

FULL PAPER

Assessment on the Occurrence of Anisakid and other Endoparasitic Nematodes Infecting Commercially-Important Fishes at Tayabas Bay

Maribeth H. Ramos, Trazarah Hanoof E. Argarin, Beatriz A. Olaivar

Bureau of Fisheries and Aquatic Resources, Regional Office 4A, Bambang Los Baños, Laguna Philippines

ABSTRACT

Anisakid nematodes are parasites commonly present in the marine environment. Parasites belonging to the family Anisakidae or the genus *Anisakis* can cause two different clinical manifestations: gastrointestinal disorders and allergic reactions known as anisakiasis. In this study, we examined 7,126 marine fishes belonging to four different commercially-important fish species; *Rastrelliger kanagurta*, *Sardinella lemuru*, *Atule mate*, and *Selar crumenophthalmus* for the presence of anisakid and other endoparasitic nematode infection. The fishes caught from Tayabas Bay were bought from three different landing sites from March 2017 to February 2018. The gonads, liver, and stomach of each fish species were incubated for 12-18 hours for rapid isolation and endoparasite evaluation. After the isolation of parasites, anisakid nematodes were fixed in vials with 70% ethanol for morphological analysis under the microscope. Six anisakid groups of genera, including *Hysterothylacium*, *Terranova*, *Anisakis*, *Contraecum*, *Raphidascaris*, and *Camallanus*, and a non-anisakid group *Echinorhynchus* were identified. The results showed that the prevalence of anisakid infection in all species was 24.18 %, with a mean intensity of infection of 1.91. *Rastrelliger kanagurta* (Dalahican), *Atule mate*, and *Selar crumenophthalmus* were the most infected with 50.90%, 38.98%, and 30.52% prevalence rate, respectively, followed by *Rastrelliger kanagurta* (San Francisco) (24.18%) and *Sardinella lemuru* (7.46%). The collected data suggest that commercially-important fish caught in the Tayabas Bay waters are susceptible to parasitization by larvae of the genus *Camallanus* followed by *Hysterothylacium* and *Terranova* in their visceral organs. The prevalence of anisakid infection was almost similar between female (45.3 %) and male (47.21 %) fishes with a mean intensity of 1.95 & 1.96, respectively. Also, larger fishes were heavily infected with anisakid larvae than small fishes. Thus, the intensity and prevalence of the fish parasite can be used as a biological tag for benchmarking and stock assessment purposes.

*Corresponding Author: nsap4a@yahoo.com
Received: January 28, 2020
Accepted: November 19, 2020

Keywords: Endoparasites, Anisakid nematodes, prevalence, intensity, biological tag, Tayabas Bay

1. INTRODUCTION

Marine fish are known to be infected by many different parasites. Parasitic nematodes constitute one of the earliest known groups of helminths in fishes (Molnár et al. 2006). Anisakid nematodes (Nematoda: Anisakidae) are common parasites of marine fish used as definitive, intermediate, or paratenic hosts (Cruz et al. 2007). Some nematodes are endoparasites in marine mammals, sea birds, and fish, with four major anisakids known to infect marine fish (Dadar et al. 2016). Larvae of anisakid nematodes are a significant problem for commercial

fishing industries and are potential human health hazards, both as causative agents of anisakiasis and potential food-borne allergens (Daschner and Pascual 2005). Anisakids with economic and public health importance are *Anisakis*, *Pseudoterranova*, *Contraecum*, genera of family Anisakidae (EFSA 2010) while *Hysterothylacium*, the genus of family *Raphidascarididae*, is commonly considered not zoonotic (Iglesias et al. 2002), except for sporadic cases (González-Amores et al. 2015; Yagi et al. 1996). These are commonly found in the viscera and musculature of many teleost fish species (Costa et al. 2003).

In Europe, anisakid nematodes are the most relevant group of parasites in terms of consumer health risk and product quality, with *Anisakis* and *Pseudoterranova* as the genera of greatest concern because several species are considered a human health hazard (Mattiucci et al. 2017). Recent data on the possible use of anisakid nematodes have been presented as biological indicators of a) the definition of fish stocks within a multidisciplinary approach, b) integrity and stability of trophic webs, and c) habitat disturbance (Mattiucci and Nascetti 2008). They are also playing an essential role in marine ecosystems by affecting their hosts' population dynamics (Rohde 1993). The information on marine fishes' food habits, such as the predator-prey relationship, is useful to assess the role of marine fishes in the ecosystem (Bachok et al. 2004).

With increasing interest in fish production, parasite infestations are becoming a threat to fish health management. Thus, limited studies on the infection of endoparasitic nematodes in Philippine waters, especially in Tayabas Bay, encouraged the authors to perform parasitological studies.

The general objective of this study is to generate baseline data on the anisakid and camallanid worms found in commercially-important species as biological indicators in the formulation of policies for the sustainable management of Tayabas Bay. Specifically, the authors aimed to identify the occurrence of parasites infecting the commercially-

important marine fishes; to assess the prevalence, abundance, and intensity of endoparasitic nematodes infecting marine fishes at Tayabas Bay; and to correlate the sex, length, weight, and gonadal stage of the marine fishes with the number of parasitic infections at Tayabas Bay.

2. MATERIALS AND METHODS

2.1 Study area

Tayabas Bay is one of the major fishing grounds in the CALABARZON Region. It has the second largest area in the region by 2,213 km² next to Lamon Bay with 2,838 km². The bay covers 16 municipalities and one city in three provinces: Quezon, Marinduque, and Batangas. In Quezon Province, Lucena City is the only city along Tayabas Bay; the 11 municipalities are Agdangan, Catanauan, General Luna, Macalelon, Mulanay, Padre Burgos, Pagbilao, Pitogo, San Francisco, Sariaya, and Unisan. It consists of 121 coastal barangays, which include the study area, namely Barangay Matandang Sabang Kanluran of Catanauan (13°58'65.89" N latitude & 122°27'11.33" E longitude), Silongin and Inabuan, Poblacion and Cawayan I of San Francisco (13°34'33.21" N latitude & 122°51'82.19" E longitude), and Barangay Dalahican of Lucena, as shown in Figure 1.

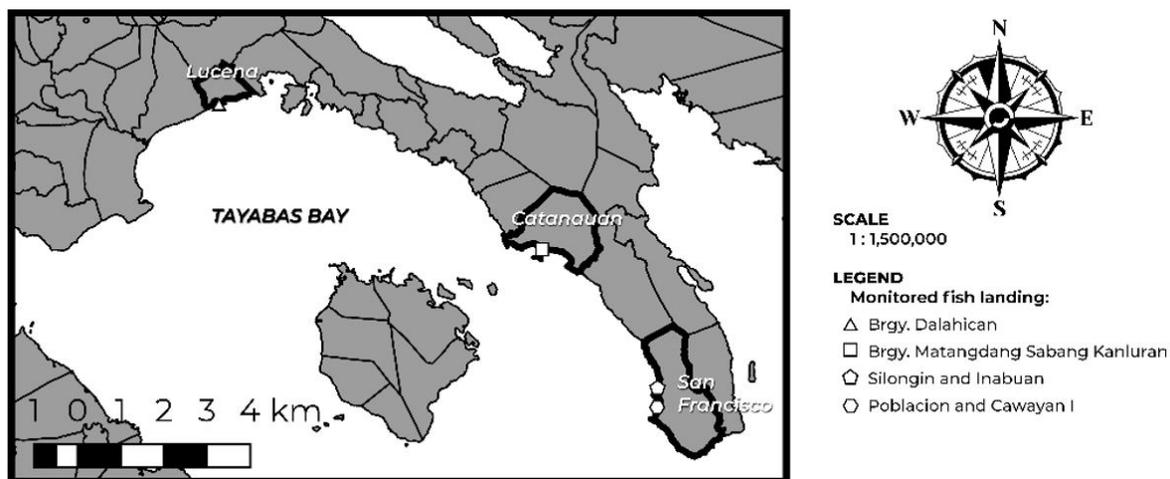


Figure 1. Map of Tayabas Bay showing commercially-important fish sampling sites where sampling for this study was undertaken.

