

BENTHIC HARMFUL DINOFLAGELLATE ASSEMBLAGES IN A FRINGING REEF OF SAMPADI ISLAND, SARAWAK, MALAYSIA

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ABSTRACT

A study on the presence and relative abundance of benthic harmful algal bloom (BHAB) forming dinoflagellate species was carried out in the coral reefs of Sampadi Island, Sarawak, Malaysia. The study involved deployment of fiberglass screens as an artificial substrate for the benthic epiphytic microalgae. The screens were placed for 24 h above the seafloor along a 100 m transect at 10 m intervals. BHAB species attached to the screens were identified and cell abundances were enumerated under a light microscope. The BHAB community at the study site was dominated by *Prorocentrum* spp. and *Coolia* spp. Other BHAB species collected included *Amphidinium* spp., *Gambierdiscus* spp. and *Ostreopsis* spp. Total cell densities collected on the screens ranged from 5 to 100 cells per 100 cm². The two BHAB groups of primary concern, *Gambierdiscus* spp. and *Ostreopsis* spp. were detected at relatively low abundances of 0.6–4.2% and 1.8–16% respectively. This study has shown that potentially toxic BHAB species were present in the coral reef and the artificial substrate approach could provide a convenient quantitative method for the collection of clean samples for identification and enumeration purposes.

Keywords: *Amphidinium*, benthic harmful algal bloom, *Coolia*, *Gambierdiscus*, *Ostreopsis*, Sampadi Island.

INTRODUCTION

Benthic dinoflagellates are one of the important components in benthic microalgae assemblages of tropical and subtropical coastal environments. The ecology of five major genera of benthic dinoflagellates, viz. *Amphidinium*, *Coolia*, *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* has been well-studied (Tindall and Morton, 1998; Tosteson et al., 1998; Vila et al., 2001). These species are found in benthic ecosystems associated with substrates such as seaweeds, coral rubbles and sand particles. Species in some genera are known to be producers of biotoxins related to ciguatera food poisoning (CFP), diarrhetic shellfish poisoning (DSP), palytoxin (PITX) seafood poisoning and clupeotoxism (Shimizu et al., 1982; Holmes et al., 1995; Lawrence et al., 2000; Holmes and

Teo, 2002; Durando et al., 2007; Aligizaki et al., 2011). Increase in incidences of these poisonings (e.g., Ciminiello et al., 2006; Barroso Garcia et al. 2011; Tubaro et al., 2011) and toxin discovery (Ciminiello et al., 2008, 2010) in the recent years have led to concomitant increase in studies of harmful benthic microalgae (BHAB) worldwide.

In Malaysian waters, long-term data on BHAB occurrence and its relationship to environmental conditions is lacking. Even though occurrences of BHAB species have been reported in some selected reefs and islands (Leaw et al., 2001, 2010, 2011; Mohammad-Noor et al., 2009), the data obtained were limited in spatial and temporal coverage. The lack of such basic information makes it difficult to gauge the potential significance of BHAB related

seafood poisonings and BHAB dynamics in the country.

Sampling methods that have been used in benthic algae studies varied widely among researchers. The most common method used in field sampling is to collect the hosts/substrates and dislodge the cells attached from the natural substrates (e.g., Villa et al., 2001; Leaw et al., 2010). However, the technique is sometimes unfeasible in samples with high silt or muddy sand content, as samples collected are often heavily overlaid with sediment, making cell enumeration difficult. Furthermore, the methods that have been commonly used are only semi-quantitative at best. This makes comparative studies on species abundance difficult. In this study, we adopt a sampling approach that utilizes artificial substrates as a means for collecting BHAB species from the natural environment (Kibler et al., 2010). The aims of this study are (1) to evaluate the feasibility and efficiency of this technique in BHAB study, and (2) to determine the occurrence and distribution of BHAB dinoflagellate species in a selected fringing

reef in Sampadi Island, Sarawak, southwest of Borneo.

MATERIAL AND METHODS

Study site

Sampadi Island is located in Sarawak, east Malaysia, to the southwest of Borneo ($1^{\circ}44'10''\text{N}$, $110^{\circ}05'06''\text{E}$) (Fig. 1). The 30-ha island is located 3.2 km from the mainland. This island is adjacent to Satang-Talang Island Marine Park, having typical equatorial climate affected by the northeast and southwest monsoon with surface water of 27 to 29°C (The Metrological Department, Sarawak). The triangular island has a rocky beach at the southern part of the island facing the mainland, while the northeast is a rocky cliff, and sandy beach on the northwest.

