 ISSN: 1656-0264

Parasite Infestation on *Oreochromis niloticus* (Linnaeus, 1758) from Selected Fish Farms In Cebu, Philippines

Gloria G. Delan[1], Raamah C. Rosales[1] , Rachel Luz V. Rica[1], Christine M. Corrales[1] and Anthony S. Ilano[2]
[1]ICRM-Cebu Technological University-Main Campus
[2]Department of Biology, University of San Carlos

ABSTRACT

This study was conducted to determine parasite infestation on *Oreochromis niloticus* Linnaeus (1758) cultured in the selected fish farms in the province of Cebu, Philippines. A total of 20 fish samples were collected from the selected fresh and brackish water fish farms and dissected on the day of collection. Microscopic examination was conducted on 100 specimens comprising of liver, muscle, intestines, gills, skin and scales dissected from the fish samples. A total of 806 organisms were collected from these organs of the fish host. Allorganisms identified were classified under 14 genera. The 12 genera belong to parasitic groups dominated by protozoans of ciliates and flagellates such as *Piscinoodinium*, *Trichodina*, *Cryptobia, Icthyophthirius, Chilodonella,* and*Ambiphrya.* Organismsbelonging to other genera of parasitic groups were *Dactylogyrus, Ergasilus,Myxozoa*, *Coccidia,* Microsporidiaandalarval fluke. There were two other organisms classified as *Navicula* and *Pleurosigma*, but were not in the parasitic group. Higher diversity of parasites occurred in fish samples from freshwater farms than in brackish water environment. The species diversity of parasites could have been influenced by some environmental factors.

Keywords: aquaculture, water quality, parasitism, fish host, mortality, protozoan

INTRODUCTION

Aquaculture has become a sustainable food source in the Philippines (Guerrero, 2007). It has generated jobs to thousands of local residents and provided livelihood program among the coastal communities (BFAR, 2013). However, some fish farms have reported economic loss due to some infections of fish diseases (Lio-Po, 1984). According to Cruz-Lacierda (2001), parasite infestation was cited as among the causes of fish mortality with debilitating effects. Health risk for human infections by eating raw and semi-raw fish has also been reported in Japan with an estimated 1 million individuals infected annually (Yasunaga *et.al.* 2010).

One common feature in parasite infestation is their variability among the populations of the same host species (Bagge *et. al.,* 2003). Detection of parasite is a great challenge since most of the organisms are microscopic such as parasitic organisms of the protozoan group.Previous studies have also described some fish parasites as host specific (Whittington et al., 2000). *Ecto* parasites infect the external parts while *endo* parasites infect internal organs.

 ISSN: 1656-0264

Environmental factors may facilitate parasite proliferation in a given culture system. Water qualities such as salinity, temperature and water pH are some of the variables that are associated with parasite abundance. Mud substrate was also identified as a reservoir of cystic parasite (Komar and Wendover, 2007). Fish population was also described as a determining factor of parasite infection as it increases the transmission of parasites by the increase of contact rate between and among the infected and uninfected fish (Arneberg, 2002).

This study presented a baseline information on the occurrence and prevalence of microscopic parasite infestation for cultured *Oreochromis niloticus*on some fish farms in the island of Cebu, Philippines.The growing population in the whole island demands more supply of food to sustain the ever growing need, hence food safety and sustainable aquaculture livelihood programs become imperative.

MATERIALS AND METHODS

Study Sites

The fish samples of *Oreochromis niloticus* Linnaeus (1758) were collected at the selected fish farms located in the municipalities of Moalboal, Alcantara, Barili, Daanbantayan and Carmen in the province of Cebu, Philippines (Fig. 1). Water quality parameters were determined from all the study sites which include pH, temperature, salinity, ORP (Oxidation Reduction Potential) and D.O. (Dissolved Oxygen). The two latter parameters were recorded using Hanna multi-parameter apparatus and Horiba apparatus, respectively. Geographical coordinates for the study areas were identified and recorded with Garmin GPS76CX.

Sample Collection

The collection of fish samples were done from the selected fish farms using a gill net. The culture systems used in the identified study sites were fish pen and pond with mud or concrete substrate constructed in either freshwater or brackish environment using semi-intensive culture system.

