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Combined Effects of Hypoxia and Hydrogen Sulfide on Early Developmental Stages of White Shrimp *Metapenaeus monoceros*

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Abstract Acute toxicity of hydrogen sulfide to larval stages of white shrimp *Metapenaeus monoceros* was measured by using the continuous flow through system. Survival rates of white shrimp larvae exposed to hypoxia ($\leqq 3.4\ mgO_2\ell^{-1}$) and hypoxia ($2.5, 3.4\ mgO_2\ell^{-1}$) with two different levels of hydrogen sulfide ($5, 10\ \mu gS\ell^{-1}$) were also determined. The 48hr-LC_{50} values of hydrogen sulfide were 8.7, 11.4 and 18.5 $\mu gS\ell^{-1}$ for zoea, mysis and juvenile, respectively. Survival rates of zoea, mysis and juvenile exposed to hypoxia were significantly affected by $\leqq 3.4\ mgO_2\ell^{-1}$, $\leqq 1.5\ mgO_2\ell^{-1}$ and $\leqq 1.5\ mgO_2\ell^{-1}$, respectively. Combined effects of hypoxia and hydrogen sulfide were stronger than each single effect. Namely, survival rate was reduced under the combined exposure to $\leqq 3.4\ mgO_2\ell^{-1}+10\ \mu gS\ell^{-1}$, $\leqq 3.4\ mgO_2\ell^{-1}+10\ \mu gS\ell^{-1}$ and $\leqq 2.5\ mgO_2\ell^{-1}+10\ \mu gS\ell^{-1}$ levels to zoea, mysis and juvenile, respectively. From these results, it could be concluded that combined effects of the hypoxia and hydrogen sulfide reported in the coastal region of the Seto Inland Sea, Japan in summer stratification would have affected the larval population of white shrimp in the area.

Key words: developmental stage, hydrogen sulfide, hypoxia, toxicity, white shrimp

INTRODUCTION

Marine habitats with oxygen-deficiency present only a few possibilities for existence of marine organisms. Survival of marine organisms is affected by the availability of oxygen (DETHLEFSEN and VON WESTERNHAGEN, 1983; ROSSIGNOL-STRICK, 1985). Tolerance to low dissolved oxygen concentration differs from species to species, but survival is limited in most cases by persistent anoxia or hypoxia in combination with high summer temperature (SANTOS and SIMON, 1980).

In aquatic environment, the depletion of oxygen is often correlated with the formation of hydrogen sulfide. Species survival during hypoxia or anoxia may be decreased further by the presence of unusually high concentrations of hydrogen sulfide in sediment or by its diffusion to the sediment surface (JØRGENSEN, 1980). Although the release of hydrogen sulfide occurs in combination with oxygen depletion, combined effects of oxygen deficiency and hydrogen sulfide on marine organisms have not been extensively studied, especially on larval stages of marine organisms.

White shrimp *Metapenaeus monoceros* is one of the important shrimp in the commercial fisheries of the Seto Inland Sea, Japan and seed production of the white shrimp and stocking of the seed are widely operated in the area. While, the sediment and the overlying water of it in the coastal areas of the Seto Inland Sea are reported to contain significant levels of hydrogen sulfide, as do the lower parts of the water column after severe periods of oxygen deficiency (OCHI and TAKEOKA, 1986; KANG *et al.*, 1993).

In the present study, in order to evaluate the combined effects of hypoxia and hydrogen sulfide on the developmental stages of white shrimp in the Seto Inland Sea, laboratory toxicity experiments were carried out by using different developmental stages of white shrimp as test organisms.

MATERIALS AND METHODS

Test animals

The larvae of white shrimp *M. monoceros* were obtained from Hiroshima Prefecture Fish Farming Center. Freshly hatched larvae were immediately transferred to the laboratory and reared at 20.0-22.5℃ as was described by ANGER *et al.* (1984). Salinity, pH and dissolved oxygen were maintained at 32±0.5‰, 8.2-8.4 and 7.0-7.2 $mgℓ^{-1}$, respectively. Twenty-five larvae of zoea, mysis and juvenile of white shrimp were used as test organisms in each treatment and in the control experiment.

