Determination of Seasonal Occurrence of *Camallanus* sp. and *Ligula intestinalis* on Fresh Usipa, *Engraulicypris sardella* from Selected Mzuzu Markets, Malawi

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Abstract This study assessed the seasonality of two parasites, *Camallanus* sp. and *Ligula intestinalis* on fresh *E. sardella* from selected Mzuzu markets in Malawi. Samples were collected during the cold, dry and rainy seasons and the determination of the parasites was conducted by quantifying by calculation of prevalence rates and mean intensities of the parasites during the three seasons. These parasites were located in gills and the gastrointestinal cavity respectively. No parasites were observed during the cold season. A total of 46 parasites were observed during the dry season and 28 parasites were observed during the rainy season out of two hundred samples per season. The prevalence rates for the cold, dry and rainy seasons were 0%, 21% and 13% respectively and corresponding mean intensities of 0, 1.1 and 1.08 respectively. The study revealed seasonal variations of prevalence rates and the mean intensities of the parasites. The cold season registered the lowest prevalence rate and mean intensity. These findings indicate a strong interaction of parasite with the environment, the host fish and other fish behavioural factors such as feeding and foraging. The findings suggest that the proliferation of some parasites in *E. sardella* is influenced by ecological dynamics of the habitats as well as the seasonal variations.

Keywords *Ligula intestinalis*; *Camallanus* sp.; Prevalence rates; Mean intensity; Seasonal variations

Background
Parasitism is the relationship between organisms in which the parasite benefits at the expense of the host organism (Roberts and Janovy, 2005) and it is a common phenomenon in most ecological systems (Madenire-Moyo and Barson, 2010). Fish may be final or intermediate hosts of the parasites like cestodes (Sharma, 2016) with intensity of infection varying from one species to another due to physiological and ecological differences (Nimbalkar et al., 2010). The presence of parasites debilitates the fish and affects their quality leading to economic losses on the market (Bhuiyan et al., 2007; Maguza-Tembo and Mfitilodze, 2008; Sumuduni et al., 2015). Khurshid and Ahmad (2012) attribute the severity of the parasite infection to climate of a particular area. The fish under study, *E. sardella* have a fast growing short-life history pattern, endemic to Lake Malawi and spawns throughout the year mainly during the rainy season (Maguza-Tembo et al., 2009). Although some study was conducted on *Ligula intestinalis* infection in *E. sardella* in southern Lake Malawi by (Rusuwa et al., 2014), no work has been done on the parasitic fauna of *E. sardella* that is sold in Mzuzu city from northern Lake Malawi in relation to seasonal population dynamics. Cestodes, *L. intestinalis* cause ligulosis in humans if they are infected when they consume raw or undercooked fish meat (Urdes and Hangar, 2013; Ahmadiara, 2017). Therefore, this study was conducted to determine the parasitic load of fresh *E. sardella* in relation to different seasons of the year with focus on the prevalence rate and the mean intensity of the identified parasite species.

1 Materials and Methods
1.1 Study area and sample collection
The study took place in the city of Mzuzu which has three distinct seasons namely, cold (June and July), dry (October and November) and rainy (January and February). A total of two hundred fish were collected during each season randomly from the selected markets shown in Figure 1. They were taken fresh to the laboratory for examination.
1.2 Fish examination and parasite identification

Specimen preparation and examination were done following procedures by Akinsanya et al. (2007), Biu et al. (2014), Sumuduni et al. (2015) and Edeh and Solomon (2016) while parasites identification were done according to Parpena (1996), Klinger and Floyd (2002) and Poudre et al. (2005).

The skin, gills, internal parts, the eye and the gastrointestinal cavity were examined by scraping using a scalpel. Dissecting and compound microscopes up to 400× magnification were used to examine the specimen on the slides. The recovered parasites were preserved in 70% ethanol for further identification.

Skin scrape was put on the slide with water drop added and examined under the compound microscope. The eye and the intestines were dissected and contents mounted on the microscope for observation. Gill and heart tissues were also placed on the slide for examination while bigger parasites were removed using forceps and identified. The parasites species and location in the host species were recorded.

The calculations of prevalence rate and mean intensity were done according to Ezenwanji et al. (2005), as follows:

\[
\text{Prevalence rate} = \frac{\text{Number of fish infected}}{\text{Number of fish examined}} \times 100\%
\]

\[
\text{Mean intensity} = \frac{\text{Total number of parasites}}{\text{Number of fish infested}}
\]
1.3 Data analysis
The data obtained from the parasitic load determination was analyzed by F-test using SPSS statistical package version 16.0 at 5% level of significance. Tukey HSD and Bonferroni Post Hoc multiple comparisons tests were also conducted to verify the significance of the differences.

