A novel fluorometric method of cell abundance estimation on harmful algae (*Karenina brevis*), the concept and lab experiment

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Abstract

Harmful algal blooms (HABs) are common and significant worldwide problems, causing mass mortality of marine organisms, especially fish in aquaculture cages or shellfish hanging from rafts, and shellfish contamination can sometimes lead to human fatalities. In situ monitoring of cell abundance of harmful algae is an urgent necessity to reduce the damages caused by HABs. A new method was developed that measures a fluorescence ratio (FSI, Fluorescence spectral Shift Index) between two wavelengths (670 nm and 690 nm) to characterize each plankton species' unique fluorescence characteristics. It was found that some 'harmful' algae (such as *K. mikimotoi*, *K. brevis* etc.) have higher FSI (usually > 2.0) than other algae (such as diatoms, usually with FSI < 1.9). Based on the FSI concept, a sensor called HAI (Harmful Algae Indication) was developed and used in lab experiments to test this method on *K. brevis* and *K. selliformis*. The method provides not only dominance rates of 'harmful' algae in natural phytoplankton communities, it may also estimate cell abundances once fluorescence intensities from a monoculture of a species is tested at various concentrations. The comparison with microscopic HAB cell counts showed consistent results with the HAI sensor measurements. The minimal level of cell density estimation by the HAI sensor for detecting 'harmful' algae from other species in natural phytoplankton communities is less than 20 cells/ml. This can provide earlier detection of HABs and allow actions to be made that can prevent any further damages.

Introduction

The measurement of chlorophyll a in vivo fluorescence was introduced to oceanography by Lorenzen (1966). The fluorescence intensity emitted by phytoplankton is related to the algal biomass. A light excitation induced fluorescence instrument (Fluorometer) has been widely used for quantification of phytoplankton in freshwater and marine environments. Although using a fluorescence generated by a single wavelength excitation in the blue region of the light spectrum has been a mature technology to assess total phytoplankton biomass, it is not able to provide algal classification as done by microscope counts, thus we cannot understand the algal species composition in the water.

Beutler *et al.* (2002) used a submersible instrument which excited chlorophyll fluorescence with five distinct wavelengths using light emitting diodes (LED) to identify spectral groups of microalgae. This produced some consistent results for coarse taxonomic groups only when the assumptions for setting up this technology are met. Algal classification down to species is difficult for multi-wavelength spectra methods. Instead of using an approximation of spectrum with limited wavelength band, Yoshida *et al*. (2021) reported a new method by considering a fluorescence ratio (FSI, Fluorescence spectral Shift Index) between two wavelengths (670nm and 690nm) to characterize each plankton unique fluorescence characters. *Karenia mikimotoi* (Dinophyceae) and *Chattonella marina* var. *antiqua* (Raphidophyceae) have been monitored daily for long periods of time as the main species contributing to HAB disasters to aquaculture and fisheries in Japan, mostly by sampling water and microscope counts. In order to find an alternative monitoring technology to microscopic cell counting, the FSI method was developed and tested with *Karenia mikimotoi* and *Chattonella marina var. antiqua*. Lab experiments to verify FSI technology showed that some toxic species (including *K. mikimotoi* and *C. marina* var. *antiqua*) have obvious higher shifts in spectrum than those of diatoms, such as *Skeletonema* sp. Field experiments together with comparison of microscopic counts demonstrated their consistent relationship. The FSI values higher than a threshold (1.95, taken as threshold) are usually accompanied with harmful algae appearance. However, FSI estimation only provides a criterion when the specific 'harmful' algae appear in the water. A new method to estimate cell abundance of specific 'harmful' algae based on FSI measurements was developed by JFE Advantech (US2022/0163451A1). The new method is not only able to provide an estimate of the cell abundance of a specific algae, but it also calculates its ratio in the algae population composition.

In the Gulf of Mexico, *Karenia brevis* blooms have occurred almost annually. In this study, we conducted a lab experiment on K. brevis with this new method to prove its validity before *in-situ* application.

