Monogenean and cestode infestation in the gills and intestines of *Clarias gariepinus* (African catfish) in Zarraga, Iloilo and *Chanos chanos* (Milkfish) in Dumangas, Iloilo

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Abstract

In the Philippines, *Clarias gariepinus* (African catfish) and *Chanos chanos* (milkfish) are two economically important fish species. Studies conducted by Echem et al. (2018) and Agbabiaka et al. (2017), respectively have suggested that both of these species have high mean intensity and prevalence of parasites which can cause mortalities in fish. The study aimed to identify and determine the prevalence and mean intensity of parasites present in *Clarias gariepinus* and *Chanos chanos* in Zarraga and Dumangas, Iloilo, respectively. The gills and intestines of 30 fish samples from each species were dissected and examined under a microscope. It was found that *Dactylogyrus spp.* and *Gyrodactylus spp.*, both monogeneans, infected the gills of both species. Meanwhile, varied species of cestodes were only present in the intestines of *Clarias gariepinus*. A positive correlation was found between the number of parasites present, and the length and weight of *Clarias gariepinus,* while no correlation was observed in *Chanos chanos*. This may be explained by the difference in the environmental preference of the fishes.

Keywords: *Cestodes, correlation, mean intensity, monogenean, prevalence*

Introduction. Fish is among the most widely cultured food items in the country as it is a common source of protein. Diseases brought about by parasites are one of the main reasons for the decrease in fish supply [1]. This usually results in huge economic losses among fish farmers and fish hatchery owners. In the country, *Chanos chanos* is generally considered as one of the main aquaculture products [2]. On the other hand, *Clarias gariepinus* (African catfish) is also one of the most abundant cultured species in the country [3].

Clarias gariepinus [4] and *Chanos chanos* [1] are known to be susceptible to parasites. However, the degree of parasitism varies among these species. The diversity and composition of parasites that infest fish such as *Clarias gariepinus* and *Chanos chanos* vary depending on several factors such as water quality, diet, habitat, and weight and length of the fish [5,6].

Zarraga and Dumangas, Iloilo are the two main producers of *Clarias gariepinus* and *Chanos chanos* in the province, respectively. Several studies have been conducted concerning the parasitism in *Clarias gariepinus* and *Chanos chanos*. However, those that address parasite prevalence in the province are limited. This study aims to determine the mean intensity and prevalence rate and identify the types of parasites present in *Chanos chanos* from Dumangas, Iloilo and *Clarias gariepinus* from Zarraga, Iloilo. The data obtained in this study may help local fish farmers in selecting treatments for their farms.

The study aimed to identify the types of parasites present in *Clarias gariepinus* in Zarraga, Iloilo, and *Chanos chanos* in Dumangas, Iloilo.

It specifically aimed to:

(i) identify the parasites present in *Clarias gariepinus* and *Chanos chanos*;

(ii) determine the prevalence of parasites present;

(iii) determine the mean intensity of parasites present;

(iv) correlate the weight of the fish to the number of parasites present; and

(v) correlate the length of the fish to the number of parasites present.

Methods. The data gathering was composed of three phases: sample collection, sample preparation, and fish dissection and examination. Sample collection involved the purchase, collection, and transportation of *Clarias gariepinus* and *Chanos chanos* samples from their respective fish farms to the Microtechnique Laboratory in Southeast Asian Fisheries Development Center (SEAFDEC), Tigbauan, Iloilo. Sample preparation involved euthanizing, measuring, and identifying the sexes of the fish samples before dissection. Fish dissection and examination involved the dissection and evisceration of the fish samples, examination of the gills and intestines, and data recording. Prevalence

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and mean intensity of parasites in each of the fish samples were calculated by formulas provided by Bush et al. [7]. Pearson Correlation Test was then performed using IBM Statistical Package for the Social Sciences Statistics Version 22 (SPSS) to determine the relationship between the number of parasites present and the length and weight of the fish samples from each fish species.

