# Parasitic Fauna of Rock Oyster (*Saccostrea forskali*) Cultured in Thailand

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## ABSTRACT

Rock oysters (*Saccostrea forskali)* were sampled assuming 2% disease prevalence from two sites at the upper part of the Gulf of Thailand every four month for one year. *Marteilia* sp*., Perkinsus* sp. and trematodes in the family Plagiochiidae were found in the oyster samples. Histopathological observations revealed that *Marteilia* sp. occurred in the digestive gland, metacercaria of digenean were found in gonads and a few sporocysts containing rediae were located in the gills. *Perkinsus* sp. was histologically observed only in oysters over one year of age that were sampled from one location in November. Digenean was detected at both sites year round but histopathological observations suggested that it was not pathogenic to the host. Infection of oysters by these parasites had no apparent effect on the production of oysters at these sites. The prevalence of these parasites was low and further investigations should be undertaken to clarify their specific taxonomic identity.

Taveekijakarn, P., Somsiri, T. Puttinaowarat, S., Tundavanitj, Chinabut, S. and Nash, G. 2008. Parasitic fauna of rock oyster (*Saccostrea forskali*) cultured in Thailand, pp. 335-342. *In* Bondad-Reantaso, M.G., Mohan, C.V., Crumlish, M. and Subasinghe, R.P. (eds.). Diseases in Asian Aquaculture VI. Fish Health Section, Asian Fisheries Society, Manila, Philippines. 505 pp.

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## INTRODUCTION

Culture of rock oyster (*Saccostrea forskali*) in Thailand is distributed along the eastern coast of the Gulf of Thailand as well as in some parts of the southern coastal areas. Most of the oyster farms are small-scale using natural spat collection. The most popular method of oyster culture in Thailand is the hanging method, with oyster spats attached to a roll of cement disc (one inch diameter) that are joined together by a rope. Oysters are harvested at 10-12 months of age depending on size. Yields from production areas average 10 tonnes per hectare. Thailand exports oysters to many countries, including Australia, Canada and Japan. A total of 302,636 kilograms worth US$875,187 were exported in 2004.

In 1999, a *Marteilia*-like species was found in the rock oyster (*Saccostrea forskali)* sampled from the east coast of Thailand (Taveekijakarn *et al.*, 2002). *Marteilia refringens* is the causative agent of marteiliosis, one of the notifiable mollusk diseases in the aquatic animal pathogen list of the Office International des Epizooties (OIE). Little information on this oyster disease is available in Thailand and the Southeast Asian region.

Two *Marteilia* spp. are known, *M. refringens* from flat oysters (*Ostrea edulis*) in Europe, and *M. sydneyi* from rock oysters (*Saccostrea glomerata*) in Australia (Bondad-Reantaso *et al*., 2001; Berth *et al*., 2004). The *Marteilia* sp. found in the rock oyster in Thailand has not yet been identified up to a species level. Further study is required not only for classification purposes but also for epidemiological data.

Other parasitic diseases have been reported from other parts of the world where they cause significant damage to the industry (Bower *et al*., 1994; Carnegie and Cochennec-Laureau, 2004). Those include *Mikrocytos mackini* infection in Pacific oysters (*Crassostrea gigas*) cultured in western Canada and the west coast of the United States, infections with *Bonamia ostreae* and *B. exitiosa*, respectively reported in flat oysters cultured in Europe and New Zealand, *Perkinsus marinus* and *Haplosporidium nelsoni* in the Eastern oyster (*Crassostrea virginica*) populations along the mid-Atlantic region of the United States. However, less information on diseases of oysters is available in Thailand. The aim of this present work was to conduct a preliminary investigation of parasitic diseases of oysters cultured in Thailand. This study will provide a better understanding of the parasitic diseases situation of cultured oysters in Thailand, and also suggest disease prevention and control measures.

