*Ocean Science Journal, Vol. 43 No. 2, 107-113(2008)*

**Article**

Ocean Science Journal

http://osj.kr

# Effects of Sodium Cyanide (NaCN) on the Endogenous Rhythm of the Oxygen Consumption Rate in the Black Rockfish *Sebastes schlegeli*

**Wan Soo Kim[1]*, Jong Wook Kim[1], Jae Hak Lee[1], and Sung Hoe Huh[2]**

[1]*Marine Living Research Department, KORDI, Ansan P.O. Box 29, Seoul 425-600, Korea*

[2]*Department of Oceanography & Korea Inter-University Institute of Ocean Science, Pukyung National University, Pusan 608-737, Korea*

Received 4 April 2008; Revised 16 May 2008; Accepted 13 June 2008

**Abstract** – Laboratory bioassays were conducted to test the acute toxicity effects of sudden exposure to sodium cyanide (NaCN) on the endogenous rhythm of the oxygen consumption rate (OCR) in the black rockfish *Sebastes schlegeli*. The OCR of the black rockfish ($n$ = 14, total length = 20.4 ± 1.16 cm, wet weight = 158 ± 25 g) was measured with an automatic intermittent-flow-respirometer. OCR decreased significantly when experimental fish were exposed to NaCN. When exposed to 10 ppb NaCN, fish were able to recover their OCR rhythmic activities. When fish were exposed to 20 ppb, however, the metabolic activity rhythms were not recovered. These results suggest that exposure to NaCN concentrations over 20 ppb cause severe physiological damage to the endogenous rhythms of black rockfish.

**Keywords** – black rockfish, endogenous rhythm, NaCN, oxygen consumption, *Sebastes schlegeli*, sodium cyanide, anesthetic

## 1. Introduction

Cyanide is introduced into surface waters mainly from industrial wastewaters such as those due to metal working/finishing operations and chemical processing, as well as from biological nitrogen metabolism and decomposition of complex- and organo-cyanides in water by bacteria, fungi, and algae (Eisler 1991). Because it is fast acting, easy to use, and relatively inexpensive, sodium cyanide (NaCN) has been widely applied as an anesthetic to capture marine aquarium fish (Rubec 1988).

Although cyanide is a potent and rapidly acting asphyxiant toxic to most organisms (Egekeze and Oehme 1980), the toxic mode of action of cyanide is reversible at certain concentrations and exposure times (Lewis 1960). When an organism is exposed to sublethal levels of a toxicant, physiological adaptation to the toxicant can make it difficult to interpret sublethal effects. While detecting toxicant-induced changes within an animal is relatively easy, determining whether the effects are deleterious or merely an expression of the organism's normal range of adaptation is often difficult (Mount and Stephan 1967). This question is of basic importance in research seeking to determine the toxicant exposure time that causes a 50% death rate in various organisms exposed to a toxicant for 24, 48, or 96 h. To overcome the numerous shortcomings of this experiment, we used pre-mortality symptoms as a means of recording rapid response to toxic stress. The most important factor in the experiment was in designing the method, especially the practical continuous online automated monitoring system (Thomas *et al*. 1996). Recently, limitations imposed by earlier procedures were alleviated by new techniques that allow noninvasive monitoring of certain rate processes in animals using computer-aided data acquisition and storage systems (Kim *et al.* 1996, 2001, 2003). An automatic intermittent-flow respirometer (AIFR) employs such techniques and was used to eliminate some of the problems associated with the traditional $LC_{50}$ technique.

Many marine organisms have endogenous rhythms that enable synchronization of their behavior and physiology with cyclic changes in the environment (Kim *et al*. 1999, 2003). The rhythms of the oxygen consumption rate (OCR) are good indicators of the general condition of an animal

*Corresponding author. E-mail: waskim@kordi.re.kr

(Kim *et al*. 1996, 1997, 1998, 2005) and have been correlated with stress from factors such as exposure to NaCN, commonly found in blast furnace operation waste (Pablo *et al*. 1997). Previous studies on the effects of sudden exposure to NaCN have focused on the survival rate or lethal levels (Carballo *et al*. 1995; Pablo *et al*. 1997; Gacsi *et al*. 2005), growth rate (Kimball *et al.* 1978), production (Chew *et al.* 1998), fertilization (Billard and Roubaud 1985), and oxygen consumption (McKenzie and Taylor 1996; Simcock *et al*. 2006). However, the recovery of physiological processes, particularly endogenous rhythms in marine animals after dampening (*i.e*. the effects of NaCN on the rhythms of the OCR), is not well documented.

