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# Human Endoparasites Present in the Digestive Tracts of Two Species of Cichlidae Fish: *Oreochromis Niloticus* (Linnaeus, 1758) and *Tilapia Tholloni* (Sauvage, 1884) Caught in the Malebo Pool (Congo River), D.R. Congo

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## Abstract

Parasitic diseases of fish are very rarely highlighted in comparison with certain parasitic diseases transmitted to humans through animals or plants such as echinococcosis, bilharziasis or fasciolosis. The aim of this study is to provide knowledge on endoparasites of the human digestive tract that also infest the digestive tracts of two species of Cichlidae fish: *Oreochromis niloticus* Linnaeus, 1758 and *Tilapia tholloni* Sauvage, 1884 caught in the Congo River (Malebo Pool). Microscopic observations were made on 42 specimens of *O. niloticus* fish and 42 of *T. tholloni*. The results obtained showed that the digestive tracts of fish *Oreochromis niloticus* host 8 species of parasites grouped in 6 genera, 6 families, 4 classes and 2 branches. The species belonging to the phylum Nemathelminths branch were more abundant (58.8%) than Plathelminths (41.2%). Species *Trichurus trichiura* (23.5%), *Ascaris lumbricoides* (20.6%), *Strongyloides stercoralis* (14.7%), *Schistosoma haematobium* and *Schistosoma intercarlantum* (11.8%) were the most frequently observed. On the other hand, microscopic analyses of the digestive tract of *Tilapia tholloni* revealed the presence of five species of parasites belonging to 3 branches, 4 classes, 4 families and 4 genera. Nemathelminthes (58.3%) and Plathelminthes (25%) are the most represented phylum than Amoebians (16.7%). *Enterobius vermicularis* (33.3%), *Trichocephalus trichuris* (25%), *Schistosoma haematobium* (20.8%) and *Entemoeba hystolica* (16.7%) were frequently observed. These results confirm the presence of endoparasites in congolese freshwater fish. This information is important in setting up a strategy to protect fish and consumers against these pathogens.

**Keywords:** Endoparasites, freshwater, *Oreochromis niloticus*, *Tilapia tholloni*, Congo River, Malebo Pool, Kinshasa

## 1. Introduction

Aquatic ecosystems provide many goods and services that are often not fully appreciated (Brummett *et al.,* 2008). Water pollution from a variety of natural, domestic, agricultural and industrial causes is the source of many diseases related to infectious or toxic factors (Delolme *et al.,* 1992). Generally, fish from these polluted environments are infested, their consumption could transmit the parasitosis to humans, from which fecal parasites can probably be observed from humans to fish and from fish to humans whose reservoir is the water. According to Thillement (2015), more than 100 pathogens are likely to parasitize humans, but only a small number represent a public health problem. In water, pathogens are easily transmitted from one fish to another through the skin and gills. Fish are sensitive to stress, can be disturbed by poor water quality, poor diet, rough handling or a disturbed environment. This can result in a decrease in immune system activity, which can lead to the sudden onset of disease. Fry and juveniles are the most vulnerable; they need to develop their immunity (Edéa *et al.,* 2019).

There is a multitude of possible diseases (parasitic, bacterial, viral, etc.). Stressed fish can often be identified by abnormal behaviour such as decreased appetite, nervous or waddling swimming, upright position at the surface, or by clinical symptoms or pathologies such as morphological abnormalities (barbels or damaged fins, large belly, blocked mouth, goiters, etc.), eye disorders (protruding eyes, glassy eye, loss of eye, etc.) or by the presence of a large number of other diseases. ), gill disorders (degeneration, whiteheads, etc.), internal organ disorders (dark spots on the liver, heart, spleen, inflammation of the intestine; etc.), skin disorders (foamy lips, blackish tumours, etc.), behavioural disorders (fish piping air on the surface, uncoordinated swimming movements, etc.), undesirable hosts (algae, hydrous, chlorinated water, etc.). Nevertheless, these symptoms are not specific, therefore, precise laboratory techniques are required to diagnose the disease (Lacroix, 2004).

In Kinshasa, in the Democratic Republic of Congo, watercourses are considered to be the drain through which all waste is evacuated (faeces, urban, industrial and domestic waste). One third of the fresh fish consumed by the people living along the Congo River in the Pool Malebo comes from artisanal fisheries. Offering consumers fresh fish free of parasites and other types of pollutants is a real challenge, especially since the Congo River is considered a dumping ground by the same population. This situation constitutes a real threat to the human health of Kinshasa's population (Masua *et al.,* 2020; Munganga *et al*., 2020).