## MATERIALS AND METHODS

Rock oysters (*Saccostrea forskali)* were collected in order to ensure detection of pathogens occurring at 2% prevalence (150 oysters per site). They were collected from two farms in Chonburi Province: Bangplasoy (site 1) and Bangsai (site 2, estuary of the Bangplakong River) in March, July and November 2004. In addition, 150 of over-one year-old oysters (18 months) were collected for comparison with the younger stocks (10 months). Water temperature and pH at the surface level of culture sites were also recorded. Clinical signs and macroscopic parasites of the oysters were observed. The samples infected with macroscopic parasites were freshly chopped to isolate the parasite for a further identification. For the histological study, the samples were fixed in 10% buffered formalin

for 24 hrs and then embedded in paraffin blocks. The samples were cut at a 5-μm thickness and mounted on glass slides. The sections were stained with haematoxylin and eosin (H&E) and observed under a light microscope.

## RESULTS

In March and November, two kinds of parasite were found: a *Marteilia* sp.-like and a digenean trematode (Table 1). The light microscope observation revealed *Marteilia* sp. in the digestive gland. Young and mature sporulation stages of *Marteilia* sp., with refringent strongly eosinophilic inclusion bodies, occurred in the epithelium of the digestive diverticulae (Figure 1). Typically, each sporangiosorus had 2 to 6, or possibly more sporonts which contained two spores (Figure 2). Haemocytic infiltration was also found surrounding the infected area. Extra sporogonic proliferation was observed in the gill of some of the infected oysters.

The digenean trematodes were found at the both sites throughout the year (Table 1). In average, 1 to 6 digenean worms were commonly found in one oyster. Metacercaria were usually found in the connective tissue of the gonad, and a few sporocysts containing rediae were observed in gill tissues (Figures 3 and 4). Inflammatory response was not observed in association with the infection. The acetabulum of the digenean was bigger than the oral sucker and lay over the midline of the body (Figure 5). They were identified as Plagiorchiidae following Yamaguti (1958). Some samples showed focal haemocytic infiltration with no pathogenic organism.

The third sampling conducted in November was infected with *Marteilia* sp., a digenean and also a *Perkinsus* sp. in the older oyster samples. In these infected samples, focal necrosis was observed with haemocytic infiltration in the ovary. The variously-sized trophozoites were scattered in the necrotic area and among the connective tissue between degenerated tubules of digestive glands (Figure 6). Some trophozoites were also observed in the tissue as a typical signet ring cell structures, although many of them were present as balls of schizonts (Figure 7).

**Table 1.** Parasitic infection in rock oyster (*Saccostrea forskali),* sampled from Chonburi province, Thailand at different times of the year.

| Month (2004) | Site | pH | Salinity (ppt) | Temp (°C) | Marteilia (%) | Perkinsus (%) | Digenean trematode (%) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Histo[1] | Macro[2] |
| March | 1 | 7.6 | 29 | 30 | 0 | 0 | 3.2 | 10.1 |
| | 2 | 7.6 | 27 | 30 | 1.2 | 0 | 0 | 11.0 |
| July | 1 | 7.7 | 20 | 29 | 0 | 0 | 4.0 | 10.0 |
| | 2 | 8.0 | 20 | 28.5 | 0 | 0 | 11.3 | 6.0 |
| November | 1 | 8.1 | 26 | 29 | 4.3 | 0 | 19.6 | 15.0 |
| | 1 (>1 yr) | 8.2 | 24 | 29 | 3 | 2 | 8 | 50 |
| | 2 | 8.3 | 28 | 29 | 0 | 0 | 4 | 12 |

[1] Histological observations
[2] Macroscopic observations

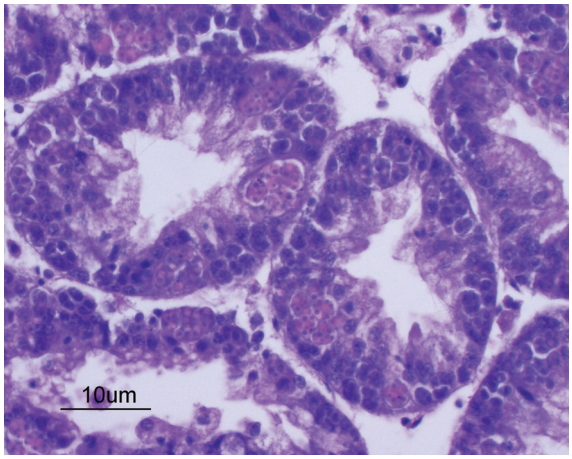


**Figure 1**. Severe infection of *Marteilia* sp. in digestive glands showed young and mature sporont within sporangiosorus.

