistosomiase étaient enregistrées dans les hôpitaux de ces trois sites. Pour effectuer les analyses des données, les tests ANOVA et Kruskal Wallis ont été utilisés grâce au logiciel R et les indices Shannon, Simpson et Fisher-alpha à l'aide du logiciel PAST.

3763 spécimens ont été capturés et sont répartis dans 11 espèces. Ces dernières sont respectivement: *Biomphalaria pfeifferi* (30.75%), *Melanoides tuberculata* (25.64%), *Melanoides sp* (18.89%), *Pila ovata* (17.06%), *Lymnaea natalensis* (3.19%), *Bulinus tropicus* (2.84%), *Bulinus globosus* (1.44%), *Bulinus forskalii* (0.05%), *Lymnaea sp* (0.05%), *Biomphalaria sp* (0.05%) et *Bellamya sp* (0.03%). Seulement, *B. pfeifferi* a été trouvé dans la plaine de la Ruzizi comme l'espèce la plus abondante. Les étangs et canaux d'irrigation creusés pour les fins agricoles offrent une forte diversité spécifique des mollusques que les rivières. Mais il n'y a pas une différence significative sur le nombre total de mollusques collectés dans ces trois types d'habitats dulcicoles ($p= 0.4819$).

Les résultats montrent que la bilharziose est répandue dans toute la plaine de la Ruzizi, mais Luvungi est une localité où la prévalence de la bilharziose est très élevée ($p < 0.001$). Seulement, *B. pfeifferi* propageait les cercaires de *S. mansoni,* mais le *B. globosus* et *B. forskalii* n'ont pas été trouvés comme vecteurs des cercaires de schictosome humain au cours de notre enquête. Les taux d'infestation de *B. pfeifferi* à Luvungi, Sange and Kiliba étaient 24.5%, 18.3% and 15.4% respectivement.

**Mots clés :** Mollusques d'eaux douces, taux d'infestation, foyers de bilharziose, plaine de la Ruzizi

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# CHAPTER ONE: INTRODUCTION

## 1.1. Background

Essential component of freshwater ecosystems, the Molluscs provide exploitable resources for both fish and humans (Thiam & Diallo, 2010). The phylum Mollusca is an enormously diverse taxon second only to Arthropoda in number of extant species (Agaba, 2013). Gastropoda is the largest and most diverse molluscan taxon comprising snails and slugs (Ruppert *et al*., 2004 in Agaba, 2013). The knowledge of the total freshwater malacological biodiversity is incomplete especially in tropical latitudes supporting the largest number of species of the world. However, the sustainable management of freshwater ecosystems in our country needs a follow-up programme of study, control and classification of these (Kankonda, 2001 & 2008).

Molluscs are present in most African freshwater environments. They are distinguished from other aquatic organisms by the presence of a limestone shell consisting of one-piece in gastropods group and two parts articulated to the Lamellibranchs (called Pelecypoda or bivalves) (Lévêque and Durand, 1980) .

Most freshwater molluscs play a significant medical and veterinary role of a snail as known from the natural transmission of parasites (Lévêque and Durand, 1980; De Clercq, 1987; Brown, 1994; Campbell *et al.,* 2000; Day & De Moor, 2002; Ntonifor and Ajayi, 2007; Kane *et al.*, 2008; Darwall *et al*., 2011; Kotchi *et al.*, 2013; Zaghloul *et al.*, 2013). They are necessarily place of multiplication of the larval stages of trematode parasites. Also, some molluscs are used in biological control against the vectors of these parasitic diseases (Kotchi *et al.*, 2013). Freshwater molluscs are also involved in the determination of several indices of biotic integrity as the Global Standardized Biological Index (IBGN) (Kotchi *et al.*, 2013).

There are just fewer than 5,000 freshwater molluscs for which valid descriptions exist, in addition to a possible 4,000 undescribed gastropod species (Bogan 2008; Strong *et al*. 2008 in Darwall *et al*., 2011). Of these, only a small number had their conservation status assessed before this assessment, with just 14% of known species (679) on the IUCN Red List at the time (Darwall *et al*., 2011).

Gastropods have been divided commonly into two major groups, the Streptoneura (including those known as the Prosobranchia or prosobranchs) and the Euthyneura (pulmonates and opisthobranchs). Almost all African freshwater prosobranchs have an operculum of horny or calcareous material attached to the foot and closing the aperture to a varying degree (the operculum is much reduced in *Soapitia* and apparently lacking in *Valvatorbis*). All the freshwater pulmonates lack an operculum (Brown, 1994).

Schistosomiasis is a parasitic disease of major public health importance in many countries of Africa. The disease is caused by trematodes of the genus *Schistosoma* (Bennike *et al*, 1976; Cooke *et al.*, 1999; Stauffer and Madsen, 2012) that require specific freshwater snail species to complete their life cycles. People contract schistosomiasis when they come in contact with water containing the infective larval stage (cercariae) of the trematode (Stauffer and Madsen, 2012).

It was in 1934 that the intermediate host mollusc of schistosome was discovered by Fischer at Stanleyville (called currently Kisangani) (Fain, 1952). The validity of this species has been questioned by Van den Berghe, 1939 in Fain (1952). This author, following an in-depth comparative study of the different species of schistosomes collected by him in human and animals in the Belgian Congo, comes to the conclusion that the species described by Fischer is only *Schistosoma haematobium* (Fain, 1952). A new classification of the snails of the Belgian Congo took place in 1948 by Schwetz.

### 1.2. Research problem

Located in the Albertine Rift, the Ruzizi plain is a natural area rich in malacological biodiversity (Darwall *et al.*, 2011; Schultheiβ *et al.*, 2011). Unfortunately, there is very little information about the freshwater gastropods of the Ruzizi Congolese plain. A last work realized at Kiliba four years ago on the intermediate host snails of schistosomiasis (Muhigwa *et al.*, 2011) did not unfortunately cover the whole of Ruzizi plain area. Furthermore, the conservation status of many freshwater snails from Ruzizi plain cannot be assessed adequately due to lacking of recent data.

In the Ruzizi plain, intestinal schistosomiasis due to *Schistosoma mansoni* is one of the most important public health problems and its intermediate host mollusc is *Biomphalaria pfeifferi* (Gryseels, 1985; Baluku, 1990; Baluku *et al.*, 1999). This pathology is widely distributed and the incidence is very high in the sites where agricultural activities are applied (Baluku, 1990).

The real situation of schistosomiasis in this area remains currently unknown due to insufficient large investigations and lacking of data on the rate of infestation and the outbreak of infection.

Staff working to fight against bilharzia in this region is aware that there is insufficient information on the systematic, biology and ecology of freshwater molluscs.

It is again still necessary to collect for a full assessment of all freshwater mollusc vectors and no vectors of bilharzia in the Ruzizi area, and to identify and classify in an efficient way the species responsible of schistosomiasis.

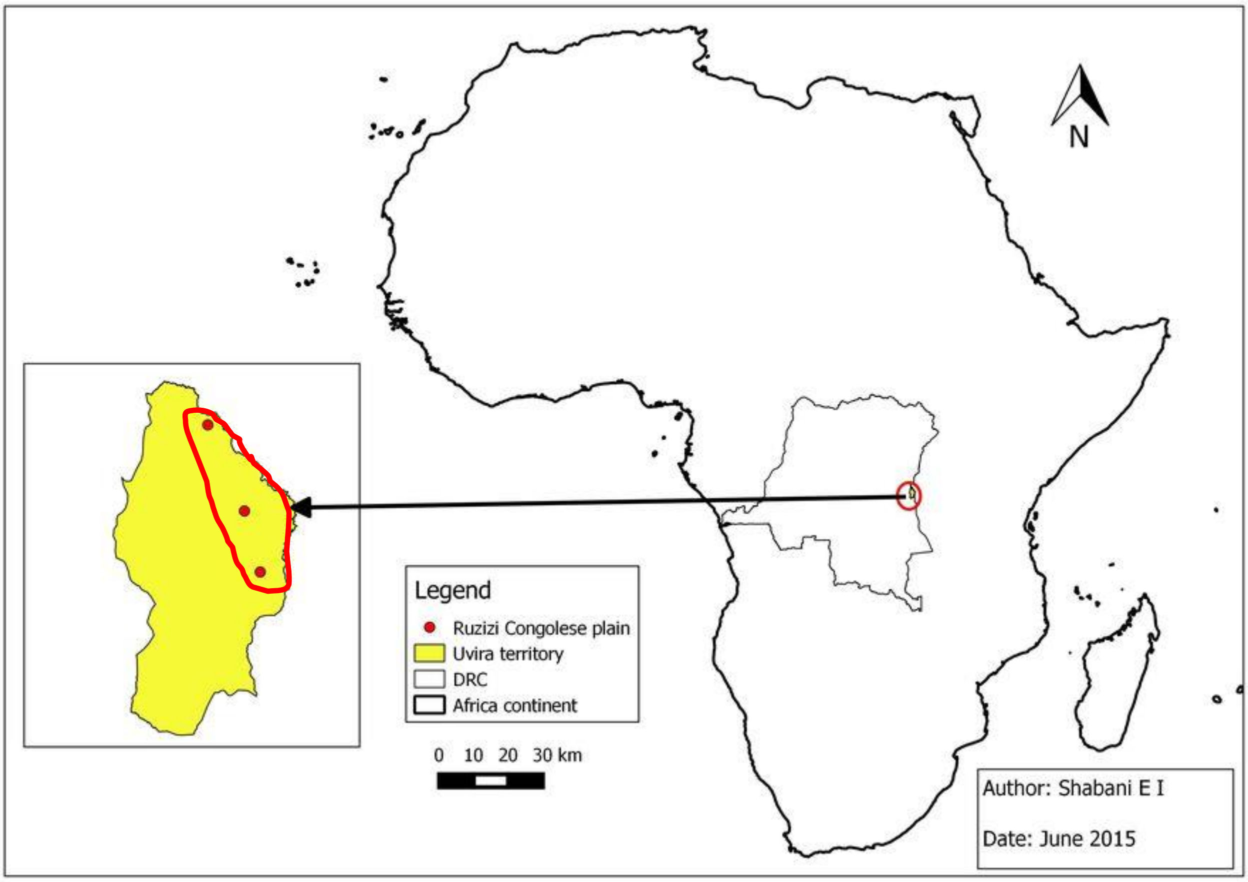



Figure 1: Map showing the Ruzizi Congolese plain. The insert represents the territory of Uvira within the Africa continent

## 1.3. Research Hypotheses

- Ponds and irrigation canals dug for the agricultural needs provide a higher diversity of molluscs than rivers;
- Among the freshwater intermediate host snails of schistosomiasis, the *Biomphalaria pfeifferi* species is the most abundant in the Ruzizi Congolese plain;
- Schistosomiasis is widespread in all Ruzizi Congolese plain.

## 1.4. Research aim and objectives

**1.4.1. Aim:** to assess the freshwater malacological biodiversity in the Ruzizi Congolese plain.

### 1.4.2. Specific objectives

- To determine the diversity and specific abundance of molluscs in ponds, irrigation canals dug and rivers in the Ruzizi Congolese plain;
- To identify the species of intermediate host molluscs of schistosomiasis in the Ruzizi Congolese plain and their rates of infestation;
- To assess the prevalence of schistosomiasis in the Ruzizi Congolese plain; and
- To assess the conservation status of each species.

