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Fields of interest include: all forms of microscopy. Image acquisition and improvement techniques, along with computer-aided microscopy and analysis are included.

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ISSN: 0219-2209
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Harmful Algal Species in the Tebrau Strait: An SEM Observation of the Dinoflagellate Assemblage

Toh-Hii Tan¹, Po Teen Lim², Mohd Razali, Roziawati³ and Chui-Pin Leaw¹*

¹ Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
² Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
³ Fisheries Research Institute Batu Maung, Department of Fisheries Malaysia, 11960 Batu Maung, Pulau Pinang, Malaysia

* Corresponding author:
Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
Email: cpleaw@ibec.unimas.my, chuipinleaw@gmail.com Tel: +6082 583005 Fax: +6082 583004

ABSTRACT

Harmful algal bloom (HAB) is a natural phenomenon due to the increase of algal cell density in the water column that subsequently causes deleterious effects to natural environments as well as mankind. HABs in the country mainly occurred when a particular group of dinoflagellate cells proliferate in the eutrophied semi-enclosed coastal water body. In this study, dinoflagellate species composition in the Tebrau Strait was determined by scanning electron microscope (SEM). Plankton samples were collected by a 20-micron plankton net haul at several locations of the strait. Samples were undergone fixation, serial dehydration and followed by critical point drying. Samples were then observed under a JEOL analytical SEM. Total of 11 dinoflagellate species were identified, with 7 species known to be associated with HABs events. The occurrence of a fish-killing unarmoured dinoflagellate, Karlodinium veneficum was reported for the first time from Malaysian waters. The presence of this and other potentially harmful dinoflagellate species in the strait should be taken seriously by the respective authorities in future expansion of aquaculture industry in the strait.

Keywords: Dinoflagellates, Tebrau Strait, SEM, morphology, Karlodinium veneficum.

INTRODUCTION

Malaysia is a country surrounded by waters with a total coastline of 4,675 km, and Johore is one of the states in the Peninsula that has the longest coastline. Aquaculture industry in the state is rapidly growing particularly in the Strait of Tebrau. Other than cockles and shrimp farming, culture of marine fishes in floating cages is one of the common aquaculture activities in the strait.

Harmful algal blooms (HABs) are not uncommon to the country, with increasing frequency and distribution over the last decade. New records of HAB species and expansion of places affected with these events have been reported (Lim et al., 2004; Usup et al., 2002). Parallel elevation of both aquaculture activities and HAB events in the country has triggered the issues of seafood safety as well as environmental deteriorations due to aquacultural activities. The current knowledge on the occurrence of HAB species in the country particularly in the Strait of Tebrau is far lacking. The only reported HAB outbreak in the strait was the blooms of Prorocentrum minimum in 2002 (Usup et al., 2004). This information will be of crucial importance for future assessment and mitigation purposes.

In the present study, we aim to document the dinoflagellate assemblages as a species inventory in the Straits of Tebrau, particularly of those that are harmful. Plankton samples were collected from two selected sites and the dinoflagellate species were examined by using scanning electron microscope (SEM). This study was carried out at locations with intensive aquaculture activities on-going. By using this species inventory of harmful dinoflagellates we hope to provide further information to respective country authorities in monitoring and mitigating HABs as well as selection of aquaculture sites.
MATERIALS AND METHODS

Samples were collected using a 20-µm plankton net from the waters in the Tebrau Strait between July and December, 2009 (Fig. 1). Samples collected through net hauls were preserved in Lugol’s solution in the field. Samples brought back to the laboratory were kept at 4ºC in the dark for further analysis. Samples were prepared for SEM observation as described in Leaw et al. (2010). In brief, preserved samples were fixed in 5% glutaraldehyde. The fixed samples were filtered on a 0.2 µm black polycarbonate membrane filter, and rinsed a few times with cacodylate buffer (0.1 M, pH 7) through a vacuum manifold. Samples were then enclosed in a filter paper envelope and dehydrated with a graded series of ethyl alcohol concentration (30%, 50%, 70%, 80%, 90%, 95%, and 100%) for 15 min each, and twice in the intermedium, amyl acetate for 15 min each. Samples were then undergone critical point drying (CPD). Dried samples were mounted on to a stub, and coated with gold-palladium using a JEOL JFC-1600 magnetron sputter coating instrument (JEOL, Japan). Samples were then viewed under a JEOL JSM-6510 analytical scanning electron microscope (JEOL, Japan).