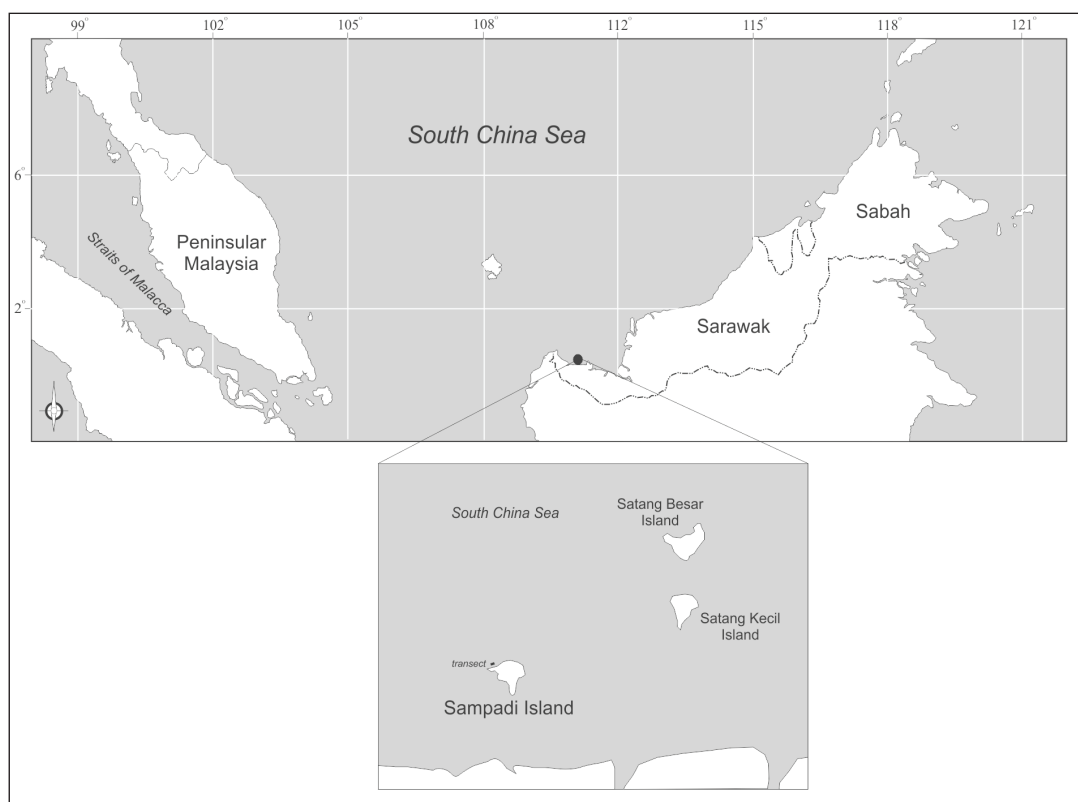


Figure 1. Malaysia map showing the location of Sampadi Island, Sarawak (inset: schematic map of Sampadi Island with the location of sampling transect).

Samples

Benthic dinoflagellates samples were collected using grey/black fiberglass window screens (~1.6 mm mesh size, 310 cm²; 4'×6'; Fig. 2A) that were deployed under the water for 24 hours along a transect line parallel to the northwest of the island, with screens deployed in a 10-m interval apart from each other (Fig. 2C–E). Total of ten screens were deployed along the 100 m-transect line. Each screen was carefully removed and placed into a 1-liter wide-mouth jar underwater. The jar was shaken vigorously to detach the epiphytic dinoflagellate cells. The materials were sieved through a 500- μ m mesh sieve, and cells retained in a 20- μ m mesh sieve were back-washed into a 50 mL centrifuge tube. Samples were preserved in 1% acidic Lugol's solution or 2% phosphate buffered paraformaldehyde for cell enumeration. Live samples designated for culture were brought back to the laboratory for further cell isolation.

Habitat mapping and classification

An underwater video survey was conducted along the transect line, and the surface of sediments were photographed during SCUBA dive. Seaweed community composition in the 5-m radius area from the screen deployed was determined 'by eye'

percentage cover estimates. The video footage was reviewed, surficial sediments described based on Wentworth grain size classification (Wentworth, 1922), and conspicuous vegetation identified, both by the visual appearance. Habitat types classification was then performed subjectively using the data obtained.

Species identification and enumeration

Samples were counted under a normal light microscope with magnification of 100 to 600 \times . Benthic dinoflagellates under the genera *Amphidinium*, *Coolia*, *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* were counted. Digital images were captured using an Olympus IX51 inverted microscope equipped with a CX30 cooled charge-couple device (CCD) camera (Olympus, Tokyo, Japan). Further identification was performed under epi-fluorescence equipped on the same microscope by observing the calcofluor-stained cells.

