# Comparison on the Cell Abundance of Benthic Dinoflagellates in Macrophytes and Water Column Collected from Open Coastal Waters and Semi-Enclosed Lagoon (Perbandingan Kelimpahan Sel Dinoflagelat Bentos pada Makrofit dan Turus Air yang Diambil dari Pesisir Terbuka dan Teluk Semi Tertutup)

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# ABSTRACT

Benthic dinoflagellates are known to occur in the water column. The reason they can be found in different parts of the ecosystem is not clear. This study aims to determine the species and the cell abundance of benthic dinoflagellates in the water column and macrophytes collected from two different locations i.e. semi-enclosed lagoon and open coastal waters. The physico-chemical parameters (temperature, salinity, pH, dissolved oxygen) and nutrients (nitrate and phosphate) were determined. Results showed that in the lagoon, the most abundant dinoflagellate species on the macrophytes was also the most abundant dinoflagellate species in the water column. The species that dominated the water column and marophytes in the lagoon was Bysmatrum caponii. In the coastal area the trend was not clear. Coolia dominated the macrophytes whereas Peridinium quinquecorne dominated the water column. The physico-chemical parameters determined were similar at both sites except for nutrients. Results show that type of substrates and different geomorphology effects benthic dinoflagellates cell abundance in the macrophytes and water column.

Keywords: Benthic dinoflagellates; hydrodynamic; planktonic; seaweed; water column

# ABSTRAK

Kehadiran dinoflagelat bentos di dalam turus air sememangnya diketahui. Sebab kehadiran organisma ini di dalam bahagian ekosistem yang berbeza tidak diketahui. Tujuan kajian ini ialah untuk mengenal pasti spesies dan kelimpahan sel dinoflagelat bentos di dalam turus air dan makrofit di dua lokasi berbeza iaitu di teluk separa tertutup dan di kawasan pesisir yang terbuka. Parameter fizikal-kimia (suhu, kemasinan, pH, oksigen terlarut) dan nutrien (nitrat dan fosfat) ditentukan. Keputusan menunjukkan di teluk, spesies yang banyak ditemui pada makrofit juga dijumpai banyak di dalam turus air. Spesies yang mendominasi makrofit dan turus air di teluk ialah Bysmatrum caponii. Namun, di kawasan pesisir, trendnya tidak jelas. Coolia didapati mendominasi makrofit tetapi Peridinium quinquecorne mendominasi turus air. Bacaan parameter fiziko-kimia di kedua-dua kawasan adalah sama kecuali nutrien. Keputusan menunjukkan bahawa jenis subtrak dan geomofologi yang berbeza mempengaruhi kelimpahan spesies yang hadir dalam makrofit dan turus air.

Kata kunci: Dinoflagelat bentos; hidrodinamik; planktonik; rumpair laut; turus air

## INTRODUCTION

From early reports, benthic dinoflagellates species were thought to exist in the water column. The type species of Ostreopsis, O. siamensis Schmidt was first described from water collected from the Gulf of Siam (Thailand) (Schmidt 1902). Later it was learned that benthic dinoflagellates can inhabit various bottom substrates such as the macrophytes and sediment and also be found in the water column. The first description of O. labens reports that this species was found in the water column, in sand and associated with the macroalgal (Faust 1995). Ostreopsis marinus were identified from samples collected from the water column, on surface of colored sand, on coral rubble and associated with marcoalgal (Faust 1999). The occurrence of benthic dinoflagellates in the water column has been correlated with number of cell on the macrophytes (Mangialajo et al. 2008; Vila et al. 2001). Prorocentrum levis and P. rhathymum were recorded in the water column during the same

period as the species was found highest in the sediment and/or macrophytes (Aligizaki & Nikolaidis 2006). Benthic dinoflagellate assemblages on macroalgaewere thought to follow the seasonal pattern of the macro algae (Mohammad-Noor et al. 2006; Vila et al. 2001). Vila et al. (2001) has reported that *O. ovata* was found in the water column during warm months. The occurrence of benthic dinoflagellates in the water column may be as a part of their life cycle. Study by Bravo et al. (2012) on the life cycle stages of *Ostreopsis* cf. *ovata* showed that the species produce different forms of cysts during sexual and asexual reproduction. However, the complete life cycle is still not fully understood. Finally, benthic dinoflagellates can also be found in the water column after storms.

The purpose of this study was to compare cell abundance of benthic dinoflagellates in the water column and macrophytes collected from two different geomorphologies i.e. a sheltered lagoon and an open coastal water area. Physico-chemical parameters including temperature, dissolved oxygen, pH, nitrate and phosphate were also determined. Results of this study increase our understanding on the ecology of benthic dinoflagellates particularly in the tropical areas.

