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Infection of Acanthocephalan Parasite *Acanthogyrus* sp. in Nile Tilapia *Oreochromis niloticus* Collected from Agusan Marsh, Philippines

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ABSTRACT

Infection of the acanthocephalan parasite *Acanthogyrus* sp. in Nile Tilapia *Oreochromis niloticus* from Agusan Marsh, Philippines, was carried out last December 2019 – January 2020 in a semi-purposive sampling in municipalities surrounding Agusan Marsh, Caraga Region, Philippines. A total of 180 *O. niloticus* individuals were collected from Bunawan, San Francisco, Rosario, Talacogon, La Paz, and Loreto. Prevalence of infection among various sampling sites were significant ($p<0.05$). Acanthocephalan infection, particularly *Acanthogyrus* sp. in San Francisco, was reported highest 76%, followed by Loreto (53.3%), Talacogon (50%), La Paz (40%) and Rosario (30%) while Bunawan (20%) recorded lowest. Juveniles exhibited low prevalence (23.9%), while adults recorded highest 71.1%; ($p < 0.05$). Moreover, the prevalence rate was higher in females (49%) than in males (39.7%) but not significant ($p > 0.05$). This study confirms that *O. niloticus* in Agusan Marsh are definitive hosts of *Acanthogyrus* sp. Moreover, results showed that *Acanthogyrus* sp. had a highly aggregated distribution pattern in *O. niloticus* ($p <0.05$; $k = 0.43$, variance = 16.99 > mean =2.49). The aggregated distribution of the parasite has implication on the regulation of host population. This study provides baseline information for future investigation on the ecology and infection dynamics of acanthocephalans in fish in the area.

Keywords: *Acanthogyrus* sp., Fish parasite, *Oreochromis niloticus*, Agusan Marsh

1 Introduction

Agusan Marsh is considered a Philippine treasure in terms of biodiversity as this habitat harbors unique species of wildlife. The marsh has international significance in providing ecosystem function and services such as water cycling, and carbon sequestration, regulating local climate, while providing an economic resource to local communities (RAMSAR 2003, and Relox and Camino 2018). Nonetheless, Agusan Marsh is also in threat of anthropogenic activities such as pollution, overutilization of natural resources, and changes in natural flood regimes, increasing its vulnerability to invasion and dominance of introduced species (Junk 2002, and Jumawan and Seronay 2017). Habitat alterations in the environment may result in the emergence of infectious disease which contributes to a significant impact for the transmission of parasitic diseases between animals and humans (Lacey et al. 2003, and Jones et al. 2008).

Acanthocephala is a monophyletic group of spiny-headed parasitic worms that play a vital role in shaping ecosystems by regulating their hosts' community structure (Thielen et al. 2004). They may impact the food web patterns and community composition of their hosts. Acanthocephalans respond to changes, especially in aquatic habitats, making them good bio-indicators of environmental degradation (Sures 2003, and Paller et al. 2016). They are considered a highly successful group of parasites, for they infect all vertebrates, including humans (Amin 1998, and Kennedy 2006). Studies have shown their potential as agents of disease upon reaching epizootic levels and as biomonitors of environmental changes. Acanthocephalans are often studied to demonstrate several aspects of parasite biology to understand the host-parasite relationship in an ecological and evolutionary perspective as well as its implication to public health (De la Cruz et al. 2013). Information on freshwater fish parasites in the Philippines is scarce, and no study was conducted on the occurrence of acanthocephalans in Agusan Marsh. The study aimed to determine the infection of acanthocephalan parasites in Nile tilapia and provide baseline information for future studies in the area.

2 Materials and Methods

Study area

Fish Collection and Processing

A total of 180 Nile tilapia *Oreochromis niloticus* were collected in Agusan Marsh, specifically from the municipalities of San Francisco, Talagocon, La Paz, Loreto, Bunawan and Rosario, Agusan del Sur, Caraga Region (Figure 1). The collection of fish was done once in each sampling site between December 2019 and January 2020, in a semi-purposive sampling considering the accessibility of the area and the presence of fresh *O. niloticus*. Stations were mapped out with Quantum GIS 3 version 2.18.23.

