HAI sensor: Harmful Algal Indication sensor and its development and performance as a continuous harmful algal bloom monitoring tool

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Harmful Algal Blooms (HABs) can cause serious damage to fisheries and aquaculture industries. HABs produce a variety of toxins that cause heavy mortality of fish and other marine organisms during their bloom, which in turn can affect these organisms stocks and disrupt business activities (Furuya *et al.* 2018). In Asia, *Karenia mikimotoi* and *Chattonella antiqua* are among the phytoplankton species that have caused severe damage frequently (Imai *et al.*, 2006, 2012, Sakamoto *et al.*, 2020), and because of that, their dynamics have been monitored daily (Sakamoto *et al.*, 2020). However, there is a need for new technologies that would allow enhancing monitoring coverage and efficiency in order to prevent or reduce the HABs economic impact. In this work, we address these issues through the development of a harmful phytoplankton detection sensor (Fig. 1) that can detect the presence of *Karenia mikimotoi* and *Chattonella antiqua* (Fig. 2) by making use of the specificity of the chlorophyll fluorescence spectrum of these two species. In addition, we have assessed the sensor performance during profiling and long-term moorings at sea.

Fig. 1 Harmful Algal Indication sensor (HAI sensor).

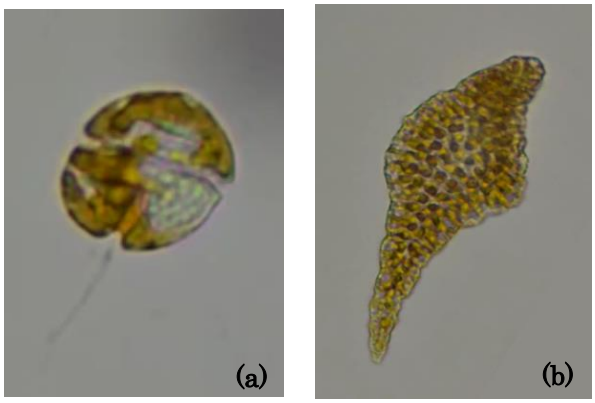

Fig. 2 Micrographs of (a) *Karenia mikimotoi* and (b) *Chattonella antiqua.*

Currently, most of the HAB monitoring is done using discrete water sampling. These samples need to be transferred from the field to a laboratory to have the present phytoplankton species identified and counted using optical and electronic microscopy. However, this procedure is costly, time consuming, and not very accurate as it is highly manual oriented and requires high skilled personnel to identify target species (Yuan *et al.* 2012). Still, frequency and coverage of observations must be increased in order to improve accuracy (particularly between initial development and logarithmic growth periods), which is very difficult at current stage.

We have discovered that *Karenia mikimotoi* and *Chattonella antiqua* have very specific characteristics in their chlorophyll fluorescence spectrum that allows our new sensor to identify their presence and infer about their concentration in situ.

Fluorescence spectral Shift Index (FSI)

We have compared the fluorescence emission spectra of several phytoplankton species obtained from illuminated incubator cultures. The fluorescence spectrum was measured with a spectrofluorometer (F2700, manufactured by Hitachi) and cell suspension was transferred to a glass cuvette and stirred. The spectrofluorometer excitation bandpass was set to 5 nm, and the fluorescence spectrum was measured at an excitation wavelength of 440 nm and a detection wavelength of 600 to 750 nm. In Fig. 3, we show that *Karenia mikimotoi* and *Chattonella antiqua* fluorescence emission spectra is clearly shifted to higher wavelengths when compared to other species. In order to express and quantify such fluorescence spectra shift, we have developed the Fluorescence spectral Shift Index (FSI). FSI is the ratio of fluorescence intensity at 690 nm to that at 670 nm and can be define as follows

$$\mathrm{FSI}=\frac{\text{Fluorescence intensity at 690 nm}}{\text{Fluorescence intensity at 670 nm}} \qquad (1)$$

FSI will be higher in species that have a very clear fluorescence intensity spectra shift, such as (but not limited to) *Karenia mikimotoi* and *Chattonella antiqua* (see Fig. 3).

