

Prevalence Of Parasites In Freshwater Fishes In The Southern Part Of Ligawasan Marsh, Philippines

Sarip Tarita Ampatuan- Ali
saripnautapma@gmail.com

Assistant Professor, Cotabato City State Polytechnic College
09218285290/09666841963

Abstract. Ligawasan Marsh, the largest river basin in Mindanao is home of common fishes such as dalag, pupuyo, hito, tilapia and gourami. Fish parasites pose a great threat to freshwater fishes to public health and economy of an area. This study aims to examine the Prevalence of Parasites in Freshwater Fishes in the Southern Part of Ligawasan Marsh; Endoparasites in the representative species; Mean intensity and abundance infection of the Endoparasites. The study utilized necropsy, dissection and microscopy method to examine Endoparasites. Trematodes *Clinostomum* sp, suspected *Camallanus* sp., a *Neocamallanus* sp. and a member of Order *Spirurida*, all nematodes were recovered. The study yielded that Fish parasites in *Channa striata* *Anabas testudineus* had prevalence in *Kabuntalan* and *Datu Piang*. Nine Acanthocephalan recovered in *Oreochromis niloticus* had also prevalence rate. Suspected trematode cysts and eggs were also recovered in *Trichopodus trichopterus* and *Clarias macrocephalus*. Thus, it is concluded that there is prevalence of nematodes and trematodes species in *C. striata*, *A. testudineus*, *O. niloticus*, *C. macrocephalus* and *T. trichopterus* The *Camallanus* sp and *Neocamallanus* sp, both common aquarium parasites are first found in the Southern part of Ligawasan Marsh.

Key Words: Prevalence of Parasites, Freshwater Fishes, Necropsy, Dissection and Microscopy, Ligawasan Marsh, Philippines

1 Introduction

Ligawasan Marsh is located in South Central Mindanao, and is recorded as the largest swamp and marsh area on the island consisting of two adjoining marshy basins-Ligawasan and Libungan Marsh where most of the population in ARMM fish for food and source of income.

There are 344 species of freshwater fishes in the country, including the native and introduced fish species. Maguindanao had their shares in the annual increase of fish production. Nineteen point two (19.2) per cent are acquired through inland municipal fishing. Tilapia, milkfish (bangus), carp, freshwater catfish (hito), mudfish (dalag), guorami and freshwater goby (biya) were just the common freshwater species caught by inland fishermen (BAS, Phil 2012).

Local studies show the prevalence and occurrences of the endo- and ectoparasites in Ligawasan Marsh. A Study made by Salcedo et al (2009) on fishes sold at the Kabacan Market to which one of the sources is the northern part of Ligawasan Marsh. showed a frequency rate of parasitic occurrence of 1.87% (Ligawasan Marsh) and 6.97% from Pulangi River which geographically is connected to Ligawasan Marsh. Other local studies previously done on fishes examined coming from Kabacan Public Market recorded absence of trematodes and nematodes

and prevalence of tapeworms (Fernando & Gonzaga 2004). Same results were recorded on the study done in Midsayap Public Market. Among the three fish species examined, cestodes (tapeworms) were the only collected fish parasites.

Recently, Fortínez, (2016) conducted a study on Kuyapon side of Ligawasan Marsh and revealed fecal contamination as well as presence of zoonotic nematodes such as *Trichuris* sp and *Ascaris* sp. A trematode species *Clinostomium philippinensis* and *Ascaris testudines* were recorded to be infesting fish species of *Channa striata*.

All of these studies are indicative of greater possibilities of parasite infestations in fishes in the Southern area of Ligawasan Marsh, which has been unstudied so far.

The general objective of this study was to determine the prevalence of the fish parasites in selected areas of the southern portion of Ligawasan Marsh. Specifically, this study determined prevalence of endoparasites, mean intensity of infection, and abundance of the endoparasites.

Studies on fish parasites have long been contributing worldwide to the current knowledge of the diversity and biology of the ecto- and endoparasites, protists and metazoans, of freshwater fishes. Numerous studies had already been conducted in the different lakes and bodies of water all over the Philippines, including Ligawasan Marsh. High prevalence and occurrences of parasites had been recorded in the northern part - Pagalungan side of Ligawasan marsh. However, there is no data gathered yet from the southern part of Ligawasan Marsh.