2 Results
The parasitic loads for the cold, dry and rainy seasons were determined. Seasonal variations on the parasitic load were noted during the study period. Out of the two hundred sampled fish per season, only two classes of parasites were observed. These are presented in the Table 1.

Table 1 Parasites and location in fresh *E. sardella* during cold, dry and rainy seasons

<table>
<thead>
<tr>
<th>Parasite Class</th>
<th>Parasite Species</th>
<th>Site Located</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nematoda</td>
<td><em>Camallanus</em> sp.</td>
<td>Gills</td>
</tr>
<tr>
<td>Cestoda</td>
<td><em>Ligula intestinalis</em></td>
<td>Gastrointestinal cavity</td>
</tr>
</tbody>
</table>

The two classes of parasites identified in *E. sardella* during the three seasons were cestoda and nematoda. The cestoda species identified was *Ligula intestinalis* and the species of nematoda identified was the *Camallanus* sp. *Ligula intestinalis* parasites were observed in the gastrointestinal cavity while the *Camallanus* sp. parasites were observed on the gills. *Ligula intestinalis* parasites are shown in the Figure 2.

![Figure 2 Ligula intestinalis parasites from E. sardella gastrointestinal cavity](image)

The seasonal parasitic loads are shown in the Table 2

Table 2 Parasitic loads of fresh *E. sardella* during cold, dry and rainy seasons

<table>
<thead>
<tr>
<th>Season</th>
<th>Cestoda</th>
<th>Nematoda</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold season</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dry season</td>
<td>40</td>
<td>6</td>
<td>46</td>
</tr>
<tr>
<td>Rainy season</td>
<td>25</td>
<td>3</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>9</td>
<td>74</td>
</tr>
</tbody>
</table>

Out of the two hundred sampled fish per season, no parasites were observed during the cold season. Instead, parasites were observed during the dry and the rainy seasons. A total of 46 parasites were observed during the dry season and a total of 28 parasites were observed during the rainy season. Out of the 46 parasites observed during the dry season, 40 parasites were cestoda and 6 were nematoda. During the rainy season, 25 parasites were cestoda and 3 were nematoda. This entails that 86.96% and 13.04% of the recovered parasites during the dry season were cestoda and nematoda respectively. During the rainy season, cestoda comprised of 89.21% and nematoda comprised of 10.79% of the total for the season. A total of 65 cestoda and 9 nematoda were recovered during all
the three seasons. Therefore, 74 parasites were observed in total over the study period. The analysis of variance ($F$ test) showed that the effect of seasons on the prevalence of the parasites was significant, $F (2,597) = 24.04$, ($p<0.05$). The prevalence rates of the observed parasites during the three seasons are shown in the Figure 3.

![Figure 3](image3.png)

Figure 3 The prevalence rates of parasites during the cold, dry and rainy seasons

The lowest prevalence rate was observed during the cold season. The prevalence rate for the cold season was 0% for the parasites targeted by the study. On the other hand, the highest prevalence rate of 21% was noted during the dry season indicating high infection rate during that time of the year. The rainy season registered a prevalence rate of 13%. This indicates that parasites were also observed during the rainy season in fresh *E. sardella* species. The seasonal changes also showed variations in the mean intensity of the parasites in fresh *E. sardella*. Figure 4 shows the mean intensities of the parasites during the study period.

![Figure 4](image4.png)

Figure 4 The mean intensity of parasites during the cold, dry and rainy seasons

The cold season had mean intensity of 0 while the dry season had a mean intensity of 1.1. The rainy season had a mean intensity of 1.08 which is almost the same as the dry season. Finally, multiple comparisons tests using the Tukey’s HSD and Bonferroni criteria for significance indicated that the differences of the parasitic loads among the seasons were statistically significant. The pair-wise comparisons of the parasitic loads were significant ($p<0.05$).
3 Discussion

