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Detection of *Neoechinorhynchus* sp. (Acanthocephala: Neoechinorhynchidae) in the Gut of Wild Nile Tilapia (*Oreochromis niloticus*, L.) from Lake Mainit, Caraga, Philippines

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ABSTRACT

Wild-caught freshwater Nile tilapia is potentially carrying parasites. Acanthocephalan parasites occur in almost market-sized tilapia, which frequently infect its gut. This infection presents a compelling subject for study, as it gains insights that extend beyond mere public health concerns to encompass broader environmental health implications. This study aimed to detect acanthocephalan parasites from the gut of wild Nile tilapia from Lake Mainit, Philippines. A total of 60 randomly sampled fishes were collected and examined for acanthocephalan parasites under the compound microscope. The results of the study revealed that 40% of the fish sampled were infected with *Neoechinorhynchus* sp. (Acanthocephala: Neoechinorhynchidae). *Neoechinorhynchus* sp. primarily infects fish and other aquatic organisms but does not pose a significant risk to human health. There is no significant correlation between *Neoechinorhynchus* sp. intensity and fish length and weight, suggesting that the morphology of tilapia, specifically its length and weight, is not influenced by the parasite's low intensity. This indicates that while the fish may harbor these parasites, their growth and physical development remain largely unaffected under low parasitic loads. Such findings are important for aquaculture practices, implying that a low-intensity of *Neoechinorhynchus* sp. infections might not impact the overall fish productivity and market value. Nonetheless, ongoing monitoring and management of parasite levels are recommended to ensure the continued health of the fish populations. While not a major public health threat, awareness and education on prevention can help reduce the risk of zoonotic infections, especially in regions where people consume raw or undercooked fish.

Keywords: *: fish parasites, bioindicator, prevalence, parasitic helminths*

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1 Introduction

Acanthocephalan parasites are known as spiny-headed worms due to the presence of eversible proboscis, armed with spines that are mainly for piercing and anchorage to the host gut wall (Taraschewski 2000). Several studies have shown the susceptibility of fish as hosts to acanthocephalan parasites however they are of little importance as an agent of disease but their role in the determination of the environment health specifically as biomonitors of heavy metals is promising, as acanthocephalan parasites were found to be accumulated more heavy metals than its fish host and sediments (Thielen et al. 2004; De la Cruz et al. 2013; Paller et al. 2022). The acanthocephalan parasites isolated from the gut of Nile tilapia in Laguna Lake, Philippines, exhibit significantly higher levels of lead (Pb) compared to fish tissues and ambient waters (Paller et al. 2016). Additionally, recent research has documented acanthocephalan parasites infecting fish in various aquatic systems in Mindanao, Philippines (Munda & Estaño 2020; Ali 2021; Villahermosa et al. 2022). These findings provide valuable insights and present an excellent opportunity for conducting ecological studies related to environmental health.

Lake Mainit in the Caraga Region, Mindanao was declared with exceptional biodiversity resources such as fish and shellfish (LMDA 2007). The lake was considered one of the Key Biodiversity Areas (KBA) in the Philippines but was recently reported as at risk due to some anthropogenic activities which include forest degradation, conversion of forested land to agriculture, shifting cultivation, and small-scale mining operated near the lake (Paylangco et al. 2020; Demetillo et al. 2016). Moreover, zoonotic parasites were also documented in the surrounding ricefield of the lake such as from the wild rats (Abao-Paylangco & Nepa 2021), *Oncomelania hupensis quadrasi* snail (Abao-Paylangco et al. 2019), and bovines as a reservoir host (Jumawan et al. 2020; Estaño & Jumawan 2023;). Lake Mainit produces the edible fish tilapia both wild and cultured. Due to its abundance, it served as a food source and livelihood for some residents living near Lake Mainit. Hence, this study aims to detect acanthocephalan parasites in the gut of wild Nile tilapia from Lake Mainit and its association with fish weight and length.