Fish consumption is increasingly important on a global scale: it was 9.9 kg per capita per year in 1960 and 20.1 kg per capita per year in 2014 (FAO, 2016). Despite its economic and dietary importance, this food is a biotope that is very favourable to the development of a large number of parasites (Nchoutpouen & Fomena, 2011; Falaise, 2017). Given that fishing is the main activity of the riparian populations of the Malebo Pool (Congo River), this situation is a problem for the maintenance of fish in their ecosystem but also for human health. This study aims to identify human endoparasites in the digestive tracts of two species of freshwater fishes of the family Cichlidae (*Oreochromis niloticus* Linnaeus, 1758 and *Tilapia tholloni* Sauvage, 1884) caught in the Malebo Pool (Congo River) in the Democratic Republic of Congo. The results of this study will make it possible to warn the population about the risks incurred following the consumption of fishery products from polluted environments but also to fill the gaps in knowledge of pathogens harmful to human health through the consumption of fish.

## 2. Material and Methods

### *2.1 Study Environment*

This study took place in the Malebo Pool (Congo River) at the Kinkole fishing station (figure 1). The Malebo Pool, formerly known as the Stanley Pool, is the widening of the Congo River located at the border between the Democratic Republic of Congo (Kinshasa city province) and the People's Republic of Congo (Brazzaville prefecture) (Pwema *et al*., 2019). It is located between 04° 05' - 04° 20' South and 15° 19' - 15° 33' East, at an altitude of 275 m. This part of the river is about 35 km long and 25 km wide. Its average depth is 3 m with a maximum of 20 m and a surface area of 500 km$^2$ (Burgis & Symoens, 1987; Muzigwa *et al*., 1994). The Malebo Pool enjoys a hot and humid tropical climate of AW$_4$ type according to the Köppen classification, characterised by two types of seasons: the long rainy season (between September and mid-May) and the dry season (between mid-May and August). The average temperature varies between 22.5 °C and 25 °C. The hydrography of Pool Malebo includes the Congo River and mainly the N'sele and N'djili rivers which are its tributaries (Mbadu, 2001; Munganga *et al*., 2020).

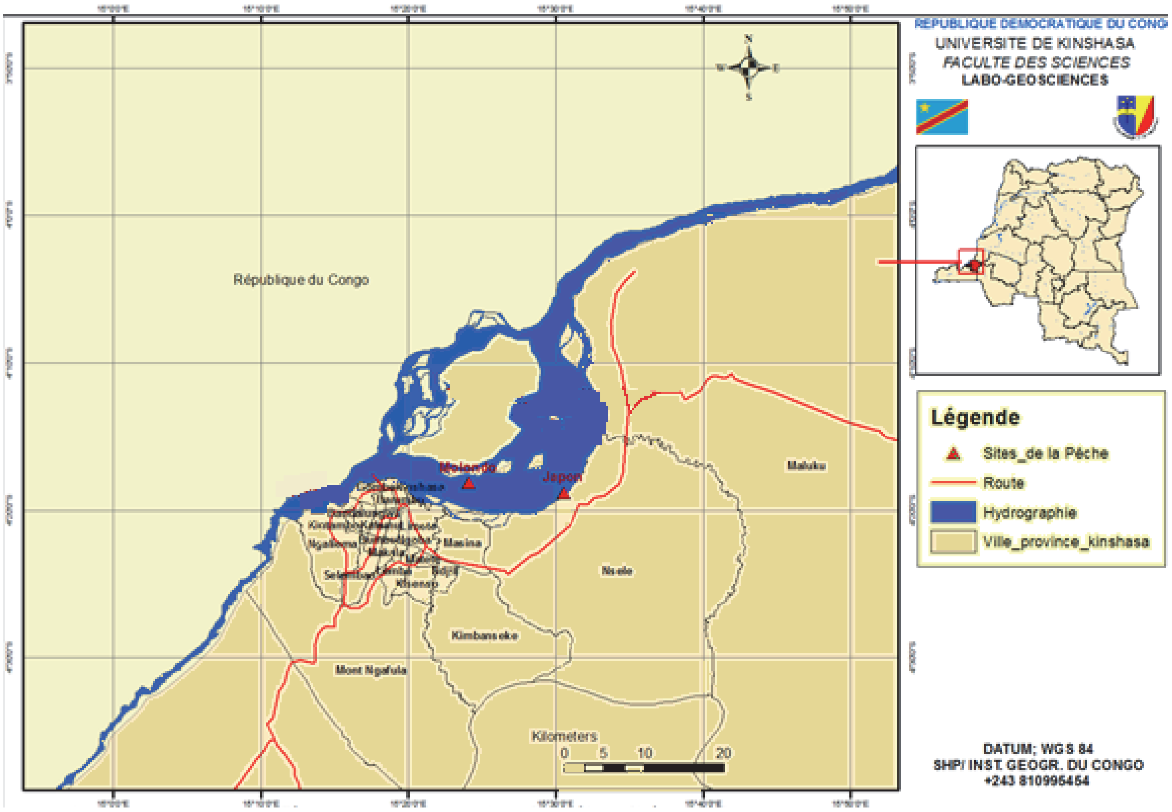

Figure 1. Map of the Malebo Pool (Congo River) showing the fishing sites at the Kinkole station

