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SHORT COMMUNICATION

Notes on the Mass Occurrence of the Ciliate *Mesodinium rubrum* (non-toxic red tide) in Malacca River, Malaysia

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Malacca is a famous tourist spot in Malaysia which have been listed as the World Heritage site by the UNESCO in 2008 (UNESCO). The Malacca Tourism Board conducts cruising and sight seeing activities along the Malacca River. Hundreds of wild marine and freshwater fishes (catfish, pomfret, mullet and scat) were reported floating and dead in Malacca River, Malaysia on 22 April 2014 (Berita Harian 2014). Environmental Board of Malacca authority had analyzed the water quality and no harmful chemicals were detected that may have caused the fish kill (Berita Harian 2014). Nonetheless, the authority claimed that the amount of dissolved oxygen was significantly low (Berita Harian 2014). Interviews with the local tourist guide and fishermen reported that the incidence has been re-occurring several times yearly. Hence, preliminary surveillance of plankton sample from the site was conducted for presence of nuisance creating organism. Observation under microscope revealed high relative abundance of the ciliate *Mesodinium rubrum* (syn: *Myrionecta rubr*a). *Mesodinium rubrum* is distributed worldwide and forms non-toxic red tide when the condition is permissible (Cloern *et al.* 1994; Whites *et al.* 2011; Yih *et al.* 2013). High abundance of these ciliates was suspected to have caused the decreased of dissolved oxygen that lead to the fish kill. Blooms of *M. rubrum* has not been reported elsewhere in Malaysia, as dinoflagellate-related blooms were more common (e.g. Roy 1977; Ting & Wong 1989; Usup *et al.* 2002). This article is suggesting for periodic water quality and phytoplankton monitoring in Malacca River to avoid revenue loss for local fishermen and deteriorate aesthetic value for tourism activity.

Plankton sample was collected from the Malacca River (N2° 12' 14.80" E102° 12' 05.17") water surface (less than 10 meters) during the fish kill event using 20 mm plankton net. The salinity was determined using hand refractometer (Atago, Japan). Samples were preserved with Lugol's solution and observed directly under 50× dissecting microscope (Nikon SMZ645, Japan). The morphology of *M. rubrum* was further examined using epifluorescence microscope (Olympus U-LH100-3, Japan). Cell micrographs were captured using built in camera (Olympus U -TV1x, Japan) and measured with analysis LS Professional software by Olympus. References used of morphology identification were based on Garcia-Ceutos *et al.* (2012).

Malacca River upstream exhibits a distinct odor of hydrogen sulfide (H_2S) (personal observation). The salinity at the river mouth was 8.0 PSU and brownish patches displayed in the water column. The water quality was affected by the extensive development projects along the river bank. Samples observed under light microscopy showing relatively high abundance of zooplankton and non-toxic red-tide forming ciliate *M. rubrum*. Other bigger ciliates and rotifers were also present in high density which is apparently grazing on the *M. rubrum,* as can be seen directly through their transparent gut. Under light microscope, the *M. rubrum* cell is reddish brown with many big chloroplasts (Figure 1A, B, white arrow). *Mesodinium rubrum* (Lohmann) Jankowski body consists of two hemispherical portions with long stiff cilia at the pre-equatorial girdle (Figure 1A, red arrows). The cells were 16-27 mm long and 13-18 mm (n = 47) in width, slightly smaller than morphology

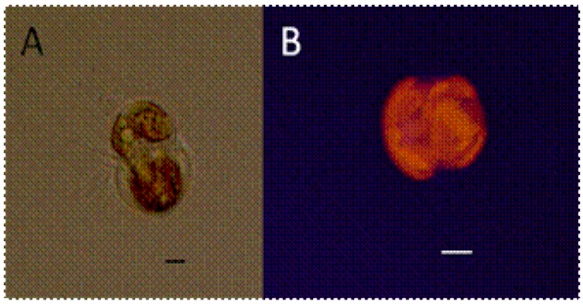

Figure 1. Light microscopy of *M. rubrum* (A-B). A: Tentacles are present at the oral end (black arrow), stiff cilia at the girdle (red arrows) and chloroplasts (white arrow), scale: 10 mm. B: Epifluorescence image of the chloroplasts (white arrow), scale: 10 mm.

