Occurrence of Endoparasite in Rohu Carp (*Labeo rohita*) in Buluan, Maguindanao, Philippines

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Abstract: *Lake Buluan is the third largest lake in the island of Mindanao, located in Buluan, Maguindanao, Philippines, which is dominated by freshwater fishes, including the Rohu carp (Labeo rohita), the most economically important species in the inland fisheries of the Municipality of Buluan. This fish is widely distributed to nearby cities and municipalities. However, cases of parasitic infections may affect fish health through reproductive and physiological damage which may result to economic loss. Furthermore, presence of zoonotic parasites in fishes is of public health importance. Thus, there is a need to determine the parasite load of L. rohita from this source. A total of 100 samples of L. rohita were examined during the study period (November 2018 to February 2019). Internal parts (stomach, eyes, muscles, brain, liver, gills, kidney and heart) were examined through necropsy, artificial digestion and microscopy. Parasites were subjected to staining for identification. Results revealed that 8% of the samples were infected with helminth parasites specifically, the nematode Camallanus sp. (2%) in the intestine and trematodes Clinostomum philippinesis (6%) in the gills. Furthermore, increased parasitism was observed in smaller fish than larger fish samples. The mean values of condition factor were in the limit of 0.928 to 1.373. This study is the first report of the occurrence of helminth parasites in L. rohitain Lake Buluan, Philippines.*

Keywords–Endoparasite**,** Freshwater fishes, *Labeo rohita*, Lake Buluan, Rohu carp

INTRODUCTION

Rohu carp (*Labeo rohita*) is one of the most important species of inland fisheries of the municipality of Buluan, Maguindanao. As indicated by the fisheries statistics of the Philippines, a total of 653.56 metric tons of carp was also accounted from the province. Rohu Carp (*L. rohita)*, commonly known as "Tarok," is one of the major fish species from Lake Buluan, Maguindanao. However, due to limited assessment conducted, the per capita consumption for this commodity is not yet established. Due to its large production, it is widely distributed to nearby cities and municipalities and an important protein source among locals in the area.

The parasite fauna of freshwater fishes has not yet been comprehensively explored in the Philippines [1]. A comprehensive knowledge of the prevalence of fish parasites, the causative factors, and type of environment that promotes their proliferation are of fundamental importance for disease control.

There is no existing record on the parasites of *L. rohita*, thus, this study provides baseline information addressing the problems related to the absence of this information. Issues concerning health may arise considering the fact that this species are commonly consumed by locals. Also, results of this study can be used in fish health management if and when *L. rohita* will be commercially produced in the future.

OBJECTIVE

This study aimed to detect the occurrence of parasites in *L. rohita*; characterize and identify different parasites found in *L. rohita*; and determine the prevalence of parasites in *L. rohita*.

METHOD

Location of the Study

Lake Buluan located in Buluan, Maguindanao, Philippines, with an estimated area of 62 square kilometers and has an average elevation of 4.5 meters. *L. rohita* is one of the most important species for

inland fisheries in the municipality of Buluan, Maguindanao. In this study, the site for fish collection was located in Brgy. Poblacion, Buluan, Maguindanao alongside Lake Lanao, where the only fish port in the municipality is located. In this site, fish from Lake Lanao are transported to the markets of the nearby barangays and towns. Furthermore, because of its very rich wildlife, the lake has considerable potential for nature tourism. However, due to some insurgent activities and presence in the area, access is restricted.

Collection of fish samples

Freshly caught *L. rohita*were obtained from the fishermen in the area. A total of one hundred samples were collected and placed individually in resealable zip-locked bags and immediately transported to the laboratory for examination. In the laboratory, each of the specimens were measured according to their size or length (cm), width (cm), and weight (g) using a Vernier caliper and weighing scale, respectively. The first batch sampling period for *L. rohita* was done during the dry season on the months of November – December 2018. The second batch sampling period was conducted during the rainy season on the months of January – February 2019. Considerable water levels at Lake Lanao varies during the wet and dry season, and water levels affects the *L. rohita* production.

