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ECTOPARASITES AND VIBRIOS ASSOCIATED WITH FATTENING CULTURED MUD CRABS [*Scylla serrata* (Forsskal, 1775)] FROM PEMALANG COAST, INDONESIA

Sarjito[a*], Alfabetian Herjuno Condro Haditomo[a], Desrina[a], Ferdian Bagus Ferinandika[b], Lilik Setyaningsih[c], Slamet Budi Prayitno[a]

[a]Aquaculture Study Program, Fisheries Department, Fisheries and Marine Science Faculty, Diponegoro University, Jl. Prof. Soedarto, SH Tembalang, Semarang – 50275, Indonesia
[b]PT. Perindo, Jl. Muara Baru, Penjaringan Jakarta Utara 14440, Indonesia
[c]Post Graduate Student of Aquaculture Study Program, Fisheries Department, Fisheries and Marine Science Faculty, Bogor Agriculture Institute,Bogor, Jl. Lingkar Akademik, Kampus IPB, Dermaga Bogor 16680, Indonesia

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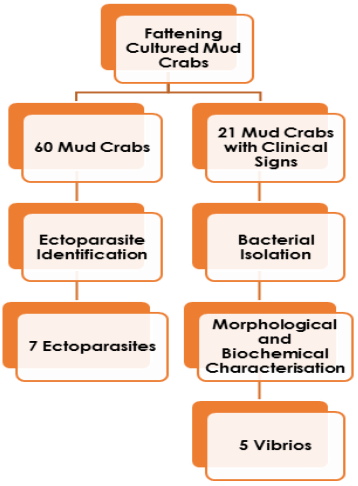
*Corresponding author
sarjito_msdp@yahoo.com

Graphical abstract



Abstract

Infectious of ectoparasites and vibrios were found as a problem in fattening mud crab. The aims of this study were to determine the clinical sign of infected mud crabs and to identify the ectoparasites and generic vibrio in the fattening mud crabs from Pemalang Coast, Indonesia. Ectoparasites were collected by smear method. Whereas, 20 isolates were collected from hepatopancreas, gills, hemolymph and injured carapace of the mud crabs. Thiosulphate Citrate Bile Salt Sucrose (TCBS) medium was used to isolate suspected vibrios. The clinical signs of mud crabs infected by ectoparasites were shown through the damaged gills and the attachment of other organisms on the gills, whereas the clinical signs of mud crabs infected by vibriosis were shown through red spot on carapace and wound in carapace, claw and abdomen, followed by weak condition. It also indicated that seven ectoparasites were *Ichthyobodo* sp., *Epistylis* sp., *Carchesium* sp., *Vorticela* sp., *Octolasmis* sp., *Lepeophtherius* sp, and *Copepodit* sp., whilst five *Vibrios were V. harveyi*, *V. Cholerae*, *V. parahaemolyticus*, *V. alginolyticus* and *V. fischeri*.

Keywords: Ectoparasites, fattening, mud crabs (*Scylla serrata* [Forsskal, 1775]), vibrios

1.0 INTRODUCTION

Mud crabs (*Scylla serrata* [Forsskal, 1775]) is one of aquaculture products which has a high value in domestic and international markets [1]. To meet the mud crabs market needs, fattening culture of mud crabs was widely implemented to increase the production. However, disease outbreaks often attack in fattening culture of mud crab, especially in Pemalang district, Central Java, Indonesia. Disease of mud crab may be caused by bacterial and parasites [2, 3]. According to Chen *et al.* [4], vibriosis

becomes a big concern in mud crabs culture. Therefore, both of them become seriously concerned in mud crab culture because they will decrease the price and production of mud crabs.

