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Effects of Hydrogen Sulfide on the Early Developmental Stages of Javanese Carp, *Puntius gonionotus* (Bleeker)

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Abstract

The toxicity of hydrogen sulfide (H_2S) to the larvae and eggs of the Javanese carp, *Puntius gonionotus* (Cyprinidae), was determined using a flow-through bioassay. The eggs and larvae showed similar low tolerances to H_2S. The EC_{50} value for the eggs was 2.8 µg l^{-1} H_2S and the 96h-LC_{50} (median lethal concentration) for the larvae was 2.7 µg l^{-1} H_2S. One hundred percent of the eggs failed to hatch at H_2S levels higher than 3.4 µg l^{-1} H_2S, while a 100% mortality of the larvae occurred at 5.4 µg l^{-1} H_2S. The growth rate of the larvae exposed to H_2S level higher than 0.6 µg l^{-1} was significantly reduced. Newly hatched larvae from eggs exposed to more than 0.6 µg l^{-1} H_2S showed tail deformities. Histological studies showed increasing degrees of hyperplasia in fish gills at increasing H_2S concentra-tions. This study indicates that *P. gonionotus* is vulnerable to organic pollution in the aquatic environment.

Introduction

Hydrogen sulfide (H_2S) is a toxic compound that occurs in natural waters as a result of the anaerobic decomposition of organic matter in sediments, domestic sewerage, pulp-mill wastes and other industrial effluents. In thermally stratified lakes, high concentrations of H_2S are found in hypolimnetic waters (Reynolds and Haines 1980). Many species of fish spend all or part of their life in shallow waters and may experience sulfide toxicity near the bottom. Many fishes lay eggs and spend their early and critical development stages at the bottom. Smith and Oseid (1974) reported that H_2S levels commonly occurring in nature could be lethal to eggs and larvae of several freshwater fish species. Lethal concentrations of H_2S would limit fish survival and reduce fishery production (Smith *et al.* 1976). Porter *et al.* (1986) reported that sulfide level in the aged pond sediment could be as high as 500 $\mu g\ l^{-1}$. Unionized hydrogen sulfide above 0.3 – 5.0 $\mu g\ l^{-1}$ H_2S is toxic to freshwater catfish *Mystus*

nemurus, Bagridae (Hoque *et al.*, unpubl. data) and shrimp *Penaeus monodon*, Penaeidae (Law 1988).

Little work has been done on H_2S toxicity in tropical fish (Law 1988; Hoque *et al.* 1996a, 1996b; Hoque *et al.* 1998) compared to fishes in temperate region (Bonn and Follis 1967; Aldelman and Smith 1970, 1972; Smith and Oseid 1972, 1975; Broderius *et al.* 1977; Reynolds and Haines 1980; Bagarinao and Vetter 1993). As H_2S is toxic and frequently occurs in fish ponds, this study was carried out to determine the acute and chronic toxicity of H_2S to *Puntius gonionotus* (Bleeker), Cyprinidae, an important commercial species in Malaysia, in its early developmental stages.

Materials and Methods

Test Organisms

P. gonionotus larvae were obtained from the Bukit Tinggi Fish Breeding Station (Department of Fisheries) in Pahang, Malaysia. The larvae were acclimatized for one week in the Aquatic Ecology Laboratory in Universiti Putra Malaysia (UPM) before they were used in the experiments. The mean weight and total length of the larvae were 0.05±0.02 g and 1.6±0.2 cm, respectively. The one-hour-old fertilized eggs were collected from the Fish Hatchery Unit, UPM, and used immediately for the toxicity tests.

Experimental Set-up and Hydrogen Sulfide Assay

A flow-through bioassay system for hydrogen sulfide was employed in this study (Law and Yusoff, unpubl. method). Under this system, hydrogen sulfide levels were maintained within 10% of the mean value throughout the experimental period. Hydrogen sulfide stock solutions in the ranges between 1,000-10,000 mgl^{-1} total hydrogen sulfide, in oxygen-free deionized water at pH 9 were prepared daily and kept at 4°C. The analytical grade sodium sulfide ($Na_2S.9H_2O$) (Fluka, Switzerland) was used as the source of H_2S. The stock solutions were pumped into bioassay flasks (1-l Erlenmeyer flasks) using DESAGA peristaltic pumps and instantly mixed with a large volume of seasoned oxygenated water at room temperature. To avoid rapid disappearance of hydrogen sulfide from the bioassay flasks, no aeration was given to the test solutions. The incoming oxygenated seasoned water was able to maintain the dissolved concentrations of the test solutions higher than 5 mgl^{-1}. The total hydrogen sulfide in the test solutions was analyzed daily according to the standard method and the unionized hydrogen sulfide concentrations were calculated from pH and the equilibrium constant (Cline 1969; APHA-AWWA-WPCF 1989; Boyd 1990). Water quality parameters such as temperature, pH and dissolved oxygen were monitored routinely throughout the experiments to ensure that water quality was acceptable for the growth of the fish larvae. Dissolved oxygen was measured with a YSI oxygen meter (Model 57) and pH with a Corning pH meter (Model SA520). No significant difference ($p>0.05$) of water temperature (means ranged from 27.8-28.0 °C), pH (7.13-7.57) and dissolved oxygen (7.0–8.1

mgl^{-1}) values in different treatments were detected throughout the study period. During the experiment, sulfide concentrations fluctuated at ±18.9. According to Sprague (1990), fluctuations of toxicant concentration within ±20% of the mean during flow-through bioassays are acceptable.

