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Cochlodinium Red Tide Effects on the Respiration of Abalone, *Haliotis discus hannai* Ino

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Cochlodinium polykrikoides -related red tide is the most notorious tidal bloom, resulting in mass mortality to marine animals. This study aimed to test the effect of *C. polykrikoides* on the lethality to *Haliotis discus hannai* under controlled conditions. The oxygen demand of *C. polykrikoides* increases to reach its peak during the night, while the oxygen usage by *H. discus hannai* was continuously decreased with a threshold of 2 mg L^{-1}. The addition of *C. polykrikoides* did not effect the respiration of the *H. discus hannai*. However, the usage of oxygen by *C. polykrikoide*s during the night may lead to anoxia in the animal. With aeration, the level of dissolved oxygen (D.O.) was between 6.06 and 7.28 mg L^{-1}; 90% of abalones survived even with a high concentration of *C. polykrikoides* (9000 cells mL^{-1}). Without aeration (3 mg L^{-1} of D.O.), however, the *H. discus hannai* suffocated immediately. Once 20 hours had elapsed, all of the abalones were dead. The density of the *H. discus hannai* population contributed to their mortality. Therefore, aeration during the night and maintaining lower abalone densities is the best way to promote the survivorship of *H. discus hannai* during a *C. polykrikoides* red tide.

Key Words: *Cochlodinium polykrikoides*, dissolved oxygen, harmful algal bloom, *Haliotis discus hannai*, lethality test, red tide, respiration rate

INTRODUCTION

Harmful algal blooms (HAB) have brought enormous economic and environmental damage to the world (Flleweling *et al*. 2005; Paperzak 2005). Among the dinoflagellates, *Cochlodinium polykrikoides* is the most frequently occurring harmful phytoplankton and is responsible for mass kills in farmed marine animals in Asia (NFRDI 2004).

Abalone (*Haliotis discus hannai*) is a commercially important shellfish worldwide, including Asia, South Africa, and Austria (Sales and Britz 2001). Through the recent introduction of commercial cultivation techniques, the number of abalone farms among fisheries has rapidly increased (NFFC 2001). In 2003, during a *C. polykrikoides*-related red tide, a great number of farmed abalones on the south coast of Korea were killed. The economic damage to abalone farms along the southern coast of Korea reached 8 million United States Dollars (NFRDI 2004).

This study documents a bioassay experiment to determine the oxygen consumption of and mortality to *Haliotis discus hannai* affected by the addition of *C. polykrikoide* cells.

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MATERIALS AND METHODS

Experimental organisms

Haliotis discus hannai (wet weight, approximately 50g) were supplied from the Shellfish Research Center in Namhae, Korea. The animals were subjected to a three days of acclimation. The average water temperature and salinity during the acclimation period were maintained at 22°C and 32‰, respectively. Filtered seawater from submerged recirculating filter beds installed in the Shellfish Research Center was used for all experiments.

Cochlodinium polykrikoides was collected in Namhae (latitude 34°43'63" N; longitude 128°02'89" E) during the blooming of this species in 2003. The initial density of *C. polykrikoides* was measured (32,500 cells mL^{-1}) and changes in the density were monitored, using a Sedgewick-Rafter chamber under a light microscope.

Experiments were conducted with or without a supply of air circulation. During aeration, the level of dissolved

oxygen during the tests was maintained at a concentration between 6.08 and 7.28 mL^{-1}, while the concentration of dissolved oxygen with no air supply fluctuated from 0.17 to 7.16 mg L^{-1} over 45 hours. During the bioassay, the physical conditions in the tank remained constant, with a temperature of 23-24°C, 32-33‰ water salinity, and a dissolved oxygen level of approximately 5 mg L^{-1}.

Respiration test

Respiration was determined through measurement of decreased levels of dissolved oxygen. The changes in dissolved oxygen level were monitored using an YSI-55 Oxygen and Temperature Meter (YSI Environmental, USA). For 45 hours, the change in dissolved oxygen (mg L-1) by *C. polykrikoides* was monitored in four 8 L tanks, each containing different cell densities: 0, 3000, 3500 and 5000 cells mL^{-1}.

The background oxygen consumption was measured by using blanks (i.e. no *Haliotis discus hannai*) as a control. The respiration rate (RR) was calculated as the oxygen consumption rate (mgO_2 wet $weight^{-1}$ h^{-1}):

$$RR = (D.O.0 - D.O.t) \cdot V / (t / n)$$

where V is the volume of the chamber (8 L), n is the weight of the animal (mg wet weight), t is the duration of the experiment (in hours), $D.O._0$ is the dissolved oxygen level at t = 0 and $D.O._t$ is the dissolved oxygen level at time t in vessels with animals.

