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Spectral fluorometric characterization of phytoplankton types in the tropical coastal waters of Singapore

Victor S. Kuwahara [a,*], Sandric Chee Yew Leong [b]

[a] Graduate School of Engineering, Soka University, 1-236 Tangi-machi, Hachioji-shi, Tokyo 192-8577, Japan
[b] Tropical Marine Science Institute, National University of Singapore, 18 Kent Ridge Rd., Singapore 119227, Singapore

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ABSTRACT

Developing rapid in situ optical detection techniques for discriminating the potential for harmful algal blooms is an ongoing goal for scientists around the world. The utility of a profiling 9-wavelength Multi-Exciter fluorometer in concert with other bio-optical measurements was examined using isolate cultures in the laboratory and a field survey in the East Johor Strait, Singapore. Background bio-optical measurements of field isolate cultures confirmed the difficulty in discerning between diatoms and dinoflagellates, although raphidophytes and the dinoflagellate *Karlodinium* sp. did show unique bio-optical signatures. Significant relationships were found between the Multi-Exciter 470 nm signal and traditional chlorophyll *a* pigment measurements both in the laboratory and field ($p < 0.001$). Chlorophyll *a* specific-absorbance and -fluorescence relationships showed the potential for identifying phytoplankton groups based on the slope of the relationship between them. The 6-station field survey in the East Johor Strait displayed relatively shallow depths (5.06 ± 0.81 m), warm temperatures (29.88 ± 0.26 °C), low salinity (25.81 ± 1.48) and high phytoplankton biomass (17.6 ± 11.7 μg Chl-*a* L^{-1}). Absorbance and Multi-Exciter fluorometric measurements from the field did not reveal similar optical signatures from studied laboratory isolates, although the 435 nm:570 nm and 470 nm:525 nm fluorometric ratios displayed distinct profile responses. However, these wavelength-to-wavelength ratio signatures were not identified, and further investigation is required. The results suggest that in situ fluorometry coupled with phytoplankton absorption properties could assist in defining bloom parameters and enhance our ability in determining and detecting pre-bloom conditions.

1. Introduction

Problems associated with episodic blooms of phytoplankton and cyanobacteria particularly within natural and aquaculture systems worldwide appear to be increasing in severity and extent for reasons that are not completely understood (Anderson et al., 2008, 2012; Gilbert et al., 2005). Harmful algal blooms (HABs) are the most problematic among planktonic blooms due to their severe economic, ecological and human health impacts (Landsberg et al., 2005). The increases of episodic HABs in tropical estuaries and coastal areas such as Singapore are also an increasing and prevalent concern (Gin et al., 2000, 2006; Holmes et al., 2002; Kok and Leong, 2012; Tang et al., 2007). For example, a significant HAB causing massive fish kills and economic losses was reported to have occurred in the East Johor Strait in Dec 2009 (Quek and Lim, 2010), and more recently in Feb 2014 (Lee, 2014). However, the trigger, identification and monitoring of these episodic tropical HABs remain elusive.

Historically, the inability to effectively forecast or hindcast HABs can be traced to the limitations of adequate monitoring methodologies and technologies. Although remote sensing capabilities are evolving rapidly, current field efforts typically rely upon traditional microscopy, flow cytometry, molecular analysis or pigment signatures which are costly, laborious, highly specialized (leading to variable data) and limited in synoptic spatial and temporal coverage (e.g. Babin et al., 2008; Jefferey et al., 2011; Moisan et al., 2012). From this perspective there remains a high demand to develop alternative methods to simplify and quantify phytoplankton communities for targeting and monitoring purposes, particularly in situ, using innovative techniques (Anderson et al., 2012; Leong et al., 2012; Millie et al., 2002).

Bio-optical measurements using various analytical techniques continue to offer new ways to characterize general phytoplankton biomass and composition. Spectral absorption properties from commercially available in situ sensors and traditional quantitative filter-pad techniques (QFTs), and spectral excitation/emission fluorescence have emerged as potential first-order screening methods towards more accurate and robust sampling strategies (Bidigare et al., 1989; Chase et al., 2014; Craig et al., 2012; Houliez et al., 2012; Johnsen et al., 1994, 1997; Millie et al., 2002). These methodologies are based on the understanding that phylogenetic groups and species have distinct pigment

* Corresponding author.
E-mail addresses: victor@soka.ac.jp (V.S. Kuwahara), tmslcy@nus.edu.sg (S.C.Y. Leong).

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compositions and concentrations, and can be quantified and grouped based on these signature characteristics in mixed populations for a given water mass. Although the spectral absorption properties hold promise in delineating general phytoplankton characteristics, the solution to quickly identify functional groups remains challenging due to overlapping pigment absorbance signatures, complications associated with cell size and fitness, and overall light acclimation conditions (Cullen et al., 1997; Johnsen et al., 1994; Kirkpatrick et al., 2000; Lorenzen, 1966). The spectral absorption method, however, still remains complimentary and facilitative to remote sensing and light microscopy analysis when broad synoptic spatial and temporal scales are processed.

