Helminth Parasites in Slippers-Shaped Oyster, *Crassostrea iredalei* (Taustino, 1932) (*Bivalvia:Ostreoida*)

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**ABSTRACT**

Disease caused by viruses, bacteria and parasitic worms is one of the causes in the decline of *Crassostrea eridalei*. Since previous studies mostly focused on microscopic parasites, this study aimed to identify the helminthic parasites of *C. eridalei*. Randomly collected samples from Cambuilao, Bais City Negros Oriental were categorized according to size, depurated, dissected and examined for the presence of parasites. Collected parasites were then counted and identified to lowest possible taxon under the light microscope. Results of this study showed that out of 90 samples examined, a total of 53 *Crassostrea iredalei* were found to be infected with parasites. Fifty of which were infected with annelids with a prevalence of 45% in shell length 1-3cm, 37% in shell length 4-6cm, and 69.5% in shell length 7-10cm. Two were infected with Platyhelminthes with a prevalence of 5% and 4.16 in shell lengths 1-3cm and 4-6cm, respectively; only one individual was infected with Nemertea with a prevalence of 4.16%. A total of 80 annelid parasites were collected from the infected *C. iredalei* individuals with a mean infection intensity ranging from 0.69-2. The annelid parasites observed in this study were under Class Polychaeta, Families Neridae, Opheliidae, Hesionidae, Spionidae, Syllidae, Capitellidae, Phyllodocidae, and Polynoidae.

**Keywords:** Helminth, parasites, *Crassostrea*, oyster, Bais, Polydora.

**INTRODUCTION**

Oysters are bivalve mollusks that are classified under Phylum Mollusca, Class Bivalvia, Order Ostreoida, Family Ostreidae. Oysters are widely distributed and found in habitats like lagoons, estuaries, and brackish water in both temperate and tropical regions (Angell, 1986; Raj, 2008). They are considered as one of the foundation species which help maintain biodiversity, population, and food web dynamics, nutrient cycling, and water quality (Raj, 2008). There are three known genera of oysters: Ostrea, Sacosstrea, and *Crassostrea* (Angell, 1986).

The *Crassostrea* variety is considered as the most important commercial species of oysters found throughout Southeast Asia (Angell, 1986). In the Philippines, *Crassostrea iredalei* is one of the four species cultivated along with *Crassostrea cucullata*, *C. malabonensis*, and *C. palmipes*. Of these four species, *C. iredalei* is the most commercially wanted because it grows at a faster rate to a larger size and has straight shell margins which make them easier to open (Food and Agriculture Organization of the United Nations, 2016).

According to Robledo et al. (2014), the population of *Crassostrea iredalei* suffers a significant decline due to overharvesting, environmental pollution or diseases. Disease of bivalve mollusks is mostly caused by biological agents such as viruses, bacteria, fungi,
protists, digenean trematodes, polychaetes and copepods (Boehs et al., 2010). Niajah et al. (2008) reported that sampled oysters from Setiu Wetland, East Coast Peninsular Malaysia, harbor Shewanellaputrifaciens, Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio cholerae, Enterobacter cloacae, Escherichiacoli as well as Chromobacteriumviolaceum bacteria. A protozoan parasite, Nematosis sp. was found to infect C. iredalei in Palau Bentong, West Coat of Penang Malaysia. The same species, together with the cestode parasite, Tyloceplahumsp, was also found to infect C. iredalei in Ivisan, Capiz, Philippines (Ezaro et al., 2010). These protozoan parasites were also observed to infect other species of Crassostrea. The Haplosporidiumnelsoni, another protozoan parasite, causes the Multinucleated SphereX or MSX disease which affects mollusks; the MSX first appeared in 1957 at the Delaware Bay (Ewart& Ford, 1993). Other protozoan parasites like the Perkinususmarinus, have caused problematic oyster disease particularly in Crassostrea virginica varieties in Georgia, USA (Power et al., 2006). A protist, Marteiliasyndeyi, causes a QX disease that is described by Dang et al. (2013) to decrease meat quality, reproductive capacity and massive mortalities in Crassostrea virginica.

Though Crassostrea has been subject to many parasite related studies, (Boehs et al., 2010; Dang et al., 2013; 2005 De Vera et al., 2005; Ezaro et al., 2010), most of these focused on microscopic parasites such as bacteria, protozoans, and ciliates. Knowledge about the helminth parasites of oysters and its impact on Crassostrea iredalei culture in the Philippines is still scarce.

This study aimed to identify the helminth parasites found in Crassostrea iredalei and determine the parasitic load in terms of prevalence and intensity. Results of this study will serve as the basis for identifying the macroscopic parasites of the Crassostrea iredalei and therefore will help in improving the meat quality, reproductive capacity, marketability, and production of farmed Crassostrea iredalei.