Test apparatus

All tests were conducted by using the flow-through apparatus as was described by KANG *et al.* (1993). Hypoxia was achieved by continuous bubbling of N_2 gas into the test medium, and the flow rate was adjusted with flow meters. Stock solution of hydrogen sulfide was prepared by dissolving known amounts of sodium sulfide in distilled water. The concentration of hydrogen sulfide in each test chamber was maintained by adjusting the introduction of stock solution and actual test concentrations of hydrogen sulfide during the experiment were also monitored by methylene blue method (FONSELIUS, 1983). Test medium was analyzed twice a day for hydrogen sulfide, dissolved oxygen (Winkler's method), pH (pH meter D-11), salinity (YSI Model 133) and temperature (thermometer).

Toxicity of hydrogen sulfide

Acute toxicity of hydrogen sulfide to zoea, mysis and juvenile of white shrimp were determined for a period of 48 hours. Experimental setup to determine the median lethal concentration (LC_{50}) consisted of four hydrogen sulfide concentrations (5.5±0.7, 10.2±1.5, 19.5±1.2, 32.3±1.8 $\mu gSℓ^{-1}$) and a control was maintained without hydrogen sulfide. Three chemical species of hydrogen sulfide (H_2S, HS^- and S^{2-}) exist in sea water depending on the pH (GOLDHABER and KAPLAN, 1975; POWELL *et al.*, 1979), but for the purposes of the present experiments, no distinction was made between the species. Dissolved oxygen concentration was maintained at 7.3-7.5 $mgℓ^{-1}$ for control test. Larvae were not fed during the toxicity tests. The 48hr-LC_{50} value was estimated for each bioassay by the graphical method (APHA, 1989).

Combined effects of hypoxia and hydrogen sulfide

Survival rates of different developmental stages of white shrimp exposed to hypoxia ($\leq$3.4 $mgℓ^{-1}$) and hypoxia (2.5, 3.4 $mgℓ^{-1}$) with two different levels of hydrogen sulfide (5, 10 $\mu gSℓ^{-1}$) were also determined. Hypoxia conditions (1.5, 2.5, 3.4 $mgℓ^{-1}$) were maintained by introducing O_2 gas and N_2 gas. Dissolved oxygen concentrations in the hypoxia treatments were monitored using Winkler's Method. Hydrogen sulfide levels were maintained by mixing hydrogen sulfide in oxygen-free water at the entrance of the test chambers with a proportional amount of oxygen-saturated water to achieve the desired oxygen and hydrogen sulfide concentrations. Control experiment was carried out at 7.4 mgℓ^{-1} dissolved oxygen without hydrogen sulfide. The zoea were fed with a microalga *Nannochloropsis* sp., while the mysis and juvenile were fed with hatched nauplii of *Artemia salina*. Differences of survival rates between replicate treatments and control were analyzed by analysis of variance (ANOVA).

RESULTS

Toxicity of hydrogen sulfide

Experimental results of hydrogen sulfide on white shrimp were shown in Fig. 1 and Table 1. Survival rate of white shrimp decreased with increasing hydrogen sulfide concentration, and that of zoea was lower than that of mysis or juvenale (Fig. 1). The 48hr-LC_{50} values of hydrogen sulfide for different developmental stages were 8.7, 11.4 and 18.5 μgS

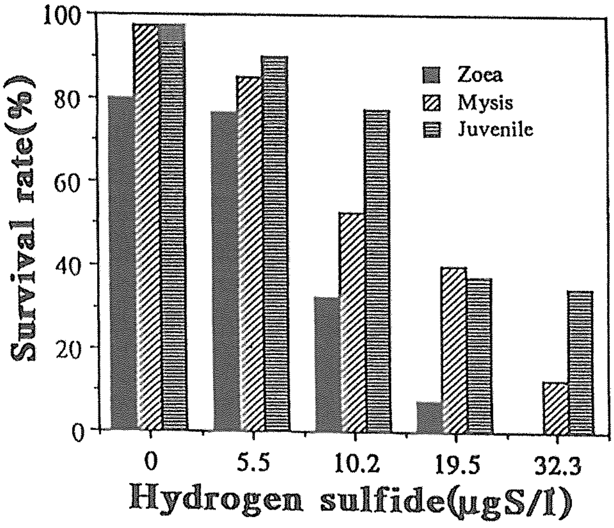

Fig. 1 Survival rate of *M. monoceros* after 48 -hr exposure to different concentrations of hydrogen sulfide.

Table 1. Experimental conditions and 48 hours median lethal concentration (LC_{50}) of hydrogen sulfide for three developmental stages of *M. monoceros*. Data are presented as ^{A}range or ^{B}mean±SD (n=6).