Thirty (30) samples were obtained from each sampling station. The samples were placed in an icebox and transported to the laboratory and processed immediately. The standard length (cm) and weight (g) of each fish were measured using a caliper and a digital weighing scale. The age of fish was allocated to three age classes based on body weight. Fish <10 g were assigned to juvenile age class; individuals weighing 10 to <25 g as sub-adult class; and individuals >25 g as adults (El-Sayed 2006, and Lim and Webster 2006). The prominent appearance of urogenital papillae for sex identification in *O. niloticus* was noted wherein males have tapered shape below the anus. In contrast, females have a more rounded shape with a triangular indentation in the center (Gómez-Márquez et al. 2008). The intestine was isolated into a Petri dish containing a physiological saline solution and examined for the presence of adult acanthocephalan parasite under a dissecting stereomicroscope (Leica EZ4 China) using the key to species of Amin (1998). The parasites were observed under a compound microscope for further analysis of morphological features and then photographed under scanning view (20x) using a digital camera (Canon A2300).

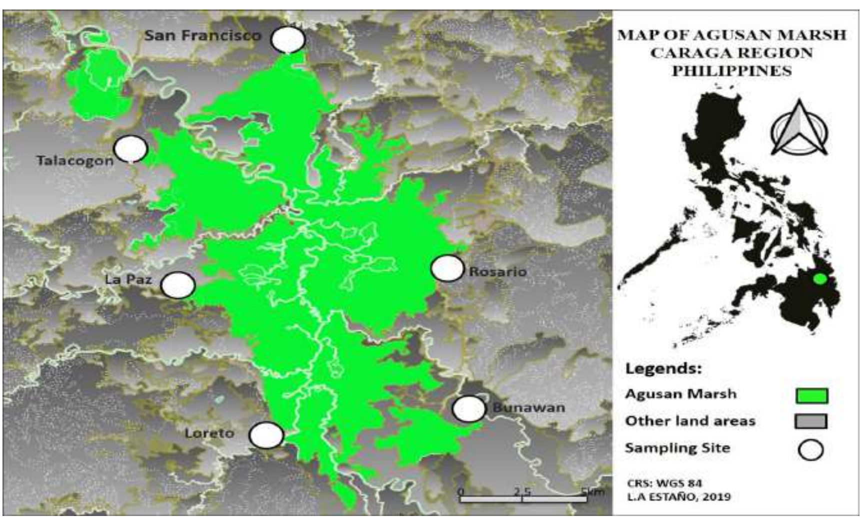

Figure 1. Map showing the sampling sites: Agusan Marsh, Agusan del Sur, Caraga Region, Philippines.

Statistical Analyses

The prevalence rate of acanthocephalan infection was computed by dividing the number of infected hosts by the total number of samples and multiplying with 100. Differences in the prevalence rate among age, sex, and location sites were analyzed using Chi-square of independence test. Moreover, acanthocephalan parasites mean intensity is the total number of parasites found in the infected host divided by the total number of examined samples. Nonparametric Kruskal–Wallis test was performed to determine differences of location sites and ages classes on acanthocephalan parasites intensity. Mann-Whitney U test was done for sex differences on acanthocephalan intensity.

Pearson correlation analysis was used to determine the association between parasite intensity, and fish length and weight. The distribution of the parasites in their fish hosts was characterized using goodness-of-fit, including Kolmogorov-Smirnov test and Shapiro-Wilk Test, and negative binomial distribution (NBD) models. Where appropriate, the k parameter of the negative binomial and was also interpreted to estimate the degree of aggregation. Moreover, the dataset of parasite intensity was examined using Poulin's discrepancy index of variance and mean (Poulin 1993).