2.2 Sample Collection and Preparation of Fish Sample

We collected samples of Bali sardine (*Sardinella lemuru*), Yellowtail scad (*Atule mate*), Bigeye scad (*Selar crumenophthalmus*), and Indian mackerel (*Rastrelliger kanagurta*) (Figure 2) in three landing sites of Tayabas Bay, specifically at Dalahican, Catanauan, and San Francisco, Quezon Province (Figure 1). The selection of fish samples depends on the abundant catch of the most operated fishing gears in the respective sampling area that are available whole-year-round. Sampling size varied among fish species and sampling localities. In general, smaller sized species like sardines were sampled and examined in large quantities compared to medium-sized species such as scad and mackerel (Table 1).

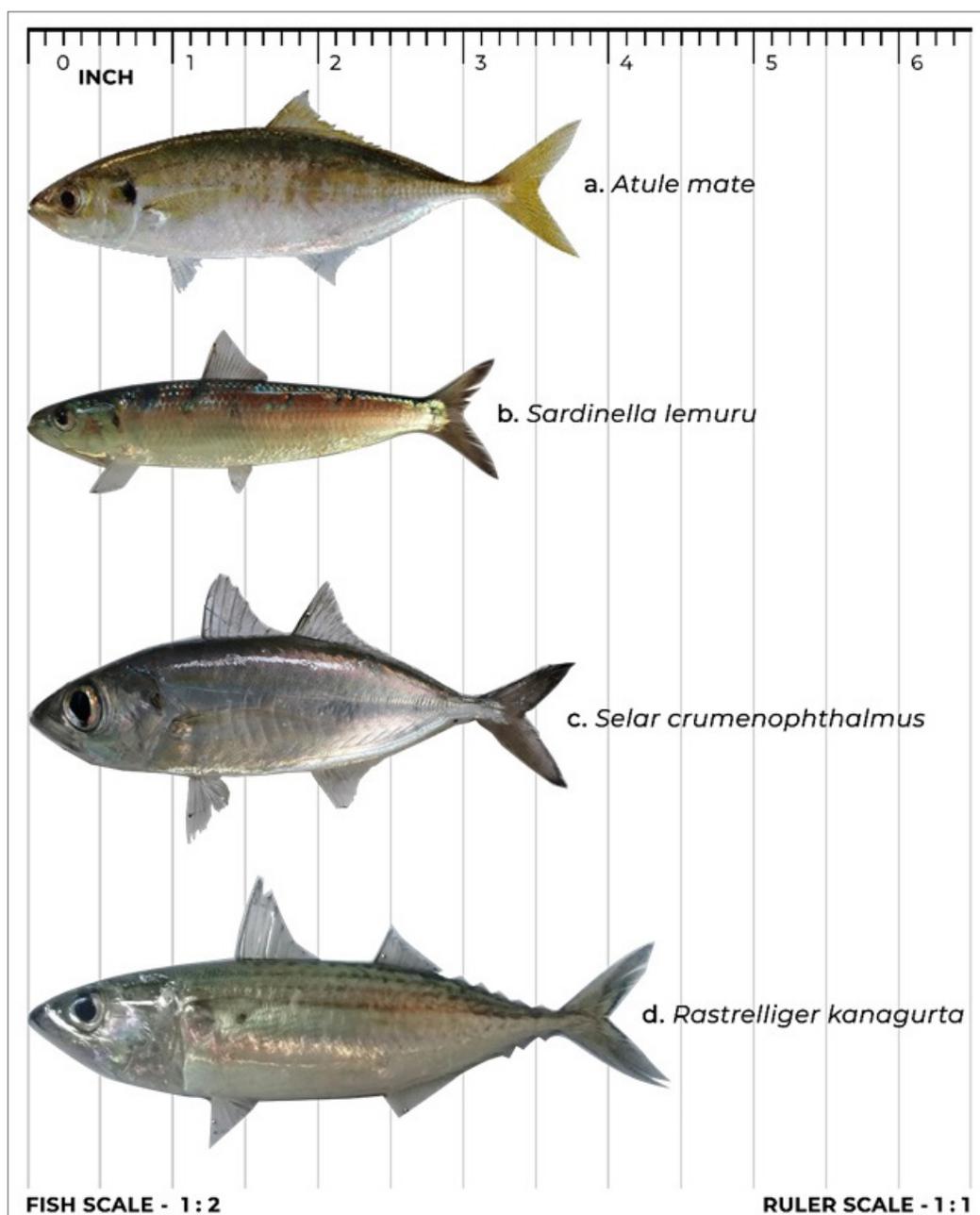


Figure 2. Commercially-important fishes of Tayabas Bay.

Table 1. Summary of the statistics of fishes: number of fish examined (N), number of infected fish (NI), sex ratio, mean length (cm) and mean weight (g), number of parasites (NP), prevalence (P%), mean abundance (MA) & mean intensity (MI) (standard error of the mean), median and infection rate (IR%) of larval anisakid, and camallanid nematodes parasites from the examined fish species in Tayabas Bay; D (Dalahican), SF (San Francisco).

Fish species	N	NI	Sex Ratio (M/F)	Mean length (cm)	Mean weight (g)	NP	P (%)	MA	MI ± SEM	Median	IR (%)
<i>Atule mate</i>	744	290	1.52	20.43±0.21	107.25±50.48	711	38.98	0.96	2.53 ± 0.09	2	95.56
<i>Rastrelliger kanagurta</i> (D)	719	366	0.76	23.26±0.11	143.99±41.84	946	50.90	1.32	2.56 ± 0.08	2	131.57
<i>Rastrelligerkanagurta</i> (SF)	827	200	1.22	24.13±0.16	156.79±49.26	302	24.18	0.37	1.77 ± 0.09	1	36.52
<i>Sardinella lemuru</i>	2,641	197	0.95	13.62±0.14	23.15±11.96	224	7.46	0.08	1.22 ± 0.08	1	8.48
<i>Selar crumenophthalmus</i>	2,195	670	1.04	20.95±0.08	115.93±38.31	884	30.52	0.40	1.52 ± 0.05	1	40.27
OVERALL	7,126	1,723	1.04	20.89±3.78	114.62±54.95	3,067	24.18	0.43	1.91 ± 1.88	1	43.04

The fish were randomly collected twice or thrice a month from each landing center to reach the ideal target of samples of 2-5 kg per species. Sampling was done for 12 months, starting March 2017 to February 2018. Commercially-important fishes in the area were bought from three landing sites and were preserved and transported to the laboratory for examination using plastic bags containing water with broken pieces of ice. Each fish sample was weighed to the nearest gram (g) and total length (cm) measured from the snout's tip to the longest caudal fin. The sex of the fish was also recorded before the parasitological examination.

2.3 Parasite Sampling

We removed the fish samples from the plastic bags and carefully dissected them for isolation of endoparasites. Each species of fish was opened dorsoventrally to collect the entire visceral organs. They are stored in box containers and kept at room temperature (37°C maximum) for 12-18 hours to simulate its final hosts' body temperature until it decomposes for easy collection of parasites.

2.4 Parasite Collection and Fixation

After the decay period, the partly decomposed visceral organs were

removed from plastic bags and placed immediately in a cylindrical tube or beaker and washed thrice with Physiological Saline Solution (PSS). These were then transferred in a petri dish and thoroughly examined for the collection of nematodes using an improvised needle. After the isolation of parasites, anisakid nematodes were fixed in 70% ethanol for morphological analysis under the microscope. Nematoda and Acanthocephala were dehydrated in a graded ethanol series and transferred to 100% glycerine for further identification purposes (Riemann 1988).