MATERIALS AND METHODS

Collection of Crassostrea iredalei Samples

Crassostrea iredalei (Faustino, 1932) or slipper-shaped oyster is a bivalve mollusk that can be distinguished by the presence of a black scar in its adductor muscle (Figure 1). A total of 90 samples were randomly collected from Cambuilao, Bais City, Negros Oriental. The samples were then slightly washed, placed in an icebox and transported to the Biology Laboratory, Negros Oriental State University. In the laboratory, each bivalve sample was measured for its length and height using a Vernier caliper and was sorted out by size. Each sample was placed in individual containers with seawater from the actual site of collection. After which, the bivalves were allowed to depurate for 3-4 days. Depuration is a process by which shellfish are put in tanks of clean seawater under conditions which maximize the natural filtering activity which outcomes in the expulsion of intestinal contents, which enhances separation of the expelled contaminants from the bivalves, and prevents their recontamination (Ronald et al., 2008). In this study, plastic containers were used as an alternative for tanks to contain both oysters and seawater, and to capture any impurities including parasites as the oysters depurate. Each oyster sample was individually dissected using dissecting sets for helminth parasite examination. Depurated water was also examined for the presence of parasite.
Collection and Identification of Helminth Parasites

The method described by Gardner et al. (2012) was adapted and modified for parasite collection. Helminth parasites were collected from the oysters' mantle and depurated water. Collected parasites were sorted out into lowest possible taxon, counted and placed in plastic vials with labels and containing 70% ethanol solution for storage.

The parasites were identified under the compound microscope using the guides of Handley (1999; 2000); SAORC, 2003; Rodrigo (2006); Martin et al. (2012); Cinar et al. (2015) and online databases http://www.annelida.net/, http://species-identification.org/, and http://www.marinespecies.org/. Identified parasites were then verified by Melih Cinar, Ph.D., a Marine Biology professor at Ege University, Izmir, Turkey, who specializes in taxonomy and ecology of polychaetes. The same samples were verified by Jerry A. McLelland, Ph.D., from Gulf Benthic Taxonomy Assessment, Mississippi.

Data Analysis

The method used by Mergo & Crites (1986) was adopted for the determination of parasitic prevalence and the mean intensity of the shell length of the slipper-shaped oyster. Prevalence is the proportion of infected hosts among all the hosts examined while infection intensity is the total number of parasites found in each host.

RESULTS

In this study, the shell length of Crassostrea iredalei was ranged from two to ten cms. Out of 90 samples examined, a total of 53 Crassostrea iredalei were found infected with helminth parasites; and almost all infected samples were found with blisters from the periostracum to the nacreous layer of the shell (Figure 2). Fifty out of 53 infected samples harbored annelids with...
a prevalence of 45% in shell length 1-3cm, 37% in shell length 4-6cm, and 69.5% in shell length 7-10cm. Two were infected with Platyhelminthes with a prevalence of 5% and 4.16% in shell lengths of 1-3cm and 4-6cm, respectively. There was only one individual infected with Nemertea with a prevalence of 4.16% (Table 1).

A total of 80 annelid parasites were collected from the infected C. iredalei individuals: two Platyhelminthes parasite and one nemertean parasite. Data shows that the annelids bear the highest infection intensity with a mean intensity ranging from 0.69-2 (Table 2).

Table 1. The number of infected individuals and prevalence of helminth parasites in each size range of Crassostrea iredalei

<table>
<thead>
<tr>
<th>Shell Length (cm)</th>
<th>No. Examined</th>
<th>Number of Infected C. iredalei</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Annelida</td>
<td>Platyhelminthes</td>
</tr>
<tr>
<td>1-3</td>
<td>20</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>4-6</td>
<td>24</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>7-10</td>
<td>46</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>50</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2. Mean infection intensity of helminth parasites in Crassostrea iredalei

<table>
<thead>
<tr>
<th>Shell Length (cm)</th>
<th>No. of Parasites collected</th>
<th>Mean Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Annelida</td>
<td>Platyhelminthes</td>
</tr>
<tr>
<td>1-3</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>4-6</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>7-10</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 2. Blisters and parasites found in the shell of Crassostrea iredalei. (BL=blister) (P=parasite).
The annelid parasites observed in this research were under Class Polychaeta, Families Neridae, Opheliidae, Hesionidae, Spionidae, Syllidae, Capitellidae, Phyllodocidae, and Polynoidae (Figure 3).

Family Nereididae is distinguished by the presence of rectangular to a triangular head. A pair of short antennae between a pair of jointed palps with stout bases is also present followed or surrounded by four pairs of long tentacular cirri (Read, 2004). Two pairs of large eyes are also observed (Figure 3A).

