Pertanika J. Trop. Agric. Sci. 36 (3): 217 - 224 (2013)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

A Case Study of Protozoan Parasite Gregarine *Nematopsis* spp. (Apicomplexa: Sporozoa) Infestation in Mangrove Oyster *Crassostrea belcheri* Imported from Thailand

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ABSTRACT

One hundred mangrove oysters (weight: 248.41 ± 28.41g and length: 10.69 ± 0.73cm) from Thailand meant for human consumption were examined for parasite infestation. Gross observation showed that the infestation comprised mostly cliona (43%) followed by mud (1%) and water blister (1%). Histopathological study revealed high prevalence of gregarine *Nematopsis* sp oocysts (99%), metaplasia (92%), inclusion cells (15%), brown cells (14%), oedema (5%), ceriod (8%) and abscess (1%). A large number of phagocytes with gregarine oocysts inside the gill and connective tissues were observed. Most of the oocysts were located within parasitophorous vacuoles and phagocytes. The oocysts appeared ellipsoidal at 73.55µm wide and 140.56µm long with a thick outer surface. The clusters of oocysts consisted of a single vermiform sporozoite engulfed by phagocytes. The number of oocysts in each phagocyte varied ranging from 1 to 9 with an average of 4.9. The findings revealed that gregarine oocysts did not cause much pathological damage as there was no irregular arrangement and disruption of gill filament or loss of cilia. Similar histopathology was also observed in four molluscs (i.e. *Anadara granosa, Perna viridis* and *Arcuatula arcuatula* and *Paphia undulate*) as reported in Thailand, and three cultivated molluscs (*A. granosa, P. viridis* and *C. iredalei)* as reported in Malaysia. The present study provides significant baseline information on the health profile of the important molluscan species, particularly on *Nematopsis* spp. infestation in oysters.

ARTICLE INFO

Article history:
Received: 5 July 2010
Accepted: 26 January 2013

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ISSN: 1511-3701

Keywords: Mangrove oyster, *Crassostrea belcheri*, protozoa, *Nematopsis* sp., oocysts

INTRODUCTION

Crassostrea belcheri, known locally as mangrove oyster or white-scar oyster in Thailand, is mainly cultured in Asia, particularly in Thailand and Malaysia. Four species of oysters are found in Malaysia, namely, *Crassostrea belcheri, C. rivularis, Saccostrea cucullata* and *Ostrea folium*. Among the four species, *C. belcheri* is mainly cultured with an average production of 1000 tones per year. The production of oysters in Malaysia is low compared to the production of other bivalve species (Table 1) (Annual Fisheries Statistics 2006, 2007, 2008, 2009 & 2010). *Crassostrea belcheri* is in high demand from the local Malaysian seafood market, and generally, the demand is higher than the local supply. The importation of oysters from Thailand is an alternative measure by local suppliers to fulfill the high demands. Generally, the oysters are sold in shell-on forms which are consumed fresh.

In Malaysia, a monitoring programme on the molluscan health focusing on the Office International des Epizooties (OIE) revealed that gregarine was the most frequently listed parasite since 1999 until 2009, which showed that gymnospores of gregarine (*Nematopsis* sp.) were the parasites most frequently encountered in tropical oysters (*Crassostrea iredalei*) and blood cockles (*Anadara granosa*). Although they can be observed in most of the specimens in both cultured species, no significant health effects have been found. Bower and McGladdery (2001) reported that gymnospores and oocysts of gregarines are usually associated with a focal, benign inflammatory response and there is no serious effect on or damage to the host. Gymnospores need marine arthropods such as crabs or shrimps in order to complete their life cycle. Hence, multiplication of gregarines is limited to bivalves.

Mass mortality caused by gregarine *Nematopsis* sp. has not been reported in Malaysia although mass mortality of cockles and clams in Portugal had been associated with it (Azevedo & Cachola, 1992). Infection of Apicomplexan protozoan *Nematopsis* has been regularly reported in molluscs, which acts as the intermediate host with crustaceans as the definitive host (Spraque, 1970; Lee *et al*., 2000; Tuntiwaranuruk *et al*., 2004 and 2008). In Thailand, infestation of gregarine *Nematopsis* spp. has been widely reported in bivalve species, such as cockles (*A. granosa*), mytilid mussels (*P. viridis* and *A. arcuatula*) and venerid

TABLE 1
Total production of cultured molluscan (tonnes) in Malaysia from 2006 to 2010.