The study indicated that seasonal variations had an effect on parasitism. Although the sample size was limited, the total number of parasites, prevalence rate and mean intensity varied across the three seasons of the study. The results observed in the study indicated that the cold season registered the lowest number of parasites while the dry season registered the highest number of parasites. The observations agreed with the findings of Ali et al. (2010) and Ahmad and Ahmad (2013). They reported similar results of higher prevalence rate in summer and lowest in winter. Ahmad and Ahmad (2013) reported highest infection levels in summer months and least infection levels in winter months for helminths parasites in Schizothorax species (S. plagiostomus, S. labiatus, S. ecosinus, S. curvifrons) in Shallabugh Wetland. Khurshid and Ahmad (2012) also observed higher prevalence rate of fish parasites in summer and least in winter in some species of Schizothorax from River Sindh in their study. Koyun (2012) supported the trend that some species of helminths parasites had highest prevalence during different seasons of the year. Fish are hosts to different parasites whose diversity, strategy and abundance depends on several factors including seasonality (Violente-Gonzalez et al. 2008; Neves et al. 2013) while parasites also respond to the seasonal changes of the environment (Yufa and Tingbao, 2011). In contrast, Khanum et al. (2011) reported that parasites were more abundant during the rainy season and lowest during the winter season. Sinare et al. (2016), observed a higher prevalence rate in rainy season as compared to the dry season. According to Poulin (2004) biotic factors such as body size of the host, population density, social behaviours, lifespan, biogeography and diet, and abiotic factors such as temperature, size and geographical range are responsible for the changes in the prevalence and mean intensity of the parasites. Iyaji et al. (2009) observed that as much as the chemical and the physical factors affect the cycles of the organisms, the biotic factors were the ones that mainly affected the prevalence and the abundance of the parasites. According to Khanum et al. (2011), the lower rate of parasite infection during the cold season could be due to reduced feeding tendency of the host fish. On the other hand, Ali et al. (2010) observed that during summer and autumn, the higher incidence could be due to the rise in the temperature that resulted in favouring the development of the larvae in the secondary host. This suggests that the seasonal fluctuations of the parasites may be due to temperature changes, availability of the intermediate host and the host feeding behaviours. In the winter, the low prevalence rates were due to low availability consumption of the intermediate hosts (Ali et al., 2010). Additionally, Dash (2015) highlighted that most parasites had seasonal cycles that were definite and were influenced by the seasonal changes that affected the environment and the physiology of the host fish. Observations by Ibiwoye et al. (2004) revealed that during the dry season evaporation led to reduced water volume, which in turn, resulted in the contraction of the habitat thereby increasing the densities of the host and the parasites. Consequently it resulted in more contact between the host and the parasite. Therefore this could result in the higher infection during the dry season. The increase of the parasites during the rainy season was attributed to rainfall, flood and reduced immunity of the hosts and prevalence of the insects that had an impact on the water bodies (Nhiwatiwa et al., 2009; Khanum et al., 2011). Similarly, the exchange of materials between the terrestrial and the aquatic ecosystems affect the quantities of substances and physical chemical aspects of the water bodies (Affonso et al., 2011). Sumuduni et al. (2015) suggested that slow moving fish were more susceptible to parasites than the fast moving fish while Munoz and Cribb (2005) reported that larger hosts had higher parasites load than the smaller hosts. Therefore by taking in more food and having more space, chances of infection were also high.