Data analysis

One-way analysis of variance (ANOVA) was performed to test for statistical differences among stations. Similarity matrix was constructed using Bray-Curtis similarity based on species relative abundance data. Relationships between stations

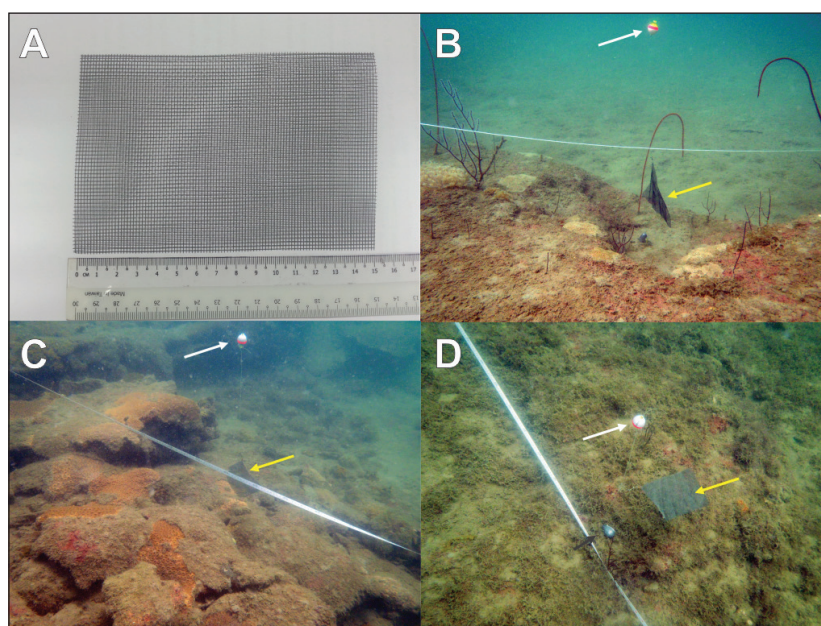


Figure 2. Artificial substrate used in this study for BHAB sampling. (A) Black fiberglass screen. (B–D) Underwater images showing the line transect and the screen deployed. White arrows indicate buoys, yellow arrows are screens.

were visualized using cluster analysis. The analyses were performed using PAST (Hammer et al., 2001).

RESULTS AND DISCUSSION

Benthic habitats along the transect

A 100-m transect line was placed parallel to the shore at the northwest of Sampadi Island, as samplings can be conducted along isobaths (constant depths and current strength). The benthic habitats along the transect-line differ among stations, with habitats of high epiflora coverage to no visible flora observed (Table 1). Seaweeds, *Dictyota dichotoma* and *Padina* sp. were the two common epiflora found (Fig. 3). Corals included sea fan (*Gorgonia* sp.), sea whip (*Ellisella* sp.), branching corals, star corals, brain corals (*Diploria* sp.), and *Acropora* sp. (Fig. 3). Some parts of the bottom substrates were overlaid with muddy silt (Figs 2, 3).

The benthic habitat along the transect-line was classified into three habitat types (Table 2) based on bottom substrates and coverage of epiflora (% seaweeds) subjectively. Station S1, S2, and S3

were grouped as habitat type A, with seaweed coverage of >50%; stations S5 to S9 were grouped as habitat type B, with seaweed coverage <50%; station S4 was classified as habitat type C where no obvious epiflora was observed (Table 2).