Several researches had been conducted to find out the diversity of ectoparasite [2] and vibrios in mud crab [1, 2]. There are a number of parasites that have been found, such as *Vorticella* sp., *Epistylis* sp., *Zoothamnium* sp., *Acineta* sp., *Octolasmis* sp., *Euphlotes* sp., *nematodes* [2], *Dermocystidium* sp. and *Rhinosporidium seeberi* [5]. In addition, some studies have reported various bacteria from genus *Vibrio* in mud crabs, namely *V. parahaemolyticus, V. vulnificus, V. splendidus, V. orientalis* [2], *V. campbelli, V. nereis,* and *V. fischeri* [3]. However, based on observation conducted, there are limited information about ectoparasites and vibrios infected on the fattening mudcrabs culture in Indonesia. This present study aimed to know the clinical signs of fattening cultured of mud crabs infected by ectoparasite and vibrios and identify the ectoparasite and vibrios that were infected in fattening culture of mud crabs.

2.0 EXPERIMENT

2.1 Mud Crab Sampling

A total of 81 mud crabs (*S. serrata*), presumably infected by parasite and bacteria with body length of (17.53 ± 0.82) cm were collected from brackish water pond of fattening culture in coast of Pemalang, Central Java, Indonesia. In this study, 60 mud crabs were used to evaluate the ectoparatites infection while the rest was used to determine the diversity of vibrios. Identification of mud crabs infected by ectoparasite and bacteria was referred to method proposed by Lavilla and De La Pena [2]. After collection, the mud crabs samples were kept in a container and brought to the Integrated Laboratory of Diponegoro State University for ectoparasite and bacterial isolation.

2.2 Parasites and Bacterial Isolation

To collect and investigate the ectoparasites from mud crab, smearing all the surface body was applied. Smearing was applied to the carapace, swimming legs, walking legs, claws and gills. After that, the smearing result was observed under a microscope.

Bacteria were isolated from hepatopancreas, gills, haemolymph and wound or injured carapace of the mud crabs by streaking plates on specific media for vibrio, TCBS (Thiosulphate Citrate Bile Salts Sucrose). In addition, bacterial isolation was conducted by pouring plate method from wound carapace and pale gill that were scrape off with a sterile knife. Then, a dose of 1 mL of a dilution of 10^{-1}, 10^{-3} and 10^{-5} CFU mL^{-1} was spread on the TCBS and was incubated at room temperature for 24 h to 48 h [6, 7]. Then, based on the morphological feature, colonies were randomly picked and purified using streak plate method. In order to obtain pure isolates, purification was performed, then pure isolates were stored in nutrient agar (NA, Merck).

2.3 Identification of Ectoparasite and Bacteria

Identification of ectoparasites was based on method of Kabata [8], Grabda [9] and Moller and Anders, [10]. Meanwhile, clinical signs of moribund mud crabs were described according to Lavilla and De La Pena [2]. Based on the morphological performance, to estimate diversity of the vibrios on the mud crab, some isolates were chosen for further investigation. Identification of isolates was carried out with morphological and biochemical tests approach at the Laboratory of Fish Quarantine, Quality Control and Safety of Fishery Class II Semarang. The test results were then compared for similarity to the genus *Vibrio,* based on method of Buller [11], and Austin and Austin [12].

3.0 RESULT AND DISCUSSION

3.1 Ectoparasites

Clinical signs of mud crab (*S. serrata*) infected by ectoparasites can be seen in Figure 1. The clinical signs were shown through the fouling of other organisms that resemble sprouts (*Octolasmis* sp.) on the gills and crab's injured body parts (a), the appearance of thin fibers such as moss fouling on the carapace, white spots on the carapace (b) and gill colour change into black or pale (c).

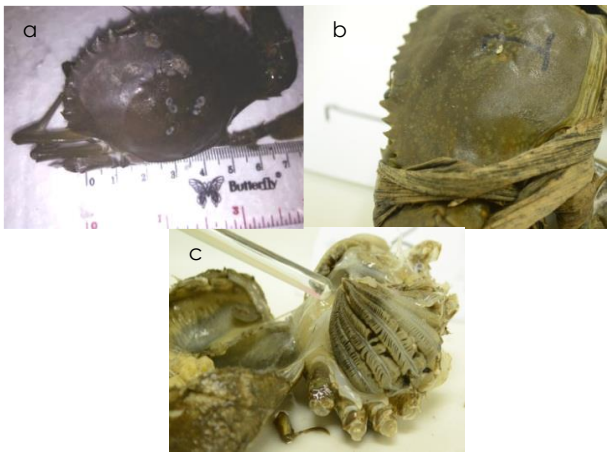


Figure 1 Clinical sign of mud crab infected by Ectoparasites: a. Fouling of other sprouts on the injured body parts; b. White spots on the organisms that resemble carapace; c. Gill colour change into black or pale