In this study, the level of infestation and occurrences of parasites in the two sampling sites- municipalities of Kabuntalan and Datu Piang, all under ARMM were taken into account. Expectedly the outputs of this study would educate the local residents on the implications on this health presence of zoonotic parasites, in their local fish commodities, on their health. Moreover, this study also served as the basis for the Local Government of Kabuntalan and Datu Piang to come up with a resolution to preserve the natural environment of Ligawasan Marsh by educating the residents on proper waste disposal and for the Department of Health to spread awareness campaign as to the impact of parasites on health issues and importance of proper sanitary practices. Also by knowing the level of infection of the parasites on the health of the fish, something can be done to address them, thus increase the income of the fishfarming communities in that part of Ligawasan Marsh. The study also integrated new findings regarding parasites in Ligawasan Marsh with some information and references gained from prior studies in order to add to the understanding of the parasite existence as well as on its ecological relationship and importance.

This study was conducted purposely to examine and assess the existence of zoonotic endoparasites in freshwater fishes in the southern part of Ligawasan Marsh. However was only limited to the occurrence and incidence of the endoparasitic infestation in freshwater fishes on species of *Channa striata* (dalag), *Oreochromis niloticus* (tilapia), *Clarias macrocephalus* (hito), *Trichopodus trichopterus* (guorami), and *Anabas testudineus* (pupuyo) which are the most consumed fishes in the area.

2 Methodology

Research Design

The research design used in the study was survey. Experimental processes were done in standard laboratory procedures.

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Locale of the Study

Ligawasan Marsh is an extensive swamp region with a circumference of 25mi (40km) long and 20mi (30 km wide along the Pulangi River in South Central Mindanao, Philippines. It is a vast complex of river channels, small freshwater lakes and ponds, extensive freshwater marshes. It is potentially diversified in freshwater fishes of great economic values and importance.

Collection of Samples and Identifications

The two ARMM municipalities namely, the municipalities of Kabuntalan, and Datu Piang served as sampling sites where fish samples were collected.

Fresh fish were purchased from the contracted local fishermen, on a daily basis.

The purchased live fishes were placed in storage ice chest, transported to the laboratory of the Department of Biology in University of Southern Mindanao, identified using pictorial keys, recorded length in centimeter (cm) and weighed in gram(gm), photodocumented and were processed for parasitic examination. The total purchased of each type of fishes were approximately thirty (30).

Sample Preparation, Identification and Fixation of the Parasites

A. Internal Examination

Each fish examined was laid on one side and was opened from the anal region to the mouth via ventral incision. A pair of blunt/sharp scissors was used, with the blunt end going into the body cavity to minimize damage to the internal organs. The internal organs as well as the entire body cavity and intestine were inspected for the parasites. The collected suspected parasites were soaked in 9% saline solution.

B. Fixation

In this study, the parasites extracted from the fishes were fixed with 70% ethanol and/or formalin.

B.1. Staining for Nematodes

The nematodes were fixed in glacial acetic acid overnight and stored in 70% ethanol with 10% glycerin. Nematode morphologies were studied in temporary mounts by then removing from alcohol and clearing with glycerin. Before returning the nematodes to storage, it was rinsed away with clearing fluid using 70% ethanol. Staining was done for detailed identification.

B.2. Staining for Trematodes and Cestodes

The trematodes and suspected trematode metacercariae were released, pressed between glass slides and stored in 70% ethanol.

Flukes (trematodes) were stained using acetocarmine staining technique. Metacercariae were stored in 70% ethanol directly. Flatworms were usually stained and mounted as permanent mounts.

B.3. Staining for Acanthocephalans

Acanthocephalans were placed into 70% ethyl solution until the staining procedure. The specimen were stained using the acetocarmine staining procedure. The specimen was placed in an epindomorph tube. By pipetting solutions in and out of the vial containing the parasites, staining was accomplished.

The staining technique used was the Semichon's Acetocarmine Technique.

C. Identification of Parasites

The parasite identification was done using identification dichotomous keys and pictorial guides. The pre-identified parasites were then confirmed by the experts of the USM Biological Departments of the College of Arts and Sciences.

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Analysis for the Degree of Parasitic Infestation in the Freshwater Fishes

The following terminologies formulae were used to describe the degree of parasitic infestation in freshwater fishes in this study, based on the formula of Miah, et al 2013 and Margolis, et al 1982.