2 Materials and Methods

Study site and fish collection

The wild Nile tilapia was collected and purchased from Lake Mainit, Barangay Beltran, Jabonga, Agusan del Norte, Philippines (Figure 1). The lake is the fourth largest and the deepest lake in the country and is located between 9°15’ and 9°35’ North Latitude and 125°28’ and 125°35’ East Latitude (Tumanda et al. 2003; LMDA 2007), and is considered one of the Key Biodiversity Areas in the Philippines (Seronay et al. 2020). The study was also conducted due to the presence of other intestinal parasites found in other commercially produced fish, *Channa striata* in Lake Mainit (Paglinawan et al. 2022). The fish sample collection took place in a more accessible barangay during the months of October (N=30 fishes) and November (N=30 fishes) and was chosen due to the reported high likelihood of capturing market-sized tilapia in the lake during this period,

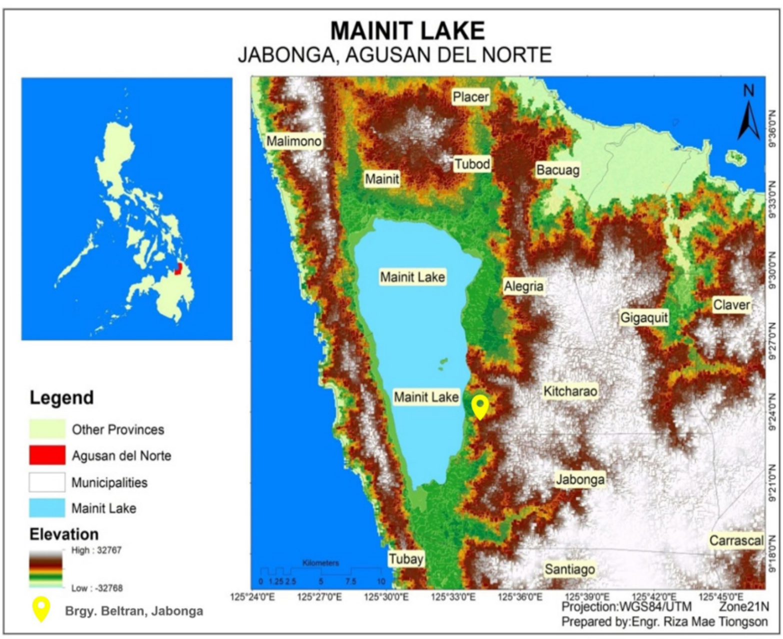

Figure 1. Map showing the source of the wild Nile Tilapia, in Brgy. Beltran, Jabonga, in Lake Mainit, Agusan del Norte, Philippines

as indicated by local knowledge and experience. Limited resources and the large size of the fish, coupled with their high market price, constrained the ability to examine larger sample sizes. Consequently, the fish collection was restricted to only 30 fish samples, each month. Inside the plastic coolers with ice blocks, fresh fish samples were then transported immediately to the Laboratory for acanthocephalan parasite examination.

Isolation and identification of acanthocephalan parasites

Before parasite examination, the fish weight and body length were measured using triple beam balance (g) and ruler (cm), respectively. Identification of juvenile and adult fishes was not assessed, instead, a relative market-sized fish was only taken for parasite examination. The isolation and preservation methods of the fish parasites were adopted by Paller et al. (2016). The fish was laid on the dissecting board on one side and was opened from the mouth to the anal region. The gut was removed and placed in a petri dish with saline solution. The gut was then stretched out in the petri dish and cut longitudinally to expose the gut contents. The contents in the gut were then examined for the presence of an acanthocephalan adult or juvenile stage under a stereo microscope (Motic, China), then photomicrograph at 100x and 200x and further confirmed under compound microscope (Motic, China). Identification of isolated acanthocephalan was based on its morphological characteristics and locating its reported geographical distribution as tilapia fish as their host (De la Cruz & Paller 2012; De la Cruz et al. 2013; Briones et al. 2015). The isolated acanthocephalan parasites were stored in a clean glass vial containing distilled water and then refrigerated for up to two hours for the eversion of proboscis then later transferred to 10% formalin for longer storage. Voucher specimens were kept and deposited in the Biology Laboratory at the Caraga State University, Ampayon, Butuan City, Philippines.