The clinical sign of mud crab infected by *Ichtyobodo* sp. was the invisibility of white spot on the carapace. Clinical signs in the mud crabs marked the appearance of thin fibers such as moss green gray on the carapace, suggesting that crabs are suspected of being infected ectoparasites *Carchesium* sp. and *Vorticella* sp. The clinical signs has rarely reported in the mud crab, however reported in black tiger shrimp [13]. Clinical signs of fouling of other organisms like sprouts found in the claws, carapace and gills of mud crab infected *Octolasmis* sp. It was resulted on damaged on the carapace and gills, so the gills infected *Octolasmis* sp. turned to pale colour , and resulted on difficulty breathing [14]. Irvansyah *et al.* [15] reported that *Octolasmis* sp. could infect wounds on carapace.

A total of seven ectoparasites (Figure 2) were found in this research namely *Ichthyobodo* sp. (a), *Epistylis* sp. (b), *Carchesium* sp. (c), *Vorticella* sp. (d), *Octolasmis* sp. (e), Copepodit sp. (f) and *Lepeophtheirus* sp. (g). Four ectoparasites (*Ichthyobodo* sp., *Epistylis* sp., *Carchesium* sp. and *Vorticella* sp) belong to the class of protozoea, while three others (*Octolasmis* sp., *Copepodit* sp. and *Lepeophtheirus* sp.) were included in the class of crustacea.

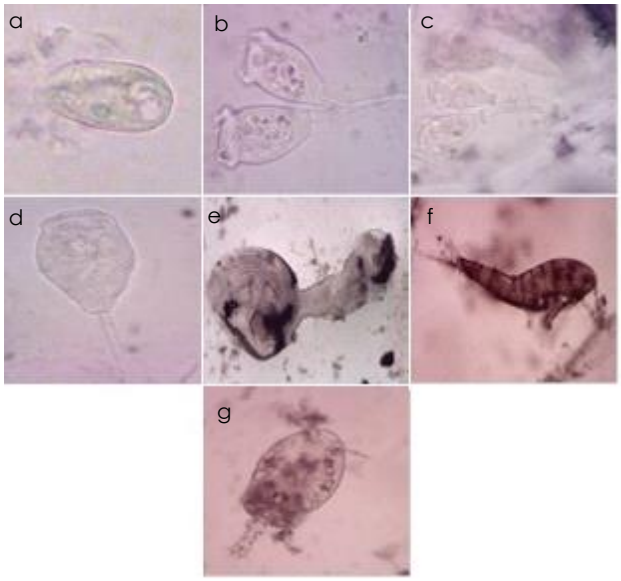


Figure 2 Ectoparasites: a. *Ichtyobodo* sp.; b. *Epistylis* sp.; c. *Carchesium* sp.; d. *Vorticella* sp.; e. *Octolasmis* sp.; f. *Copepodit* sp.; g. *Lepeophtheirus* sp.

Ectoparasites from class protozoae and crustacean were commonly found on mud crabs [16]. *Ichthyobodo* sp. was found in oval form, translucent color and has four pieces of flagella at the pointed end [17]. *Ichthyobodo* sp. can injure the host body with a tapered shape and use flagella to attach to the host. *Ichthyobodo* sp. was ectoparasites with the highest number found in this study. It was believed to be related to environmental conditions and reproduction binary fission in these ectoparasites. According to Sarjito *et al.* [17], the ectoparasites can breed in very much the same way of binary fission so that when the environmental conditions were very favorable for the life of the parasite, the parasite would be able to proliferate in the very large number and within 1 wk to 2 wk can heavily infect host. *Ichthyobodo* sp. can tolerate temperatures up to 38 °C and will actively reproduce at a temperature of 24 °C to 25 °C, while the low pH waters (pH 4 to pH 5) did not inhibit the reproduction of these ectoparasites [8].