# MATERIALS AND METHODS

Samples were collected at Dinawan Island, Sabah, Malaysia (Figure 1). Two sites were chosen i.e. the lagoon area and the coastal water. The lagoon area was dominated by sea grass, Enhalus sp. Other seaweed species present were viz. Sargarssum, Halimeda and Padina. The seawater flow-in and out during high tide and low tide through entrance point A (Figure 1). Twice a month, the seawater flow out from the lagoon to the open sea during spring tide through entrance B (Figure 1). Therefore the lagoon is regarded as semi-enclosed area. The coastal area was characterized by seaweeds including Sargassum, Padina, Halimeda and seagrass, Enhalus. Sargassum dominated the area. To determine the cell abundance of benthic dinoflagellates in the water column, 500 mL integrated seawater samples were collected randomly at each site. The depth of each sampling point at both areas ranged from 2 to 3 m. These water samples were pulled together from many sampling points to make up final volume of 30 L. Samples were preserved immediately with Lugol's iodine. In the laboratory, the samples were concentrated into 50 mL. To determine benthic dinoflagellates on macrophytes, all seaweed and seagrass present at the sampling areas were collected and placed in a plastic bag separately. Each seaweed or seagrass was collected in two replicates. The

plastic bags containing the seaweed or seagrasses were shaken vigorously before filtering through sieves of 125 and 20  $\mu$ m mesh in size. Samples collected on a 20  $\mu$ m sieve and transferred into containers and preserved with Lugol's iodine. For enumeration, 1 mL of each sample was counted using Sedgwick Rafter counting chamber under light microscopy. Samples were counted twice to ensure the better accuracy. Identification was based on Mohammad-Noor et al. (2006).

For nutrient determination, 1 L of integrated seawater samples were collected in two replicates at each site. Samples were filtered through membrane filter GF/C. Nitrate and phosphate concentrations were determined according to standard method by Parson et al. (1984). Physico-chemical parameter such as salinity, temperature, pH and dissolved oxygen were recorded *in-situ* using Hydrolab. Statistical analysis was done using SPSS (Statistical Package for the Social Sciences) Volume 15.

# RESULTS

In the lagoon, relative abundance of benthic dinoflagellates was more abundant (79.6% of the total cell numbers) compared to planktonic dinoflagellates (20.4%). In the coastal water planktonic dinoflagellates were higher (80.2%) than benthic dinoflagellates (19.8%) (Figure 2). The difference of relative abundance for benthic dinoflagellates between these areas was significantly different (p<0.05).

Comparison between benthic dinoflagellates found in the substrates and in the water column in the lagoon showed similar trends (Figure 3). The species that were found in



FIGURE 1. Map of the study area. A. Malaysia B. Sabah. C. Dinawan Island showing the sampling areas



FIGURE 2. Relative abundance of dinoflagellates at the lagoon and coastal areas

high abundances on the substrates were also found high in cell number in the water column. On the substrates, Bysmatrum caponii has the highest cell number  $(1.3 \times 10^4 \pm$  $1.6 \times 10^3$  cells/g wet weight) followed by *Coolia* (2.2 ×  $10^3 \pm 4.3 \times 10^2$  cells/g wet weight) and *P. rhathymum* (1.7)  $\times 10^3 \pm 2.9 \times 10^2$  cells/g wet weight) (Figure 3). In the water column, the cell density of Bysmatrum caponii was  $532 \pm 116$  cells/L, P. rhathymum was  $287 \pm 64$  cells/L and Coolia was  $136 \pm 51$  cells/L (Figure 3). Whereas, in the coastal area the trend is not the same (Figure 4). Species that were high in the substrates were not necessarily high in the water column. The highest cell number in the substrates was dominated by *Coolia*  $(3.9 \times 10^3 \pm 1.3 \times 10^3 \text{ cells/g wet})$ weight) and followed by *P. rhathymum*  $(2.9 \times 10^3 \pm 7.7 \times 10^3 \pm 7.$  $10^2$  cells/g wet weight) and *P. norrisianum* ( $1.4 \times 10^3 \pm 3.6$  $\times 10^{2}$  cells/g wet weight). Meanwhile in the water column, *P. quinquecorne* has the highest cell number with the cell density of  $136 \pm 24$  cells/L followed by *P. rhathymum* with cell density of  $67 \pm 13$  cells/L.

Nutrients concentration in the seawater showed that coastal area has higher concentration of nitrate but lower concentration on phosphate compared to the lagoon area (Table 1). In coastal area, nitrate and phosphate concentrations were 4.2  $\mu$ M and 1.1  $\mu$ M, respectively whereas in lagoon, nitrate concentration was 2.7  $\mu$ M and phosphate concentration was 4.3  $\mu$ M (Table 1). This indicates that nutrients were not limiting at these areas. Physico-chemical parameters such as salinity, temperature, dissolved oxygen and pH recorded at both areas were almost similar as shown in Table 1. Generally warm water has low DO and in this study temperature is almost the same in lagoon and coastal area. Based on statistical analysis, no correlation was found among cell densities of benthic dinoflagellates at both places with physico-chemical parameters studied.