Morphology of BHABs species from Sampadi Island

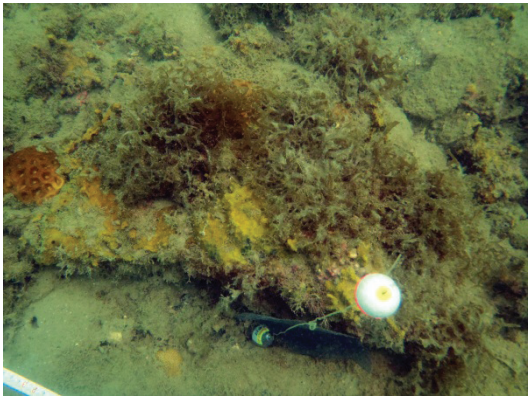
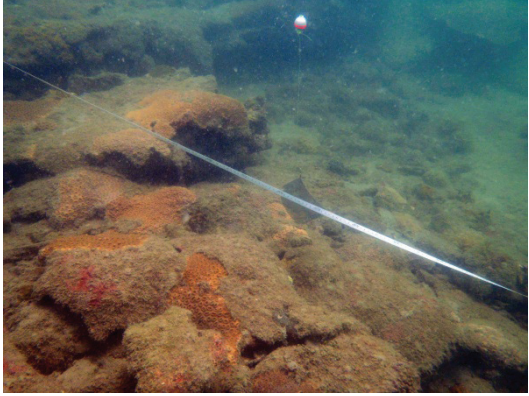
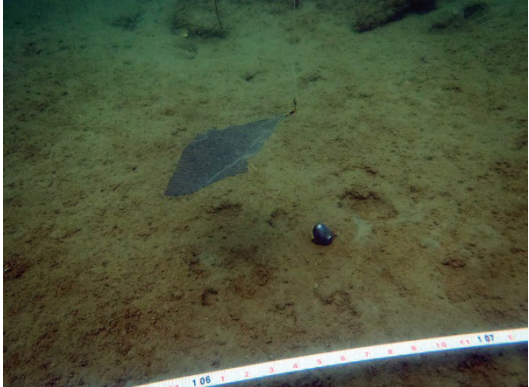
The five genera of BHABs species were easily distinguished under light microscopy, as they possessed distinct cell shapes and sizes (Fig. 4). Per se, *Amphidinium* was a naked free-living cell with cell size $30.4 \pm 3.4 \mu\text{m}$ in length and $25.4 \pm 2.9 \mu\text{m}$ in width. Cells were globular to fusiform, laterally compressed with a large hypocone. Cingulum circular or slightly displaced was located in the anterior part of the cell. Cells are easily distinguished by its small cap-like epicone which was one third or less of the total cell length. The species was identified as *Amphidinium cartarae* (Fig. 4A, B).

Cells of *Coolia* were thecate, biflagellate, with cell sizes ranged $24.0 \pm 0.8 \mu\text{m}$ in length and $25.0 \pm 1.8 \mu\text{m}$ in width. Cells appear to be rounded in apical and antapical views. Cells could be distinguished under a light microscope

Table 1. Descriptions of habitats from the video survey along the 100 m-transect line in Sampadi Island, where the screens deployed (10 m-interval)

Transect (m)	Station	Habitat description
10	S1	Screen was placed beside a branching coral between star corals, silt overlying. Patches of seaweed, dominated by <i>Dictyota dichotoma</i> (~80%).
20	S2	Screen was placed on star corals, <i>D. dichotoma</i> (~80%) with silt overlying.
30	S3	Screen was placed beside <i>D. dichotoma</i> (~50%). Silty sand.
40	S4	Screen was placed on the sediment floor. Silty sand. No obvious epiflora species.
50	S5	Screen was placed among star corals, partially covered by silt. <i>D. dichotoma</i> (~20%) in muddy mixed sediment.
60	S6	Screen was placed among star corals. Anemone, brain corals, patches of seaweed, <i>D. dichotoma</i> (~10%).
70	S7	Screen was placed on the sediment with small patches of <i>D. dichotoma</i> (~20%) around it. Small branching coral.
80	S8	Screen was placed on a star coral covered with silt. <i>D. dichotoma</i> (~20%) present on the sediment.
90	S9	Screen was placed on a star coral covered with silt. Patches of <i>D. dichotoma</i> (~30%), brain coral, sea whip, and sea fan.
100	S10	Screen was placed among anemone, sea whip, sea fan and some branching corals. Patches of <i>D. dichotoma</i> (~30%). Silt overlying.

Table 2. Habitat types classified along the 100 m-transect line in Sampadi Island where the screens deployed.

Habitat Description	Habitat type	Station	Seafloor image
Silty sand. Seaweed coverage >50%.	A	S1, S2, S3	
Silty sand. Seaweed coverage <50%.	B	S5–S9	
Silty sand, no obvious epiflora.	C	S4	

by observing the displacement of the epicone and hypocone at the lateral view (Fig. 4C). The species, *C. malayensis*, was identified under the epi-fluorescence microscopic observation (Fig. 4D).

Cells of *Gambierdiscus* observed in this study were heavily thecated and apical-antapically compressed (Fig. 4E, F), with cell sizes $55.0 \pm 14.4 \mu\text{m}$ in length and $57.4 \pm 10.9 \mu\text{m}$ in width. Cells of *Ostreopsis* were apical-antapically compressed with a unique tear-drop shape (Fig. 4G, H). Cell

dimension were in the range of $44.7 \pm 5.5 \mu\text{m}$ long and $31.5 \pm 5.7 \mu\text{m}$ wide.

