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Notes

Mortality of Fishes and Shellfishes to Harmful Algal Blooms

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Mortality of several species of fish and shellfish exposed to Harmful Algal Blooms (HABs) caused by *Cochlodinium polykrikoides*, *Heterosigam akashiwo*, *Alexandrium tamarense*, *Eutreptiella gymnastica*, *Heterocapsa triquetra* and *Prorocentrum micans* was studied. When fish were exposed to a cell density of 8,000 cells mL^{-1} in *C. polykrikoides*, 35% of flatfish and darkbanded rockfish died within 48 hrs. However, jacopever rockfish had mortality of higher than 85%. Rock bream, filefish and red sea bream showed 100% mortality within 10 hrs with an exposure cell density of 8,000 cells mL^{-1}. The rest of HABs except for *C. polykrikoides* showed that there was no fish and shellfish death throughout the 48 hrs even in the maximum cell density of 100,000 cells mL^{-1}. These results imply that *C. polykrikoides* can have a serious impact on fish mortality and it is regarded as an ichthyotoxic dinoflagellate. The fish death may be attributed to anoxia caused by a combination of the production of reactive oxygen species (ROS) and polysaccharide from *C. polykrikoides* during blooms.

Key Words: *Cochlodinium polykrikoides*, HABs, Mortality, ROS, Polysaccharide, Red tide

Introduction

Harmful algal blooms (HABs) in Korea have been significantly increased in scale, duration, and frequency since 1981. *Cochlodinium polykrikioides* is known to be the most harmful red tide dinoflagellate, which has caused serious damage to the fish farming industry in Korean waters (Kim et al. 1997). To protect fish from attack by the red tide of *C. polykrikoides*, its toxic mechanisms should be elucidated, as well as the factors included in the red tides caused by the dinoflagellates.

This study investigated the immediate effects of red tide waters caused by *C. polykrikoides* and several species of dinoflagellates on the mortality of marine fish and shellfish living in the area where the red tides often occur.

Materials and Methods

Harmful dinoflagellates

HABs in this study were *C. polykrikoides*, *Heterosigam akashiwo*, *Alexandrium tamarense*, *Eutreptiella gymnastica*, *Heterocapsa triquetra* and *Prorocentrum micans* which occurred annually in the Korean coastal waters in the monospecific blooms from 1996 to 2002. The ratio of their cell number to total phytoplankton were much higher than 97%. They were collected from the surface on the blooming spots with a vacuum suction pump and diluted with filtered seawater to control the densities.

Bioassay test

Six species of fish, flatfish (*Paralichthys olivaceus*), jacopever rockfish (*Sebastes schlegeli*), red sea bream (*Parus major*), darkbanded rockfish (*Sebastes inermis*), rock bream (*Oplegnathus fasciatus*) and filefish (*Aluterus monoceros*), and four species of shellfish, oyster (*Crassostrea gigas*), mussel (*Mytilus edulis*), arch shell (*Scapharaca broughtonii*) and abalone (*Norditus discus*) were purchased from the markets of the Tongyoung area in the southern parts of Korea. The body length in fish and shellfish ranged from 19 to 25 cm and from 4.5 to 7 cm, respectively. The conditions of the seawater were as follows: water temperature: 20℃, salinity: 31 psu, pH: 8.4, dissolved oxygen (DO): 6-7 mg L^{-1}, cell density: 1,000-100,000 cells mL^{-1}. The blooming water was poured into a

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transparent tank of a 50 L capacity with air supply, then the test animals were introduced 5-15 individuals for each species. Observations on the mortality of the animals were done at the interval of 4 hrs during the period of 48 hrs.