described by (Garcia-Ceutos *et al.* 2012). It has rounded oral end and its aboral part is conical. Tentacles were observed at the oral end (Figure 1A, black arrow). The cell floats in water and movement is by rapid jumping and swimming. Their photosynthetic ability is the result of retained plastids from ingested cyptomonads (Johnson *et al.* 2007). *Mesodinium rubrum* in this study fall within the size range of *M. chameleon* (Garcia-Ceutos *et al.* 2012) but differs in terms of pigment and number of chloroplasts. The lifestyle of the latter is benthic as opposed to the planktonic *M. rubrum*. *Mesodinium major* on the other hand share similar color, shape and number of chloroplasts as *M. rubrum* but *M. major* has greater body length and width i.e. 40-55 mm in length and 35 -50 mm wide (Garcia-Ceutos *et al.* 2012). *Mesodinium pupula* and *M. pulex* were not included in comparison as both species are heterotrophic and do not possess any chloroplasts (Garcia-Ceutos *et al.* 2012).

Fish-kills in Malacca River is not an isolated case as several incidents has been reported since February 2014 in east and west Malaysia including Tanjung Kupang, Johor, and Likas, Sabah (unpublished data). In Tanjung Kupang, Johor, the HAB outbreak was caused by the first occurrence of toxic naked dinoflagellate *Karlodinium australe* (Lim *et al.* 2014) and the non-toxic thecate dinoflagellate *Scrippsiella trochoidea* which lasted for several weeks (personal observation). Soon after the report, the recurring bloom of dinoflagellate *Noctiluca* sp., *Pyrodinium* sp. and *Gonyaulax* sp. took place in Sabah coastal waters (personal observation). Frequent HAB blooms were preceded by the heat wave phenomenon which in general affected the water level in rivers, raised temperature and shift in ecosystem equilibrium. During dry period, the dam at Malacca River is closed to maintain water level for tourist regular cruising activities. A longer than usual period of dry season before the fish kill incidence was followed by heavy rainfall. Nutrient run-offs from upstream particularly the river bank project sites that concentrated at the blocked estuary outlet may have fuel the proliferation of existing *M. rubrum*. Red tides caused by this ciliate in San Francisco Bay, California (Cloern *et al.* 1994) occurred in about the same condition at the Melaka River. Anoxic water condition is not a limiting factor for *M. rubrum* (Crawford & Lindholm 1997).

Frequent fish kill in Malacca River is a concern as *M. rubrum* may have been the causing organism. Information on factors affecting the temporal abundance and spatial distribution of *M. rubrum* in Malacca River is important in order to foresee potential excessive oxygen consumption pertaining from increased grazing activities. *M. rubrum* is often left out in phytoplankton counts due to confusion in their trophic grouping, thus lead to potential underestimation Crawford (1989). Small and fragile cell of *M. rubrum* is also susceptible to passing through net mesh and may disintegrate totally during handling (Lindholm 1985) as cited in Crawford (1989). Series of bloom events in Korea were documented in Yih *et al.* (2013). It is also worth to note that the diarrhetic shellfish poisoning (DSP) causing dinoflagellate *Dinophysis fortii* may co-exist as it was reported to predate on *M. rubrum* as well (Nagai *et al.* 2008). This may indicate the Malacca River is a potential HAB site.

Although the presence of *M. rubrum* in Malaysian water is common, red tide occurrence attributed to this ciliate have not been reported. Seasonal and spatial distribution of *M. rubrum* in Malacca River deserved better attention as the site has been receiving concerns from tourists about the sighting of floating dead fish during their visits. Therefore, adequate monitoring and sampling of watermass character (e.g., nutrients,

temperature, and salinity) that may stimulate mass occurence is crucial to better understand the bloom dynamics of *M. rubrum* in Malacca River.

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