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Investigation of Parasites and Ecto-Symbiont in Wild Mud Crab, Genus *Scylla* from Terengganu Coastal Water, Malaysia: Prevalence and Mean Intensity

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ABSTRACT

Seventy three sample of wild mud crab genus *Scylla* were collected from setiu wetland, Terengganu coastal waters, Malaysia with the measured of Carapace Width (CW) and Body Weight (BW) 8.9±0.1 and 103.9±3.8, respectively. The percentage prevalence of parasites from these studies is 81.9% and the mean intensity was 13. The total number of 887 parasites that were collected includes barnacle (*Octolasmis* spp.), ciliates, sessile protozoan (*Epistylis* sp.), nematode and copepod. The higher number of parasites that were found is barnacle with prevalence of 71.1% and mean intensity 14.4 followed by ciliates, nematode, copepod and sessile protozoan with prevalence of 18.1, 8.4, 2.4 and 1.2% and mean intensity 1.1, 2.1, 2.0, 1.0, respectively. Results show that pedunculate barnacle genus *Octolasmis* show the most abundance species of parasites, compared to others.

Key words: Wild mud crab, genus *Scylla*, *Octolasmis* spp., parasites and ecto-symbiont

INTRODUCTION

Mud crabs in the genus *Scylla* spp. inhabit brackish water, i.e., mangrove areas and estuaries. There are highly named as an important source for small scale fishers throughout the Asia Pacific region. Mud crabs (*Scylla* spp.) are the most traded seafood commodity in Asia and the culturing practiced is already done in most of Asian country a few years ago. The demand for mud crabs has increased rapidly over the last decade, providing great potential for the development of the mud crab aquaculture industry.

There were three species of mud crab in Malaysia that have been identified which is *Scylla olivacea*, *Scylla paramamosain* and *Scylla tranquebarica* (Ikhwanuddin, 2001, 2013). *Scylla olivacea* is the most common species compared to the other species (Ikhwanuddin, 2001, 2013). *Scylla paramamosain* is solitary species compared to others species which is found in the certain area (Ikhwanuddin, 2001). However, the taxonomy and species status of mud crab in Malaysia are yet to be characterized (Ikhwanuddin, 2013).

In this region, most of the techniques that have been applied for the mud crab culture are fattening and soft shell producing. But most of the crabs are captured from wild. The breeding technologies is not done proper because of the survival rate of larvae is too low. However, the parasites of mud crabs attracted less attention from researchers maybe because of lack of

description of their infections as compared to other commercial exploited crustacean and other aquaculture species. The studies of mud crab parasites is not proper done yet in Asia country because of the new introduces mud crab culture techniques that has been newly started since past year before.

With increasing interest in mud crab aquaculture and growing requirements to manage the health of mud crab population, especially in culturing aspects to consider the effect of parasites in cultured and wild population to ensure long term sustainability of this resources. Therefore, improved understanding of the parasites profile of mud crab is crucial (Jithendran *et al*., 2010).

Parasite is the main organism that can contribute to the disease. Normally bacteria can consider as secondary infection after affecting by parasites. According to Anwar (2005), the parasites can obtain food or shelter from the other organism and derives all the benefit from the association. Thus, parasites can be explain as organisms which live on the external or internal parts of a living host, which derive nutrients from the host and gives nothing in return (Ihwan *et al*., 2013; Scholz, 1999; Anwar, 2005).

Scholz (1999) observed that parasite can caused the apparent damage in the wild organism population and become causative agents of disease that were affected the ecosystem. Parasites are divided into two groups such as "ectoparasites" and "endoparasites". Ectoparasites are parasite that lives on the gill and surface of a host and dependent on at least one gene or its product from that host to complete their cycle (Schmidt and Roberts, 2000; Anwar, 2005). The number of parasites found showed the severity of the infection (Ihwan *et al*., 2009, 2013). Endoparasites is a parasite that lives inside the body of the host organism (Anwar, 2005; Ihwan *et al*., 2013). They live inside the body of the host and damage the tissue during the feeding activity such as nematode or digenea (Ihwan *et al*., 2013).

The information for the parasites is largely extensive with extremely low input gathered. There have parasites species being reported with significant pathogenicity and adverse effect on the production of mud crabs in Asia region. In the present review, the viral, bacterial, fungal, protozoan, parasitic infections and metazoan pathogens of mud crabs and their impact on the aquaculture are discussed. This review is only focused on parasitic infections especially in Asia region i.e., Singapore, Thailand, Philippines, India, etc.