Fig. 1 Map of Tebrau Strait showing two sampling locations in this study.

RESULTS & DISCUSSION

A total of 11 species of dinoflagellates were identified in this study. Phytoplankton bloom was observed in the field samples collected during July and December 2009. Among the species identified, 7 were harmful either as fish killers or toxin producers (Table 1) which encountered more than half of the species found (60%). Morphology of each species found in the strait is described herewith based on the SEM observation of the outer cell structures. It is notable that a fish-killing unarmoured dinoflagellate, Karlodinium veneficum is found for the first time in Malaysian waters. This record of occurrence is a first record ever in Malaysia.

Species description
Karlodinium veneficum (D. Ballantine) J. Larsen 2000

Synonyms
The first name ever given to this species (basionym) was Gymnodinium veneficum (Ballantine, 1956). This athecate species was also known as Karlodinium micrum (Leadbeater et Dodge) Larsen (Daugbjerg et al., 2000), Gymnodinium galatheanum (Braarud sensu Kite et Dodge), Gymnodinium micrum (Leadbeater et Dodge) Loeblich III, Gyrodinium galatheanum (Braarud) Taylor and Woloszyńska micra (Leadbeater et Dodge) (Bergholtz et al., 2006).
Table 1 Harmful species of dinoflagellates found in the Tebrau Strait.

<table>
<thead>
<tr>
<th>Species</th>
<th>Impact</th>
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</thead>
<tbody>
<tr>
<td><em>Dinophysis acuminata</em></td>
<td>Diarrhetic shellfish poisoning (DSP)-toxins producer</td>
</tr>
<tr>
<td><em>Dinophysis caudata</em></td>
<td>DSP-toxins producer</td>
</tr>
<tr>
<td><em>Karenia mikimotoi</em></td>
<td>Fish killer</td>
</tr>
<tr>
<td><em>Karlodinium veneficum</em></td>
<td>Fish killer (ichthyotoxin, karlotoxin producer)</td>
</tr>
<tr>
<td><em>Neoceratium furca</em></td>
<td>Fish killer</td>
</tr>
<tr>
<td><em>Prorocentrum micans</em></td>
<td>Bloom forming species, shellfish killer</td>
</tr>
<tr>
<td><em>Scrippsiella trochoidea</em></td>
<td>Non toxic fish killer</td>
</tr>
</tbody>
</table>

**Diagnosis**

Cells are small and ovoid without dorso-ventral compression (Fig. 2). Cells are with length of 18 – 22 \( \mu \text{m} \) and width of 14 – 18 \( \mu \text{m} \) \( (n = 4) \). Cells are slightly bigger than those reported which ranged in 9 – 18 \( \mu \text{m} \) in length and 7 – 14 \( \mu \text{m} \) in width (Ballantine, 1956; Dodge, 1982; Taylor et al., 1995).

The epicone and hypocone are equal in size (Fig. 2A, B). The cell’s anterior end is slightly pointed. Ventral pore is situated on the left side of the apical groove. The ventral pore is not rounded but slightly elongated ventrally (Fig. 2A). Apical groove present at the epicone starting from the apical-ventral of the cell towards the dorsal side (Fig. 2C).

Hypocone is rounded with a slight indentation at its posterior end. The deep cingulum is displaced in a descending spiral of 3 times its width. The sigmoid-shaped sulcus slightly invades the epicone (Fig. 2B). The sulcus is deeply excavated and displaced 3 times into the hypocone on the right ventral side (girdle width = 2.0 \( \mu \text{m} \), girdle displacement = 5.03 \( \mu \text{m} \), \( n = 4 \)) (Fig. 2B).