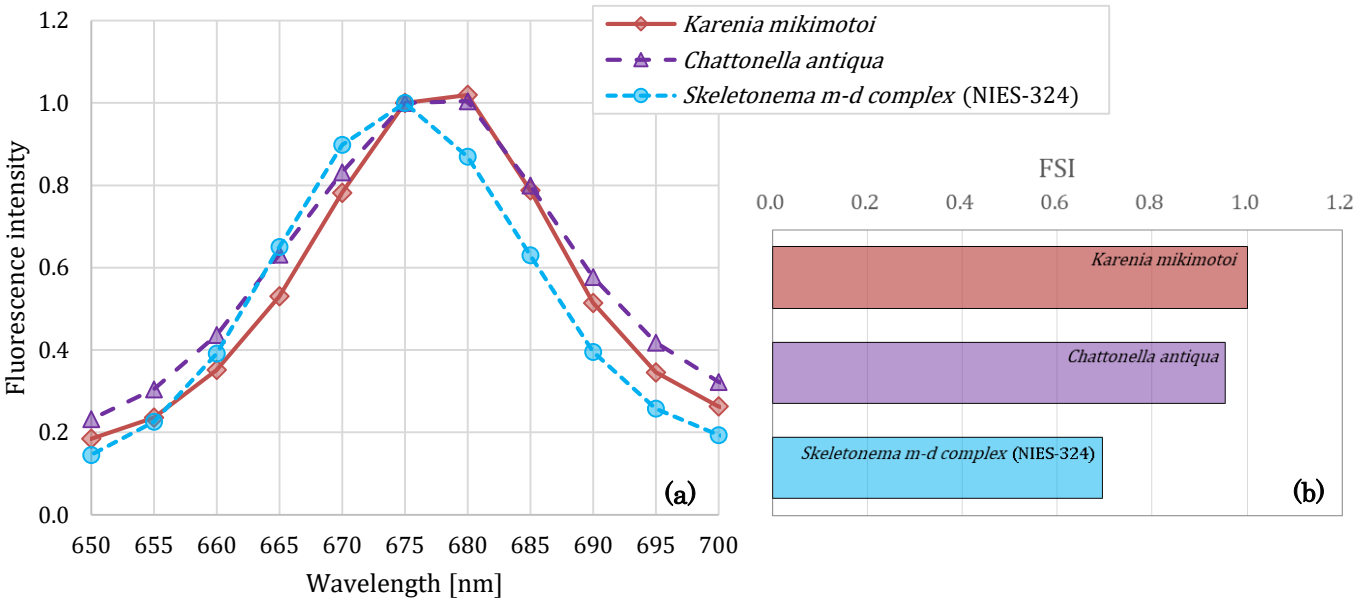

Fig. 3 (a) Normalized fluorescence intensity spectra (with detection wavelength of 675 nm being 1) from *Karenia mikimotoi, Chattonella antiqua* (shown in red solid line and purple dashed line respectively) and *Skeletonema m-d complex* (blue dashed line). (b) FSI results for these three species normalized using *Karenia mikimotoi* FSI results. *Skeletonema m-d complex* was chosen as a representative typical spectrum for several phytoplankton species.

Underwater spectrophotometry

In order to simplify and reduce costs of HABs observations, we have developed an underwater spectrophotometer to be used *in situ* (see Fig. 4). We have deployed this instrument in different Japanese regions since 2014, in which HABs frequently occur, and investigated these events. We have been presenting our findings in different opportunities, such as at conferences from The Oceanographic Society of Japan. These observations lead us to discover *Karenia mikimotoi* and *Chattonella antiqua* specific characteristics that we mentioned above.

Based on the *in situ* data obtained from our underwater spectrophotometer and laboratory experiment data, we have developed a low-cost alternative for HABs monitoring and early warning systems.

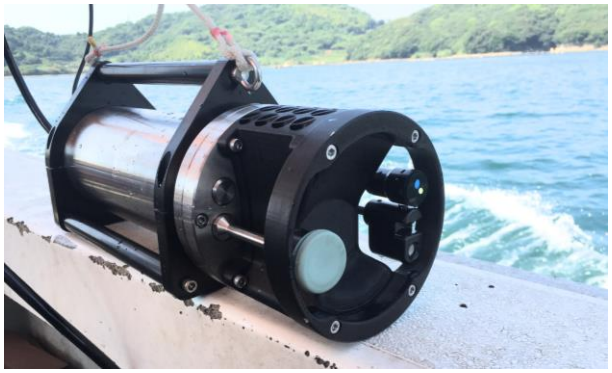

Fig. 4 Underwater spectrophotometer developed by JFE Advantech Co., Ltd. The instrument carries additional temperature, conductivity, chlorophyll-a fluorescence and fast optical dissolved oxygen sensors.