## CHAPTER TWO: MATERIAL AND METHODS

### 2.1. Study sites

The Ruzizi Congolese plain is located between 2.5°-3°24' S latitude and 28.5°-29° E longitude where our work was carried out from Luvungi to Kiliba. It is limited to the North-west by high mountains of Kivu, in the East by the Ruzizi river and on the South side by lake Tanganyika. The altitude varies between 773m and 1000m (Vancoppenolle *et al.*, 1984). Its climate is tropical dry characterized by a rainy season of 7 months (October-April) and a dry season of 5 months (May-September). The average annual rainfall is about 763 mm, with a monthly average of 63.3 mm (Ntakimazi *et al.*, 2000). The average annual temperature is 22.5°C (Baluku, 1990). It is drained by many rivers of which the most important is the Ruzizi river, marshes and ponds. The main activities of population are agriculture, farming and fisheries. Sampling was carried out at three sites, including Luvungi, Sange and Kiliba. The vegetation that dominated all sites was *Phragmites mauritanus* Adanson 1763, *Typha, Nymphaea, Ceratophyllum, Cyperus, Hyphaene ventricosa* Kirk 1966 and *Acacia polyacantha* Willdenow 1806. The selection of sampling sites was influenced by both the accessibility and anthropogenic activities.

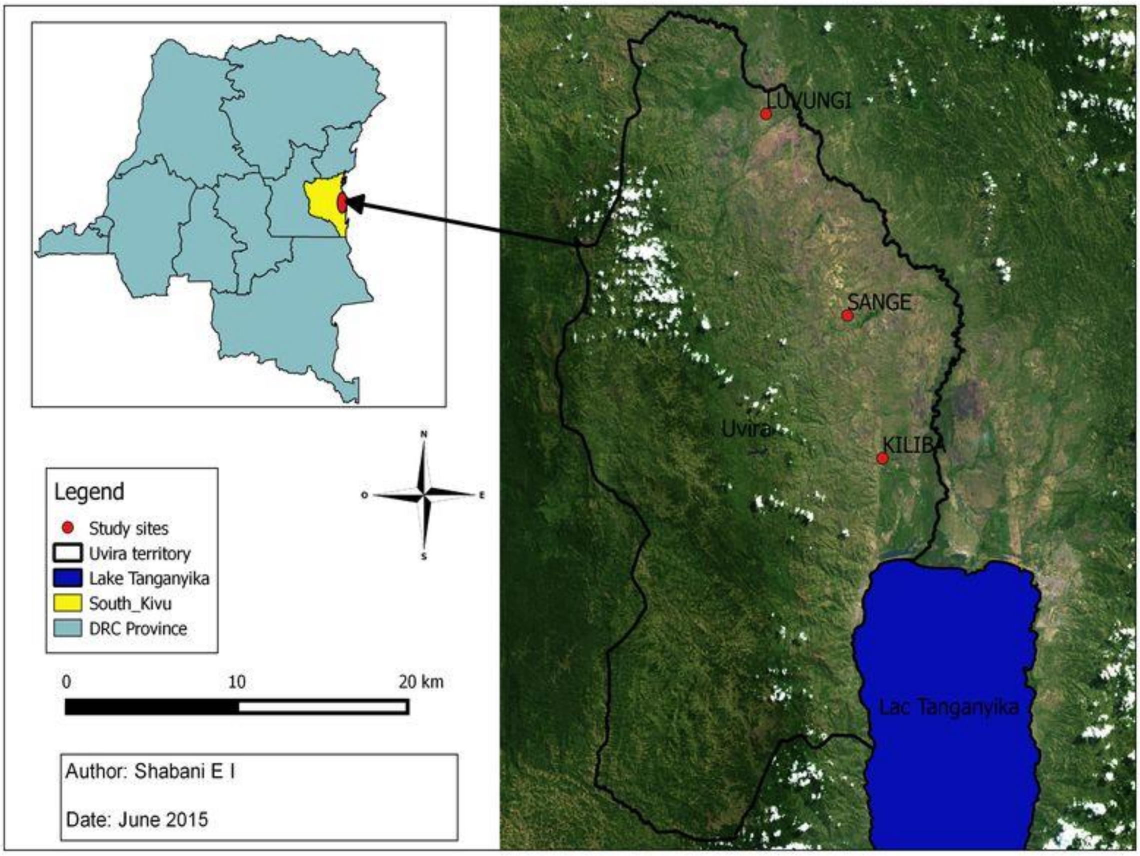



Figure 2: Map showing the sampling sites of investigation in the Ruzizi Congolese plain

## 2.2. Field sampling

The samples were collected from 2nd May 2015 to 2nd June 2015. The sampling carried out from 6.00 am to 10.00 am and from 2.00 pm to 4.00 pm. Each of the sites includes 2 ponds, 2 irrigation canals and 2 rivers. In total, 18 collecting points have been visited twice each one. All sites were dominated by aquatic vegetation and the substrate type of the former was detritus, sand and clay. Molluscan samples were collected using a dip scoop net of 0.5 mm of mesh, sieves or plastics container.

Figure 3: The sampling points: (A) pond at Kiliba, (B) irrigation canal at Kiliba, (C) Kiliba river, (D) pond at Luvungi, (E) irrigation canal at Luvungi, (F) Luvungi river, (G) pond at Sange, (H) irrigation canal at Sange and (I) Sange river

The collected specimens were grouped according to the sites and dates and placed in labelled plastic containers filled with freshwater and deposited at the Biology laboratory of Hydrobiological Research Centre (CRH) in Uvira for further processing. After some analyses at the CRH laboratory, material collected was preserved in 70% ethanol and then transported to Hydrobiology laboratory of faculty of sciences of University of Kisangani for further analyses.

The medical statistics data of outbreaks of infection of schistosomiasis from 2010 to 2014 were recorded from hospitals in these sites. As well as the schistosomiasis cases recorded from 2$^{nd}$ May to 2$^{nd}$ June 2015 were also collected. This enabled us to make a correlation between *B. pfeifferi* collected and schistosomiasis cases identified during data collection.

All sampling sites were geo-referenced using a hand-held Global Positioning System (GPS). Some physicochemical factors influencing the distribution of species were measured such as water temperature, pH, dissolved oxygen and conductivity (Mohamed *et al.*, 2011; Muhigwa *et al.*, 2011; Saddozai *et al.*, 2013; Zaghloul *et al.*, 2013) using a Multiparameter apparatus.

Figure 4 : Sampling of water conditions using Multiparameter apparatus

### 2.3. Identification of freshwater gastropods

In the laboratory, all mollusc specimens were identified using systematic keys based on recognisable shell morphological characteristics (WHO, 1982 and Brown, 1994). For snails of small size, a binocular microscope (Model: 210, Eyepiece: 10) has been used to identify them. Among the captured snails, only *Biomphalaria pfeifferi* know intermediate hosts of *Schistosoma mansoni* have been examined. All *B. pfeifferi* were checked for the presence of trematode cercariae using the emerging method (Abdel *et al*., 2006). Each living individual was placed into a small plastic cup containing distilled water and exposed to the sun or intense artificial light for at least four hours. Therefore the small plastic cup was regularly examined to the naked eye to detect the cercariae of *Schistosoma*. The snail did not produce cercariae after 4 hours of experience was seen as negative. The infestation rate has been calculated on all population of *B. pfeifferi* tested in each site.

Figure 5: Pictures showing some steps of emerging method

Red List Categories of the International Union for Conservation of Nature (IUCN) are provided for each molluscan species where available referring to the Pan Africa freshwater mollusc species list (Table 4).

### 2.4. Data analysis

Statistical analyses were performed using ANOVA and Kruskal Wallis tests in R software (version 3.1.2) to compare variables, as well as we computed a Principal Component Analysis (PCA) to visualize snail community responses to physicochemical parameters.

To account for species diversity within the freshwater habitats, we used Shannon index, as well as Simpson 1-D and Fisher alpha diversity indices.

a. Shannon index is calculated as:

$$H = -\sum_{i=1}^{S} Pi \ln pi$$

Where: Pi= fraction of the entire population made up of species i; S= number of species encountered and Σ = sum from species 1 to species S.

b. Simpson's index:

We considered the Simpson's Index of Diversity 1–D which represents the probability that two individuals randomly selected from a sample will belong to different species.

$$D = 1 - \frac{\sum_{i=1}^{S} n_i(n_i - 1)}{N(N-1)}$$

Where: *S* is the number of species, *N* is the total percentage cover or total number of organisms and *n* is the percentage cover of a species or number of organisms of a species. In this form, D ranges from 1 to 0, with 1 representing infinite diversity and 0 representing no diversity.

c. Fisher's alpha is defined by the formula: $S = a * \ln(1 + \frac{n}{a})$, where *S* is number of taxa, *n* is number of individuals and *a* is the Fisher's alpha. Its advantage is an index showing the variation of species diversity within a habitat. It varies from 0 to n; it can even reach 60 or more within a habitat (Marcon, 2013). These diversity indices were evaluated using PAST (Hammer and Ryan, 2008) software.

## CHAPTER THREE: RESULTS

### 3.1. Abundance of molluscs

A total of 3763 snails were collected belonging to 11 species, 6 genera, 5 families, 3 orders, and 1 class were reported in the freshwater habitats of Ruzizi Congolese plain (Table 1). *Biomphalaria pfeifferi* is the highest abundant, with 30.75% (n= 1157) of all individuals followed by *Melanoides tuberculata* with 25.64% (n= 965). The lowest number with 0.03% belonged to the genus *Bellamya*.

Table 1: Systematic broad outline and relative abundance of freshwater snails collected in the Ruzizi Congolese plain

| **Class** | **Order** | **Family** | **Genus** | **Species** | **No. of individuals** | **%** |
|---|---|---|---|---|---|---|
| Gastropoda | Architaenioglossa | Ampullariidae | *Pila* | *Pila ovata* Olivier, 1808 | 642 | 17.06 |
| | | Viviparidae | *Bellamya* | *Bellamya sp.* | 1 | 0.03 |
| | Sorbeoconcha | Thiaridae | *Melanoides* | *Melanoides sp.* | 711 | 18.89 |
| | | | | *Melanoides tuberculata* Müller, 1774 | 965 | 25.64 |
| | Hygrophila | Lymnaeidae | *Lymnaea* | *Lymnaea natalensis* Krauss, 1848 | 120 | 3.19 |
| | | Planorbidae | | *Lymnaea sp.* | 2 | 0.05 |
| | | | *Bulinus* | *Bulinus forskalii* Ehrenberg, 1831 | 2 | 0.05 |
| | | | | *Bulinus globosus* Morelet, 1868 | 54 | 1.44 |
| | | | | *Bulinus tropicus* Krauss, 1848 | 107 | 2.84 |
| | | | *Biomphalaria* | *Biomphalaria sp.* | 2 | 0.05 |
| | | | | *Biomphalaria pfeifferi* Krauss, 1848 | 1157 | 30.75 |
| **Total** | | | | | **3763** | **100** |

### 3.2. Species richness and diversity

A total of 11 species of gastropods were identified that 9 species identified in the ponds, 8 species in the irrigation canals and 5 species in the rivers. The ponds showed the highest abundance of specimens, with 46.69% (n= 1757) followed by the irrigation canals with 41.59% (n= 1565). The rivers showed the lowest number with 11.72% (n= 441, Figure 7). *Biomphalaria pfeifferi* with 30.64% (n= 1153) was most collected in the ponds. However, there was an observed increase in the abundance of *Melanoides sp* with 18.82% (n= 708) in the irrigation canals (Figure 6). The mean values of ponds, irrigation canals and rivers did not show a significant difference (p= 0.4819, Figure 8) in the total numbers of gastropods collected.