Stage	Experimental conditions				48hr-LC_{50} ($\mu gS\ell^{-1}$)
	Temp.^{A} (℃)	Sal.^{A} (‰)	pH^{A}	DO^{B} (mgℓ^{-1})	
Zoea	21.5±1.3	32±0.5	8.2±0.2	7.3±0.3	8.7
Mysis	21.0±1.4	32±0.5	8.1±0.3	7.4±0.2	11.4
Juvenile	22.2±1.2	32±0.5	8.1±0.1	7.3±0.3	18.5

ℓ^{-1} to zoea, mysis and juvenile, respectively (Table 1).

Combined effects of hypoxia and hydrogen sulfide

Water quality of the test medium used in the experiments were shown in Table 2. Dissolved oxygen concentrations varied little during the experiments, showing the standard deviation (SD) between 0.12 and 0.24 $\text{mg}\ell^{-1}$ in the dissolved oxygen treatment. The SD of hydrogen sulfide concentration fluctuated between 0.14 and 0.18 $\mu\text{gS}\ell^{-1}$. Survival rates of white shrimp under hypoxia are presented in Fig. 2. During the 72-hr experiments, survival rate decreased with decreasing oxygen concentration. After 64-hr exposure, there was no survival of zoea at the lowest test concentration of dissolved oxygen (1.5 $\text{mg}\ell^{-1}$). Significant decrease in the survival rate was observed between control and the test concentrations of $\leqq 3.4\ \text{mgO}_2\ell^{-1}$, $\leqq 1.5\ \text{mgO}_2\ell^{-1}$ and $\leqq 1.5\ \text{mgO}_2\ell^{-1}$, respectively for zoea, mysis and juvenile (ANOVA, $P<0.05$).

Combined effects of hypoxia and hydrogen sulfide were generally estimated to be stronger than the single effect of hypoxia (Fig. 3). Namely, no survival of zoea after 56 hours occurred under the combination of 2.5 $\text{mg}\ell^{-1}$ dissolved oxygen and 10 $\mu\text{gS}\ell^{-1}$ hydrogen sulfide. Significant decrease in survival rate of zoea, mysis and juvenile occurred at $\leqq 3.4\ \text{mgO}_2\ell^{-1}+10\ \mu\text{gS}\ell^{-1}$, $\leqq 3.4\ \text{mgO}_2\ell^{-1}+10\ \mu\text{gS}\ell^{-1}$ and at $2.5\ \text{mgO}_2\ell^{-1}+10\ \mu\text{gS}\ell^{-1}$, respectively (ANOVA, $P<0.05$).

DISCUSSION

Depending on the developmental stage, survival rate of zoea, mysis and juvenile of *M. monoceros* exposed to hypoxia was significantly affected by the test concentrations $\leqq 3.4\ \text{mg}\ \text{O}_2\ell^{-1}$, $\leqq 1.5\ \text{mgO}_2\ell^{-1}$ and $\leqq 1.5\ \text{mgO}_2\ell^{-1}$, respectively. Minimum dissolved oxygen requirements for various aquatic organisms vary widely among species. For example, summer flounder showed stress at 5.1 $\text{mg}\ell^{-1}$ dissolved oxygen, while goldfish tolerated oxygen levels as low as 0.5 $\text{mg}\ell^{-1}$ (DAVIS, 1975). Brown shrimp, *Penaeus aztecus*, and another white shrimp, *Penaeus setiferus* were able to tolerate oxygen levels as low as 2.0 $\text{mg}\ell^{-1}$ in rearing ponds, although they showed some indications of stress (BROOM, 1971). But non visible stress was noted at or above 4.0 $\text{mg}\ell^{-1}$ of dissolved oxygen. However, these

Table 2. Experimental conditions of the survival experiments under hypoxia and hypoxia with hydrogen sulfide. Temperature, 20.2～22.5℃; Salinity, 31.2～32.5‰. Data are presented as mean±SD (n=6).

	Test chambers				
	1	2	3	4	5
Hypoxia					
DO ($\text{mg}\ell^{-1}$)	1.54±0.12	2.47±0.21	3.44±0.19	7.32±0.24	–
pH	7.82±0.23	7.87±0.28	7.81±0.32	7.85±0.24	–
Hypoxia+H_2S					
DO ($\text{mg}\ell^{-1}$)	2.48±0.16	2.53±0.17	3.42±0.21	3.38±0.14	7.36±0.15
H_2S ($\mu\text{gS}\ell^{-1}$)	5.43±0.14	10.17±0.18	5.38±0.21	10.22±0.18	0.00
pH	7.78±0.28	7.80±0.23	7.83±0.20	7.75±0.26	7.72±0.16