The black rockfish *Sebastes schlegeli* is a coastal species that inhabits rocky reefs in waters 5-100 m in depth (Boehlert *et al*. 1986; Chung 1997). This fish is one of the most intensively cultured and commercially important species in Korea, Japan, and China, and has been showing sharply increased production in recent years (Kim and Chin 1995). The purpose of the present study was to evaluate the effects of sudden exposure to NaCN on the endogenous rhythm of OCR in black rockfish. This study is part of a broader project investigating NaCN toxicity to estuarine and marine organisms in coastal waters. The data will enable reevaluation of water quality guidelines for Korean coastal water with respect to NaCN.

## 2. Materials and Methods

### The chemical

Stock solutions were prepared by dissolving technical grade NaCN (F.W. 49.00, Assay 95.0% purity; Duksan Pure Chem. Ind. Ltd., Seoul, Korea) in distilled water. The black rockfish were exposed in a 5 liter respirometer chamber with three replicates ($n = 9$) for each exposure concentration (10, 20, and 30 ppb) and control ($n = 5$) without NaCN.

### Experiment material

The black rockfish used in this experiment were reared in a culture tank (500 liter) at 31.2-31.5 psu for 10 months at the Korean Ocean Research and Development Institute (KORDI). Fish were held continuously under laboratory conditions (12 h light:12 h dark) at 20 °C before the actual experiments, which were carried out from January to August 2006 under constant temperature and darkness conditions (CC). Before the experiments, fish were fed commercial food pellets once daily between 09:00 h and 10:00 h. Feeding was stopped 48 h before the fish were placed in a 4 liter test chamber. The mean body length (mm) and total body weight (g WW) of the experimental black rockfish ($n = 14$) was 20.4 ± 1.16 cm and 158 ± 25 g (mean ± SD), respectively (Table 1).

### Experimental design

The OCR of black rockfish was measured with an automatic intermittent-flow-respirometer (AIFR, one system with two chambers). Measurements were conducted in a constantly darkened incubator (RI-50-1060; Revco, Asheville, NC, USA) under a constant temperature of 19.7-21.2 °C (SD = 0.07-0.13). Experimental waters were filtered free of bacteria using sterile membrane filters (with two Sartorius capsule filters, input 0.2 μm and output 0.07 μm). Background oxygen consumption by bacteria was measured by running blanks (i.e., no fish) for 6-12 h before the experiments.

**Table 1.** Oxygen consumption rate (OCR) in the black rockfish *Sebastes schlegeli* after sodium cyanide (NaCN) exposure. Statistical values were computed for each batch from 5286 to 7579 measurements. Values are means ± standard deviation (SD).

| NaCN exposure | Control | 10 ppb | 20 ppb | 30 ppb |
|---|---|---|---|---|
| Total length (cm) | 21.0 ± 2.2 | 22.0 ± 2.0 | 20.3 ± 4.0 | 20.2 ± 1.6 |
| Mean wet weight (g wet weight) | 147.3 ± 4.6 | 173.3 ± 3.5 | 133.3 ± 2.3 | 143.3 ± 4.9 |
| Temperature (°C) | 19.7 ± 0.1 | 20.6 ± 0.7 | 19.9 ± 0.2 | 20.7 ± 0.7 |
| Salinity (psu) | 32.1 ± 0.1 | 32.2 ± 0.1 | 32.1 ± 0.2 | 32.0 ± 0.2 |
| Chamber volume (liter) | 5 | 5 | 5 | 5 |
| Flow rate (mL/min) | 690 | 690 | 690 | 690 |
| Duration of the experiment (h) | 137-184 | 221-230 | 168-208 | 108-139 |
| Number of test fish (n) | 5 | 3 | 3 | 3 |
| Number of experiments (N) | 5 | 3 | 3 | 3 |
| Mean decreasing rate (mL $O_2$ $g^{-1}$ WW $h^{-1}$) of oxygen consumption (%) between before (A) and after (B) exposure to NaCN = (A-B)/A | | 6.2 | 28.9 | 50.1 |

