

Bloom of a freshwater green alga *Botryococcus braunii* (Botryococcaceae, Trebouxiophyceae) and the associated mass fish mortality in a man-made lake, Sarawak, Malaysia

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Received 4 August 2020; Accepted 15 December 2020 Responsible Editor: Kazumi Matsuoka

doi: 10.3800/pbr.16.59

Abstract: Mass mortality of fish (~8,500 fishes), mainly *Oreochromis placidus*, was noted in a man-made lake located at Kuching, Sarawak (Malaysia). A field investigation was conducted to collect water samples and fishes. Patches of discoloration in brick red were observed in the lake and clear oil layer was found on the surface of the water. Microscopic observation and enumeration of the water samples showed that the plankton composition was dominated by a green algal species *Botryococcus* sp., with the colony densities ranging 1.2×10^3 – 7.4×10^6 colonies L⁻¹. Detailed morphological assessment by light microscopy revealed the dominant species as *Botryococcus braunii* Kützing. Molecular characterization using an rDNA marker further supported the species identity as *B. braunii* in the L race. Fish gill observation showed that cells of *B. braunii* and the oily substances were found in the dead fish gills. The race-L *B. braunii* bloom was reported, for the first time, to be associated with a fish kill event in a freshwater lake in Malaysia and confirmed the species as one of the algal types causing harmful effects to the environment.

Key words: Black tilapia, *Botryococcus braunii*, fish kill, harmful algal bloom, hydrocarbon

Introduction

The green alga *Botryococcus braunii* Kützing is widely distributed in freshwater system worldwide. This species has been considered a slow-growing species (Banerjee et al. 2002, Melis 2013) but the species has also been reported to form massive blooms (Mitchell 1975, Labib et al. 2012). *Botryococcus braunii* has attracted the world's attention in the last decade due to its ability to produce hydrocarbon compounds (Metzger et al. 1991, Banerjee et al. 2002).

The alga *Botryococcus* was first described by Kützing in 1849, with *B. braunii* as the type species (Kützing 1849). Up to this date, there are a total of 14 taxonomically accepted species in the genus worldwide, including one

fossil species, *B. balkachicus* Zalessky (Guiry and Guiry 2020). Komárek & Marvan (1992) described the species of *Botryococcus*, including five new species, from environmental populations (Komárek and Marvan 1992); however, species delineation in this genus was not clearly unravelled, as the morphology of single isolates could resemble several described species depending on the growth stage (Plain et al. 1993). Notwithstanding this confusion, the type species *B. braunii* has often referred to in many studies related to this green alga (Senousy et al. 2004).

As morphological differences to delimit the species of *Botryococcus* are debatable, several studies have adopted molecular techniques to classify the species of *Botryococcus* and to infer the relationships among the species (Kawachi et al. 2012, Hegedüs et al. 2015). The gene marker in the region of the small subunit ribosomal RNA gene (SSU rDNA) has been commonly used to infer phylogenetic relationships between the species of *Botryococcus* (Sawayama

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