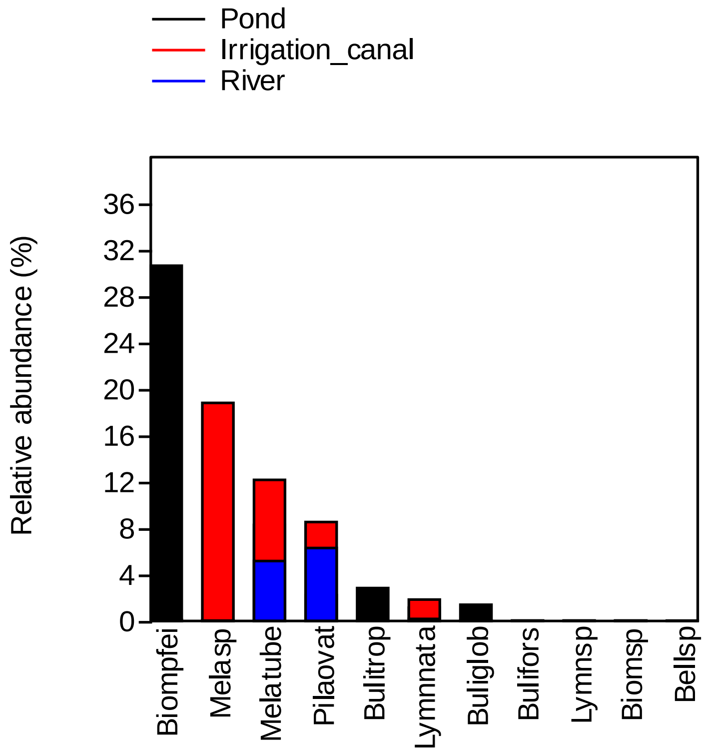



Figure 6: Number of snails of each species collected in the freshwater of Ruzizi plain

**Legend:** Biompfei= *Biomphalaria pfeifferi*, Melasp= *Melanoides sp*, Melatube= *Melanoides tuberculata*, Pilaovat= *Pila ovata*, Bulitrop= *Bulinus tropicus*, Lymnnata= *Lymnaea natalensis*, Buliglob= *Bulinus globosus*, Bulifors= *Bulinus forskalii*, Lymnsp= *Lymnaea sp*, Biomsp= *Biomphalaria sp*, Bellsp= *Bellamya sp.*

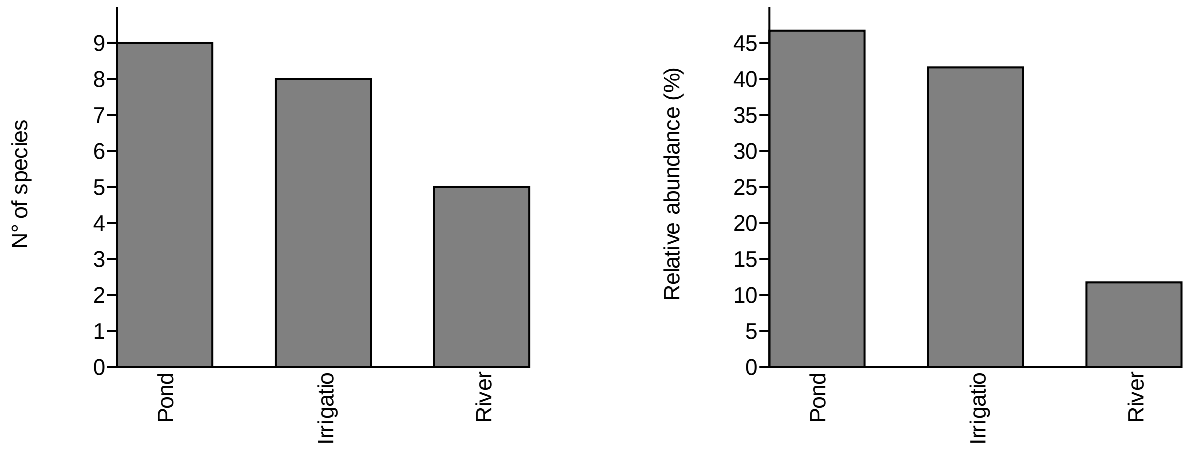



Figure 7: Number of species and number of individuals collected in each habitat

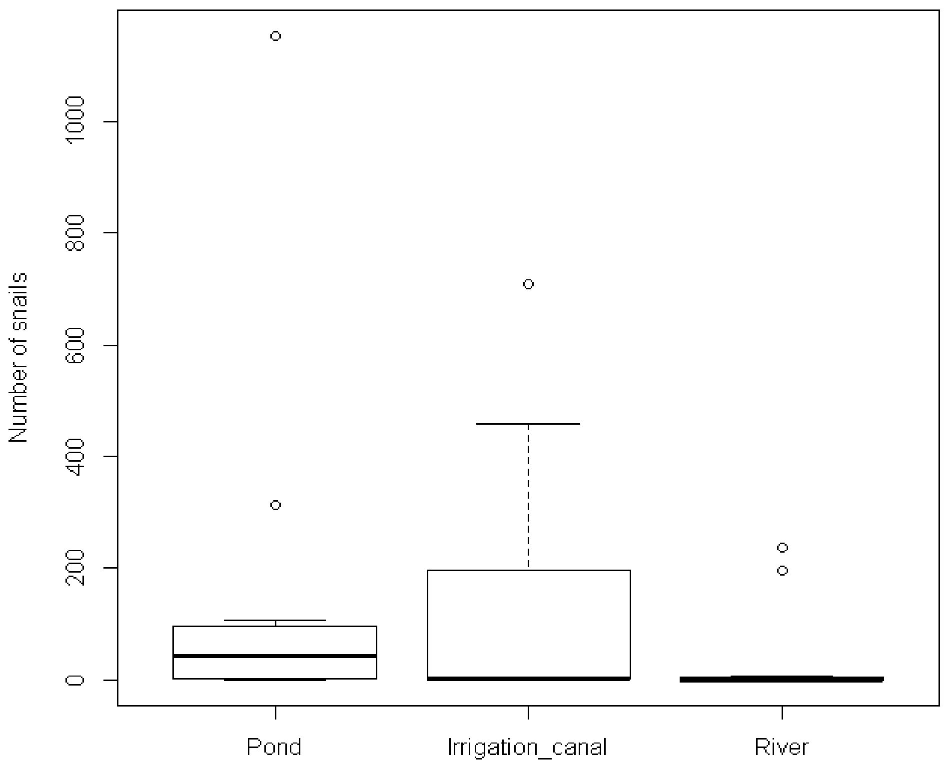


Figure 8: Comparison of mean values of total numbers of gastropods collected in the ponds, irrigation canals and rivers (p-value = 0.4819)

Table 2: Species diversity in the freshwater habitats

| Species richness | Freshwater habitat | | |
|---|---|---|---|
| | Pond | Irrigation canal | River |
| Simpson_1-D | 0.53 | 0.67 | 0.52 |
| Shannon_H | 1.12 | 1.22 | 0.79 |
| Fisher_alpha | 1.24 | 1.10 | 0.79 |

In table 2, diversity indices among the freshwater habitats are indicated. A comparison of diversity within these 3 freshwater habitats of Ruzizi Congolese plain, using the Shannon index, showed a no significant difference (p= 0.9565).

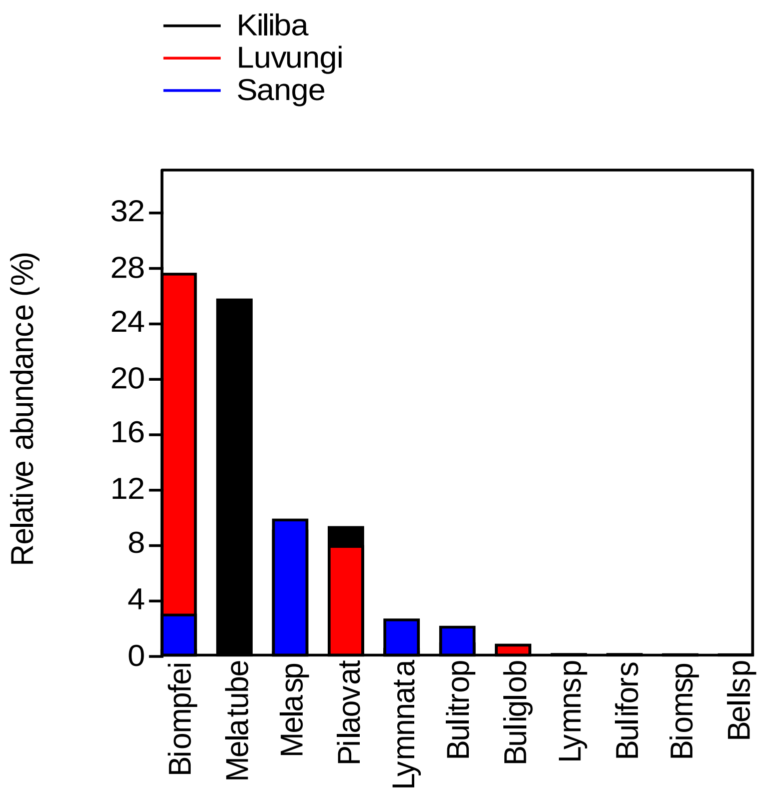



Figure 9: Number of molluscs of each species captured in the three localities

*Biomphalaria pfeifferi* with 27.5% (n= 1035) was found in great abundance at Luvungi while *Melanoides tuberculate* with 25.64% (n= 965) was high captured at Kiliba. Luvungi showed the highest abundance of specimens, with 46.32% (n= 1743) belonged to 7 species followed by Kiliba with 36.43% (n=1371) belonged to 9 species. Sange showed the lowest number with 17.25% (n= 649) belonged to 5 species. The sites of Luvungi, Sange and Kiliba did not show a significant difference (p= 0.66) in the total numbers of snails captured (Figure 10).

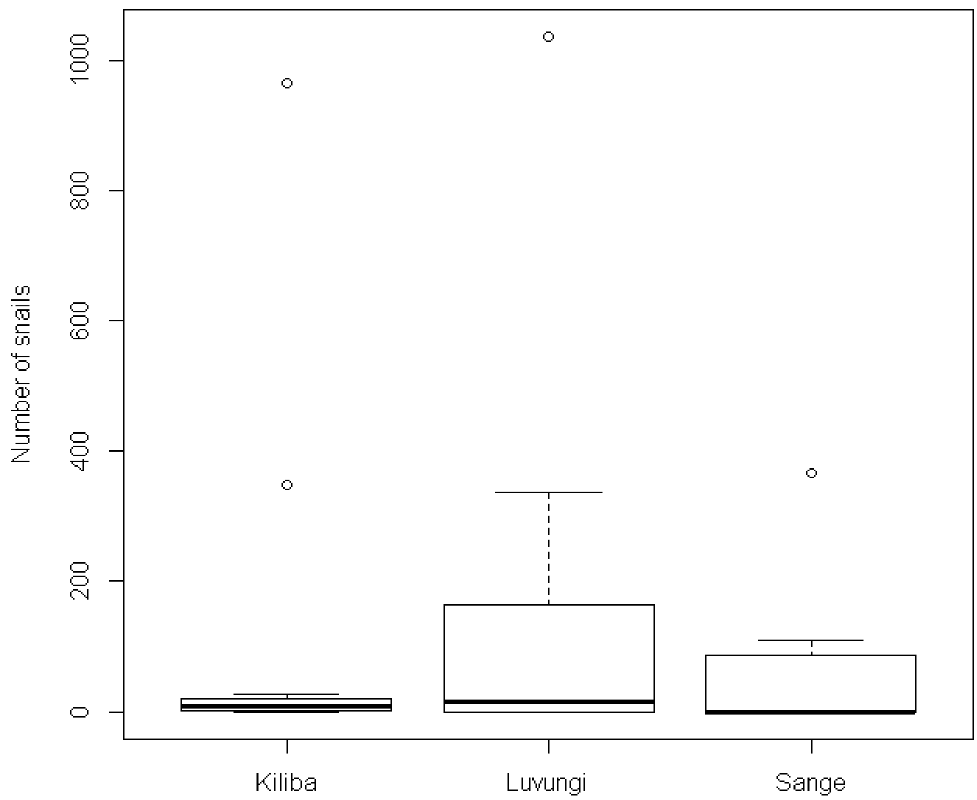



Figure 10: Comparison of mean values of all snails captured in the localities of Kiliba, Luvungi and Sange (p-value= 0.66)

### 3.3. Physicochemical factors of water

The mean values of main water's physicochemical features (temperature, pH, dissolved oxygen and conductivity) measured in different biotopes of Ruzizi Congolese plain are given in figures 11, 12, 13 and 14.