Salinity (psu) was measured with a salinometer (LF 320; Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Water for the experimental tank was passed through the 5 liter respirometer chamber using a magnetic drive gear pump (Reglo-ZS; Ismatec SA, Glattbrugg, Switzerland) at a constant water flow rate (690 mL $min^{-1}$). Thick-walled Tygon tubing was used to connect the chambers to dissolved oxygen probes and a three-way valve assembly, which allowed the respirometer to operate in an open flow-through or closed mode. Oxygen levels in the Plexiglas experimental chamber were maintained between 85% (lowest) and 95% (highest) saturation level. No measurements were made while flushing the chamber with oxygen-saturated seawater from a storage tank (20 liter) to restore the oxygen saturation level to 95%. When the oxygen level dropped below the predetermined limit, the drive gear pump and the actuator valve (TX 350-1DA-1/2; Ilyoung, Seoul, Korea) automatically supplied the system with oxygen-saturated seawater until the selected oxygen level was reached. To minimize stress, the fish were maintained in the respiration chambers for about 5 days before the exposure tests began. The difference in oxygen solubility with decreasing salt content was calculated with a computer program using a formula by Weiss (1970). The OCR was calculated from the changes in oxygen saturation level in the test chamber over time. The saturation concentration $KO_2$ [mL/L] was calculated for standard conditions (atmospheric pressure $P_{atm}$=1 atm = 1013 mbar) as a function of temperature and salinity using the Weiss (1970) formula:

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

where T is temperature [K], S is salinity [psu] at the time of measurement, and A and B are the following constants: $A_1$ = -173.4292; $A_2$ = 249.6339; $A_3$ = 143.3483; $A_4$ = -21.8492; $B_1$ = -0.033096; $B_2$ = 0.014259; $B_3$ = -0.0017000. To obtain the concentration in mg $L^{-1}$, the following conversion of gas volume $V_{Std}$ measured under the standard conditions into gas volume $V_R$ measured under experimental conditions was used:

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

with T [K] and $P_{atm}$ [mbar] being taken at the time of measurement (Mortimer 1983). Following this, $KO_2$ [mg/L] was calculated (Forstner and Gnaiger 1983):

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

Three replicate measurements were made on each exposure level (10, 20, and 30 ppb) of NaCN, using one rockfish for each experiment. Five replicate measurements were made for the control (0 ppb). More detailed descriptions of calculation methods and a schematic of the apparatus are given in Kim *et al*. (1996, 1998, 2001).

**Analysis of oxygen consumption records**

The rhythmicity of the OCR was determined by a maximum entropy spectral analysis (MESA) program using raw data transformed into 10-min lag intervals. The time series were analyzed for periodicity using MESA spectra following the procedures and algorithms described in Dowse and Ringo (1989). The analysis of the OCR rhythm was performed using the weighted smooth curve procedure of 2%. To plot a best-fit smooth curve through the center of the data, the locally weighted least squares error method was used (KaleidaGraphy custom program for Macintosh; Synergy Software, Tokyo, Japan). The value of 2% obtained from the repeated tests showed a best-fit curve. Statistical values were computed for each batch from the data points measured (Table 1). Significant differences of mean values were examined using Student's *t*-test. The values presented in this study are the means ± standard deviation.

## 3. Results

**Oxygen consumption rate (OCR) of the control group**

The OCR of the single black rockfish exhibited rhythmicity throughout the experiments for 137 h (Fig. 1A). The frequencies of rhythmicity observed were about three cycles at 24.9-h intervals with a period of 72 h (Fig. 1A). The mean oxygen consumption rate (mOCR) averaged over the entire duration of the experiment and over the entire range of oxygen levels (85.6–94.4%) was 20.1 ± 4.2 mL $O_2$ $g^{-1}$ WW $h^{-1}$ under constant darkness and a temperature of 19.7 ± 0.07 °C (CC). MESA spectra of the data set presented in Fig. 1A indicated that peaks of the OCR mainly occurred at 24.9-h intervals, corresponding to a circadian rhythm of the fish (Fig. 1B). Instantaneous OCRs also showed minor peaks at 12.5-h intervals. The five replicate experiments of varying lengths (137-184 h) corroborated the results presented in Fig. 1B, showing diurnal periodicity.