However, the preliminary study on parasites infection in wild and cultured crab populations and seeds production facilities receive little attention. Furthermore, the infected mud crab that potentially affected and maybe transmit to other crabs but not much data are not available except for others crustaceans (Jithendran *et al*., 2010). Against this background, the present compilation is an overview of the potential parasites encountered in mud crab *Scylla* spp. based on the currently available literature besides the work carried out by the Researcher from Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Kuala Terengganu, Malaysia. The objectives of the study, are to determine the prevalence and mean intensity species of parasites and ecto-symbiont found in three species (Ikhwanuddin, 2013) of wild mud crab, genus *Scylla*.

MATERIALS AND METHODS

Seventy three wild mud crabs were sampled from Setiu Wetland (5°40'47.93"N, 102°46'45.04"E) of Terengganu coastal water, Malaysia from September, 2011 to March, 2012 (Fig. 1), which consist of three mud crab species such as *Scylla olivacea*, *S. tranquebarica* and *S. paramamosain*. The crab were brought back to the Parasitological laboratory at Institute of Tropical Aquaculture, Universiti

Fig. 1: Maps of sampling site at setiu wetland, terengganu coastal water, Malaysia

Malaysia Terengganu and kept maintain on aerated aquaria fill with brackish water. The Carapace Width (CW) and Body Weight (BW) were measured by using caliper and electronic balance, respectively.

The mud crab samples were kept in the refrigerator to kill the crab. Observations of parasite were started from outside of its body by using dissecting microscopes. All ectoparasites parasites from external body of the mud crab were collect. Then the carapaces were pulled out and the internal organ and gill was sort and separated into petri dish and observed under dissecting microscopes. The counting process is according to Ihwan *et al*. (2013). The basis of a direct count is the actual counting of every organism (or every living organism). Direct counts include a direct microscopic count which a determination of the number of parasite found within a demarcated region of a slide. By using this total count method, all parasites are counted whether dead or alive. All suspected parasites were recorded in the data sheet. The numbers of parasites or ecto-symbiont were count for further analysis.

The samples that have been collected were directly fixed in bijou bottle. All samples found were fixing in the 70% alcohol for further study. The samples were mounting using glycerin jelly to make permanent slide. Every mounting slide was cover with plain nail polish to make sure the samples were not collapse. The procedures for mounting pedunculate barnacle were modified from Berland (2005) because till now they are not specific procedure for fixing the barnacle.

Digital shots of parasites were taken using Advanced Compound Microscope (Nikon Eclipse 80i) and Advanced Stereo Microscope (Nikon AZ100). For each species of mud crab, the prevalence and mean intensity of parasitic infection as well as prevalence of each parasite species was recorded. The prevalence and mean intensity of parasitic infection were calculated using the formula follows by Margolis *et al*. (1982):

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

RESULTS AND DISCUSSION

The mean mud crab Carapace Width (CW) and Body Weight (BW) sampled from the present study were 8.9±0.1 and 103.9±3.8, respectively. The percentage prevalence of parasites from the present study is 81.9% and the mean intensity was 13 (Table 1). A total individual number of 887 parasites were collected includes barnacle (*Octolasmis* spp.), ciliates, sessile protozoan (*Epistylis* sp.), nematode and copepod (Fig. 2). The higher number of parasites that were found is barnacle with prevalence of 71.1% and mean intensity 14.4 followed by ciliates, nematode, copepod and sessile protozoan with prevalence of 18.1, 8.4, 2.4 and 1.2% and mean intensity 1.1, 2.1, 2.0, 1.0, respectively (Table 1). Results show that pedunculate barnacle, genus *Octolasmis* show the most abundance species of parasites compared to others.

From this study, we can assume that wild mud crabs are less infected with the parasites except pedunculate barnacle. The natural environment of the growth of barnacle contribute to the highly infection on the mud crab especially in the gill of this crab species. Disease management is an important component of mud crab aquaculture as cultured of mud crab are afflicted by range of bacteria, parasitic disease agent, ecto-symbiont organism and this is major problem especially for larvae culture (Jithendran *et al*., 2010).