2.5 Parasite Identification

After clearing the parasites in glycerine, larvae were identified at the genus level by a compound microscope, according to the morphological criteria (Berland 1961; Gygi et al. 1995). Moreover, the identification of fish parasites was traditionally carried out using a series of classical keys based upon the morphology of the whole organism (Bruno et al. 2006). Further morphological examination revealed that larval specimens observed belong to families Camallanidae (genus *Camallanus*); Rhabdiascaridae (genus *Rhabdiascaris* and *Hysterothylacium*); Anisakidae (genus *Terranova*, *Anisakis* and *Contraecium*); and Coreidae (genus *Echinorhynchus*).

Santos and Moravec (2009) classified the genus *Camallanus* as medium-sized nematodes with thick, finely transversely striated cuticle and

large, orange-brown capsule typical on the genus, i.e., consisting of two sclerotized lateral valves internally supported by longitudinal ridges and each bearing two short sclerotized plates at anterior margin, short basal ring, and well-developed dorsal and ventral tridents.

The genus *Raphidascaaris* were identified as whitish nematodes with well-defined lips, cuticle with transverse striations and mucron, absent inter labia, muscular esophagus, and broader posteriorly than anteriorly representing 8.5-13.31% of its body length (Jahantab et al. 2014). *Hysterothylacium* is considered one of the largest of the ascaridoid genera parasitizing fish; these species have lips with lateral flanges, deep post labial grooves, inter labia and elongate intestinal caecum, pyriform ventriculus, long sac-like ventricular appendix, an expanded filamentary excretory system, and excretory pore near the nerve ring (Raffel and Anderson 2009).

The genus *Anisakis* were classified mainly based on the shape and length of the esophagus's glandular part (ventriculus) and the absence of the intestinal caecum and ventricular appendix (Abou-Rahma et al. 2016). *Contracaecum* spp. are parasitic nematodes known to have highly pathogenic impacts on wildlife (fish, birds, marine mammals) and humans. As the genus' name suggests, these nematodes have two oppositely-directed caecae as part of their digestive system. They also have an excretory pore located at their anterior end. These should be considered the most significant morphological characteristics when differentiating *Contracaecum* species from the rest of the anisakid nematodes because they are the most consistent at all developmental stages (Shamsi 2019). British explorers Leiper and Atkinson established the genus *Terranova* in 1914. Features of this nematode were large simple labia, an intestinal caecum that is slightly longer than the cylindrical ventriculus (Sprent 1970), and the absence of inter labia (Shamsi et al. 2019).

The genus *Echinorhynchus* is a member of the Palaeacanthocephala, an order which can be differentiated from the others by the following characteristics: definitive host habitat, two to eight multinucleated cement glands; sub-cuticular nuclei as numerous amitotic fragments or few highly branched nuclei; closed proboscis receptacle with two muscle layers and a single ligament sac (Wayland et al. 2015).

2.6 Statistical Analysis

The prevalence, mean intensity, and abundance of parasites present in different marine fishes were calculated using the formula proposed

by Margolis et al. (1982) and Bush et al. (1997). The measures of parasitic infection referred to in this study are prevalence (which is the number of fish infected divided by the number of fish examined, expressed here as a percentage) and mean intensity (the average intensity of a particular species of parasite among the infected members of a specific host species). In other words, it is the total number of parasites of a particular species found in a sample divided by the number of hosts infected with that parasite. The mean abundance is the total number of parasites of a specific taxon found divided by the total number of fish examined. The infection rate was calculated by dividing the total number of the larvae detected with parasite species by the total number of the host species (infected + uninfected) in the sample, multiplied by 100.

Summary statistics of prevalence, intensity, abundance, and infection rate of the parasites were generated. For further analysis, the Kruskal-Wallis test was used to determine the significant differences in the median number of parasites per sex, gonadal stage, and fish species. If the Kruskal-Wallis test is significant, Dunn's test was performed to determine which levels of the independent variable differ from each other; it is the pairwise comparison of the median test. Similarly, the Chi-square test of independence was used to determine if the species of fish and parasites are associated with each other. Furthermore, the relationship between explanatory variables (sex, species, gonadal stage, weight, and length of fish) and dependent variable (number of parasites) was tested using Quantile Regression Analysis.

The obtained data were then analyzed using Kruskal-Wallis, Dunn's test, Chi-square test (R version 4.0.1 package), and Quantile Regression Analysis in Stata Version 12.0.

3. RESULTS

The study results focused mainly on the identification, occurrence, and distribution of zoonotic anisakid species in the visceral parts of fish. The results showed that the prevalence of infection across all fish samples examined was 24.18% (1,723 infected out of 7,126 fish examined). We identified seven parasitic groups belonging to four families (Camallanidae, Rhabidascaaridae, Anisakidae, and Coreidae); six anisakid nematodes (roundworms) and one acanthocephalan (thorny-headed worms) (Figure 3). All fish species infected were particularly parasitized by those under the family Anisakidae. These were found mostly in the body cavity attached to the surface of visceral organs.

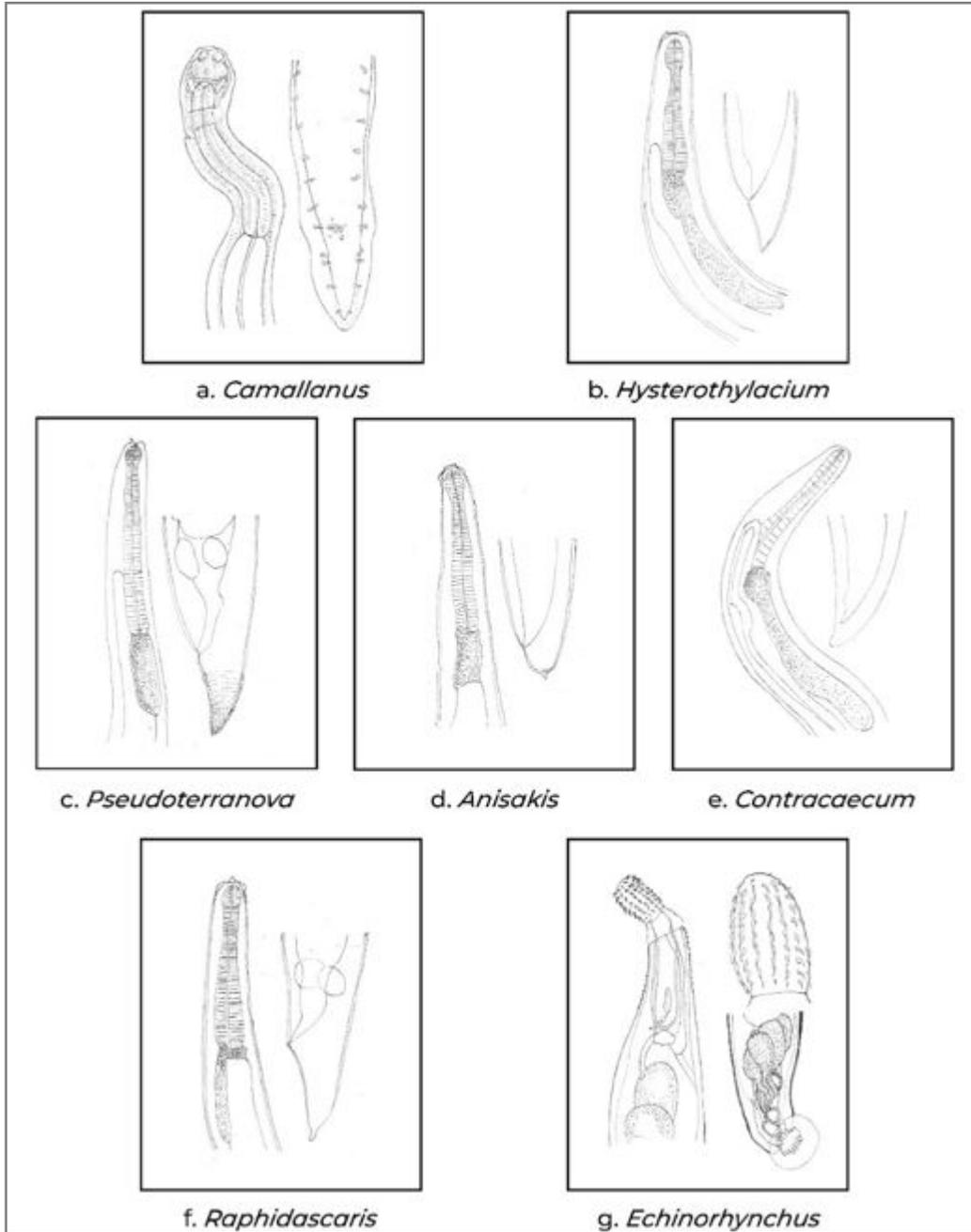


Figure 3. Images for the representative genera of parasites.

