aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Animal and Veterinary Advances 7 (2): 173-179, 2012
ISSN 1683-9919 / DOI: 10.3923/ajava.2012.173.179

Parasites of Slipper-cupped Oyster *Crassostrea iredalei* from Pulau Betong, West Coast of Penang, Malaysia

M. Jasim Uddin, N.B. Kamsol, Zulfigar Yasin and Aileen Tan Shau-Hwai
Marine Science Laboratory, School of Biological Sciences, University, Sains Malaysia, 11800 Penang, Malaysia

Corresponding Author: M. Jasim Uddin, Marine Science Laboratory, School of Biological Sciences, University, Sains Malaysia, 11800 Penang, Malaysia Tel: (6)016-427-3404 Fax: (604) 653-3500

ABSTRACT

Until now, knowledge about bivalve mollusk diseases in tropical waters of Asia is scarce. A histopathologic survey was performed on the slipper-cupped oyster, Crassostrea iredalei from the Malaysian waters for the first time to investigate the presence of parasites and their effects on host tissues. C. iredalei samples were collected from Pulau Betong, west coast of Penang, Malaysia. A total of 60 oysters were examined from October 2010 to January 2011. After shell height measurement, the samples were dissected. A transverse section (3 mm) was cut through the anterior part of the soft body containing gills, mantle, digestive glands, gonads and foot tissue. The sections were processed by standard histological techniques, stained with hematoxylene and eosin and examined under microscope. A protozoan parasite, Nematopsis sp. was detected from the connective tissue of digestive gland tubules, gills, mantle and gonads. The prevalence of infection of Nemaptosis sp. were 15% each during October and November and 30% during January. The maximum number of hemocytes containing Nematopsis sp. per histological section was 5. The oocysts were oval in shape and maximum no. of oocysts per hemocyte was 7. No pathological threat was detected from the histopathological survey performed. This preliminary survey will be useful for planning a health monitoring programme for the natural and cultivated slipper-cupped oyster and other bivalves of commercial importance in Malaysian waters.

Key words: *Slipper-cupped oyster, Crassostrea iredalei,* histopathology, Malaysia, *Nematopsis,* prevalence

INTRODUCTION

Aquaculture production is increasing at a faster rate to meet up the demand of the rapidly increasing population. Undoubtedly, global aquaculture is playing a vital socio-economic role in relation to nutrition, employment opportunities and income generations (Berthe, 2008). In 2007, shellfish contributed over 26% of the global aquaculture production. Asia contributed over 80% of the global mollusk aquaculture production during this time and bivalves including oysters, clams, mussels, cockles and scallops accounted for 90% of such production (FAO, 2009). Although Asia is the main contributor in mollusk aquaculture production in the world, the study of mollusk diseases still remained a neglected field in most of the tropical parts of Asia. There are many examples of bivalve disease outbreaks associated with mass mortalities in many parts of Asia. Therefore, it is high time to focus on this field for the protection of the increasingly active mollusk aquaculture sector in this region.

Aquaculture production in Malaysia has increased by 450% in the past 16 years from 1993 to 2009 (DOF, 2011). Due to gradual decrease in production from natural stocks over time, DOF introduced oyster culture in Malaysia to fulfill the demand. Diseases and parasites affecting bivalves in Malaysian waters have not been systematically investigated so far. However, a number of parasites have been reported causing diseases in farmed and natural bivalve stocks from neighboring countries (Tuntiwaranuruk *et al*., 2008; Taveekijakarn *et al*., 2008; Erazo-Pagador, 2010). Therefore, it is necessary to investigate the presence of parasites of farmed *Crassostrea iredalei* in Malaysian waters from their economic and epidemiological point of view. From the above context, present survey was conducted to investigate the occurrence of parasites in farmed slipper-cupped oyster, *Crassostrea iredalei* from Pulau Betong, west coast of Penang, Malaysia using histopathology.

MATERIALS AND METHODS

Sampling efforts: *Crassostrea iredalei* samples were collected from Pulau Betong, west coast of Penang, Malaysia (5°31 N 100°18 E) (Fig. 1). Sampling was drawn from an oyster farm in October 2010, November 2010 and in early January 2011. Twenty *C. iredalei* were processed monthly for the current histopathology survey. Upon arrival, the shell height of each oyster was measured using a slide calipers and the soft body was exposed using an oyster opening knife. For histological preparations, a transverse section (3 mm) was cut through the anterior part of the soft body in such that it contained gills, mantle, digestive glands, gonad and foot tissue.

