

Gastrointestinal Parasite Infection of Commercially Important Benthic Fishes in Manila Bay

Kexya O. Gustilo¹, Kennesa Klariz R. Llanes¹, Victor S. Ticzon^{1,2}

¹Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, Laguna

²University of the Philippines Los Baños Museum of Natural History

ABSTRACT

Parasites are ubiquitous, infecting a diverse range of hosts including commercially important species. Despite being commercially exploited, very few literatures on fish parasitism are published in the Philippines. This study is one of the few conducted in a major fishing ground in the country that aims to survey the prevalence and diversity of helminth parasites in fishes caught in the municipal waters of Orani, Bataan and Sta. Cruz, Pampanga, Manila Bay. Fish samples from family Leiognathidae (n=86, 2 species), Mullidae (n=9, 3 species), Nemipteridae (n=13, 2 species), and Terapontidae (n=42, 2 species) were collected for dissection. The isolated gastrointestinal tract of collected fish samples were then examined for parasites using a dissecting microscope. Out of 150 fish samples collected, 79 samples were found to be infected with helminths. In these infected samples, a total of 576 parasites (Acanthocephala= 312, Nematoda= 264) were isolated. Infection rate varied among fish families with Family Leiognathidae recording the highest infection level. High intensity of infection and dual infection were observed only in *Leiognathus equulus*, while other species were infected only with either acanthocephalans or nematode parasites. Overall, acanthocephalan infection was observed only in Family Leiognathidae, while nematode infection was observed in all fish families sampled. Interestingly in mullids, parasitic nematode infection showed a significant positive correlation with body length ($p=0.012$). The differential parasitic infection observed across demersal fish families sampled could be attributed to the host's size, feeding behavior, and parasite biology. Initial results of the study suggest wide distribution of gastrointestinal parasites in commercially important food fishes caught in Manila Bay.

KEYWORDS:

Endoparasites, Dual-infection, Acanthocephala, Nematoda

INTRODUCTION

Manila Bay is one of the most industrialized and overfished bays in the Philippines (NEDA 2020). It is estimated that currently, 45% to 50% of the surrounding coastline of the bay are populated urban centers that enact multiple land and water use, which contributes to the continuous physico-chemical degradation of the bay (Santos et al. 2017; NEDA 2018). Manila Bay is also overfished, and the overextraction of fishery resources has resulted in the depletion of commercially important species (Bendaño et al. 2017). Overall, the poorly planned and implemented land use policies, pollution, and resource overextraction have resulted in the disruption of ecosystem balance and function. Among the multitude of impacts that resulted from

the degradation of the bay, the least studied is the impact of the disruption of ecosystem processes on the quality and health of the fish stock that supplies the coastal communities and beyond (Diwa et al. 2021). Specifically, fish-borne parasitic zoonoses are possibly the least studied in the region and are one of the neglected tropical diseases (NTDs) in the country. In Manila Bay, fecal matter from industrial, agricultural, and domestic areas is a potential source of parasite eggs that can find its way into the bay which could allow the continuation of the parasite's life cycle via the trophic system. Intestinal Trematodiasis, Anisakiasis, Diphyllbothriasis, Corynosomiasis, and many others result from accidental ingestion of parasite infective stages (Chai et al. 2005; Katahira et al. 2017; Butters et al. 2019; Shamsi 2019). In the Philippines, there are very few reports and records of helminth infection let

*Corresponding Author:

Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, Laguna; Email address: kogustilo@up.edu.ph

alone of fish-borne zoonoses. Consumer perception of parasites, underdiagnosed cases of infection in humans, lack of parasite prevalence studies on fish, and lack of fish parasitologists could be existing barriers to why literature on parasites is limited (Bao et al. 2018; Shamsi and Sheorey 2018).