Cells of *Prorocentrum* were bilateral thecate, oval shape in general. Some cells had a pyrenoid in the centre valves (Fig. 4K) and some absent (Fig. 4L). Two species of *Prorocentrum* were identified in this study. Cells with oval shape and a pyrenoid were identified as *P. lima* (Fig. 4K, L); cells with a slight V-shaped indent at the periplagellar area were identified as *P. emarginatum* (Fig. 4I, J). It was interesting to note that some common tropical *Prorocentrum* species, such as *P. concavum*, *P.*

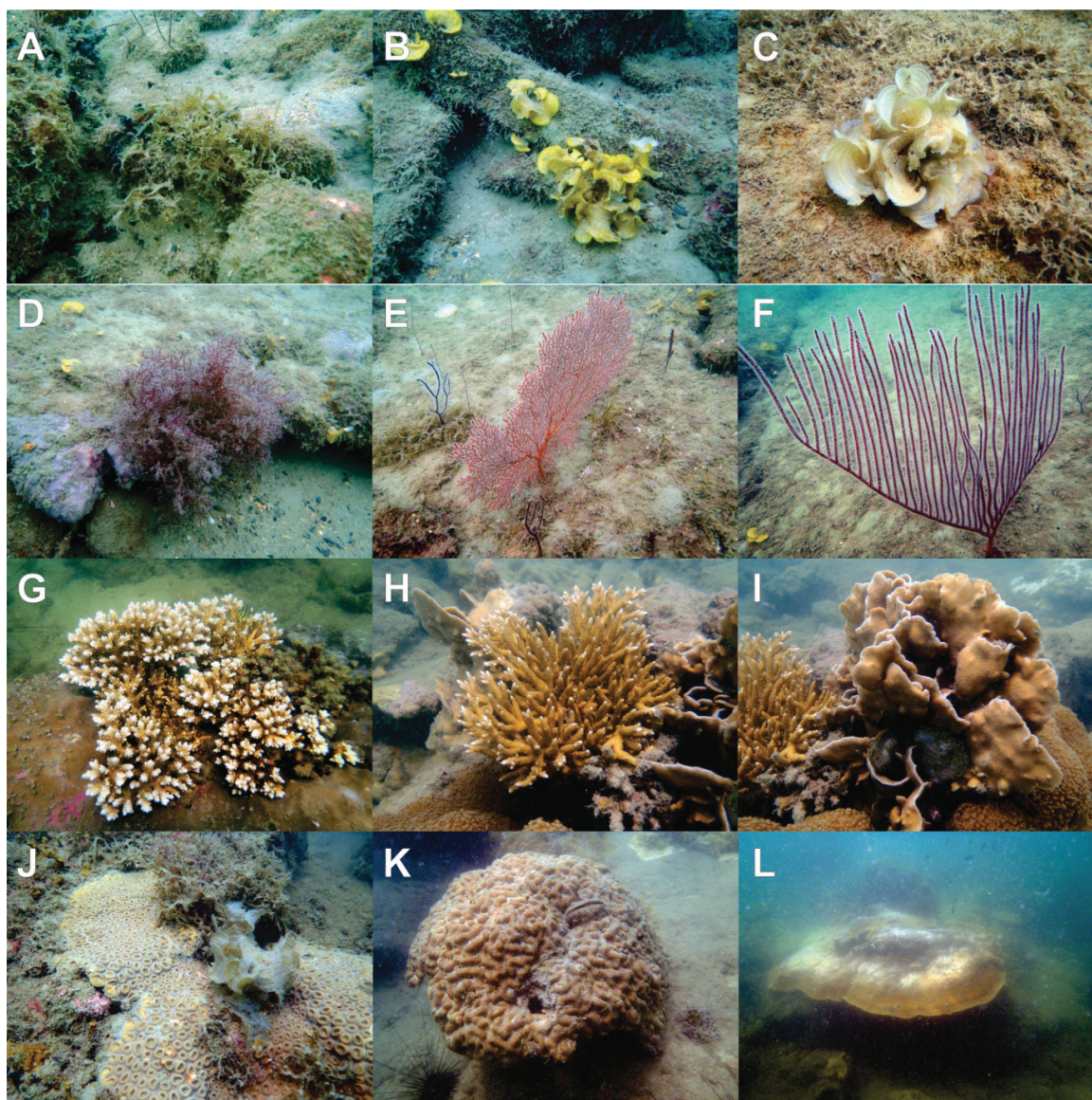


Figure 3. Seaweeds and coral substrates found in Sampadi Island, Sarawak: Brown seaweeds, *Dictyota dichotoma* (A) and *Padina* spp. (B, C), red seaweeds (D), sea fan (E), sea whip (*Ellisella* sp.) (F), branching corals (G, H), star corals (J), brain corals (*Diploria* sp.) (K), and *Acropora* sp. (L).

mexicanum and *P. rhathymum*, were not found in the samples, although cells of *P. rhathymum* were often observed in the plankton samples of Kuching waters (Tan et al., 2012).