Harmful Algal Indication sensor (HAI sensor)

The HAI sensor was designed to be used in the field that can easily detect the degree of fluorescence spectrum shift due to the presence of Karenia mikimotoi or Chattonella antiqua. Fluorescence intensity spectrum shift estimation and direct evaluation requires analyzing emitted fluorescence using a spectrometer with sufficient wavelength resolution to identify the shift. However, if we use FSI as a proxy for fluorescence spectrum shift, fluorescence will need to be measured in only two wavelengths. This fact allowed us to design a compact and inexpensive device that measures fluorescence intensities using a bandpass optical filter centered at 670 nm and 690 nm (see Fig. 5). The main specifications of the HAI sensor are shown in Table 1.

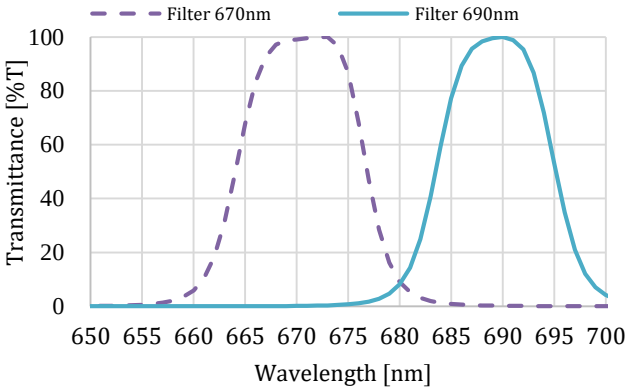

Fig. 5 Bandpass optical filter centered at 670 and 690 nm.

Performance

(a) Laboratory

We have verified the performance of the HAI sensor when estimating FSI using different phytoplankton strain cultures as shown in Fig. 6. Each one of the strains had their FSI estimated in different cell densities. We have confirmed that *Karenia mikimotoi* and *Chattonella antiqua* FSI values are independent from cell densities and we were able to distinguish these two species FSI for concentrations as low as 10 cells ml^{-1}.

(b) Field test

We have performed several tests in Saiki Bay, Oita prefecture, Japan during the years of 2016, 2017, 2018 and 2019. Several profiles were made using the HAI sensor during two HABs formed by *Karenia mikimotoi* in the summer of 2017 and 2018. We have also collected water samples for microscopic identification and cell density estimations during these HAB events. The FSI calculated by HAI sensor was then compared with optical microscope analysis results as shown in Fig. 7a and Fig. 7b. We have established FSI equal to 1.9 as a threshold to determine the presence of *Karenia mikimotoi* in cell densities between 25 to 50 cells ml^{-1}. During one of the HABs events, FSI exceeded the threshold between 4 and 5.5 meters, and again at approximately 9.5 meters depth (see Fig. 7a). The microscopic analysis showed high cell densities of *Karenia mikimotoi* (higher than 100 cells ml^{-1}) at 5, 6 and 10 meters depth (see Fig. 7b), which is consistent to what was observed using the HAI sensor. It is important to highlight that even when other phytoplankton species are present in high concentrations (up to 1000 cells ml^{-1}), FSI only exceed the 1.9 threshold when *Karenia mikimotoi* is present. Thus, FSI variation depends on the presence or absence of *Karenia mikimotoi* (this is also true for *Chattonella antiqua*) regardless of the chlorophyll fluorescence intensity.

Table 1 Main specifications of HAI sensor.

Sensor	Chlorophyll	Temperature	FSI	Pressure
Range	0 to 400 ppb	-3 to 45°C	---	0 to 50 dbar
Initial accuracy	± 1% FS (0 to 200 ppb)(1)	±0.02 °C (3 to 31 °C)	±0.05 (0 to 200 ppb)(2)	±0.3% FS (Repeatability) ±0.1% FS (Non-linearity)
Communication	RS-485 (through Hand-held unit)			
Weight	0.8 kg (in air and excluding cable)			
Dimensions	Φ70 mm × 176 mm (excluding cable)			
Current drain	less than 120 mA (using DC12 V)			
Material	Titanium (grade 2)			
Cable length	30 m (maximum of 50 m)			
Depth rating	50 m depth equivalent			

(1) Non-linear, calibration using Fluorescein Sodium Salt (Uranine)
(2) Repeatability using Fluorescein Sodium Salt (Uranine)

