First application of one-class support vector machine algorithms for detecting abnormal behavior of marine medaka Oryzias javanicus exposed to the harmful alga Karenia mikimotoi

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First application of one-class support vector machine algorithms for detecting abnormal behavior of marine medaka *Oryzias javanicus* exposed to the harmful alga *Karenia mikimotoi*

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Abstract

It is empirically known that fish exposed to harmful algal blooms (HABs) exhibit abnormal behavior. This might serve as a method for early detection of HABs. There has been no report of the detection of behavioral abnormalities of fish exposed to harmful algae using machine learning. In this study, the behavior of *Oryzias javanicus* (Java medaka) exposed in a stepwise manner to the HAB species *Karenia mikimotoi* at densities of 0 cells mL^{-1} (control), 1×10^3 cells mL^{-1} (nonlethal), and 5×10^3 cells mL^{-1} (sublethal) was recorded for 30 min at each cell density using two digital cameras connected to a software that tracked behavioral metrics of fish. The level of anomaly in the behavior of Java medaka was then analyzed using one-class support vector machines (OC-SVM) to determine whether the behavioral changes could be considered abnormal. The results revealed abnormal swimming behavior evidenced by an increase of swimming speed, a decrease of shoaling behavior, and a greater depth of swimming in Java medaka exposed especially to the sublethal *K. mikimotoi* density. The medaka exposed to *K. mikimotoi* also displayed physical deformities of their gills that were thought to have caused their abnormal behavior. This supposition was confirmed by further analysis using OC-SVM because the behavior of groups exposed to nonlethal and sublethal densities of *K. mikimotoi* were considered abnormal compared with that of the control groups. The results of this study show the possibility of using this system for early and real-time detection of HABs.

Protection of marine biological resources is necessary to secure food resources from sustainable fisheries. There are, however, many factors that may contribute to damaging the resources and inducing undesired side effects on economic activities. One such threat to the stability of marine resources is increased frequencies of harmful algal blooms (HABs) occurrences. HAB species such as *K. mikimotoi* are known to cause mass mortalities of fish and shellfish (Yamaguchi 1994; Shi et al. 2012). These blooms have occurred in many countries including Norway (Tangen 1977), Scotland (Davidson et al. 2009), Ireland (Ottway et al. 1979; Silke et al. 2005; O'Boyle et al. 2016), Japan (Honjo 1994; Yoshimatsu 2008; Juyanagi), India (Robin et al. 2013), and Singapore (Kok and Leong 2019). The damages caused by *K. mikimotoi* blooms have caused massive economic losses from the killing of both wild and farmed fish. In Imari Bay of Japan, for example, the estimated economic losses from *K. mikimotoi* blooms in 2017 reached US$6,000,000 approximately and have led to the mass mortalities of fish including tiger pufferfish and bluefin tuna (Aoki et al. 2019).

The mechanisms responsible for the toxicity of *K. mikimotoi* to fish are currently not well understood. Observations made thus far have noted that exposure to *K. mikimotoi* induced severe damage to fish gills (Wang et al. 2001; Mitchell and Rodger 2007), abnormal swimming behavior (Jones et al. 1983; Mitchell and Rodger 2007; Niu et al. 2021), and mass mortalities (Davidson et al. 2009; Aoki et al. 2019). Abnormal swimming is thought to be caused by pain due to contact of *K. mikimotoi* cells with the gills and lack of oxygen due to decreased functionality of gas exchange by the gills,

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but the mechanism by which gills are damaged is unknown. Whereas, these effects are all thought to be correlated, the changes of fish swimming behavior are considered to be the tell-tale signs that *K. mikimotoi* may have infiltrated and/or affected an area. In the same article, Jones et al. (1983) have recorded surfacing and circling behavior of fish exposed to *K. mikimotoi* prior to their sinking and turning over. In a field report conducted by Mitchell and Rodger (2007), surfacing behavior was detected in farmed salmons exposed to 3.0×10^3 cells mL^{-1} of *K. mikimotoi*. Additionally, exposure toward *K. mikimotoi* led to lower swimming velocity and swimming distance in *Danio rerio* larvae at 1×10^3 cells mL^{-1} and above (Niu et al. 2021). Observations of such behavioral changes are crucial because often times, it may not be immediately apparent when fish are ailing. In turn, such observations may also be used as a method of monitoring water quality in a variety of situations.

Early detection of *K. mikimotoi* cells can be an effective way to mitigate damages to fisheries. Evaluation of water quality by observing fish behavior has been assessed in many reports. Devices are being developed for this specific purpose because observations of fish behavior are highly reliable for long-term environmental assessments, and they are cost-effective for general application compared with using physiochemical sensors (Gerhardt et al. 2006). The Japanese medaka (*Oryzias latipes*) is an example of a model organism commonly used in ecotoxicological assessments such as those proposed by the Organization of Economic Cooperation and Development (OECD 2019). To date, there have been many studies of the effects of chemical stressors such as insecticide (Khalil et al. 2017) or residues of pharmaceutical and personal care product (Nassef et al. 2010) on Japanese medaka behavior. This approach can be applied for environmental monitoring of various toxic materials that may possibly enter aquatic systems (Kang et al. 2009). The applicability of such device for monitoring fish behavior suggests that there is potential for the application of the same technology for HAB detection because there is a suitable marine model organism.

The advances of behavioral studies for environmental monitoring can be facilitated with the use of algorithms for machine learning. Within the vast array of machine learning techniques, detection of anomalies has been a frequent goal. Machine learning can be used to detect discrepancies within certain preconceived patterns (Chandola et al. 2009) such as outliers or other peculiarities. An example would be the one-class support vector machine (OC-SVM), which identifies one class of items and detects everything else that falls outside that class (Pimentel et al. 2014). There have been many instances wherein machine learning has been applied to the analysis of animal behavior to help behaviorists analyze more complex data from image recognition (Valletta et al. 2017) to decoding of brain neurons (Gajadhar et al. 2018). We hypothesized that the same technology might also be used to facilitate detection of anomalies or changes of fish behavior upon exposure to HAB.