Sample Collection. A total of 30 live fish samples per fish species were directly purchased from their respective fishponds. Samples were purchased in groups of 10 as these are the maximum number that could be dissected per day. Fish samples were stored in aerated fry bags, along with water from the pond where the samples were harvested [8]. Fry bags were transported in large buckets, with a total number of five (5) samples per bucket [8]. This method was repeated until 30 *Clarias gariepinus* and 30 *Chanos chanos* samples were collected and examined. *Clarias gariepinus* and *Chanos chanos* samples were then transported to the Microtechnique Laboratory of SEAFDEC/AQD in Tigbauan, Iloilo for the dissection and examination of the gills and intestines.

Sample Preparation. The fish samples were sedated using 2.4 mL phenoxyethanol with a concentration of 200 ppm diluted in five (5) liters of water inside the fry bags [9]. The total length (TL) of each fish was then taken using a ruler with a 1-mm precision [8] Each fish sample was then weighed using an Asuki TB-300 digital weighing scale with a one (1) mg precision [8].

Fish dissection and examination. Fish samples were dissected by first cutting the left and right operculae open. The gill arches were then cut and removed from the cavity using dissecting scissors and tweezers. The gill filaments were cut from the gill arches, placed on a glass slide, and covered with a cover slip. An incision was then made from the anus of the fish up to its mouth, exposing the digestive tract [10]. The fish samples were then eviscerated. The small and large intestines were removed, and placed on a petri dish filled with freshwater for *Clarias gariepinus*, and seawater for *Chanos chanos to* mimic the salinity of the fish pond where the fish species were collected. The gill filaments were examined under the Olympus BX51 compound light microscope, and the Howell Binocular Compound Microscope at 40x and 100x magnification. Photographs of parasites in the gills were taken at 100x magnification. The intestines were examined under the Howell Binocular Stereomicroscope. Parasites found in the gills and intestines were identified up to the genus level based on morphology, using the book Health Management in Aquaculture by Cruz-Lacierda et al. [11], and further verified by the Fish Health Section of SEAFDEC/AQD. After identification, parasites were counted based on their types.

Data Analysis. The parasites were then quantified by calculating the prevalence and mean intensity. The formula for prevalence is shown below.

$$Prevalence = \frac{Number\ of\ infected\ samples}{Number\ of\ samples}\ X\ 100$$

Mean intensity can be calculated using the equation:

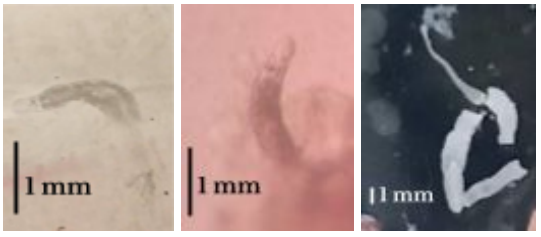
$$Mean\ intensity = \frac{Total\ number\ of\ parasites}{Number\ of\ infected\ samples}$$

The length and weight of the fish samples were then correlated to the number of parasites present in each fish species using the Pearson Correlation Test in SPSS.

Safety Procedure. Before dissection of *Clarias gariepinus* samples, spines located along the pectoral fins were removed by cutting them using dissecting scissors. The spines contain venom that causes swelling and increased blood flow to the affected area. Improper handling of *Clarias gariepinus* could result in potential injuries during the dissection of the fish. After dissection and examination, used fish samples were properly disposed according to SEAFDEC protocols. Samples were buried in a vacant lot in SEAFDEC/AQD, Brgy. Buyu-an, Tigbauan, Iloilo.

Results and Discussion. The results and discussion is composed of six (6) parts: parasites present, prevalence, and mean intensity; attachment sites; *Clarias gariepinus* weight and length-parasite relationship; *Chanos chanos* weight and length-parasite relationship; parasitism comparisons between *Clarias gariepinus* and *Chanos chanos*; and water parameters that may have affected the extent of parasitism in each fish species.