Generally, the features are similar to the description of *K. veneficum* by Ballantine (1956), Dodge (1982), and Taylor et al. (1995). Cell dimensions are in the range of *K. armiger* Bergholtz, Daugbjerg & Moestrup, *K. australis* de Salas, Bolch & Hallegraeff, *K. conicum* de Salas, but dissimilar from other *Karlodinium* species described (Table 2). It differs from *K. armiger* by the cingulum displacement where *K. armiger* is with two cingulum widths and approximately one-third of the cell length. Whereby the cells observed in this study have 3 times cingulum width, displaced roughly 20% of the cell length. The cells differ from *K. australis* and *K. decipiens* by the deeply excavated cingulum, where both latter species possess shallow cingulum. No longitudinal furrow is observed in the cells which is the distinctive feature of *K. corrugatum* de Salas.

![Fig. 2 Scanning electron micrographs of Karlodinium veneficum from the Tebrau Strait, Johor. (A) Apical-dorsal view showing elongated ventral pore (vp) and apical groove (ag). (B) Dorsal view of cell showing the cingulum displacement (cd) descending to the right. (C) Ventral view of cell showing the starting of apical groove. Scale bar = 5 \( \mu \text{m} \).](image-url)
Toxicity/harmful effect

The species was reported to produce karlotoxin, an ichthyotoxin (Peng et al., 2010), that are lethal to fish through damage of gill epithelia (Deeds et al., 2006; Hernández-Becerril et al., 2000). A study by Kempton et al. (2002) reported that blooms of *K. veneficum* had caused fish kills in the hybrid striped bass aquaculture pond in Chesapeake Bay (Kempton et al., 2002).

*Dinophysis acuminata* Claparède and Lachmann, 1859

Cell is oval or elliptical shape and rounded from the posterior. Cell has a high cingulum with list giving a small cap-like epitheca and a large hypotheca. The theca surface is covered with pores. The sulcus list extends to half the body length. There are lesser and smaller pores at the megacytic zone that looks smoother compared to the pores in the middle of the hypotheca (Fig. 3A). The left sulcal list is well developed and extends beyond the midpoint of the cell and is of equal depth.

Toxicity/ harmful effect

This species has been reported to produce okadaic acid (Cembella & Therriault, 1989; Lee et al., 1989) and associated with DSP cases (Kat, 1985).

*Dinophysis caudata* Saville-Kent, 1881

The cell is laterally compressed with a small cap-like epitheca and a very large hypotheca (Fig. 3B). Cells are around 81-91 µm in length and 45-65 µm in dorso-ventral width (at base of LSL). The cell’s dorsal side curve gradually to the anterior part of the hypotheca. There is a large and long projection at the hypotheca towards the posterior end. The cingulum is narrow with two well-developed lists. The anterior cingular list is wider than the posterior cingular list. Both cingular lists are supported by ribs and form a funnel shape which hides the epitheca. There is a long and big left sulcal list which contains three ribs and its length is almost half the total length. The thecal plate is heavily aerolated and each contains a pore.

Toxicity/ harmful effect

This species had been reported to produce pectenotoxins (Miles et al., 2004).

*Diplopsalis lenticula* Bergh, 1881

The cells are subspherical to lenticular shape (Fig. 3C), with length of 23-48 µm and diameter of 32-68 µm. The epitheca and hypotheca are divided equally by the circular, median cingulum. Cell surface is smooth with scattered pores. Cingular lists are supported by fine ribs which are broad and projecting laterally. The left sulcal list is bigger than the right sulcal list and curved to the right. The species is non-toxic.

*Gyrodinium spirale* (Bergh, 1881) Kofoid et Swezy, 1921

Cell is large with the length of 100 µm and width of 35.7 µm (Fig. 3D). Cingulum is excavated and narrow. The displacement is more than one-third of the body length (displacement = 42.8 µm). It has a pointed apex curved to the right side. There are body ridges from the apical to antapical. The cell observed is slightly longer compared to the original description with the cell length of 70 – 80 µm and width of 30 µm (Rangel et al., 2004). A characteristic feature of this species is the longitudinal surface ridges.