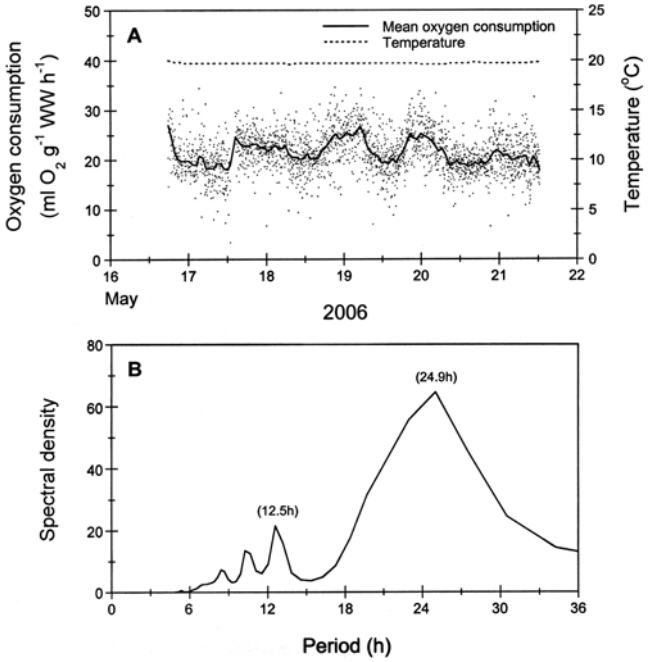


**Fig. 1.** (**A**) Time series of oxygen consumption rate (OCR) in the black rockfish *Sebastes schlegeli* not exposed to sodium cyanide (NaCN) (control). The black rockfish were kept in constant darkness at 19.7 ± 0.07 °C and oxygen levels of 94.4 to 85.6%. Curves of mean OCR were fitted to a weighted smooth curve of 2%. Dots represent mean oxygen consumption rate at 90-s intervals. (**B**) Maximum entropy spectral analysis (MESA) spectra of the black rockfish. Period lengths (h) corresponding to the dominant peaks in the MESA plots are given in parentheses.

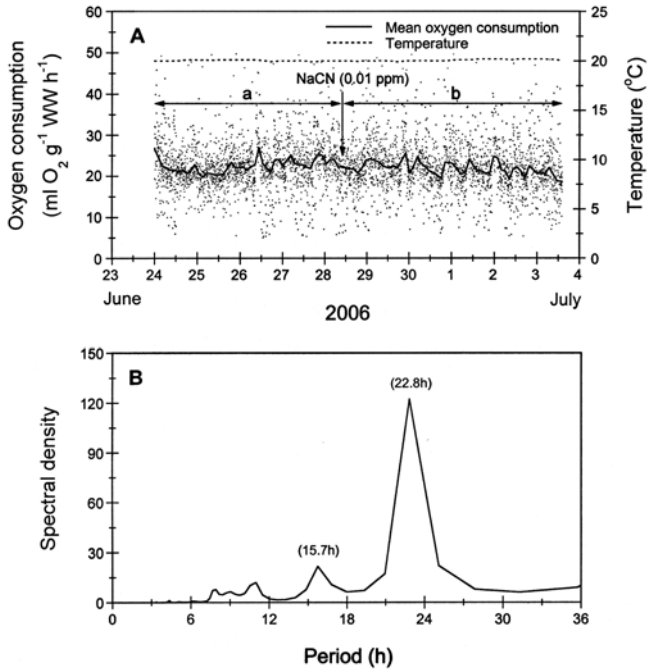


**Fig. 2.** (**A**) Patterns of oxygen consumption rate in the black rockfish *Sebastes schlegeli* before (a) and after (b) exposure to 10 ppb sodium cyanide (NaCN). (**B**) Maximum entropy spectral analysis (MESA) spectra of the black rockfish. Period lengths (h) corresponding to the dominant peaks in the MESA plots are given in parentheses.