Species	2006	2007	2008	2009	2010
Blood cockle	45.674	49.620	61,138	64,938	78,024
Green mussels	6.904	4.034	8,993	10,596	10,529
Oysters	915	869	275	2,128	812

clams (*P. undulate*) except rock oysters (*Saccostrea cucullata*) during a survey of infestation of *Nematopsis spp* sporozoites in Thailand (Tuntiwaranuruk *et al*., 2004). *Nematopsis* sp., *Tylocephalum* sp., *digenetic trematodes* and *ciliates* were also reported from *C. iredalei* in the Philippines (Erazo-Pagador, 2010). In Malaysia, our survey of OIE listed parasites in tropical oysters (*C. iredalei*) in 2000 and 2003 found a prevalence of gregarine *Nematopsis* sp. oocyst, ranging from 33.33% to 73.33% (Kua & Taha, 2004).

Despite the commercial value and gradual increase in the production of *C. belcheri* in Malaysia, there has been no report or any specific study on problematic diseases of the oysters. Several reports on the diseases, particularly those caused by parasites, have been described from various shellfish in other countries (Sindermann, 1990). Itoh (2002) reported the paramysean ovarian parasite in pacific oysters and the economic loss caused by the disease, which was estimated to be a few hundred million Japanese yen in Okayama Prefecture. Imanaka *et al*. (2001) reported that the ovarian parasite may kill wild oysters. Taveekijakarn *et al*. (2008) also highlighted on the occurrence of *Marteilia* sp., *Perkinsus* sp., and trematodes in oyster samples from the Gulf of Thailand. In Malaysia, a molluscan health monitoring programme has been initiated focusing on the OIE listed parasites in three main species (*C. iredalei*, *P. viridis* and *A. granosa)*. In 2007, we had an opportunity to investigate 100 specimens of mangrove oysters (*C. belcheri*) imported from Thailand as provided by the Fish Quarantine Centre, Bukit Kayu Hitam, Kedah. Thus, the main objective of this study was to examine the presence of parasites in these imported mangrove oysters. The findings were significant and contributed new knowledge to the research community, especially on *Nematopsis* infestation in the commercially important mangrove oysters, *C. belcheri*.

MATERIALS AND METHODS

All the 100 specimens of *C. belcheri* were measured, opened and examined for colour, conditions (fat, medium or watery), macroparasites, as well as shell and tissue abnormalities. The body was removed and fixed in 10% buffered formalin for 4 hours. The fixed specimens were then cut in cross-section at the gills, stomach, digestive gland and intestine with 2-3 cm of thickness. The specimens were fixed again with the same fixative for another 24 hours before being processed by an automatic tissue processor (Leica ASP 300, Germany) and embedded in paraffin wax. The embedded specimens were sectioned at 5 µm thick, stained with haematoxylin and eosin (H&E), and finally mounted with DPX before being examined under a compound microscope (Leica DM5000B, Leica Microsystems, Germany) connected to a digital camera (Leica DFC 320, Leica Microsystems, Germany) and equipped with a computer software (Leica QWin, Leica Microsystems, Germany). The histological techniques were based on the method suggested by Humason (1979).

RESULTS AND DISCUSSION

Gross observation on the mangrove oysters showed mostly cliona (43%) followed by mud (1%) and water blister (1%). Fouling organisms, such as barnacles, ascidians and algae, were not observed during the study. The post-harvest cleaning process should have largely reduced the fouling organisms on the oysters before being exported to Malaysia, therefore, only a few fouling organisms were observed in the present study. Apart from the fouling organisms, small crabs and polychaete worms were also found on the soft tissues of the oysters. The cleaning process after the mangrove oysters were harvested from the culture sites before exporting to Malaysia resulted in the observation of a few fouling organisms.