### *2.2 Methodology*

#### 2.2.1 Fish Data Collection and Laboratory Handling

The fish specimens *Oreochromis niloticus* Linnaeus, 1758 and *Tilapia tholloni* Sauvage, 1884 were caught in the Malebo Pool (Congo River) at the Kinkole station (04°18′,26″ S, 15° 30′,33″ E) located in the commune of N'sele. Specimens were collected monthly for 6 months between January and June 2017. The catches were obtained by artisanal fisheries while using hawks nets of 2 cm mesh size and 5 m in diameter. One hundred and six (106) specimens (juveniles and adults) of Cichlidae fish, consisting of 42 individuals *Oreochromis niloticus* Linnaeus, 1758 and 64 *Tilapia tholloni* Sauvage, 1884. These fish were caught during artisanal fishing campaigns using a hawksbill net with a mesh size of 2 cm and 5 m in diameter. After fishing, the specimens were kept in 10 kg plastic bags and then placed in a glacier to facilitate their transport to the laboratory. The date of sample collection was marked on each batch of specimens and kept cold in a freezer.

The fish caught were identified using identification keys proposed by Boulenger (1911); Poll (1939a & b, 1959); Poll & Gosse (1995a et b) available at the Limnology, Hydrobiology and Aquaculture Laboratory of the Biology Department of the Faculty of Sciences of the University of Kinshasa. The fish were measured (with an electronic ichthyometer, Digital Caliper 200 mm-8 mark) according to Lévêque *et al*., (1990 & 1992) and weighed (with an electronic balance, Salter mark) to the nearest millimetre and gram respectively. For size accuracy of the specimens of *Oreochromis niloticus* and *Tilapia tholloni* studied, the size class of the fish was calculated according to Sturge's rule (Pwema, 2014).

#### 2.2.2 Measurement of Environmental Parameters

Measurements of the environmental variables (temperature (°C), conductivity (µS/cm), turbidity (NTU: Nephelometric Turbidity Unit) and pH) were carried out at each sampling site with the HANNA pH/ORP/EC/DO No. HI 9828 multiparameter Combo pH meter probe.

### 2.2.3 Investigation and Identification of Intestinal Endoparasites

The fish were dissected to isolate the intestines, which were first examined with the naked eye and then with a binocular magnifying glass (Olympus brand TOKYO 323693). Each digestive tract thus isolated was placed in a pre-numbered glass vial containing 4 drops of physiological water. Fresh smears of fragments of these stomach contents were observed with a Euromex photomicroscope at the laboratory of the Mont-Amba hospital center. The endoparasites observed were identified using the identification guide for intestinal parasites proposed by WHO (1994). This identification was done at the species level.

### 2.2.4 Statistical Analysis and Data Processing

The various data were analysed and encoded on the Excel 2010 spreadsheet. The results obtained after treatment were expressed in the form of tables, graphs and figures to facilitate their interpretation. Origin 6.1 software was used to generate the graphs.

## 3. Results

### *3.1 Physico-Chemical Parameters of Water*

Table 1. Physico-chemical parameters of the waters of the Congo River at Kinkole

| Sites | Température (°C) | Conductivity (µS/cm) | Turbidity (ppm) | pH |
|---|---|---|---|---|
| I | 29,3±0,13 | 27,9±0,97 | 13,73±0,1 | 6,27±0,11 |
| II | 28,9±0,18 | 27,5±0,83 | 14,21±0,38 | 6,62±0,28 |
| Mean | 29,1±0,2 | 27,7±0,2 | 13,97±0,24 | 6,44±0,17 |

The results of the environmental parameters shown in table 1 above show that the surface water of the Congo River in the Malebo Pool has an average temperature of 29.1±0.2 °C and is low in mineral salts (average conductivity equal to 27.7±0.2 µS/cm). This water is slightly turbid at the sampling sites and has an average turbidity of 13.97±0.24 ppm. The hydrogen potential shows that this water is slightly acidic (pH: 6.44±0.17).