Toxicity/ harmful effect

*Gyrodinium spirale* was reported to produce PSP toxins and cause fish mortality in Luanda Bay, Angola in September 2002 (Rangel et al., 2004).

*Karenia mikimotoi* Miyake et Kominami ex Oda, 1935

Cell is without thecal plates. Cell is small, broadly oval to almost round, slightly longer
Table 2  Morphological comparison of species in the genus of *Karlodinium*. Morphological characteristics of each species were compiled from literatures (Bergholtz et al., 2006; Daughjerg, 2000; De Salas et al., 2005; De Salas et al., 2008; Siano et al., 2009).

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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Elongated and ovoid</td>
<td>ovoid</td>
<td>ovoid</td>
<td>Small &amp; ellipsoidal</td>
<td>Pentagonal outline</td>
<td>Biconical to pentagonal</td>
<td>ellipsoidal</td>
<td>Broadly elliptical, circular cross-section</td>
<td>Ovoid, small</td>
</tr>
<tr>
<td>Length (μm)</td>
<td>15-24</td>
<td>12-22</td>
<td>19-26</td>
<td>11-18</td>
<td>19-29</td>
<td>13-21</td>
<td>18-25</td>
<td>8-18</td>
<td>7-18</td>
</tr>
<tr>
<td>Width (μm)</td>
<td>10-14</td>
<td>8-18</td>
<td>16-22</td>
<td>8-14</td>
<td>15-25</td>
<td>11-17</td>
<td>13-19</td>
<td>8-14</td>
<td>7-14</td>
</tr>
<tr>
<td>Apical groove</td>
<td>very long, extending through most of the ventral side and halfway down the dorsal side of the epicone</td>
<td>Extends above sulcal extension on ventral side of cell. Slightly curve, bypasses apex. Extends one-forth the length of the epicone on dorsal side</td>
<td>Apical groove short and straight, extending only briefly onto the dorsal side of the epicone</td>
<td>very short and linear, extending less than halfway down the ventral epicone and very briefly down the dorsal side</td>
<td>short, covering 1/3 of the ventral epicone and 1/4 of the dorsal side</td>
<td>medium length, originating above the anterior end of the sulcus and extending 1/4 of the way down the dorsal epicone</td>
<td>linear, originating parallel to the level of the apical margin of the cingulum and extending to halfway down the dorsal epicone</td>
<td>Straight descending one-seventh down the dorsal epicone</td>
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</tr>
<tr>
<td>Ventral pore</td>
<td>Pore located high on ventral side</td>
<td>Elongate ventral pore on left side of epicone</td>
<td>Left of sulcal extension</td>
<td>Absent or inconspicuous</td>
<td>Large, approx halfway between the anterior sulcal termination and beginning of apical groove</td>
<td>A thin, long slit located well to the left of the sulcal region.</td>
<td>Anterior pore at the junction of the left side of the girdle and sulus, posterior pore at the junction of right side girdle and sulcus, deeply impressed, narrower on the ventral side</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cingulum</td>
<td>Shallow and wide</td>
<td>Anterior side delineated sharply from epicone, posterior extending smoothly into hypocone</td>
<td>Deeply excavated</td>
<td>Wide and deeply excavated, with prominent margins</td>
<td>Shallow and wide</td>
<td>Equatorial, deep and narrow</td>
<td>Shallow and wide</td>
<td>Two girdle widths</td>
<td></td>
</tr>
<tr>
<td>displacement</td>
<td>1/3-1/2 of cell length</td>
<td>2 cingulum width, 1/3 of cell length</td>
<td>1/4 of cell length</td>
<td>1/3 of cell length</td>
<td>1/4 of cell length</td>
<td>1/4 of the cell length</td>
<td>2 girdle widths</td>
<td>Two girdle widths</td>
<td></td>
</tr>
</tbody>
</table>
(19.84 μm) than wide (18.31 μm) with a characteristic long and straight apical groove to the right of the sulcal axis (Fig. 3E). Epicone is broadly conical and smaller than the hypocone. Hypocone is notched by the widening sulcus at the antapex resulting in a lobed posterior. The wide and deeply excavated cingulum is pre-median, and is displaced in a descending spiral about 2 times the cingulum width.