The size of the fish and the presence of parasites in fish products renders the value and quality to drop, thus affecting fish marketability (Abollo et al. 2001; Levsen, et al. 2005; Karl 2008; Llarena-Reino, et al. 2013; D'Amico et al. 2014; Llarena-Reino et al. 2015 as cited by Bao et al. 2019). Fish quality, especially the benthic species, have shown notable decline in recent years and are sold at lower market prices primarily due to their relatively smaller sizes (Sjöberg 2015). This is not surprising since both parasite infection and pollution result in inefficient nutrient and energy utilization, and increase in energy expenditure of fish through respiration, impacting growth and reproduction of the species (Marcogliese and Pietrock 2011; Khan 2012; Shea-Donohue et al. 2017; FAO 2020). More importantly, the risk posed by fish-borne parasitic zoonoses to human health (Quiazon 2015; Bao et al. 2019) is estimated to be high especially since fish is the staple food of fishing communities and eating raw fish is common practice in the region (Soares Magalhães et al. 2014; Tenorio and Molina 2021).

Despite the known impact of parasites in a fish individual or a population, as well as its impact on the food safety and socio-economic stability of a coastal community heavily dependent on fishery, studies on parasites of commercially important fishes are very scarce. In nearly twenty-four years, only two studies on marine parasites of Manila Bay were published (e.g., Lopez 1998; Pagoso and Rivera 2017). This study aims to contribute to building baseline knowledge to address this gap. The focus of the study is on fish helminth endoparasites found in the gastrointestinal tract of soft bottom feeders. The study is one of the few empirical assessments that record the occurrence of helminth endoparasites in commercially sold benthic fish species caught in the waters of Manila Bay. The goal of the study is to survey the prevalence and diversity of helminth endoparasites collected from the gastrointestinal tract of commercially important bottom feeders collected from the markets of Manila Bay. Specifically, the study aims to; (1) determine gastrointestinal parasite composition found in commercially sold benthic fish feeding species; and

(2) correlate host condition with parasite infection.

MATERIALS AND METHODS

Fish Sample Collection

A total of only 150 fish samples were collected from wet markets in Orani, Bataan and Sta. Cruz, Pampanga on March 11, 2020 (see **Figure 1**). The samples collected in the study were from families Leiognathidae (n=86, 2 species), Mullidae (n=9, 3 species), Nemipteridae (n=13, 2 species), and Terapontidae (n=42, 2 species). Samples were sorted by family upon purchase and placed in an ice-filled Styrofoam box for transport. Further identification by species level was done by the Aquatic Zoology Research Laboratory, Institute of Biological Sciences, University of the Philippines- Los Baños (see **Figure 2**).

