Distribution patterns of gregarine parasitism of wild marine bivalve, *Anadara cornea* (reeve, 1844) concerning seasonality and water quality

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Abstract. Gregarines are groups of protozoan parasites that ubiquitously infect arthropods worldwide including host from terrestrial and aquatic environment. To date, 1,600 gregarine species have been described, but less study for invertebrates have been recorded for these apicomplexan parasites. Wild marine bivalve species *Anadara cornea* (Reeve, 1844) populations were surveyed for gregarines at Setiu Lagoon in Terengganu, Malaysia for a year. This species of bivalve was previously unlisted as hosts. Malaysia is known as a country that experience with rainy and dry season. The trends of rainfall regime in Peninsular Malaysia is mainly affected by the seasonal monsoon i.e. Southwest (SW) monsoon (May to October) and Northeast (NE) monsoon in November and ends in April the following year. The objective of this study was to associate the incidence of gregarine in common wild bivalves, *A. cornea* of Setiu Lagoon, Terengganu in relation to water quality and seasons. Gregarine prevalence and mean intensity of infections were compared between seasons. Samples were collected at seagrass beds which contain sandy muddy area which is a suitable habitat for this bivalve. The study was conducted from January to December 2018 with a total of 480 sample bivalve collected (n=40 per month). Total numbers of parasites were counts during this study were 33,239 phagocytes. The highest intensity of the infection of the parasite was found in November (n=5014) and the lowest number of infection was in January (n=767). The confidence interval for proportions normal approximation in the prevalence of dry season was 48.9% with 95% CI=45.3%–54.3%. The confidence interval for proportions normal approximation in the prevalence of rainy season was 47.7% with 95% CI=43.2%–52.2%. Risk estimation for odds ratio (OR) was 0.087 with 95% CI=0.01-0.68 Pearson Chi-Square showed the significant value (p≤0.05) cases via season. Most of the site infections of this parasite were on gill and digestive tract. Prevalence of parasites was considerably the same for both seasons. The mean intensity levels of infections were slightly high during the rainy season, which was assumed characterized by low surface seawater temperature, low salinity, and high levels of rainfall. Regression analyses revealed that season was significantly related to gregarine prevalence and intensity. This result emphasizes the importance of considering season and habitat as a part of the consideration factor while studying gregarine in bivalve ecology.
1. Introduction

Gregarines are a group of protozoan parasites that ubiquitously infect arthropods worldwide including host from terrestrial and aquatic environment. The phylum Apicomplexa is a cluster of more than 6000 protozoan parasites that cause morbidity and mortality in a widespread of vertebrates and invertebrates. To date, 1,600 gregarine species have been described, but less study for invertebrates have been recorded for these apicomplexan parasites. Shellfish such as scallops, clams, cockles, mussels, and oysters reportedly host a wide range of apicomplexans [1–5]. Wild marine bivalve species *Anadara cornea* (Reeve, 1844) populations were surveyed for gregarines at Setiu Lagoon in Terengganu, Malaysia for a year. This species of bivalve was previously unlisted as hosts (figure 1). Malaysia is known as a country that experience with a rainy and dry season. The trends of rainfall regime in Peninsular Malaysia is mainly affected by the seasonal monsoon i.e. Southwest (SW) monsoon (May to October) and Northeast (NE) monsoon in November and ends in April the following year.

For the past 16 years from 1993 to 2009, aquaculture production in Malaysia has reportedly increased by 45% [6]. Due to the gradual demand for seafood for consumption and production from natural stocks over time, the Malaysian government through DOF introduced bivalve culture. OIE (Office International des Epizooties) [7] has listed that gregarine as risk parasites that infect in the shellfish especially for consumption and productions worldwide. Therefore, report of diseases and parasites infecting bivalves in Malaysian waters have not been systematically investigated respectively [8]. A study of parasites has been reported causing diseases in farmed and natural bivalve stocks from neighboring countries [9–12] The objectives of this study are to associate the incidence of gregarine in common wild bivalves, *A. cornea* of Setiu Lagoon, Terengganu relation to water quality and seasons.