Table 1: Prevalence and mean intensity of mud crab parasites

No. crab infected (N)	Type of parasites	No. of parasites	Prevalence (%)	Mean intensity
15	Ciliates	17	18.1	1.1
59	Barnacle	852	71.1	14.4
7	Nematode	15	8.4	2.1
1	Sessile	1	1.2	1.0
1	Copepod	2	2.4	2.0

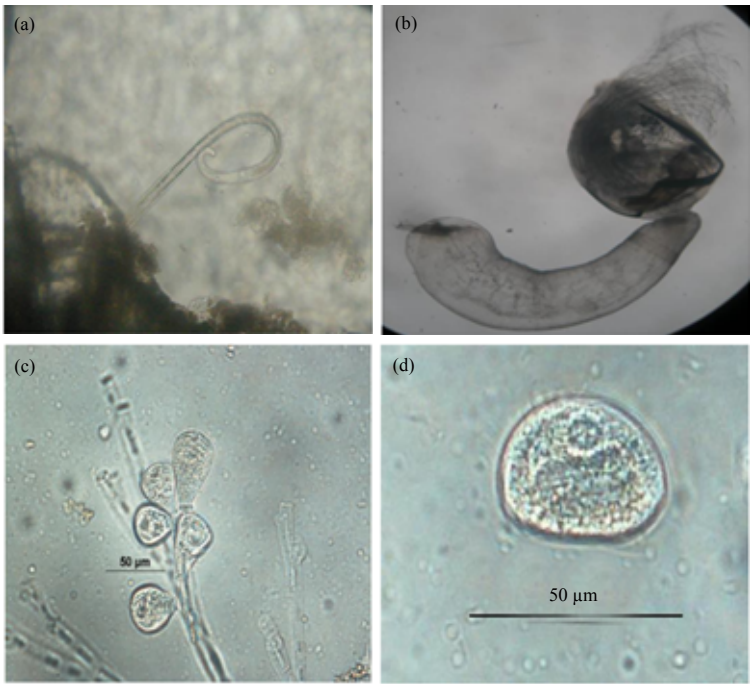

Fig. 2(a-d): Parasites and ecto-symbiont that were found during the present study, (a) Nematode, (b) Pedunculate barnacle, (c) Sessile and (d) Ciliates

Nematode and copepod that were found during this study maybe free living organism those trap during the respiration of the mud crab. This nematode normally lives in the mud and this is assumed that the nematodes were filtered during the respiration of the mud crab. From the previous research, blood parasites, *Hematodinium* are one of the suspected parasites that were found commonly in cultured condition (Lavilla-Pitogo and de la Pena, 2004; Lio-Po *et al*., 1982). But in this present study, no blood parasites were found and this maybe because of the mud crab were caught from the wild.

The present study clearly shows that all the wild mud crab species were infected with pedunculate barnacle genus *Octolasmis*. This factor maybe because of the barnacle larvae are already developed in the natural environment. Mud crab is currently one of the potential species for aquaculture in Malaysia. This host-species are distributed in tropical environment around the tropical country. Therefore, it appears that acclimation of mud crab from wild into captivity will lead to the transfer of parasitic and ecto-symbionts and that effective quarantine measures will be required to avoid introducing these parasites into aquaculture (Ihwan *et al*., 2013).

Parasites are generally found aggregated among their hosts (Kvingedal *et al*., 2006; Morado, 2011; Morado *et al*., 2012). Aggregation can be caused by numerous factors. Morand and Guegan (2000) reported that among the parasites, there have relationships between sources of infection and hosts and/or differential susceptibility of hosts to parasites attacks. Barber (2005) and Ihwan *et al*. (2013) stated that parasites depend on host-derived energy for growth and development and potentially affected by host's ability to acquire nutrients under competitive foraging scenarios.

A wide variety of parasitic organisms such as blood parasite, nematode and ciliated protozoan have been reported as causing significant problem in aquatic organisms especially mud crab (Hudson and Lester, 1994; Anderson and Prior, 1992; Kvingedal *et al*., 2006; Quinitio and Parado-Estepa, 2003; Jithendran *et al*., 2010; Jeffries and Voris, 1979; Jeffries *et al*., 1982, 1989, 1995).

Ihwan *et al*. (2003) observed the relationships between parasites of some wild and cultured aquatic organism. In the wild environment, parasitic diseases of host-species are cause predominantly by protozoan, particularly ciliates (Cruz-Lacierda and Erazo-Pagador, 2001). Due to the complexity and enormity of the marine ecosystem, disease problem in wild aquatic organisms are difficult to approach (Sindermann and Lightner, 1988). Meanwhile to prevent the parasitic infection, identification and diagnosis are important (Ihwan *et al*., 2015).

