J. Fish. Sci Tech.. 6(2), 59-65, 2003

JOURNAL OF
FISHERIES SCIENCE AND TECHNOLOGY

Critical Thermal Maximum (CTM) of Cultured Black Rockfish, *Sebastes schlegeli*

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The critical thermal maximum (CTM) of black rockfish, *Sebastes schlegeli*, was evaluated. Black rockfish were acclimated at 24℃, and then exposed to temperatures from 24 to 33℃. Black rockfish were kept in constant darkness and subjected to a gradual temperature increase (1℃ 12 h^{-1}). The oxygen consumption rate (OCR) was measured using an automatic intermittent-flow-respirometer (AIFR) during the exposure period (from 119.3 to 143.5 h). The OCR increased from 94.5 to 214.2 mL O_2 kg^{-1} ww h^{-1} as the temperature rose from 24 to 29.4-30.9℃. Subsequently, the OCR increased abruptly, reaching 245.8-412.7 mL O_2 kg^{-1} ww h^{-1} at 32℃. This study suggests that the CTM for black rockfish is 29.4-30.9℃ when temperature is increased at 1℃ 12 h^{-1} following acclimation at 24℃.

Key words: *Sebastes schlegeli*, Black rockfish, Critical thermal maximum, CTM, Oxygen consumption

Introduction

Sebastes are ovoviviparous species in which the females carry fertilized eggs for one or two months and then spawn pelagic larvae (Larson, 1980). The black rockfish, *Sebastes schlegeli*, is a coastal species that inhabits rocky reefs and can be found at depths between 30 and 100 m (Boehlert et al., 1986). Since 1990, its commercial production has increased rapidly with the development of hatchery techniques, the use of farmed fingerlings for cage cultures in shallow coastal waters, and restocking programs. This species is one of the most intensively cultured and commercially important species in Korea and Japan. There is often massive mortality of black rockfish in aquaculture farms during the summer, which is caused by thermal discharge from power plants along the coast. Not-withstanding the considerable economic importance of the black rockfish, there have been only a few investigations of the thermal limits of *S. schlegeli* (Taka, 1981). Most attention, thus far, has been directed toward survival rates (Kang and Chang, 1998), feeding rates (Lee et al., 1993, 1994; Myeong et al., 1997), growth rates (Yi and Chang 1994; Myeong et al., 1997; Kang and Chang, 1998; Park et al., 1998), gametogenesis (Chung and Chang, 1995), reproduction (Boehlert et al., 1986), and oxygen consumption (Taka, 1981; Kim and Chin, 1995) in juvenile black rockfish. The optimum temperatures for the growth of juvenile black rockfish are between 17 and 20℃ (Taka, 1981), although the experiments that identified these limits were conducted for relatively short periods or within a narrow range of temperatures. To conclusively determine the thermal limits, long observation periods are essential. An automatic intermittent-flow-respirometer (AIFR) is a suitable device for measuring the exact thermal limits on respiratory activities (Kim et al., 1996, 1997, 2001).

This study determined the critical thermal maximum (CTM) of oxygen consumption of black rockfish exposed to a range of temperatures (24-33℃) using an AIFR. The results were used to evaluate whether the mass mortality of wild and aquaculture black rockfish is caused by high temperatures.

Materials and Methods

Animals and treatments

The black rockfish used in this study were reared

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in a 2000-L tank at the Korea Ocean Research and Development Institute (KORDI). The fish were held under laboratory conditions continuously (12 h light: 12 h dark) at 24-25℃. The fish (n=6, total length 24.7 ± 2.4 (mean ± SD) cm, wet weight (ww) 207.5 ± 83.4 g) were held in water at 32.9 psu (Table 1), and acclimated to temperatures of 24-25℃ for at least two weeks before the experiments. The fish were fed a commercial pelleted diet once daily, until 48 h before the experiments.

Experimental design

Fish were placed in a chamber individually and the oxygen consumption rate (OCR) was measured from 119.3 to 143.5 h. At the end of the tests, the individuals were removed from the metabolic chamber. To avoid "handling stress" or "non-steady-state" effects, the animals were maintained in the respiration chambers for two days before the tests.