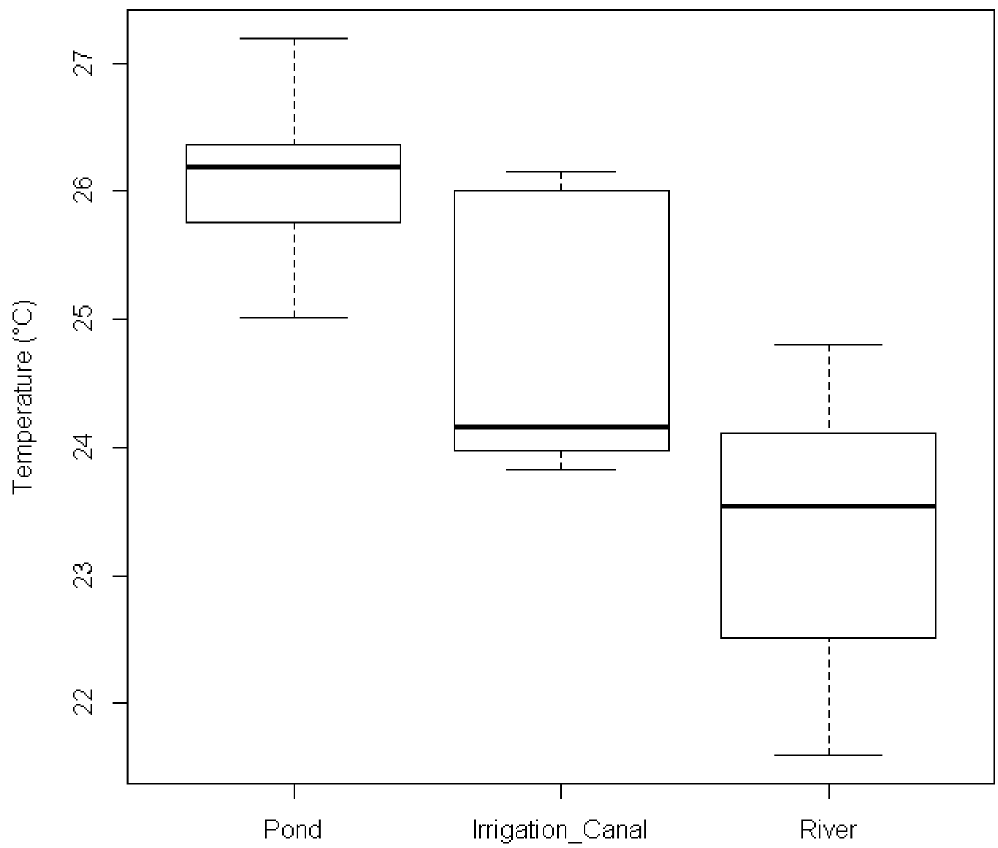



Figure 11: Values of temperature in all biotopes

The temperature of water varies between 21.6°C and 27.19°C. The mean values of temperature showed a significant difference (p= 0.00105) between the sites. Highest values of temperature were observed in the ponds and lowest in rivers.

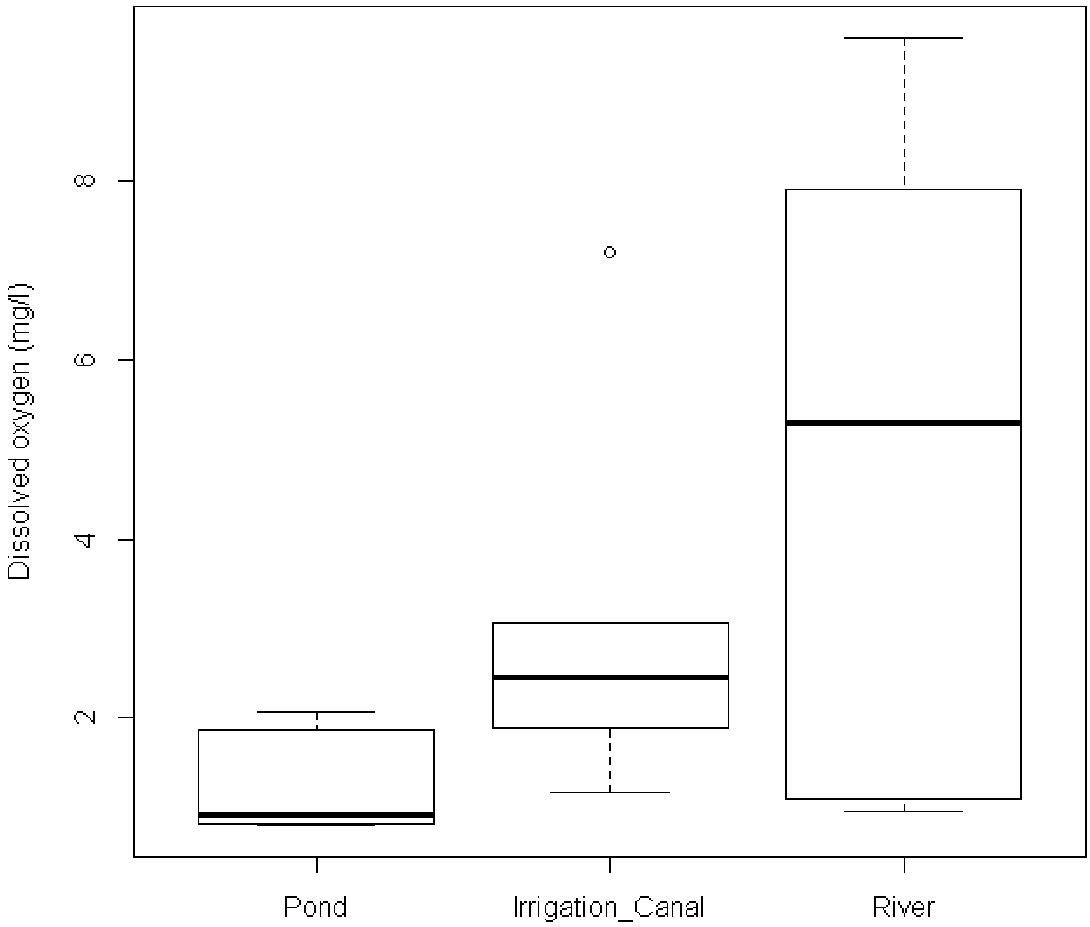



Figure 12: Values of dissolved oxygen in all habitats

In comparing however biotopes of the Ruzizi Congolese plain, figure 12 showed that the mean values of dissolved oxygen do not differ significantly (p= 0.0549). It is important to note that highest changes of dissolved oxygen were observed in the rivers.

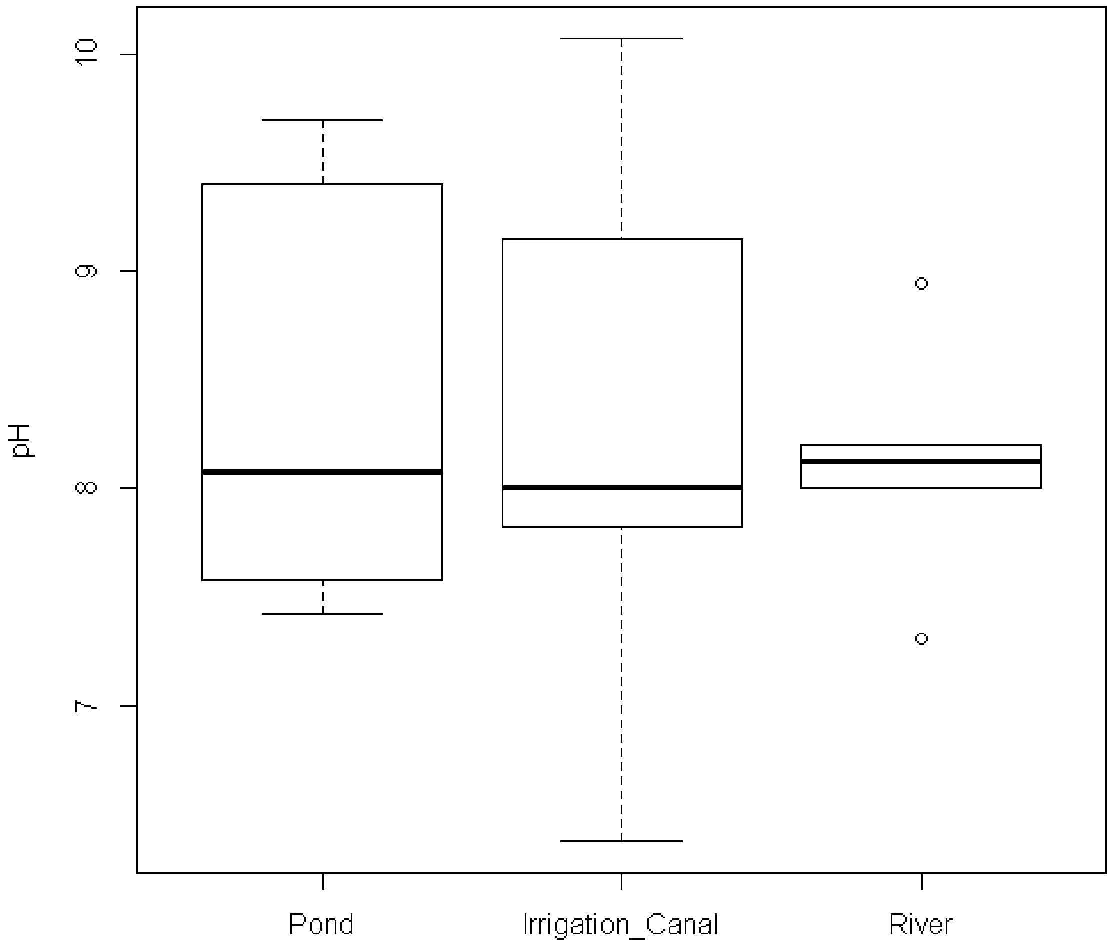



Figure 13: Values of pH in these three biotopes

The mean values of pH showed that water is alkaline in all biotopes. There's no significant difference between the biotopes (p= 0.899).