Results showed a 43.04% infection rate in all the fishes examined. It was established that among the number of fishes infected by parasites, 3,067 (2,508 nematodes; 51 acanthocephalans; 508 composed of not identified and broken parasites) were recorded. The highest prevalence (50.90% and 38.98%), mean intensity (2.56 and 2.53), and mean abundance (1.32 and 0.96) were observed in *Rastrelliger kanagurta* (Dalahican) and *Atule mate*, respectively, which were found to be dominantly infested with genus *Hysterothylacium* and *Camallanus*. A total of 914 specimens belonged to the genus *Camallanus* from the Camallanidae family, which was reported to be the most dominant family in terms of parasite infestation, followed by Rhapidascaridae, Anisakidae, and Coreidae.

The *Rastrelliger kanagurta* (San Francisco) was the longest and heaviest, with length and weight of 24.13 centimeters and 156.79 grams, respectively. The *Sardinella lemuru* has the least length and weight, with a mean length and weight of 13.62 centimeters and 23.15 grams, respectively. For the intensity of infection, both *Atule mate* and *Rastrelliger kanagurta* (D) have a median number of parasites of two (2 parasites). In contrast, *Rastrelliger kanagurta* (SF), *Sardinella lemuru*, and *Selar crumenophthalmus* have a median number of parasites of one (1 parasite).

The infection of anisakid parasites varied according to fish body weight. A high percentage was

detected with body weight ranges from 129-153 grams (20%) followed by body weights 105-129 grams (18%). Similarly, fishes with lengths 21.7-23.9 cm, 19.4-21.6 cm, and 23.8-26.1 cm showed a higher percentage of parasites, 29%, 25%, and 21%, respectively.

At a 5% level of significance, we have sufficient evidence to say that at least one of the sexes, gonadal stages, and species had a different median number of parasites. Moreover, it was found that the median numbers of parasites of male and female fishes do not significantly differ using Kruskal-Wallis and Dunn's test. Subsequently, the median numbers of parasites at gonadal stage 5 do not significantly differ from the other gonadal stages. The median number of parasites of *Atule mate* and *Rastrelliger kanagurata* do not significantly differ; only *Sardinella lemuru* and *Selar crumenophthalmus* differ from the other species.

4. DISCUSSION

Mixed infections had been reported among other helminthic parasites in different fish species (Varjabedian 2005). The present study observed that all species examined were infected with anisakid larvae (Figure 5). However, two fish species were infected by the family Anisakidae. This agrees with previous studies that reported that Anisakidae Skrjabin and Karokhin 1945 is a major family among the most reported larval parasites in fishes (Mattiucci and Nascetti 2008; Klimpel and Palm 2001).

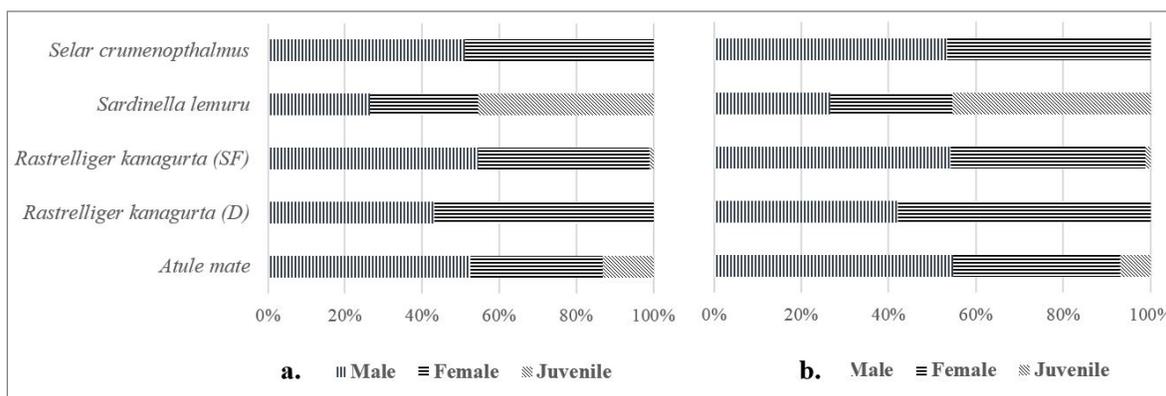


Figure 4. Frequency of a) infected fishes and b) number of parasites based on sex and species.

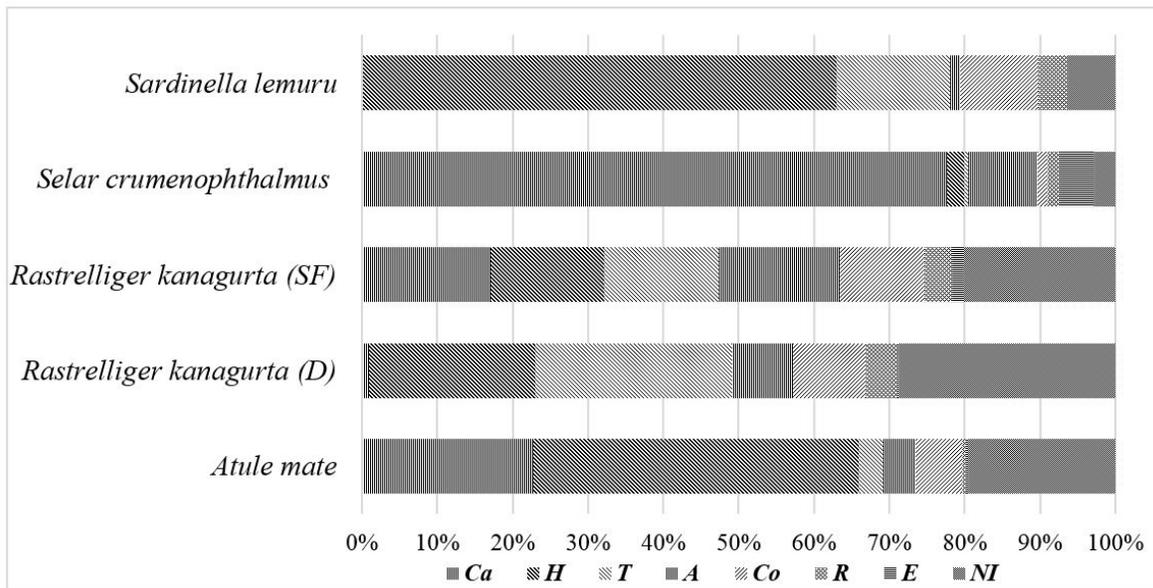


Figure 5. Summary of preliminary helminth data from commercially-important fishes collected at Tayabas Bay from March 2017-February 2018. *Camallanus* (Ca); *Hysterothylacium* (H); *Pseudoterranova* (P); *Anisakis* (A); *Contracaecum* (Co); *Raphidascaris* (R); *Echinorhynchus* (E); including the not identified (NI) species.

In the present study, the prevalence of four fish species examined reached 24.18%. It was revealed that the prevalence of anisakid infection was almost similar between female (45.3%) and male (47.21%) fishes with a mean intensity of 1.95 and 1.96, respectively.