Histology: The transverse section of the oyster was fixed in Davidson's solution with proper labeling. The tissue was subsequently dehydrated and embedded in paraffin. The paraffin blocks were sectioned at 6 μm thickness and stained with Harris's haematoxylene and eosin Y. The histological preparations were examined under a light microscope to investigate the presence of parasites in different tissues. Parasite prevalence (i.e., number of animals infected divided by the number of animals examined multiplied by 100) and distribution in each host were evaluated for each month.

Statistical analysis: All the data were analyzed statistically using SPSS statistical package version 11.0 to calculate the mean and standard deviation. In all cases, differences were considered significant when $p<0.05$.

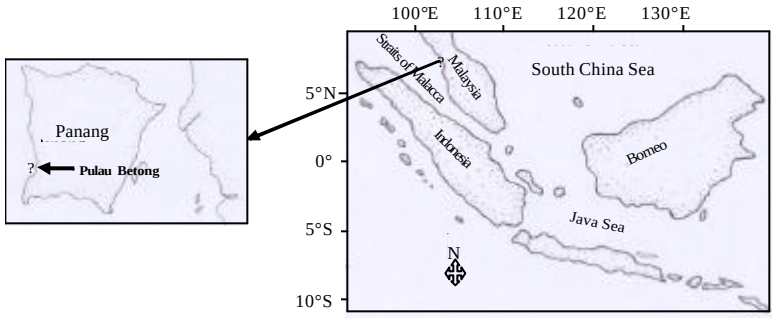

Fig. 1: Sampling site at Pulau Betong, Penang, Malaysia (5°31 N 100°18' E)

RESULTS

Morphological measurements: Shell height was the only morphological measurement taken in the present investigation. Table 1 shows the mean shell height (±SD) of *C. iredalei* examined during the 3 sampling dates; October 2010, November 2010 and January 2011. The highest mean shell height was measured in October (105.49±10.57 mm) and the lowest mean value was noted in November (86.36±7.84 mm). The shell height ranged from 92.0-127.8 mm in October 2010, 70.8-103.5 mm in November 2010 and 73.7-110.8 mm in January 2011.

Gross examination: Gross examination of *C. iredalei* revealed the presence of barnacles, oyster spat and crab in the shells. Figure 2 shows the presence of a crab in the shell of the oyster. The screening of *C. iredalei* by gross observation did not reveal any evidence of disease or parasite in the samples.

Histopathology: This is the first report on histopathological survey on oysters from Malaysian waters. Histological examination confirmed the presence of protozoan parasite, *Nemaptosis* sp. with a very light intensity of infection. The maximum number of hemocytes containing Nemaptosis per histological section was 5. The prevalence of infection of *Nemaptosis* sp. *oocysts parasitized C. iredalei* varied among sampling dates. The prevalences of infection of *Nematopsis* sp. were 15% each in October and November 2010 while the values were 30% in January 2011 (Table 1). The parasite was detected in connective tissue of digestive gland tubules, gills, mantle and gonads (Fig. 3). Figure 3a displays the occurrence of *Nematopsis oocysts* in the connective tissue of the digestive gland tubules. No clear host tissue damage was detected due to the presence of the parasites. No pathological damage was also evident due to the presence of *Nematopsis* sp. *sporocysts* in the gill tissue of C. iredalei investigated in the current study (Fig. 3b). Only some hemocytic

Table 1: Shell height (±SD) (range), prevalence (%) and site of infection of *Nematopsis* sp. in *Crassostrea* iredalei collected from Pulau Betong, west coast of Penang, Malaysia during October, November 2010 and January 2011

Sampling date	Shell height (mm) (±SD) (range)	Prevalence of *Nematopsis* sp.	Site of infection
22 October (2010)	105.5±10.6 (92.0-127.8 mm)	15	Connective tissue, gills and digestive glands
25 November (2010)	86.4±7.8 (70.8-103.5 mm)	15	Connective tissue, gills and mantle
3 January (2011)	88.1±8.1 (73.7-110.8 cm)	30	Connective tissue, gills, mantle and digestive glands