### DISCUSSION

Higher cell abundance of benthic dinoflagellates was recorded in the lagoon area compared to in the coastal area. The likely reason is that the lagoon area is a semi-enclosed area whereas the coastal water is opened water. Due to limited access of seawater flow-in and out in the lagoon area, therefore, we hypothesized that the lagoon has weak hydrodynamic water movement and calm environment. The limited source of planktonic dinoflagellates introduced



FIGURE 3. The cell density of benthic dinoflagellates found in the macrophytes (cell/g) and water column (cell/L) at the lagoon area



FIGURE 4. The cell density of benthic dinoflagellates found in the macrophytes (cell/g) and water column (cell/L) at the coastal area

TABLE 1. Physico-chemical parameters recorded at the sampling sites

Parameter	Lagoon	Coastal
Nitrate, µM	$2.6 \pm 1.5$	$4.2 \pm 2.0$
Phosphate, µM	$4.3 \pm 1.2$	$1.1 \pm 0.4$
Salinity	$30.5 \pm 2.4$	$30.4 \pm 1.9$
pH	$8.2 \pm 0.1$	$8.2 \pm 0.1$
Temperature, °C	$30.9 \pm 1.4$	$30.9 \pm 0.1$
Dissolved oxygen, mg/L	$5.8 \pm 1.2$	$6.4 \pm 1.4$

to the lagoon will reduce the species diversity. For benthic dinoflagellates, the lagoon creates a suitable environment. Totti et al. (2010) has reported the finding on the significantly abundance of Ostreopsis ovata was observed in sheltered area compared to exposed area. Study by Vila et al. (2001) also indicated that each species of benthic dinoflagellates has their own niche environment. They reported that Ostreopsis preferred turbulent to slightly turbulent waters whereas, C. monotis preferred slight turbulence to calm waters. In the coastal area, the hydrodynamic activity is much stronger and the source of planktonic dinoflagellates comes continuously from the open sea. This allows more planktonic dinoflagellates to be present but unfortunately this type of environment is not preferable for the benthic dinoflagellates. This indicates that the environmental condition plays a vital role to sustain particular dinoflagellates in a specific area.

The positive correlation between number of cell of benthic dinoflagellates living in the macrophytes and in the water column has been reported elsewhere. Vila et al. (2001) and Mangialajo et al. (2008) for example have reported on the abundance of *Ostreopsis* in the water column coinciding with high number of cells on macrophytes. In this study, similar trend was observed particularly in the lagoon area but not in the coastal area. In lagoon area, *Bysmatrum* dominated the water column and the macrophytes. Whereas in the coastal area *P. quinquecorne* dominated the water column and *Coolia* dominated the macrophytes. The different species dominating in different areas is believed to be the reason of different substrates availability and abundance. Study on substrates preferences has suggested that benthic dinoflagellates, in this case Ostereopsis spp., prefers substrates with three-dimensional and high surface area (Vila et al. 2001). But Totti et al. (2010) suggested that Ostreopsis ovata preferred seaweed with branched thalli to non-branched seaweed .In the lagoon, Enhalus dominated thus provides a high surface area and this has created a suitable habitat for this species to grow. Furthermore, Bystmatrum caponii was first described from tide pool and this species is considered as tycoplanktonic species. The ability to perform vertical migration compared to other species will provide better access to favorable growth conditions and therefore, occur in high number both in water column and macrophytes. The other two species that were found high in the water column were P. rthathymum and Coolia sp. P. rhathymum also known as tycoplanktonic species and this give a reason for high cell number in the water column. In the coastal water, Coolia sp. that was high in abundance in seaweed was not found in the water column. Nevertheless, P. quinquecorne which was present in low number in the seaweed dominated the water column. The difference in species found on seaweed and water column may be due to the fact that *P. quinquecorne* is not a truly benthic species. This species is commonly associated with benthic dinoflagellates species and a fast swimmer. P. rhathymum was also found in the water column.

Physico-chemicals parameters including nutrients recorded have no correlation with the cell number of benthic dinoflagellates in both study areas. Studies on the importance of these parameters have been reported from temperate countries. Several parameters such as temperature, salinity and nutrient enrichment have been suggested to affect the growth of benthic dinoflagellates (Pistocchi et al. 2011; Salco et al. 2012; Vidyarathna & Granéli 2013). However, the correlation is said to be strain specific and subjective to the area which the species occur (Totti et al. 2010). But other studies (Okolodkov et al. 2007) indicated negative correlation between physicochemical parameters and benthic dinoflagellates. In the tropical area whereby monsoons play an important role, the relationship or parameter influencing the growth of benthic dinoflagellates might be different (Mohammad-Noor et al. 2006). Therefore, studies on the importance of these parameters in the tropical areas are needed to be addressed these metrics on benthic dinoflagellates, especially since they can cause human health problem.

Generally, it can be concluded that the high number of benthic dinoflagellates cells was found in the water column when the species was abundance in the macrophytes. However, this depends on which species dominated the macrophytes. Nevertheless, the presence of benthic dinoflagellates in the water column and macrophytes depends on many abiotic and biotic factors and therefore long term data is needed to increase our understanding on the ecology of benthic dinoflagellates particularly in the tropical area.

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