A total of 20 fish hosts were investigated for *Oreochromis niloticus* from five (5) sampling sites. The fish samples were randomly selected from unknown number of population from the fish farms. Each fish was weighed using a weighing scale in grams and measured from the tip of the snout to the tip of the caudal fin that constituted the total length in centimeter (Khan *et. al*., 2003).

 ISSN: 1656-0264

Figure 1. Map of Cebu located at the center of the Philippine archipelago with the study sites: Moalboal, Barili, Alcantara, Carmen and Daabantayan

Most of the fish samples were dissected on site while some were brought and dissected at the laboratory room of the Integrated Coastal Resources Management (ICRM) of the Cebu Technological University (CTU). The organs removed from each fish were intestines, liver, muscle, gills, skin and scales. The skin and scale were scraped from the back of the head to the tip of the caudal fin using a scalpel while the rest of the specimens were removed through a dissecting tool and kept in a sampling bottle with 90% ethyl alcohol ready for analysis.

Microscopic examination was conducted on 100 specimens (20 fish samples with 5 specimens/fish) for parasite infection on intestines, liver, muscle, gills and skin and scales. The slides were placed under a binocular microscope with 40x objective magnification using wet mount preparations and histological sections (Klinger and Floyd, 2013). The procedure for microscopic examination adapted by Alam and Alam (2014) was followed. This study has adapted the taxonomic identification keys of Klinger and Floyd (2013) supplemented with the study of Iyaji and Eyo (2008). Planktons were identified using the book of Suthers and Rissik (2008). The identification of parasites and other species was on genus level only.

RESULTS AND DISCUSSION

Prevalence and Identification of the Parasitic Species

A total of 806 organisms were collected from the specimens of *O*. *niloticus* found in the different organs of the fish hosts (Table 1). Parasites variability was higher in freshwater culture system than in brackish water. All the identified organisms were classified into 14 genera with 12 genera classified as parasitic while 2 genera were classified as plankton organisms. The collected organisms belong to parasitic groups of protozoan such as ciliates and flagellates. Other parasites identified were parasitic crustacean, monogenean and digenean.

 ISSN: 1656-0264

Table 1. Collected organisms in *Oreochromis niloticus* in freshwater and brackish water environment.

Species	Freshwater	Brackish water	TCO
Piscinoodinium Lom and Schubert 1983	+		382
Coccidia Leuckart 1879	+		160
Trichodina Ehrenberg 1830	+		154
Cryptobia Leidy1846	+		22
Ambiphrya Thompson, Kirkegaard and Jahn, 1974	+		21
Pleurosigma Smith 1890		+	20
Navicula Hendey 1958		+	15
Microsporidia	+		15
Myxozoa Jurine 1825	+		6
Icthyophthirius Fouquet 1876	+		5
Chilodonella Zacharias 1894	+		3
Ergasilus von Nordman 1832	+		1
Dactylogyrus Diesing1850	+		1
Larval fluke	+		1
Total			806

TCO = total number of collected organisms

Most of the parasitic organisms with high occurrence rate were the protozoan (Table 2). Protozoan has a simple life cycle with mostly have a direct or without the need of intermediate host (Klinger and Floyd, 2013). In relatively good environmental conditions, parasitic protozoan can rapidly multiply in short span of time utilizing the nutrients of the infected host (Stromberg, 1997). The occurrence of parasites have indicated species diversity infecting the different organs of a fish host.

Figure 2 shows the parasites identified that were infecting the different dissected organs of *O*. *niloticus* which include the intestine, liver, muscle, gills, skin and scales. The highest frequency of parasite infestation was recorded in gills of the fish samples collected from the fish farm from Barili followed by skin and scales of the fish samples collected from Moalboal. However, lower frequency of parasite infestation was recorded in liver, muscles and intestines of fish samples collected from the fish farms in Alcantara, Daanbantayan and Carmen.