**Effects of NaCN on the oxygen consumption rate (OCR)**

**Exposure to 10 ppb NaCN:** The OCR of the single black rockfish exhibited rhythmicity throughout the experiments for 230 h under constant darkness and a temperature of 20.8 °C (SD = 0.07) (Fig. 2A). The OCR of the fish exposed to 10 ppb NaCN was somewhat depressed for about 4-5 h. After this brief dampening of the OCR, the rockfish appeared to resume a normal metabolic rhythm, resuming the original OCR pattern. Rockfish exposed to 10 ppb consumed about 4.8% (mean = 6.2%, $p < 0.05$) less oxygen than they did before exposure (control) (Table 1). MESA spectra of the data presented in Fig. 2A indicated that peaks in the OCR mainly occurred at 22.8-h intervals, corresponding to a circadian rhythm (Fig. 2B). Instantaneous OCRs also exhibited minor peaks at 15.7-h intervals. The three replicate experiments of varying lengths (221-230 h) corroborated the results presented in Fig. 2B, showing a rhythmic pattern.

**Exposure to 20 ppb NaCN:** The instantaneous OCR of single black rockfish was observed for 136 h under constant darkness and a temperature of 19.7 °C (SD = 0.06). The observed OCRs were highly variable, ranging from 5 to 31 mL $O_2$ $g^{-1}$ WW $h^{-1}$. The rates were fitted to a weighted smooth curve of 2% (Fig. 3A). The OCR of black rockfish showed similar rhythmicity patterns: a diurnal rhythm on the first 4 days and a dampened pattern thereafter (Fig. 3A, B). The mOCR after exposure to 20 ppb NaCN was 11.2 ± 3.1 mL $O_2$ $g^{-1}$ WW $h^{-1}$, which was 20.0% (mean 28.9%, $p < 0.05$) lower than that before exposure (Table 1). MESA spectra of the data presented in Fig. 3A indicated that OCR peaks mainly occurred at 24.8-h intervals, corresponding to a circadian rhythm (Fig. 3B). Instantaneous OCRs also showed minor peaks at 12.4 and 9.2 h. The three replicate experiments of varying lengths (168-208 h) corroborated the results presented in Fig. 3A, showing a rhythmic pattern.

**Exposure to 30 ppb NaCN:** The instantaneous OCR of single black rockfish was observed for 127.0 h under constant darkness and a temperature of 21.1 °C (SD = 0.01) (Fig. 4). The OCR of the rockfish exposed to 30 ppb decreased rapidly. The mOCR of rockfish after exposure to 30 ppb was 48.6% lower (mean 50.1%, $p < 0.05$) than that of the control (Table 1), and the fish died after 4 h.

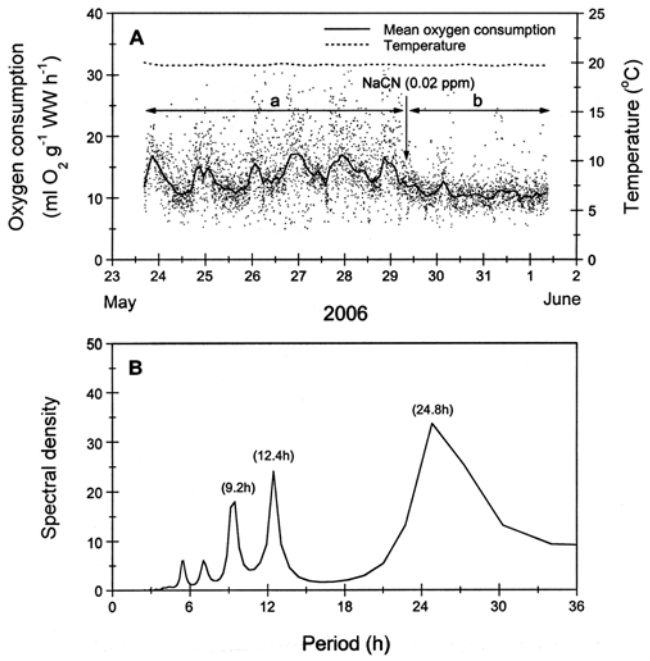


**Fig. 3.** (**A**) Patterns of oxygen consumption rate in the black rockfish *Sebastes schlegeli* before (a) and after (b) exposure to 20 ppb sodium cyanide (NaCN). (**B**) Maximum entropy spectral analysis (MESA) spectra of the black rockfish. Period lengths (h) corresponding to the dominant peaks in the MESA plots are given in parentheses.