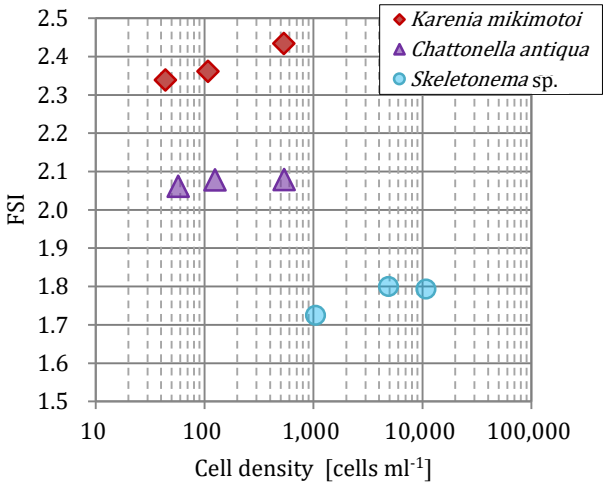

Fig. 6 FSI results for several phytoplankton species in different cell density conditions

(c) Long-term monitoring

In order to reliably and effectively detect early stages of a bloom, which is important for HABs monitoring and early warning systems, observations should be automatic and continuous (with intervals between 30 minutes to few hours). The HAI sensor, equipped with an anti-fouling wiper, can be moored and connected to a telemeter system for long-term deployments. We performed a continuous monitoring test of *Karenia mikimotoi* in Saiki Bay, Oita prefecture, Japan between April and September 2019. The HAI sensor was moored at middle of the water column (6 meters depth) and performed observations at every 15 minutes. We have also performed microscopic analysis from water samples collected periodically during the experiment. FSI results of this long-term experiment are partially shown in Fig. 8 (only few days in August 2019). FSI values observed in the mid-water were higher during the day when compared to FSI values at night. These results are likely showing diurnal vertical migration of *Karenia mikimotoi*, which tends to migrate towards the surface before dawn and return to deeper layers at night as a strategy to optimize nutrient and light utilization for growth throughout the water column (Koizumi *et al.*, 1996). Still on Fig. 8, on August 9th, FSI was significantly higher than the predetermined threshold, evidencing that *Karenia mikimotoi* was present in the mid-water. This was also confirmed by microscopic analysis from water samples obtained at 6 m around noon in that same day, with cell densities equal to 27 cells ml^{-1}. These results show that HAI sensor can detect (automatically and continuously) *Karenia mikimotoi* presence even at low cell densities not only in laboratory, but in the field as well.

(d) Karenia brevis

Although most of the results shown here are focused in the responses from *Karenia mikimotoi* and *Chattonella antiqua*, we are also investigating other species within the same genus that could share same characteristics. At the moment, preliminary results are promising using *Karenia brevis* in laboratory. We have obtained very similar FSI response from *Karenia brevis* to those obtained using *Karenia mikimotoi* (see Fig. 9). These results indicate that HAI sensor may help early monitoring of *Karenia brevis* blooms. Specially in regions in which this species is well-known for causing serious damage, such as in the Gulf of Mexico, where frequent blooms of *Karenia brevis* causes large fish kills, human health respiratory distress, and significant economic impacts (Magaña et al., 2003; Steidinger, 2009).

