

PREVALENCE OF PARASITE INFESTATIONS AMONG FOUR COMMERCIALY EXPLOITED WILD SHELLFISH SPECIES IN SETIU WETLAND, TERENGGANU, MALAYSIA

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Abstract: The lagoon of Setiu Wetlands has high biodiversity of bivalve's species. The majority of villagers are fisherman and bivalves are one of their income-generating activities. Studies on parasites of bivalves are important to our knowledge for maintenance of natural resources. This study investigated the parasite prevalence among four commercially exploited shellfish species from Setiu Wetland, Malaysia. A total of 120 samples were collected during the low tide time for four shellfish species which were *Polymesoda expansa*, *Meretrix meretrix*, *Anadara* sp. and *Crassostrea iredalei*. The bivalve specimens were measured on its length with and without shell, width, height, weight and microscopically examined on the presence of parasites focused at gills, muscle and digestive tract. The discovered macroparasite were fixed into 70% ethanol solution for preservation. In this study, *Anadara* sp. was highest infestation of parasites, while *Polymesoda expansa* has the least infestation of parasites. There were occurrence of copepod and *Nematopsis* sp. in *Anadara* sp., *M. meretrix* and *C. iredalei*. Apart from that, there were other parasites observed in four bivalve species such as metacercaria, cestode larvae, *Panopeus* sp., *Pinnotheres* sp., *Balanus* sp. and unidentified ciliates. Ecological factors, feeding activity, season and abundance of definitive host were known to be elements that altered the prevalence of parasites in host. Upon the observation, a high prevalence of *Nematopsis* sp. were observed in three species of bivalves except *P. expansa*. Whereas for other parasites were occurred in low prevalence and intensity, without causing significant damage towards host.

Keywords: *Polymesoda expansa*, *Meretrix meretrix*, *Anadara* sp., *Crassostrea iredalei*, parasite, Setiu wetland.

Introduction

Mollusk bivalve is key component in the environment of marine and brackish water. As filter feeder, bivalve plays vital role in maintaining water quality and perform certain ecosystem services such as carbon sequestration and turbidity reduction of marine water (National Academy of Sciences, 2010).

Owing to the increase demand and global production of bivalve for human consumption, the parasite infestation on bivalve has been gradually reported in worldwide as it is the major limiting factor in aquaculture venture (Robledo *et al.*, 2014). Anthropogenic activities,

growing of international trade and demographic change has accelerated the spreading of parasitic disease (Marcogliese, 2002).

Bivalve aquaculture such as oyster and cockle culture has been promoted by Malaysia government under the support of Department of Fisheries as aquaculture production is continuously rose in rapid rate to meet the demand of exponential growth of human population due to the dwindling of natural stock from time by time (Tan, 2015). Yet, the parasite and disease that prompt negative reverberation on bivalve in Malaysia water and susceptibility of parasitic disease among some shellfish species

are not methodically scrutinize (Uddin *et al.*, 2012). Bivalve-associated disease outbreak has been occurred in neighboring countries and developed country has contributed to extensive economic loss and mortality (Potasman *et al.*, 2002).

Determining parasites in bivalve mollusks is competent for ensuring the long-term sustainability in aquaculture venture. Furthermore, there are no comprehensive study on the prevalence of parasite infestation of four bivalve species in Setiu Wetland. In this study, parasite prevalence among four commercially exploited shellfish species in Setiu Wetland has been determined to ensure the maintenance of resources.

Materials and Methods

Sampling area

Setiu Wetland was selected as the sampling site for the collection of four commercial bivalve species in Terengganu, Malaysia. Setiu Wetland is located in east coast region of Peninsular Malaysia with the latitude 5° 39' 36" N along with longitudes 102° 43' 48" E.

Sample Collection

Thirty aquatic mollusks per species were collected for scrutinizing parasites prevalence and net intensity for each bivalve. Proper handling was performed to ensure the freshness of the bivalve species by storing it in the polystyrene box with water sample, sand and gravel from inhabited area of the bivalve species itself (Duobinis-Gray *et al.*, 1991). Those samples were transported to the laboratory within one day of collection. Aeration was inserted into the polystyrene box for air circulation and avoid suffocation of bivalve specimens.