Methodology

FSI (Fluorescence spectral Shift Index) method suggested by Yoshida *et al.* (2021)

$$\mathrm{FSI} = \frac{\textit{Fluorescence intensity emitted at 690 nm}}{\textit{Fluorescence intensity emitted at 670 nm}} \quad (1)$$

Dominance rate (R_H) by harmful algae based on fluorescence,

$$R_H = \frac{F_H}{F_{all}} \quad (2)$$

or

$$R_H = \frac{F_{all} - F_N}{F_{all}} \quad (3)$$

where, the total fluorescence of sample (F_{all}) is sum of fluoresece of a 'harmful' species(F_H) and fluoresence of all other 'Non-harmful' species (F_N) .

Dominance rate estimated by FSI

Considering a unit volume of water, where multiple species of algae exist, the fluorescence from a species (i) over a wavelength band λ is

$$F_i(\lambda) = f_i(\lambda) \cdot a_i \quad (4)$$

f_i is the fluorescence intensity emitted from individual cell of species (i), a_i is total cell numbers of species (i).

The total fluorescence emitted by all species of algae ($\mathrm{i} = 1, 2, 3 \ldots$) in the unit volume of water, $F(\lambda)$, will be,

$$F(\lambda) = \sum_i f_i(\lambda) \cdot a_i\,, \quad \mathrm{i} = 1, 2, 3 \ldots \quad (5)$$

If we consider fluorescence emitted only from wavelength of λ_{670} and λ_{690}, similarly we have,

$$F(\lambda_{670}) = \sum_i f_i(\lambda_{670}) \cdot a_i\,, \quad \mathrm{i} = 1, 2, 3 \ldots \quad (6)$$

$$F(\lambda_{690}) = \sum_i f_i(\lambda_{690}) \cdot a_i\,, \quad \mathrm{i} = 1, 2, 3 \ldots \quad (7)$$

The FSI concept is to distinguish 'harmful' algae from others. In other words, only two groups are considered i.e. 'Harmful' algae and 'Non-harmful' algae. Hereafter, we refer 'Harmful' algae as 'H' algae and 'Non-harmful' alga as 'N'. Therefore the above formula can be rewritten as,

$$F(\lambda) = f_H(\lambda) \cdot a_H + f_N(\lambda) \cdot a_N \quad (8)$$

$$F(\lambda_{670}) = f_H(\lambda_{670}) \cdot a_H + f_N(\lambda_{670}) \cdot a_N \quad (9)$$

$$F(\lambda_{690}) = f_H(\lambda_{690}) \cdot a_H + f_N(\lambda_{690}) \cdot a_N \quad (10)$$

Then,

$$\mathrm{FSI} = \frac{F(\lambda_{690})}{F(\lambda_{670})} = \frac{f_H(\lambda_{690}) \cdot a_H + f_N(\lambda_{690}) \cdot a_N}{f_H(\lambda_{670}) \cdot a_H + f_N(\lambda_{670}) \cdot a_N} \quad (11)$$

$$\mathrm{FSI}_H = \frac{f_H(\lambda_{690}) \cdot a_H}{f_H(\lambda_{670}) \cdot a_H} \quad (12)$$

$$\mathrm{FSI}_N = \frac{f_N(\lambda_{690}) \cdot a_N}{f_N(\lambda_{670}) \cdot a_N} \quad (13)$$

Based on this, we may deduce a formula to calculate dominance rate by harmful algae from FSI measurement as following,

$$R_H = \frac{FSI - FSI_N}{(1-\alpha) \cdot FSI - (FSI_N - \alpha \cdot FSI_H)} \quad (14)$$

In which, $\alpha = \frac{f_H(\lambda_{670})/f_H(\lambda)}{f_N(\lambda_{670})/f_N(\lambda)}$, is a model parameter. It is empirically detected by lab experiments.