Histopathological sections revealed high infestation of gregarine *Nematopsis sp*. oocysts (99%), metaplasia (92%), inclusion cells (15%), brown cells (14%), oedema (5%), ceriod (8%) and abscess (1%). Large numbers of phagocytes with gregarine oocyts were seen in the gills and connective tissues (Fig.1). Most of the gregarine oocysts were located within parasitophorous vacuoles and phagocytes (Fig.2). The clusters of gregarine oocysts showed individual gregarine oocysts, which consisted of a single vermiform sporozoite engulfed by phagocytes. The number of gregarine oocysts in each phagocyte varied from 1 to 9 with an average number of 4.9. The gregarine oocysts appeared ellipsoidal (73.55 ± 24.69 µm wide and 140.56 ± 27.77 µm long) with a thick outer surface. The comparison of the diameter of gregarine oocysts showed that there was a slight difference in size. The diameter of the single ellipsoidal oocyts in the study was bigger compared to *Nematopsis mytella* (12.9 – 13 µm long with 8.0 – 8.6 µm wide) as reported for *C. rizophorae* from Brazil (Padovan *et al*., 2003) and *Nematopsis spp.* (10 - 15 µm long with 6.4 - 12.7 µm wide) from Thailand (Tuntiwaranuruk *et al*., 2004).

Infestation of gregarine *Nematopsis sp* oocysts in other bivalve species has been documented (Table 2). However, gregarine *Nematopsis sp.* oocyst infestation of cultured or wild oyster *C. belcheri* from Thailand and Malaysia has never been reported. This study reports a prevalence of 99% of gregarine oocysts in *C. belcheri* from Thailand, which provides baseline information on health profile of the important molluscan species, particularly on *Nematopsis sp.* infestation in oysters. If high prevalence occurs under the condition of food shortage or reduced water flow, the abundant presence of parasites in the gills of oysters could further reduce gas exchanges and food intake, thus weakening the infected oyster.

However, there are several reports on high prevalence of gregarine oocyst infestation in mollusc, which do not show significant health effects. Carballal *et al*. (2001) mentioned the prevalence (76%) of *N. veneris*, *N. ostrearum*, and *N. schneideri*, *Nematopsis spp.* with low intensity occurring in clam, *Cerastoderma edule,* from 34 locations along the coasts of Galicia, Spain, which showed no impact on the health of the clam. Desser and Bower (1997) highlighted little evidence of pathological

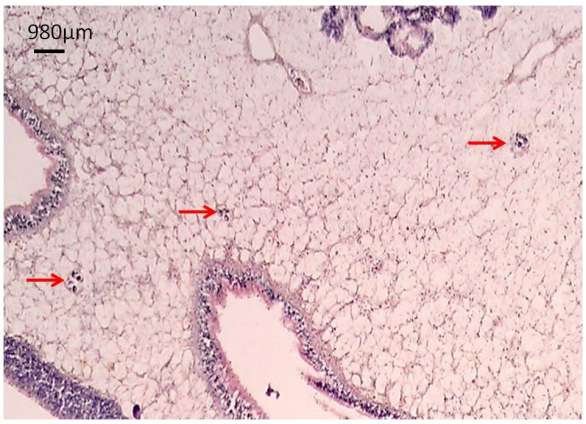

Fig.1: Phagocytes with gregarine oocyts (arrow) in connective tissues of mangrove oyster infested with *Nematopsis* sp. Hematoxylin and Eosin. Magnification: x40. Scale bar: 12 mm = 980 µm.