Parasites Examination

Prior to sample analysis, bivalve specimen was weighted with and without shell by electronic balance. Their length was measured simultaneously using vernier caliper. Bivalve

samples were euthanized using the scalpel blade to cut off the adductor muscles. The structure of muscle, gill and digestive tract were observed under dissecting microscope and light microscope (Danford & Joy, 1984). The muscle part of bivalve specimen was squashed between 2 glass plates along with a drip of seawater for parasite examination (Rantanen *et al.*, 1998). Whereas the gill part of shellfish was scrutinized using smear method, while wet mount method was utilized for investigating the parasite prevalence on the digestive tract of the bivalve specimen. The discovered macroscopic parasites were put into 70% ethanol solution for storage (Leron & Bantoto-Kinamot, 2015).

Data Analysis

Bivalve parasite identification was aided by several parasitic disease journals and books (Gosling, 2004; Erazo-Pagador, 2010; Leron & Bantoto-Kinamot, 2015; Uddin *et al.*, 2012, etc.) For each species of bivalve mollusk, the prevalence and mean intensity of parasite pervasiveness were calculated. The prevalence and mean intensity were determined by using the formulas below by Margolis *et al.* (1982):

$$\text{Prevalence} = \frac{\text{No. of samples infected}}{\text{No. of samples examine}} \times 100$$

$$\text{Mean Intensity} = \frac{\text{No. of parasite collected in a sample}}{\text{No. of infected hosts}}$$

Results and Discussion

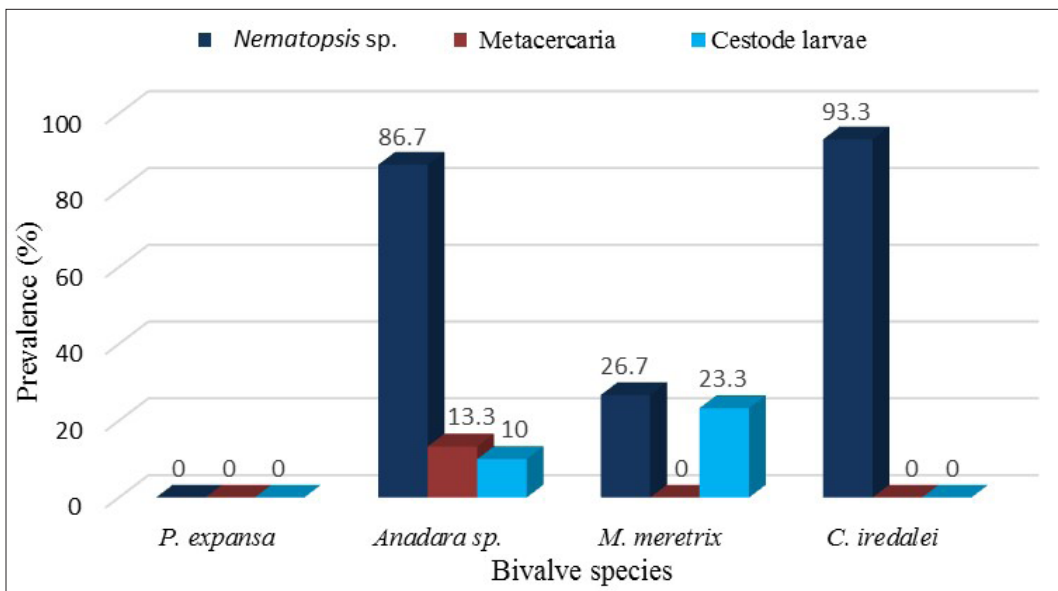
A total of 120 bivalve mollusks were comprehended in analyses, incorporated with *Polymesoda expansa* (n=30), *Anadara* sp. (n=30), *Meretrix meretrix* (n=30) and *Crassostrea iredalei* (n=30). Parasite detection was underwent determining on the structure of gill, muscle and digestive tract. Of the 120 bivalves sampled, *Polymesoda expansa* indicated the lowest parasite infestation in its organs, while *Anadara* sp. exhibited the highest presence of parasites. There were unidentified ciliates and peacrab (*Pinnotheres* spp.) discerned in the gill and muscle parts of *P.*

expansa respectively. Parasites were discovered in digestive tract and periostracum shell of *P. expansa*. The aforementioned *Anadara* sp. were heavily infected by many parasitic species. Acorn barnacle, *Balanus* sp. was discovered at the periostracum shell. Copepod was discerned in the gill part of *Anadara* sp. The apicomplexa oocyst of *Nematopsis* sp. were found in gills, muscles and digestive tract. While metacercaria and cestode larvae were found to be infected the muscles and digestive tract. For *M. meretrix* sample, undoubtedly *Nematopsis* sp. had the higher rate of infection in gills, muscles and digestive tract. Copepod and peacrab (*Pinnotheres* spp.) were infected in the gill