The *Octolasmis* sp. morphological structure has tergum, carina, capitulum, scutum and legs [18]. Irvansyah *et al.* also described that the morphological structure of *Octolasmis* sp. consists of a liquid that serves to coat the inside of organs, capitulum which serves as the stomach which can destroy the nutrients of food that can be digested by the body, and the legs are used to attach themselves to the gills of host. Host of *Octolasmis* sp. in this study was found in large numbers in the gills. It is believed that this was to be related to the abundance of *Octolasmis* sp in this environment. These ectoparasites have been reported to infect mud crab [14, 15, 19], *Portunus pelagicus* [20] and blue crabs (*Callinectes sapidus*) [21].

Epistylis sp. were identified in this study in shape like a bell, with flagella and appeared as colony (about two to five visible colonies). The ectoparasites colony size and number was varied [8]. In addition, these ectoparasites are characterised in colonies and non-contractile or not moving [17, 22]. Nicolou *et al.* [23] reported that these ectoparasites are found in many substrates areas. Waters with dissolved organic matter contained high and low disolved oxygen (DO) concentrations can increase growth *Epistylis* sp. Quinitio *et al*. [24] found that *Epistylis* sp. can reproduce optimum water with a temperature of 10 °C to 25 °C, pH 6.5 to pH 7 and salinity 15 ng L^{-1} to 31 ng L^{-1}. These ectoparasites have been reported to infect young mangrove crabs in District Sedati, Sidoarjo regency [15] and wild mud crab from Trengganu, Malaysia [19].

Similar to the statement of Kabata [8], present study also observed that *Carchesium* sp. was found in shape like bell, cilia and appeared as visible colonies (more than three colonies). According to Nicolau *et al.* [23], *Carchesium* sp. and other protozoa groups were found in substrates areas and waters with high dissolved organic matter content. Darwish [14] also reported that these ectoparasites has been found in mud crabs from the mangrove forest area of Tarakan, East Kalimantan, Indonesia.

Vorticella sp. were found in shape like a bell or bells and yellowish, with contracted cells, macronucleus, adoral membrane, long stems and do not colonize. Ectoparasites shape is almost like *Epistylis* sp. and *Carchesium* sp., but *Epistylis* sp. and *Carchesium* sp. live in colonies while *Vorticella* sp. solitary.

Morphology of *Vorticella* sp. was also found by Ihwan *et al.* [8] who stated that *Vorticella* sp. have solitary life with contracted cells, macronucleus, and adoral ciliated membrane. This movement of this ectoparasite resembles with *Carchesium* sp. wherein the stalk on *Vorticella* sp. can be shortened and rolled, allowing *Vorticella* sp. to move. Azis *et al*. [13] stated that stalks in *Vorticella* sp. can be shortened and rolled when stimulated by movement. According to Nicolou *et al.* [23], substrates, waters with high organic matter content was very supportive for life of *Vorticella* sp. These ectoparasites have been reported to infect mud crab [14, 15]. *Vorticella* sp. was also occasionally detected in mud crab.

Copepodit sp. was found to have a segmented body with eight segments and has two tails behind abdomen. This is in accordance with Moller and Anders [10] who argued that *Copepodit* sp. has a segmented body up to 16 segments. *Lepeophtheirus* sp. was found to have a pair of antennas, legs and genital segment. This is in accordance with some studies which revealed that *Lepeophtheirus* sp. has a pair of antenna 1, a pair of antenna 2, a pair of maxilla 1, a pair of maxilla 2, a pair of maxiliped, five pairs of arms, genital and abdominal segments [9, 10, 26]. The result of this research shows that the ectoparasites were found in small amount and do not cause clinical signs in mud crab. This result is contrary to Moller and Anders [10] who stated that *Copepodit* sp. of this type is included in the group of parasites that cause serious infections in organisms cultivation. Most organisms of *Lepeoptheirus* sp. impact on the damage of mud crabs including head and muscle, and destroying the surface of the body.