Members of Family Opheliidae sand burrowers; they are short-bodied, cigar-shaped, and muscular. Their head is sharply conical, sometimes with a knobbed tip and its mouth is just a ventral slit (Figure 3B). Opheliids are also considered as deposit feeders, but they are probably selective in their intake of particulate material. Adult species measures up to 20 mm in length and slender, while other species are more desirable and maggot-like and may reach up to 30 mm long (Read, 2004).

Hesionidaepolychaetes have prostomium that is simple or bilobed containing four eyes, two to three antennae and two palps that are each divided into two sections (Allen, 1904). Its first segment dorsally reduced and with up to eight pairs of tentacular cirri present on cephalized segments. Its pygidium is with two cirri (Figure 3C).

Family Spionidae is generally small and slender active worms, with head palps used for feeding. Their palps arise dorsally on either side of a narrow prostomium. Spionidpygidial structures are varied and include short cirri (one to three pairs or many), lobes, cups, and plates (Figure 3D). Adult size ranges from 150 mm long and 10 mm diameter, but more usually 10-30 mm (Read, 2004).

Syllids are typically of small size and delicate in appearance; they are characterized by having forms with long thin antennae and noticeable dorsal cirri that stand partly upright from the body and could be annulated. Their prostomium has three antennae: the median one is the posteriormost; a pair of palps directed somewhat ventrally, and with one or two pairs of eyes present. A proventriculus or a gizzard-like structure is present, which is usually visible through the body wall. One or two pairs of tentacular cirri are possessed by its peristomium (Figure 3E). Adult size is around 20 mm or less in length (Read, 2004).

Members of Family Capitellidae are thread-like deposit dwellers that live in unlined, rambling burrows and are considered to be relatively non-selective particle feeders. Capitellid worms are long, delicate, and difficult to collect intact. The prostomium is conical to pointed, and the first segment usually lacks chaetae (Figure 3F). Adult size reaches up to 50 mm long, and is very thin (Read, 2004).

The Phyllodocids are long, slender, and very active carnivorous worms characteristically possessing enlarged dorsal and ventral cirri which are often flattened and leaf-like and is characteristically colourful family of polychates (Read, 2004). The anterior of their head is often somewhat shovel-shaped and bears a group of four 'antennae' (the ventral pair is probably modified palps). Further back, a fifth median antenna may be present (Plate 3G). Jaws are absent, but with an anterior gut that has a very long eversible proboscis which is usually papillose and with terminal papillae; 2-4 pairs of tentacular cirri on 2-3 variably-fused segments behind the head are also observable (Read, 2004). Adult size members of this family are less than 20 mm long.

Polynoidae scale worms are dorsoventrally flattened, often rather oval-shaped. Their upper surface is typically fully protected by a number of large overlapping plates, known as elytra, each of which may be adorned and fringed with papillae,
tubercles, and hairs (Figure 3H). Located near its center is the mushroom-like attachment point of each elytron. Adult size is up to 100 mm long (Read, 2004).

Helminths under Phylum Nemertea were distinguished by the presence of retractable proboscis, a long muscular tube that is thrust out swiftly when grasping their prey (Figure 3I). Platyhelminthes on the other hand, have dorsoventrally flattened and bilaterally symmetrical body (Figure 3J) (Hickman et al., 2008).

Figure 3. Images of parasites identified from Crassostrea iredalei under LPO (100X) A. Nereididae B. Opheliidae. C. Hessionidae D. Spionidae E. Syllidae F. Capitellidae G. Phyllodocidae H. Polynoidae I. Nemertean J. Platyhelminthes.
DISCUSSION

Annelids are helminths that are less known to be parasitic; however, there are cases that involve polychaete infection (Handley et al., 2003; Struck, 2008) which affected the physiological condition and marketability of their host. For instance, the presence of polychaete in Saccostrea commercialis affected its marketability in Australia (Skeel, 1979). In Hawaii, another polychaete, the Boccardiaproboscidea, was found to heavily infect the oysters (Bailey-Brock, 2000). These polychaetes affect cultured mollusks, including oysters by reducing their flesh condition through delayed growth rates and thus increasing mortality (Simon et al., 2009). In New Zealand, polychaete under the genus Polydora has been reported to damage commercially important bivalves such as the Crassostrea gigas, Perna canaliculus, and Pectennozelandiae (Read, 2010). In this study, it was found out that Crassostrea iredalei is also susceptible to annelid infection. Out of 90 annelid parasites identified, polychaete specifically under the genus Polydora was observed to infect most of the oyster individuals. According to Lleonart et al. (2003), Polydora is known to be a parasitic polychaete that infects mollusks such as abalones and oysters. This species usually burrows into a substrate that contains calcium carbonate such as the shells of oysters found in muddy sediments (Hill, 2007).