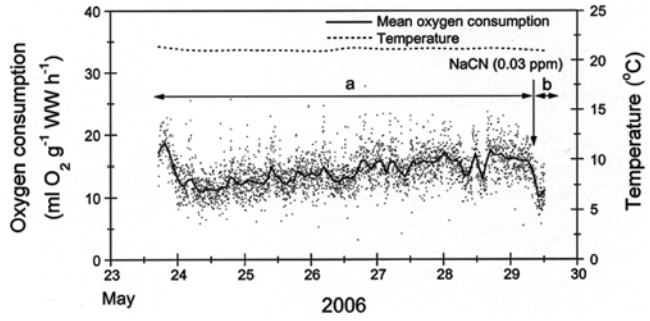


**Fig. 4.** Patterns of oxygen consumption rate in the black rockfish *Sebastes schlegeli* before (a) and after (b) exposure to 30 ppb sodium cyanide (NaCN).

## 4. Discussion

Many studies have examined observed rhythmic patterns in fish behavior and metabolic processes in relation to the ebb and flow of tides as well as in response to light conditions. In this study, black rockfish were isolated from external stimuli that might affect their rhythmic activity, such as light, temperature, food, salinity, and tides. The differences in OCR patterns between wild and cultured fish were mainly caused by differences in the environmental conditions between tidal and nontidal habitats (Yoon *et al.* 2003). In this study, as shown by the MESA (spectral density), peak OCR intervals for cultured black rockfish under constant conditions occurred mainly in a diurnal pattern, corresponding to a circadian rhythm.

Data on the toxicity of NaCN that could be used for comparison with our metabolic activity rhythm results are sparse. However, sublethal exposure to NaCN is known to significantly alter the tolerance of black rockfish *S. schlegeli*. Preexposure did not necessarily confer tolerance when black rockfish preexposed to NaCN were initially sensitized, but they later regained control-level tolerance.

After exposure to 10 ppb NaCN, fasted black rockfish maintained normal metabolic activity and OCR returned to its original rhythmic pattern. NaCN has been widely used for many years to capture marine aquarium fish because it is a fast-acting anesthetic (Rubec 1988). The present results indicate that black rockfish can recover normal metabolic activity from an anesthetized state after exposure to 10 ppb NaCN.

Figure 3 highlights the remarkable NaCN detection performances of aquatic fish compared to other biomonitors. When exposed to 20 ppb NaCN, the metabolic activity of black rockfish was depressed (28.9%) and did not recover to its preexposure state, although the fish survived for 3 days after exposure. When exposed to 30 ppb NaCN, however, the black rockfish died 4-5 h after exposure. The literature on preexposure of fish to cyanide is confusing at best; all studies have been based on the less meaningful survival time instead of the lethal threshold. For example, survival time of minnows, *Phoxinus phoxinus*, at 500 ppb HCN increased after 2 days of preexposure at 100 ppb (Malacea 1968). Moreover, an extremely toxic dose of 50 ppb NaCN immediately kills about 50% of exposed fish in the sea, and less than 40% of the fish that survive such fishing practices end up in aquariums (Simpson 2001). Other species, such as the Australian prawn (*Penaeus monodon*), showed estimated 96-h $LC_{50}$ = 110 ppb as cyanide (Pablo *et al.* 1997). These results suggest that the effect of exposure to cyanide on marine organisms is species-specific. The present results were somewhat different, with survival time after exposure to 30 ppb NaCN considerably less than 4–5 h. Therefore, the observed OCR pattern was great enough to affect the metabolic activity of the black rockfish. It appears that NaCN exposure between 20 and 30 ppb caused severe physiological damage to the metabolic activity of the black

rockfish. In the Manila clam (*Ruditapes philippinarum*), however, metabolic activity rhythm was dampened by 150–300% upon exposure to 2-4 ppm NaCN (unpublished data) compared to the black rockfish. Therefore, it appears that the physiological damage due to toxic exposure is stronger for fish than for bivalves given the same toxicant.