3 Results and Discussion

Isolated acanthocephalan parasite

Laboratory analysis revealed that Nile tilapia individuals from the six sampling stations of Agusan Marsh harbor *Acanthogyrus* sp. This species is a member of phylum Acanthocephala-- endoparasitic worms with a characteristic retractile proboscis bearing rows of thorny hooks (Golvan and De Buron 1988). The most common characteristic of this acanthocephalan is the presence of an anterior protrudable proboscis that is usually covered with spiny hooks (Figure 2). The proboscis is used to pierce the final host's gut wall and holds it tightly to the intestinal wall of the host, allowing the parasite lodge to complete the life cycle (Robert et al. 2009).

Out of 180 *O. niloticus* individuals, 81 (45%) were found to be infected with *Acanthogyrus* sp. The distribution pattern of *Acanthogyrus* sp. in *O. niloticus* host followed a non-random pattern of dispersal ($p<0.001$). The prevalence rate of *Acanthogyrus* sp. in *O. niloticus* was observed highest in San Francisco (76.7 %), followed

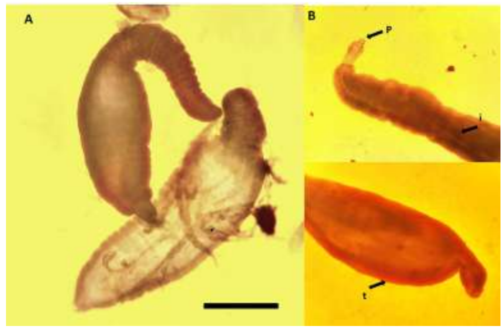

Figure 2. *Acanthogyrus* sp. infecting Nile tilapia in Agusan Marsh, Philippines. (20 x, scalebar = 1 mm). (A) Whole structure (B) Labelled parts: Globular proboscis (p) at anterior view, Intestinal tube (i) and Tegumental surface (t) at the body surface posterior view.

by Loreto (53.3 %), Talacogon (50%), La Paz (40%) and Rosario (30%) while Bunawan (20%) was recorded lowest. Likewise, results from the Chi square-independent test were found to be significant ($p<0.001$). The recorded infection of *Acanthogyrus* sp. in *O. niloticus* in Agusan Marsh may pose a possible transmission of the parasite to other fauna in the food web involving *O. niloticus*. Acanthocephalans, in general, are of less essential agents of disease while some species serve as indicators of the health of the ecosystem (Latham and Poulin 2002, and De la Cruz et al. 2013). Acanthocephalans have an increased capacity to accumulate heavy metals concentrations higher than in host tissues (Mehana et al. 2020). Paller et al. (2016), reported that the acanthocephalan parasite *Acanthogyrus* sp. was found inhabiting *O. niloticus* as biosink of Lead (Pb) contamination in Sampaloc Lake, Laguna, Philippines.

The variability of infection among sites (Figure 3) may indicate differences in water quality. Study of Sures (2003), Thielen et al. (2004), and Paller et al. (2016), reported that in the aquatic ecosystem, polluted with heavy metals and threatened inland water, *Acanthogyrus* sp. was recovered from Nile tilapia. Breward (1996) mentioned some of the anthropogenic activities in Agusan Marsh, such as unregulated small-scale artisanal mining, dumping of solid waste, and chemical waste from both agricultural and industrial activities done in the area. Roa et al. (2010), reported lead and mercury accumulation in some fish and aquatic plants in some areas downstream of Agusan Marsh.

While the prevalence rates of *Acanthogyrus* sp.

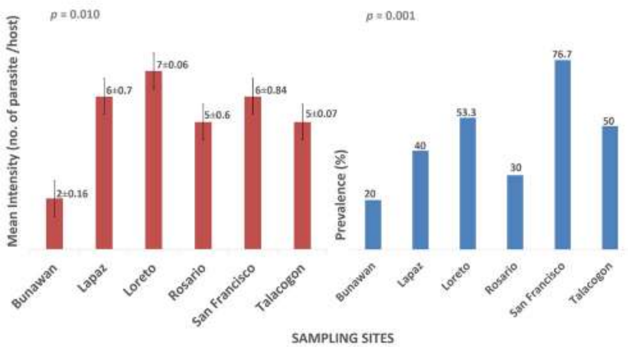

Figure 3. Intensity and prevalence of *Acanthogyrus* sp. in *O. niloticus* among various sites in Agusan Marsh, Philippines.