Abundance and species composition of BHAB assemblage in Sampadi Island

Species composition of BHAB community was consistent throughout the sampling site, with all five BHAB taxa, viz. *Amphidinium*, *Coolia*, *Gambierdiscus*, *Ostreopsis* and *Prorocentrum*, observed in all sampling stations (S1–S9) except

S10. Data for station S10 was excluded because the screen was masked up with thick layer of silt which prohibited reliable cell enumeration.

Coolia was observed with the highest cell density (62 ± 26 cells/100 cm² screen), followed by *Prorocentrum* (39 ± 10 cells/100 cm²), *Ostreopsis* (11 ± 9 cells/100 cm²), *Amphidinium* (5 ± 3 cells/100 cm²); and *Gambierdiscus* with the lowest cell density (3 ± 1 cells/100 cm²) (Fig. 5). Total cell densities along the transect line ranged from 60 to 170 cells/100 cm² screen, with the highest cell density recorded at station S1.

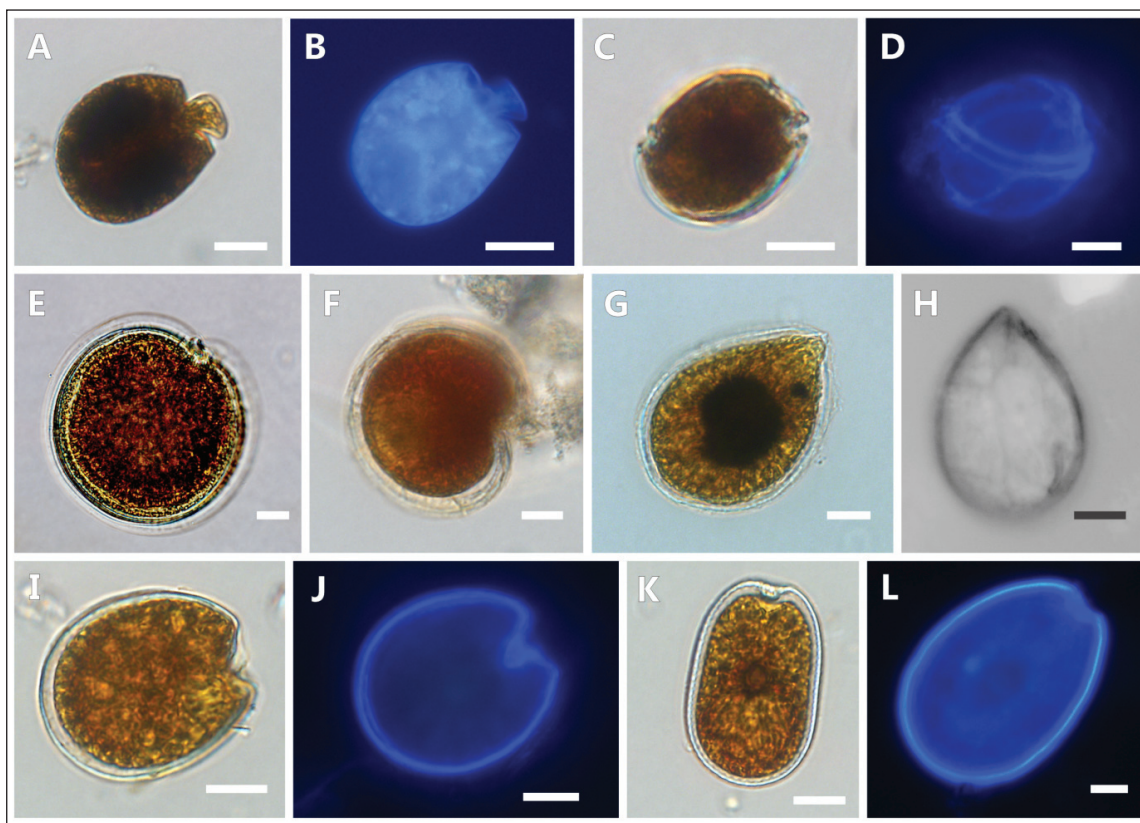


Figure 4. Light and epi-fluorescent micrographs of benthic harmful dinoflagellate cells observed from Sampadi Island, Sarawak. (A, B) *Amphidinium cartarae*, (C, D) *Coolia malayensis*, (E, F) *Gambierdiscus* sp., (G, H) *Ostreopsis* sp., (I, J) *Procentrum emarginatum*, (K, L) *P. lima*. Scale bar = 10 µm.