To measure the animal's oxygen utilization, five individuals of *Haliotis discus hannai* were introduced to each 8 L tank. Decreasing levels of dissolved oxygen by *H. discus hannai* were measured for 20 hours. Elapsed RR values were compared between two different sets of conditions, such as having only *H. discus hannais* or having both *H. discus hannais* and *C. polykrikoides* at a cell density of 7000 cells mL^{-1}. Data were summarized based on triplicated experiments.

Lethality test

To measure the impact of *Cochlodinium polykrikoides* density on the animal's mortality, *C. polykrikoides* cell density values of 3000, 5000, 7000, and 9000 cells mL^{-1} were chosen on the basis of the knowledge that the peak density in the field was 10,000-15,000 cells mL^{-1} and that the concentration for a red tide alarm in Korea was 7000 cells / mL. Next, ten abalones were introduced into the tanks to observe the impact of the *C. polykrikoides* organisms over a time period of 72 hours. A short-term bioassay was conducted, since extended aquarium-format bioassays may cause mortality in part due to technical reasons, such as the degradation of water quality and overgrowth by other microorganisms, rather than the lethality of *C. polykrikoides* (Lovko *et al.* 2003).

To test the impact of the density of *Haliotis discus hannai* on their own respiration and mortality, a series of 5, 10, 15, 20, 25, and 30 abalones were placed into 20 L tanks. The decreased levels of dissolved oxygen and mortality were measured for 36 hours.

The effects of diet on abalone mortality were tested with supplies of the natural food (chopped kelp in 0.5 cm^2 size) versus artificial pellets (Alitec Co., water: 24.8%, protein: 32.2%, lipids: 2.6%, fiber: 1.5%, calcium: 2.69%, phosphate: 0.81%, and nitrogen-free-elements: 30.6%). Food was supplied into the tanks filled with 3000, 5000, 7000, or 9000 cells mL^{-1} of *C. polykrikoide* to determine whether there is a desensitization or enhancement of the affect of *C. polykrikoides* on abalones. Starved individuals of *Haliotis discus hannai* were used as the control group.

Data were summarized based on triplicated experiments and statistical differences between treatments in any given experiment were examined using ANOVA (analysis of variance test) and Tukey's test. Values in parenthesis represented 95% confidence limits.

RESULTS

Change of dissolved oxygen level by red tide organism and abalone

The temporal changes in dissolved oxygen levels by *Cochlodinium polykrikoides* were recorded with cell concentrations of 0, 3000, 3500, or 5000 cells mL^{-1}. Dissolved oxygen in tanks was correlated closely to the density of *C. polykrikoides* (Fig. 1). The oxygen demand of *C. polykrikoides* increased until the end of the dark period. The level of dissolved oxygen increased during the light period, as a result of photosynthesis by *C. polykrikoides*. The levels reached their maximum at the end of the light period.

In the tanks with abalones, the level of dissolved oxygen decreased below a threshold as low as 2 mg L^{-1} within a few hours; the RR values of *Haliotis discus hannai* also decreased (Fig. 2). The addition of 7000 cells mL^{-1} of *Cochlodinium polykrikoides* to the tanks having *H. discus hannai* did not result in any significant change in the RR values of the abalones ($p = 0.179$, $R = 0.981$), while the oxygen consumption by *H. discus hannai* correlated with

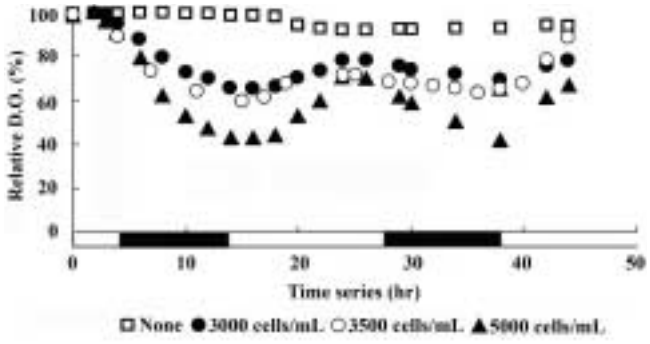

Fig. 1. Diurnal alternation of dissolved oxygen level by *Cochlodinium polykrikoides*. Note that the black and white bars on the X-axis denote the night and day phases, respectively.