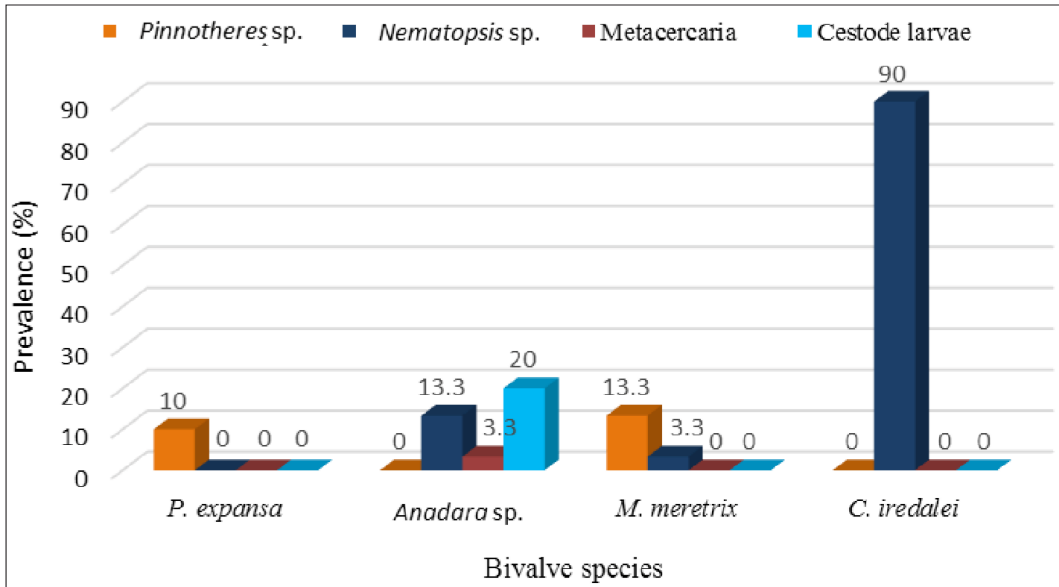
and muscle parts of *M. meretrix* respectively. Comparing to *Anadara* sp. samples, *M. meretrix* has lower cestode larvae infestation in organs, which digestive tract was the solely targeted area for settling down. In addition, the acorn barnacle, *Balanus* sp., *Panopeus* sp. and copepod were discerned at the periostracum shell, aperture of oyster's shell margin, and gills of *C. iredalei* respectively. Protozoan parasite, *Nematopsis* sp. was detected in the gills, muscles and digestive tract of slipper-cupped oyster, *C. iredalei*. The graphical and table presentation of the parasite prevalence in periostracum, gills, muscles and digestive tract of four samples have been illustrated in Table 1, 2 and Figure 1.

Table 1: Parasite species in four commercially exploited bivalve samples

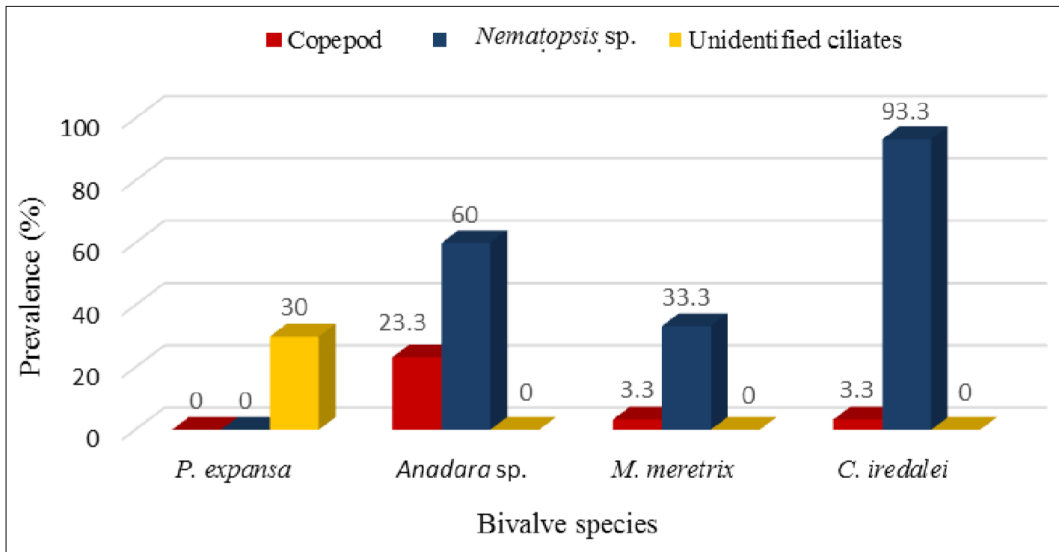
Bivalve species	Ectoparasite		Endoparasite										
	<i>Balanus</i> sp.	<i>Panopeus</i> sp.	Gill			Muscle				Digestive Tract			
			Copepod	Unidentified ciliates	<i>Nematopsis</i> sp.	<i>Pinnotheres</i> sp.	<i>Nematopsis</i> sp.	Metacercaria	Cestode larvae	<i>Nematopsis</i> sp.	Cestode larvae	Metacercaria	
<i>Polymesoda expansa</i>	-	-	-	+	-	+	-	-	-	-	-	-	-
<i>Anadara</i> sp.	+	-	+	-	+	-	+	+	+	+	+	+	+
<i>Meretrix meretrix</i>	-	-	+	-	+	+	+	-	-	+	+	-	-
<i>Crassostrea iredalei</i>	+	+	+	-	+	-	+	-	-	+	-	-	-