The fluorescence from single cell of *K. brevis*

$$\mathrm{C}_{sd} = \frac{\textit{Fluorescence generated by K. brevis}}{\textit{cell density of K. brevis (by microscope)}} \quad (15)$$

Cell abundance estimation of *K. brevis* (HAD, Harmful Algae Density)

$$HAD = \frac{F(\lambda) \times R_H}{\mathrm{C}_{sd}} \quad (16)$$

Lab experiment and Results

The experiment on K. brevis was conducted in the lab of University of Texas using the HAI sensor designed and manufactured by JFE Advantech Co., Ltd. (Figure 1)

Figure 1, HAI sensor from JFE Advantech Co., Ltd., The detail of sensor specification are described in reference [3]

The marine water (no *K. brevis* existing) from the research pier of University of Texas at Port Aransas along the coast of Gulf of Mexico was used as background solution. An amount of cultured *K. brevis* was also prepared for the test. The experiment procedures are,

a. Measurement of marine water by HAI sensor, the averaging FSI_{marina}= 1.78

b. Adding single species of *K. brevis* in a filtered sea water and measured by HAI sensor, the averaging $FSI_{K.\,brevis}$= 2.42.

c. Adding cultured sample of *K. brevis* into marine water step by step. Total 6 samples with different cell densities of *K. brevis* were prepared, i.e. 6.5 ,19, 40.5, 89.5, 174.5, 292 (cells/ml, confirmed by microscope), and were measured by HAI sensor.

Results:

1. Dominance rate of *K. brevis* in marina water (Fig. 2)

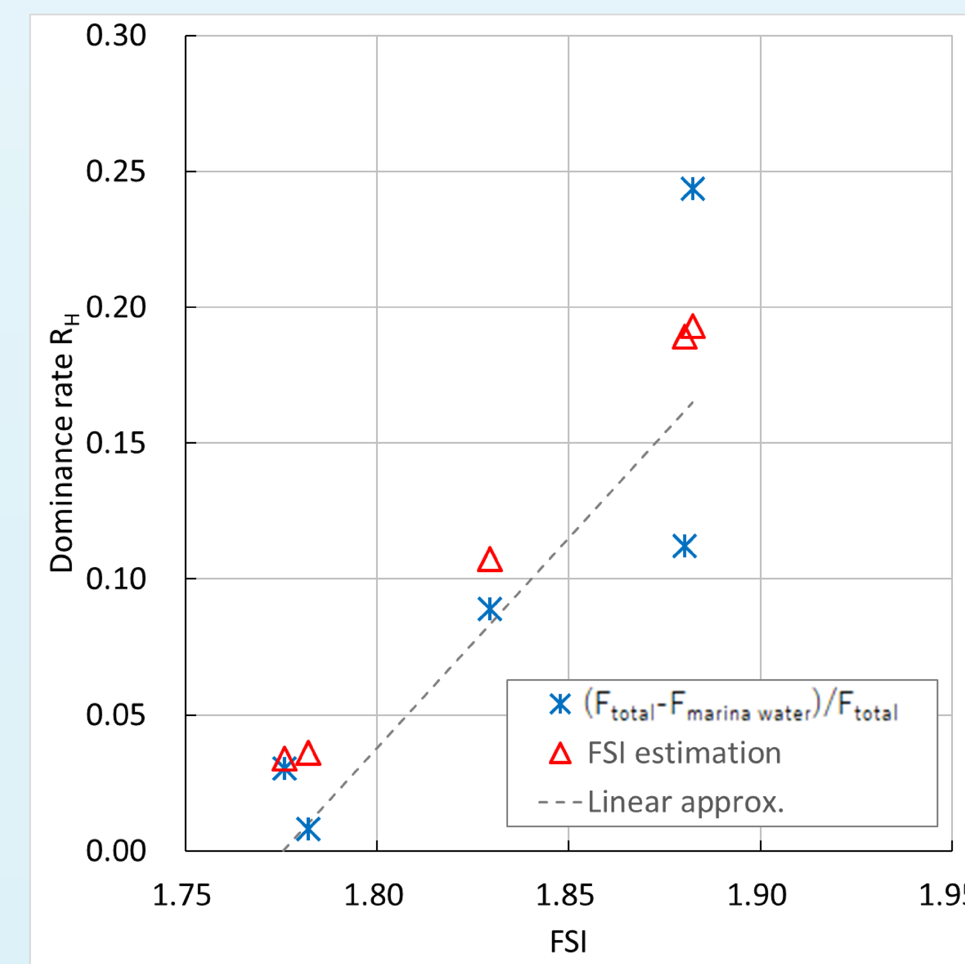

Figure 2, Dominance rate by *K. brevis* in marina water.