Sample preparation and parasite identification

Internal examination

Endoparasite examination was done by observing the gills, internal view of the eyes, muscle, liver, kidney, heart, brain and the digestive tract of the fish samples. Each fish was dissected by cutting along the lower lateral line using a scalpel down the anus. The internal organs were observed using a hand lens while detailed examination for each observed organ with suspected parasites was done under a compound microscope. The intestines and the stomach were stretched out in a Petri dish and were cut open longitudinally. Internal organs (kidneys, heart, stomach, brain, eyes, liver, and muscles) were placed in saline solution in covered plastic containers. The contents were examined under a compound microscope at 100x and 400x magnification.

Muscle examination

The muscular tissues of the fish were collected close to the abdomen of the fish and were sliced in a fillet, then pressed tightly in a glass slide. Samples were then examined under a compound microscope under 100x and 400x magnification.

Fixation of Parasites

Trematodes

Fixation of Trematodes [2]. Adult trematode were carefully removed from the infected fish samples, and placed directly into distilled water. The collected trematode samples were then transferred to 70% ethanol for preservation. For staining, specimens from 70% ethanol were placed directly into dishes containing acetocarmine solution for about 2 hours. The staining duration depends on the size of the specimen and the age and concentration of the stain.After staining, the specimens were again placed in 70% ethanol for 20-30 minutes. The stain is then washed afterwards using 70% acid ethanol (70% ethanol with 1 ml 1 N HCl/10 mL) until the stained trematode samples attained pinkish-red color. Then, the specimens were again submerged successively in different solutions; 70% ethanol for 10 min and 95% ethanol for 20-30 minutes. Specimens were then submerged in xylene, the solution being replaced by a new solution every 20-30 minutes. This procedure was done twice before placing the specimen on a glass slide along with a drop of xylene. A generous drop of Canada balsam was added, making sure that mounting medium was not too thick and not too runny. Coverslip was carefully placed without smashing the sample.

Nematodes

Fixation of nematodes [3]. Nematodes were stored in a container containing 4% formalin. The container is immediately placed in the incubator: 95 °C for 2 minutes, 65 °C for 10 minutes, 75 °C for 10 minutes, 85 °C for 10 minutes, 95 °C for 10 minutes. When the tube has reached room temperature, it was shaken and its contents were transferred to a glass container. The container was then rinsed several times with distilled water, and water was placed inside the container afterwards. Nematodes were picked out and transferred to a glass slide with a mixture of glycerin and distilled water in proportion 1:20. Slide preparation was done according to the standard technique for glycerin collection slides: nematodes were transferred into a minute drop of pure glycerin on a slide glass with the paraffin wax. Then covered by a cover slip. The cover slip was placed at an angle to the slide glass above the drop of glycerin with nematode. The slide glass was gently heated on a hot

plate at 80-85 °C until the paraffin wax melts and seals the glycerin drop with nematodes.

Condition factor (K)

The condition factor (K) was determined using length and weight data of fish samples. The condition factor was calculated as per the standard method of [8].

$$Condition\ Factor\ (K) = \frac{weight \times 100}{length^3}$$

Statistical analysis

Results were presented using descriptive statistics. The prevalence, intensity, and abundance of parasites in *L. rohita*were calculated using the following formulas:

$$Prevalence\ of\ Parasites = \frac{total\ numer\ of\ infected\ samples}{total\ number\ of\ samples\ examined} \times 100$$

$$Intensity\ of\ Parasites = \frac{total\ number\ of\ parasite\ found}{total\ number\ of\ infected\ samples}$$

$$Abundance\ of\ Parasites = Number\ of\ parasites\ found$$

Parasite documentation and identification

All parasites that were collected from the fish samples were documented and sketched and were identified up to the lowest level of identification.

RESULTS AND DISCUSSION

Endoparasite of Rohu carp

In this study, two helminth phyla were identified in *L. rohita* samples, a nematode (*Camallanus*sp.) and a trematode (*Clinostomum philippinensis*) (Fig. 1). Fishes act as final hosts to parasites and are intermediate hosts for larval stages of many parasites [5]. In *L.rohita*, common parasites belong to ciliophora, platyhelminthes, arthropod, and aschelminthes wherein heavy infections are observed in the intestine and stomach [6]. Moreover, ciliophoran parasites mostly occur in gills and skin, and more often than not, these parasites are mainly dominated by trematodes.

Eight percent (8%, N=100) of the examined samples were infected with parasites (Table 1). Subsequently, 6% of the fish samples were positive for *Clinostomum philippinensis*, while 2% were infected with *Camallanus*sp.