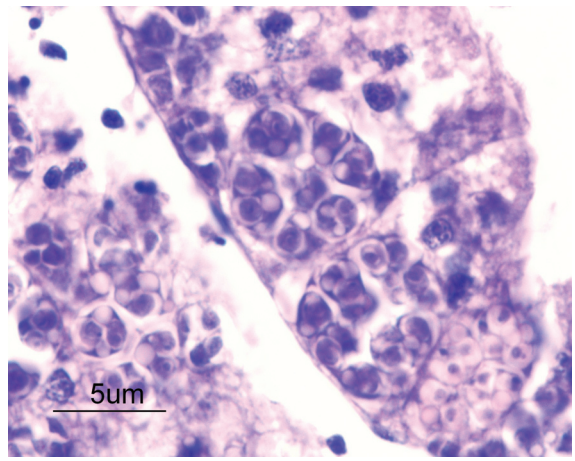


**Figure 2**. Sporangiosorus of *Marteilia* sp. showed 6 sporonts with 2 spores inside.

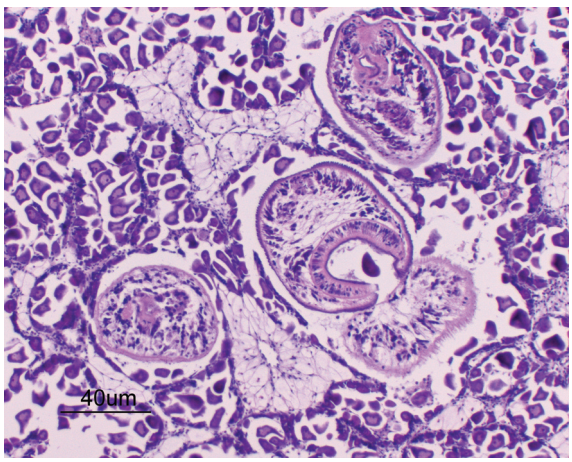


**Figure 3**. Digenean metacercaria was found in gonad.


**Figure 4.** Sporocysts containing rediae within the gills.

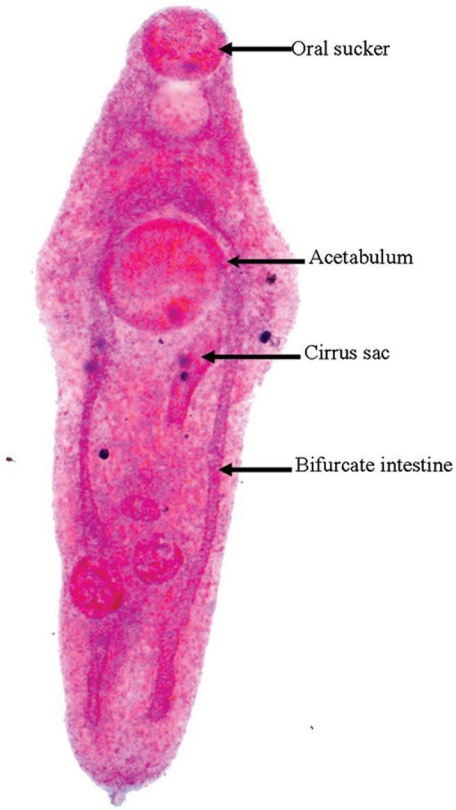


**Figure 5**. Metacercaria showed cirrus sac and long bifurcate intestine.

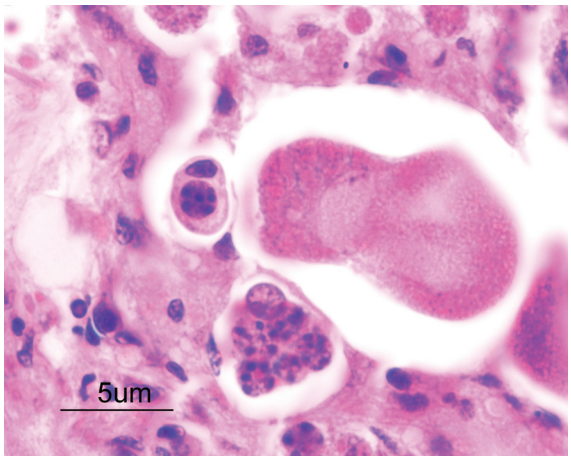


**Figure 6.** Tomonts of the *Perkinsus* sp. in the gonad with necrotic lesion.

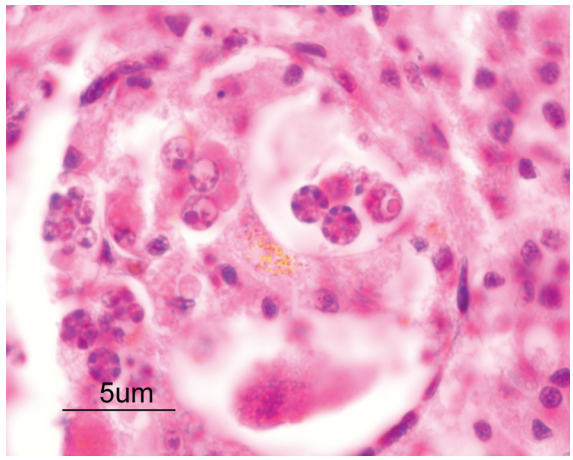


**Figure 7.** Trophozoites and tomonts of *Perkinsus* sp. in the connective tissue between the digestive gland tubules.

## DISCUSSION AND CONCLUSION

Rock oyster sample were collected three times per year during the dry (March), rainy (July), and cool (November) season at two locations. Three kinds of parasites (*Marteilia, Perkinsus* and digenetic trematodes of the family Plagiochiidae) were observed.

Digenetic trematodes were detected in all samples. *Marteilia* infection was found only during the dry and cool seasons at 2% prevalence. *Perkinsus* infection was also observed in cool season but was only detected in the older oysters (more than one year old). In general oysters farmers keep the stocks for only 8-10 months (approximately from September to July). Only when the oysters have slow growth, farmer would keep them longer than 10 months.

Some environmental parameters such as pH and temperature were similar among the three seasons, but salinity dropped to 20 ppt during the rainy season. Interestingly, there was no *Marteilia* infection in the samples collected from neither site 1 nor 2 during the

rainy season. Salinity is believed to play an important role in the *Marteilia* infection. It is premature to make such a conclusion in our study. This proposal was supported by the report of Wolf (1979), who studied *M. sydneyi* infections in the Australian oyster, *Saccostrea commercialis*. In contrast, Berthe *et al*. (1998) suggested that temperature was a main parameter governing the life cycle and sporulation of *M. refringens*; and its high infection