Prevalence is equal to the number of individuals of a host species infected with a particular parasite species divided by the number of host examined.

$$\text{Prevalence rate} = \frac{\text{No. of Host infected}}{\text{Total No. of Host Examined}} \times 100$$

Mean Intensity is the total number of a particular parasite species in a sample of a host species divided by the number of infected individuals of the host species in the sample.

$$\text{Intensity of Infection} = \frac{\text{No. of Parasites Collected in a Sample}}{\text{No. of Infected Host}}$$

Relative Density or Abundance is equal to the total number of individuals of a particular parasite species in a sample of hosts divided by the total number of individuals of the host species (both infected and uninfected) in the sample.

$$\text{Density of Infection} = \frac{\text{No. of Parasites Collected in a Sample}}{\text{Total Host Examined}}$$

Data was analyzed using the formula based on the formula of Miah, et al 2013 and Margolis, et al 1982 in analyzing the degree of parasitism in an organism.

3 Results and Discussion

Endoparasites in the Five Representative Fishes

Fishes are intermediate hosts of transient forms of a vast number of parasites (Amar, 2014). The muscle of fishes are habitat of larvae whereas adult parasites very often occur in their gastrointestinal tract, liver and other internal organs as well as on their skin. The most dangerous are those which, when ingested with courses which include fish meat, may undergo developmental stages in the internal organs or muscles of humans, hence may include and influence onset of life-threatening human diseases.

Table 1 shows the parasites present in the species of *Channa striata*, *O. niloticus*, *C. macrocephalus*, *T. trichopterus* and *A. testudineus* in the different sampling sites.

Table 1: Endoparasites in freshwater fishes in Ligawasan Marsh

Endoparasites	Freshwater fishes									
	C. striata		A. testudineus		O. niloticus		C. macrocephalus		T. Testudineus	
	Kab	DP	Kab	DP	Kab	DP	Kab	DP	Kab	DP
Trematode										
<i>Clinostomum</i> sp.	√	√	√	√	–	–	–	–	–	–
<i>Posthodiplostomum</i> sp.	–	√	√	√	–	–	–	–	–	–
Un. Trematode 1	–	√	√	√	–	–	–	–	√	–

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Un. Trematode 2	-	-	-	-	-	-	-	√	√	√
Un. Trematode 3	-	-	-	-	-	-	-	-	-	√
Nematode										
<i>Camallanus</i> sp.	-	-	-	√	-	-	-	-	-	-
<i>Neocamallanus</i> sp.	√	√	-	√	-	-	-	-	-	-
Order Spirurida	-	-	-	√	-	-	-	-	-	-
Acanthocephalan	-	-	-	-	√	√	-	-	-	-
Unidentified										
Eggs 1	-	-	√	√	-	-	-	-	-	-
Eggs 2	-	-	-	-	-	-	-	√	-	-
Eggs 3	-	-	-	-	-	-	-	-	-	√

Legend: (√) means presence of parasites and (-) negative for the parasite. Kab = Kabuntalan, DP=Datu Piang

There were 7 species of parasites identified. Of them 2 were trematodes, 2 were nematodes and 1 acanthocephalan. In addition, there were 3 unidentified parasites including suspected trematode, suspected nematode and some suspected parasite eggs.

Prevalence, Mean Intensity and Percentage Abundance of Parasites in Each of the Representative Species of Fish

Table 2 shows parasite prevalence in both Kabuntalan and Datu Piang sites. Prevalence is the percentile representation of infected hosts divided by hosts examined multiplied by 100.

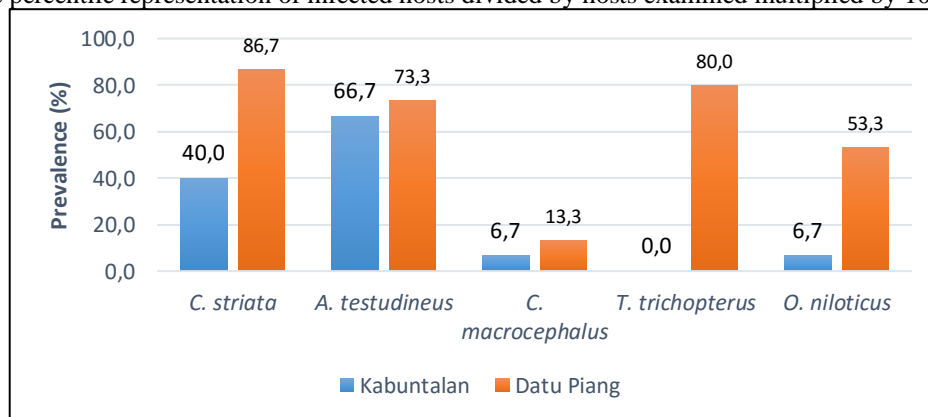


Table 2. The graph showing the prevalence rate of parasites in the fish representatives