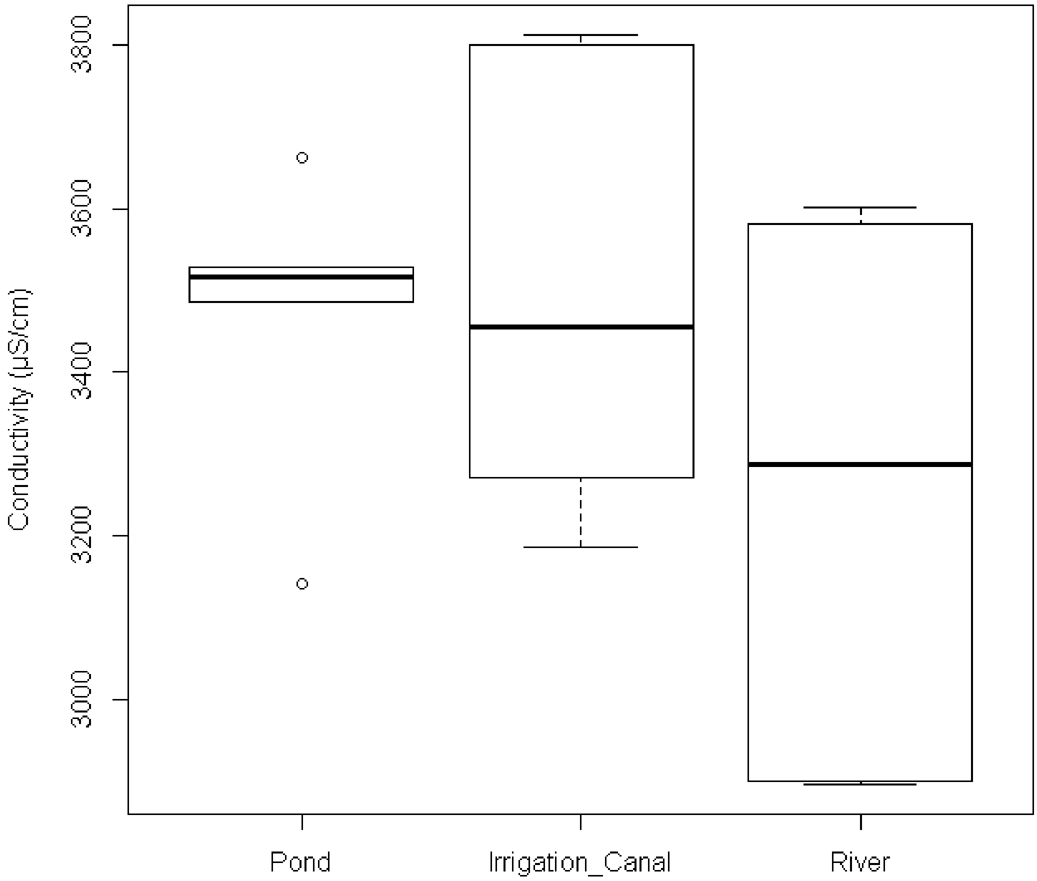



Figure 14: Values of electrical conductivity

The electrical conductivity values showed a no significant difference between the sites (p= 0.2478). The lowest conductivity values were observed in the rivers.

The figure 15 shows the Principal Component Analysis (PCA) of the distribution of the main species of molluscs and physicochemical features with the freshwater biotopes.

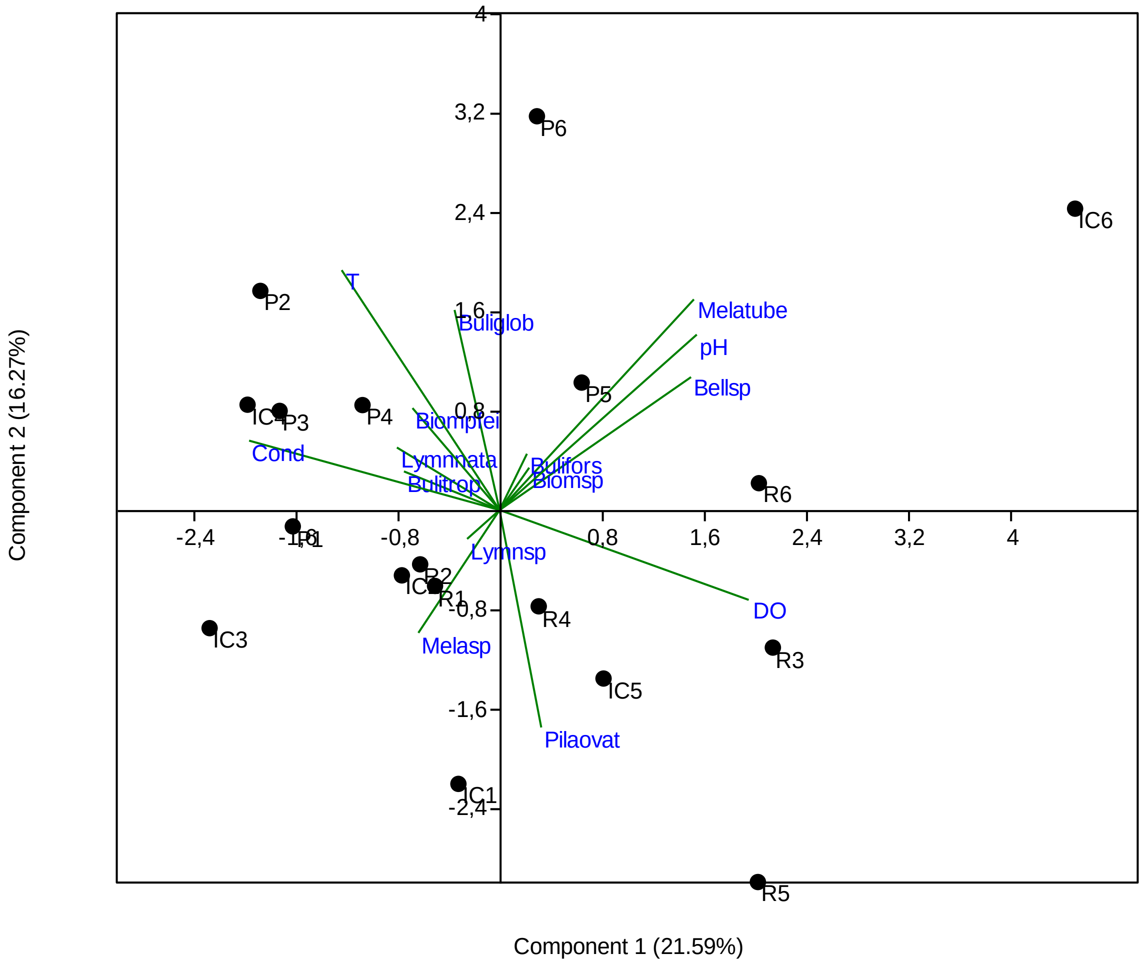



Figure 15: Principal Component Analysis expressing the physicochemical parameters effect on mollusc communities. **DO** = dissolved oxygen, **T** = temperature, **Cond** = conductivity, **P** = pond, **IC** = irrigation canal, **R**= river, **Biompfei**= *Biomphalaria pfeifferi*, **Melasp** = *Melanoides sp*, **Melatube**= *Melanoides tuberculate*, **Pilaovat** = *Pila ovata*, **Bulitrop** = *Bulinus tropicus*, **Lymnnata** = *Lymnaea natalensis*, **Buliglob**= *Bulinus globosus*, **Bulifors**= *Bulinus forskalii*, **Lymnsp** = *Lymnaea sp*, **Biomsp**= *Biomphalaria sp*, **Bellsp** = *Bellamya sp.*

The percentage of variances of the first two axes (37.86%). pH most influenced the presence of *M. tuberculata* and *Bellamya sp*, but the presence of *Biomphalaria sp* and *B. forskalii* is less influenced by this parameter. We note that *P. ovata* is strongly associated with the dissolved oxygen, while the *B. globosus* seems to be highly influenced by temperature and conductivity; however species like *B. pfeifferi*, *L. natalensis* and *B. tropicus* are less influenced by these two parameters. Note that other parameters were not measured due to the short duration of sampling.

### 3.4. Infestation rates of intermediate host molluscs of schistosomiasis due to *Schistosoma mansoni*

Table 3: The rates of infestation in prospected sites

| | Prospected sites | | |
|---|---|---|---|
| | Kiliba | Sange | Luvungi |
| *Biomphalaria pfeifferi* collected | 13 | 109 | 1035 |
| *Biomphalaria pfeifferi* infested | 2 | 20 | 254 |
| Infestation rate (%) | 15.4 | 18.3 | 24.5 |

The rates of infestation among prospected sites are indicated. Luvungi locality showed the highest infestation rate (24.5%). It is a site where *B. pfeifferi* is the most abundant with 27.5% (Figure 9).

### 3.5. Prevalence of schistosomiasis in the Ruzizi Congolese plain

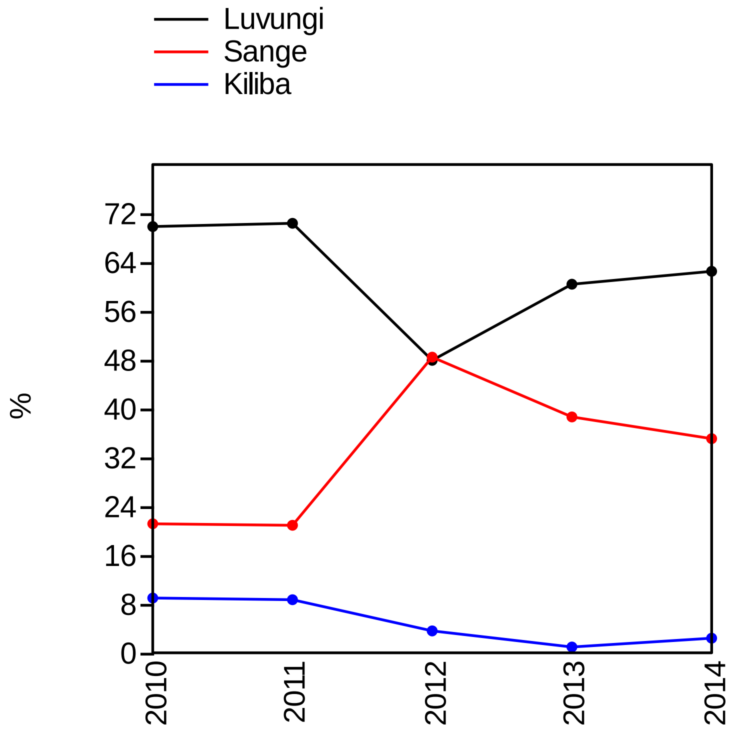


Figure 16: Outbreaks of infection of schistosomiasis

Figure 16 showed that the outbreaks of schistosomiasis in locality of Luvungi remains high with the important peaks observed in years 2010 and 2011. This is a site where individuals of *Biomphalaria pfeifferi* infested with trematode parasites are the most abundant (Table 3). However, from 2012 there was a decrease in Sange locality after an increase from 2011 to 2012.

The figure 17 shows Pearson's correlation between *B. pfeifferi* abundance and infection rate of schistosomiasis in three localities.