in European flat oyster (*Ostrea edulis*) was correlated with high water temperature (>17ºC). However, those considerations were conducted under very different environmental conditions. Importantly in our study, there was no mass mortality associated with in the samples infected with *Marteilia* sp. in Thailand unlike other outbreaks in Europe and Australia where high mortalities occurred (Alderman, 1979; Robert *et al*., 1991; Wolf, 1972; Adlard and Ernst, 1995). This might have been due to the environmental habitat, which was much different from those countries or it might have been simply due to the virulence of the pathogen. The species found in this study appears to be more similar to *M. sydneyi* than *M. refringens* because they have two spores in each sporont.

The digenean trematode was commonly found in most samples collected at different times of the year regardless of sampling sites. They could be only identified to the family Plagiorchiidae, which is closely similar to those of family Gymnophalloidae, a frequently reported group in most bivalves (Bower *et al*., 1994). The digenean found in this study showed cirrus sac and long bifurcate intestine, which are the main distinct character of the family Plagiorchiidae. Moreover, Plagiorchiidae is normally found only in the gonad, whereas the Gymnophalloidae can be found in other organs such as mantle and adductor muscle (James, 1964; Soon-Hyung and Chai, 2001). The digenean trematodes from these two families have a marine bird as a final host. This may be an important consideration for further management of this parasitic infection. Culture methods may be adapted to limit the capacity for the parasites to complete their life-cycle.

*Perkinsus* sp. infections are usually recognized as significant. Two species, *Perkinsus marinus* and *P. olseni,* are notifiable to the OIE. This latter species has been reported from Thailand although it was from various species, not oysters. It would be important to continue monitoring this parasite in order to better forecast potential impact on the development of oyster farming.

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