### *3.2 Fish Size Classes*

Table 2. Different size classes of fish specimens studied

| Species of fish | Size classes | Size range (mm) | Number of fish |
|---|---|---|---|
| *Oreochromis niloticus* | I | [64,3 - 98,3 [ | 6 |
| | II | [99 – 133 [ | 5 |
| | III | [134 – 168 [ | 6 |
| | IV | [169 – 203 [ | 10 |
| | V | [204 – 238 [ | 9 |
| | VI | [239 – 273 [ | 15 |
| | VII | [274 - 308,1 [ | 13 |
| | Total | | 64 |
| *Tilapia tholloni* | I | [66,4 – 104,4 [ | 5 |
| | II | [105 – 143 [ | 7 |
| | III | [144 – 182 [ | 4 |
| | IV | [183 – 221 [ | 9 |
| | V | [222 – 260 [ | 7 |
| | VI | [261 - 298,3 [ | 10 |
| | Total | | 42 |

The size classes of the different specimens of *Oreochromis niloticus* and *Tilapia tholloni* studied were established according to Sturge's rule. A total of 64 specimens of *O. niloticus* fish were grouped into 7 size classes. The sixth class (size range: 239mm to 273 mm) is the one with a large number of individuals (i.e. 15 fish) and the second class (size range: 99 mm to 133 mm) has fewer specimens (i.e. 5 individuals). The fish *Tilapia tholloni* has 42 specimens grouped in six size classes, of which the sixth class (size between 261 mm and 298.3 mm) has the majority of fish (i.e. 10 specimens) and the third class (size range: 144 mm to 182 mm) has fewer fish (i.e. 4 individuals).

### *3.3 Parasites Observed in the Stomach Contents of the Fish Studied*

#### 3.3.1 *Oreochromis Niloticus* Linnaeus, 1758

Table 3. Endoparasites identified in the digestive tract of fish *Oreochromis niloticus*

| Phylum | Class | Family | Genus | Species |
|---|---|---|---|---|
| *Némathelminthes* | *Adenophorea* | *Trichuridae* | *Trichocephalus* | *Trichocephalus trichiurus* |
| | *Secernetea* | *Ascarididae* | *Ascaris* | *Ascaris lumbricoides* |
| | | *Stronglodidae* | *Strongloides* | *Strongloides stercoralis* |
| *Plathelminthes* | *Cestode* | *Taenidae* | *Taenidae spp* | *Taenidae spp* |
| | *Trématode* | *Schistosomidae* | *Schistosoma* | *Schistosoma mensoni* |
| | | | | *S. intercalantum* |
| | | | | *S. haematobium* |
| | | *Tronglomatidae* | *Paragonimus* | *Paragonimus westermani* |
| 2 | 4 | 6 | 6 | 8 |

Microscopic observations made on 42 digestive tracts of *Oreochromis niloticus* fish reveal the presence of 8 species of endoparasites that have infested these poisons. These parasites are divided into 6 genera, 6 families, 4 classes and 2 branches. Of the 42 digestive tracts of fish analysed, only 32 are positive and 10 others are not infested.

##### 3.3.1.1 Relative Abundance of Branches of the Identified Endoparasites

The majority of the *Oreochromis niloticus* fish analysed are infested by endoparasites of the Nemathelminthes phylum (58.8%) than the Plathelminthes phylum (41.2%) (figure 2).

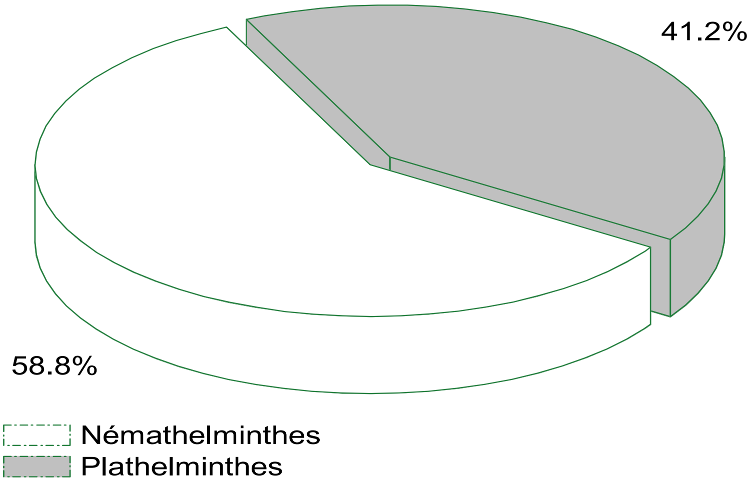

Figure 2. Relative abundance of the orders of parasites identified in the digestive tract of fish *Oreochromis niloticus*

### 3.3.1.2 Relative Abundance of the Genera of the Identified Pests

Of all the genera of endoparasites identified in the digestive tract of fish *Oreochromis niloticus*, those of the genus Schistosoma are in the majority (with 37.5%) and those of the genera Ascaris, Strongloides, Taenia spp and Pragonimus are in the minority (representing 12.5% respectively) (figure 3).