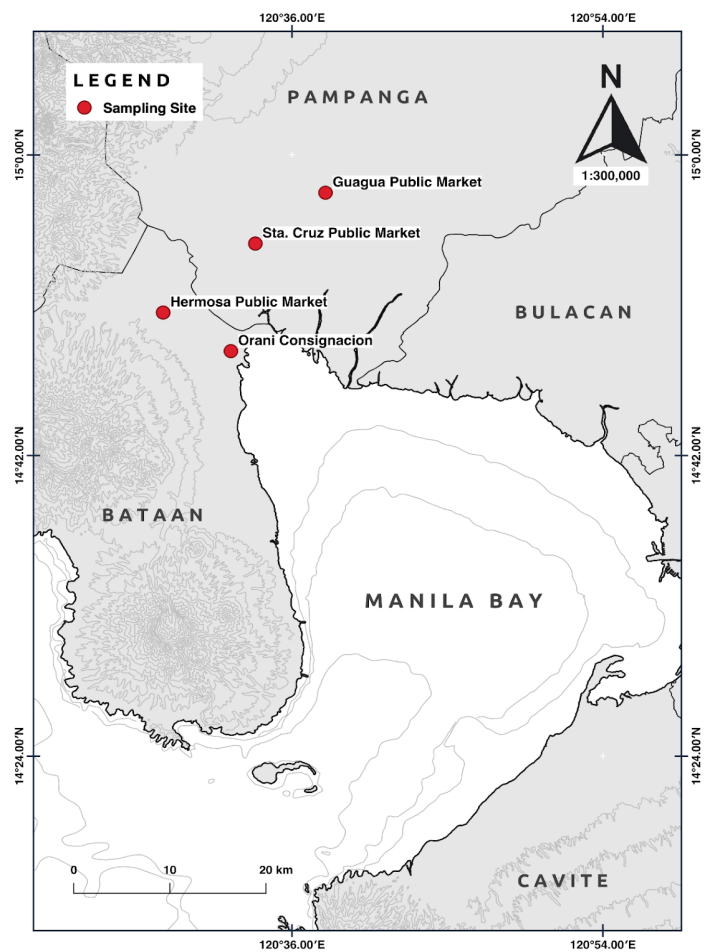


Figure 1. Location of fish markets where fish samples for analysis were collected. Only fish caught in Manila Bay were collected on March 11, 2020.