We selected Java medaka, *Oryzias javanicus*, as a potential bioindicator for detection of *K. mikimotoi* blooms. Java medaka (Bleeker 1854) is a marine relative of the Japanese medaka that was first discovered in Java, Indonesia and inhabits the brackish waters of the Southeast Asian regions (Angel et al. 2019). Java medaka is known to tolerate water salinities ranging from freshwater to seawater conditions (Inoue and Takei 2002). There is also a history of using Java medaka in ecotoxicological monitoring of chemicals (Koyama et al. 2008), microplastics as vectors for pollutants (Takai et al. 2023), and toxic heavy metals (Khodadoust et al. 2013; Puspitasari 2016) in marine environmental settings. Their resilience to high salinity environments and history in marine ecotoxicological monitoring was crucial for this study because most blooms of *K. mikimotoi* are reported to occur in seawater. Another reason why Java medaka would make a good test organism is that they grow rapidly and reach sexual maturity at an age of ~100 d (Kakuno et al. 2001). Many studies on the ichthyotoxicity of *K. mikimotoi* have been conducted on marine aquaculture fish as red sea bream (Shin et al. 2023), turbot (Li et al. 2017), and sheepshead minnow (Mooney et al. 2010), which generally require more time to reach full maturity and resources for maintenance, which generally require more time to reach full maturity and resources for maintenance. The ease with which they can be maintained under laboratory conditions enhances their appeal as test organisms and thus highlights their representability as a marine model organism. Although we see a potential for using Java medaka for detection of algal blooms based on these traits, there is a need for investigations of their sensitivity to *K. mikimotoi* blooms because there is currently very little information related to such sensitivity.

In this study, we therefore observed the changes in the behavior of Java medaka upon being exposed to a nonlethal, low density and a sublethal high density of *K. mikimotoi*. We hoped to be able to confirm their viability as a bioindicator for early onset detection of algal blooms. First, the shoaling behavior, average swimming speed, and average vertical swimming position of the Java medaka were measured to obtain a clear picture of their behavioral changes under different exposure conditions. Subsequently, the level of anomaly in their behavior was analyzed using OC-SVM to determine whether or not these changes could be considered abnormal. Lastly, cross-sections of gills of Java medaka exposed to *K. mikimotoi* were taken to observe the extent of the damages in hopes that it could explain the reasons behind the changes in their swimming behavior.

Materials and methods

Fish culture

O. javanicus were maintained in glass tanks. The water temperature was kept at a temperature of 25 ± 1°C and a salinity of 35 with an aerating filter system in each tank. Feeding with brine shrimp was conducted twice a day, once in the morning (09:00 h) and once in the evening (18:00 h). The fish were maintained on a 16:8-h light: dark cycle. The fish tanks were cleaned by replacing half of the water every 2 weeks.

Algae culture

A non-axenic strain of *K. mikimotoi* isolated from Omura Bay (Nagasaki, Japan) was obtained from the Seikai National Fisheries Research Institute. The *K. mikimotoi* cells were kept in culture flasks suspended in modified SWM-3 medium (Yamasaki et al. 2007) at a pH of 7.9 and a salinity of 30. The cells were maintained at 20°C and an irradiance of 100 ± 10 μmol photons m^{-2} s^{-1} provided on a 12:12-h light: dark cycle.

Determination of lethal density of *K. mikimotoi*

The experiment to determine the lethal density of *K. mikimotoi* was conducted for 24 h inside a plankton growth chamber set to a temperature of 25°C under a continuous irradiance of 100 ± 10 μmol photons m^{-2} s^{-1}. Juvenile Java medaka (1-month posthatch, 0.95 ± 0.3 cm body length; mean ± SD) were transferred into individual, screw cap glass vials filled (6 cm in height and 3.2 cm in diameter) with 20 mL of artificial sea water at a salinity of 35. *K. mikimotoi* cells were then added to reach cell densities of 2×10^2, 5×10^2, 1×10^3, 5×10^3, and 1×10^4 cells mL^{-1}. Seawater was used as a negative control. Three individual fish were used for each condition, with the exception of the treatment with 1×10^4 cells mL^{-1} and the control groups, for which five individual fish were used. Dead fish were removed immediately, and the cell density of *K. mikimotoi* was counted afterwards.

Analysis of *O. javanicus* swimming behavior upon exposure to *K. mikimotoi* strains

Based on the results of the lethal density experiments, the experimental fish were exposed to 0, 1×10^3, and 5×10^3 cells mL^{-1} as the control, non-lethal, and sub-lethal exposure conditions respectively. Three randomly selected adult fish (6-months posthatch, 2.8 ± 0.3 cm body length) from our stock aquarium were starved for 24 h prior to the start of the observation. The starved fish were then transferred to an experimental aquarium (10 cm length, 10 cm width, and 10 cm height) with two cameras: one facing the front of the observation tank and the other facing a mirror angled to the left side of the tank, a red light, and a water-circulating system (flow rate of 500 mL min^{-1}, Fig. 1). The camera lenses were positioned 24.5 cm in front of the observation tank sides. The side view camera was positioned 16.5 cm in front of the angled mirror, which was 8.0 cm away from the tank side, totaling to 24.5 cm in distance. Both cameras were mounted vertically where the lenses would align to the midpoint of the observation tank side. After an acclimatization period of 90 min, the swimming activity of the control fish was recorded using two digital video cameras connected to a software that tracked behavioral parameters (Seiko Electric, Fukuoka, Japan) for 30 min. The 30 min recording time for each treatment was selected based on the qualitative observation of the density determination, which was enough time to observe the behavioral changes without necessarily inflicting prolonged stress to the Java medaka. This tracking involved mapping the positions of the fish within the experimental aquarium. The positions were recorded at a frequency of 10 frames per second. After a resting period of 10 min postrecording, sufficient *K. mikimotoi* cultures were introduced to produce a cell density of 1×10^3 cells mL^{-1}, and the test water was circulated for 5 min before recording of the swimming activity was resumed using the same group of fish. This process was then repeated for the high-density exposure condition, which involved adding sufficient *K. mikimotoi* cultures to produce a cell density of 5×10^3 cells mL^{-1}. This experimental procedure was conducted with three independent groups of three fish each. All recordings were conducted during the time interval 12:30–14:30 h Japan standard time to minimize errors stemming from time-dependent behavioral differences among the experimental fish.