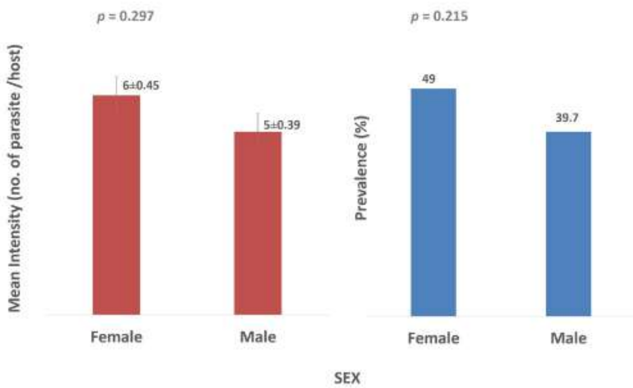

Figure 4. Intensity and prevalence of *Acanthogyrus* sp. in *O. niloticus* from Agusan Marsh, Philippines based on sexes.

infection between male and female *O. niloticus* are not statistically significant (Figure 4), females have a higher prevalence and mean intensity than males. Several studies reported the differences in host sex and parasitic loads in intensity and prevalence (Winternitz et al. 2012, Morrill et al. 2017, and Sures et al. 2017). The current findings indicate that the parasite is not selective of the sex of the *O. niloticus* as an intermediate host.

In terms of host maturity based on size (Figure 5), it is evident that there is a higher prevalence and intensity observed in adult fish (71.1%; 6±0.3), followed by sub-adults (44.8%; 5±0.4) and juveniles (23.9%; 3±0.3)(p=0.001). Adult fish hosts may also have a higher infection due to an assumed wider range for foraging and breeding purposes, which makes them more susceptible to acquiring infective stages of the parasite in the environment (Rascalou and Gourbiere 2015, and Chaisiri et al. 2015). Previous studies on acanthocephalan parasites by De la Cruz et al. (2013), indicate that the size-intensity profile is another factor that influenced the association between the host's size and degree of infection of *Acanthogyrus* sp. in Nile Tilapia in Sampaloc Lake. Correlation analysis showed a significant but weak positive relationship between intensity and length (r=0.32; $p < 0.001$) as well as the weight (r=0.30; $p < 0.001$). The result suggests that the parasite load increase with increasing size indicates that parasite appeared to aggregate in the larger fish host. Mature fish may encounter more intermediate hosts such as copepods and shrimps and may feed on these unselectively as part of their age-related diet preference. Additionally, it could be implied that parasite recruitment is evident during the juvenile stage of the fish. A greater number of adult parasites were observed as the fish became older and larger.

The distribution pattern of *Acanthogyrus* sp. in *O. niloticus* followed a non-random pattern of dispersal, which supports that the *Acanthogyrus* sp. in *O. niloticus* in Agusan Marsh follows the

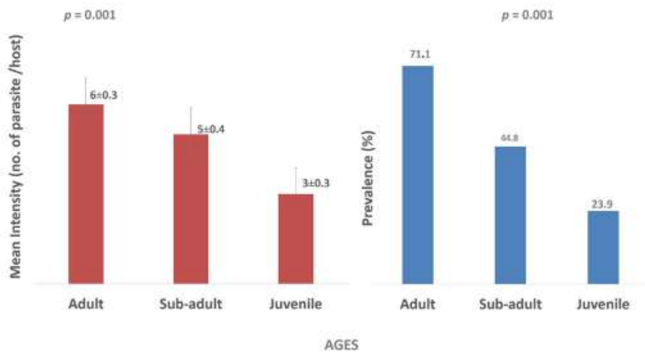

Figure 5. Intensity and prevalence of *Acanthogyrus* sp. in *O. niloticus* in Agusan Marsh, Philippines at varying stages of maturity.

aggregated distribution. The resulting variance of (16.99) was higher than the mean (2.49) this showed a significant departure from the uniform distribution. This result supports that the *Acanthogyrus sp*. in *O. niloticus* in Agusan Marsh follows the aggregated distribution. An aggregated distribution is indicated when the variance is significantly higher than the mean (Poulin 1993).