The OCR was measured with an AIFR (one system with two chambers) (Fig. 1) following the procedures described by Kim et al. (1996, 1998, 2002). The water was filtered through two sterile membrane filters (Sartorius capsule filters, 0.2 and 0.07 μm; Sartobran pH, Sartorius, Göttingen, Germany) to remove bacteria. Oxygen levels in the 9.6-L chamber were maintained between 85 and 95% saturation to minimize any stress caused by hypoxia. When the oxygen levels were below a predetermined limit, a drive gear pump (Regle-ZS, Ismatec Sa., Wertheim, Germany) and actuator valve (TX 350-1 DA-2/1, Ilyoung, Seoul, Korea) automatically supplied the system with oxygen-saturated seawater until the desired oxygen level was reached. Fish were supplied with a constant flow of water (690 mL min^{-1}) driven by the gear pump. Thick-walled Tygon tubing (Nalgene 8000, Nalge Co., NY, USA) was used to connect the chambers to dissolved oxygen probes and a 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 water (20 L). Measurements were made in a dark incubator (RI-50-1060, Revco, NC, USA) with increasing temperatures (1℃ 12 h^{-1}) or at a constant temperature, 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 90-s intervals and all data were graphically displayed and recorded in real time.

The oxygen content, KO_2 [mg L^{-1}], 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).

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

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^{-1}, the following formula was used to convert the gas volume under standard conditions, V_{std}, into the gas volume under measured conditions, V_R:

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

Where T (K) and P_{atm} (mbar) were taken at the time of measurement (Mortimer, 1983). Then, KO_2 (mg L^{-1}) was calculated using (Forstner and Gnaiger, 1983):

$$KO_2 (\text{mg } L^{-1}) = KO_2 (\text{mL } L^{-1}) \times 1.429$$

Data readings, including local and experimental time (sec), temperature (℃), air pressure (hPa),

Table 1. Experimental parameters and "Critical thermal maximum (CTM)" of black rockfish *Sebastes schlegeli* (Values are means ± SD)

	1	2	3	4	5	6	Average (x ± SD)
Experimental temperature (℃)	24.3-31.3	24.1-31.4	24.0-32.1	24.0-32.1	24.0-31.0	24.1-32.0	
Total length (cm)	27.2	26.9	22.7	21.2	25.7	24.6	24.7±2.4
Weight (g)	330	290	189	171	129	136	207.5±83.4
Duration of the experiment (h)	119.3	143.5	138.5	135.2	128.5	136.2	133.5±8.5
Number of points measured (n)	1853	2813	3173	3583	2747	2353	2754
Level of oxygen saturation (%)	85.6-95.6	85.8-95.9	85.5-95.6	85.4-95.4	85.7-95.6	85.7-95.2	
CTM (℃)	29.4	29.4	30.9	30.9	29.6	30.0	30.1±0.7