It indicates *C. striata*, *A. testudineus*, *T. trichopterus* and *O. niloticus* from Datu Piang and *C. striata* and *A. testudineus* from Kabuntalan exceeded more than 50 percent prevalence. It is also remarkable to note the high prevalence rate of the parasites in four (4) of the fishes examined in Datu Piang as compared to low prevalence of parasites in the fishes in the Kabuntalan area except for *A. testudineus*

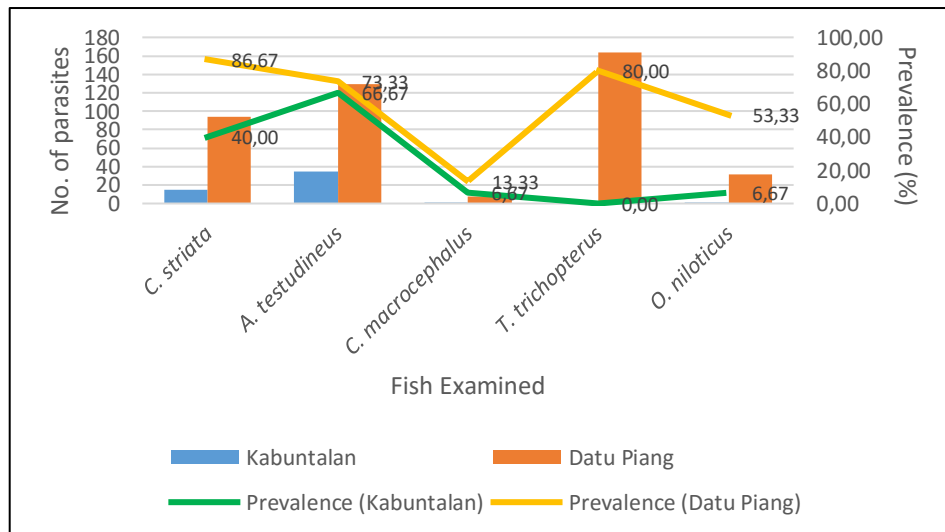


Table 3. Graph showing both the abundance of parasites and its prevalence in the common fishes in Ligawasan Marsh

Abundance of parasites are determined by the number of parasites collected in a sample over the total number of fish examined. Table 3 illustrates the high abundance rate of *C. striata* in terms of parasites collected in Datu Piang site, followed by *T. trichopterus* and *A. testudineus*. There was a tremendous drop in the graph as it illustrates the lowest abundance in *C. macrocephalus* as well as the absence of collected parasites in the *T. trichopodus* and *O. niloticus* in *C. punctatus* in Kabuntalan site.

In the study of Miah, et al., 2013, *C. punctatus* was infected by 7 species of parasites. Of these, four were trematodes (*Genarchlopsiis bangladnesis*, *Allogamtitrema attu*, *Phyllodistomum* sp., *Neoppecocline saharanpuriensis*); two were nematodes (*Ascaridia* sp., *Procamallanus* sp.) and one was an Acanthocephalan (*Pallisentis nandai*). The findings of this study is almost the same with the findings of Miah, et al., 2013. Furthermore, in this study there were five trematodes: *Clinostomum* sp., *Posthodiplostomum* sp. and three unidentified trematodes; three nematodes: *Camallanus* sp and *Neocamallanus* sp. and a member of Order *Spirurida* as well as 1 Acanthocephalan were found. But, the trematodes and nematodes found in *C. striata* were also abundant in *A. testudineus* of this study and Order *Spirurida* sp was only found in the *A. testudineus*. In addition, unidentified trematodes of *Clinostomum* sp. and *E. heterostomum* resemble those recovered in *T. trichopterus* but Acanthocephalan sp. were only present in *O. niloticus* and were not found in *C. striata* and *A. testudineus*. However the dissimilarities in fish hosts of the mentioned trematodes, nematodes and acanthocephalan in both studies may be attributed to the factors such as feeding habits of the hosts, water quality in terms of habitat and availability of infected intermediate hosts (Kundu & Bhuiyan et al., 2016).