Because the black rockfish is one of the most important fish species cultured in northeastern Asia, the observed toxic effect of NaCN could be of practical importance when evaluating industrial wastewaters associated with iron-making operations. Black rockfish is mainly cultured in large tanks of circulating natural seawater along the coastlines of Korea, where the temperature varies from 2.5 to 30 °C throughout the year (Kim *et al.* 2003).

Although the current study did not focus on $LC_{50}$, when published $LC_{50}$ values of fisheries including prawns are compared, the results suggest that much lower concentrations are lethal to the fish subjected to the current experiment. We found that fish died or experienced serious damage to physiological activities at 20-30 ppb. However, the current experimental results were obtained using different techniques than used to measure $LC_{50}$ critical concentrations after 24-48 h, and are therefore not directly comparable. However, the current experimental technique is anticipated to be a very useful tool for analyzing biological response to toxic exposures by comparing physiological rhythms pre- and postexposure to toxic chemicals, such as cyanide for 5 days using the black rockfish.

Industrialized development, such as by metal processing industries along many coastlines, has resulted in the closure of many black rockfish aquaculture farms. More recently, untreated and unfiltered wastewaters containing cyanide from metal processing industries have become contentious issues among aquaculture communities and environmental NGSs because of the overflow of contaminated effluent into coastal waters in the summer rainy season. Such issues are expected to become more common, not only for cyanide, but also for many other toxic chemicals, as marine organisms are vulnerable to such exposure.

## References


Billard, R. and P. Roubaud. 1985. The effect of metals and cyanide on fertilization in rainbow trout (*Salmo gairdneri*). *Water Res.,* **19**(2), 209-214.

Boehlert, G.W., M. Kusakari, M. Shimizu, and J. Yamada. 1986. Energetics during embryonic development in kurosoi, *Sebastes schelgeli*. Hilgendorf. *J. Exp. Mar. Biol. Ecol.,* **101**, 239-256.

Carballo, M., M.J. Muñoz., M. Cuellar, and J.V. Tarazona. 1995. Effects of waterborne copper, cyanide, ammonia, and nitrite on stress parameters and changes in susceptibility to saprolegniosis in rainbow trout (*Oncorhynchus mykiss*). *Appl. Environ. Micro.,* **61**, 2108-2112.

Chew, S.F., E. Goh, C.B. Lim, and Y.K. Ip. 1998. Cyanide exposure affects the production and excretion of ammonia by the mudskipper *Boleophthalmus boddaerti*. *Comp. Biochem. Physiol.*, **C120**, 441-448.

Chung, M.K. 1997. The Fishes of Korea. Iljisa, Seoul, Korea.

Dowse, H.B. and J.M. Ringo. 1989. The search for hidden periodicities in biological time series revisited. *J. Theor. Biol.,* **139**, 487-515.

Egekeze, J.O. and F.W. Oehme. 1980. Cyanides and their toxicity: A literature review. *Vet. Quart*., **2**(2), 104-114.

Eisler, R. 1991. Cyanide hazards to fish, wildlife and invertebrates: A synoptic review. U.S. Department of the Interior. Fish and Wildlife Service, *Biol. Report,* **85**, 1-23.

Forstner, H. and E.Gnaiger. 1983. Polarographic oxygen sensors. Springer–Verlag, Berlin.

Gacsi, M., I. Czegeny, G. Nagy, and G. Banfalvi. 2005. Survival of fish upon removal of cyanide from water. *Environ. Res*., **97**, 293-299.

Kim, C.H. and P. Chin. 1995. The effects of dietary energy/protein ratio on oxygen consumption, ammonia nitrogen excretion and body composition in juvenile rockfish, *Sebastes schlegeli. J. Korean Fish. Soc.*, **28**, 412-420.

Kim, W.S., H.T. Huh, S.-H. Huh, and T.W. Lee. 2001. Effects of salinity on endogenous rhythm of the Manila clam, *Ruditapes philippinarum* (Bivalvia: Veneridae). *Mar. Biol*., **138**, 157-162.