Results

The experimental fish were exposed to *C. polykrikoides* of a different cell density, and their mortality was shown in Fig. 1. When flatfish was exposed to a cell density of 8,000 cells mL^{-1}, only 35% of the fish died. However, more than 85% on flatfish and jacopever rockfish died when exposed to the cell density of 15,000 cells mL^{-1}. When exposed to 8,000 cells mL^{-1}, 100% of rock bream, filefish and red sea bream died within 10 hrs. In contrast to the mortality of flatfish and darkbanded rockfish, there was no mortality of jacopever rockfish exposed to the cell density of 8,000 cells mL^{-1}. In darkbanded rockfish exposed to 8,000 cells mL^{-1}, there was a similar mortality as in the flatfish. Unlike the *C. polykrikoiaes*, the rest of the dinoflagellates, *H. akashiwo*, *A. tamarense*, *E. gymnastica*, *H. triquetra* and *P. micans*, showed no mortality, although these fish were exposed to an even higher concentration of 20,000 to 100,000 cells mL^{-1}. The experimental shellfish treated by all dinoflagellates used in this study showed no mortality throughout the experimental period.

Discussion

Onoue and Nozawa (1989a) suggested that *C. polykrikoiaes* produced a toxic fraction with hemagglutinative properties and then secreted ichthyotoxic substances outside of the cell. Subsequently, two unique paralytic shellfish poisionings (PSP) were identified by Onoue and Nozawa (1989b). However, these findings are still not enough to explain the toxic mechanisms on fish deaths. In this study, it was observed that most of the fish exposed to red tide waters caused by *C. polykrikoides* died within 10 hrs under the cell density of more than 8,000 cells mL^{-1}, although mortality was different depending on the species of fish. Thus, it is assumed that exposure cell density of 8,000 cells mL^{-1} caused an acute toxic effect of fish. Considering our present results, it is assumed that vigorously swimming fish such as rock bream, filefish and red sea bream are more sensitive to red tide waters caused by *C. polykrikoides* than slow moving fish (flatfish, darkbanded rockfish and jacopever rockfish). To clearly understand why there is a different mortality, we need to examine a morphological and histochemical study on ultrastructural changes in gill lamellae and consider a physiological observation on olfactory responses in the future.

Recently, Kim et al. (1999) suggested that *C. polykrikoides* produced a reactive oxygen species (ROS) such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), associated with oxidative damage leading to fish deaths. Subsequently, Cho et al. (1999) reported that the total content of polysaccharides in *C. polykrikoides* appeared to have a higher value than any other tested microalgae. In particular, the highest rate to generate the ROS and polysaccharides was determined in the exponential phase of *C. polykrikoides* (Cho et al., 1999: Kim et al., 1999). There were some reports on the ROS production caused by *Chattonella* species, and most of researchers suggested it a causative factor for the death of fish (Shimada et al., 1991, 1993; Oda et al., 1992; Tanaka et al., 1992, 1994). While the production of the ROS from *C. polykrikoides* was first described by Kim et al. (1999), the ROS was possibly enough to markedly inhibit the osmoregulation and gaseous exchange in the gill lamellae and fish were on the verge of death due to an oxygen deficiency. It is known that polysaccharide produced by marine algae and gelatinous products secreted from the epithelia membranes of fish gills coated with the algal mucilage may cause the clogging of the gills in fish (Hallegraeff et al., 1995). Hasui et al. (1995) reported that extracellular sulfated polysaccharides from *C. polykrikoides* might play a role in a potential therapeutic utilization against the human immunodeficiency virus. Likewise, polysaccharide substances released from *C. polykrikoides* may also contribute to an impact to the epithelia membranes of fish gills during dense blooms.

Although *A. tamarense* was reported as a fish killing species by Montoya et al. (1996), our results showed that there were no fish death when exposed to an even higher concentration (10,000 cells mL^{-1}) of *A. tamarense*. This result indicated that fish can accumulate PSP toxins directly from the ingestion of *A. tamarense* cells, but its toxic effect on fish is less severe in acute exudative and degenerative changes in the epithelial gill structure than that of *C. polykrikoides*. Dense blooms caused by *H. akashiwo* in New Zealand, Canada, USA, Great