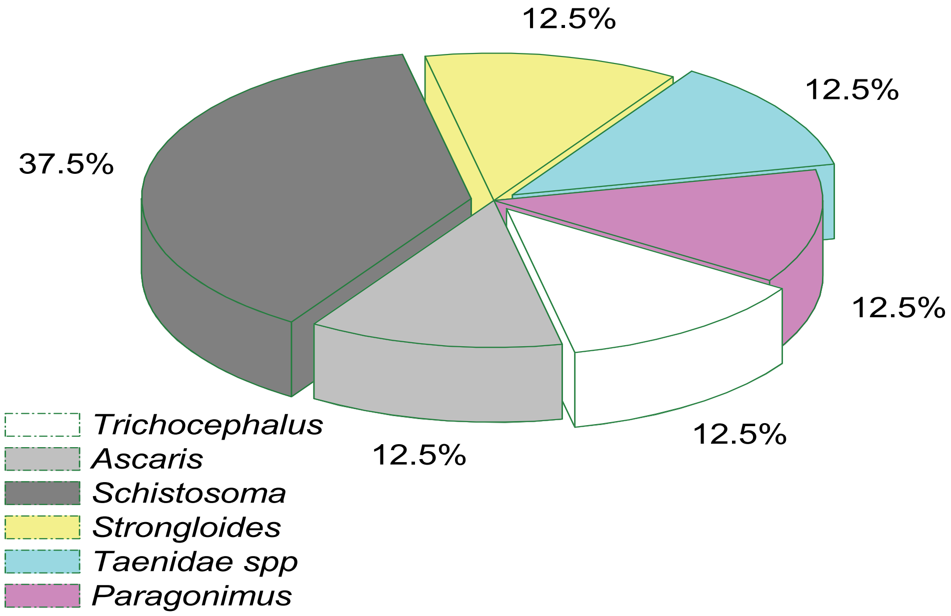

Figure 3. Relative abundance of the genera of parasites identified in the digestive tract of fish *Oreochromis niloticus*

### 3.3.1.3 Relative Abundance of Identified Endoparasite Species

*Trichurus trichiura* (23.5%) is the most common endoparasite found in the digestive tracts of fish analysed. *Ascaris lumbricoides* (20.6%) came second followed by *Strongyloides stercoralis* (14.7%), *Schistosoma haematobium* and *Schistosoma intercarlantum* (with 11.8% respectively). *Schistosoma mensoni* accounts for 9% while *Taenia spp* was analysed at 5.9% and *Paragonimus westermani* at 2.9% (figure 4).

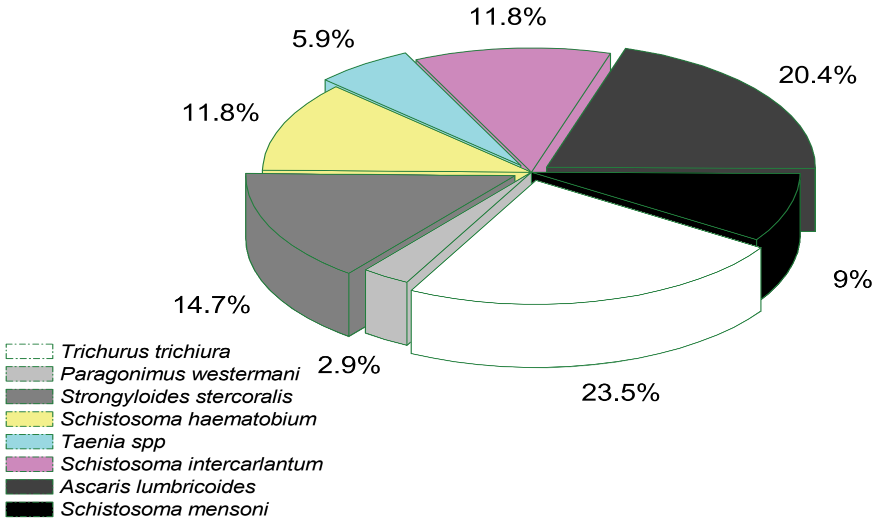

Figure 4. Relative abundance of species of parasites identified in the digestive tract of fish *Oreochromis niloticus*