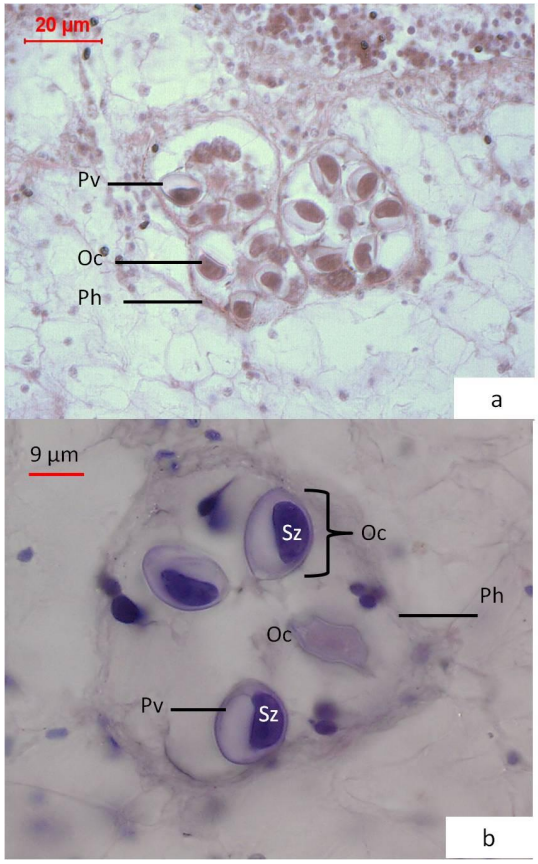

Fig.2: Histological section of connective tissue in mangrove oyster infected with *Nematopsis* sp. (a). Three phagocytes (Ph) of *C. belcheri* showing oocyts (Oc) within parasitophorous vacuoles (Pv) and (b). One phagocyte (Ph) with three oocysts (Oc), each one in a parasitophorous vacuole (Pv) with sporozoite(Sz). Hematoxylin and Eosin. Magnification: (a).x200, (b).x1000, Scale bar: (a). 3cm = 20 µm and (b). 1cm = 9 µm.

effect on *Protothaca staminea* even though cyst infestations were 70 and 100%. Uddin *et al*. (2011) showed that the blood cockles from the Straits of Malacca were infected with *Nematopsis sp*. without any sign of pathological damage. Despite wide reports of no pathogical impact of *Nematopsis sp.* infestation on bivalves, there were a few reports on the pathological significance of *Nematopsis sp.* (Azevedo & Cachola, 1992, Tuntiwaranuruk *et al*., 2004 and 2008).

In this study, pathological damage was caused by gregarine *Nematopsis* sp. Oocysts, which was comparatively minimal as compared to some irregular arrangement and disruption of gill filaments and loss of cilia in severe infection as reported by Tuntiwaranuruk *et al*. (2008). According to them, high infestation of *Nematopsis* sp. oocysts could cause a large number of phagocyte presence in the gill lumen of *Perna viridis* leading to the obstruction of water flow and indirectly reducing the filtering efficiency and food intake (Tuntiwaranuruk *et al*., 2004). The histopathology of this study was similar to previous cases on *A. granosa, P. viridis* and *A. arcuatula* and *P. undulate* reported in Thailand (Tuntiwaranuruk *et al*., 2004) and three cultivated bivalves (*A. granosa, P. viridis* and *C. iredalei)* in Malaysia .

TABLE 2
Nematopsis spp. reported from wild or cultured bivalves.

Host species	Bivalves group	Reported Nematopsis species	Location	References
Crassostrea rizophorae	*Oyster*	*Nematopsis mytella*	Brazil	Padovan *et al*., 2003
Callista chione	*Clam*	*Nematopsis* sp.	Italy	Canestri-Trotti *et al*., 2000
Anadara granosa	*Blood cockle*	*Nematopsis* sp.	Thailand	Tuntiwaranuruk *et al*., 2004
Anadara granosa	*Blood cockle*	*Nematopsis* sp.	Malaysia	Uddin *et al*., 2010
Perna viridis	Green Mussel	*Nematopsis* sp.	Thailand	Tuntiwaranuruk *et al*., 2004
Perna viridis	Green Mussel	*Nematopsis* sp.	Malaysia	Kua and Taha, 2004
Cerastoderme edule	Clam	*Nematopsis* sp.	Portugal	Azevedo and Cachola, 1992
Ruditapes decussatus	Clam	*Nematopsis* sp.	Portugal	Azevedo and Cachola, 1992
Arcuatula arcuatula	Clam	*Nematopsis* sp.	Thailand	Tuntiwaranuruk *et al*., 2004
Paphia undulate	mussel	*Nematopsis* sp.	Thailand	Tuntiwaranuruk *et al*., 2004
Crassostrea iredalei	*Tropical oyster*	*Nematopsis* sp.	Philippines	Erazo-Pagador, 2010
Crassostrea iredalei	*Tropical osyter*	*Nematopsis* sp.	Malaysia	Kua and Taha, 2004

CONCLUSION

In this study, high prevalence (99%) of mild to moderate gregarine *Nematopsis* infestation with phagocytic response was observed in the *C. belcheri* specimens imported from Thailand. This study provides important baseline information on health profile of the mangrove oyster *C. belcheri* to the research community, especially on *Nematopsis* infestation in oysters.

ACKNOWLEDGEMENTS

The authors are grateful to the Fish Quarantine Centre, Kedah, for supplying the specimens for this study.

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