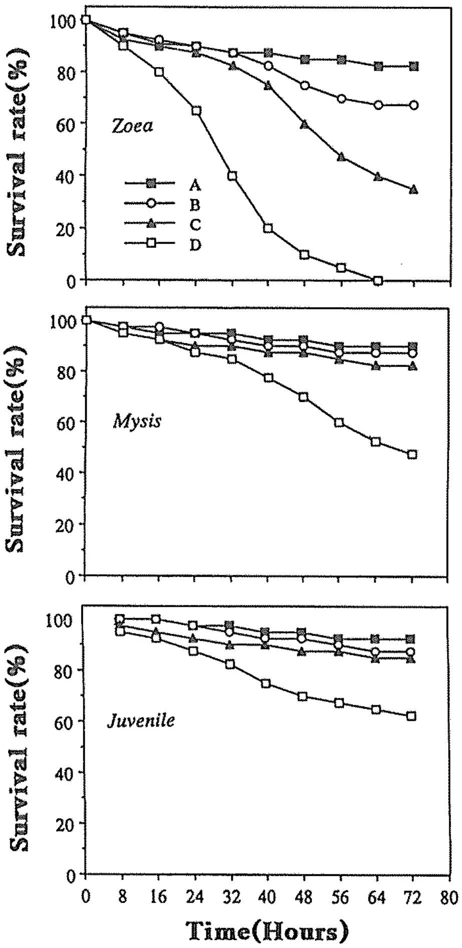

Fig. 2 Survival rate of *M. monoceros* exposed to different concentrations of dissolved oxygen during 72 hours. A, Control (7.3 mg ℓ^{-1}); B, 3.4 mg ℓ^{-1}; C, 2.5 mg ℓ^{-1}; D, 1.5 mg ℓ^{-1}.

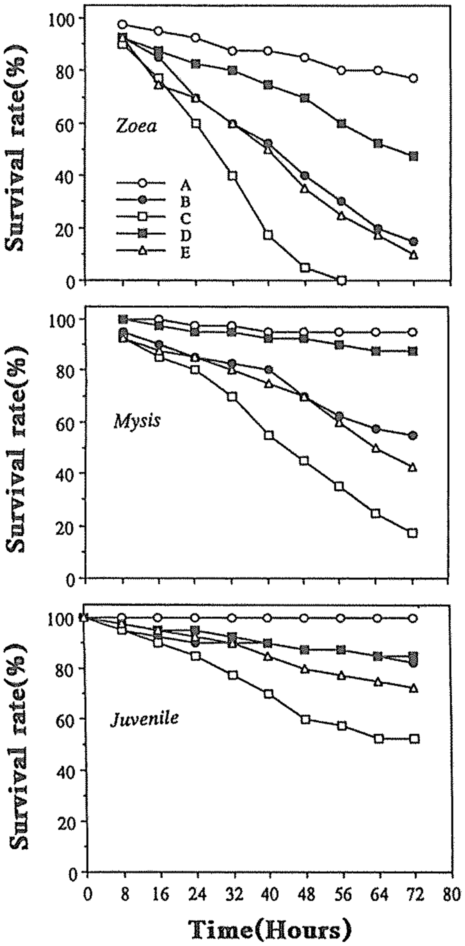

Fig. 3 Survival rate of *M. monoceros* exposed to different concentrations of hypoxia and hydrogen sulfide during 72 hours. A, Control (7.4 $mgO_2\ell^{-1}$); B, 2.5 mgO_2 ℓ^{-1}+5 μgS ℓ^{-1}; C, 2.5 mgO_2 ℓ^{-1}+10 μgS ℓ^{-1}; D, 3.4 mgO_2 ℓ^{-1}+5 μgS ℓ^{-1}; E, 3.4 mgO_2 ℓ^{-1}+10 μgS ℓ^{-1}.