This study revealed that most of 60 fattening mud crab samples were infected with ectoparasites. The ectoparasites, number of infected crabs, intensity, prevelance and dominance can be seen in Table 1.

Based on the Table 1, the total number of 37 514 ectoparasites were found in the present study. The highest number of parasites that were found is *Ichthyobodo* sp. with prevalence of 78.3 %, intesity of 535, and dominance of 66.9 %, followed by *Octolasmis* sp. *Epistylis* sp., *Vorticella* sp., *Carchesium* sp., *Copepodit* sp. and *Lepeophtheirus* sp with prevalence of 66.9 %; 41.6 %, 10 %, 3.3 %, 1.6 %, 1.6 %, intensity of 238.9, 147.2, 179.5, 21.5, 10, 3, and dominance of 15.9 %, 15.6 %, 0.9 %, 0.31 %, 0.3 %, 0.01 % respectively. Therefore, the result found that *Ichtyobodo* sp., *Octolasmis* sp. and *Epistylis* sp. show the most abundant species of parasites in mud crabs from Pemalang Coast, Indonesia, compared to others.

Table 1 Ectoparasites, intensity, prevalence and dominance of Ectoparasites on mud crabs (*S. serrata*) from Pemalang Coast

∑ Sample	∑ Infected Sample	Ectoparasite	∑ Ectoparasite	I	P (%)	D (%)
60	47	*Ichtyo bodo* sp.	25 145	535.0	78.3	66.90
60	25	*Epistylis* sp.	5 973	238.9	41.6	15.90
60	46	*Octolamis* sp.	5 895	147.2	66.6	15.60
60	2	*Charchesium* sp.	359	179.5	3.3	0.90
60	6	*Vorticella* sp.	129	21.5	10.0	0.30
60	1	*Copepodit* sp.	10	10.0	1.6	0.02
60	1	*Lepeophtheirus* sp.	3	3.0	1.6	0.01

Notes : I = intensity, P = Prevalence, D = Dominance

Results of this identification also indicated that four ectoparasites (*Ichthyobodo* sp., *Epistylis* sp., *Carchesium* sp. and *Vorticella* sp) were included in the class of protozoea, while three others (*Octolasmis* sp., *Copepodit* sp. and *Lepeophtheirus* sp.) were included in the class of crustacea. Ectoparasites are generally found aggregated among their hosts [5]. A diversity of parasitic organisms such as blood parasite, nematode and protozoan have been reported as causing significant problem in mud crab [16].

The present study clearly shows that most of the fattening cultured mud crab from Pemalang coast were infected by ectoparasites, mainly *Ichthyobodo* sp, *Octolasmis* sp., and *Epistylis* sp. This may be caused by the development of the ectoparasites in the natural environment. Suitable environment for the growth of *Ichthyobodo* sp., *and Epistylis* sp. may contribute to this ectoparasite infection on the mud crab. The seeding of seed mud crabs which was captured from wild into fattening cultured brackish pond will be able to transfer the parasitic. Therefore, quarantine will be required to avoid proliferation of these parasites into aquaculture [27].

Based on the microhabitat, ectoparasite showed that only *Octolasmis* sp. was detected on all part of mud crab surface body (swimming leg, walking leg, claw, carapace and gills), but it was mostly found in the gills. Whereas, *Ichthyobodo* sp. was found in swimming leg, walking leg, claw and carapace. Number of ectoparasites found was based on the microhabitat presented in Table 2.