Kim, W.S., H.T. Huh, J.G.. Je, and K.N. Han. 2003. Evidence of two-clock control of endogenous rhythm in the Washington clam, *Saxidomus purpuratus*. *Mar. Biol.,* **142**, 305-309.

Kim, W.S., H.T. Huh, J.H. Lee, H.Rumohr, and C.H. Koh. 1999. Endogenous circatidal rhythm in the Manila clam *Ruditapes philippinarum* (Bivalvia: Veneridae). *Mar. Biol.,* **134**, 107-112.

Kim, W.S., J.K. Jeon, S.H. Lee, and H.T. Huh. 1996. Effects of pentachlorophenol (PCP) on the oxygen consumption rate of the river puffer fish *Takifugu obscurus*. *Mar. Ecol. Prog. Ser.,* **143**, 9-14.

Kim, W.S., J.M. Kim, M.S. Kim, C.W. Park, and H.T. Huh. 1998. Effects of sudden changes in salinity on endogenous rhythm of the spotted sea bass *Lateolabrax* sp. *Mar. Biol.,* **131**, 219-225.

Kim, W.S., J.M. Kim, S.K. Yi, and H.T. Huh. 1997. Endogenous circadian rhythm in the river puffer fish *Takifugu obscurus*. *Mar. Ecol. Prog. Ser.*, **153**, 293-298.

Kim, W.S., S.J. Yoon, J.M. Kim, J.W. Gil, and T.W. Lee. 2005. Effects of temperature changes on the endogenous rhythm of oxygen consumption in the Japanese flounder *Paralichtys*

*olivaceus*. *Fish. Sci.,* **71**, 471-478.

Kimball, G.L., L.L. Jr. Smith, and S.J. Broderius. 1978. Chronic toxicity of hydrogen cyanide to the bluegill. *Trans. Am. Fish. Soc.,* **107**(20), 341-345.

Lewis, W.M. 1960. Sodium cyanide in fish management and culture. *Prog. Fish-Culturest*, **22**, 177-180.

Malacea, I. 1968. Studies on the acclimatization of fishes to high concentrations of toxic substances. *Archiv Für Hydrobiologie,* **65**, 74-75.

McKenzie, D.J. and E.W. Taylor. 1996. Cardioventilatory responses to hypoxia and NaCN in the neotenous axolotl. *Resp. Physiol.,* **106**, 255-262.

Mortimer, C.E. 1983. Chemie. Georg Thieme Verlag, Stuttgart.

Mount, D.I. and C.E. Stephan. 1967. A method for establishing acceptable toxicant limits for fish-malathion and the butoxyenthanol ester of 2, 4-D. *Trans. Am. Fish. Soc.*, **96**, 185-193.

Pablo, F., R.T. Buckney, and R.P. Lim. 1997. Toxicity of cyanide, iron–cyanide complexes, and a blast-furnace effluent to the banana prawn, *Penaeus monodon*. *Bull. Environ. Contamin. Toxi*., **58**, 822-829.

Rubec, P.J. 1988. The need for conservation and management of Philippine coral reefs. *Environ. Biol. Fish*., **23**, 141-154.

Simcock, D.C., S. Brown, J.D. Neale, S.M.C. Przemeck, and H.V. Simpson. 2006. L3 and adult *Ostertagia* (*Teladordagia*) *circumcincta* exhibit cyanide sensitive oxygen uptake. *Exp. Parasitol.,* **112**, 1-7.

Simpson, S. 2001. Fishy business. *Sci. Am*., **85**, 82-89.

Thomas, M., A. Florion, D. Chrétien, and D. Terver. 1996. Real-time biomonitoring of water contamination by cyanide based on analysis of the continuous electric signal emitted by a tropical fish: *Apteronotus albifrons*. *Water Res.,* **30**(12), 3083-3091.

Weiss, R.F. 1970. The solubility of nitrogen, oxygen, and argon in water and seawater. *Deep-Sea Res*., **17**, 721-735.

Yoon, S.J., C.K Kim, J.G Myoung, and W.S. Kim. 2003. Comparison of oxygen consumption patterns between wild and cultured black rockfish *Sebastes schlegeli*. *Fish. Sci.*, **69**, 43-49.