Rastrelliger kanagurta (Dalahican) showed a high infection rate (131.57%) due to a high number of parasites detected over the total examined fish, followed by *Atule mate* (95.56%) and *Selar crumenophthalmus* (40.27%). Arthur et al. (1982) reported that the infection rate of larval anisakids varied with geographic location. Results showed that *Rastrelliger kanagurta* from Dalahican was higher than in San Francisco in terms of prevalence, intensity, and infection rate. It could be due to the uneven number of infected fish. MacKenzie et al. (2008) agreed that when the parasite fauna of two populations of the same fish species sampled from two different geographic areas is diverse, it means that the life-histories of those fish samples were different. It was revealed in the results of this study that different infection levels of the same fish species are different from each geographic location. Many studies gave variations in infection intensity among individual fish within a certain geographic area. Several ecological factors and host attributes can influence the number and diversity of parasites infecting hosts at the individual level (Cirtwill et al. 2015). In fish, these factors may include age or size, the number of different prey consumed, prey selectivity,

habitat, etc. (Poulin 2000; Johnson et al. 2004; Locke et al. 2014). Cipriani et al. (2017) reported that specific oceanographic or ecological factors at the actual fishing area have a greater effect on the certain parasite infection level than specific fish host characteristics such as body size.

The results indicated no significant differences between the prevalence of anisakid infection and the body length of fishes. This finding agreed with Olurin et al. (2012) but disagreed with others (Khanum et al. 2011; Dan-Kishiya et al. 2012; Yakhchali et al. 2012; Esiest 2013; Idris et al. 2013). The relationship between parasite infection and host body length varied according to host and parasite (Hila Bu and Leong 1999). The study revealed that parasite diversity depends on the size of its host. The larger the fish host, the larger sizes of parasites collected compared to small fishes. Among the four commercially-important fish sampled, *Sardinella lemuru*, measuring 9.4-19.3 cm in total length, had the smallest number and size of parasites compared to larger fishes like *Rastrelliger kanagurta*, *Selar crumenophthalmus*, and *Atule mate* measuring 12.1-30.6 cm, 15.7-27.1 cm, and 11.3-28.9 cm, respectively. This was attributed to the variation of fish length and the feeding upon crustacean as intermediate hosts or due to an accumulation of parasites in the host in its life (Bussmann and Ehrich 1979) or variations of fish diet (Valero et al. 2006). The shift in the dietary preference with age or size of the fish samples illustrates that size can also be an essential

determinant of helminth richness and abundances in fish hosts (Johnson et al. 2004; Poulin and Leung 2011).

The body weight had an effect on the prevalence of anisakids and total infected fishes. This finding agreed with Yakhchali et al. (2012). Based on the quantile regression plot (Figure 6a), the explanatory variables (sex, species, gonadal stage, weight, and length of fish) do not contribute to the variability in the intensity of infection for the fishes that do not have a high number of parasites. This implies that the

mentioned variables of fishes having small numbers of parasites and the infection intensity are independent. However, for fishes having a high number of parasites, the weight, species, and gonadal stage of fish started to have a significant effect on the intensity of infection. These variables have positive effects on the intensity of infections except for *Sardinella lemuru*, which has a negative effect on the intensity; that is, the number of parasites is less on this species. On the other hand, fishes' sex and weight do not have a significant contribution to the intensity of infection of fishes at any number of parasites.

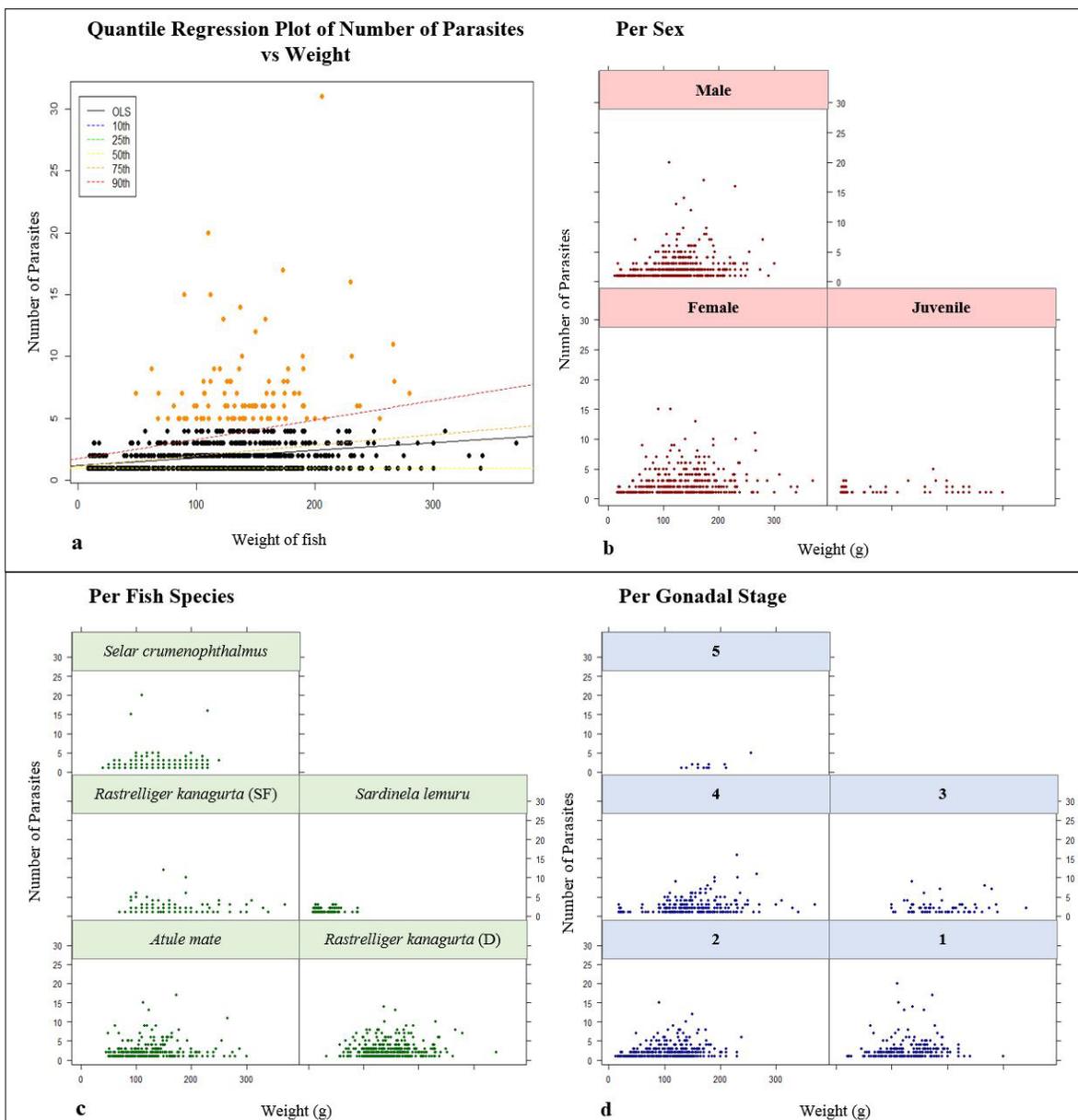


Figure 6. Scatterplot of a) weight (g) vs number of parasites, b) weight vs number of parasites per sex (Female=F, Juvenile=J and Male=M), c) weight vs number of parasites per species, and d) weight vs number of parasites per gonadal stage.

For fish with low numbers of parasites, plots are scattered (Figure 6c). The weight does not seem to contribute to the number of parasites; however, for *Atule mate* with a high number of parasites, the weight seems to positively affect the number of parasites. Thus, body weight affects the prevalence of anisakid larvae parasites and total infected fish. Other illustration shows that when fish have low numbers of parasites, weight does not contribute to the number of parasites. However, for fish with a high number of parasites, the weight seems to have a positive effect on the number of parasites for both male and female fish (Figure 6b). In Figure 6d, the plots are scattered for fish with low numbers of parasites. The weight does not seem to contribute to the number of parasites. However, except for gonadal stage 5, fish with a high number of parasites, the weight seems to have a positive effect on the number of parasites. The prevalence increased with increasing the fish body weight, which may be due to the increase and growth of the internal organs of the hosts leading to the increase in the surface areas of infection as suggested by Hagrais et al. (1995), or could be due to exposure time of infection (Muzzall et al. 1990).