Data Analyses

The acanthocephalan parasite infection rate was computed as the total number of infected fish over the total number of fish examined and then multiplied by 100 to get the prevalence percentage (de la Cruz et al. 2013). The total number of acanthocephalan parasites recovered over the total number of infected tilapia was also obtained for the mean intensity. To determine the relationship between the intensity of acanthocephalan infection and the fish length-weight measurement, spearman correlation analysis was used (Carpio-Hernández et al. 2020). A normality test was obtained before Spearman correlation analysis. Statistical analysis was done through the use of IBM SPSS version 20 and performed at a 5% level of significance.

3 Results and Discussion

Acanthocephalan parasite in wild Nile tilapia

The acanthocephala parasites were carefully isolated from the fish samples and identified as *Neoechinorhynchus* species of the Neoechinorhynchidae family (Briones et al. 2015; De la Cruz et al. 2013; De la Cruz & Paller 2012). These parasites are characterized by their distinct morphology, including a cylindrical body and a retractile proboscis armed with hooks used for attachment to the host's intestinal wall. This holdfast organ is one of the key structures in distinguishing acanthocephalan worms from other parasitic organisms and is unique to every species of acanthocephalans. The attachment of the spiny proboscis to the intestine can cause irreversible mechanical damage in the architecture of the intestinal tissues leading to pathological changes (Herlyn 2021). These changes include deterioration and or damage of the intestinal villi, formation of granular tissues, and capsule formation. Upon these changes, the response of the host’s immune system seriously affects the digestive and absorptive efficacy of the animal. When heavy infections occur in the organism they can result in intestinal obstruction and migration of the parasites into infrequent locations has also been recounted (Nickol 2006). The body of the isolated worms in the study is composed of major parts: Figure 2A: everted proboscis (P) and neck (N); Figure 2B: trunk (T); Figure 2C: everted bursa (B) of the male worm is visible; Figure 2D: whole mount of the acanthocephalan parasite with a scale bar of 100 µm.

Acanthocephalan parasites have a unique morphology that aids in the specialized absorption of nutrients from their fish hosts. The trunk which is the main body of the acanthocephalan is generally cylindrical in shape which became

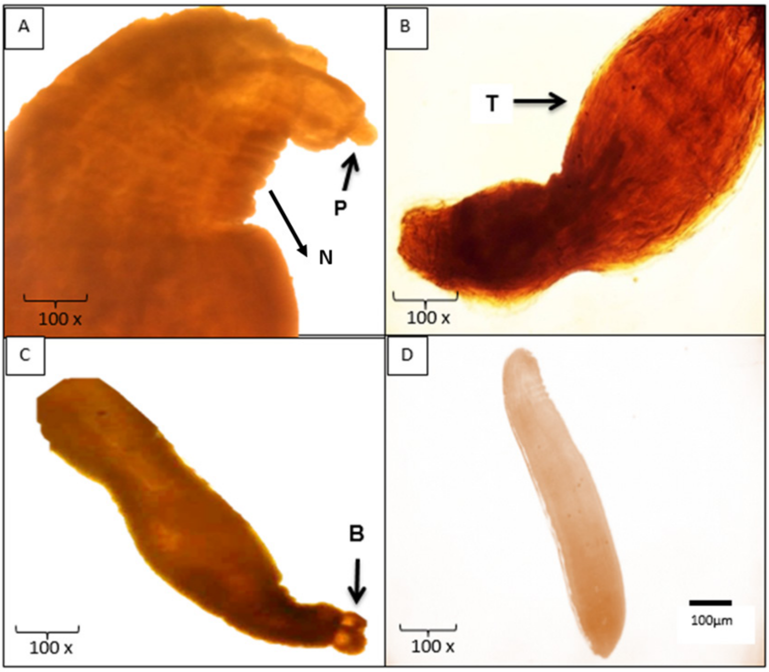

Figure 2. The recovered Acanthocephalan parasites from the gut of wild Nile tilapia from Lake Mainit, Jabonga, Agusan del Norte, Philippines. A: everted proboscis (P) and neck (N); B: trunk (T); C: everted bursa (B) of the male worm; D: whole mount of the acanthocephalan parasite. Scale bar=100um