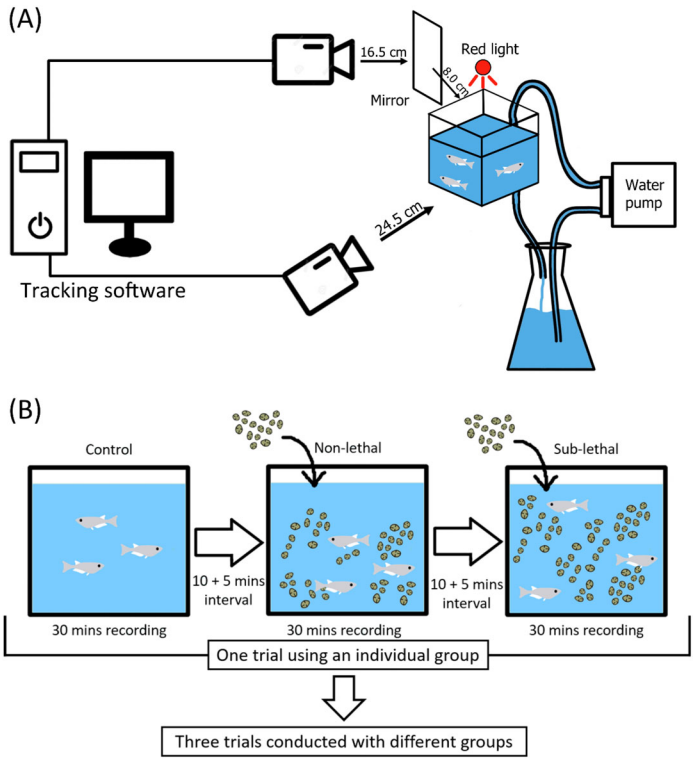

Fig. 1. Experimental setup for behavior analysis (**A**) and the flow of each trial in the experiment (**B**). Cameras were connected to a computer with behavioral-parameter-tracking software (Seiko Electric, Fukuoka, Japan). The observation tank was connected to a water pump and an external water tank for circulation. Java medaka were acclimated in the observation tank for 90 min prior to the start of the control condition recording.

Anomaly detection using OC-SVM

Anomalies were detected by OC-SVM (Pimentel et al. 2014) using medaka behavioral data measured under three conditions: control, nonlethal (1×10^3 cells mL^{-1}), and sublethal (5×10^3 cells mL^{-1}) *K. mikimotoi* densities. The measured data

consisted of 3D orthogonal coordinates of three points, one point for each fish. Two cameras were used to simultaneously observe the (x, y) axes from both the front and the side of the observation tank. Adjusting the two x-axes to the y-axis obtained in both footages retrieves the three-dimensional (x, y, and z) axes. The x and z axes represented the horizontal directions, and the y axis represented the vertical direction. The downward direction was considered positive. In accordance with the (x, y, and z) axes obtained through the simultaneous recording, the position of each fish was mapped to these axes to obtain their respective (X, Y, Z) coordinates. The coordinates of the three points obtained from the three fish were averaged to obtain the central coordinates $C = \{C_x, C_y, C_z\}$ of the medaka group.

$$C_x = \frac{X_1 + X_2 + X_3}{3}$$

$$C_y = \frac{Y_1 + Y_2 + Y_3}{3}$$

$$C_z = \frac{Z_1 + Z_2 + Z_3}{3}$$

The analysis included (1) average swimming position obtained from the center coordinates of medaka group, (2) average degree of aggregation of the medaka group $D = \{\mathcal{D}_x, \mathcal{D}_y, \mathcal{D}_z\}$,

$$D_x = \sqrt{\frac{1}{3}\sum_{i=1}^{3}\left(X_i - \overline{X}\right)^2}$$

$$D_y = \sqrt{\frac{1}{3}\sum_{i=1}^{3}\left(Y_i - \overline{Y}\right)^2}$$

$$D_Z = \sqrt{\frac{1}{3}\sum_{i=1}^{3}\left(Z_i - \overline{Z}\right)^2}$$

and (3) the average speed of movement of the central coordinate of the medaka group $V = \{V_x, V_y, V_z\}$.

$$V_x = \frac{C_{x\cdot n} - C_{x\cdot n-1}}{fps}$$

$$V_y = \frac{C_{y\cdot n} - C_{y\cdot n-1}}{fps}$$

$$V_z = \frac{C_{z\cdot n} - C_{z\cdot n-1}}{fps}$$

We used the OC-SVM (Supplementary Material S1) algorithms in our study to analyze the degree of behavioral abnormality in *K. mikimotoi* exposed Java medaka based on their shoaling behavior, swimming speed, and surfacing tendencies. The algorithm analyses the patterns in the experimental data and tests it against a normal classification boundary to give a ratio of how anomalous the data are. The classification boundary was generated using data from the 10 min after the start of measurements on the control group, and the range of normal behavior was assumed to be within that boundary (learning process). The measurement data for the three conditions (control, nonlethal, and sublethal densities) were used as test data, and the ratio (abnormality rate) of the amount of data outside the classification boundary to the number of test data for 1 min was calculated every minute. From the abnormality rates, anomaly detection with the OC-SVM was conducted using the Python module svm from scikit-learn (Pedregosa et al. 2011). An anomaly threshold to determine the range of behavior considered to be normal was equated to the sum of the mean anomaly ratio of the control group and three times the standard deviation of that anomaly ratio ($\mu + 3\sigma$).

