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Effect of Yellow Clay on the Oxygen Consumption Rate of Korean rockfish, *Sebastes schlegelii*

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Abstract : *Yellow clay dispersion has been applied to minimize fisheries impact by the red tide Cochlodinium polykrikoides blooms in Korean coasts since 1995. The present preliminary study documents the effect of yellow clay on Korean rockfish, Sebastes schlegelii, in terms of oxygen consumption rate (OCR). The OCR in the low clay suspension (0.05 and 0.23 %, w/w) showed normal level compared to the control. In contrast, the OCR for each one of three replicates in the high clay suspension (1.16 and 5.58 %, w/w) was not returned to the previous level that clay was not treated, indicating that high clay suspension ($\geq$1.16%, w/w) might give negative effect on Korean rockfish. Overall, this result suggests that field application of clay to control Harmful Algal Blooms (HABs) may not give impact on Korean rockfish once the clay is dispersed in a low concentration ($\leq$0.23 %). In order to understand the changes of OCR in the repeated exposure to clay, it is required to do further studies on the changes of OCR when the fish is exposed to clay repeatedly after recovery in the normal seawater.*

Key words : *Yellow clay, Fisheries impact, HABs, Oxygen consumption rate, Korean rockfish, Sebastes schlegelii*

1. Introduction

Korean rockfish, *Sebastes schlegelii*, is one of the most intensively cultured and commercially important species in Korean waters(Ministry of Maritime Affairs and Fisheries, 2003). There has been massive fish kills including Korean rockfish in aquaculture farms caused by red tide in Korean coasts since 1993(Kim, 1998; Kim et al., 2012). Particularly, there was a huge scale red tide caused by *Cochlodinium polykrikoides* in 1995, resulting in 95 million US dollars' fisheries damage (Fig. 1, sourced 2008 Korean annual red tide counterplan meeting). Thereafter, the practical field application of red tide control technique by yellow clay has been applied to minimize fisheries damages by *C. polykrikoides* blooms in Korea since 1996(Choi et al., 1998; Bae et al., 2000). Anyhow, the clay control method has been used successfully in Korea(Choi et al., 1998; Kim et al., 2012), Japan(Shirota, 1989) and China(Yu et al., 2001; Cao et al., 2012) to minimize fisheries impact by red tide.

There are several reports on HABs (Harmful Algal Blooms) mitigation techniques by clay control methods. Many of the papers emphasized the use of coagulants in addition to wild clay(Sengco et al., 2001; Pierce et al., 2004; Sengco and Anderson, 2005), modified clay(Cao et al., 2012), mixed clay(Sun et al., 2004; Lee et al., 2008), or modified clay dispenser(Bae et al., 2000) to enhance removal rate of clay aiming at the decrease of clay amount to be introduced into the sea.

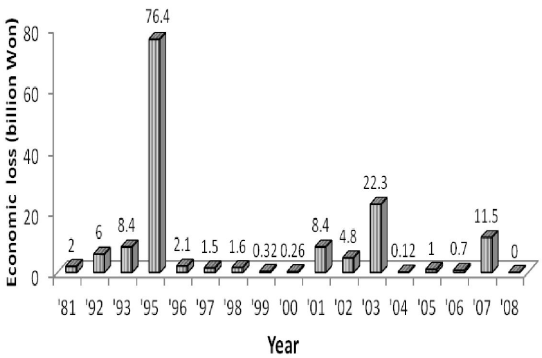

Fig. 1. Fisheries damage by red tide in Korean coasts since 1981, sourced from 2008 Korean annual red tide counterplan meeting.

While there have been relatively few studies on the impact of clay on marine organism, up to date several papers reported side effects on shellfish(Stevens, 1987; Quinn et al., 1992; Cranford and Gorden, 1992; Cranford, 1995; Archambault et al., 2002; Shumway

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et al., 2003; Seo et al., 2008) and marine bottom fauna(Howell and shelton, 1970).