The identification of parasitic nematodes on the commercially-important fishes of Tayabas Bay and an indication of its abundance reveals that these zoonotic parasites contribute as a biological tag for fish stock assessments. Quiazon (2015) discussed that the basic principle on the use of parasites as a biological tag is that a fish becomes infected by a parasite species only when it is in the endemic area of that parasite. The endemic area of the parasite is the geographical region where the abiotic (temperature and salinity) and biotic (presence of suitable intermediate and definitive hosts) factors are ideal for the transmission and completion of its life-cycle (Quiazon 2015). All sample species in this study are inhabited in coastal bays (pelagic fishes) but vary by the use of fishing gears operated in the area. Thus, the results showed a higher infection rate of anisakids on fishes caught by gillnets (bottom set gillnet, drift gillnet), particularly in *R. kanagurta* from Dalahican, followed by *A. mate* from Catanauan, which also uses gillnets and hook and lines. A higher prevalence of anisakid infestation depended on the hosts' availability in the region, the parasite's ability to complete its life cycle, its food ingested, and to the water column layer inhabited; bottom versus pelagic (Palm et al. 2007).

Quiazon (2015) assumes that when a fish population is found infected by a parasite species, it means that the fish has spent part of its life-history

in the parasite's endemic area, where fish behavior and feeding habits could result in different infection levels by that parasite species. In terms of parasite infestation, at a 5% level of significance, this study has sufficient evidence to say that the fish species and the identified parasites are not independent. This means that a certain type of fish species is likely to have more of a certain parasite type. Hence, a parasite can be used as a suitable biological tag for fish stock identification when its geographical distribution and life-cycle are known and when the parasite's residence time in the host is long enough compared to the lifespan of the fish host (Quiazon 2015). In this sense, the parasite as a biological marker reflects the fish population's geographic origin on a spatial scale. Indeed, the genetic or molecular markers define the stock on the evolutionary temporal scale, while the parasite taxa characterize the stock on a geographical scale (Mattiuci et al. 2014).

Since nowadays, the use of parasites as "biological tags" has become a useful tool, mainly concerning species with a high commercial value in fisheries (Quiazon 2015), parasites can provide ecological information on the origin, migration, nursery ground, and life-history of the fish species (Thomas et al. 1996). Parasites have also been considered favorable biological tags for discriminating different stocks or populations of marine fishes. Anisakid larvae have been reported as a useful tool for stock recognition of fishes in Europe and Asia (MacKenzie 2002; Quaizon et al. 2011; Mattiuci and Nascetti 2008).

5. CONCLUSION

In conclusion, it is found that the commercially-important fishes caught in Tayabas Bay harbor seven distinctly identified helminth parasites belonging to the genus *Anisakis*, *Hysterothylacium*, *Contracaecum*, *Pseudoterranova*, *Raphidascaris*, *Camallanus*, and *Acanthocephala*. Nematode larvae of the genus *Camallanus* and *Hysterothylacium* are common parasites in *Rastrelliger kanagurta*, *Selar crumenophthalmus*, *Atule mate*, and *Sardinella lemuru*, all of which are consumed mostly by the people in the area.

The infection rate of *Rastrelliger kanagurta* at Dalahican is higher than in San Francisco, which may be caused by the urbanization affecting the water quality in the area. Further studies on geographical characteristics should be conducted to assess the intensity and infection rate of different areas.

The identification of parasitic nematodes on the commercially-important fishes of Tayabas Bay, as well as an indication of its abundance, reveals that these zoonotic parasites contribute as a biological tag for fish stock assessments. Hence, it is needed to correctly identify the parasites before they can be used as biological tags. Generally, anisakid larvae are very difficult to identify to species level using morphology due to the lack of character variation. The larva will then be identified at species-level when they reached the adult stage, wherein they are already described and genetically categorized. The results of this study suggest that commercially-important fishes caught in Tayabas Bay waters are morphologically identified and susceptible to parasitization of larvae of genus *Camallanus* followed by *Hysterothylacium* and *Terranova* in the visceral organs.

At present, these parasites do not cause a significant public health problem among the population in Quezon Province, particularly those residing near the Tayabas Bay waters. This is due to locals' dietary habits not favoring the consumption of raw or improperly cooked fish. However, it is necessary to be more careful in using them for beneficial purposes in improving fishery products, food safety, and security. Also, another year of sampling with an even quantity of samples is recommended for further studies. Following the recommendations on using a combination of morphological and molecular analysis may, therefore, be more robust for identifying and discriminating final species of parasite taxa and should be considered in future studies using parasites as biological tags.

6. ACKNOWLEDGMENT

We express our profound gratitude to those who offer time, effort, and passion for supporting this research. To the NSAP Assistant Project Leaders and Analysts, Louie V. Gatlabayan, Catherine N. Amican, Rosalie L. Abreu, Philip Jesse Jay M. Dicediquin, Ma. Sena S. Erta, Mark John B. Rosales, and Princess Marie Alynagen L. Roque thank you for your untiring effort in encoding, data processing, and consolidating.

To the NSAP enumerators in the selected landing sites of Tayabas Bay, Arlene A. España, Jesusa P. Balbin, Glenn R. Siasa, Christopher H. Bautista, Angelo O. Baliza, Norberto D. Villaflor, Arnel M. Arsolon, Allan Ray L. Abesamis, and Monchito M. Villanea, who bravely faced the unexpected challenges to perform their responsibilities as data gatherers, we sincerely thank you.

Gratitude was also extended to Joshua P. Crisostomo and Dennis B. Macalindong for sharing their expertise in photo editing; to Dr. Karl Marx A. Quiazon, for his assistance in the identification of parasitic infestation and his valuable advice on the research; and to Mr. Josel F. Afunggol for mentoring in statistical analysis.

7. REFERENCES

- Abou-Rahma Y, Abdel-Gaber R, Kamal Ahmed A. 2016. First Record of *Anisakis simplex* Third-Stage Larvae (Nematoda, *Anisakidae*) in European Hake *Merluccius merluccius lessepsianus* in Egyptian Water. *J Parasitol Res.* [cited 2020 June 8]; 2016: 1-8. Available from: <https://doi.org/10.1155/2016/9609752>
- Arthur JR, Margolis L, Whitaker DJ, McDonald TE. 1982. A quantitative study of economically important parasites of walleye Pollock (*Theragra chalcogramma*) from British Columbian waters and effects of postmortem handling on their abundance in the musculature. *J. Fish. Res. Bd. Can.*, 39(5): 710-726. DOI: 10.1139/f82-100
- Bachok Z, Mansor MI, Noordin RM. 2004. Diet composition and food habits of demersal and pelagic marine fishes from Terengganu waters, east coast of Peninsular Malaysia. *NAGA, World Fish Center Quarterly.* [cited 2020 May 7]; 27(3-4): 41-47. Available from: <https://pubs.iclarm.net/Naga/naga27-3n4/pdf/article08.pdf>
- Berland B. 1961. Nematodes from some Norwegian marine fishes, *Sarsia*, 15(2): 1-50. DOI: 10.1080/00364827.1961.10410245
- Bruno DW, Nowak B, Elliott DG. 2006. Guide to the identification of fish protozoan and metazoan parasites in stained tissue sections. *Diseases of aquatic organisms.* 70(1-2): 1-36. [cited 2020 June 24]. DOI: 10.3354/dao070001
- Bush AO, Lafferty KD, Lotz JM, Shostak AW. 1997. Parasitology meets ecology on its own terms: Margolis et al revisited. *Journal of Parasitology.* [cited 2020 May 7]; 83(4): 575-583. Available from: <https://doi.org/10.2307/3284227>
- Bussmann B, Ehrlich S. 1979. Investigations on infestation of blue whiting (M-P) with larval