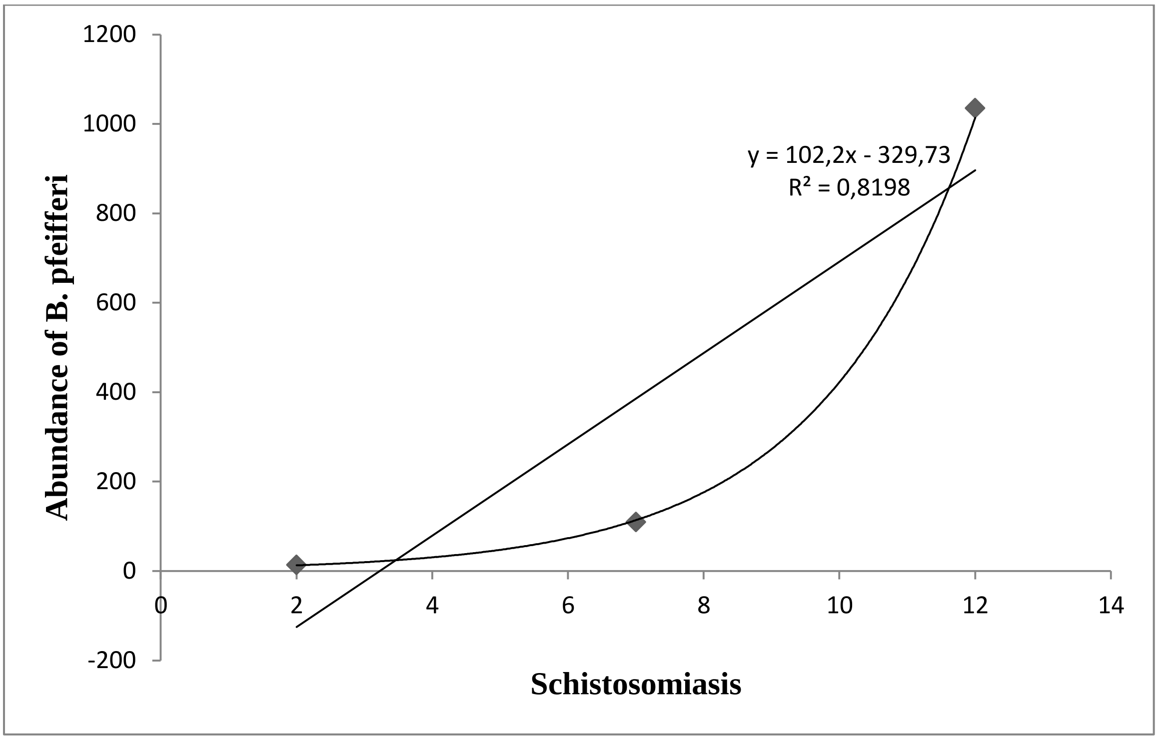



Figure 17: Importance of *B. pfeifferi* in the schistosomiasis caused by *Schistosoma mansoni*

Positive significant Pearson's correlation between species abundance and schistosomiasis is shown. The infestation rate of schistosomiasis is high with the abundance of *B. pfeifferi*.

Figure 18: Schistosomiasis case identified during data collection at Kiliba locality (photo: Shabani, May 2015)

The encountering of our data to the IUCN Red List gives the situation presented in table 4.

Table 4: International Union for Conservation of Nature Red List Category (IUCN, 2011)

| **N°** | **Species** | **Red List Category** |
|---|---|---|
| 1 | *Bellamya sp* | - |
| 2 | *Biomphalaria sp* | - |
| 3 | *Biomphalaria pfeifferi* | Least Concern |
| 4 | *Bulinus forskalii* | Least Concern |
| 5 | *Bulinus globosus* | Least Concern |
| 6 | *Bulinus tropicus* | Least Concern |
| 7 | *Lymnaea sp* | Least Concern |
| 8 | *Lymnaea natalensis* | Least Concern |
| 9 | *Melanoides sp* | - |
| 10 | *Melanoides tuberculata* | Least Concern |
| 11 | *Pila ovata* | Least Concern |

Table 4 shows that the snail species collected in the freshwaters of Ruzizi plain are not threatened.

## CHAPTER FOUR: DISCUSSION

### 4.1. Freshwater molluscan biodiversity

The freshwater malacological fauna inventory presents 11 species belonging to 6 families of class Gastropoda, two of them (*Bulinus tropicus*, *Bellamya sp*.) being a first record for the study area. This is consistent with the general observation of malacological fauna in the Ruzizi Congolese plain freshwaters. Species such as *Biomphalaria pfeifferi*, *Pila ovata*, *Biomphalaria sp*, *Bulinus tropicus*, *Bulinus globosus*, *Bulinus forskalii*, *Lymnaea natalensis*, *Melanoides tuberculata* and *Melanoides sp* were recorded from the ponds. However, eight molluscan species were captured in the irrigation canals such as *P. ovate*, *Bellamya sp*, *Melanoides sp*, *M. tuberculata*, *L. natalensis*, *Lymnaea sp*, *Bulinus globosus* and *Biomphalaria pfeifferi* while five species among eleven were identified in the rivers such as *P. ovate*, *Melanoides sp*, *M. tuberculata*, *L. natalensis* and *Biomphalaria sp*. The present results indicated that the ponds showed a highest abundance of freshwater gastropods compared to irrigation canal and rivers although the numbers were not statistically significant (p= 0.4819, Figure 8).

Of the 11 species recognised here, only *B. pfeifferi* (30.75%, Table 1) was found in the highest abundance in Ruzizi Congolese plain and only collected in the ponds and irrigation canals. This could be attributed to the fact that *B. pfeifferi* has more a preference for stagnant waters than running waters (OMS, 1957; Lévêque and Durand, 1980; Gryseels, 1985; Brown, 1994**).** Lévêque and Durand (1980) and Sarr *et al.* (2011) note that in stagnant waters, the pulmonate vectors of schistosomiasis are usually dominant and often very abundant in the vegetation. *B. pfeifferi* is common throughout Africa (Kotchi *et al.*, 2013).

Among the three locating study sites, Kiliba showed the highest number of species (n= 9) such as *Pila ovate*, *Bellamya sp*, *Melanoides sp*, *M. tuberculata*, *L. natalensis*, *B. forskalii*, *B. globosus*, *Biomphalaria sp* and *B. pfeifferi* followed by Luvungi (n= 7 species) such as *Pila ovate*, *Melanoides sp*, *L. natalensis*, *B. globosus*, *Lymnaea sp*, *B. tropicus* and *B. pfeifferi* while Sange showed the lowest number (n= 5) such as *Melanoides sp*, *L. natalensis*, *B. tropicus*, *Biomphalaria sp* and *B. pfeifferi*. There was no significant difference in the total numbers of snails collected from the three localities (Figure 10).

The presence of invasive species in our collecting localities was indeed affect local molluscan fauna. This is the case of *Melanoides tuberculata* who is a very effective competitor of some medical interest planorbids (Kotchi *et al.*, 2013; Van Bocxlaer *et al*., 2015).

Nearly similar observations at Kiliba were reported from these found in 2011 by Muhigwa *et al.* on freshwater snails of Kiliba such as *P. ovata, L. natalensis, B. pfeifferi, Biomphalaria sp, M. tuberculata, Physa acuta, Bulinus truncatus trigonus, Planorbis sp*.

The molluscan gastropods inventoried in the prospected sites of Kiliba, Luberizi, Lubarika and Sange in the Ruzizi plain from 1979 to 1980 were *L. natalensis, B. pfeifferi, B. sudanica*, *B. forskalii*, *B. truncatus*, *Gyraulus costulatus* and *P. ovata* (Baluku, 1990).

Gryseels (1985) recorded in the Ruzizi valley (Burundi) *P. ovata*, *L. natalensis*, *B. pfeifferi*, *B. sudanica*, *B. forskalii*, *Bulinus coulboisi*, *Segmentorbis angustus* and *Ceratophallus natalensis*. Gryseels concluded that *B. pfeifferi* is present in all types of freshwater biotope except in the rivers and sole intermediate host of *Schistosoma mansoni* in Ruzizi valley of Burundi.

Shabani and Gembu (2014) collected three species in the freshwaters of Mambasa (Oriental Province, DRC) such as *L. natalensis, B. pfeifferi* and *B. forskalii*. They observed the highest abundance of these species within the ponds and irrigation canals.

Checklist of freshwater snails recorded in Ituri (June 1986 to August 1988) showed the presence of *L. natalensis*, *L. truncatula*, *Lymnaea sp*, *Bulinus africanus*, *B. truncatus*, *B. forskalii*, *Gyraulus sp*, *Gyraulus costulatus*, *Segmentorbis angustus*, *Ceratophallus natalensis*, *Lentorbis benguelensis*, *Afrogyrus coretus*, *B. pfeifferi*, *B. sudanica*, *B. stanleyi*, *Ferrissia sp*, *Ferrissia eburnensis*, *Potadoma liricincta*, *M. tuberculata*, *Cleopatra bulimoides*, *Gabbiella matadina*, *Bellamy sp* and *Pila wernei* (Chartier *et al*., 1992). Our results are similar to these of Chartier *et al*. (1992) concerning the distribution of *B. pfeifferi* in Ituri.

De Clercq (1987) found six species in the streams of Kinshasa such as *Physa acuta*, *B. forskalii*, *M. tuberculata*, *L. natalensis, B. pfeifferi* and *B. globosus*. Fain (1951); De Clercq (1987), Day and De Moor (2002), Ndassa and Mimpfoundi (2005), Ntonifor and Ajayi (2007), Sarr *et al.* (2011), Stauffer and Madsen (2012) note that *B. pfeifferi*, is the main intermediate host of *Schistosoma mansoni* in tropical Africa. *B. forskalii*, intermediate host of *S. intercalatum*. *B. globosus*, intermediate host of *S. haematobium*. *L. natalensis*, intermediate host of *Fasciola gigantica* and *F. hepatica*. *L. natalensis* is common in perennial streams in small dams unpolluted, in impoundments shallow but rarely in temporary pools (Kotchi *et al.*, 2013).

The investigations of Schultheiβ *et al*. (2011) on freshwater molluscs in Eastern Congo showed the presence of Gastropoda like *P. ovate*, *Gabbiella humerosa*, *G. h. edwardi*, *Potadoma ignobilis*, *P. liricincta*, *M. tuberculata*, *L. natalensis*, *B. pfeifferi*, *B. smithi*, *B. stanleyi*, *Bulinus forskalii*, *B. truncatus*, *Ceratophallus kigeziensis* and *Gyraulus sp*. Schultheiβ *et al*. (2011) noted that *B. pfeifferi* was collected from irrigation channels watering fields.
The findings of Agaba (2013) from lakes Mburo and Nakivale (Uganda) during the wet and dry seasons indicated five genera: *Biomphalaria*, *Bulinus*, *Lymnaea*, *Melanoides* and *Pila* were collected that the highest number of snails belonged to genus *Biomphalaria*.
Checklist of freshwater molluscs reported in the Ouro-Doukoudje rice fields (Cameroon) by Ndassa and Mimpfoundi (2005) indicated the presence of *B. pfeifferi*, *C. natalensis*, *B. senegalensis*, *B. forskalii*, *B. globosus*, *Burnupia sp*, *L. natalensis*, *Cleopatra bulimoides*, *M. tuberculata*, *Pila wernei*, *Lanistes ovum*, *Mutela sp*, *Aspatharia* and *Caelatura sp*. Only *B. pfeifferi* was the most abundant and shedding *S. mansoni* cercariae at Ouro-Doukoudje (Ndassa and Mimpfoundi, 2005).
Ntonifor and Ajayi (2007) found in the Bauchi State (Nigeria) five medically important snail species such as *B. globosus*, *B. truncatus*, *B. forskalii*, *B. pfeifferi* and *L. natalensis*. They observed *B. globosus* was the most abundant and most widely distributed followed by *B. pfeifferi*.
During the investigations of Mohamed *et al*. (2011) on freshwater Gastropoda at Qena Governorate, Upper Egypt, 13 species were caught such as *Lanistes carinatus*, *Bellamya unicolor*, *Cleopatra bulimoides*, *M. tuberculata*, *Theodoxus niloticus*, *Gabbiella senaariensis*, *L. natalensis*, *Succinea cleopatra*, *Physa acuta*, *Bulinus truncatus*, *Biomphalaria alexandrina*, *Gyraulus ehrenbergi* and *Helisoma duryi*. The most abundance of snails belonged to *Cleopatra bulimoides*. In this checklist, *B. pfeifferi* was not reported.