Table 2 Ectoparasite associated with fattening mud crabs (*S. serrata*) based on microhabitat

Ectoparasite	Swimming Leg	Walking Leg	Claw	Carapace	Gills
Ichtyobodo sp.	4 707	8 694	2 728	9 016	0
Epistylis sp.	73	102	2 805	2 993	0
Octolamis sp.	41	7	4	3	5 959
Charchesium sp.	0	0	329	30	0
Vorticella sp.	31	58	0	40	0
Copepodit sp.	0	1	1 234	0	9
Lepeophtheirus sp.	0	2	0	0	1

3.2 Vibrios

This present research found that fattening cultured pond caused bacterial diseases on mud crabs. It can be seen in Figure 3 which shows the clinical signs of mud crab affected by bacterial diseases from the fattening cultured pond. The clinical signs were gill opening, dry and dark, brown spots and wounds on the carapace (a), claws (b) and the ventral (c).

Figure 3 Clinical signs of mud crab affected by bacterial diseases: a. Brown spots and wounds in the carapace; b. Wounds in the ventral; c. Wounds in the claws

Infection of vibrios in the fattening mud crab was characterized by dark spots on the carapace, wounds in the abdomen, claws and ventral. The similar clinical signs have been reported by Sarjito *et al.* [1], Lavilla and De La Pena [2], Jithendran *et al.* [16], and Wang [32].

Bacterial isolation results in total of 20 bacteria isolates were obtained from hepatopancreas, gills, haemolymph and injured or wounded carapace of the 21 moribund fattening mud crabs (Table 3). Based on the morphological perfomance differences of isolates, such as: colony colour, source, form, characteristic and medium used, five isolates (SJP2; SJP3; SJP7; SJP10; SJP15) chosen for further investigation was represented on Table 4.

The present study found that the diversity of vibrios associated with fattening mud crab from Pemalang coast, Central Java, Indonesia was *V. harveyi* (SJP2), *V. cholerae* (SJP3), *V. parahaemolyticus* (SJP7), *V. alginolyticus* (SJP10) and *V. fischeri* (SJP15). Therefore, the vibrios diversity on fattening mud crab from Pemalang coast was found higher than mud crab from Semarang Gulf [1] and WSSV infected mud crabs [33]. However, this diversity was lower than the diversity that was reported by Jithendran *et al.* [16] in cultured and wild crabs in India.

Table 3 Characteristic of isolate bacteria associated on mud crab from fattening cultured pond

No.	Isolate code	Media	Source	Colony		
				Color	Form	Characteristic
1	SJP1	TCBS	Gill	Yellow	Rounded	Convex
2	SJP2	TCBS	Gill	Yellow	Rounded	Convex
3	SJP3	TCBS	Wound	Yellow	Rounded	Convex
4	SJP4	TCBS	Wound	Yellow	Rounded	Convex
5	SJP5	TCBS	Hepatopancreas	Yellow	Rounded	Convex
6	SJP6	TCBS	Gill	Green	Rounded	Convex
7	SJP7	TCBS	Hepatopancreas	Green	Rounded	Convex
8	SJP8	TCBS	Hepatopancreas	Green	Rounded	Convex
9	SJP9	TCBS	Haemolymph	Yellow	Rounded	Convex
10	SJP10	TCBS	Hepatopankreas	Yellow	Irregular	Convex
11	SJP11	TCBS	Gill	Grey	Rounded	Convex
12	SJP12	TCBS	Hepatopancreas	Yellow	Rounded	Convex
13	SJP13	TCBS	Gill	Grey	Irregular	Convex
14	SJP14	TCBS	Gill	Yellow	Irregular	Convex
15	SJP15	TCBS	Wound	Grey	Rounded	Convex
16	SJP16	TCBS	Wound	Grey	Rounded	Convex
17	SJP17	TCBS	Gill	Yellow	Rounded	Convex
18	SJP18	TCBS	Gill	Yellow	Rounded	Convex
19	SJP19	TCBS	Hepatopancreas	Yellow	Rounded	Convex
20	SJP20	TCBS	Gill	Yellow	Rounded	Convex