The two identified endoparasites were usually observed in smaller than larger Rohu carp. Similarly, findings showed that parasitic diseases may lead to the reduction in growth of smaller Rohu carp as they are more susceptible to parasitism, and their body cannot support parasite burden which could result to increased susceptibility [7].

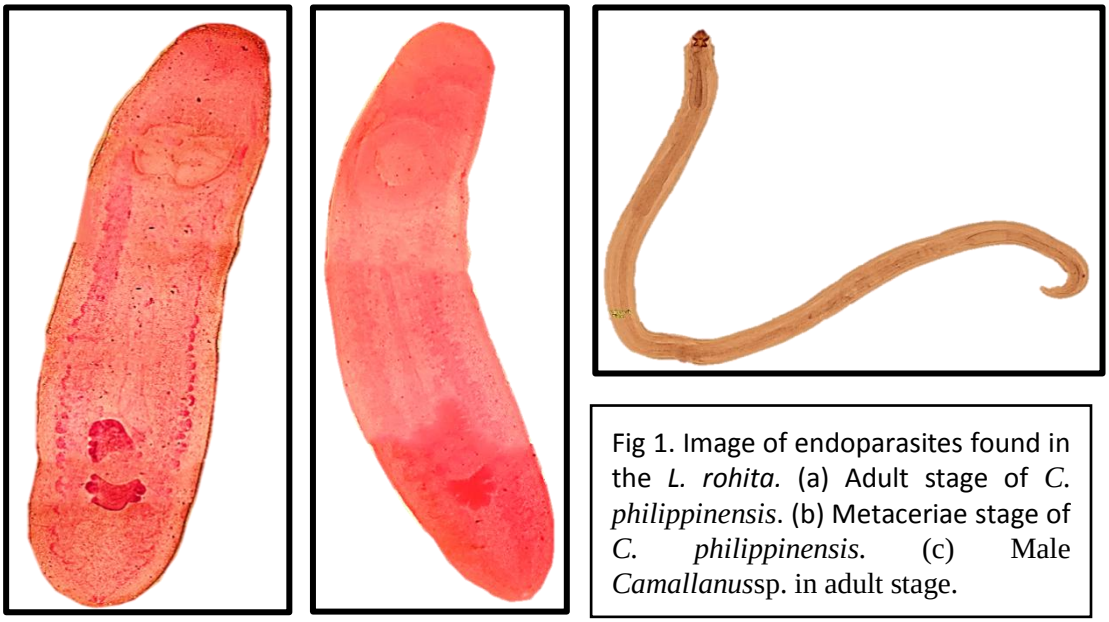

Fig 1. Image of endoparasites found in the *L. rohita.* (a) Adult stage of *C. philippinensis*. (b) Metaceriae stage of *C. philippinensis.* (c) Male *Camallanus*sp. in adult stage.

Table 1. Parasite species recovered in the internal organs of the samples freshwater fish, *Labeo rohita*from Lake Lanao, Buluan, Maguindanao.

Parasite	Internal Organs					
	Heart	**Intestine**	**Muscle**	**Kidney**	**Liver**	**Gills**
Nematode: *Camallanus*sp.	0	2	0	0	0	0
Trematode: *Clinostomum philippinensis*	0	0	0	0	0	6

Furthermore, parasites were commonly observed in the gills and intestines. Several fish studies have observed that trematodes were commonly found on gills, skin, and fin base, while nematodes were commonly found abundantly attached in the stomach and intestine which is in agreement with the result of the study [6], [8-10].

As observed in the study, infected *L. rohita* were characterized by the presence of pale gills, compared to uninfected samples. The body was also less slimy with scales that are easily detached while the eyes have yellowish to dark red color. Infected fish samples were also observed to have discoloration of the intestines, liver, and stomach accompanied by having a foul odor.

Prevalence, abundance and intensity parasites per batch samples of *Labeo rohita*

Table 2 shows that batch 9 samples have the highest prevalence of *C. philippinensis* infection with 22.22 % while batch 8 samples have the highest prevalence of infection of *Camallanus sp.* with 11.11%. On the other hand, highest abundance of *C. philippinenses* were observed in batch 9 samples and batch 11 samples while *Camallanus sp.* was abundant in batch 8 samples and batch 9 sample. Batch samples 8-11 have higher intensity as compared to other batch samples.

Table 2. The abundance, intensity and prevalence of endoparasites per batch samples freshwater fish of *Labeo rohita* from Lake Lanao, Buluan, Maguindanao.