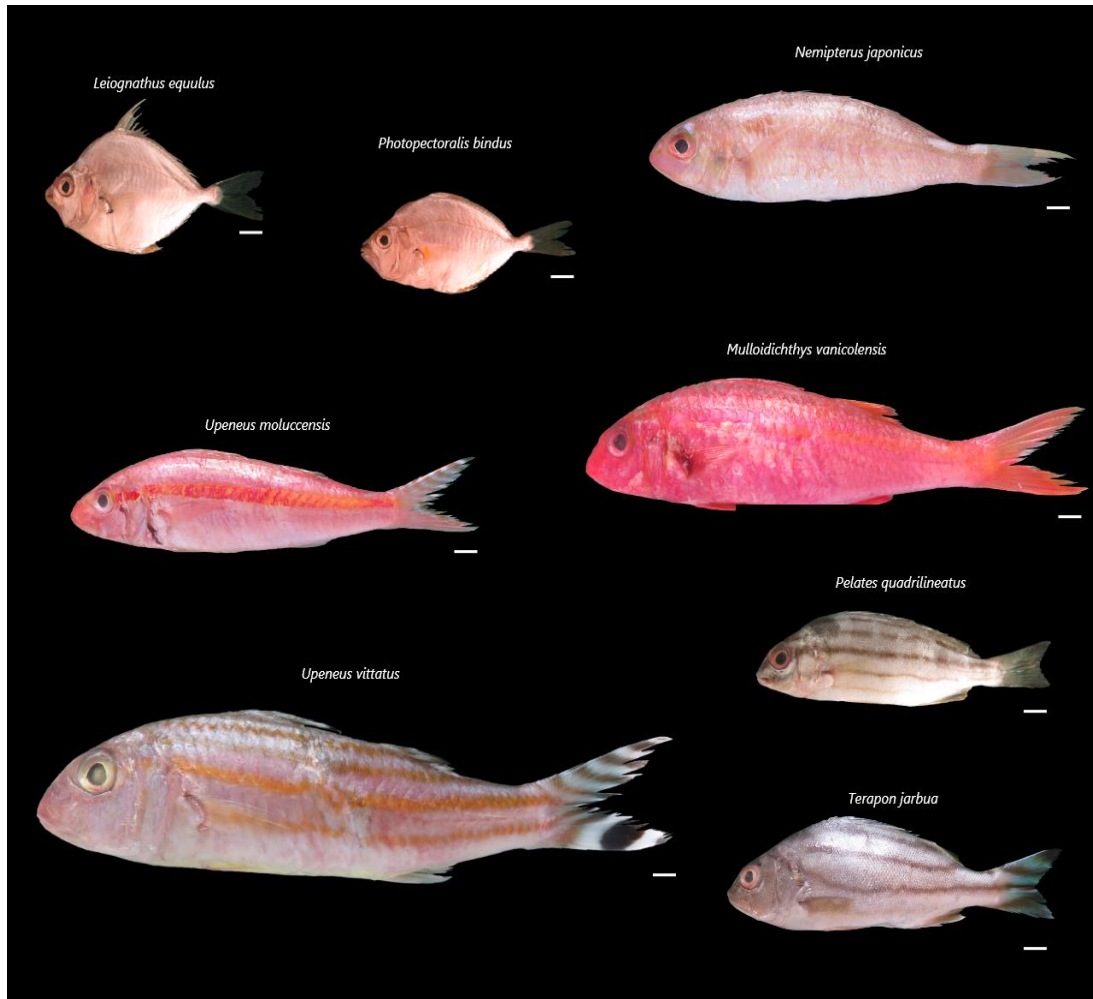


Figure 2. Commercially important benthic fishes (Scale = 1 cm) collected from the wet markets of Manila Bay on March 11, 2020.

Fish and Parasite Samples

Individual fish samples were identified at the species level, tagged, weighed (wet weight in g), and photo-documented. Fish total length (TL) was measured using the ImageJ software (Andrialovanirina et al. 2020). Fish samples were gutted, weighed (gutted weight in g), and stored in the freezer. The GIT was tied at both ends using thread to prevent parasites from leaking to other organs. Collected internal organs were stored in 50 ml Falcon tubes with 70% ethanol. After 24 hours in ethanol, the organs were removed from the Falcon tube and dissected. Due to the strict implementation of the safety protocols in the University, the processing of helminth samples was limited to basic specimen preparation. Furthermore, access to the use of advanced digital imaging instruments was restricted. The limited time to work on the samples collected

made it impossible to observe parasites found in other organs (liver, gonad, etc.) and collection focused on the fish gastrointestinal tract (GIT). The GIT (stomach, pyloric caeca, small intestine, and large intestine) was observed using a dissecting microscope. The organ was sliced or opened gently under the microscope using forceps to look for helminth parasites.

The collected parasites were placed in separate microcentrifuge tubes with 70% ethanol. Gut contents were recorded for future reference. For each fish species, parasites were separated according to phyla. Parasites were transferred in a solution of 70% ethanol with 5% glycerin for clearing in preparation for temporary mounts. Parasites were examined and photographed using NIKON Light microscope and ToupView Application (see **Figure 3**). Macroscopic examination and micrographs of collected parasites were used for parasite identification.



Figure 3. Sample light micrograph of nematodes: (A) Anisakidae (*Contraecum* sp.), (D) Camallanidae (*Camallanus* sp.); and acanthocephalan parasite: (F) Neoechinorhynchidae (*Neoechinorhynchus* sp.) (Scale= 200 μ m). Whole specimen (A and F); showing the anterior portion (B, D, and G); and the posterior end (C, E, and H) of the parasites.

While percent prevalence, mean intensity, and correlation analysis were done using Pacman and psych packages in R version 4.1.0, calculations of length-weight relationship, relative condition factor and visualization of frequency distribution were done using Excel.

Relative condition factor (K_n)

Relative condition factor (K_n) reflects the general health of a fish individual derived from the computed length-weight relationship (Le Cren 1951, Khristenko and Kotovska 2017). Length-weight relationship in itself provides an overview of fish growth patterns, condition of the environment, and life history. The length-weight equation

$$W = aL^b$$

was subjected to logarithmic transformation into

$$\log(W) = \log(a) + b \log(L)$$

to calculate for K_n using the following equation:

$$K_n = \frac{W}{aL^b}$$

where a (intercept) and b (slope) coefficients are derived from the logarithmic length-weight equation. K_n value closer to or equal to 1.0 will indicate a fit individual (in good growth condition).

RESULTS

Gastrointestinal parasite composition

A total of nine fish species were collected in the wet markets of Orani, Bataan and Sta. Cruz, Pampanga on March 11, 2020. Gastrointestinal parasites were found in all species sampled except in *T. jarbua* and *M. vanicolensis* (**Table 1**). These two were also the species which had the fewest representative samples collected at one a piece. Hence, there still remains a large probability that these fish species are also host to parasites.