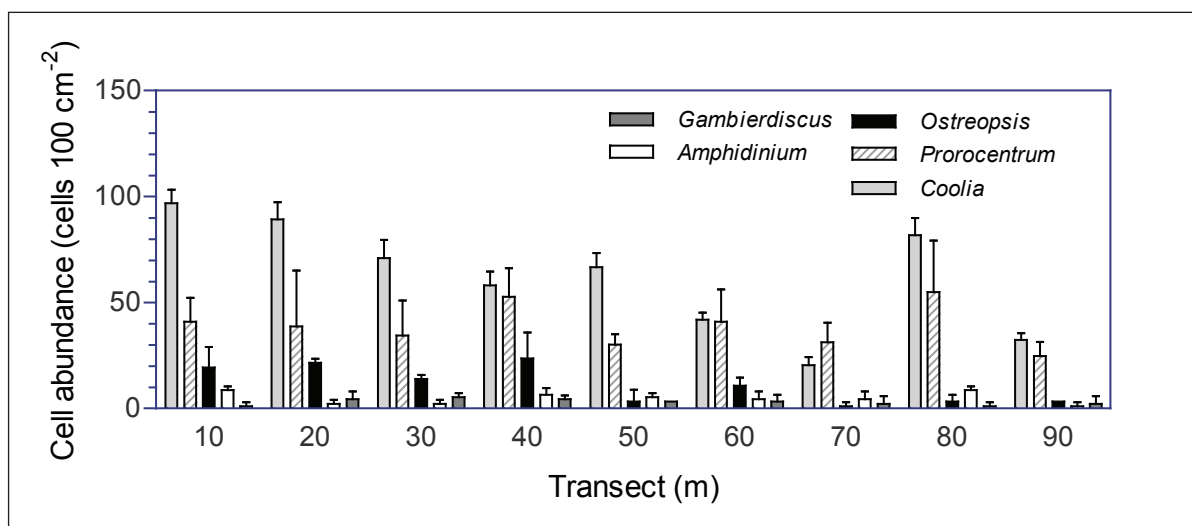


Figure 5. Total cell densities (cells 100 cm⁻¹) of five major benthic harmful dinoflagellate genera along the 100 m-transect line.

The distribution and relative abundances of BHAB species along the transect line changed slightly among sampling stations ($P < 0.001$, Fig. 6), however species composition and dominance were relatively constant. Species evenness among sampling stations was in the range of 0.6–0.8. Two taxa, *Coolia* and *Prorocentrum* were found dominating the BHAB community (34–50% mean relative abundances). Other taxa were found with very low mean relative abundances; *Gambierdiscus* had a mean relative abundance of $3 \pm 1\%$; while *Ostreopsis* and *Amphidinium* with $8 \pm 5\%$ and $4 \pm 2\%$ respectively.

Our preliminary results of cluster analysis using Bray-Curtis similarities revealed a degree of interaction between algal community (in a function of relative abundance) and benthic habitat condition (Fig. 6B). The analysis resolved three major groups congruent with the habitat type classification. Station S1–3 were clustered due to the dominance of *Coolia* and *Ostreopsis*, accounted up to 70% of relative abundance. The two taxa were known to be commonly associated with seaweeds; the abundance of these two taxa in these stations is not surprising as high abundance of macrophytes was observed in the stations (habitat type A, >50% seaweeds). Station S5–9 were grouped together, and this is congruent with the classification of habitat type B (<50% seaweeds). Station S4 was split out from other stations likely due to slightly higher abundance of *Ostreopsis* (16%) compared to other stations (Fig. 6A). This splitting was somehow congruent with habitat classification, where the station was classified as habitat type C. No obvious epiflora and corals were observed in this station, with silty sand as the benthic substrate. *Ostreopsis* spp. were commonly found in three different habitats; associated with macroalgae and sand, as well as in the water column (e.g., Faust and Morton, 1995; Vila et al., 2001). A study by Vila et al. (2001) on BHAB habitat preference demonstrated that *Ostreopsis* sp. was the dominant species not only as epiphyte to macroalgae, but also in the water column and sand. However *C. monotis* and *P. lima* were found in low cell densities in the water column, and mostly absent from sand (Vila et al., 2001).

Cell densities of BHAB species found in this area are incomparable to other previous studies as the quantification benthic epiphytic dinoflagellates abundances is usually expressed in cell g^{-1} fresh/dry weight macrophytes. However, this technique provides a comparison of abundances from different sampling sites, and allows quantification of cell abundances regardless of the type of substratum.