To understand the possible impact of yellow clay on the fish, the effect of respiratory activities on Korean rockfish along with clay concentration was examined by using automatic intermittent-flow-respirometer (AIFR), a suitable device for measuring oxygen consumption rate.

2. Materials and Methods

2.1 Test fish and clay preparation

The juvenile Korean rockfish were acclimated to water temperature of 24～25 ℃ at least for 2 weeks under natural light condition with the supply of commercial pelleted diet once a day until 48 h before the experiment. Totally 15 juveniles (8.3 ± 0.6 cm in length and 9.0 ± 2.1 g in weight) were used for the experiment (Table 1). The fish were maintained in the test chambers (1.4 L) for 3-5 days until clay treatment to minimize environmental stress by non-steady-state effects. One individual fish was contained in each of two test chambers and three replicate experiments, each varying in duration between 7 and 9 days (168-216 h) were conducted. The chamber was kept under dark condition and constant temperature without feeding to fish during the experimental period to minimize the effect by light, temperature and feeding.

Table 1. Experimental parameters for measuring oxygen consumption rate of Korean rockfish at the different clay concentrations

Parameters	Values
Temperature (℃)	23.8-25.2
Salinity (psu)	32.7- 2.9
Number of fish (ind.)	15
Total length (cm)	8.3±0.6
Body weight (g)	9.0±2.1
Duration of experiment (hour)	168-216
Level of oxygen saturation (%)	85.5-94.8

The clay used for this experiment was collected from Tongyeong, Korea. The clay consists of quartz and elements including Si (211.7 mg/kg), Al (842.2 mg/kg), Fe (62.3 mg/kg) and Mg (337.3 mg/kg). After all discernible lumps and friable particles had been broken, yellow clay was ground and sieved with a 0.125 mm sieve following the guideline for yellow clay evaluation by National Fisheries Research and Development Institute of Korea (NFRDI, 2007). The clay was then placed in a dry oven (60±5 ℃) for two days. After cooling and weighing, powdered clay was used to make clay suspensions for 0.05, 0.23, 1.16 and 5.58% (w/w) of clay concentration. Each clay suspension was prepared based on the calculation of total water volume (21.5 L) for reservoir container (20 L), test chamber (1.4 L) and tube (0.1 L). Stirrer was equipped within the reservoir tank to prevent from clay sedimentation. Clay sedimentation within the test chambers was not detected during the experiment due to the continuous water circulation by pumping.

2.2 Experimental design

The OCR was measured with an AIFR (one system with two chambers) (Fig. 2) following the procedures described by Kim et al.(1996, 1998, 2002).

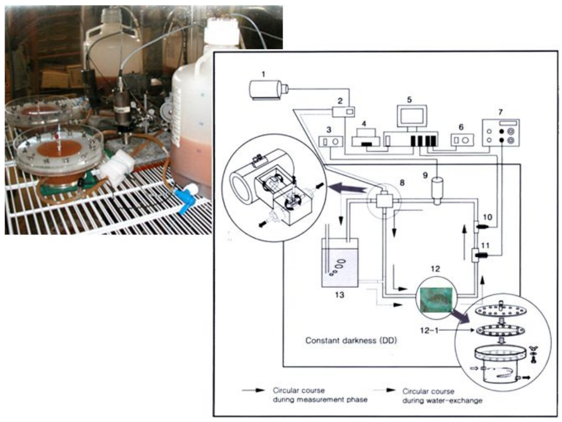

Fig. 2. Schematic diagram of automatic intermittent flow respirometer (AIFR) for measuring oxygen consumption of Korean rockfish *Sebastes schlegelii*. 1: air compressor; 2: air valve controller; 3: pump controller; 4: printer; 5: computer for control and data storage; 6: air pressure sensor; 7: picoammeter; 8: three-way valve; 9: toothed wheel pump; 10: temperature sensor; 11: oxygen sensor; 12: chamber; 12-1: silicon ring; 13: reservoir container.