an interest of many scientists due to its unique function. One of them is the absorption of nutrients to its body wall as acanthocephalan parasites have no known gut (Klimpel et al. 2019; Cheng et al. 2022). Their teguments consist of various pores, canals, and several structurally distinct layers, which perform both a protective and absorptive function (Nabi et al. 2016). Acanthocephalans are dioecious and it is in the trunk where most of the reproductive organs are found, while mating in acanthocephalans happens in the vertebrate host's intestine. Acanthocephalan parasites of fishes are found either as adults in the intestine or as larvae (post-cystacanths) in fish tissues. The male acanthocephalans are distinguished from females because of the presence of copulatory bursa. A fluid-filled called Saefftigen's pouch is connected to the bursa and is responsible for the eversion of the bursa (Nickol 2006; Kimpel et al. 2019).

The study recorded positive samples of fish infected with Acanthocephala. Out of 60 fish samples examined, a total of 42 *Neoechinorhynchus* sp. worms were isolated from the fish guts, resulting in a 40% infection prevalence. The distribution and infection rate of *Neoechinorhynchus* sp. in freshwater fish like Nile tilapia, are influenced by several factors. The host species diversity, as this parasite infects a wide range of fish species, both marine and freshwater. An inventory of aquatic organisms was recorded in Lake Mainit. A total of 41 fish species and several invertebrate species were listed in the Lake (Uy et al. 2015). This parasite was also recorded infecting a diverse range of fish hosts including the commercially important species in the Philippines (Gustillo et al. 2022). Additionally, a higher intestinal infection rate (72%) of *Neoechinorhynchus* sp. was recorded in tuna fish in Indonesia (Uliya & Yushi 2020).

Environmental conditions can also significantly impact the life cycle and distribution of *Neoechinorhynchus* sp. Optimal environmental conditions can enhance the development and survival of both the parasites and their intermediate hosts such as the crustaceans. However, previous studies have shown that crustacean distribution is likely influenced by the soil substrate and

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riparian vegetation in Lake Mainit, resulting in low diversity (Paylangco et al. 2020). Moreover, a distinct seasonal fluctuation also influences *Neoechinorhynchus* sp. high prevalence rate (71.7%) in freshwater fishes (Okoye et al. 2014) and even in marine water fishes as well (Chine et al. 2020).

Ecological interactions, such as the presence of predators and competitors can affect the population dynamics of both the fish hosts and the intermediate hosts, indirectly influencing the infection rates of *Neoechinorhynchus* sp. (Thomas 2002). Anthropogenic factors can also alter the aquatic environment, affecting both the hosts and the parasites. Declining water quality, open-access fisheries, and the introduction of exotic species are among the most pressing environmental issues jeopardizing the sustainability of Lake Mainit's biodiversity (Uy et al. 2015). Pollution can impact the health of fish, making them more susceptible to infections, while habitat modification can influence the distribution and abundance of both the fish hosts and the intermediate hosts. A high susceptibility of fish with recorded prevalence of 83.3% and histopathological analysis of *Neoechinorhynchus* to fishes was observed and showed complete desquamation of the intestinal epithelium with severe evidence of inflammatory reaction associated with fish host immune response (Martins et al. 2001). Similar results revealed a substantial mucus production in the fish intestine indicating a tissue inflammation. Moreover, microscopic examinations revealed that *Neoechinorhynchus* sp. induced disruption, necrosis, and degeneration of the host epithelial cells (Chine et al. 2020). Understanding these factors is crucial for managing the spread of *Neoechinorhynchus* sp. and mitigating its impact on fish populations, particularly in aquaculture settings where the health of fish is directly linked to economic outcomes. Effective monitoring and management strategies should consider these variables to control the distribution and infection rates of this parasite.

Acanthocephalan parasite infecting tilapia has an ecological impact as this was known to bio-accumulate more heavy metals than its fish host tissues (Paller et al. 2016). Interestingly, their abundance and distribution among fishes could serve as bioindicators for a healthier animal and environment. The intensity of parasite infection is crucial in assessing animal health because it might relate to fish morphology and physiology. However, some instances could be due to the interactions between conspecific parasites that act as regulators on the number of parasites per host (Barber 2007). In many acanthocephalan parasites infecting fish, studies have shown that the number of acanthocephalan worms declines when a certain number of host parasitic load is reached (Labaude et al. 2020). Although the hosts are of the same species and live in the same habitat, the variation in number of accumulations of parasitic organisms can still be observed. Furthermore, it was stated that parasitic infections are more common in the wild where ecological requirements for intermediate hosts and parasite transmission are met.