Histopathological examination of *O. javanicus* gills

Two Java medaka (2.0 ± 0.1 cm body length) were exposed to *K. mikimotoi* cultures at a cell density of 1×10^4 cells mL^{-1}, and two additional fish were sampled as controls for comparison with the exposure group. The fish in the exposure group were sampled prior to their deaths, followed by the control group, and the fish were dissected just below their gill flaps. The heads were then preserved in a 10% formalin solution for 48 h and stored in 80% alcohol for an additional 24 h. The samples underwent further dehydration via immersion in increasing alcohol gradients of 80%, 90%, 95%, and 99%, followed by three immersions in 100% ethanol, with each immersion lasting for 10 min. Following these immersions, the samples were treated and embedded with Technovit® 7100 (Kulzer-Technik GmbH., Hanau, Germany) according to the directions provided by the manufacturer. After the samples were fully embedded, they were processed into sagittal-plane histopathological section and the tissues were stained using hematoxylin–eosin staining protocols. The stained tissue samples were then observed under a light microscope, and digital images were taken.

Statistical analysis

The experimental data (positions of fish) were obtained relative to the midpoint of the shoals in each group ($n = 3$). Assumptions of homogeneity of variance was tested using Levene's Test. If the variances were found to be homogenous, Student's t-test was used to compare the exposure groups to the controls during the same timeframe with Bonferroni correction method for p-value adjustment. However, if the assumption of homogeneity was rejected, the nonparametric Wilcoxon rank sum test, followed by a post hoc analysis using Dunn's test, was used instead to compare the means within the individual groups. We also conducted a cross-sectional analysis using generalized linear model (GLM) with a Gaussian family and identity links. These analyses were conducted using RStudio version 2022.12.0 (Posit, PBC, Massachusetts, USA) under R version 4.2.1 (The R Foundation for Statistical

Computing, Vienna, Austria) and statistical differences were considered to be significant at $p < 0.05$.

Results

Determination of lethal density of *K. mikimotoi*

At the highest *K. mikimotoi* density of 1×10^4 cells mL^{-1}, all but one of the Java medaka died within the first 3 h of the observation period. Prior to their deaths, the fish displayed erratic behavior such as continuous, rapid surfacing (Fig. 2). The Java medaka exposed to 5×10^3 cells mL^{-1} were able to survive for a considerably longer time. There was one death 19 h from the start of the observation period, and the fish displayed similar erratic behavior, but to a lesser degree. No deaths were observed in the groups exposed to no more than 1×10^3 cells mL^{-1} of *K. mikimotoi*. Rapid swimming and surfacing were briefly observed in the group exposed to 1×10^3 cells mL^{-1} during the early observation period, but those behaviors dissipated after some time had passed. The behavior of the groups exposed to 5×10^2 and 2×10^2 cells mL^{-1} did not change noticeably throughout the exposure period.

Analysis of *O. javanicus* swimming behavior upon exposure to *K. mikimotoi* cultures

Exposure to *K. mikimotoi* resulted in changes of the distance of individual Java medaka from the midpoint of their shoal (Fig. 3). In the groups exposed to 1×10^3 cells mL^{-1}, the variance of the distance of Java medaka from the midpoint of their shoal changed very little compared with the control group up until 25–30 min of exposure when the distance grew wider, albeit not significantly. The range of distances of individuals from the midpoint of the shoal in the groups exposed to 5×10^3 cells mL^{-1}, however, was much higher than the ranges of the other groups: some distances exceeding 50 mm. Nevertheless, when compared with the control group during the same timeframes, none of the changes were considered significant in either exposure groups.

The swimming speed of Java medaka in the groups exposed to 1×10^3 cells mL^{-1} displayed a gradually increased throughout the exposure period (Fig. 4). This increase continued for the remainder of the observation period though the changes were not considered significant. A decrease, however, was observed once the fish were exposed to 5×10^3 cells mL^{-1} *K. mikimotoi*. The swimming speeds of fish exposed to sublethal concentration of *K. mikimotoi* were initially much higher than those of the controls but gradually decreased as time passed. Despite this decrease, significant difference of swimming speed vs. the control group during the corresponding timeframes were maintained throughout the first 5 min of the recording session; the swimming speeds were similar to those of the control groups during the second half of the recording session.

The vertical position during swimming of Java medaka (Fig. 5) was also used as a metric of their behavior. The median position of the groups exposed to 1×10^3 cells mL^{-1} was visually lower than that of the control group, but their vertical position during swimming was not significantly different from that of the control group. However, the average positions during swimming of fish exposed to 5×10^3 cells mL^{-1} were lower throughout the exposure compared with both the control and the nonlethal group. The vertical positions during swimming of fish exposed to 5×10^3 cells mL^{-1} were significantly different from those of the control group throughout the observation periods.

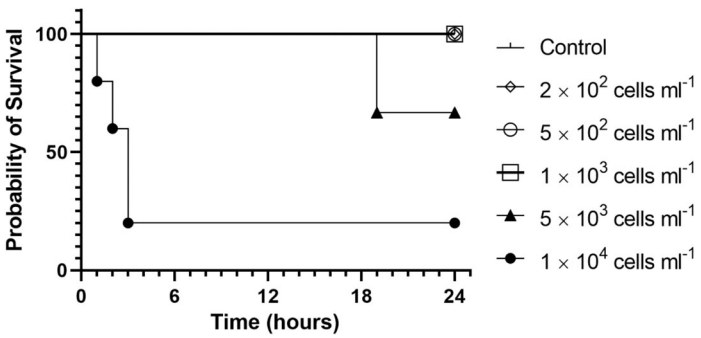

Fig. 2. Survival rate of Java medaka upon 24-h of continuous exposure toward varying levels of *K. mikimotoi* densities.