reported results are for adult aquatic organisms, and responses of larval exposure to hypoxia are poorly understood. KANG *et al.* (1993) found that survival of blue crab *Portunus trituberculatus* under hypoxia was correlated with developmental stages. Zoea stage was significantly sensitive than megalopa and juvenile crab. In the present study similar results were also obtained. Namely, higher mortality of *M. monoceros* exposed to hypoxia occurred in zoea stage exposed to hypoxia than mysis and juvenile. These results indicate that resistance of marine crustacean larvae to hypoxia increased with progressing developmental stages. It could be suggested that zoea population of *M. monoceros* in their natural habitats could be affected by the exposure to $\leqq 3.4\ mg\ell^{-1}$ dissolved oxygen. The presence of hydrogen sulfide (10 $\mu gS\ell^{-1}$) during hypoxia significantly increased mortality in all stages of *M. monoceros* tested in this study. Several toxicity studies have been conducted on marine invertebrates in which hydrogen sulfide exposure was coupled with hypoxia. Studying the coot clam *Mulinia lateralis*, SHUMWAY *et al.* (1983) found that survival during hypoxia with hydrogen sulfide was much lower relative to survival under hypoxia alone. Of two congeneric species of mudflat clams, *Macoma nasuta* showed higher tolerance to hydrogen sulfide than *Macoma secta*, and both showed lower tolerance to hypoxia in the presence of hydrogen sulfide (LEVITT and ARP, 1991). The burrowing intertidal polychaete *Cirriformia tentaculata* survived 10 days under anoxia and 5 days under anoxia plus $200\,\mu gS\ell^{-1}$ hydrogen sulfide (BESTWICK *et al.*, 1989). KANG *et al.* (1993) observed that survival of blue crab *Portunus trituberculatus* during hypoxia ($10\,\mu gS\ell^{-1}$) with hydrogen sulfide is much lower than under hypoxia alone. In the present study, similar results were obtained, *i. e.* survival rate of *M. monoceros* larvae was lower than that exposed to hypoxia alone. These results indicated that population of *M. monoceros* larvae in their natural habitats could be affected further by hypoxia ($\leqq 3.4\,mg\ell^{-1}$) in combination with hydrogen sulfide ($\geqq 10\,\mu gS\ell^{-1}$) Several studies were reported that high hydrogen sulfide concentrations ($\geqq 10\,\mu gS\ell^{-1}$) in the bottom water of the Seto Inland Sea associated with low dissolved oxygen ($\leqq 3.2\,mg\ell^{-1}$) during the summer stratification (OCHI and TAKEOKA, 1986; KANG *et al.*, 1993). The results of the present study suggest that toxicity of hypoxia and hydrogen sulfide were found to differ according to the developmental stages of *M. monoceros*. Namely, survival rate was affected by the combined exposure to $\leqq 3.4\,mgO_2\ell^{-1}+10\,\mu gS\ell^{-1}$, $\leqq 3.4\,mgO_2\ell^{-1}+10\,\mu gS\ell^{-1}$ and $\leqq 2.5\,mgO_2\ell^{-1}+10\,\mu gS\ell^{-1}$ levels to zoea, mysis and juvenile, respectively. Zoea and mysis stages of *M. monoceros* are presumably affected by the critical bottom water conditions during vertical migration. Juveniles tend to dive into sediments where more serious effects of hypoxia and hydrogen sulfide are resulted compared with that in the bottom water. From the experimental data of the present study, it may be concluded that hydrogen sulfide observed in bottom water in combination with low dissolved oxygen during summer may affect the larval population of white shrimp *M. monoceros* in the Seto Inland Sea, Japan.

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ヨシエビの初期発達段階に及ぼす貧酸素と硫化水素の複合的影響

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貧酸素と硫化水素が単独あるいは複合的にヨシエビの初期発達段階に及ぼす影響を実験的に明らかにした。ヨシエビのゾエア期，ミシス期，稚エビ期幼生に対する硫化水素の 48hr-LC_{50} は，それぞれ 8.7, 11.4, 18.5 $\mu gS\ell^{-1}$ であった。貧酸素条件下でヨシエビの幼生の生存率は，ゾエア期では DO 濃度 3.4 $mg\ell^{-1}$ 以下，ミシス期と稚エビ期では DO 濃度 1.5 $mg\ell^{-1}$ で有意に減少した。貧酸素と硫化水素の複合的な条件下でヨシエビ幼生の生存率は，ゾエア期とミシス期では DO 濃度 3.4 $mg\ell^{-1}$ 以下と硫化水素濃度 10$\mu gS\ell^{-1}$，稚エビ期では DO 濃度 2.5 $mg\ell^{-1}$ 以下と硫化水素濃度 10 $\mu gS\ell^{-1}$ で貧酸素単独条件下よりも有意な減少を示した。以上の結果を瀬戸内海の夏季の底層水中の溶存酸素と硫化水素濃度に適用してみると，そこに生息するヨシエビ幼生の資源は海域によって大きな悪影響を受けるものと考えられる。

キーワード：毒性，発達段階，貧酸素，ヨシエビ，硫化水素