The findings of this study is consistent with the previous studies, that recovered common intestinal nematode *Camallanus anabantis* from *A. testudineus* (Mandal et al., 2016). The intestinal parasites was also reported to be harbored by many other indigenous fishes such as *C. striata* and *T. trichoptrus* and the *Puntius* sp. only caught in Datu Piang site.

The relative density of infection (Table 4) derived from the number of collected parasites in a sample over the total host examined reflects a higher account on *T. trichopterus* in Datu Piang site followed by *A. testudineus*, still in Datu Piang site. All in all, the Datu Piang

site shows higher densities of parasitic infection, which is in fact more than a half of the densities of infection in the Kabuntalan site.

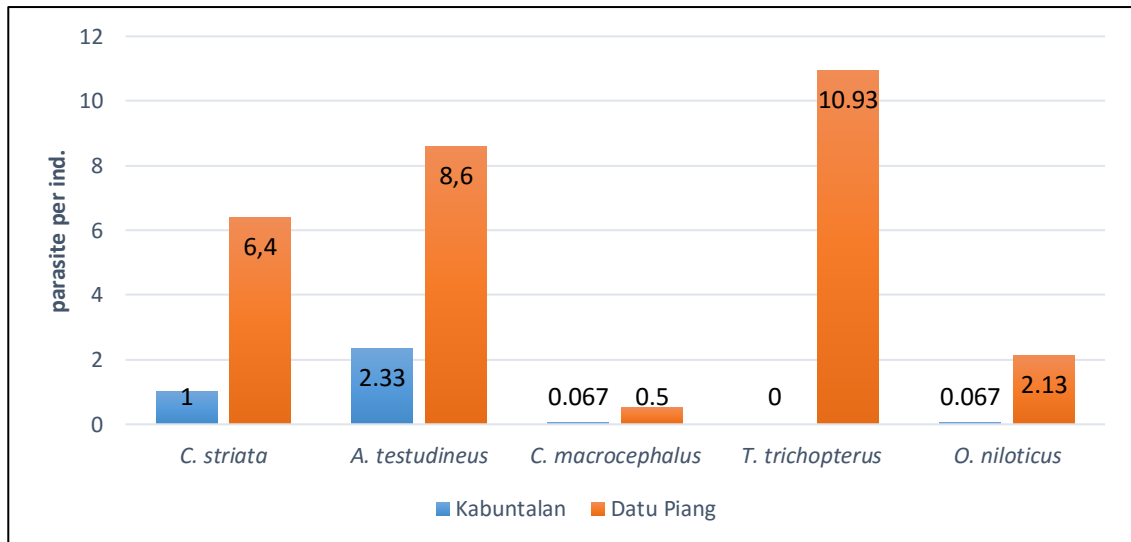


Table 4. A graph showing the relative density of parasites in freshwater fishes in Ligawasan Marsh.

During the study of Dhole et al., 2010 in India, they observed variations in parasitic infection with sampling stations, which is quite similar in this study, Kabuntalan site and Datu Piang showed big difference in terms of intensity and density of parasitic infection as reflected in the figures.

In the study of Dhole et al. in 2010, *Mastacembalus armatus* a fish species in India, constantly showed higher parasitic infection. In comparison, the fish they used, and the *T. trichopterus* used in the present study, were heavily infected with parasites in one site but showed absence of parasites in the other site. Moreover, in this study the intensity of infection of parasites was so high in the fishes caught in the Datu Piang site. Some workers concluded that fish from more polluted waters tend to harbor more parasites than from less polluted area. Looking at the areas of Kabuntalan and Datu Piang in the map (Fig. 17) Barangay Kalipapa (yellow) in Datu Piang has lesser area and the risk to pollution is higher than Brgy Butiren (pink) in Kabuntalan. Furthermore, according to Dhole' et al., 2010 as cited from Polanski (1961a) the main factors determining the variety of parasite fauna as well as the intensity and incidence of infection can be summarized as follows: the diet of the host, life span of the host, the mobility of the host throughout its life including the variety of habitat it encounters, it's population density and the size attained, where large host provides more habitats suitable for parasites than small ones. The last statement though was contradictory to the findings of this study where prevalence, intensity and abundance of parasites recorded were high in *A. testudineus* and *T. trichopterus* which were low *C. striata* and *C. macrocephalus*.