Cross-sectional analysis using GLM

All three dependent variable (shoal size, swimming speed, and swimming depth) were statistically correlated with density as the independent variable. In comparison, only the shoal size was shown to have a positive statistical correlation with time whereas the other variables had negative associations (Table 1).

Anomaly detection using OC-SVM

The swimming patterns of all groups of fish exposed to either 1×10^3 cells mL^{-1} or the 5×10^3 cells mL^{-1} were abnormal based on the analysis of anomalies using OC-SVM (Fig. 6). The fish exposed to 1×10^3 cells mL^{-1} in Group 1 displayed abnormal swimming patterns that exceeded the normal behavior threshold during the first 10 min of *K. mikimotoi* though this eventually subsided for the remainder of the recording period. An increase of abnormal swimming patterns by Group 2 and Group 3 was also apparent over time; their swimming abnormalities exceeded the threshold after 5 and 10 min of exposure, respectively. The initiation of exposure to 5×10^3 cells mL^{-1} of *K. mikimotoi* almost immediately after the exposure to 1×10^3 cells mL^{-1} had lapsed led to higher rates of abnormality at the initiation of the observations. During this exposure to 5×10^3 cells mL^{-1} of *K. mikimotoi*, all three groups displayed highly unstable patterns of abnormality that greatly exceeded the normal behavior threshold. The anomaly ratio of Group 2 consistently exceeded 50%.

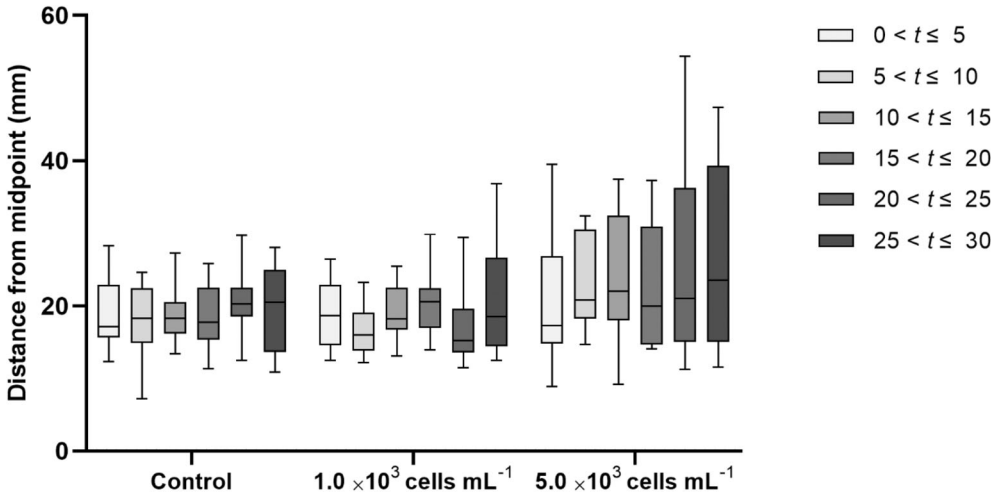

Fig. 3. Analysis of Java medaka shoaling behavior upon exposure to *K. mikimotoi* cells. Time intervals (*t*) are measured in minutes from the start of the observation. Significant differences between the exposure group and the control group during the same time interval are labeled with asterisks (*$p < 0.05$; **$p < 0.01$).

Histopathological examination of *O. javanicus* gills

Histopathological investigations revealed that Java medaka gills were deformed after exposure to *K. mikimotoi* cultures (Fig. 7). There were cases of cellular hypertrophy among the epithelial cells of the secondary lamellae that led to the enlarging of filaments and consequently blocking of interlamellar gaps. Several secondary lamellae were also seen to have fused tips with adjacent lamellae as a consequence of this enlargement. In some cases, the lamellae within the entire side of the filament became completely fused.

Discussion

Previous studies have shown the extent of damage that *Karenia* blooms can cause to marine wildlife and the fisheries industry (Itakura and Imai 2014). The damage caused by these blooms has led to further research into the problem. There have been efforts to understand the mechanisms of by which the blooms kill (Dorantes-Aranda et al. 2015; Li et al. 2017) and to develop methods to mitigate the damages. The goal of the present study was the latter. We specifically sought to identify a method to detect HABs that would allow problems to be addressed sooner. Monitoring of HABs has traditionally been conducted via in situ ship surveys and laboratory analyses, though these methods are often considered inefficient. Satellite imaging has also been used and is a more preferred method to monitor HAB occurrences. The spectral characteristics of the blooms along with oceanographic conditions can be observed to provide further insights into the occurrence of the blooms. There are, however, limitations to the use of