Parasites present, prevalence, and mean intensity. Overall, all fish samples of *Clarias gariepinus* were infected with at least one type of parasite, while no parasite was recorded for 16 out of 30 samples of *Chanos chanos*. Table 1 shows that *Clarias gariepinus* was parasitized by three different parasites, namely, *Dactylogyrus spp.* (monogenean, Fig. 1A), *Gyrodactylus spp* (monogenean, Fig. 1B), and Cestode (Fig. 1C). Among the parasites that were recorded, two types were also found to parasitize *Chanos chanos*, which include *Dactylogyrus spp.* (monogenean), and *Gyrodactylus spp* .(monogenean) as summarized in Table 2. Cestodes were only found to be present in *Clarias gariepinus*.



A B C

Figure 1. Parasites present in the fish samples: (A) Monogenean [*Dactylogyrus spp.*]; (B) Monogenean [*Gyrodactylus spp.*]; and (C) Cestodes.

Attachment sites. Attachment sites of the parasites in each fish species are shown in Tables 1 and 2. *Dactylogyrus spp.* (monogenean), and *Gyrodactylus spp* . (monogenean) were found attached to the gills of all fish samples for both *Clarias gariepinus* and *Chanos chanos*. Cestodes were only found in the intestines of *Clarias gariepinus*.

Table 1. Parasites found in *Clarias gariepinus.*

Parasites recorded	Site of attachment	TP*	inf**
Dactylogyrus spp. (monogenean)	Gills	1157	30
Gyrodactylus spp. (monogenean)	Gills	61	15
Cestode	Intestines	32	5

**TP* number of parasites, ***inf* number of infected samples

Table 2. Parasites found in *Chanos chanos.*

Parasites recorded	Site of attachment	TP*	inf**
Dactylogyrus spp. (monogenean)	Gills	19	12
Gyrodactylus spp. (monogenean)	Gills	1	1

**TP* number of parasites, ***inf* number of infected samples

The prevalence (%) and mean intensity (MI) of parasites present in *Clarias gariepinus* and *Chanos chanos* are shown in Table 3. *Dactylogyrus spp.* (monogenean) was found to be the most prevalent parasite infecting both fish species, followed by *Gyrodactylus spp.* (monogenean), and finally Cestodes for *Clarias gariepinus*. *Dactylogyrus spp.* (monogenean) had the greatest MI among other types of parasite for *Clarias gariepinus* and *Chanos chanos*. The parasite exhibited an MI of 39 parasites per sample in *Clarias gariepinus* and two (2) parasites per sample in *Chanos chanos*. *Dactylogyrus spp.* (monogenean) has an MI of four (4) parasites per sample in *Clarias gariepinus*, and an MI of one (1) parasite per sample in *Chanos chanos*, the least MI of parasites in both species. Meanwhile, Cestodes exhibited an MI of six (6) parasites per fish sample in *Clarias gariepinus.*

Table 3. Prevalence and mean intensity of parasites present.

	Clarias gariepinus		*Chanos chanos*	
Parasites present	%*	MI**	%*	MI**
Monogenean (*Dactylogyrus spp.*)	100	39	40	2
Gyrodactylus spp. (monogenean)	50	4	3.33	1
Cestode	16.67	6	-	-

*% prevalence, ***MI* mean intensity

Clarias gariepinus weight and length-parasite relationship. Among the two fish species, *Clarias gariepinus* was the most infected, hosting three types of parasites, and a total of 1250 parasites in 30 samples. The correlation of catfish weight and length to the number of parasites present in the fish are shown in Figure 2. The correlation coefficients (r) of the length and weight to the number of parasites were 0.639 and 0.622 respectively. A positive correlation of the weight and length to the number of parasites present was found to be significant at α=0.01. With this, the correlation of the weight and length of catfish to the number of parasites present can be described as moderate since the values of r fall between the range of 0.40-0.69 [12].