Correlation between the fish weight and length and intensity of Neoechinorhynchus sp.

The fish length and weight were recorded to determine its association with the intensity of *Neoechinorhynchus* sp. Correlation analysis (Figure 3) showed no significant relationship between the intensity and length ($r= -2.53$, $p= 0.177$) as well as the weight ($r= -2.42$, $p= 0.198$). This suggests that the fish morphology such as the length and weight are not affected or altered by the low intensity of acanthocephalan parasites. Similar results reported that *Neoechinorhynchus* sp. has no relationship between its intensity and fish host length and weight (Olurin et al. 2012). However, some studies showed that more parasite burden can be seen in larger fishes due to the larger surface area available for attachment (Akinsanya & Otubanjo 2006).

Parasitic infection tends to increase with the size and age of the fish. Larger fish provide more space for parasites, have a greater food supply, and live longer, which increases their exposure to parasitic infections over time (Brickle et al. 2005). The smaller/younger fish on the other hand had a high incidence of parasitic infection and this is mainly due to the less developed immunity against parasitic infection (Emde et al. 2012). Moreover, a significant relationship between the fish sex and size to the parasitic helminth infections in fishes was also recorded (Koyun 2012). However, in some instances, the zooplankton copepod, *Lernaeenicus sprattae* did not establish a host-size preference

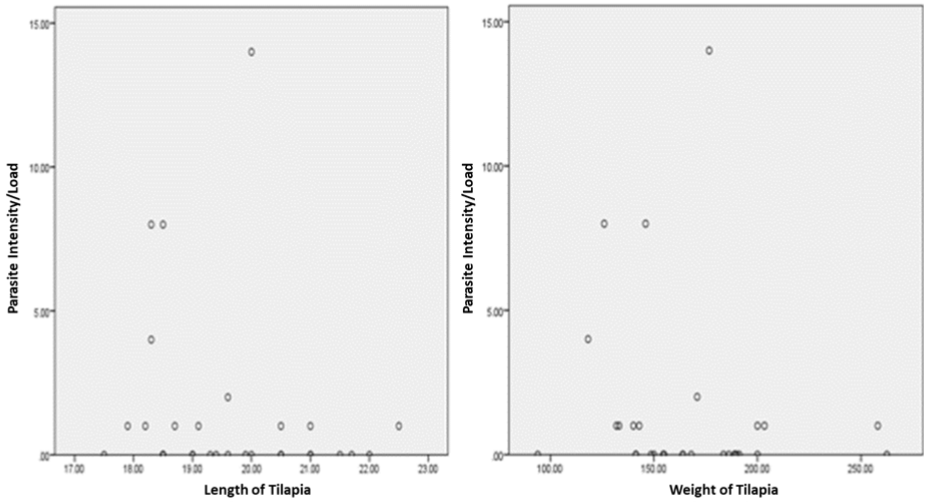

Figure 3. Plot graphs showing the relationship of parasite intensity/load and the length and weight of wild Nile tilapia from Lake Mainit, Agusan del Norte, Philippines

hence there is always a negative correlation of parasitic load to the fish size because of this shifting feeding habits in fish (Pulkkinen et al. 2000; Otachi 2009).

4 Conclusions and Recommendations

The wild Nile tilapia in Lake Mainit showed susceptibility to acanthocephalan parasites, particularly to *Neoechinorhynchus* sp. The presence of these parasites has significant implications for consumers and environmental health, particularly when the host fish are exposed to contaminated water. Parasites do not necessarily have a host-size preference, as several factors might influence their distribution. Future studies are highly recommended to adopt a more holistic approach, including socio-economic aspects, assessment of heavy metal contamination in the study area, and electron microscopy photomicrography for the isolated parasites.

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5 Statement of Conflict of Interest

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

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