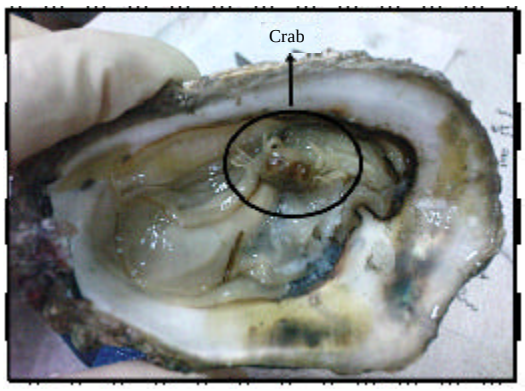

Fig. 2: Presence of crab inside the shells reported during gross observation

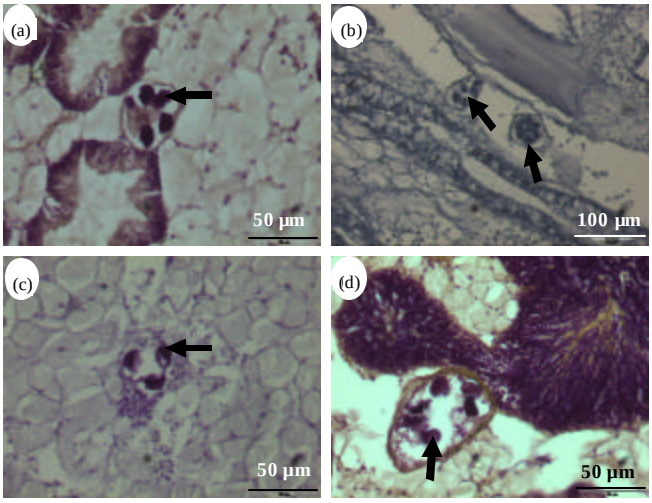

Fig. 3 (a-d): Oocysts of *Nematopsis* sp. in different tissues of *Crassostrea iredalei* collected from Pulau Betong, west coast of Penang, Malaysia. a. infection in connective tissue of digestive gland tubules; b. in gills; c. infection in mantle tissue associated with hemocytic responses; and d. in connective tissues of male gonad

responses were associated with *Nematopsis* sp. infection in the mantle tissue (Fig. 3c). No other pathological threat was detected from the current histopathology survey. In Fig. 3d, the presence of parasites did not show any anomaly to the connective tissue of the male gonads and no clear host response was also observed due to *Nematopsis* sp. infection.

The oocysts of this Gregarines protozoan appeared to be oval shape. Each phagocyte contained variable number of oocysts with 3-4 being the most frequent condition. The length of the phagocyte was within the range of 30 µm to 60 µm. The maximum number of *Nemaptosis oocyts* per phagocyte was 7 (Fig. 3b). Each oocyst contained a single uninucleate sporozoite. The mean oocyst length was 15.04 µm and the width was 12.22 µm.

DISCUSSION

Research concerning diseases of oyster in Malaysia is scarce. A number of parasites have been reported causing diseases in farmed and natural bivalves from the neighboring countries. In Thailand, *Nemaptosis* sp., *Maeteilia* sp., *Perkinsus* sp. and *trematodes* were detected in cultivated and locally harvested bivalves from the Gulf of Thailand (Tuntiwaranuruk *et al*., 2008; Taveekijakarn *et al*., 2008). *Nemaptosis* sp., *Tylocephalum* sp., *digenetic trematodes* and *ciliates* were reported from slipper-cupped oyster, *Crassostrea iredalei* from the coast of the Philippines (Erazo-Pagador, 2010). For the sustainable oyster farming in Malaysian waters, it is timely to monitor the health status of farmed bivalves on regular basis.