- Anisakis* sp. (Nematoda: Ascaridida) Arch. Fischer Eiwissens Chaf. 29:155-65.
- Cipriani P, Sbaraglia GL, Palomba M, Giulietti L, Bellisario B, Bušelić I, Mladineo I, Cheleschi R, Nascetti G, Mattiucci S. 2017. *Anisakis pegreffii* (Nematoda: *Anisakidae*) in the European anchovy *Engraulis encrasicolus*, from the Mediterranean Sea: fishing ground as a predictor of the parasite distribution. Fish. Res. <http://dx.doi.org/10.1016/j.fishres.2017.03.020>.
- Cirtwill A, Stouffer D, Poulin R, Lagrue C. 2015. Are parasite richness and abundance linked to prey species richness and individual feeding preferences in fish hosts? Parasitology. 143: 1-12. [cited 2020 May 7]; DOI: 10.1017/S003118201500150X
- Costa G, Pontes T, Mattiucci S, D'Amelio S. 2003. The occurrence and infection dynamics of *Anisakis* larvae in the black-scabbard fish, *Aphanopus carbo*, chub mackerel, *Scomber japonicus*, and oceanic horse mackerel, *Trachurus picturatus* from Madeira, Portugal. Journal of Helminthology. 77(2): 163-166. [cited 2020 June 8]. Available from: <https://doi.org/10.1079/JOH2002156>
- Cruz C, Barbosa C, Saraiva A. 2007. Distribution of larval anisakids in blue whiting off Portuguese fish market. Helminthologia. [cited 2020 May 7]; 44: 21-24. Available from: <https://doi.org/10.2478/s11687-006-0051-8>
- Dadar M, Alborzi A, Peyghan R, Adel M. 2016. Occurrence and Intensity of Anisakid Nematode Larvae in Some Commercially Important Fish Species in Persian Gulf. Iranian Journal of Parasitology. [cited 2020 June 7]; 11(2): 239-246. Available from: <http://ijpa.tums.ac.ir> <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5236102/pdf/IJPA-11-239.pdf>
- Dan-Kishiya AS, Oboh1 A, Ibrahim UB. 2012. The prevalence of Helminthes parasites in the gastro-intestinal tract of wild African sharp tooth cat fish *Clarias gariepinus* (Siluriformes: Clariidae) in Gwagwalada, Nigeria. J. Cuadernos de Invest. Uned. 5(1):83-87. [cited 2020 June 25]. Available from: <https://doi.org/10.22458/urj.v5i1.189>
- Daschner A, Pascual C. 2005. *Anisakis simplex*: sensitization and clinical allergy. Curr Opin Allergy Clin Immunol. 5:281-285. Available from: <https://doi.org/10.1097/01.all.0000168795.12701.fd>
- EFSA Panel on Biological Hazards (BIOHAZ). 2010. Scientific Opinion on risk assessment of parasites in fishery products. EFSA Journal. 8(4):1543. [91 pp.]. [cited 2020 June 25]; Available from: www.efsa.europa.eu. DOI: 10.2903/j.efsa.2010.1543
- Esiest ULP. 2013. Length-weight relationship and parasites of *Chrysichthys nigrodigitatus* in Cross River Estuary Itulocal government area Akwa Ibom State, Nigeria. J. Agric. Sci. [cited 2020 June 24]; 2(7): 154-165. Available from: <http://www.basicresearchjournals.org>
- González-Amores Y, Clavijo-Frutos E, Salas-Casanova C, Alcain-Martínez G. 2015. Direct parasitological diagnosis of infection with *Hysterothylacium aduncum* in a patient with epigastralgia. Rev Esp Enferm Dig. 107(11):699-700. [cited 2020 June 24]; Available from: <http://scielo.isciii.es/pdf/diges/v107n11/imagenes2.pdf> PMID: 26561913
- Gygi D, Rahman M, Lai H, Carlson R, Petter J, Hughes C. 1995. A cell - surface polysaccharide that facilitates rapid population migration by differentiated swarm cells of *Proteus mirabilis*. Molecular Biology. Available from: https://doi.org/10.1111/j.1365-2958.1995.mmi_17061167.x
- Hagras AEM, El-Naggan MM, Mansour MFA, El-Naggan AM. 1995. Influence of age, length and sex of the catfish *Clarias lazera* on infestation with Six Monogenean parasites. Mans. ScL Bull. (Biol). [cited 2020 June 16]; 22(2):37-55. Available from: http://online.reed.es/Revistas/REED_2015_107_11/Contenido/pdf/vol107num11_en_9.pdf
- Hila Bu S, Leong TS. 1999. Spatial distribution of Gill Monogeneans in a Tropical Cyprinid from Cenderuh Reservoir, Perak, Malaysia. Malay. Nat. J. 53:239-247.
- Idris HS, Balarabe-Musa B, Osawe SO. 2013. The

- Incidence of endoparasites of *Clarias gariepinus* (sharp tooth Catfish) (Burchell, 1822) and *Oreochromis niloticus* (Tilapia fish) (Linnaeus, 1758) in Jeremiah Usein river, Gwagwalada, Nigeria. *Int. J. Biol. Sci.* 1(1):1-5.
- Iglesias L, Valero A, Galvez L, Benitez R, Adroher, FJ. 2002. In vitro cultivation of *Hysterothylacium aduncum* (Nematoda: Anisakidae) from 3rd-stage larvae to egg laying adults. *Parasitology*.125 (Pt 5): 467-475. DOI: 10.1017/S0031182002002263
- Jahantab M, Haseli M, Salehi Z. 2014. Morphological and genetic characteristics of the anisakid nematode *Raphidascaris acus* from the southwest Caspian Sea: evidence for the existence of sibling species within a species complex. *Parasitology research*. [cited 2020 June 25]; 113(9):3419–3425. Available from: <https://doi.org/10.1007/s00436-014-4007-5>.
- Johnson MW, Nelson PA, Dick TA. 2004. Structuring mechanisms of yellow perch (*Perca flavescens*) parasite communities: host age, diet, and local factors. *Canadian Journal of Zoology*. 82: 1291-1301. Available from: <https://doi.org/10.1139/z04-092>
- Khanum H, Begum S, Begum A. 2011. Seasonal prevalence, intensity, and organal distribution of the helminth, parasites in *Macroglythys aculeatus*. Dhaka University. *J. Biol. Sci.* 20(2): 117-22. Available from: <https://doi.org/10.3329/dujbs.v20i2.8971>
- Klimpel S, Palm HW. 2001. Anisakid nematode (Ascaridoidea) life cycles and distribution. Increasing zoonotic potential in the time of climate change? *Parasitology Research Monographs*. 2:201-222. Available from: https://doi.org/10.1007/978-3-642-21396-0_11
- Locke SA, Marcogliese DJ, Valtonen ET. 2014. Vulnerability and diet breadth predict larval and adult parasite diversity in fish of the Bothnian Bay. *Oecologia*. [cited 2020 May 7]; 174(1), 253-262. Available from: <https://doi.org/10.1007/s00442-013-2757-x>
- MacKenzie K. 2002. Parasites as biological tags in population studies of marine organisms: An update. *Parasitology*. [cited 2020 May 8]; 124(7): 153-163. Available from: <https://doi.org/10.1017/S0031182002001518>
- Mackenzie K, Campbell N, Mattiucci S, Ramos P, Pinto AL, Abaunza P. 2008. Parasites as biological tags for stock identification of Atlantic horse mackerel *Trachurus trachurus* L. *Fisheries Research*. [cited 2020 June 7]; 89(2): 136-145. Available from: <https://doi.org/10.1016/j.fishres.2007.09.031>
- Margolis L, Esch WG, Holmes JM, Kuris AM, Shad GA. 1982. The use of ecological terms. (Report of an Ad Hoc Committee of the American Society of Parasitologists). *J. Parasitology*. [cited 2020 May 9]; 68(1): 131-133. Available from: <https://www.jstor.org/stable/3281335>. DOI: 10.2307/3281335.
- Mattiucci S, Nascetti G. 2008. Advances and trends in molecular systematics of anisakid nematodes, with implications for their evolutionary, ecology and host-parasite co-evolutionary processes. Academic Press. *Advances in Parasitology*. 66: 47–148. Available from: [https://doi.org/10.1016/S0065-308X\(08\)00202-9](https://doi.org/10.1016/S0065-308X(08)00202-9)
- Mattiucci S, Cimmaruta R, Cipriani P, Abaunza P, Belisario B, Nascetti G. 2014. Integrating *Anisakis* spp. parasites data and host genetic structure in the frame of a holistic approach for stock identification of selected Mediterranean Sea fish species. *Parasitology*. 142(1): 1-19. Available from: <https://doi.org/10.1017/S0031182014001103>
- Mattiucci S, Cipriani P, Paoletti M, Levsen A, Nascetti G. 2017. Reviewing biodiversity and epidemiological aspects of anisakid nematodes from the north-East Atlantic Ocean. *Journal of Helminthology*. 91(4):422–439.
- Molnár K, Buchmann K, Csaba S. 2006. Phylum Nematoda. In: PTK Woo, editors, *Fish Diseases and Disorders. Protozoan and Metazoan Infections*. Oxfordshire: CABI Publishing. 2 ed. 1:417-443.
- Muzzall PM, Sweet RD, Mijewski CL. 1990. Occurrence of *Diplostomum* sp. (Trematoda: Diplostomatidae) in pond-reared walleyes