The left frequency distribution of *Acanthogyrus* sp. using the NBD model is characteristic of fish acanthocephalan populations that are overdispersed under most conditions (Kennedy 2006). Besides, only 45% of the fish samples were infected with *Acanthogyrus* sp. and among the infected hosts sub-population, most harbor few parasites while the remaining succumb to heavy infection. The low value of *k* also supports this distribution of parasites suggesting that the parasite follows the aggregated distribution (Figure 6). It is worth noting that highly aggregated, or overly-disperse parasites are known to stabilize population dynamics in which increased parasite burden usually results in increased morbidity and mortality for its hosts (Kennedy 2006, and Yakob et al. 2014).

The occurrence of acanthocephalan infection is an excellent indicator to monitor habitat status and ecosystem health in Agusan Marsh. However rare, the widespread transmission of the acanthocephalan

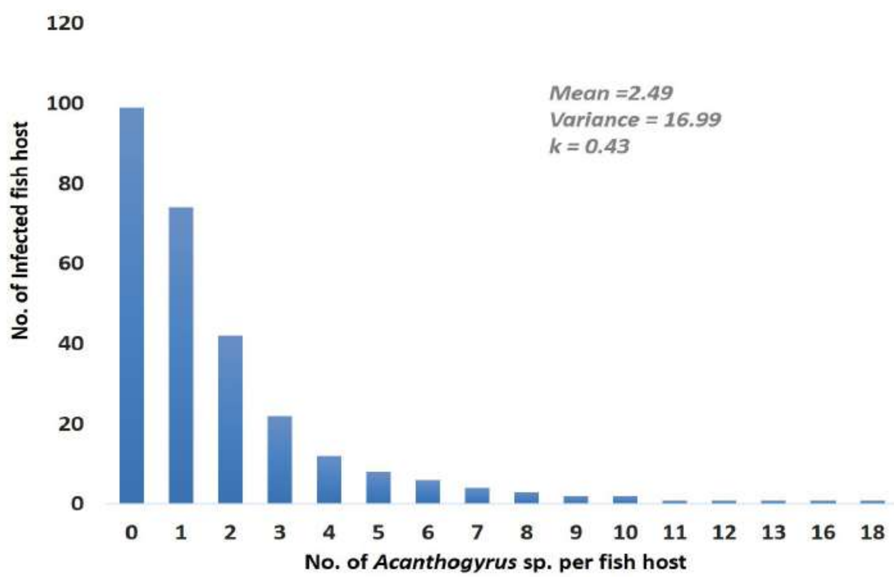

Figure 6. Frequency distribution of *Acanthogyrus* sp. in *O. niloticus* collected in Agusan Marsh, Philippines.

parasite may be possible in other animals and humans when raw, and semi-raw fishes are ingested (Schmidt 1971, Tada et al. 1983, and Haustein et al. 2010). However, no reports on human infection of this parasite were documented in the Philippines.

4 Conclusion and Recommendations

The present study provides essential information on the infection of *Acanthogyrus* sp. in *O. niloticus* in Agusan Marsh. Furthermore, the parasite has aggregated distribution in their fish host; the spatial distribution of the infective stage, host susceptibility, and age-factor influence the distribution pattern of the parasite. The study provides baseline evidence on the understanding of ecological concepts with regards to fish-acanthocephalan interaction and epidemiology. Molecular and ultrastructural analyses would be helpful in highlighting morphological features of the parasite. Surveys and case studies on the occurrence of this parasite in humans and other animals would also be useful in assessing the impact of these parasites in their host.

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Statement of Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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