Parasite prevalence in fish was also high in the study sites. Overall, 79 of the collected 150 fish samples sold in the wet markets of Orani, Bataan and Sta. Cruz, Pampanga yielded gastrointestinal parasites (**Table 2**). In total, 576 parasites (Acanthocephala= 312, Nematoda= 264) were collected from all the fish samples collected in the two wet markets. Among the isolated helminths, *Camallanus sp.*, *Contracaecum sp.*, and *Neoechinorhynchus sp.* were identified (**Figure 3**). However, the majority of the parasites encountered remain unidentified at this taxonomic level. Dual infection was observed only in *L. equulus* collected from Sta. Cruz public market, while other samples were infected only with either acanthocephalan or nematode parasites (**Tables 1 and 2**). Interestingly, acanthocephalan infection ($n=312$) was observed only in Leiognathidae samples (**Figure 4**). In contrast, nematode infection was observed in all fish families sampled (**Table 2**).

Table 1. Detected endoparasites in nine fish species collected from wet markets in north Manila Bay, Philippines on March 11, 2022

Sampling Site	Family	Fish Species	n	Acanthocephala	Nematoda
Orani	Leiognathidae	<i>Leiognathus equulus</i>	62	+	-
		<i>Photopectoralis bindus</i>	8	+	-
	Terapontidae	<i>Pelates quadrilineatus</i>	41	-	+
		<i>Terapon jarbua</i>	1	-	-
Sta. Cruz	Leiognathidae	<i>Leiognathus equulus</i>	16	+	+
		<i>Mulloidichthys vanicolensis</i>	1	-	-
		<i>Upeneus moluccensis</i>	2	-	+
	Nemipteridae	<i>Nemipterus cf. bathybius</i>	4	-	+
		<i>Nemipterus japonicus</i>	9	-	+

+ Presence - Absence

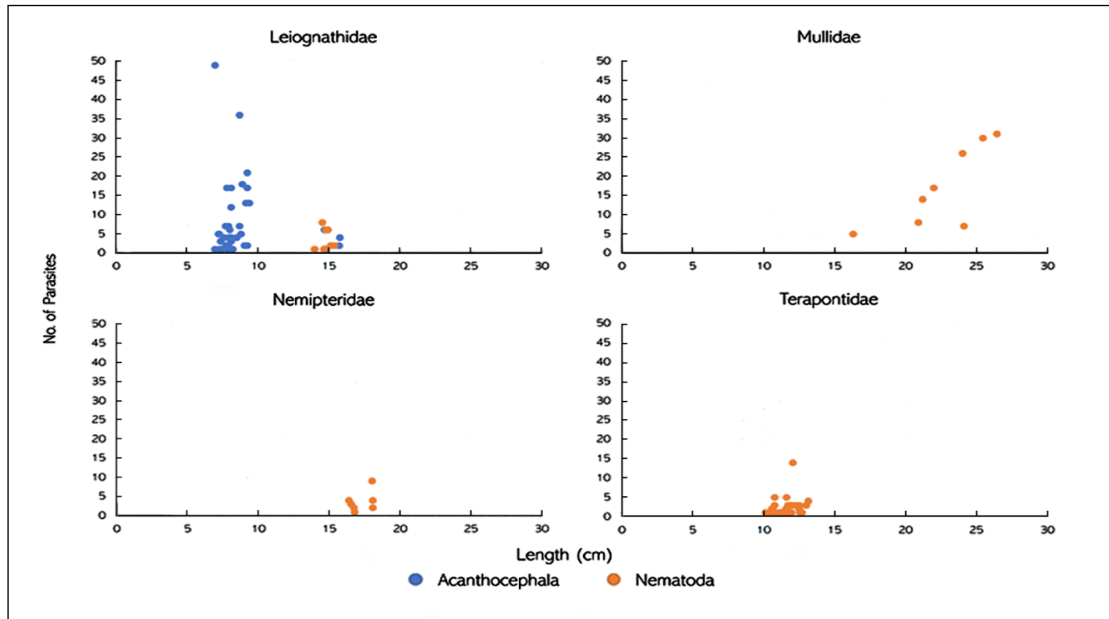


Figure 4. Helminth endoparasite distribution among host fish families and across size range.