- from Michigan. *Progr. Fish-Cult.* 52(1):53-56. Available from: [https://doi.org/10.1577/1548-8640\(1990\)052<0053:OODSTD>2.3.CO;2](https://doi.org/10.1577/1548-8640(1990)052<0053:OODSTD>2.3.CO;2)
- Olurin K, Okafor J, Asiru A, Ademilua J, Owonifari K, Oronaye O. 2012. Helminthes parasites of *Sarotherodon galilaeus* and *Tilapia zillii* (Pisces: Cichlidae) from River Oshun, Southwest Nigeria. *Int. J. Aqua. Sci.* 3(2):49-55. Available from: <https://pdfs.semanticscholar.org/3eb1/e2a22e9e52200ae4f96557eb84a90db435cc.pdf>
- Palm HW, Klimpel S, Walter T. 2007. Demersal fish parasite fauna around the South Shetland Islands: high species richness and low host specificity in deep Antarctic waters. *Polar Biology*. [cited 2020 May 11]; 30(12):1513-1522. Available from: <https://doi.org/10.1007/s00300-007-0312-0>
- Poulin R. 2000. Variation in the intraspecific relationship between fish length and intensity of parasitic infection: Biological and statistical causes. *Journal of Fish Biology*. [cited 2020 May 9]; 56(1): 123-137. Available from: <https://doi.org/10.1111/j.1095-8649.2000.tb02090.x>
- Poulin R, Leung TLF. 2011. Body size, trophic level, and the use of fish as transmission routes by parasites. *Oecologia*. [cited 2020 May 10]; 166 (3): 731-738. Available from: <https://doi.org/10.1007/s00442-011-1906-3>
- Quiazon KMA, Yoshinaga T, Ogawa K. 2011. Distribution of *Anisakis* species larvae from fishes of the Japanese waters. *Parasitol Int.* 60(2):223-226. Available from: <https://doi.org/10.1016/j.parint.2011.03.002>
- Quiazon KMA. 2015. Updates on Aquatic Parasites in Fisheries: Implications to Food Safety, Food Security and Environmental Protection. *Journal of Coastal Zone Management*. [cited 2020 May 8]; 18(1): 396. Available from: <https://doi.org/10.4172/2473-3350.1000396>
- Raffel TR, Anderson TK. 2009. A new species of *Hysterothylacium* (Nematoda: *Anisakidae*) from the stomach of the red-spotted newt, *Notophthalmus viridescens*, from Pennsylvania fishless ponds. *The Journal of parasitology*. [cited 2020 June 30]; 95(6): 1503-1506. Available from: <https://doi.org/10.1645/GE-2092.1>
- Riemann F. 1988. Introduction to the study of meiofauna. Higgins RP, Thiel H, editors. Washington, DC: Smithsonian Institution Press. 488 p.
- Rohde K. 1993. Ecology of Marine Parasites. An Introduction to Marine Parasitology. 2nd Edition. Wallingford, Oxon: CABI. 304 p. Available from: <http://www.cabdirect.org/abstracts/19930807939.html>
- R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: <https://www.R-project.org/>.
- Santos CP, Moravec F. 2009. *Camallanus tridentatus* (Drasche) (Nematoda: Camallanidae): new taxonomically important morphological data. *Mem Inst Oswaldo Cruz.* 104(1):93-99. Available from: <https://doi.org/10.1590/s0074-02762009000100014>
- Shamsi S. 2019. Parasite loss or parasite gain? Story of *Contracaecum* nematodes in antipodean waters. *Parasite Epidemiology and Control.* 4: e00087. Available from: <https://doi.org/10.1016/j.parepi.2019.e00087>
- Shamsi S, Barton DP, Zhu X. 2019. Description and characterisation of *Terranova pectinolabiate* n. sp. (Nematoda: *Anisakidae*) in great hammerhead shark, *Sphyrna mokarran* (Rüppell, 1837), in Australia. *Fish Parasitology*. [cited 2020 June 25]; 118(7): 2159-2168. Available from: <https://doi.org/10.1007/s00436-019-063604>
- Sprent JFA. 1970. Some ascaridoid nematodes of fishes: Heterocheilinae. *Systematic Parasitology.* 16: 149-161. Available from: <https://doi.org/10.1007/BF00009613>
- StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP.
- Thomas F, Verneau O, de Meeu T, Renaud F. 1996. Parasites as to host [corrected] evolutionary prints: Insights into host evolution from

- parasitological data. *International Journal for Parasitology*. 26(7): 677-686. Available from: [https://doi.org/10.1016/0020-7519\(96\)00023-9](https://doi.org/10.1016/0020-7519(96)00023-9)
- Varjabedian GK. 2005. Parasitic Nematodes of Freshwater Fish in Egypt. Publication of the National Biodiversity of Egypt.
- Valero A, Paniagua MI, Hierro IR, Diaz V, Valderrama MJ, Benitez R, Adroher FJ. 2006. Anisakid parasites of two fork beards (*Phycisblennoides* and *Phycisphycis*) from the Mediterranean coasts of Andalu1'a (Southern Spain). *Parasitol. Int.* 55(1):1-5. Available from: <https://doi.org/10.1016/j.parint.2005.07.001>
- Wayland MT, Vainio JK, Gibson DI, Herniou EA, Littlewood TDJ, Väinölä R .2015. The systematics of *Echinorhynchus* Zoega in Müller, 1776 (*Acanthocephala*, Echinorhynchidae) elucidated by nuclear and mitochondrial sequence data from eight European taxa. *ZooKeys*. [cited 2020 on June 25]. 484:25-52. Available from: <https://doi.org/10.3897/zookeys.484.9132>
- Yagi K, Nagasawa K, Ishikura H, Nakagawa A, Sato N, Kikuchi K, Ishikura H. 1996. Female worm *Hysterothylacium aduncum* excreted from human: a case report. *Jpn. J. Parasitol.* 45(1): 12-23.
- Yakhchali M, Tehrani AA, Ghoreishi M. 2012. The occurrence of helminthes parasites in the gastrointestinal of catfish (*Silurus glanis* Linnaeus 1758) from the Zarrine-roud River, Iran. *Vet. Res. Forum* 3(2):143-145. [cited 2020 June 29]; Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4312810/pdf/vrf-3-143.pdf>
-