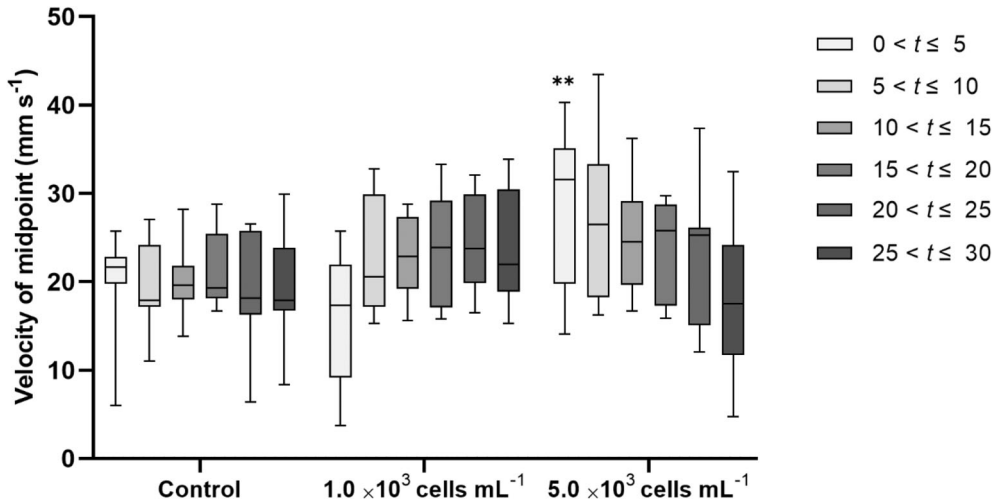

Fig. 4. Analysis of behavior of Java medaka average midpoint swimming speed upon exposure to *K. mikimotoi* cells. Time intervals (*t*) are measured in minutes from the start of the observation. Significant differences between the exposure group and the control group during the same time interval are labeled with asterisks (*$p < 0.05$; **$p < 0.01$).

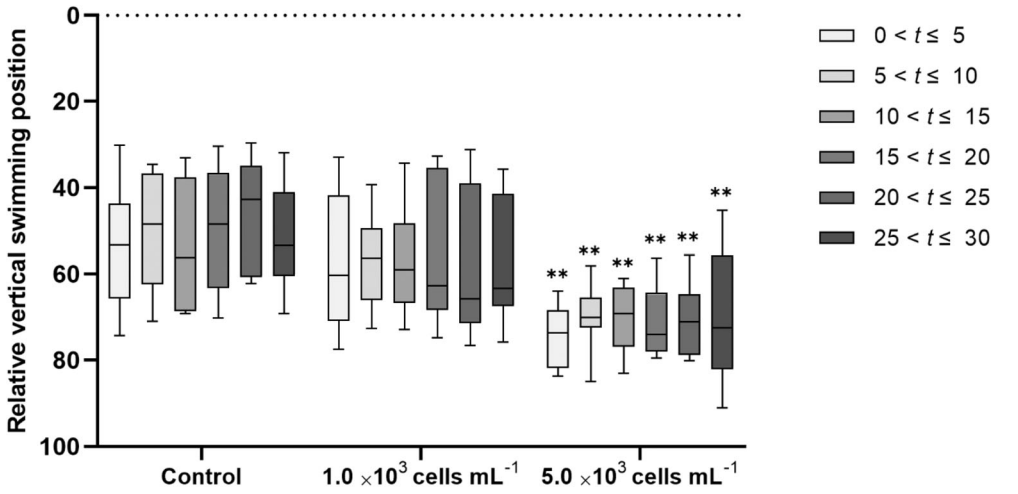

Fig. 5. Analysis of behavior of the relative average vertical swimming position of Java medaka upon exposure to *K. mikimotoi* strains. Time intervals (t) are measured in minutes from the start of the observation. Significant differences between the exposure group and the control group during the same time intervals are labeled with asterisks ($^*p < 0.05$; $^{**}p < 0.01$).

satellite method. It is difficult to conduct real-time monitoring of dynamic HABs in coastal regions via satellites and the quality of the projected images may depend on cloud conditions.

The present study was conducted to help overcome these limitations by investigating an alternative method for real-time bloom detection; namely, the analysis of behavioral changes in a model marine organism. Studying behavioral changes allowed us to discern possible reasons how changes of in the normal environment of the organism could cause significant changes of their behavioral patterns. Observing changes of the behavior of Java medaka enabled us to evaluate the quality of the water in an area and rapidly detect substances that might pose a threat to local waters.

Our observations revealed changes in the distance between individual Java medaka during shoaling, their average swimming speeds, and their average vertical position during swimming when exposed to *K. mikimotoi* cultures. Java medaka is a species of shoaling fish that tends to swim in groups called shoals. Exposure to *K. mikimotoi* changed this tendency. Individual Java medaka were seen swimming in increasingly erratic patterns. The result was an increase of the distance between the Java medaka and the midpoint of their shoal. This increase in distance was especially apparent in the groups exposed to 5×10^3 cells mL^{-1} wherein the fish swam furthest apart from each other. The average swimming speed of the Java medaka increased sometime after the group was exposed to a nonlethal density of *K. mikimotoi*. After an extended period of exposure, especially to a relatively high density of *K. mikimotoi*, the Java medaka displayed the opposite reaction and swam at a lower speed that was almost the same level as the speed of the control conditions. The average position of the Java medaka during swimming was also seen to be deeper when the fish were exposed to even higher densities of *K. mikimotoi*. Whereas the low-density group of Java medaka barely displayed any differences in their vertical position while swimming compared with the control fish, the groups exposed to 5×10^3 cells mL^{-1} swam at a greater depth compared with the other groups and remained at that depth for the remainder of the observation periods. The deeper average position during swimming of the high-density group displays was correlated with their decreased swimming speed because the fish were swimming in place, closer to the tank floor. Cross-sectional comparison using GLM also denotes the impact of *K. mikimotoi* density toward the parameters as statistical significance were observed despite the seemingly low fixed value estimates. On the other hand, elapsed time were not seen to have as much statistical impact toward the parameters compared with density. This may explain that HAB densities are more strongly correlated to the changes in fish behavioral parameters compared with the period of observation.

Table 1. GLM analyses. (a) Shoal size (shoal), (b) swimming speed, and (c) vertical swimming position (swim height) were used as the response of interests (y). Time from the beginning of the control exposure (time) and *K. mikimotoi* cell density were considered the predictor variables (x_1 and x_2).