### 3.3.2 *Tilapia tholloni* Sauvage, 1884

Table 4. Parasites identified in the digestive tract of fish *Tilapia tholloni*

| **Phylum** | **Class** | **Family** | **Genus** | **Species** |
|---|---|---|---|---|
| Amoebiens | Lobosea | Amiboidae | Entamoeba | Entamoeba histolyca |
| | Adenophorea | Trichuridae | Trichocephalus | Trichocephalus trichiurus |
| Némathelminthes | Secernetea | Oxyuridae | Enterobius | Enterobius vermicumlaris |
| Plathelminthes | Trématode | Schistosomidae | Schistosoma | Schistosoma haematobium<br>S. intercalantum |
| **3** | **4** | **4** | **4** | **5** |

Microscopic analyses of the stomach contents of *Tilapia tholloni* fish reveal that their digestive tracts are infested with 3 branches, 4 classes, 4 families, 4 genera and 5 species of endoparasites. Of the 42 fish specimens analysed, the digestive tracts of 23 were positive and 9 others showed no evidence of endoparasites.

#### 3.3.2.1 Relative Abundance of Branches of the Identified Parasites

Figure 5 below shows that the digestive tracts of *Tilapia tholloni* fish are mostly infested by Nemathelminths (58.3%), followed by Plathelminths (25%) and Amoebians are less abundant (16.7%).

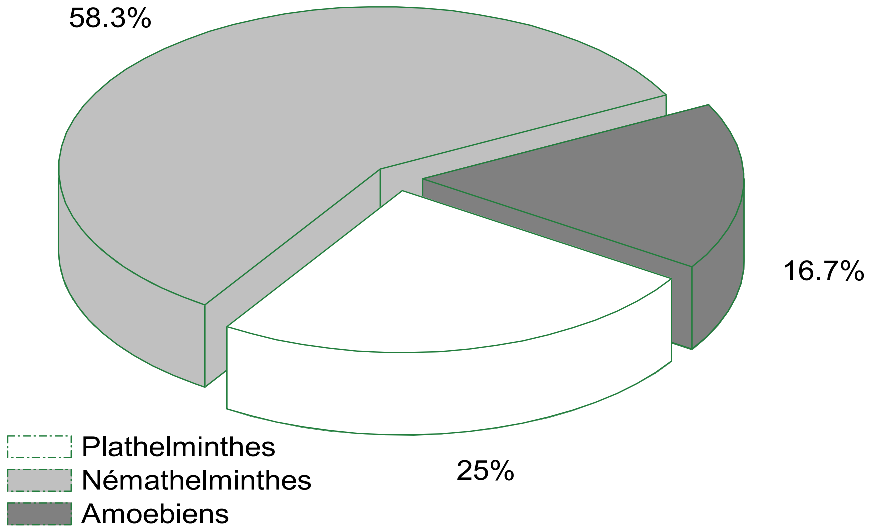

Figure 5. Relative abundance of branches of the parasites identified in the stomach contents of fish *Tilapia tholloni*

#### 3.3.2.2 Relative Abundance of the Genera of the Identified Pests

Endoparasites of the genus Schistosoma infest the majority of the *Tilapia tholloni* fish analysed (i.e. 40%), followed by those of the genera Entamoeba, Trichocephalus and Enterobius (with 20% respectively) (figure 6).

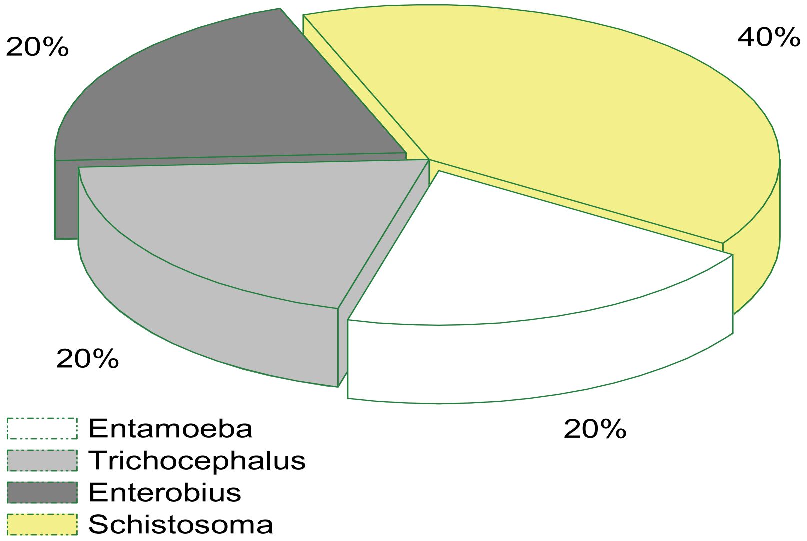

Figure 6. Relative abundance of the genera of parasites identified in the stomach contents of fish *Tilapia tholloni*

3.3.2.3 Relative Abundance of Species of Identified Parasites

In the digestive tract of *Tilapia tholloni* fish, *Enterobius vermicularis* is the most common species (33.3%), followed by *Trichocephalus trichuris* (25%), *Schistosoma haematobium* (20.8%), *Entamoeba hystolica* (16.7%) and *Schistosoma intercalantum* (4.2%) (figure 7).