Our findings on mean values of water's physicochemical parameters are near to these recorded from September 1982 to July 1983 in freshwaters dominated by *Cyperus laevigatus* in the Ruzizi valley (Burundi) (Mpawenayo, 1996).

**4.2. Prevalence of schistosomiasis**

The prevalence of schistosomiasis was carried out in three representative localities. The outbreaks of infections within the localities were located. The medical statistics data of prevalence of schistosomiasis reported from 2010 to 2014 in three sampling localities showed that there was the highest observed prevalence of schistosomiasis in the locality of Luvungi. The localities of Luvungi, Sange and Kiliba showed a high significant difference ($P < 0.001$, Figure 16). This could be explained to the fact that Luvungi is a locality where *B. pfeifferi* populations infested with trematode parasites are the most abundant (infestation rate= 24.5%, Table 3). The prevalence of schistosomiasis recorded from 2$^{nd}$ May to 2$^{nd}$ June 2015 showed a positive significant Pearson's correlation between the abundance of *B. pfeifferi* and schistosomiasis ($R^2$= 82%, Figure 17).

The investigations of Baluku (1990) on the outbreaks of schistosomiasis caused by *Schistosoma mansoni* of years 1979 and 1980 indicated followed percentage of prevalence in the sites of Lubarika (6.4% and 10.2%), Luberizi (52% and 49%), Sange (14.9% and 0%) and Kiliba (75% and 60%) respectively. He concluded that the prevalence of schistosomiasis was highest at Kiliba, locality where infestation rate of *B. pfeifferi* reached highest, with 25% and 18% respectively.

The prevalence of schistosomiasis in Kiliba locality is today lower than that reported in years 79 and 80 (Buluku, 1990). This could be due to the fact that Kiliba is today much less populated among these three localities, while it was most populated in years 1979 and 1980 when the Sugar Manufacturing Company (Société de Sucrerie de Kiliba) worked properly.

The preliminary studies of Gryseels (1984) on intestinal schistosomiasis in five villages of the Ruzizi plain (Burundi) showed the prevalence varied from 27 to 50%. He noted that the prevalence and the intensity of infections reached a maximum in the age-group from 10 to 19 years old. Adult men are more infected than women. These results are nearly similar to these reported by De Clercq (1987) in Kinshasa, the prevalence varied from 28 to 58% and the intensity of infections affected the age-group from 0 to 9 and 10 to 19 years respectively.

## CONCLUSION AND RECOMMENDATIONS

This study was mainly focused on the molluscan biodiversity in the freshwaters of Ruzizi Congolese plain, infestation rates and the prevalence of schistosomiasis.

Shell morphological characteristics of the molluscs collected from ponds, irrigation canals and rivers in three representative localities (Luvungi, Sange and Kiliba) revealed eleven gastropod species such as *Biomphalaria pfeifferi, Melanoides sp, Melanoides tuberculata, Pila ovata, Bulinus tropicus, Lymnaea natalensis, Bulinus globosus, Bulinus forskalii, Lymnaea sp, Biomphalaria sp* and *Bellamya sp*.

The most common species in all three localities were *Melanoides sp, L. natalensis* and *B. pfeifferi*. Specimens belonging to the species *P. ovata*, *Melanoides sp*, *M. tuberculate* and *L. natalensis* were found in all three freshwater biotopes i.e. ponds, irrigation canals and rivers. Of them, only *B. pfeifferi* was found in the Ruzizi plain as the highest abundant species, with 30.75%. It is a species intermediate host of schistosomiasis due to *Schistosoma mansoni*. Schistosomiasis is one of neglected tropical diseases (Hotez and Kamath, 2009), however it is a disease of major public health importance in many countries in Africa, Asia, and South America, with an estimated 200 million people infected worldwide (WHO, 2002).

The ponds and irrigation canals dug for the agricultural needs provide a higher abundance of molluscs than rivers (Figure 7).

Schistosomiasis is reported in all Ruzizi Congolese plain, but Luvungi is a locality where the prevalence of schistosomiasis becomes greatest increased (Figure 16). That confirming our three research hypotheses.

Only *Biomphalaria pfeifferi* was shedding *S. mansoni* cercariae in the Ruzizi Congolese plain; *Bulinus globosus* and *Bulinus forskalii* were never found shedding human schistosome cercariae during our survey. The infestation rates of *B. pfeifferi* at Luvungi, Sange and Kiliba were 24.5%, 18.3% and 15.4% respectively.

*S. mansoni* is highly endemic to the irrigated rice culture villages in the Ruzizi plain (Gryseels, 1984). The conditions favourable to proliferation and infestation of *B. pfeifferi* are respectively a maximum illumination, abundance of aquatic vegetation, stagnant water and abundance of human excreta (Gillet *et al.*, 1960 and Baluku, 1987).

From the results stressed above of this study, the following recommendations are made:

- More research should be done to collect mollusc populations over a longer period of time for example up to three years and at regular time intervals in order to monitor changes in the mollusc populations and the mollusc infection rate;
- Further research should be conducted to determine at what time during the day *Biomphalaria pfeifferi* releases trematode cercariae in the water in order to implement a control program against schistosomiasis;
- Future surveys should be carried out on seasonal and climatic factors influencing the biological cycle of snails;
- A detailed study of the DNA barcodes of selected representatives of the freshwater gastropod species identified, as well as the trematode cercariae present in the freshwaters of Ruzizi plain should be carried out based on both molecular and morphological characteristics;
- Project to supply drinking water, starting with the sites most affected by schistosomiasis in the Ruzizi Congolese plain should be established;
- Regulation of the establishment of latrines and waste disposal;
- Drainage water collections which are not needed and prevent stagnant water in some areas of irrigation and drainage canals;
- Construction of sanitary facilities in the irrigated fields;
- Use of waterproof, boots and gloves during the field work.

## REFERENCES


Abdel, A.M.A., Nidal, A.I. and Mohamed, A.I. 2006. Laboratory Studies on the Prevalence and Cercarial Rhythms of Trematodes from *Bulinus truncatus and Biomphalaria Pfeifferi* Snails from Khartoum State, Sudan. Sultan Qaboos *University Medical Journal.*6 (2): 65–69.

Agaba J. J., 2013. An integrative approach to biodiversity assessment of freshwater gastropods in the lake Mburo-Nakivale system: implications for transmission of potential snail-borne diseases, South Western Uganda. Matser thesis of science of Mbarara University. 39P

Anonymous: Medical Registers of Luvungi, Sange and Kiliba hospitals (2010-2014)

Baluku B., 1987. Contribution à l'étude de hôtes intermédiaires des bilharzioses : écologie des mollusques dulcicole dans deux cours d'eau du Zaïre oriental. Thèse es sciences, U. L. B. 421P

Baluku B., 1990. Répartition spatio-temporelle de la bilharziose à *Schistosoma mansoni* (Sambon, 1907) dans la plaine de la Ruzizi (Est du Zaïre). *Revue des Sciences Naturelles, vol.1* : 1-9

Baluku B., Bagalwa M. and Basabose K. 1999. Enquête malaco-schistosomique dans des camps de réfugiés situés dans la plaine de la Ruzizi en RDC. Laboratoire de Malacologie du Centre de Recherche en Sciences Naturelles de Lwiro. *Vol. 59* : 39-42

Bennike T., Frandsen F. et Mandahl-barth G., 1976. La bilharziose à Kinshasa, données actuelles et danger pour l'avenir. Etudes malacologiques, biologiques, cliniques et épidémiologiques. *Ann.Soc.belge Méd. Trop*: 56, 6, 419

Brown D. S., 1994. Freshwater Snails of Africa and their Medical Importance. Second Edition. Department of Zoology, the Natural History Museum, London. 687P

Campbell G., Catherine S. Jones1, Anne E. Lockyer, Sarah Hughes, David Brown, Leslie R. Noble and David Rollinson, 2000. Molecular evidence supports an African affinity of the Neotropical freshwater gastropod, *Biomphalaria glabrata*, Say 1818, an intermediate host for *Schistosoma mansoni*. *Proc. R. Soc. Lond. B*: 267, 2351-2358, DOI 10.1098/rspb.2000.1291

Chartier C., Mulinda B., Kristensen T. K., Sikavei N., Missona L. et Cabaret J. 1992. Inventaire des mollusques d'eau douce en Ituri (Haut-Zaïre) et Conséquences sanitaires pour l'homme et le bétail. *Rea. hydrobiol. trop.* 25 (3): 189-196

Cooke G. S., Lalvani A., Gleeson F. V. and Conlon C. P., 1999. Acute Pulmonary Schistosomiasis in Travelers Returning from Lake Malawi, Sub-Saharan Africa. From the Infectious Diseases Unit, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, and the Department of Radiology, Churchill Hospital, Oxford, England. Pp 836-839

Day JA and De Moor I.J. 2002. Guides to the Freshwater Invertebrates of Southern Africa, *The Protozoans, Porifera, Cnidaria, Platyhelminthes, Nemertea, Rotifera, Nematoda, Nematomorpha, Gastrotrichia, Bryozoa, Tardigrada, Polychaeta, Oligochaeta & Hirndinea*. *Vol 5*: Non-Arthropods, WRC Report No. TT 167/02, Pp 92-108

Darwall, W.R.T., Smith, K.G., Allen, D.J., Holland, R.A, Harrison, I.J., and Brooks, E.G.E. (eds.). 2011. The Diversity of Life in African Freshwaters: Under Water, Under Threat. An analysis of the status and distribution of freshwater species throughout mainland Africa. Cambridge, United Kingdom and Gland, Switzerland: IUCN. Pp 34, 109-119

De Clercq D. 1987. La situation malacologique à Kinshasa et description d'un foyer autochtone de schistosomiase à *Schistosoma intercalatum*. Ann. Soc. Belge. Med. Trop. Service de Parasitologie, Projet IMT-UNIKIN, Kinshasa XI. Pp 345-352

Fain A. 1951. Les mollusques transmetteurs de *Schistosoma mansoni* au lac Albert. Pp 423-439

Fain A. 1952. Description de la cercaire de *Schistosoma intercalatum* Fisher 1934 et d'une nouvelle xiphidiocercaire, du groupe Ornatae (sous-groupe Prima). Pp 433-443

Gillet J., Bruaux P. et Wolfs J. 1960. Résultats de prospections malacologiques en profondeur au lac Kivu et recherches sur la survie de *Biomphalaria* en eau profonde. Pp 643-649

Gryseels B. 1984. La schistosomiase intestinale dans la plaine de la Ruzizi (Burundi) : prospection préliminaire. Ann. Soc. Belge. Méd. Trop. Ministère de la Santé Publique du Burundi/Coopération Belge. B.P. 337, Bujumbura/Burundi. Pp249-266

Gryseels B. 1985. La répartition de *Biomphalaria* et la transmission de *Shistosoma* dans la plaine de Ruzizi, Burundi : étude préliminaire. Ann. Soc. Belge. Méd. Trop. Ministère de la Santé Publique du Burundi/Coopération Belge. B.P. 337, Bujumbura/Burundi. Pp49-58

Hammer, O. and Ryan, P. D., 2008. *PAST-PA laeontological Statistics*, ver. 1. 77

Hotez PJ and Kamath A. 2009. Neglected Tropical Diseases in Sub-Saharan Africa: Review of Their Prevalence, Distribution, and Disease Burden. *PLoS Negl Trop Dis* 3(8): e412.doi:10.1371 / journal.pntd.0000412

IUCN, 2011. Pan Africa freshwater mollusc species list. *The Diversity of Life in African Freshwaters: Under Water, Under Threat* (2011). RG next to the Red List Category indicates a regional Red List assessment. 22P

Kane R. A, J. Russell Stothard, Aidan M. Emery and D. Rollinson 2008. Molecular characterization of freshwater snails in the genus *Bulinus*: a role for barcodes? http://www.parasitesandvectors.com/content/1/1/15 *Parasites & Vectors* 1:15 DOI:10.1186/1756-3305-1-15

Kankonda B. A. 2001. Contribution à l'établissement d'une carte de pollution des eaux des ruisseaux de Kisangani par l'utilisation des macroinvertébrés benthiques comme bioindicateurs. Mémoire de DES inédit. Université de Kisangani. 90P.