![Figure 1. Internal Organ Of Anadara cornea from setiu lagoon, Terengganu, Malaysia. A. Valve, B. Gill, C. Body, D. Adductor Muscle, E. Mantle, F Gill Racker. Visceral mass and H. Digestive tract](image.png)

2. Methodology

Sampling areas were identified according to high-catch activities, mangrove area, coastal areas, and previous bivalves report on journals. A preliminary study of the infections of gregarine has been done before the selected species of bivalves was chosen for this study. Study was conducted from January to December 2018 at Setiu Lagoon, Setiu Terengganu, Malaysia with a total of 480 sample bivalve collected (N=40 per month). Gregarine prevalence and mean intensity of infections were compared between seasons (figure 2). The sample was collected at seagrass beds which contain sandy muddy area which is a suitable habitat for this bivalve. Analyses of this study were interpreted using SPSS software.
3. Results and discussion

Total numbers of parasites were counts during this study were 33,239 phagocytes. The highest intensity of the parasites was found in November ($n=5014$) and the lowest number of infection was in January ($n=767$). The confidence interval for proportions normal approximation in the prevalence of dry season was 48.9% with 95% of CI=45.3%–54.3%. The confidence interval for proportions normal approximation in the prevalence of rainy season was 47.7% with 95% of CI=43.2%–52.2%. Risk estimation for odds ratio (OR) was 0.087 with 95% CI=0.01–0.68 Pearson Chi-Square showed the significant value ($p\leq0.05$) cases via season. Most of the site infections of this parasite were on gill and digestive tract.

Rainy season showed the highest infection of this parasite. From the result, both seasons showed the highest infection of gregarine parasites. The monthly occurrence did not show slightly different between seasons throughout the year. Level value of water parameter and water quality at the sampling site was normal compared to the standard value for the aquatic ecosystem (Table 1). The analysis distribution of water parameters and water quality using SPSS in this study was significantly normal with $p\geq0.05$ (table 2).
Table 1. Comparison value of water parameter and water quality at the sampling site.

| Parameter               | Standard Level | This Study (Average 12 Month Sampling) |
|-------------------------|----------------|--------------------------------------|
| Salinity (ppt)          | 0.5-30         | 12.4                                 |
| Temperature (0°)        | 30-36          | 29.3                                 |
| Dissolved Oxygen (mg/L) | 0-14           | 3.9                                  |
| pH                      | 7.5-8.4        | 7.1                                  |
| Ammonia (mg/L)          | Less than 0.02 | 0.1                                  |
| Nitrite (mg/L)          | Below than 0.02| 0.02                                 |
| Phosphorous             | 0.005 to 0.05 mg/L | 0.02                           |

Table 2. Analysis of the distribution of water parameters and water quality

| Parameter       | Mean ± Standard Deviation |
|-----------------|---------------------------|
| Salinity        | 12.367±7.76               |
| Dissolved Oxygen| 3.917±0.84                |
| pH              | 7.075±0.34                |
| Temperature     | 29.317±1.48               |
| Ammonia         | 0.079±0.04                |
| Nitrite         | 0.023±0.01                |
| Phosphorous     | 0.018±0.01                |

Figure 4. Results of water quality throughout the year (January – December 2018)
Ammonia level slightly increased from August to September (Graph 2) maybe because of freshwater run-off from rivers. The high level of ammonia showed a decreased number of parasites infection. The previous study by Jimanez et al. (2002) [13] proves that the parasites are found with high infection level during rainy season which is supportive the finding of this study. However, no relations between water qualities are reported previously. The latest study was more focused on the identification of the parasites using DNA analysis. A study by McDevitt-Galles, et al. [14] stated that body size and length associated with an increase of parasite prevalence. The study is concern more on the effect of gregarine prevalence on the host as could be associated with morbidity and mortality in these group species under certain conditions [15]. Advance research on host density, common pathogens, host developmental stages (biotic) and temperature, pH, salinity (abiotic) factors that could cause the parasite to increase and spread an epidemic will help to clarify the parasites ecology and provide insights for establishing epidemic control and monitoring strategies.

4. Conclusions
Report on the prevalence and distribution of this parasite was not properly done. Prevalence of parasites infection was considered the same for both seasons. The mean intensity levels of infections were slightly high during the rainy season, which was assumed to be characterized by low surface seawater temperature, low salinity, and high levels of rainfall. Regression analyses revealed that season was significantly related to gregarine prevalence and intensity. The occurrence of gregarine parasite positively found in Anadara cornea (Reeve, 1844) from Setiu Lagoon, Malaysia. The intensity of this parasite was slightly higher during the rainy season. Water quality showed that the increasing concentration of ammonia gives an effect on the decreasing number of this parasites prevalence. This result emphasizes the importance of considering season and habitat as a part of the consideration factor while studying gregarine in bivalve ecology.

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