Intercept	Time	Cell density	AIC
(a) $Y_{shoal} = \beta_{time} \cdot X_{time} + \beta_{density} \cdot X_{density} + \text{Intercept}$			
$16.6 \pm 0.758^{***}$	$0.121 \pm 0.04^{**}$	$0.001 \pm 0.0001^{***}$	65.4
(b) $Y_{speed} = \beta_{time} \cdot X_{time} + \beta_{density} \cdot X_{density} + \text{Intercept}$			
$21.4 \pm 1.70^{***}$	-0.05 ± 0.08	$0.0008 \pm 0.0003^{*}$	94.8
(c) $Y_{swimheight} = \beta_{time} \cdot X_{time} + \beta_{density} \cdot X_{density} + \text{Intercept}$			
$53.8 \pm 1.71^{***}$	-0.08 ± 0.08	$0.003 \pm 0.0003^{***}$	94.8

$^*p < 0.05$;
$^{**}p < 0.01$;
$^{***}p < 0.001$.

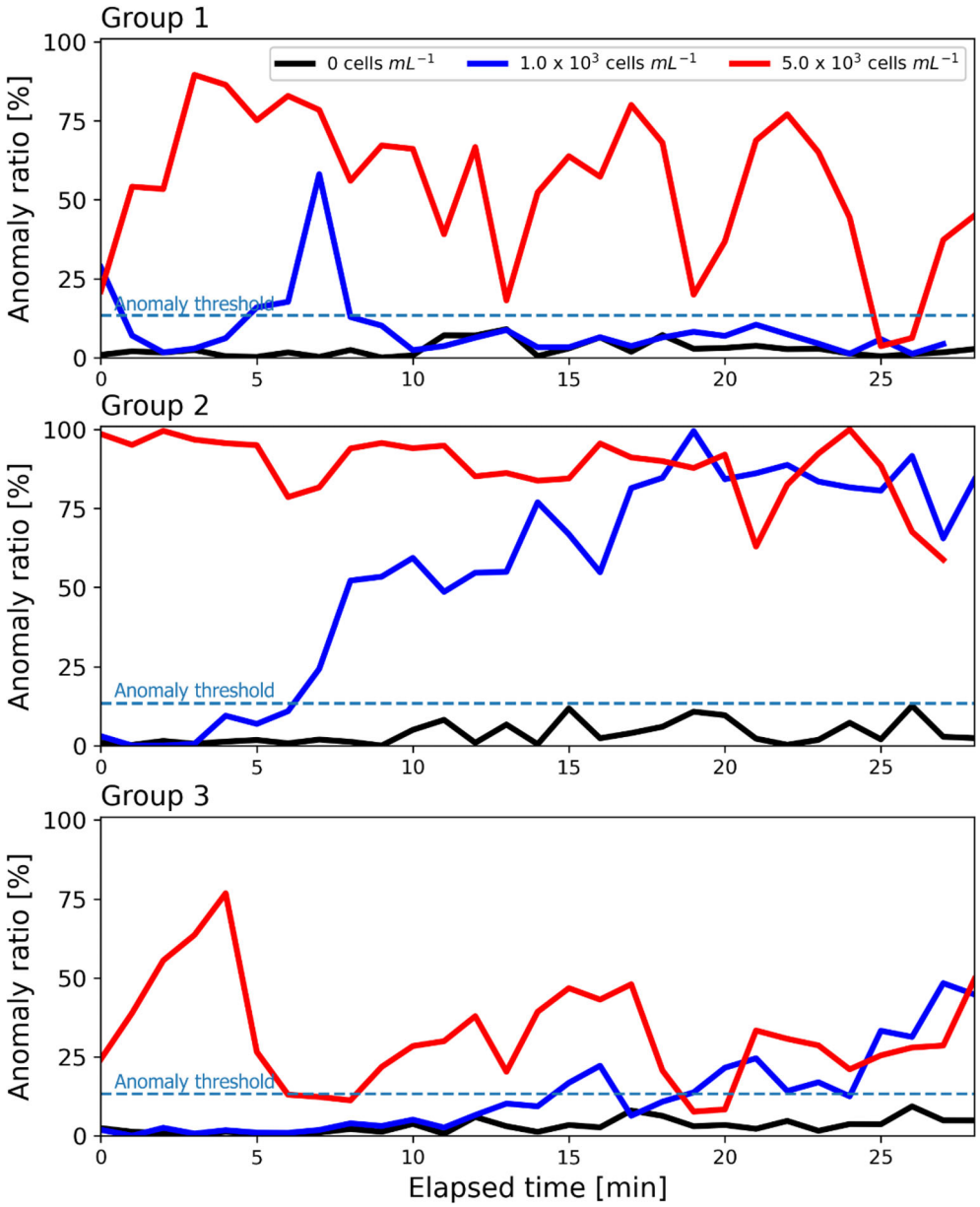

Fig. 6. Ratio of abnormal behavior in *K. mikimotoi*-exposed Java medaka as detected using OC-SVM. The anomaly threshold was obtained by taking the mean anomaly ratio of the control group and adding three times the standard deviation of the control group anomaly ratio ($\mu + 3\sigma$).

Although these patterns denoted abnormal swimming behaviors by Java medaka when exposed to *K. mikimotoi*, statistical analyses still revealed few significant differences between the behavior of the exposed fish and the controls.

To better relate the two behavior patterns observed in this experiment to exposure to *K. mikimotoi*, we used anomaly detecting machine-learning algorithms in the form of OC-SVM to determine if the swimming behavior of Java medaka in the exposure groups could be considered abnormal when compared with the control group. OCSVM is beneficial for applications in anomaly detection as it can reliably detect outliers through complex data patterns. While the method may suffer from weaknesses against higher dimensional data, this method works particularly well for the scale of our study, thus making it a suitable choice of algorithm for our scope of study. It was shown that OC-SVM can detect abnormalities that could not be detected by normal statistical processing for individual parameters, especially towards nonlethal *K. mikimotoi* densities. Exposure to 1×10^3 cells mL^{-1} of *K. mikimotoi* revealed increasingly abnormal swimming behavior over time, especially in Groups 2 and 3 (Fig. 5). The fact that this pattern of increase almost mirrored the increase in the average swimming speed of the same group, albeit insignificantly, indicated that exposure to nonlethal densities of *K. mikimotoi* caused changes in the swimming