The * are the dominance rate calculated by using formula (2) method, assuming the fluorescence from background marina water (No *K. brevis* existing) during whole experiment. △ are the dominance calculated from FSI measurements based on formula of (14)

2. Cell abundance of *K. brevis* in marina water in comparison with microscopic counting (Fig.3)

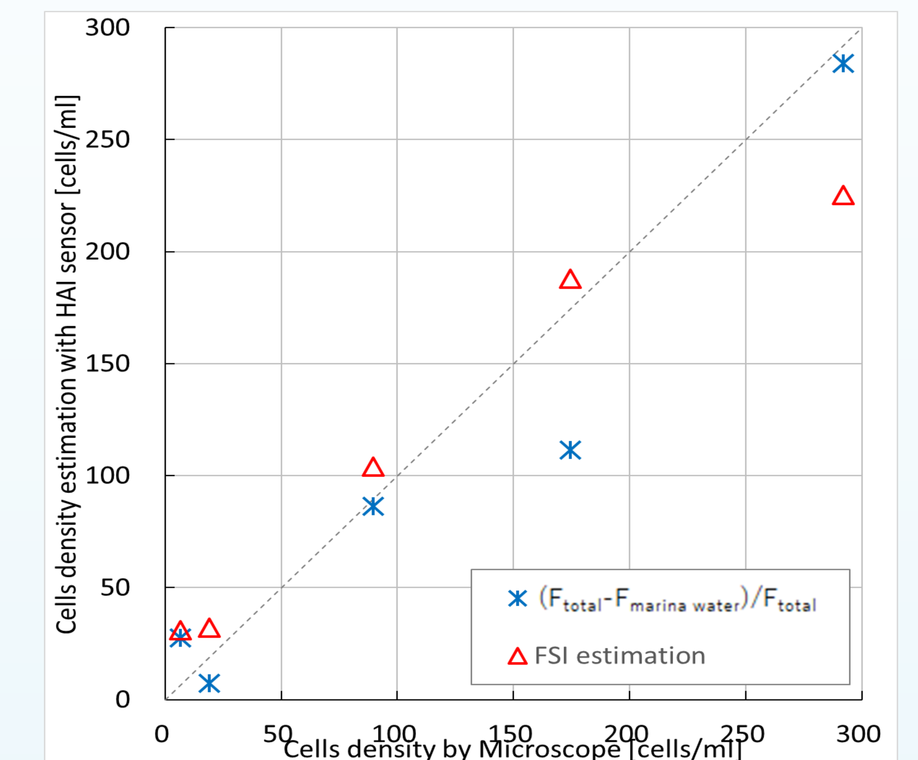

Figure 3, Cell abundance of *K. brevis* estimated by HAI sensor in comparing with microscopic counting.

In this experiment,

$$\mathrm{C}_{sd} = 0.002 \quad ppb\ cells^{-1}\ ml$$

Both the cell abundances based on FSI measurement and formula (2) have consistent results with microscopic counting.

Conclusions

- R_H estimation made by FSI have good consistent results in comparing with microscopic counts and direct fluorescence estimation.
- R_H estimation made by direct measurement of fluorescence is not available in field because it is not able to measure fluorescence contributed ONLY by *K. brevis*.
- FSI method is able to estimate cell density, and is in agreement with microscopic counts. The minimal level of cell density estimation by the HAI sensor for detecting 'harmful' algae from other species in natural plankton communities is less than 20 cells/ml.
- The fluorescence from single *K. brevis* (C_{sd}) is variable (time, location, nutrient, and algae itself), it is necessary to have correction by microscopic count.

Reference

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3. Yoshida, M. *et al.* (2021): A new fluorometer to detect harmful algal bloom species and its application as a long–term HABs monitoring tool. *J. Fac. Agr.*, Kyushu Univ., 66 (1): 37–43. https://doi.org/10.5109/4363549

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