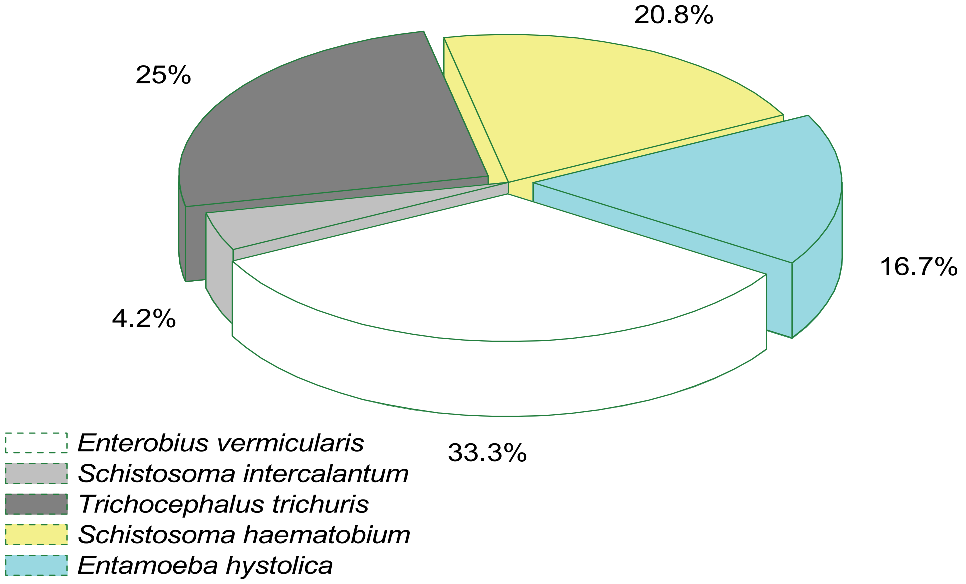

Figure 7. Relative abundance of species of parasites identified in the stomach contents of fish *Tilapia tholloni*

## 4. Discussion

The results obtained on environmental variables of the fishing sites showed that the water of the Congo River has an average temperature of 29.1±0.2 °C, with a low amount of dissolved mineral salts. This water is slightly cloudy and had a low content of suspended matter. Hydrogen potential analysis showed that this water is slightly acidic. These observations meet those made by several authors who have exploited this part of the Congo River (Mbadu, 2011; Pwema, 2014; Tembeni *et al*., 2019). Climate has a definite impact on infestation in temperate or tropical zones since the intermediate hosts are ectothermal (Van Cam, 2009; Falaise, 2017). However, thermal comfort zones are sometimes minimal and depend on the species. Petney *et al.,* (2013) state that the rate of infestation of fish by metacercariae of Opisthorchiidae varies from year to year depending on the season, age and species of the fish, geographical area, aquatic habitat and climatic factors (intensity of rainfall). Results of research conducted by Madsen *et al*., (2015) in Vietnam showed that the peak of transmission of endoparasites to fish was during the summer (May to November), particularly during the period of heavy rainfall. The prevalence and intensity of infection then decreases in winter (December-January) due to lower water levels and colder temperatures. Snails are infected but the release of cercariae into the water is slowed down. Thailand is a tropical country, the peak of faecal contamination in aquatic environments and the peak of contamination of snails by *Opisthorchis viverrini* occurs at the time of flooding. The peak of human transmission occurs just after the monsoon (October to February), when intermediate host fish are abundant and heavily parasitized. In countries with a marked cold season, peak transmission occurs during the warmer months. Indeed, the accommodation of cercariae in snails is dependent on water temperature (Falaise, 2017). In China, a study has shown that the risk of human infestation by *Clonorchis sinensis* would be greater in case of high temperatures and rainfall, but too high ambient humidity would reduce this risk (Li *et al*., 2014). Thus, the different values of physico-chemical parameters recorded in the Congo River favour the maintenance of endoparasites in this aquatic ecosystem.