V. harveyi was commonly reported as a bacterial pathogen associated with crustacean culture [30]; marine vertebrates, invertebrates [34] and grouper [31]. Therefore, *V. harveyi* was recognized as bacterial pathogen throughout the world [35]. *V. harveyi* was reported as a causative agent of vibriosis on zoea phase of mud crab [16, 33]; mud crabs [1, 2]. The incident of the bacteria was also found in mud crabs from brackish water pond surrounding Semarang Bay, Indonesia [1]; wild mud crab from Malaysia [29]; and swimming crabs, *Portunus pelagicus* [36]. The present study indicated that this bacteria was also found in the fattening of mud crab, *S. Serrata*, from Pemalang Coast.

V. cholarae was also reported associated with haemolymph blue crabs [37]. The bacterium, according to Wang [32], is generally assosiated with marine and fresh crabs. Guthrie and Daniel [38] also reported that this bacteria infected shrimps and mud crabs. The present study also revealed that this bacteria was found in the fattening mud crab. *V. alginolyticus* was commonly reported as bacterial pathogen associated with shrimps [30], and abalone [39, 40]. This bacteria is recognized as a causative agent of vibriosis on larvae of abalone, *Haliotis diversicolor supertext* [41]*;* grouper [31] and bacterial diseases of mud crabs in Malaysia. This bacteria was also found in fattening mud crabs from Pemalang coast.

Table 4 Five isolates selected of genus Vibrio assosiated with mud crab (*S. serrata*) from Pemalang Coast

No.	Isolate code	Media	Source	Colony		
				Color	Form	Characteristic
1	SJP2	TCBS	Gill	Yellow	Rounded	Convex
2	SJP3	TCBS	Wound	Yellow	Rounded	Convex
3	SJP7	TCBS	Hepatopancreas	Green	Rounded	Convex
4	SJP10	TCBS	Hepatopankreas	Yellow	Irregular	Convex
5	SJP15	TCBS	Wound	Grey	Rounded	Convex

V. fischeri was found in the fattening mud crab from Pemalang Coast. According to Sizemore and Davis [40], the bacteria were commonly isolated from sea water worldwide and it was recognized as normal microbes associated with marine organism. However, the bacteria were reported in mud crab that was infected by WSSV [3]. Sarjito *et al.* [1] also found that the *V. fischeri* was to be pathogenic for mud crab, *S. serrata*. Therefore, *V. fischeri* may accelerate outbreak of vibriosis [41]. *V. parahaemolyticus* was found in mud crab from Pemalang Coast; Grouper [31]; shrimps [30]. *V. parahaemolyticus* were also detected on mud crabs [2, 4, 29]; and blue crabs [37]. The bacteria were found as a causative agent of bacterial diseases of mud crabs in Malaysia [29].

In order to food safety, it is important to properly select and choose the raw sea food to reduce the effect of the pathogen bacteria on the human health. According to Wang [32], some bacteria pathogens, such as *V. cholerae*, should be paid attention to as they may represent potential health hazards to human beings, causing serious diseases when the crab is consumed as raw sea food.

4.0 CONCLUSION

Clinical signs of fattening mud crab (*S. serrata*) infected by ectoparasites were shown through the formation of fouling of other organisms resemble with sprouts on the gills and the injured body parts. In addition, it was also shown through the appearance of white spots and thin fibers such as moss fouling on the carapace, followed by the change of color into black or pale on the gills. Meanwhile, the clinical signs of moribund mud crabs that were infected by genus *Vibrio* were shown through the appearance of brown spots and wounds on the carapace, claws and ventral. This study also found that ectoparasites associated with fattening mud crab from Pemalang Coast were *Ichthyobodo* sp., *Epistylis* sp., *Carchesium* sp., *Vorticella* sp., *Octolasmis* sp., *Copepodit* sp. and *Lepeophtheirus* sp. Regarding to the vibrios, the fattening mud crabs from Pemalang coast, Central Java, Indonesia were associated with *V. harveyi*, *V. cholerae V. parahaemolyticus*, *V. alginolyticus* and *V. fischeri.*

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