 ISSN: 1656-0264

Table 2. Species of Parasites in *O. niloticus (*Pis: *Psicinoodinium;* Coc*: Coccidia;* Cry*:Cryptobia;* Tri*:Trichodina;* Dac: *Dactylogyrus;* Erg*:Ergasilus;* Amb*:Ambiphrya;* Ict*:Icthyophthirius;* Chi*:Chilodonella;* **Myx*: Myxozoa;* Mic*:* **Microsporidia; Lar: Larval Fluke)**

Location	Fish samples examined	Species											
		Pis	Coc	Cry	Tri	Dac	Erg	Amb	Ict	Chi	Myx	Mic	Lar
Moalboal	MO1	145	30	15								8	
	MO2	43	15		3							3	1
	MO3	40				1						4	
	MO4	13			5		1						
Alcantara	AO16				7								
	AO17		15										
	AO18							9					
	AO19							5					
Barili	BO31	15			5						6		
	BO32		75		35								
	BO33	85	25		12			7					
	BO34	41			67				5	3			
Daanbantayan	DO46												
	DO47												
	DO48												
	DO49												
Carmen	CO61				15								
	CO62			7	3								
	CO63												
	CO64				2								
Total	**20**	**382**	**160**	**22**	**154**	**1**	**1**	**21**	**5**	**3**	**6**	**15**	**1**

Figure 2 shows the parasites identified that were infecting the different dissected organs of *O. niloticus* which include the intestine, liver, muscle, gills, skin and scales. The highest frequency of parasite infestation was recorded in gills of the fish samples collected fromthe fish farm from Barili followed by skin and scales of the fish samples collected from Moalboal. However, lower frequency of parasite infestation was recorded in liver, muscles and intestines of fish samples collected from the fish farms in Alcantara, Daanbantayan and Carmen.

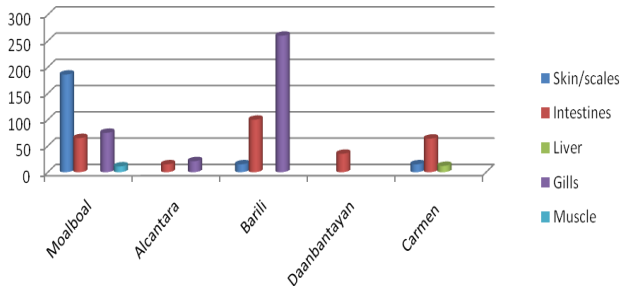

Figure 2. Frequency of parasite infestation on specific organs of *O. niloticus* from the sites.

Most parasites were tissue specific infecting specific organs or tissues of the fish hosts

 ISSN: 1656-0264

(Whittington *et al.,* 2000). It can be inferred from the result that parasites were not randomly collected in all the tissue samples. However, specific parasitic organism was collected consistently on specific tissue samples. The selective attachment of parasites can aid in identifying these organisms.

Trichodina and *Piscinoodinium* were identified as the parasites infecting the gills of the *O. niloticus* with the highest recorded frequency of infestation (Table 1). The former parasites werefound to infect the gills in all fish samples of *O. niloticus* collected in Carmen, Alcantara, and Moalboal. The parasite *Trichodina*was found to have the highest frequency and prevalence of infestations in Barili. Previous studies have revealed that *Trichodina* infects mostly the gills while *Piscinoodinium* was found to infect gills, skin and fins of the fish host (Litaker and Dykstra, 2007). These parasites can multiply rapidly and may cause serious damage to the infected organisms (Khan, 2004).

There were few numbers of *Cryptobia* species identified in this study infecting intestines and liver of *O. niloticus* collected from the fish farms of Moalboal and Carmen. These parasites normally cause granulomatous gastritis that and may develop into anemia although it was associated with low-level mortality on infected organisms (Floyd and Yanong, 2013). The microsporidians were collected in the muscle of *O. niloticus* from the fish samples coming from Moalboal. Microsporidia infects the muscle tissue of the fish host because it requires tissue for reproduction (Klinger and Floyd, 2013).

There were 3 organisms of *Chilodonella* collected in the skin of *O. niloticus* from fish samples collected in Barili. *Chilodonella* were known to be serious pathogens infecting the skin and gills of a fish host (Mitra and Haldar, 2004). Only 1 organism of genus *Ergasilus* was found infecting the gills of *O. niloticus* from the fish sample collected in Moalboal. It is a parasitic crustacean found in freshwater environment with warm weather but may cause only few problems on the infected fish host (Thatcher, 1998).