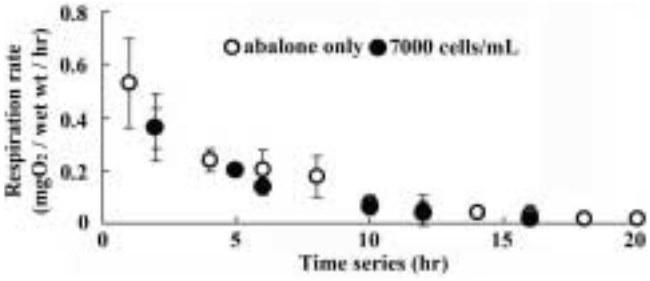

Fig. 2. Temporal decrease in the respiration rate of *Haliotis discus hannai*. Note that the total consumption of dissolved oxygen was not significantly modified by the addition of *Cochlodinium polykrikoides* (7000 cells mL^{-1}).

the increased number of the animal (Fig. 3). The impact of *C. polykrikoides* on the RR values of *H. discus hannai* is not significant when compared with the impact of adjusting the population density in the tanks.

Causes of abalone mortality

The mortality of *Haliotis discus hannai* was closely related to its own density. Eighty percent of *H. discus hannai* were alive for 20 hours in a tank with five individuals, despite the lack of an air supply (Fig. 4).

To determine if the abalone mortality was caused by an increase in *Cochlodinium polykrikoides* concentration, ten individuals of *Haliotis discus hannai* were monitored on an hourly basis in a 20 L tank filled with 3000, 5000, 7000 or 9000 cells mL^{-1} of C. *polykrikoides*, with or without a supply of oxygen, for 72 hours. With aeration, dissolved oxygen levels in the tanks were maintained between 6.06 and 7.28 mg L^{-1}, and 90% of *H. discus hannai* survived, despite *C. polykrikoides* concentrations as high as 9000 cells mL^{-1} (Fig. 5), whereas without aeration (approximately 3 mg L^{-1} of dissolved oxygen), *H. discus*

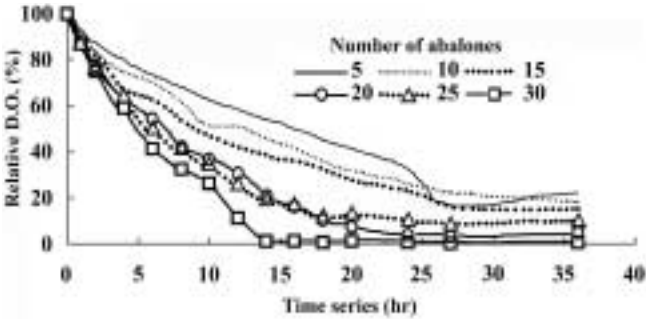

Fig. 3. The density of *Haliotis discus hannai* increases the oxygen consumption of *Haliotis discus hannai*. Note that the measurement was completed after 36 hours, when all of the tested animals were dead.

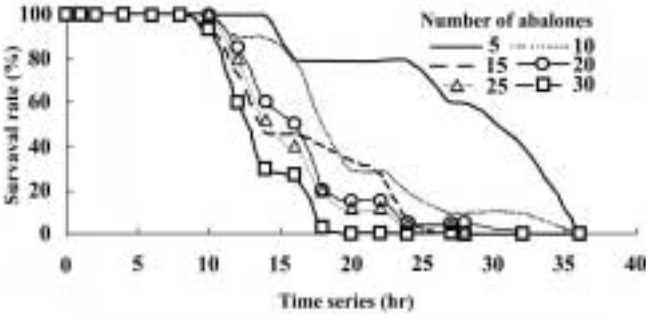

Fig. 4. Effect of the density of *Haliotis discus hannai* on the mortality. Note that the observation was completed after 36 hours, when all of the tested animals were dead.

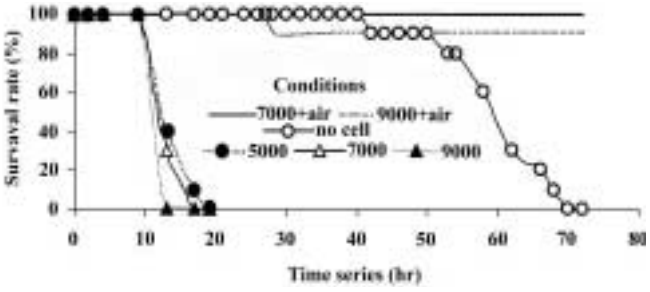

Fig. 5. Mortality of *Haliotis discus hannai* according to the amount of *Cochlodinium polykrikoides*. Note that aeration treatments, with or without *C. polykrikoides* cells, were used as control groups.

hannai started to die after five hours and all animals were lost by the time twenty hours had elapsed.