Table 2. Abundance, percent prevalence (%P), and mean intensity (mI) values of gastrointestinal helminth endoparasite infection per host fish family

Host Family	N	Ni	Abundance	Acanthocephala (n=312)		Nematoda (n=264)	
				%P	mI	%P	mI
Leiongnathidae	86	50	339	51.16	7 (1-49)	9.30	3 (1-8)
Mullidae	9	8	138	-	-	88.89	17 (1-31)
Nemipteridae	13	7	25	-	-	53.85	4 (1-9)
Terapontidae	42	14	74	-	-	76.19	2 (1-14)
TOTAL	150	79	576				

N - total number of collected host samples

Ni – total number of infected host samples

n - total number of parasites

Host condition and Parasite infection

The mean condition factor per species observed reflects that most individuals are in relatively good condition ($K_n \geq 1$) (Table 3). The results also showed that there was no significant correlation between parasite infection and fish condition. Hence, the results suggest that the general condition of each

species is attributed to other factors other than parasite infection.

Furthermore, nematode infection was found to have a significant positive correlation with Mullids ($\rho=0.012$) and Leiongnathids ($\rho<0.05$) body length (Table 4) suggesting that parasite infection may increase with an increase in host size.

Table 3. Correlation of host relative condition factor (K_n) and helminth endoparasite infection. A host's K_n approaching 1.0 indicates fitness

Host Family	Species	Mean K_n	Acanthocephala (n=312)		Nematoda (n=264)	
			<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value
Leiognathidae	<i>Leiognathus equulus</i>	1.008111	-0.1068976	0.3516	-0.1285493	0.262
	<i>Photopectoralis bindus</i>	1.004107	0.1080585	0.799	-	-
Mullidae	<i>Upeneus moluccensis</i>	1	-	-	-	-
	<i>Upeneus vittatus</i>	1.004809	-	-	-0.08916965	0.8666
	<i>Nemipterus cf. bathybius</i>	1.000399	-	-	0.7987702	0.2012
Nemipteridae	<i>Nemipterus japonicus</i>	1.004023	-	-	0.09170653	0.8145
Terapontidae	<i>Pelates quadrilineatus</i>	1.003602	-	-	-0.05941997	0.7121

Table 4. Correlation of host length with helminth endoparasitic infection. Parasitic nematode infection showed a significant positive association with increase in body length (p value <0.05).

Host		Acanthocephala (n=312)		Nematoda (n=264)	
		<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value
Total Length	Leiognathidae	-0.1285453	0.2382	0.4854763	<0.05*
	Mullidae	-	-	0.7852393	0.01216*
	Nemipteridae	-	-	0.5213261	0.0677
		-	-	0.1809129	0.2516
Terapontidae			0.1809129	0.2516	

DISCUSSION

Gastrointestinal parasite composition

Species richness of parasites in a single host can reflect host feeding behavior and imply parasite-host specificity (Palm et al. 2007). The abundance of acanthocephalans in leiognathids could be attributed to the host's feeding behavior (Palm et al. 2007). In this study, copepods were observed in high quantities in the gut of leiognathid samples, and this is seen as a potential reason why acanthocephalan infection was high in this family. Copepods are potential intermediate hosts for acanthocephalan larvae (Huys and Bodin 1997) and the parasite has developed a strategy for effective transmission to its final host. Acanthocephalan larvae alter the coloration and behavior of their copepod intermediate hosts in order to increase predation by fish which are highly visual predators (Taraschewski 2000).