behavior of Java medaka. The abnormal behavior reached a peak when the fish were exposed to higher densities of *K. mikimotoi*, (viz., Group 2), though the abnormality ratio fluctuated greatly in the other groups. This may be related with the decrease in the average swimming speed of the groups exposed to 5×10^3 cells mL^{-1} as well as to the increase in the depth at which the fish swam and the tendency of the fish to remain motionless at times. We suspected that the decrease in the postulated abnormal swimming behavior below the abnormality threshold occurred as the Java medaka returned to swimming at a speed that is similar to that of the control group. We think that this return to a normal swimming speed was the reason why the OC-SVM classified such behavior as "normal" and hence perceived fluctuations of behavior that was otherwise perceived as abnormal. Despite this, since abnormal swimming was continuously detected throughout the exposure periods, it is inferred that detection of behavioral abnormalities by OC-SVM is extremely promising for early detection of harmful red tides.

Previous studies have suggested that whereas *K. mikimotoi* does not necessarily have potent toxicities (Satake et al. 2002), high densities of *K. mikimotoi* can physically congest and damage fish gills (Mitchell and Rodger 2007). Many previous recording of *K. mikimotoi* blooms also reported abnormal behavior in fish, such as frantic swimming and frequent surfacing. Such behavior aligns with hyperventilation behavior as listed by Shen et al. (2010). We processed pathological sections of Java medaka gills in our study to further understand the relation between the gill damages and the observed behavioral changes in our study. The disfiguration apparent in Fig. 7, for example, is likely to have disrupted the respiratory process of the fish. Blockages of the interlamellar gaps in the gills and fusion of the tips of the gills reduces the surface area for gas exchange (Esmail et al. 2015). The observed hypertrophy of cells in the gill epithelium would also increase the distance of diffusion between the blood and water (Movahedinia et al. 2012) and lead to an overall reduction of the efficacy of gas exchange. This interference with gas exchange is thought to be the cause of the erratic swimming behavior and the observed rapid surfacing behavior of Java medaka in an effort to obtain more oxygen (Esmail et al. 2015). Similar behavior has also been observed in a previous study wherein marine medaka *Oryzias melastigma* displayed reactions similar to those observed in the present study upon exposure to the toxic raphidophyte species *Chattonella marina* (Shen et al. 2010).

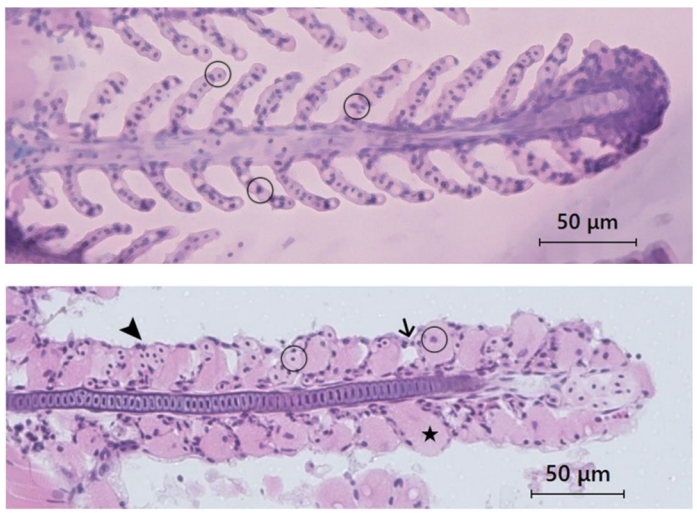

Fig. 7. Cross section of Java medaka gills from the control group (top) and the exposure group (bottom). ◦: Epithelial cell nucleus; ★: Epithelial cell hypertrophy; →: fusion of lamellar tips from epithelial lifting; ➤: complete fusion of several lamellae.

Although the specific mechanisms are still largely unknown, the mechanisms by which *K. mikimotoi* kills fish can be hypothesized. It has been suggested that cells of *K. mikimotoi* may have hemolytic effects on mammalian erythrocytes because damage to the cell membranes has been observed (Zou et al. 2010). Although there is currently no direct proof of this effect on fish species, it can be postulated that direct contact with *K. mikimotoi* may have damaged the epithelial cells of the gills and hence impaired the respiration of the fish. This impairment may also have caused the fish to rest for prolonged periods of time near the tank floor, especially during prolonged exposures. These resting periods would lower their average swimming speed and increase the average depth of swimming. The indication is that there are abnormalities in the swimming behavior of Java medaka upon exposure to *K. mikimotoi*. Use of methods such as RNA-seq should be considered to further analyze the mechanisms responsible for the toxicity of *K. mikimotoi*, especially if the specific mechanisms are to be identified at a molecular level.

Conclusions

The results of this study showed that exposure of Java medaka to *K. mikimotoi* could induce changes of their normal swimming activity and cause physical deformities in their gills, as well as how OC-SVM can detect abnormal behavior of fish under harmful algae exposure with high sensitivity. These effects are thought to be interrelated. The damages sustained by the gills inhibited the process of gas exchange, the Java medaka displayed erratic swimming behavior that was evidenced by an increase in their swimming speed. Prolonged exposure, and consequently a prolonged display of erratic behavior, exhausted the Java medaka and led to a gradual decrease in swimming speed and longer resting periods near the tank floor. This phenomenon was confirmed by further analysis using OC-SVM. The analysis revealed that the behavior of the exposure groups was abnormal compared with that of the control groups. These behavioral changes indicated that Java medaka were sensitive to *K. mikimotoi* exposure and highlighted the potential uses of Java medaka in the monitoring of algal blooms. Future studies should be carried out to identify the mechanisms responsible for the toxicity of *K. mikimotoi* blooms toward fish.

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Conflict of Interest

None declared.

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