Kankonda B. A. 2008. Ecologie des décapodes du ruisseau Masangamabe de la réserve forestière de Masako (Kisangani, RDC). Thèse de doctorat inédite. Université de Kisangani. 302P

Kotchi B. Y., Edia Oi E., Konan K. F., Kouassi n'gouan C., Diomandé D., Ouattara A. 2013. Spatial Distribution pattern of Freshwater Mollusks in Mé, Agnéby and Banco basins (Ivory Coast; West Africa). *Bull. Env. Pharmacol. Life Sci., Vol 2* (12): 146-151. Journal's URL:http://www.bepls.com

Lévêque, C. and J. R. Durand, 1980**.** Flore et Faune aquatiques de l'Afrique Sahelo-Soudanienne, Tome I. Editeurs scientifiques hydrobiologiques, O.R.S.T.O.M, Pp283-305

Marcon, E., 2013. Mesures de la Biodiversité. Retrieved from http://spip.ecofog.gf/IMG/pdf/mesures_de_la_biodiversite.pdf

Mohamed A. H., Ahmad H. Obuid-Allah, Amal A. Mahmoud and Heba M. Fangary 2011. Population dynamics of freshwater snails (Mollusca: Gastropoda) at Qena Governorate, Upper Egypt. Egypt. Acad. *J. Biolog. Sci*., 3(1): 11 -22 (2011), www.eajbs.eg.net

Mpawenayo B., 1996. Les eaux de la plaine de la Rusizi (Burundi): Les milieux, la flore et la végétation algales. Classe des Sciences naturelles et médicales, Mémoire in-8°, Nouvelle Série, Tome 23, fasc. 2, Bruxelles. Pp74

Muhigwa B. J-B., Lushombo M. J., Cirhuza K., Amundala C., Baluku B. et Cikwanine K. D. 2012. Hôtes intermédiaires de la schistosomiase et autres mollusques aquatiques autour de Kiliba, Plaine de la Ruzizi, RDC. Annales Sci. α Sci. Appl. Université Officielle de Bukavu *Vol. 3*, Pp 5-12

Ndassa A. and Mimpfoundi R. 2005. The Mollusca inhabiting rice fields in northern Cameroon and their role as intermediate hosts for schistosomes. *African Zoology* 40(2): 223–232, http://africanzoology.journals.ac.za

Ntakimazi, G., Nzigidahera, B., Nicayenzi, F. et West, K. 2000. L'Etat de la diversité biologique dans les milieux aquatiques et terrestres du delta de la Rusizi. Étude Spéciale De Biodiversité (ESBIO) Rapport. Pp 4

Ntonifor H. N. and Ajayi J. A. 2007. Studies on the ecology and distribution of some medically important freshwater snail species in Bauchi state, Nigeria. *Int. J. Biol. Chem. Sci.* 1(2): 121-127

OMS, 1957. Groupe d'études sur l'écologie des mollusques hôtes intermédiaires de la Bilharziose. Série de rapports techniques, N° 120. Palais des Nations, Genève. 42p

Saddozai S., W. A. Baloch,W. M. Achakzai and N. Memon, 2013. Population dynamics and ecology of freshwater gastropods in Manchar lake Pindh, Pakistan. *The Journal of Animal & Plant Sciences*, 23(4): 1089-1093

Sarr A., Kinzelbach R. and Diouf M. 2011. Specific diversity and ecology of continental molluscs from the lower Ferlo Valley (Senegal). *Journal MalCo* (2011) 7, 383-390

Schultheiβ R., Wembo N. O., Meni M., Marek C., Böβneck U. and Albrecht C. 2011. Freshwater molluscs of the Eastern Congo: notes on taxonomy, biogeography and conservation. *African Invertebrates, vol. 52* (2) : 265-284

Schwezt J. 1948. Une nouvelle classification des Planorbes du Congo-Belge. Pp 1, 6-11

Shabani E. I. and Gembu T. G-C. 2014. Inventaire des macroinvertebres des eaux de Mambasa (Province Orientale, RDC). Rapport de stage inédit, Fac. Des Sciences / Université de Kisangani, 22P

Stauffer J. R. and Madsen H. 2012. Schistosomiasis in Lake Malaŵi and the Potential Use of Indigenous Fish for Biological Control. www.intechopen.com Pp 120- 140

Thiam N. and Diallo A. 2010. Intégration de la biodiversité d'eau douce dans le processus de développement en Afrique. « Le suivi des Mollusques d'eau douce ». Projet de démonstration Bassin du fleuve Gambie. 44P

Van Bocxlaer B., Clewing C., Mongindo E. J-P., Kankonda A., Wembo N. O. and Albrecht C. 2015. Recurrent camouflaged invasions and dispersal of an Asian freshwater gastropod in tropical Africa. *BMC Evolutionary Biology* (2015) 15:33 DOI 10.1186/s12862-015-0296-2

Vancoppenolle R., Renard C., Sottiaux G. and Nyole H. 1984. Comprotement de *Stylosanthes guianensis* (Aubl.) Sw. Dans la Ruzizi et le Mosso (Burundi). *Tropicultura*, 2. 2. 50-55

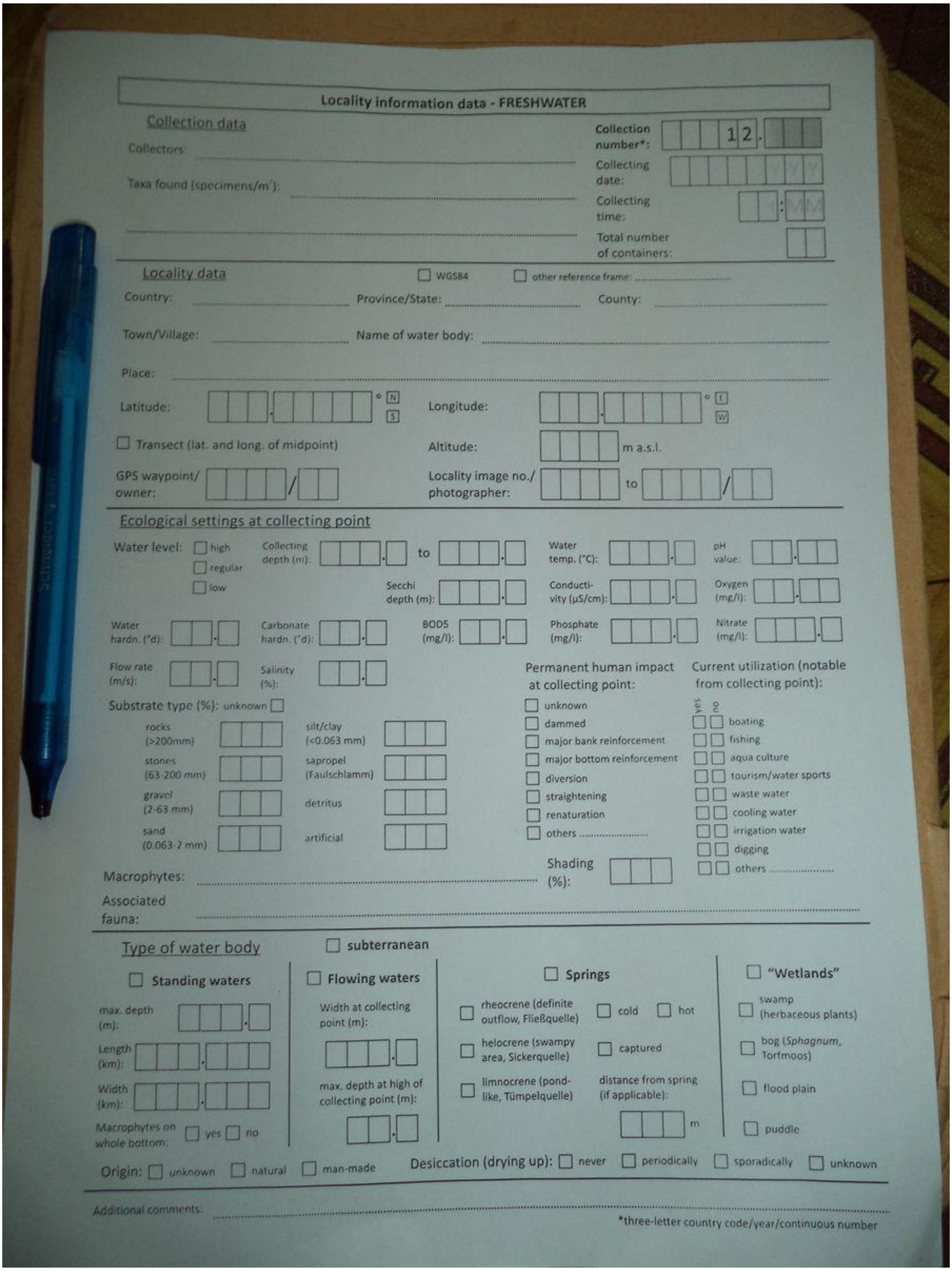
WHO, 1982 : Guide de terrain des Gastéropodes d'eau douce Africains. Centre de collaboration pour la malacologie appliquée. Laboratoire Danois de la bilharziose, Jaegersborg Alle, 1D DK 2900 Charlottenlund Danemark. 55P

WHO, 2002. Prevention and control of schistosomiasis and soil transmitted helminthiasis: Report of a WHO expert committee. Organisation Mondiale de la Santé, série de rapports techniques 912

Zaghloul Noura M. R. M., D. Grabner, Faten A.M.M. Mohamed, Hameed A.A. Sabry and Bernd Sures 2013. Effect of some Physico-Chemical Parameters on Abundance of Trematode Snails in Fayoum Governorate, Egypt. Fayoum Drinking Water and Sanitation Company, Fayoum, Egypt. Pp 8

**APPENDICES**

**Appendix 1:** Locality information data of sampling site



Locality information data - FRESHWATER

Collection data

Collectors: ..........

Taxa found (specimens/m²): ..........

Collection number*: 12.

Collecting date: YYY

Collecting time: :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

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 (>200mm) | 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

☐☐ boating ☐☐ fishing ☐☐ aqua culture ☐☐ tourism/water sports ☐☐ waste water ☐☐ cooling water ☐☐ irrigation water ☐☐ digging ☐☐ others ..........

Shading (%):

Macrophytes: ..........

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

☐ "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 2:** Genera/species of snails from the freshwaters of Ruzizi Congolese plain

Figure 1: *Pila ovate*

Figure 2: *Lymnaea natalensis*

Figure 3: *Bulinus forskalii*

Figure 4: *Melanoides tuberculata*

Figure 5: *Bulinus globosus*

Figure 6: *Biomphalaria pfeifferi*

Figure 7: *Biomphalaria sp.*

Figure 8: *Lymnaea sp.*

Figure 9: *Bulinus tropicus*

Figure 10: *Melanoides sp*