**Toxicity/ harmful effect**

The species was reported to cause massive killing of fish and shellfish. An ichthyotoxic compound was associated with the species (Silke et al., 2005).

**Neoceratium furca** (Ehrenberg) Fernando Gómez, David Moreira, Purificación López-García.

**Synonyms**


**Diagnosis**

Cell is large with two unequal parallel hypotheca horns. The right horn is shorter than the left one. Epitheca has an anterior horn that looks like a funnel. Thecal plates are thick and have linear markings (Fig. 3F). Cell is 130 μm in length and 34 μm in width which is in the range of type specimens (length is between 100 – 250 μm and width of 30 – 50 μm) (Steidinger & Tangen, 1997). The cell is similar to the original description of *Ceratium furca* (Claparède & Lachmann, 1859).

**Toxicity/ harmful effect**

This species had been reported to cause fish and invertebrate kills (Glibert et al., 2002).

**Prorocentrum gracile** Schütt, 1895

Cell is elongated almost 3 times as longer (64 μm) than wide (22 μm). The shape is pyriform with a pointed posterior end. An anterior spine around 8.3 μm is present at the periflagellar area (Fig. 3G).

Identification of *P. gracile* and *P. micans* can be confusing due to the similar trichocyst pore pattern (Steidinger & Tangen, 1997) and the similar arrangement of the apical spine (Toriumi, 1980). *P. gracile* is much longer with length: width ratio of 2 while *P. micans* has a length: width ratio of <2. Another diagnostic character is the stronger and longer apical spine compare to *P. micans* which has broader and shorter apical spine. This species is non-toxic.

**Prorocentrum micans** Ehrenberg, 1834

Cells are medium-sized of tear-drop to heart shaped when observed from the valve view. The cell is rounded at the anterior end, broadest at the middle and pointed at the posterior end (Fig. 3H). There is a short anterior spine. The surface is rugose with many trichocyst pores. The intercalary band is big and smooth. Cell dimension is 32.7 μm long and 21.36 μm wide, with the length of spine 3.18 μm (Fig. 3H).

The species was distinguished from others in the genus by the broader and shorter anterior spine. The cell is broadest in the middle with the length: width ratio of less than 2.

**Toxicity/ harmful effect**

This species is a non-toxic but bloom forming species (Pybus, 1990). It may release diatom growth inhibition substance (Uchida, 1977).
**Protoperidinium marukawai** (Abé) Balech, 1974

Cells are round and compressed apical-antapically (Fig. 3I). Cingulum with strong ribs observed. Sulcus is short, left sulcal list is larger compares to the right sulcal list. Cell surface is smooth and thecal plates are easily distinguished. Cells are 30.85 μm in transdiameter (n = 2). The species is non-toxic.

**Scrippsiella trochoidea** (Stein) Loeblich III, 1976

Cell has cone shape epitheca with a short apical convex and rounded hypotheca (Fig. 3J). Cell is small with the length of 22-25 μm and width of 18-21 μm (n = 2). Cell surface is smooth.

**Toxicity/harmful effect**

This species had been associated with fish kills (Lu & Hodgkiss, 2004).

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**CONCLUSIONS**

Among the dinoflagellates observed in the Tebrau Strait, seven are harmful or potentially harmful which were claimed to cause HAB events. However, no incidence of HABs was reported from the area thus far. This study recorded for the first time the occurrence of *Karlodinium micrum*, a fish-killing unarmoured dinoflagellate in Malaysian waters. The occurrence of this ichthotoxin producer might affect the mariculture industry in the strait. The presence of this and others potentially harmful dinoflagellate species in the strait should be taken into consideration by the related authorities in future expansion of aquaculture industry in the strait.
ACKNOWLEDGEMENTS

This study was supported by the Malaysian Government through ScienceFund and FRGS to CP Leaw and PT Lim.

REFERENCES