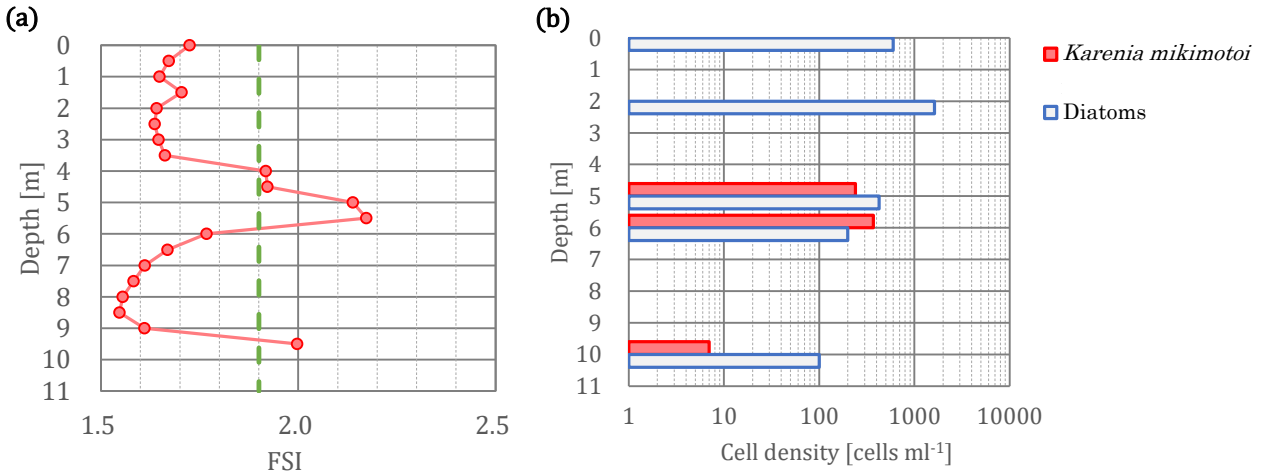

Fig. 7 (a) FSI vertical profile and (b) cell densities estimated by microscopic analysis during a *Karenia mikimotoi* HAB event in Saiki Bay, Oita prefecture, Japan. The green dashed line in (a) represents the predetermined threshold (equal to 1.90).

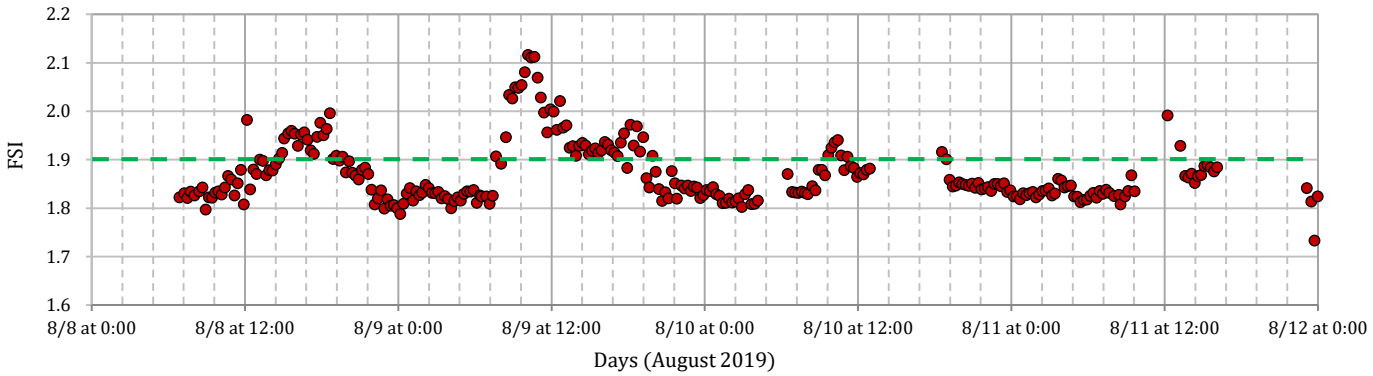

Fig. 8 FSI results during August 2019 from the long-term monitoring test of *Karenia mikimotoi*. The red circles are FSI data at mid-water (6 m depth) and the green dashed line denotes the FSI threshold (1.9).

Remarks

We have investigated the fluorescence spectra from *Karenia mikimotoi* and *Chattonella antiqua* and found that their spectra are shifted compared to several phytoplankton species. We have developed FSI in order to quantify this very particular spectrum shift, which in turn allowed us to provide a very simple and inexpensive instrument: the HAI sensor. This instrument showed a very satisfactory performance in our tests in Saiki Bay and has the potential to significantly reduce the operational costs involved in the monitoring of these two species.

We are confident that the HAI sensor can be used to identify other harmful species that have similar traits, such as *Karenia brevis* and possibly other similar species. However, we still need to confirm it through laboratory and field tests to be conducted in the near future.

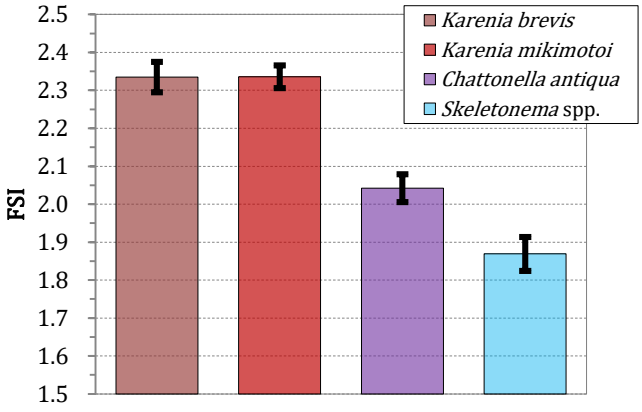

Fig. 9 FSI results (colored bars) and their associated error (black bars) measured in laboratory for *Karenia brevis* and other 3 species of phytoplankton.

Acknowledgments

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