Figure 16 shows that intensity of parasitic infection is consistently higher in Datu Piang in all fish species than intensity of infection in Kabuntalan. Higher infection rate must be attributed to the time and seasonal factors during the time of catching. In the study made by Mgwede and

Msiska (2018) on seasonal occurrence of *Camallanus* sp., Khursid and Ahmad (2012) attribute the severity of the parasite infection to climate of a particular area. In the present study, the time of catchment of fishes in Kabuntalan and Datu Piang sites varies.

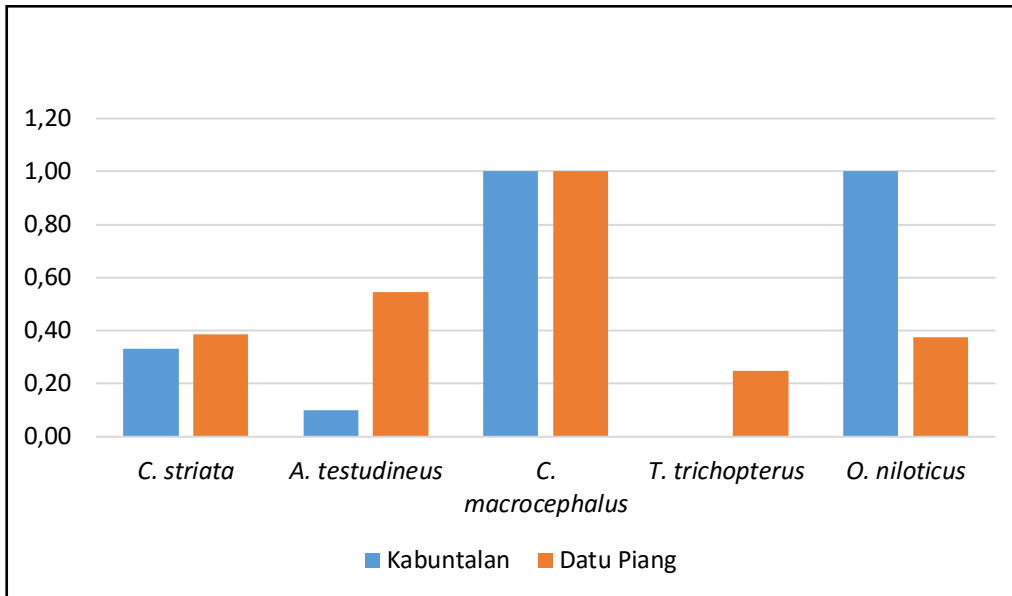


Table 5. A graph showing the intensity of infection in each representative species in Kabuntalan and Datu Piang area.

4 Conclusion

A total of one hundred fifty (150) fish samples were examined, from five common fish species, namely *Channa striata*, *Anabas testudineus*, *Oreochromis niloticus*, *Clarias macrocephalus* and *Trichopodus trichopterus*.

The two municipal sites of Ligawasan Marsh - Kabuntalan and Datu Piang were found positive for the presence of trematodes such as *Clinostomum* sp., *Posthodiplostomum* sp. and three unidentified trematodes. Nematodes were also present in both sites such as *Camallanus* sp. and *Neocamallanus* sp., and a member of Order Spirurida. There were numerous unidentified eggs recovered in both sites, and an Acanthocephalan in the Datu Piang.

The prevalence of parasite infestation in *C. striata* is 40% in Kabuntalan while it is 86.67% in Datu Piang; *A. testudineus* has 66.67% in Kabuntalan and 86.67% in Datu Piang; *C. macrocephalus* has 6.67% & 13.33% respectively; *O. niloticus* has 6.67% & 53.33%; and *T. trichopterus* has 0% but 80% in Datu Piang.

It can then be concluded that there is prevalence of nematodes and trematodes species in *C. striata*, *A. testudineus*, *O. niloticus*, *C. macrocephalus* and *T. trichopterus*. The *Camallanus* sp and *Neocamallanus* sp, both common aquarium parasites are believed to be the first to be found in the Southern part of Ligawasan Marsh.

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