An artificial diet slows the metabolism of farmed fish, since it does not support growth in terms of protein and lipid levels. The deficiency in the polyunsaturated fatty acid (PUFA) 20: 5n-3, a key nutrient for the species, may result in slow adaptation to the formulated feed (Sales and Britz 2001; Kelly and Owen 2002). An experiment was conducted to determine whether artificial feed could

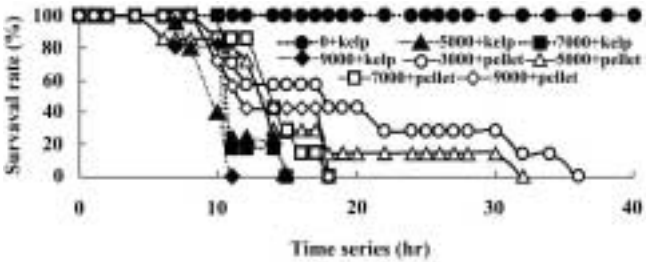

Fig. 6. Mortality of *Haliotis discus hannai* by feeding. Note that aeration treatments without *C. polykrikoides* cells were used as control groups.

contribute to desensitization of *H. discus hannai* toward the impact of *C. polykrikoides* by slowing the animal's metabolism. In this experiment (Fig. 6), the mortality of *H. discus hannai* was not significantly impacted by the supplied foods ($p = 0.078$ in the treatment with 5000 cells mL^{-1} of *C. polykrikoide*, $p = 0.26$ with 7000 cells mL^{-1}, and $p = 0.234$ with 9000 cells mL^{-1}).

DISCUSSION

Abalone is a commercially important shellfish worldwide (Sales and Britz 2001). Through the recent introduction of commercial cultivation techniques for abalones such as *Haliotis discus* Reeve and *H. discus hannai* Ino, the portion of abalone farms among fisheries has rapidly increased (NFFC 2001). In artificial cultivation, the natural mortality of abalone can reach up to 10% for many reasons (Sales and Britz 2001).

The limited survivorship of abalones may be a result of a more exposed habitat, which prevents feeding and/or creates a lack of suitable shelter (Mgaya and Mercer 1994; Kelly and Owen 2002). The survivorship is also dependent on the size of the individual: small abalone (< 8 mm) showed poor survivorship with an environmental shock such as a change in seawater temperature (Nie *et al.* 1996). Water quality, microbial contamination, prior abalone health, and variable results have been major impediments in identifying the cause and mechanism of abalone mortality (Lovko *et al.* 2003). Contamination by microorganisms introduced with water, sediment, and animals is known to be common in the aquarium environment (Burkholder *et al.* 1995, 2001; Voglbein *et al.* 2001).

Although the precise mechanisms of *Cochlodinium polykrikoides*-related red tide mortality on fish and shellfish are still poorly understood (Lim 2004), several hypothesis regarding fish kills have been proposed. In Japan, *C. polykrikoides* and another unidentified *Cochlodinium* species have been shown to produce ichthyotoxic substances consisting of three toxic fractions: neurotoxic, hemolytic and hemagglutinating (Onoue *et al.* 1985; Onoue and Nozawa 1989a, 1989b). However, the water- and chloroform-soluble fraction of methanol extracts of *C. polykrikoides* isolated from the Korean coasts did not show ichtyotoxicity (Lee 1996; Kim *et al.* 2001).

From the fish treated with *Cochlodinium polykrikoides* cells, a decrease in oxygen partial pressure (ρO_2) and the alternation of the epithelial structure and protein composition in the fish gills have been observed (Kim *et al.* 2000a, 2002). Reactive oxygen species (ROS) such as the superoxide anion (O^{2-}), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) generated from *C. polykrikoides* may also be one of the factors which induce fish kill. ROS-mediated oxidative damages are associated with the toxicity of *C. polykrikoides* (Kim *et al.* 1999, 2000b). In addition, the massive mucus production by *C. polykrikoides* may also contribute to the suffocation of the animals (Lee 1996; Cho *et al.* 1999). Future histological and biochemical studies on abalone will provide details regarding the toxicity of *C. polykrikoides*.

Many red tide organisms show a diurnal alternation of metabolism, including diurnal vertical migration (DVM), which is under the control of a biological clock (Seo and Fritz 2000; Nassaoury *et al.* 2001). For example, *C. polykrikoides* increases the amount of oxygen in the sea before dusk, when they sink downward. Increased respiration of *C. polykrikoides* is correlated with an upward migration to the surface at the end of the night period (Na *et al.* 1997; Park *et al.* 2001). Since abalones usually eat and show optimal growth during the night (Nakamura and Archdale 2001; Morikawa and Norman 2003), the use of oxygen by *C. polykrikoides* during the night would be harmful to abalones. However, the amount of dissolved oxygen used by the red tide organism was rather small. Therefore, it is reasonable to assume that the mass mortality of abalone may be attributed to suffocation from the increased cell density and mucus production by *C. polykrikoides*. This is supported by the results of this study, which showed that, with aeration, the mortality rate of abalone was reduced to 10%, of that of abalone without artificial aeration.

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