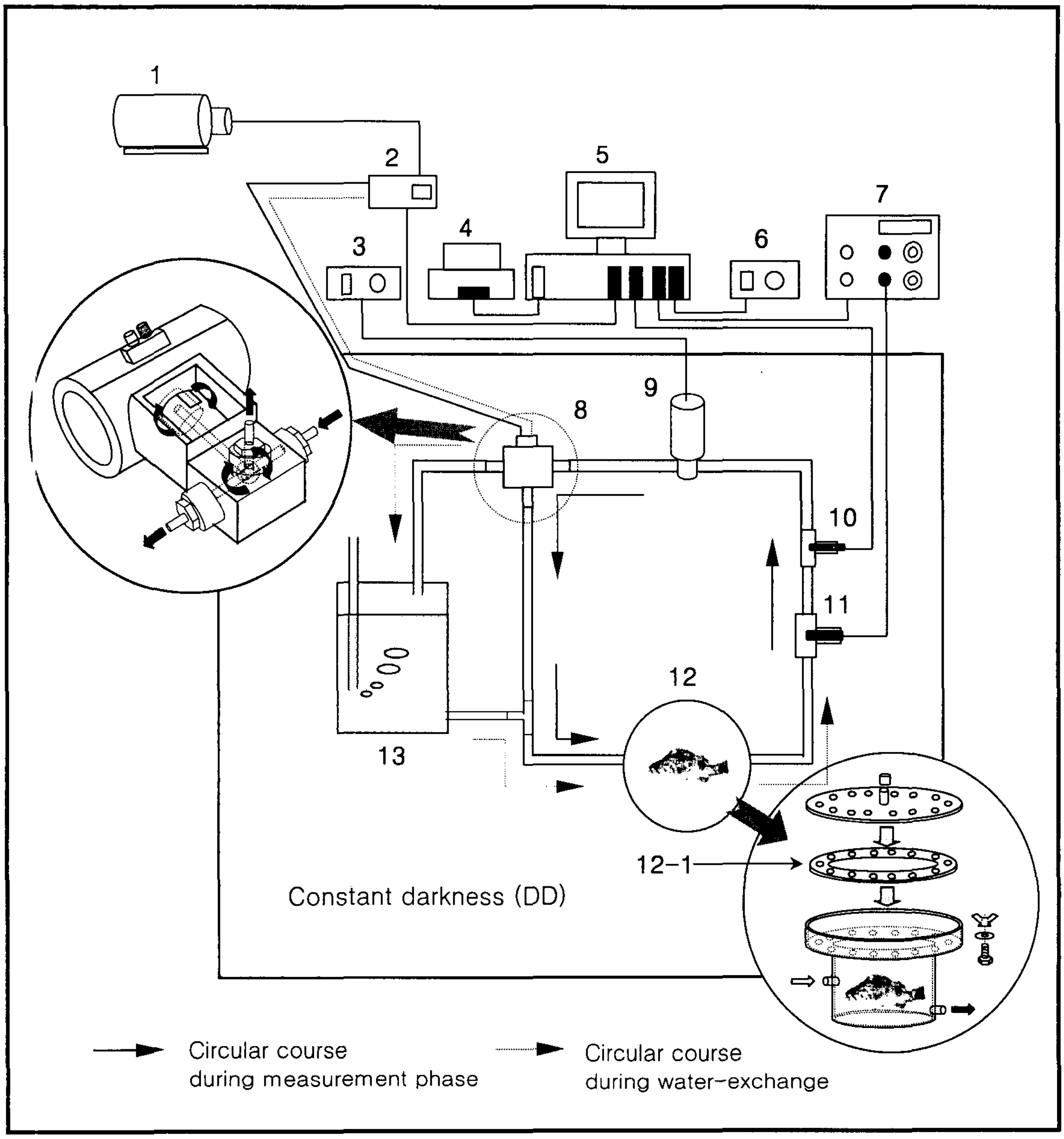

Fig. 1. Schematic of automatic intermittent-flow-respirometer (AIFR) for measuring oxygen consumption in black rockfish *Sebastes schlegeli*. 1: air compressor; 2: air valve controller; 3: pump controller; 4: printer; 5: computer for control and data storage; 6: air pressure sensor; 7: picoammeter; 8: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

oxygen consumption (mL O_2 h^{-1}), and oxygen levels (%) were stored on a hard disk for future analysis.

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 for each batch from the measured data. The data are presented as the mean ± standard deviation (SD).

Results

The metabolic rates of black rockfish were fitted to a weighted smooth curve. Fig. 2 shows a representative experiment. During the experiment, the OCR