Multi-spectral excitation/emission fluorescence also offers an alternative method to identifying phytoplankton composition by utilizing selective excitation of differing antenna and accessory pigments between taxonomic groups of algae (Houliez et al., 2012; MacIntyre et al., 2010). In other words, the spectral fluorescence response (emission) of phytoplankton from wavelength-specific light emitting diodes (excitation) depends on their photosynthetic pigment composition, or ratios of various accessory pigments to chlorophyll *a* concentrations, similar to the aforementioned absorbance signatures. However, this method has similar limitations to the absorption method in that targeted species must be sufficiently calibrated with pure culture samples, and overlapping pigment signatures among phytoplankton groups complicate analysis (Houliez et al., 2012; Millie et al., 2002). Although both spectral absorption and multi-spectral fluorometric measurements offer insight towards efficient and rapid detection of targeted phytoplankton blooms, there are still a limited number of in situ studies and even fewer conducted in tropical estuaries and coastal areas. The purposes of this study were to (1) reveal specific bio-optical characteristics of the phytoplankton community in the East Johor Strait and (2) test the field applicability of a new, commercially available in situ multi-excitation fluorometer in the field of interest. The ultimate goal of this study was to develop the possibility of using a coupled pre-evaluation bio-optical approach to HAB identification in highly turbid waters, to determine whether a more robust and detailed measurement initiative should be employed for monitoring in the coastal waters of Singapore.

2. Material and methods

2.1. Study area

Studies were conducted from December 19–22, 2012 at two locations as follows: (1) in vivo laboratory work with the HAB Group at the Tropical Marine Science Institute (TMSI), National University of Singapore, and (2) in situ field work in the East Johor Strait, Singapore (Fig. 1). The tropical coastal waters of Singapore are bordered by the Johor Strait and Peninsular Malaysia to the north, the Java Sea to the south, South China Sea to the east, and the Malacca Straight to the west. The hydrography of the region is dominated by the semi-diurnal tides and by the northeast (Dec–Mar) and southwest (Jun–Sep) monsoons (Pang and Tkalich, 2003). The shallow (20 m) Johor Strait is characterized by large terrestrial and freshwater inputs that stagnate, resulting in significant eutrophication and high phytoplankton biomass (Leong et al., 2012; Mulia et al., 2013).

2.2. Isolated cultures

Laboratory work was conducted using six locally isolated species of phytoplankton commonly found in the coastal waters of Singapore to include the following: *Chaetoceros* sp., *Skeletonema* sp., *Karlodinium* sp., *Scrippsiella* sp., *Heterosigma* sp., and *Chattonella* sp. Each species was isolated using capillary micropipette under an inverted light microscope (Olympus CK-40). All cultures were grown in ambient seawater for a few days and then transferred into f/100 media made from aged seawater collected from St. John's Island, Singapore. When cells were observed to be dividing and growing well, all cultures were then maintained in f/2 media at 30 PSU salinity, 25 °C, and 100 μmol m^{-2} s^{-1} of cool fluorescent light on a 12 h light:12 h dark cycle. However,

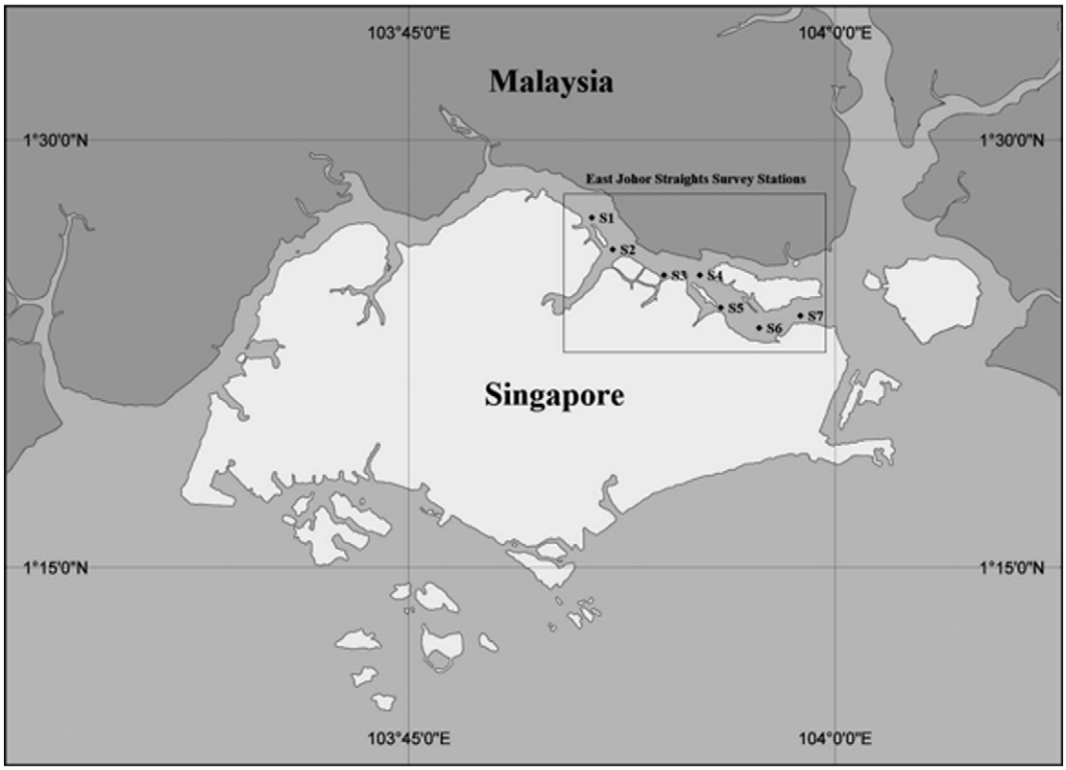

Fig. 1. Map of the study area showing survey stations S1–S7 on 21-Dec-12 in the East Johor Straight, Singapore. Note: S1 was not surveyed during this study.