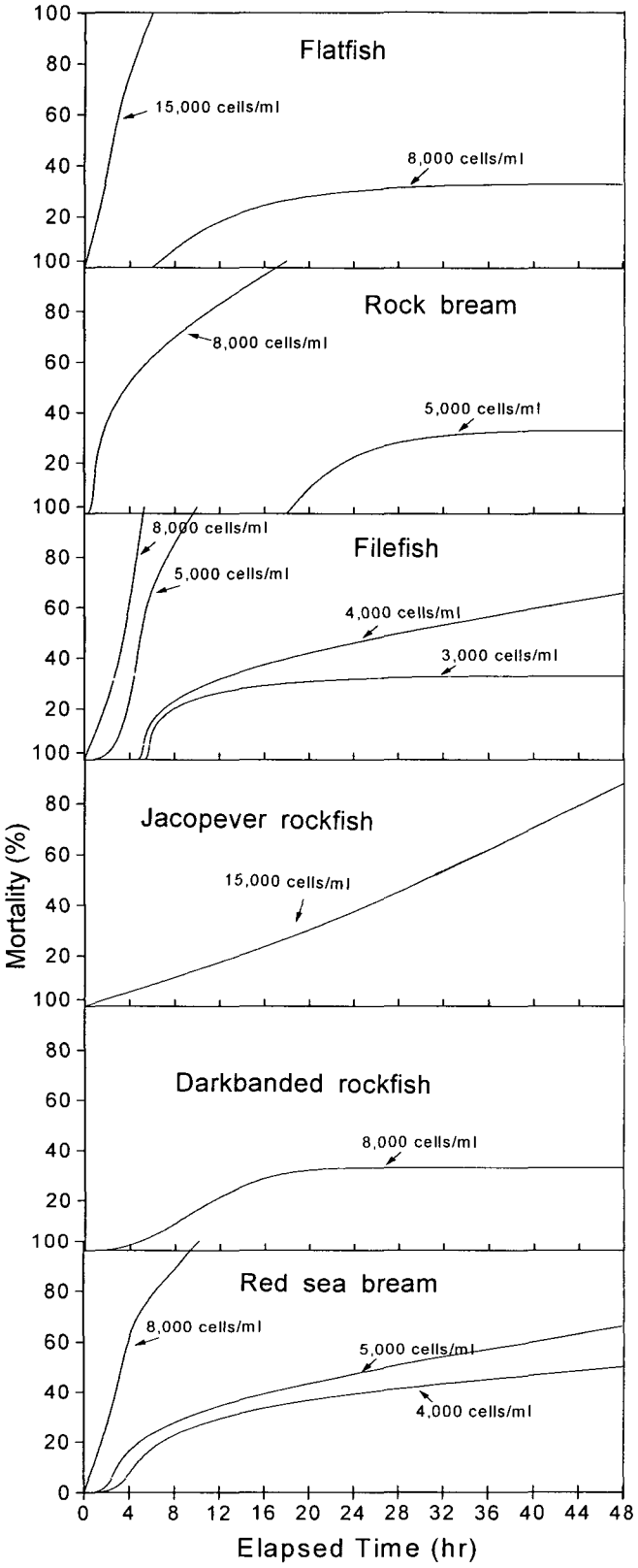

Fig. 1. Mortality of fish exposed to a different cell density of *Cochlodinium polykrikoides*.

Britain and Japan resulted in heavy fish mortality (Chang et al., 1990; Mackenzie, 1991; Tyrrell et al., 1996). The outbreaks caused by *H. akashiwo* have occurred annually in June and July in Korean coastal waters since 1980, with no reported fish deaths (Kim et al., 1996). It was also confirmed in our present study that the strain of *H. akashiwo* from Korea could not make an assurance of any impact to pathological characteristics in the fish. However, further research on the understanding of molecular discrimination of the Korean non-toxic form of ichthyotoxic *H. akashiwo* on the basis of gene sequences are required.

It is concluded that *C. polykrikoides* is associated with massive fish deaths for those exposed to a higher cell density for a few hours, although mortality is subject to the species of fish. Such damage is caused primarily by the production of the ROS and polysccarides from *C. polykrikoides* during blooms, which contribute to a severe impact of gill structure in the fish. To control mass mortality of fish caused by *C. polykrikoides*, we need to make an implementation of a comprehensive biotoxin monitoring program in Korea.

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