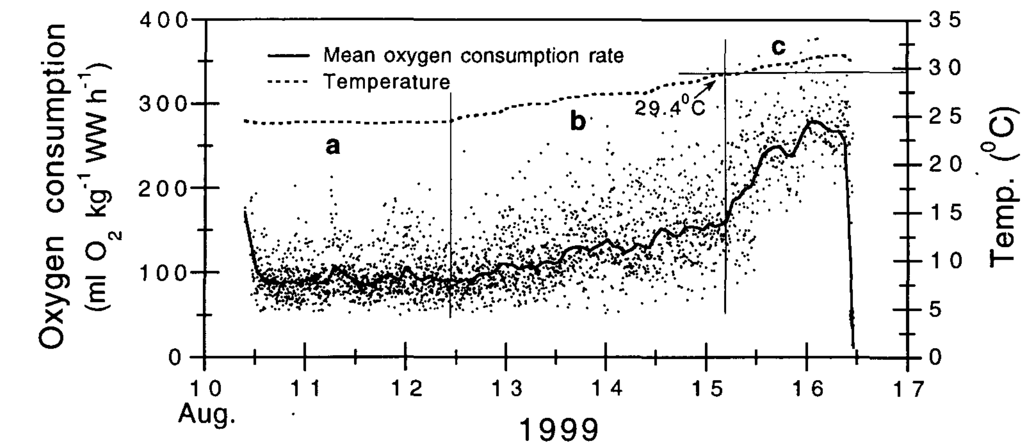

Fig. 2. Oxygen consumption by a single rockfish *Sebastes schlegeli* (290 g wet weight) acclimated at 24℃, which was subjected to a temperature increase from 24.1 to 31.4℃ during 143.5 h in constant darkness. The arrow at 29.4℃ indicates "critical thermal maximum: CTM" for oxygen consumption. The fish were kept under oxygen of 85.3 to 94.9% oxygen saturation levels. Curves of mean oxygen consumption rate and temperature (dotted line) are fitted to a weighted smooth curve of 2%. A single dot represents the mean oxygen consumption rate during 90-s intervals.

was elevated for three to four hours. The mean oxygen consumption rate (mOCR) increased in small peaks over the 48 h (Fig. 2a). The mOCR for the duration of the experiment and the entire range of oxygen levels (between 85.6 and 94.7%) was 94.5 ± 21.2 mL O_2 kg^{-1} ww h^{-1} at a constant temperature (24.1 ± 0.2℃) in darkness (Fig. 2a). The OCR increased with temperature gradually until the temperature reached 29.4℃ (Fig. 2b). Then, the OCR increased markedly from 141.7 ± 42.8 to 214.2 ± 57.4 mL O_2 kg^{-1} ww h^{-1} as the temperature increased from 24.9 to 32.1℃ (Fig. 2c). The black rockfish acclimated to 24℃ before the temperature changes survived for 31 h when exposed to temperatures of 29.4-32.1℃. Therefore, the temperature 29.4℃ appears to be the "critical thermal maximum (CTM)" for black rockfish. The OCR increased drastically, reaching a peak (maximum) at 32.1℃ (412.7 mL O_2 kg^{-1} ww h^{-1}), which was 342.2% higher than the mOCR at 24℃. The maximum OCR at temperatures above 32.1℃ was maintained for a short period, and it abruptly dropped to "near zero" with the sudden death of the rockfish.

All six replicate experiments of varying lengths produced results similar to those in Fig. 2. In black rockfish, with an increase in temperature of 1℃ 12 h^{-1}, the CTM occurred at 29.4-30.9℃ (Table 1). The OCR of black rockfish increased continuously with temperature from 24 to 29.4-30.9℃, and then rose abruptly until the temperature reached about 32℃. Therefore, 29.4-30.9℃ is the CTM of the black rockfish observed. There was a significant difference (r^2= 0.95, p<0.05) in CTM with fish size; CTM decreased gradually with increasing size (Fig. 3).

Discussion

The experimental method that we used to determine CTM differs slightly from other protocols (Jobling, 1981b; Fernandes and Rantin, 1989; Davis and Parker, 1990; Evans, 1990; Fernandes et al., 1995) in that death was used as the end point instead of a pre-lethal criterion, such as the onset of muscle spasms or loss of equilibrium. In this study, it was difficult to apply these criteria to black rockfish. No long-term studies have simultaneously monitored the OCR of fish and determined the CTM.

In this study, the OCR of black rockfish was high for the first 4 to 6 h of the experiments. This might have been due to stress caused by "handling" (Reubush and Heath, 1996; Waring et al., 1996) or "non-steady-state effects" (Jobling, 1981a; Follum and Gray, 1987).