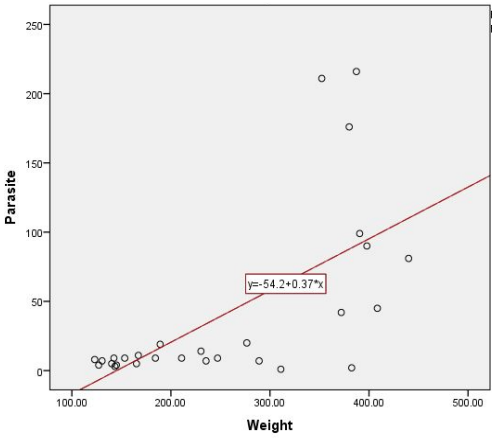


Figure 2. The graph shows the correlation of catfish weight to the number of parasites (r=0.639).

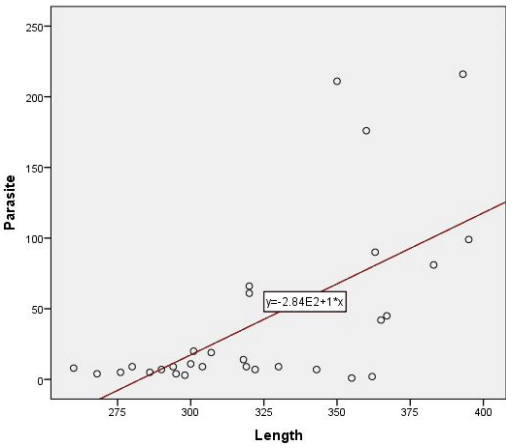


Figure 3. The graph shows the correlation of catfish length to the number of parasites (r=0.622).

Chanos chanos weight and length-parasite relationship. Chanos chanos exhibited a lower prevalence and MI of parasites present than *Clarias gariepinus*. Among the three parasites observed, *Chanos chanos* hosted only two types, with a total number of 20 parasites found for all fish samples. No significant correlation between the weight and length of *Chanos chanos* and the number of parasites was observed.

Clarias gariepinus and *Chanos chanos* both had the presence of *Dactylogyrus spp.* (monogenean) and *Gyrodactylus spp.* (monogenean) in the gills. Of the two fish species examined, only *Clarias gariepinus* had a presence of cestodes in its intestines.

The presence of parasites in the fishes may be explained by the water quality of the pond where the fishes were sourced from. When the water parameters differ from the ideal conditions of the fish species, slow growth, inability to reproduce, and the increased risk of parasite infestation due to decreased immunity is expected. Along with this, unfavorable water conditions allow for the easy proliferation of parasites in the water system. Some of these parameters include the salinity, temperature, and dissolved oxygen of the water in the pond. [13]. Also, a similarity in diet, habitat, and

feeding capacity of the host may have affected the presence and abundance of these parasites [14].

Parasites were observed to be more prevalent in the gills, than in the intestines. This is because the gills are in direct contact with the water where the fish is exposed to [15]. The gills are more likely to catch more parasites, especially monogeneans because of its threadlike filaments which makes it easy for monogeneans to hook and attach to [16]. Additionally, most parasites present in the fishes were monogeneans. Monogeneans are ectoparasitic flatworms, which mostly infect only the skin and the gills of the fish [11].

The absence of cestodes in *Chanos chanos* may be due to the salinity of the water where the fish is found. This is because cestodes usually infest freshwater fishes, such as catfish, carps, and snakeheads, while *Chanos chanos* is a euryhaline fish [11]. The difference in the water salinities in which the two organisms exist may have contributed to the minimal parasitism in *Chanos chanos*. Furthermore monogeneans exist mostly in temperatures optimal for catfish growth, which may explain the parasite prevalence in the samples [17,18,19]. In contrast, *Chanos chanos* exists in environments with high levels of dissolved oxygen. Dissolved oxygen (DO) can also affect the extent of the parasitism by supporting the immunity of the host fish against parasitic infestations [20]. Along with this, fishes that are exposed to environmental systems which have low DO and high turbidity, such as *Clarias gariepinus*, are more vulnerable to parasite infestation [21].