This is the first report on the histological screening of farmed slipper-cupped oyster, *Crassostrea iredalei* from Malaysian waters for the presence of parasites. Histopathology confirmed the presence of intrahemolytic oocysts of gregarines of the genus *Nematopsis* in the farmed stocks of *C. iredalei* along the coast of Pulau Betong, west coast of Penang, Malaysia. The prevalence and intensity of infection were low without any evidence of pathological damage to the host. The Apicomplexan protozoan parasite, *Nematopsis* spp. has been reported to infect several bivalve

locations in tropical to temperate waters. The genus *Nematopsis* was reported to infect severa species from many l bivalve species from the upper Gulf of Thailand (Tuntiwaranuruk *et al*., 2008). *Nematopsis* sp. was the most prevalent parasite of farmed slipper-cupped oyster, Crassostrea iredalei from Philippines (Erazo-Pagador, 2010). Such infection was also reported to occur along the American and European waters (Bower *et al*., 1994; Carballal *et al*., 2001; Boehs *et al*., 2010). Present findings are the confirmation of its distribution in Malaysian waters.

Nemaptosis sp. was the only parasite found in this research. The prevalence of infection was very light and did not cause any pathological damage to the farmed oyster, *C. iredalei* except some hemocytic responses. Gregarine of *Nemaptosis* sp. use marine bivalves as their intermediate host and completing their life cycle within marine arthropods' gut. In tropical waters, the prevalence of *Nematopsis* spp. infection reported were 65 to 71% in *Crassostrea iredalei* from the Philippines (Erazo-Pagador, 2010), 92% in A. arcuatala, 59% in A. granosa, 60% in Perna viridis and 70% in Paphia undulata from the Gulf of Thailand (Tuntiwaranuruk *et al*., 2004) and 73% in Mytella guyanensis from the coast of South Bahia, Brazil (Boehs *et al*., 2010). The prevalence of Nematopsis sp. infection obtained in the present study is relatively lower than the above reports which might be due to different geographic positions and host species.

Variable number of oocysts of *Nemaptosis* sp. is usually present per infected host cell (phagocyte). In the present study, we observed 1-7 oocysts per *Nematopsis* sp. infected hemocytes with 3-4 at most cases. Carballal *et al*. (2001) reported maximum four oocysts per phagocyte and 1-2 oocysts per infected phagocyte was the most common feature in Cerastoderma edule. The number of *Nematopsis oocysts* per phagocyte in Mytella guyanensis varied from 1 to 20, with 1-3 being the most frequent condition; whereas, only one oocyst per *Nematopsis* sp. infected phagocyte was seen in Anomalocardia brasiliana (Boehs *et al*., 2010). The shape and size of the oocysts measured in the present study is comparable with the findings reported by Tuntiwaranuruk *et al*. (2008), Boehs *et al*. (2010) and Erazo-Pagador (2010).

In the current study, we observed light hemocytic infiltration in the mantle tissue of some infected animals without any clear host tissue damage. Gregarines of the genus *Nematopsis* could cause focal hemocytic infiltration at most, without obvious pathogenic effects (Bower *et al*., 1994) which are in consistent with our findings. In contrast, Carballal *et al*. (2001) and Tuntiwaranuruk *et al*. (2004) reported decreased filtering efficiency and food intake of the infected animal due to the presence of large number of *Nematopsis* sp. containing phagocytes in the gill lumen which resulted in the obstruction of the water-flow between the inhalant and exhalent currents. Gills and mantle tissues were reported as the most frequent locations for *Nematopsis* spp. (Carballal *et al*., 2001; Sabry *et al*., 2007), as we observed in the current investigation. Heavy infection of *Nematopsis* sp. at gills and mantle of young bivalves may weaken the juveniles; however, such infection was found harmless in adults (Sprague and Orr, 1955; Sprague, 1970). The prevalence of *Nematopsis* sp. infection often correlates with the salinity and temperature of the seawater. In the present study, *Nematopsis* sp. exhibited lower prevalence of infection during October and November 2010 than that during January 2011. In west coast of Peninsular Malaysia, the northeast monsoon associated with heavy rainfall commence in October-November. The observed least prevalence of infection during October and November 2010 might be associated with the drop of water salinity due to heavy rainfall during this time. This finding is in consistent with Jimenez *et al*. (2002) who reported higher intensity of *Nematopsis* sp. infection in Litopenaeus vannamei during dry season and minor incidence during the rainy season. Sabry *et al*. (2007) also reported slightly lower prevalence of *Nemaptosis* sp. in mangrove oyster *Crassostrea rhizophorae*

during rainy season when both temperature and salinity decreased in that area. In agreement with the above reports, the relatively higher prevalence of infection during January might be associated with the little rainfall yielding higher salinity level in the sea water in which Nematopsis can thrive well. However, an annual survey is necessary to confirm such relationship in Malaysian waters.