a



b



c

Figure 1: Prevalence of parasite infection by structure of different bivalve species, (a) Gills, (b) Muscle, (c) Digestive Tract

Table 2: Prevalence of ectoparasite on four commercially exploited bivalve samples

Ectoparasite	Prevalence (%)			
	<i>P. expansa</i>	<i>Anadara</i> sp.	<i>M. meretrix</i>	<i>C. iredalei</i>
<i>Balanus</i> sp.	0	13.3	0	33.3
<i>Panopeus</i> sp.	0	0	0	16.7

There are at least two kinds of parasites were existed in studied bivalve species. The finding of parasites study in gills, muscle and digestive tract of bivalves were contradictory based on the abiotic factors, settlement area and geographical distribution. In this study, *Nematopsis* sp. recorded most studied shellfish species excluding marsh clam, *Polymesoda expansa* in Setiu Wetland, Terengganu, Malaysia. According to Zeidan *et al.* (2012), *Nematopsis* sp. was discovered mainly on gill and mantle of bivalve molluscs, yet the presence of *Nematopsis* sp. notwithstanding can be found at digestive gland, adductor muscle and foot of shellfish. The prevalence of *Nematopsis* sp. might ascribable to spatial variation, the exuberance of definitive hosts and the distance between infected and uninfected host as it is using bivalve as intermediate host and completes its life cycle in marine arthropod or decapod crustacean (McGladdery *et al.*, 2006). *Nematopsis* sp. is commonly found in several bivalve species, without inducing significant health effect (Cremonte *et al.*, 2005). Notwithstanding, there were several studies revealed that high abundance of *Nematopsis* sp. contributing to water flow occlusion, subsequently reducing the feeding efficiency and food intake due to the presence of great amount of phagocytes of *Nematopsis* sp. (Azevedo & Cachola, 1992; Kua *et al.*, 2013; Suja *et al.*, 2016).

The unidentified ciliates solely occurred in the gill structure of *P. expansa* with prevalence of 30%. The prevalence of ciliates infestation was different for every study as it is depends on the physiological state of the bivalves (Tharme *et al.*, 1996). High intensities of ciliates may cause xenoma, hyperplasia and epithelium vacuolization (Laruelle *et al.*, 1999).

Larval cestode was found to infect on *Anadara* sp. and *M. meretrix*, yet distinct contagion sites. Larval cestodes invaded the digestive tract of both bivalve species, however, cestode larvae also infest the muscle part of *Anadara* sp. Several studies concerned on parasitic cestode larvae in pelecypods (Cremonte *et al.*, 2005; Zeidan *et al.*, 2012; de Buron *et al.*, 2013). The species

identification of cestode was hardly to take place owing to the absence of taxonomically important characteristics and its morphological characteristics was no resemblance with adult tapeworm (Holland & Wilson, 2009). Foraging activities of elasmobranchs around the inhabitant area influenced the distribution area of larval cestode, shellfish being infected by filtering and ingesting the cestode larvae which deposited by elasmobranchs (de Buron *et al.*, 2013). The exposure of individual bivalve towards the environment surrounding might contributed to high rate of parasite infestation. Heavy infestation of larval cestodes on bivalve host leads to physiological stress that directly influence on growth, reproduction, edibility and marketability and lastly exhibited low condition index which can be characterized as poor, transparent and watery (Cremonte *et al.*, 2005). The immunological response of host towards cestode larvae was characterized by the secretion of thick fibrous capsule around the metacestode.