Parasites can adapt to any changes in their surroundings, depending on the environment and host health condition. Mud crab also have a problem of disease that are related to the environment condition including the contamination of water and soil, low dissolve oxygen, highly nutrient loaded and exposed to polluted environment (Quinitio and Parado-Estepa, 2003; Neufeld *et al*., 1980; Holliday, 1985; McLaughlin *et al*., 1996; Jithendran *et al*., 2010; Lavilla-Pitogo and de la Pena, 2004).

Although parasites might be expected to grow faster in host that are better at acquiring nutrient from natural ecosystem, it is possible that the most competitive hosts are better at countering infections, if they have an improve immune response or are able to limit the availability of nutrients to parasites (Barber, 2005). Therefore, the potential for a parasite species for spread to host individuals and accumulate in these hosts was to some extend determined by its life history strategy (Poulin, 2006). Mud crab parasites usually arise from a complex interaction of a variety of factors such as the environment, physiological aspects (Jeffries *et al*., 1989).

Diseases caused by various pathogens of fish and shrimp have been well documented, while little is known of its effect on mud crab both under hatchery and farming conditions. Mud crabs are traditionally considered as quite hardy organisms to many infectious diseases and the occurrence of diseases under culture condition is on the rise with the intensification of crab culture (Jithendran *et al*., 2010).

Much research is needed to understand the pathogens, host and environmental interaction under hatchery and farming conditions to increase the productivity and conservation of mud crab as a sustainable aqua-resource. We have limited understanding of pathogen profile of mud crab in hatchery and grow-out culture, how these pathogens are transmitted and their potential for transmission to other commercially exploited and cultured species. The cross infections between mud crab and other cultured penaeid shrimps under field conditions and the reported role of mud crab as 'carriers' of pathogens also need further elucidation (Jithendran *et al*., 2010).

This is important as culture operation of mud crab still relies on wild caught 'water crabs' or crablets. The traditional strategy of 'stamping out the pathogens' is difficult to apply in aquatic environment. A significant feature of marketing of mud crab involves the movement of live animals between capture/culture sites and market sites with potential for transmission of pathogens in situ, facilitating the spread to a relatively naïve host and/or environment. The basic knowledge concerning the pathogens of cultured mud crab and how they interact with their hosts lags behind the needs of the industry (Jithendran *et al*., 2010).

The stress organisms inclined to get any disease such as parasites, bacteria, virus and fungus. There was limited study about the disease problems in wild and cultured mud crab in Malaysia. But much information of parasite infection is report from Australia (Kvingedal *et al*., 2006), Philippines (Bian *et al*., 1979), India (Jithendran *et al*., 2010) and Thailand (Prasadan and Janardanan, 2001; Tuntiwaranuruk *et al*., 2004), such as *Hematodinium* sp. (Li *et al*., 2008), *Sacculina granifera* (Phelan and Grubert, 2007; Boschma, 1949; Knuckey *et al*., 2006). *Loxothylacus ihlei* (Phelan and Grubert, 2007; Weng *et al*., 2007), *Lagenidium callinectes* and *Haliphthoros* sp. (Bian *et al*., 1979).

Parasite could devastate mud crab population in the wild if there is no any action was taken. From the previous study, some parasites, such as *Laxothylacus ihlei* can show the symptom of enlargement of abdominal flap of males to a size comparable to female (Weng *et al*., 2007). This Sacculinid infection has an implication of stunts growth and promotes feminization of mud crab (Phelan and Grubert, 2007; Boschma, 1949; Knuckey *et al*., 2006).

The prevalence and spread of infectious disease are influenced by a variety of factors including host density, parasite transmission mode and the spatial structure of host population (Altizer *et al*., 2000; Poulin, 2006; Ihwan *et al*., 2013). According to Lavilla-Pitogo and de la Pena (2004), disease usually can be defined as any abnormality of structure, behavior or function. Disease spreading in the aquatic organism are including bacteria, parasites, virus and other environmental factor (Jithendran *et al*., 2010).

CONCLUSION

This study indicated that the parasites and ecto-symbionts were common parasites that have been found in wild mud crab, genus *Scylla*. Pedunculate barnacles, genus *Octolasmis* is the most prevalence species that have been found during this study. Nematode and copepod that accidently found are assumedly as a free living because there is sign or specific cause of infection were found during the diagnosis.

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