The water was filtered through two sterile membrane filters (Sartorius capsule filter, input-pore diameter 0.2 ㎛ and output-pore diameter 0.07 ㎛) to remove bacteria. Oxygen levels in the chamber were maintained between 85 and 95 % saturation to minimize any stress caused by low dissolved oxygen. When the oxygen levels were below a predetermined limit, a drive gear pump (Reglo-ZS, Ismatec Sa., Wertheim, Germany) and actuator valve (TX 350-1

DA-2/1, Ilyoung, Seoul, Korea) automatically supplied the system with oxygen-saturated clay water until the desired oxygen level was reached. Fish were supplied with a constant flow of clay water (690 mL/min) driven by the gear pump. Thick-walled Tygon tubing (Nalgene 8000, Nalge Co., NY, USA) was used to connect the chamber to the dissolved oxygen probe and the three-way valve assembly, allowing the respirometer to operate in open flow-through or closed modes. No measurements were made while exchanging the chamber water with oxygen-saturated clay water (20 L). Measurements were made in a dark incubator (RI-50-1060, Revco, NC, USA) at a constant temperature (24-25 ℃), while avoiding any visual or other disturbances. Using specially designed software, the oxygen levels were monitored by a digitally controlled unit via a picoammeter (M-100, Eschweiler, Kiel, Germany). The mean oxygen consumption of the test fish was calculated at every 90 second interval and all data were graphically displayed and recorded in real time.

The oxygen content, KO_2 [mg/L], was calculated for standard conditions (atmospheric pressure $P_{atm} = 1$ atm = 1013 mbar) as a function of temperature and salinity using the formula of Weiss (1970).

$$\ln KO_2 = A_1 + A_2(100/T) + A_3 \ln(T/100) + A_4(T/100) + S[(B_1 + B_2(T/100) + B_3(T/100)^2)] \quad (1)$$

Where, T is temperature (K), S is psu at the time of measurement, and $A_1 = -173.4292$, $A_2 = 249.6339$, $A_3 = 143.3483$, $A_4 = -21.8492$, $B_1 = -0.033096$, $B_2 = 0.014259$, and $B_3 = 0.0017000$. To obtain the concentration in mg/L, the following formula was used to convert the gas volume under standard conditions, V_{std}, into the gas volume under measured conditions, V_R:

$$V_R = V_{std}\ (1013\ \text{mbar}/P_{atm})\ (T/273.15\ \text{K}) \quad (2)$$

where T (K) and P_{atm} (mbar) were taken at the time of measurement (Mortimer, 1983). Then, KO_2 (mg/L) was calculated using (Forstner and Gnaiger, 1983):

$$KO_2\ (\text{mg/L}) = KO_2\ (\text{mL/L}) \times 1.429 \quad (3)$$

Data readings including experimental time (sec), temperature (℃), air pressure (hPa), oxygen consumption (mL O_2/h), and oxygen levels (%) were stored on a hard disk for future analysis.

2.3 Analysis of oxygen consumption

The OCR was analyzed using the weighted smooth curve procedure. To plot a best-fit smooth curve through the center of the data, the locally weighted, least squared error method was used. The value of 2 % obtained from repeated tests produced a best-fit curve. Statistical values were calculated from the measured data, and presented as the mean±standard deviation (SD).

3. Results and discussion

The metabolic rates of Korean rockfish were fitted to a weighted smooth curve. In general, the fish shows abrupt variation of OCR, particularly, in the early stage for 3-5 days after stocking into the test chamber due to environmental stress by non-steady-state effects, which represent general pattern of OCR occurring during stabilization period(Jobling, 1981; Follum and Gray, 1987; Waring et al., 1996). Kim et al.(1996; 1998) monitored the OCR pattern of fish usually for 3-5 days before exposure test fish to experimental conditions/factors in their experiment, which is helpful in the comparison of variation of endogenous biological rhythm for test fish between before and after impact. The OCR of fish was monitored for 3-5 days before the injection of clay into the chamber following the method by Kim et al.(1996; 1998) even though our study was not targeted on the variation of biological rhythm. Endogenous biological rhythm was, also, observed at 0.23 % of clay concentration group for three days before the clay injection in this study (Fig. 3C). In this study, therefore, the clay was injected in three or five days after stocking the fish into the test chamber to minimize experimental error happening during stabilization period.