Microscopic observations of the digestive tracts of fish specimens *Oreochromis niloticus* and *Tilapia tholloni* revealed the presence of several species of intestinal endoparasites capable of infesting humans. The digestive tracts of fish *O. niloticus* were infested with eight species of endoparasites in six genera, six families, four classes and two phyla (Nemathelminths and Plathelminths). In terms of frequency of occurrence of endoparasitic species, *Trichurus trichiura* (23.5%), *Ascaris lumbricoides* (20.6%), *Strongyloides stercoralis* (14.7%), *Schistosoma haematobium* and *Schistosoma intercarlantum* (11.8%) were the most common. On the other hand, microscopic analyses carried out on the digestive tracts of *Tilapia tholloni* specimens revealed the presence of 3 branches (Nemathelminthes, Plathelminthes and Amoebians), 4 classes, 4 families, 4 genera and 5 species of endoparasites that had infested the digestive tracts of these fish. It was found that, *Enterobius vermicularis* (with 33.3%), *Trichocephalus trichuris* (with 25%), *Schistosoma haematobium* (with 20.8%) and *Entamoeba hystolica* (with 16.7%) were frequently observed. These results are close to those obtained by Kulenduka (2015); Mondombi (2015) during research on endoparasites of the digestive tracts of certain fish in the Malebo Pool (Congo River).

Kulenduka (2015) examined the endoparasites of the fish *Oreochromis niloticus* in the Malebo Pool by reporting 6 species, 6 genera, 6 families, 5 classes and 3 branches of endoparasites. In addition, Mondombi (2015) analysed 5 species, 6 genera, 5 families, 3 classes and 2 branches of parasites in the digestive tracts *of Brycinus imberi* fish caught at the Maluku station in the Malebo Pool (Congo River). In contrast to the latter, the high proportion of parasite species observed during our analyses can be explained by the poor hygienic conditions at the Kinkole fishing station. We noticed that at the fishermen's boat sites where the fish harvesters buy, clean, gut and sell fresh fish and other fishery products, some other human activities such as washing, swimming, dumping household waste and even human and farm animal excreta are carried out. The massive presence of travellers in this fishing site waiting to reach the provinces of Mai-Ndombe, Kwilu, Equateur, Mongala, northern Ubangi and so many others by sea is remarkable. While waiting for their departure, the latter contribute to the pollution of the river waters by the discharge of all kinds of waste (liquid and solid) and consider the Congo River as a septic tank by depositing faecal matter there during their stay and journey. These activities and behaviours cause secondary contamination of the Congo River. Durand and Lévêque (1980) consider that most of the anthropic activities carried out along the countries' aquatic ecosystems constitute a real threat to aquatic resources, including fish. The presence of *Trichocephalus trichuris*, *Entamoeba hystolica* and *Enterobius vermicularis* is linked to faecal matter and that of species of the Schistomidae family is due to water contamination by the discharge of various household wastes.

The presence of the different species of endoparasites identified in the digestive tract of fish *Oreochromis niloticus* and *Tilapia tholloni* is explained by the ingestion of water and food contaminated by different types of the parasites in the fish food chain (Edéa *et al.,* 2019). Fish are intermediate hosts for most endoparasites (Nchoutpouen & Fomena, 2011; Koné, 2015; Falaise, 2017). Following the development cycle of endoparasites, humans are in the majority of cases the final host where these pathogens develop and multiply. In humans, *Trichurus trichiura* is responsible for Trichocephalosis, *Ascaris lumbricoides* is the basis of Ascariasis and *Strongyloides stercoralis* is responsible for Anguillulose or Strongyloidosis. On the other hand, intestinal amoebiasis is caused by *Entamoeba hystolica*, pinworm is caused by *Enterobius vermicularis*, *Trichocephalus trichuris* is responsible for trichocephalosis and endoparasites of the genus Schistosoma are at the base of bilharziosis. These results confirm the claims made by Gambari (2013); Mondombi (2015) on the prevalence of parasitic diseases in Sub-Saharan Africa in general and in the Democratic Republic of Congo in particular (Kulenduka, 2015).

## 5. Conclusion

This study showed that the fish *Oreochromis niloticus* Linnaeus, 1758 and *Tilapia tholloni* Sauvage, 1884 caught at Kinkole in the Malebo Pool (Congo River) are carriers of endoparasites. These organisms belong to several branches, classes, families, genera and species. The accumulation of *Trichurus trichiura*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Entamoeba hystolica*, *Enterobius vermicularis*, *Trichocephalus trichuris* and *Schistosoma sp* in fish constitutes a health hazard for consumers since fish, like other fishery products, are capable of transmitting microbial and parasitic infections to humans. In order to decrease the parasite load and reduce the risk of infestation by humans and aquatic ecosystems, the authorities of the country are called upon to take adequate measures to protect fish species. We advise the population of Kinshasa bordering the Congo River in the Malebo Pool to clean (eviscerate) immediately and freeze fish at 2°C after fishing to avoid post-mortem migration of larvae of endoparasites from the viscera to the muscles. Thorough cooking of fresh fish before consumption remains the best solution to avoid probable endoparasite infestation.

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