When fish are exposed to very low or high temperatures, their metabolic activity is reduced or increased as a result of the severe physiological stress. 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,

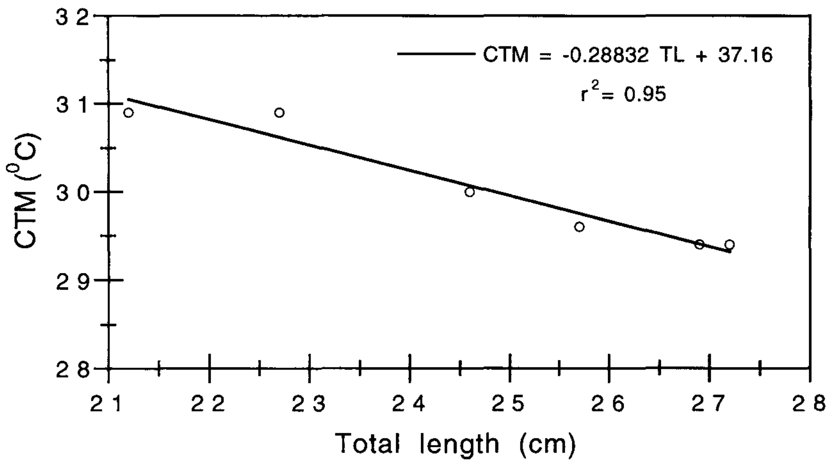

Fig. 3. Relationships between total length (cm) and "critical thermal maximum (CTM)" in black rockfish, *Sebastes schlegeli* acclimated at 24℃ and tested at 24-32.1℃.

1980), and growth rates (Jobling, 1988; Morgan, 1992). Within the thermal limit zone, the teleost OCR increases or decreases with temperature (Fry, 1971). In this study, the mOCR of the black rockfish near the CTM boundary at 29.4-30.9℃ increased by about 300%, as compared with the value of 94.5-100 ± 2.3 mL O_2 kg^{-1} ww h^{-1} at between 24 and 29℃.

Not all CTM data can be used to assess the effects of temperature changes on ecosystems. Woiwode and Adelman (1992) reported that the CTM of starved hybrid bass (*Morone saxatilis* × *M. chrysops*) averaged 0.35℃ lower than that of satiated hybrid bass. Bennett and Judd (1992) found that at temperatures slightly above the CTM, fish lose the ability to escape prevailing conditions and become vulnerable to high temperature shock.

The measured CTM is the upper boundary of the thermal resistance zone, and is determined by exposing fish to increasing water temperatures at a given rate until the fish lose equilibrium (Jobling, 1981b). CTM can vary with environmental parameters, mainly temporal and temperature changes. It may also be affected by the size (McCauley and Huggins, 1979; Barrionuevo and Fernandes, 1995), morphology, species (Lee, 1980; Menasveta, 1981), and physiological conditions (Woiwode and Adelman, 1992; Fernandes et al., 1995) of the test organisms. In this study, the CTM for 27 cm fish acclimated at 24℃ was 1.5℃ lower than that of 21 cm fish, indicating that black rockfish are more sensitive (r^2=0.95, p<0.05) to heat stress and have a lower CTM than smaller fish.

Cox (1974) found different CTM values in bluegill (Lepomis macrochirus) when fish of various sizes were subjected to different elevation rates (0.1, 0.5, and 1.0℃ min^{-1}). Cox (1974) observed a significant difference in the CTM between slower (0.1℃ min^{-1}) and faster (0.5 and 1.0℃ min^{-1}) heating rates, i.e., CTM was lower at a slower rate of heating. In our study, black rockfish were exposed to temperature increase at a much slower rate (e.g., increasing at 1℃ 12 h^{-1} from 29.8 to 32.3℃), as compared to the bluegills (Cox, 1974). This suggests that black rockfish could have an even higher CTM than the observed values (29.4-30.9℃) if acclimated at a higher temperature or subjected to faster heating rates. Since our study did not test the CTM of black rockfish acclimated at different temperatures, the effects of acclimation temperature on black rockfish cannot be compared, although it merits further study. The response of fish to fluctuating lethal temperatures is probably very complex. Temperature, rate of temperature change, and exposure time are among the factors influencing the heat resistance and tolerance of fishes.

Since the black rockfish is one of the most important fish species cultured in northeast Asia, the observed thermal limits could be of practical importance in wintering and summering the fish in aquaculture farms. This fish is mainly cultured in large tanks containing circulating natural seawater along the coast of Korea, where the water temperature varies from 2.5 to 30℃ throughout the year.

Acknowledgments

The authors are indebted to the Ministry of Science and Technology (MOST) of Korea for financial support through the National Research Laboratory (2000-N-NL-01-C-16).

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(Received December 2002, Accepted June 2003)