Batch sample	Size of Fish Sample	Season	Parasite species	Number of fish examined	Abundance		Prevalence (%)	Intensity
					Infected	Number of parasite		
Batch sample 1	Large	Dry	-	6	0	0	0	0
Batch sample 2	Large	Dry	-	7	0	0	0	0
Batch sample 3	Large	Dry	-	6	0	0	0	0
Batch sample 4	Large	Dry	-	9	0	0	0	0
Batch sample 5	Large	Dry	-	7	0	0	0	0
Batch sample 6	Large	Dry	-	9	0	0	0	0
Batch sample 7	Large	Dry	-	8	0	0	0	0
Batch sample 8	Small	Wet	Nematode*: Camallanus sp.* Trematode*: Clinostomum philippinensis*	9	2	1 1	11.11 11.11	1
Batch sample 9	Small	Wet	Nematode:*Camallanus sp.* Trematode: *Clinostomum philippinensis*	11	3	1 2	9.09 22.22	1
Batch sample 10	Small	Wet	Trematode: *Clinostomum philippinensis*	9	1	1	11.11	1
Batch sample 11	Small	Wet	Trematode: *Clinostomum philippinensis*	12	2	2	16.66	1
Batch sample 12	Small	Wet	-	7	0	0	0	0

Table 3.The condition factor (K-Factor) per batch samples freshwater fish, *L. rohita* from Lake Lanao, Buluan, Maguindanao.

Number of batch samples	Number of fish examined	Length (cm)		Mean	Weight (g)		Mean	K-Factor Mean
		Max	Min		Max	Min		
Batch sample 1	6	23.9	19.3	20.766	140	90	115.0	1.293
Batch sample 2	7	24.2	18.7	20.316	148	90	113.833	1.366
Batch sample 3	6	33.6	22.3	28.214	304	121	213.142	0.928
Batch sample 4	9	31.9	24.8	28.144	302	186	224.0	1.029
Batch sample 5	7	32.2	18.0	21.385	286	90	122.571	1.222
Batch sample 6	9	30.2	18.4	22.9	248	88	145.666	1.184
Batch sample 7	8	29.4	17.8	20.35	233	76	116.875	1.373
Batch sample 8	9	21.8	17.8	20.044	121	80	101.555	1.268
Batch sample 9	11	25.2	17.7	20.5	152	75	98.090	1.112
Batch sample 10	9	20.8	18.4	19.7	102	81	90.333	1.183
Batch sample 11	12	23.3	18.1	20.625	119	73	93.833	1.063
Batch sample 12	7	21.6	18.4	19.857	102	79	90.428	1.158

The parasite prevalence and intensity depend on many factors like parasite and its life cycle, host and its feeding habits and the physical factors of water body where the fish inhabit [11]

Condition factor (K-Factor) per batch samples of *Labeo rohita*

The mean condition factor for *L. rohita*is shown in Table 3. The condition factor computed for *L. rohita* were 1.293, 1.366, 0.928, 1.029, 1.222, 1.184, 1.373, 1.268, 1.112, 1.183, 1.063 and 1.58. Generally, these values indicate that fish samples are in good condition during the examination.

Individual fish condition is determined based on the analysis of length and weight data reflecting that the heavier fish at a given length and weight is in better condition [12]. The length-weight relationships provide basic information in fisheries biology [13] In addition, the values of these factors depend on physiological features of fish, maturity, spawning, environmental factors, and food availability in a water [14] survival, reproduction, and health of fish [4].

CONCLUSION

Two endoparasites were identified in *L. rohita*, namely *Clinostomum philippinensis* and *Camallanus*sp. Only eight percent (8%) of the samples were infected with endoparasites; 6 % for *Clinostomum philippinensis* and 2 % for *Camallanus*sp. *Clinostomum philippinensis*were found on gills while *Camallanus sp*. were found attached in the intestine. Individual fish condition is determined based on the analysis of length weight data. The mean values of condition factor were in the limit of 0.928 to 1.373 which indicate that fish samples are in good condition during the examination.

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RECOMMENDATION

To increase sample size to obtain higher chances of parasite detection and equal size of samples to really test the K-factor; it is necessary to inform the individuals in the community about public awareness of the possible parasitic diseases and also to prevent the negative effects of parasitic infection.

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