Overall, mean oxygen consumption rate of rockfish before clay injection ranged 1~2.7 mL O_2/g ww/h. Interestingly, the oxygen consumption by the fish showed a diurnal pattern even though the LD cycle was adjusted to constant darkness for the entire experimental period: decreasing of oxygen consumption in the day time and increasing in the night time with the periodicity of 25.35±3.75 h (Fig 3C). It was assumed that the fish was still regulated by the endogenous clock during the experiment by representing its wild nature that shows active behavior for feeding in the night time and the least activity in the day time.

In the 0.05 % (w/w) clay suspension, the OCR showed no effect (Fig. 3B). In the 0.23 % (w/w) clay suspension, periodic peak shown prior to clay injection was still observed for the last 3 days after clay injection (Fig. 3C).

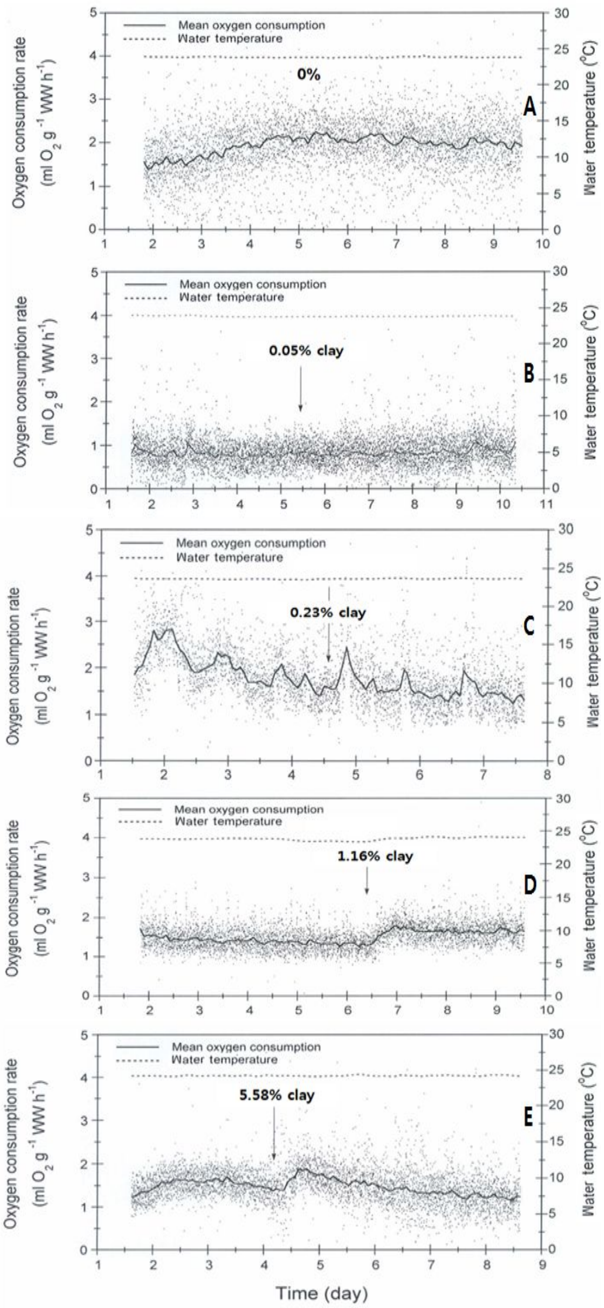

Fig. 3. Oxygen consumption by Korean rockfish, *Sebastes schlegelii* at the different clay concentrations (0, 0.05, 0.23, 1.16 and 5.58 %, w/w). The arrow indicates the time that clay was introduced. Curves of mean oxygen consumption rate (solid line) and water temperature (dotted line) are fitted to a weighted smooth curve of 2 %. A single dot represents the mean oxygen consumption rate during 90 second interval.