CONCLUSION

BHABs studies in Malaysia revolve around the report of occurrence (Usup et al., 1997; Leaw et al., 2011), morphological and molecular characterization (Mohammad-Noor et al., 2007; Leaw et al., 2010, 2011) and genetic diversity (Leaw et al., 2001). Although the existences of these potentially harmful species were reported, no abundance data was available. In this study, we initiated the data collection from the south-western Malaysian Borneo. Toxic and potentially toxic species were discovered in the recent assessment of benthic dinoflagellate composition in Sampadi Island. Cells from the five major harmful genera, *Amphidinium*, *Coolia*, *Gambierdiscus*, *Ostreopsis* and *Prorocentrum*, were found coexisting in the area. This study confirms for the first time the existence of BHAB species in Sarawak waters, albeit low cell densities.

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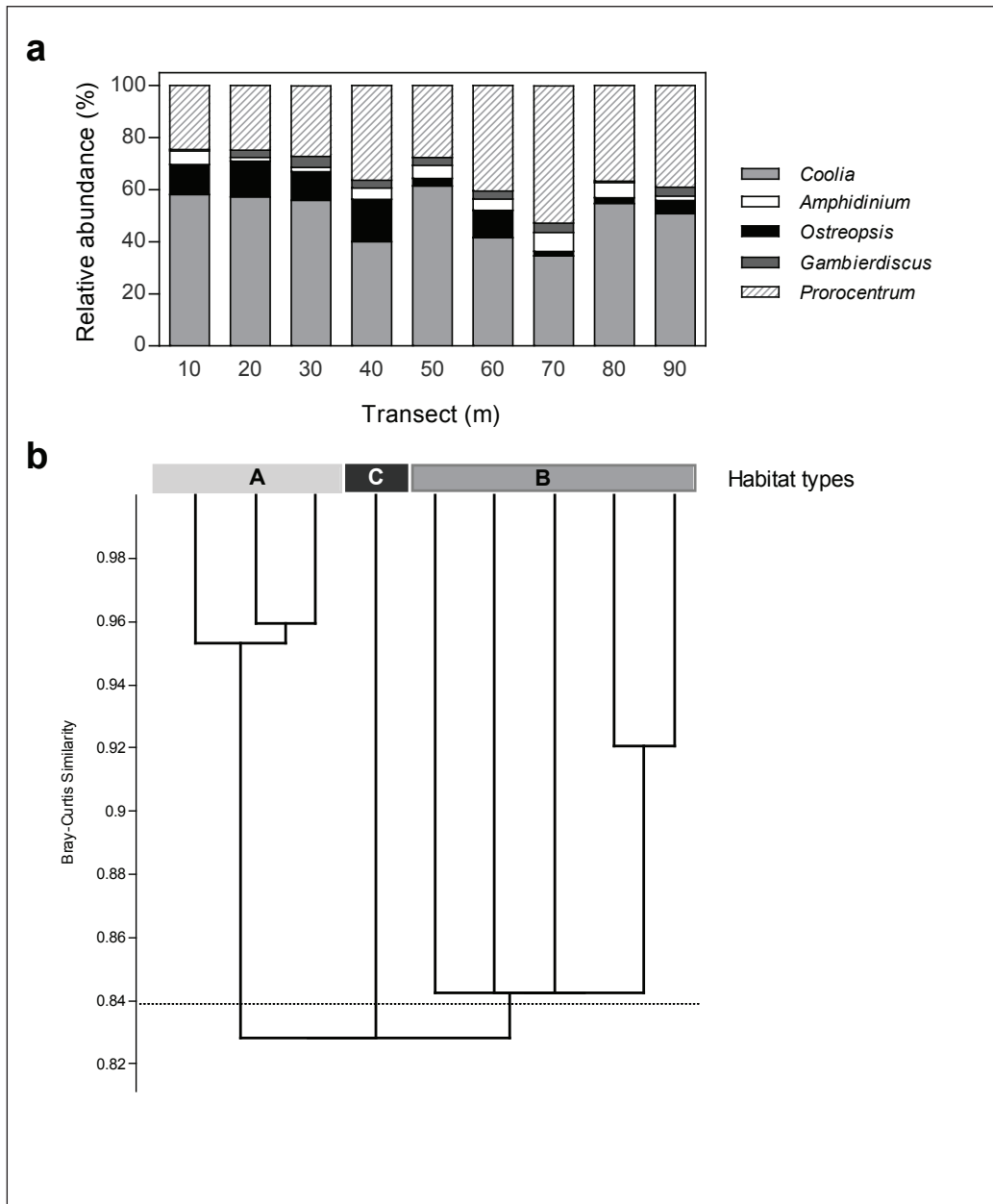


Figure 6. Relative abundance (%) of five benthic harmful dinoflagellates genera along the 100 m-transect line (A), and cluster analysis using the Bray-Curtis hierarchical agglomerative method. The horizontal dotline indicates split of three main groups (B).

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