Boring polydorids have frequently been considered as parasitic organisms from an anthropogenic rather than ecological point of view (Martin & Britayev, 1998). As soon as they infest species of mollusks such as oysters that are cultivated or harvested as a fishery resource, they are immediately regarded as pests (Martin & Britayev, 1998). It was also reported in 1979 that four polydorid parasites caused serious damage to cultured bivalves in Australia, including Crassostrea iredalei, by creating irritating and often fatal mud blisters between the shell and mantle (Skeel, 1979). The polydorid parasite has also been alarming to Australia and Chile for heavily infecting the farmed oysters (Handley, 2003; Moreno, 2006).

Polydora species infect oysters in two ways - either boring through the shell from the outside using metabolic acids (Haigler, 1969; Hadley, 1998) or by entering the oyster’s mantle cavity as a larva and thus setting within the oyster (Skeel, 1977; Handley, 1998). When parasites reach the inner nacreous layer, the oyster reacts to the irritant and secretes a layer of conchiolin at the site of contact with mantle tissue, forming a “mud blister” (Angell, 1986). It is notable in this study that most of the oysters showed mud blisters in their shell. These observations could support that these mud blisters were the results of the oyster’s reaction to the irritant caused by the parasites. Mud blister influences the physiology of the oysters which would further increase the susceptibility of oysters to parasitism.

Moreover, results showed that the size of oysters may affect its susceptibility to parasites. Parasite prevalence increased in parallel with the increase in oysters shell length (Table 1). A similar trend is found in the study of Martin and Britayev (1998). Oysters with larger size are more or less older and so have been exposed to planktonic larvae settlement or adult migration of parasites for a longer period than small sized-oysters. In addition, large oysters provide more space for parasites than small oysters (Martin & Britayev, 1998). Moreover, the susceptibility of Crassostrea iredalei to polychaete parasites could also be due to the physiological stress induced by the environmental conditions. According to several studies, susceptibility to parasitism and associated disease may increase when a host suffers from physiological stress that is brought about by extreme environmental conditions (Chapin 1991; Gustafsson et al. 1994; Urawa
1995; Lenihan et al., 1999). Stressors may include flow speed over the oysters’ reef, oxygen stress, temperature and salinity variation and poor food quality (Paynter and Burreson 1991; Burreson and Calvo 1996; Chu 1996; Lenihan et al. 1999). Oysters in low flow and reduced food quality are in poorer physiological condition and less likely to fight off parasitic infection than oysters in high flow. For example, it was found out that oysters exposed to higher levels of salinity and hypoxia showed greater susceptibility to parasite such as Perkinus marinus (Lenihan et al., 1999).

Fortunately, the occurrence of parasites in C. ireadalei is not threatening and does not pose serious health risk for human consumption because they are not zoonotic. According to Brown (2012), polychaetes specifically Polydora are sensitive to salinity and temperature. They grow well at higher temperatures rather than lower temperatures. Also, they cannot tolerate extremely low salinity; thus, exposing it to low salinity and cold water would initiate its death. Furthermore, if oysters infected with parasites are accidentally eaten, the acidic environment in the stomach of humans could result in the parasite’s death. Though this implies that oyster parasites do not impose threat in public health, these parasites should still be carefully studied as well as its eradication from its hosts due to massive destruction that it could cause to oysters which could result in the decline of oysters supply.

CONCLUSION

The helminthic parasites that infect C. ireadalei were found to be annelids, nemerteans, and platyhelminthes. Most of the individuals were infected with annelids with a prevalence ranging from 37%-69.5%. The annelids were under Class Polychaeta, under the families Neridae, Hesionidae, Syllidae, Spionidae, Phyllodocidae, Opheliidae, Capitellidae, and Polynoidae. Species under genus Polydora of the Family Spionidae was observed to greatly infect C. ireadalei. The mean infection intensity of annelid in all infected individuals ranged from 0.69-2. Oysters with larger size and shell length harbored more parasites that the small ones.

RECOMMENDATIONS

This study recommends investigating the effects of these helminth organisms to the physiology of Crassostrea ireadalei. Future studies regarding the factors which increase the risks of parasitism in C. ireadalei are also suggested. Parasitic prevalence should be observed in different age groups. Lastly, symbiotic relationship of these helminths and the oyster should also be studied.

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polychaete *Boccardiaprobsocidea* (Family Spionidae), imported to Hawai‘i with oysters. Pacific Science, 54(1), 27-30. doi:10125/1595