Accordingly, it was assumed that lower clay suspension (≤0.23 %, w/w) did not give adverse effect on oxygen consumption of Korean rockfish.

Meanwhile, in the high clay suspension (1.16 and 5.58 %, w/w), the OCR maintained its high level constantly or increased temporarily after clay injection (Fig. 3D and 3E). This results imply that high clay suspension (≥1.16 %, w/w) might give impact on the respiratory metabolism of Korean rockfish. In the 1.16 % clay suspension, one replicate showed exceptionally higher OCR than that before the clay treatment even though two replicates were almost identical. Although data is not sufficient to explain the reason, difference of fish size, primarily, might be related to the higher OCR even in the same clay concentration.

When fish are exposed to the severe environmental change, their metabolic activity is reduced or increased as a result of the physiological stress(Jobling, 1988; Morgan, 1992; Mehner and Wieser, 1994). Under these circumstances, the fish are expected to reduce their locomotion activity(Mehner and Wieser, 1994), swimming speed(Brett, 1971), acid-base regulation, osmotic balance(Reynolds and Casterlin, 1980), and growth rates(Jobling, 1988; Morgan, 1992). Also, fish secrete mucose onto the surface of their gill or skin as a defensive mechanism when they are exposed to poisonous substance or abrupt environmental changes. Herein, we cannot exclude the possibility that the mucose secreted in the gill might form coagulation with clay to deteriorate the capability of gas exchange, particularly when the test fish was exposed to high clay concentrations (≥1.16 %). Another replicate at 5.58 % (w/w) clay suspension group showed that the OCR was not recovered as long as for 3 days after clay injection likewise the test fish shown in Fig. 3D. This result might be related to the coagulation on the gill to deteriorate the capability of gas exchange during the experiment.

The effects of clay on the respiration of fish are a function of both the concentration of clay and duration of exposure. There has been reports on the possible side effects of clay on invertebrates by either enabling decrease of clearance rates and/or increase of pseudofeces production even though oyster was not so sensitive as other invertebrates(Urban and Kirchman, 1992; Shumway et al., 2003). Seo et al.(2008), also, reported that high concentration of clay (more than 0.25 %) can give negative effects on shellfish (oyster, mussel, abalone) temporarily although most of the test shellfish can recover their metabolism within a few hours after supply normal sea water. Meanwhile, there was very limited investigations on clay effects on marine organisms including vertebrates and fishes. Kaolin clay did not give any serious impact on a coral reef(Dollar and Grigg, 1981), and had little direct effect on trout gill as long as 64 days(Goldes et al., 1988).

In Korea, clay dispersion has been applied as one of the mitigation techniques, mainly, targeted on *C. polykrikoides* bloom (Choi et al., 1998; Kim et al., 2012). Therein, yellow clay has been usually dispersed into *C. polykrikoides* bloom area with 100 ~400 g/m^2 concentration, equivalent to 0.01~0.04 % based on the assumption that the water depth is 1 m, which is lower concentration than that of the lowest experimental group (0.05 %) in this study. In addition, once the clay is dispersed in the open sea, the clay concentration on aquatic animals might be much lessened. Conclusively, field application of yellow clay to control *C. polykrikoides* bloom doesn't give adverse effect on the oxygen consumption rate of aquaculture fish if the clay is dispersed under 0.23 % of clay concentration.

On the other hand, the experimental chamber was maintained with high turbidity caused by dense clay particle during the whole experimental period. Moreover, gills of test fish exposed to the high clay concentration were coated with clay. Accordingly, these conditions might be one of the possible factors that influenced on the increase of OCR, particularly at the high clay concentration. Therefore, it is required to conduct more detail experiments to clarify the interrelationship between OCR and turbidity including application to other fish based on long term observation period. In addition, it is suggested to study on the long term changes of biological rhythm of the fishes when they are exposed to clay water repeatedly after recovery from the clay exposure by stocking into the normal seawater for a certain period of time.

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