Similarly, to the cestode, digenean has complex life cycle consisting of two or more intermediate hosts to complete their life cycle. Metacercaria has been discovered in muscle and digestive tract of *Anadara* sp. Many studies reported on the occurrence of trematode larvae in different bivalve species (Cremonte *et al.*, 2005; Francisco *et al.*, 2010; El-Wazzan & Radwan, 2013; Dhrif *et al.*, 2015). The proliferation of larval trematode can be influenced by the presence of definitive host (water bird or bony fish) and increment of nutrient and sewage contaminants into water bodies due to anthropogenic factors. In addition, according to Lassalle *et al.* (2007), cockle consisting of softer tissue which was competent to carry loads of metacercaria. The infestation on gonadal space of pelecypods may contributed to castration by affecting gametogenesis of infected host (Carballal *et al.*, 2001). The contagion area such as digestive gland will be destructed by replacing by the sporocyst of digenean larvae in the host tissues, influencing the food absorptions which further resulting in mortality (Vázquez & Cremonte, 2017).

Metazoan parasite such as copepod was occurred infected the gill part of all studied species, except for *Polymesoda expansa*. The interrelation of copepod is known to be resulted in substantial desecration towards the aquaculture venture of bivalve (Kim, 2004). Parasitic copepod lays their eggs and expelled into the water column, it eventually attached to bivalve during filter feeding. The infective stage of copepod disease was the free-swimming larvae that invade into bivalve mollusks through the inhalant siphon (Gosling, 2004). Prevalence of copepod can be fluctuated among species, although all were inhabited in the same region and sampled at the same period (Kraeuter & Castagna, 2001). It can be apropos to the opportunity for encountering the copepod larvae within the area of filtration, host tolerance on parasites and the robustness of inhalant current (Gosling, 2004; Carneiro-Schaefer *et al.*, 2017). Most gill copepod appeared to be commensals, yet the attachment of copepod on gill tissue on shellfish induced localized tissue damage and gill lesion (Bower, 2002).

Pinnotheres sp. was found in mantle cavity (muscle part) of *P. expansa* and *M. meretrix*. The hypothesised factors of occurrence of this parasite in bivalve were the variegation of atmospheric condition (monsoon season and breeding period of marine organism), food availability, abundance of host, salinity, locality of host on beach along with tidal level and current. Some of the studies also stated that the host size will also be influenced on the settlement of peacrab in bivalve (Afiati-Brotohadikusumo, 2002; Watanabe & Henmi, 2009; Cuesta *et al.*, 2019). The bigger the host size, the higher the probability of *Pinnotheres* sp. inhabited within the bivalve shell.

The sessile parasitic organism, *Balanus* sp. was infected the *Anadara* sp. and *C. iredalei* situated at the escutcheon part of bivalve. The barnacle distribution pattern on bivalve mollusks can be influenced by the species of barnacle, tidal zonation in water column, water flow and presettlement of barnacle larvae (Buschbaum, 2001; 2002; Waiho *et al.*, 2017). The distribution of barnacle can prevail to be overlap with each

other, depending on species-specific response and physiological adaptation towards shore level and situation factors such as desiccation stress and wave exposure (Delany *et al.*, 2003; Shinen & Navarrete, 2010). Other than that, stronger water currents favoured by barnacle for better food foraging as it is suspension feeder. Furthermore, the balanid larvae exhibited habitat-selection behaviour which the settlement on hard substrate were proximity to the similar species of adult barnacle. The heavy infestation of barnacle contributing to mortality of bivalve as it need to put more efforts for filtering activities.

Panopeus sp. was the only parasitic crustacean species which infected *C. iredalei*. It was characterized by asymmetrical size of black-tipped claw (Mass, 2008). The interstice of the oyster valve and the habitat structure made the perfect shelter for those minuscule mobile predators made them less susceptible to predation (Kulp & Peterson, 2016). Several studies reported on the predator-prey interaction of oyster and mud crab and the effects in reefs, which reported that the survival of oyster spat were negatively corresponded with the abundance of mud crab population (Abbe & Brietburg, 1992; Hughes *et al.*, 2012). Density and abundance of mud crab population were associated with habitat factors and presence of high-trophic predator.

Conclusion

This study indicated that *Anadara* sp. was observed with the highest infestation of many parasitic species, while *P. expansa* has the least parasite infestation. Parasites such as copepod, *Nematopsis* sp., metacercaria, cestode larvae, *Panopeus* sp., *Pinnotheres* sp., *Balanus* sp. and unidentified ciliates were observed in four bivalve species. Upon the observation, *Nematopsis* sp. was the most severe parasitic infection in three species of bivalves except *P. expansa*. Nonetheless, the rest of the parasites were observed in low prevalence and intensity, without causing significant damage towards host.

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