Like acanthocephalans, most endoparasitic nematodes also require ingestion by host in order to proceed with their development, making them dependent on the trophic interaction in order to complete their life cycle (Choudhury and Dick 2000; Marcogliese 2002; Poulin and Leung 2011; Timi et al. 2011; Bhuiyan et al. 2014; Gonçalves et al. 2016; Hoshino et al. 2016; Baia et al. 2018 as cited by Neves et al. 2020). Compared to acanthocephalans, nematodes are relatively larger parasites and require several hosts that can accommodate their size and complex life cycle (Dezfuli et al. 2016; Benesh et al. 2021). Most intermediate hosts of nematodes are relatively large invertebrates such as crustaceans and small fishes (Klimpel and Rückert 2005) which were observed in the gut content (fish vertebrae, bivalves and gastropods, and crustaceans) of larger fish samples collected in this study. As such, larger individuals in the samples collected were infected by nematodes, including large leiognathids with a mean TL of 14.95 cm (13.69-15.75 cm). Larger fishes such as goatfishes are able to eat a larger variety of arthropods (Pavlov et al. 2011). This increases the chances of encounter with the parasites' intermediate hosts resulting in a strong association between the size and abundance of gastrointestinal parasites (**Figure 4**). Acanthocephalan parasites were only found in smaller leiognathid individuals with a mean TL of 7.92 cm (6.46-9.39 cm). However, it is important to note that fish GIT can be infected by both adult acanthocephalan and nematode parasites, and both are transmitted by arthropod intermediate hosts via

ingestion (Woodstock et al. 2020).

The study shows the importance of arthropod intermediate hosts for their development (Rohde 1984; Morand et al. 2005), making carnivorous and particularly invertivores more susceptible to endoparasite infections. Based on the results, most intermediate hosts would be copepod, isopod, mysid, and polychaetes. All fish samples collected are invertivores that feed near muddy substrates, and these micro- and macro- invertebrates were evident in the samples' gut content. Crustacean appendages and fish vertebrates were mostly observed in larger samples. Ontogeny plays a part in parasite transmission as the increase in prey items due to changes in diet and size increases the chances of encounter (Muñoz and Zamora 2011).

Parasitic co-infection in a single host is normally observed in fishes (Rohde 1984; Morand et al. 2005; Kotob et al. 2016). Furthermore, the abundance of parasites in relatively small fish hosts physiologically weakens the fish. Both acanthocephalans and nematodes cause lesions in the gastrointestinal walls of the fish host which impacts the overall health and lessens energy assimilation efficiency of the host. In this study, nematodes were not only found in the fish GIT but were also observed outside the intestine, embedded in the liver, and near the gonads of Mullidae, Nemipteridae, and Terapontidae. This has a significant overall negative impact on the health of the host fish. Heavy infection of parasites in an individual, or if the abundance of parasites is high enough, fish fitness in terms of immune response and net reproduction is affected (Iwanowicz 2011). The weakened condition of the host increases the chance of larger predators to consume the host. This in turn fills in the transmission gap from the paratenic host to its definitive host.

The distribution pattern observed in this study shows that there is aggregation in particular individual hosts. According to Rohde (1984) and Morand et al. (2005), parasite aggregation is advantageous to dimorphic parasites such as those found in this study due to higher chances of contacting a mate. Aggregated distribution of helminth parasites is naturally observed which could be explained by several conditions such as series of exposure, aggregation of infective stages in intermediate hosts, previous infection increases or decreases chances of infection, variation in host diet, and host "age resistance" against infection. Research on aggregation indices by Amarante et al. (2015)

states that variability in host and parasite biology in the sample population would affect the degree of aggregation in each host family and thus aggregation cannot be compared across families. The same can be concluded in aggregation across sexes, as there is no clear difference between male and female feeding behavior.