Koiri and Roy (2016) reported significant differences in the helminths (cestodes, nematodes and trematodes) loads during different seasons of their study. They observed the highest number of cestodes, followed by nematodes and lastly the trematodes. Bhure et al (2016) also observed highest number in cestodes in Channa punctatus species. On the contrary, Khurshid and Ahmad (2012) recorded the highest number of trematodes followed by cestodes with no nematodes. This suggests that different attributes could favour different parasite species proliferation. Maguza-Tembo and Mfitilodze (2008) observed the highest number of cestodes in the buccal cavity and coelomic cavity of Clarias gariepinus than in the other species of fish under study. This suggests that some species of fish could be vulnerable to some specific species of parasites. Dash et al. (2015) suggest that this could be due to host specificity nature of the species of parasites while Koiri and Roy (2017) maintain that seasons interfere with the physiology and ecology of the fish and in turn it has influence over the rate of parasitic infections.
The recruitment process of the parasitic fauna of *E. sardella* in the study was dominated by cestodes. According to Rusuwa et al. (2014), the cestodes were identified as *Ligula intestinalis*. The present study indicated that *Ligula intestinalis* (cestodes) and *Camallanus sp.* (nematodes) identified in the fresh *E. sardella* were common during the dry season and the rainy season. The cold season registered no prevalence of the parasites. Barson and Marshall (2003) observed the prevalence of *L. intestinalis* in *Barbus paludinosus* with no clear seasonal variations but suggested that the dry season had an effect on the prevalence. This is in agreement with the results of the present study which show no prevalence of parasites during the cold season and higher prevalence during the dry season. In contrast to the findings of the study, Anarse et al. (2011) reported no prevalence of cestodes during the rainy season but prevalence in the cold season and summer with the highest prevalence rate. Wetzel (2001) attributes the prevalence of *L. Intestinalis* to an increase in number of copepods which are numerous in stagnant than in running water bodies while Anarse et al. (2011) attributes the prevalence to temperatures that facilitate the hatching of the eggs. Copepods are intermediate hosts of *L. intestinalis* (Piasecki et al., 2004) while the larvae of *Camallanus sp.* are secreted into the water with the fish faeces and ingested by copepods or other crustaceans which are eaten by the other fish hosts where the larvae develops into a full adult (Yanong, 2006). On the other hand, Dejen et al. (2006) reported that during the rainy season the infection reduced due to increased turbidity that decreased the feeding efficiency on the zooplankton. They observed that the infection rate in *Barbus humilis* was high in the clear water than in the shallow turbid water. This implies that habitat changes have some significant effect on the infection rate of the fish. This could be the case because the piscivorous birds could predate more efficiently on the fish in shallow and clear waters than in the turbid and deep waters. Sharma (2016) reported the prevalence of three cestodes parasites in the intestines of freshwater fish, *Channa punctatus* with the highest infection in summer season, followed by winter and lowest in monsoon. Rusuwa et al. (2014) reported the infection of *L. intestinalis* throughout the year. This is contrary with the findings of this study where there is no infection observed during the cold season. *E. sardella* is an endemic species in Lake Malawi and breeds (spawns) throughout the year (Morioka and Kaunda, 2004). Therefore the difference between the findings of this study and that of Rusuwa et al. (2014) on the prevalence of *L. intestinalis* during the cold season could emanate from other factors like; feeding, habitat type, presence of the intermediate hosts and other environmental factors, such as temperatures, turbidity and depth of the water body. The study showed that fish were infected with large numbers of parasites in the latter part of the cold season to end of the dry season. Since most helminths parasites utilize the food chain for their transmission (Dejen et al. 2006), the differences in the prevalence rate of the parasites could be due to difference in habitat, food supply for both the intermediate host and aquatic piscivorous birds that play an important role in the completion of the life cycle of the parasites (Ibrahim and Soliman, 2010). According to Piasecki et al. (2004), the main food of *E. sardella* in the lake are zooplankton, which act as intermediate hosts of *L. intestinalis*. Proceroid larvae in copepod develop into plerocercoid in the fish abdominal cavity (Loot et al., 2006). Therefore the fluctuations in the abundance of the zooplankton could also affect the prevalence rate of the *L. intestinalis* during the different seasons of the year among other factors. The fact that the study reveals the presence of *Ligula intestinalis* should be a cause of concern in public health because it may result in the prevalence of ligulosis in humans. There are reports of human infestation with *Ligula intestinalis* (Barson and Marshall, 2003; Úrdes and Hangan, 2013). This entails that if *E. sardella* is not handled properly, humans could be affected by the parasite. However, Ljubojevic et al. (2015) emphasized that the most important risk factor for the spread of the fish-borne parasitic zoonoses is the consumption of raw and undercooked fish meat. This suggests that proper preparation of *E. sardella* which host the parasite could help avert the dangers posed by the parasite.

### 4 Conclusion and Recommendations

The study rejected the null hypothesis on the seasonal parasitic load in *E. sardella*. The results indicated seasonal variations in the prevalence rates of the parasites in the fresh *E. sardella*. The dry season had the highest prevalence rate followed by the rainy season and lastly the cold season. The study indicated a strong interaction of the parasites with the environment, the host fish and other factors that have some bearing on the cycles of the parasites. Finally, the study indicated that water parameters, variations in fish behaviour, seasonal variations and effective parasite stages were some of the contributing factors to the proliferation of the parasites. This study
recommends the need to investigate the specific factors that have a major influence on the occurrence of parasites in *E. sardella* in different locations during different seasons of the year. Therefore, further studies on different ecological aspects in different locations are essential.

**Authors’ contributions**

The authors were involved in the design of the research, analysis and interpretation of the data as well as the critical revision for important intellectual content and final approval of the version to be published. Therefore the authors are accountable for the aspects of the work published. All authors read and approved the final manuscript.

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