CONCLUSION

A histopathological survey was conducted on farmed slipper-cupped oyster, Crassostrea iredalei from Pulau Betong, west coast of Penang, Malaysia. Only protozoan parasite, Nematopsis sp. was detected from *C. iredalei* during the study period having light prevalence and infection intensity. No threat was detected to C. iredalei from the histopathological survey performed. A yearly monitoring is necessary to obtain the seasonality of parasitic infection in farmed *C. iredalei*.

REFERENCES

Berthe, F.C.J., 2008. New Approaches to Effective Mollusc Health Management. In: Diseases in Asian Aquaculture VI. Fish Health Section, Bondad-Reantaso, M.G., C.V. Mohan, M. Crumlish and R.P. Subasinghe (Eds.). Asian Fisheries Society, Manila, Philippines, pp: 343-352.

Boehs, G., A. Villalba, L.O. Ceuta and J.R. Luz, 2010. Parasites of three commercially exploited bivalve mollusc species of the estuarine region of the Cachoeira river (Ilheus, Bahia, Brazil). J. Invertebrate Pathol., 103: 43-47.

Bower, S.M., S.E. McGladdery and I.M. Price, 1994. Synopsis of infectious diseases and parasites of commercially exploited shellfish. Annu. Rev. Fish Dis., 4: 1-199.

Carballal, M.J., D. Iglesias, J. Santamarina, B. Ferro-Soto and A. Villalba, 2001. Parasites and pathologic conditions of the cockle *Cerastoderma edule* populations of the coast of Galicia (NW Spain). J. Invertebrate Pathol., 78: 87-97.

DOF, 2011. Estimated aquaculture production by state and culture system, 2009. Department of Fisheries, Malaysia.

Erazo-Pagador, G., 2010. A parasitological survey of slipper-cupped oyster (*Crassostrea iredalei*, Faustino, 1932) in the Philippines. J. Shellfish Res., 29: 177-179.

FAO, 2009. FAOSTAT fisheries data. Food and Agriculture Organization of the United Nations. http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en

Jimenez, R., L. de Barniol and M. Machuca, 2002. *Nematopsis marinus* n. sp., a new septate gregarine from cultured penaeoid shrimp litopenaeus vannamei (Boone), in Ecuador. Aquacult. Res., 33: 231-240.

Sabry, R.C., T.C. Gesteira and G. Boehs, 2007. First record of parasitism in the mangrove oyster *Crassostrea rhizophorae* (Bivalvia: Ostreidae) at Jaguaribe River estuary-Ceara, Brazil. Braz. J. Biol., 67: 755-758.

Sprague, V. and P.E. Jr. Orr, 1955. *Nematopsis ostrearum* and *N. prytherchi* (Eugregarinina; Porosporidae) with special reference to the host-parasite relations. J. Parasitol., 41: 89-104.

Sprague, V., 1970. Some protozoan parasites and hyperparasites in marine bivalve molluscs. Proceedings of the Symposium on Diseases of Fishes and Shellfishes (DFS' 1970), American Fisheries Society, Washington, DC., pp: 511-526.

Taveekijakarn, P., T. Somsiri, S. Puttinaowarat, S. Tundavanitj, S. Chinabut and G. Nash, 2008. Parasitic Fauna of Rock Oyster (*Saccostrea forskali*) Cultured in Thailaend. In: Diseases in Asian Aquaculture VI, Bondad-Reantaso, M.G., C.V. Mohan, M. Crumlish and R.P. Subasinghe (Eds.). Fish Health Section, Asian Fisheries Society, Manila, Philippines, pp: 335-342.

Tuntiwaranuruk, C., K. Chalermwat, E.S. Upatham, M. Kruatrachue and C. Azevedo, 2004. Investigation of *Nematopsis* spp. oocysts in 7 species of bivalves from Chonburi province, Gulf of Thailand. Dis. Aquat. Org., 58: 47-53.

Tuntiwaranuruk, C., K. Chalermwat, V. Pongsakchat, A. Meepool, E.S. Upatham and M. Kruatrachue, 2008. Infection of *Nematopsis* oocysts in different size classes of the farmed mussel *Perna viridis* in Thailand. Aquaculture, 281: 12-16.