Host condition and Parasite infection

In this study, there is no significant association between parasite infection and fish condition and suggests that host condition is attributed to other factors other than parasite infection. This cannot be compared across species due to the difference in body shape and life history. However, we may attribute fish condition to external factors such as food availability supported by the environmental condition and feeding activity prior to sampling (Jisr et al. 2018). Pollution, in general, has a negative effect on fishes. One example would be high concentration of microplastics (MPs) which affects growth of fish larvae due to ingestion of MPs which in turn compromises nutritional requirements for growth (Campos et al. 2021). Another would be behavioral changes and reduced survival due to intoxication caused by chemical runoffs from terrestrial areas (Naviera et al. 2021). However, the relatively good condition of fish observed in this paper could also be attributed to fish response towards multiple stressors such as the “compensation strategy”, wherein there is energy allocation towards immune response, self-repair, and self-maintenance than to growth and reproduction (Petitjean et al. 2019).

In most studies, K_n is usually negatively associated with parasite infection as parasites compete with their host for nutrition, but there are also studies that are consistent with our results. Some of the few literature reports on K_n and parasite infection that coincide with the findings of this study were on anostomid fishes infected with metazoan endoparasites by Guidelli et al. (2011), farmed *Colossoma macropomum* (Pereira and Morey, 2018) and *Piaractus brachypomus* (de Oliveira et al. 2019) infected with *Neoechinorhynchus* sp. The result of our study may have been biased since parasites were only collected from the GIT, and there were observed parasites in other visceral organs that were not accounted for in this paper. Among the published studies that coincide with our findings, observed higher K_n in individuals parasitized with endoparasites is attributed to low level of infection (de Oliveira et al. 2019), the capability of

“healthier” hosts to cope up with heavy infection, and the infection strategy of parasites species (Guidelli et al. 2011). We also consider that the heavy infection may affect the host weight influencing the calculated K_n value (Pereira and Morey 2018).

In conclusion, the study shows that there is a wide distribution of gastrointestinal parasites in commercially important food fishes caught in Manila Bay. In this study, we identified *Contracaecum* sp. and *Camallanus* sp. under phylum Nematoda and *Neoechinorhynchus* sp. under phylum Acanthocephala. Like other helminth endoparasites, *Contracaecum* sp., *Camallanus* sp., and *Neoechinorhynchus* sp. are of economic importance due to their pathological effect on their hosts (Nguyen et al. 2021; Pambudi et al. 2021). At least in this study, parasite infection is not influenced by the overall condition of the host. The results of the study emphasize the importance of arresting or at least regulating the influx of untreated domestic and animal farm wastes to Manila Bay since there are acanthocephalans and nematode species that are capable of infecting humans (Sasaki et al. 2019). For instance, among the identified parasites in this study, parasitic helminths from Genus *Contracaecum* are of public health importance due to their potential risks of zoonotic infections along with other Anisakids (EFSA 2010; Moravec et al. 2016; Mattiucci et al. 2017; Ramos et al. 2020). Hence, controlling point sources of parasite eggs and larvae in the watershed should be prioritized by the government.

The results of the study must be interpreted with caution primarily since the parasites were broadly grouped into nematodes and acanthocephalans. What it can clearly provide though is the prevalence and high infection of helminths in commercially important fish families in the bay. It is recommended that future studies on parasitic infection on fish should be conducted in major fish markets around the bay and that more samples must be collected for each family. Since the parasite samples were prepared and identified using simple microscopy wherein the study was unable to provide species-level identification, especially for larvae having cryptic features, it is also important to conduct morphological and molecular studies on the collected parasites to determine whether the helminths observed are zoonotic or may pose as a threat to consumer’s health.

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Authors:

Keyxa O. Gustilo, *Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, Laguna;*
email: kogustilo@up.edu.ph

Kennesa Klariz R. Llanes, *Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños,*
Laguna; email: krllanes@up.edu.ph

Victor S. Ticzon, *Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, Laguna;*
University of the Philippines Los Baños Museum of Natural History; email: vsticzon@up.edu.ph