It was found that both the length and weight of *Clarias gariepinus* had a positive correlation to the number of parasites present in the fish species. This is supported by the study of Khalil et al. [22], which indicated that the larger the fish, the larger the surface area that the parasites can attach to, leading to an increase in parasitism. The study of Amare et al. [1] also showed a positive correlation between the number of parasites present and the size of the fish. The results of Oscar et al. [6] also showed a high number of monogeneans present in the gills and a positive correlation between the length and weight of the fish to the number of parasites present.

No significant correlation was observed between the size and number of parasites present in *Chanos chanos*. This is inline with the findings of Echem et al. [2], which reported a negative correlation between the size of the milkfish and the number of parasites present. This is because monogeneans infesting the fish are not present in their optimum habitat for growth. Monogeneans mostly infest freshwater fishes, while *Chanos chanos* is a euryhaline fish. The difference in the tolerance to saltwater of the parasite and the fish species may have affected the extent of monogenean parasitism in the fish [11].

Treatments for the parasitism depend upon the type of parasites present. Treatments for monogenean infestation include Praziquantel (PZQ), hydrogen peroxide, formalin, potassium permanganate, hyposalinity and hypersalinity, and restocking. Meanwhile, cestode infestations are usually treated using PZQ and niclosamide.

Praziquantel (PZQ) is a selective drug used for the treatment of trematode, cestode, and monogenean infections only in ornamental fishes, and is not approved by the US Food and Drug Administration (USFDA) for the use in fishes that are being consumed [23,24]. Hydrogen peroxide, formalin, and potassium permanganate have also been used in the aquaculture industry as immersion treatments against disease-causing organisms, including ectoparasites [25,26,27]. Hyposalinity and hypersalinity are osmotic shock treatments used against parasitic infestations, especially monogenean, in certain fish species by decreasing or increasing the salinity of the water bath where the fishes are exposed to [23,28]. Disinfection in aquaculture involves emptying, drying, and disinfecting the tanks or ponds where the fishes are cultured [23]. Niclosamide is a drug which belongs to the family of medicines called anthelmintics, which are generally used to treat worm infections, like cestodes [29].

Limitations. The examination of fish parts for the presence of parasites was only limited to the gills and intestines of both fish species. The samples were also limited to *Clarias gariepinus* and *Chanos chanos* cultured and obtained from one fish farm in Zarraga, Iloilo, and one fish farm in Dumangas, Iloilo respectively. The sample size for each fish species was limited to 30 samples. *Clarias gariepinus* samples were commercially available, however, *Chanos chanos* samples were collected using aerated fry bags to transport the samples to the laboratory as suggested by the fish farm owner. This was to assure that milkfish samples were alive once they arrived at the laboratory to be dissected and examined.

Conclusion. *Dactylogyrus spp.* (monogenean) exhibited a high prevalence rate and mean intensity in *Clarias gariepinus*, while it exhibited a moderate prevalence rate and low mean intensity in *Chanos chanos*. Meanwhile, *Gyrodactylus spp*. (monogenean) exhibited moderate and low prevalence rates and mean intensities for *Clarias gariepinus* and *Chanos chanos* respectively. Cestodes were the least prevalent among the parasites, and were only found in *Clarias gariepinus*. As the length and weight of *Clarias gariepinus* increases, the number of parasites found in the fish increases with it. Meanwhile, the length and weight of *Chanos chanos* has no effect on the parasite count in the fish. Therefore, farmers culturing the fish species would be informed on possible treatments concerning parasitism of monogeneans and cestodes. Along with this, it would promote the awareness among fish farmers about how size could affect the extent of parasitism in their fish culture.

Recommendations. This study recommends to increase the number of fish samples and increase the number of fish farms where the samples are sourced from in order to obtain a more accurate representation of the fish population in the specific area. Also, more varied sizes of *Clarias gariepinus* and *Chanos chanos* samples could be used in future studies in order to have a more accurate analysis of the correlation of length and weight to the number of parasites present in each fish species. It is also recommended to take water and soil samples from the pond where the fish samples are collected to further relate the environmental parameters to the extent of parasitism.

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