Dactylogyrus was found attached to the soft tissue of the gills of *O.niloticus* from the fish sample collected in Moalboal. This monogenean parasite has been described to infect preferably on gills of the fish host and the eggs were resilient to treatment (Klinger and Floyd, 2013). Larval digenetic fluke was found on the skin/scaleof *O. niloticus* collected in Moalboal.

Myxozoans were collected in theliver of *O. niloticus* from the fish samples collected in Barili. This parasite has been considered as pathogenic and responsible for whirling disease on infected fish host. There is no known treatment against myzoxoan infections thus causing high mortality rate in fish farms (O'Donoghue, 2005). The parasite of genus *Icthyophthirius* were collected inskin/scales of *O. niloticus* in Barili. This is an obligate parasite and requires no intermediate host. Severe infection in the gills causes asphyxiation and death to the infected fish host (Coyne *et. al.,* 2011).

The organisms of genus *Ambiphrya* were collected in skin and gills of *O. niloticus* from the fish samples in Barili and Alcantara. These parasitic species are common on pond reared fish

 ISSN: 1656-0264

infecting skin, fins and gills of the fish host (Klinger and Floyd, 2013). In the study of Abdel-Baki and Al-Quraishy (2014), *Ambipryha ameurii* were collected from specimens of *Oreochromis niloticus* cultured in the central region of Saudi Arabia. It was identified with its macronucleus, cylindrical shape, oral cilia and middle bank cilia.

Plankton organisms were also identified from specimens collected in brackish water environment such as *Pleurosigma* and *Navicula.* These organisms were found infecting the intestines of *O. niloticus* collected from the fish farm of Daanbantayan. These plankton groups were not recognized as parasitic but were included in the lists for record purposes.

Plankton were described either free living or parasitic (Alves-de-Souza *et al*., 2015). Parasitic plankton can infect almost all organisms in its environment including other planktons, crustaceans and fishes (Coats*et al.,* 2008). The most widely known planktons that adapted parasitic strategies were the dinolagellates of genus *Blastodinium* responsible for algal bloom or "red tide" (Skovgaard e*t. al.,* 2012).

Water Quality Characteristics of the Fish Farms

The water quality characteristics of the fish farms for *O. niloticus* were investigated for the possibility of environmental influence of parasitism. The water parameters include D.O. (dissolve oxygen), pH, TDS (total dissolve substance), salinity, ORP (Oxidation Reduction Potential, and Temperature. The environmental conditions for the water quality of the fish farms for *O. niloticus* are presented in Table 3. The *O. niloticus* has been recognized as highly tolerant to a wide range of environmental conditions (Mjoun *et. al.* 2010).

Table 3. Water quality characteristics of the fish farms for *O. niloticus.*

Fish farms	Water quality					
	D.O. (ppm)	pH	TDS (ppm)	Salinity (ppt)	ORP	Temperature (°C)
Moalboal	3.7	7.5	157	1.47	8	28.5
Alcantara	6.7	7.6	158	1.47	77.8	27.99
Barili	5.6	7.41	145	0.41	101.3	28.85
Daanbantayan	10.1	7.65	432	4.2	32.5	32.9
Carmen	1.1	7.15	428	4.2	40.6	28.44

The lowest recorded dissolved oxygen was in Carmen with 1.1 ppm. This rate was considered low by Tsadik and Kutty (1987) in their study on the influence of ambient oxygen on the feeding and growth of *O. niloticus* with the lowest dissolve oxygen treatment at 1.3 ppm. Their study has described less feeding activity and lower growth rate of the *O. niloticus* at low dissolve oxygen levels. This species can tolerate low dissolved oxygen levels even at 0.1 mg/L (Magid and Babiker, 1975) but optimum growth can be obtained at concentrations more than 3 mg/L (Ross, 2000). It can tolerate a pH range of 3.7 to 11. These may have serious negative implication since it may weaken the defense mechanism of the fish and render it susceptible to

 ISSN: 1656-0264

parasite infection (Thatcher, 2006).

The recorded pH levels ranged from 7.5 to 7.65. According to Ross (2000), *O. niloticus* can tolerate a pH range of 3.7 to 11, but optimum growth can be achieved between pH 7 to 9 of which the recorded pH levels in this study perfectly fit. The salinity ranged from 4.2 to 4.6 ppt and it was recognized that the optimum growth of *O. niloticus* can be achieved with salinity level 0 to 18 ppt (El-Sayed, 2006).

Temperature has been considered as a major metabolic modifier for *O. niloticus*. The best temperature for growth was established at 22°C to 29°C while spawning was best at temperatures more than 22°C (Mires, 1995). In comparison with the results, the temperature for the fish farms in Moalboal, Alcantara, Barili, and Carmen has the best temperature for growth while the temperature for Daanbantayan was slightly higher which could affect the growth of the cultured fish.

The oxidation reduction potential varies in different locations. The oxidation reduction potential (ORP) may reflect the water quality since it measures the ability of a body of water to cleanse itself such as contaminants and dead plants and animals with the presence of bacteria that decomposes them (Horne and Goldman, 1994). The highest recorded ORP was in Barili and itcan be inferred that this fish farm has the highest number of bacteria with a higher presence of contaminants.

The total dissolve solids (TDS) were high in Daanbantayan and Carmen with 432 and 428 respectively. The TDS may refer to any minerals other than the pure water molecule present in the pond. This may provide the idea that the fish farms with higher TDS levels may likely have more inorganic salts, organic matter and other dissolved materials in water (Weber-Scannell and Duffy, 2007).

The water characteristics in the sampling sites have provided the basic environmental requirements for the culture of *O. niloticus*. These conditions are favorable for the growth and survival of *O. niloticus* that has also provided the needed nutrients for the parasitic organisms to survive as well with the influence of some environmental factors. Most of the ponds were using mud substrate in their culture environment. In previous study, mud substrate can facilitate the development of parasites during cystic stage (Komar and Wendover, 2007). The temperature, pH and salinity are important factors for the survival of parasitic organisms that could have been provided by the sampling sites for both the fish hosts and parasites (Stromberg, 1997).

CONCLUSION

The diversity and abundance of parasites vary evidently from the different fish farms of *O. niloticus*. Environmental factors of the different sampling areas could have influenced the variability of the parasitic organisms.

 ISSN: 1656-0264

ACKNOWLEDGMENT

We thank the personnel of the local government units for allowing us to conduct the study in their respective localities. We also thank the staff of the ICRM/CTU for their assistance in the collection of specimens and preparation of the materials. This study was supported by the Research Department of the Cebu Technological University, Cebu, Philippines.

LITERATURE CITED

Abdel-Baki, A.S. and Al-Quraishy, S., 2014. First Record of *Chilodonella* spp. (Ciliophora: *Chilodonellidae*) in Cultured Nile Tilapia (*Oreochromis niloticus*) in the Central Region of Saudi Arabia. Pakistan J. Zool., 46: 657-660.

Alam, M.H. and Alam, M.J., 2014. A comparative study of endoparasite infestation of *Oreochromisniloticus* (Linnaeus, 1758) in polluted and non-polluted water bodies of Bangladesh. International Journal of Fauna and Biological Studies. 1: 04-09.

Alves-de-Souza, C., D. Pecqueur, E. L. Floc'h, S. Mas, C. Roques, B. Mostajir, F. Vidussi, L. Velo-Suárez, M. Sourisseau, E. Fouilland, and L. Guillou, 2015. Significance of Plankton Community Structure and Nutrient Availability for the Control of Dinoflagellate Blooms by Parasites: A Modeling Approach. Journal Plos One. 10: 1-15.

Arneberg, P., 2002. Host population density and body mass as determinants of species richness in parasite communities: comparative analyses of directly transmitted nematodes of mammals. Ecography. 25: 88-94.

Bagge, A.M., R. Poulin and E.T. Valtonen, 2004. Fish population size, and not density, as the determining factor of parasite infection: a case study. Parasitology. 128: 305-313.

Bureau of Fisheries and Aquatic Resources, 2013. Philippine Fisheries Profile 2013. Department of Agriculture, Quezon City, Philippines, 65pp.