Acute Toxicity Test on the Eggs

The toxicity tests on eggs were done in 500-ml beakers using the flow-through bioassay. Thirty one-hour fertilized eggs were randomly spread on the bottom of each beaker. The H_2S concentrations in this test were 0.11±0.05, 0.62±0.16, 2.03±0.52 and 3.39±0.74 μg l^{-1} H_2S. A treatment without hydrogen sulfide served as a control. All treatments were in triplicates. The hatching rate in each beaker was recorded. The experiment was conducted for four days. The EC_{50} value to determine the hydrogen sulfide concentration at which 50% of the eggs failed to hatch was calculated using the probit method (APHA-AWWA-WPCF 1989).

Acute and Chronic Tests on the Larvae

The acute and chronic tests on the fish larvae were conducted in 1-l Erlenmeyer flasks, using the flow-through bioassay system. The H_2S concentrations were 2.07±0.61, 2.77±0.22, 3.80±0.67 and 5.35±0.72 μg l^{-1} H_2S in the acute toxicity tests and 0.03±0.01, 0.61±0.12, 1.90±0.35 and 3.41±0.50 μg l^{-1} H_2S in the chronic tests. The controls did not contain any hydrogen sulfide. Ten larvae were stocked in each flask at a total weight not exceeding one gram per liter of water. All the five treatments with three replicates were run at the same time using DESAGA peristaltic pumps to maintain the hydrogen sulfide concentrations. The acute toxicity experiment of the larvae ran for four days while the chronic toxicity test ran for 22 d.

No feeding was given to the larvae during the acute toxicity tests. In the chronic toxicity tests, the larvae were fed *ad libitum* daily with micropellets. Fish mortality was recorded at six-hour intervals and dead fish were removed immediately. Fish were considered dead when they showed no respiratory movements and no response to gentle prodding. The LC_{50} values were determined using the probit method. Both total length and weight of the fish larvae in the chronic toxicity tests were measured to determine their growth rates. Total length of the fish was measured to the nearest 0.1 cm and the body weight to the nearest 0.01 g. The differences in growth rates in the various treatments were analyzed using ANOVA and Duncan Multiple Range test according to Zar (1984) using Statistical Analysis System (SAS) computer software.

Histopathological Examination

Fish gills were dissected after 22 days of exposure to different sublethal doses of H_2S, or no H_2S. Gills were immediately preserved in 10% buffered formalin. They were then decalcified and processed by standard histopathological techniques, sectioned at 4 μm, then stained with hematoxylin and eosin.

Results and Discussion

The acute toxicity tests revealed that H_2S was highly toxic to eggs and larvae of *P. gonionotus*. One hundred percent mortality of eggs and larvae was found at H_2S levels higher than 3.4 μg l^{-1} and 5.4 μg l^{-1}, respectively (Figs. 1 and 2). The EC_{50} value of H_2S for the eggs was 2.8 μg l^{-1} while the 96-h LC_{50} for larvae was 2.7 μg l^{-1} H_2S. The effects of H_2S is generally more severe on fish larvae than on fish eggs. This is true for goldfish (*Carassius auratus*, Cyprinidae) and fathead minnows (*Pimephelas promelas*, Cyprinidae) (Smith and Oseid 1974), and northern pike (*Esox lucius*, Esocidae) (Bonn and Follis 1967). However, a reverse phenomenon was observed for goldfish and bluegills (*Lepomis macrochirus*, Centrarchidae), where the EC_{50} and 96-h LC_{50} values of H_2S for eggs and larvae were 20 μg l^{-1} and 25 μg l^{-1}, and 16 μg l^{-1} and 30 μg l^{-1}, respectively (Smith and Oseid 1974). In this study, eggs of larvae of *P. gonionotus* were equally sensitive to H_2S. The results also indicated that *P. gonionotus* was more sensitive to hydrogen sulfide than other fishes. The EC_{50} and LC_{50} of H_2S for temperate fish species are considerably higher, probably due to the lower temperatures (9-21°C) used in the former than in the present study (28°C). Adelman and Smith (1972) reported that

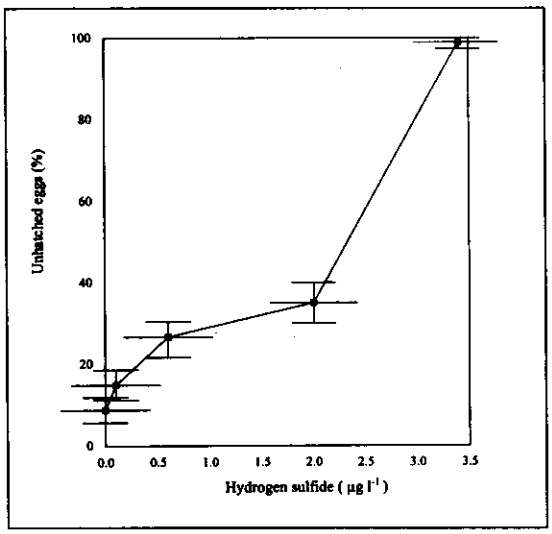
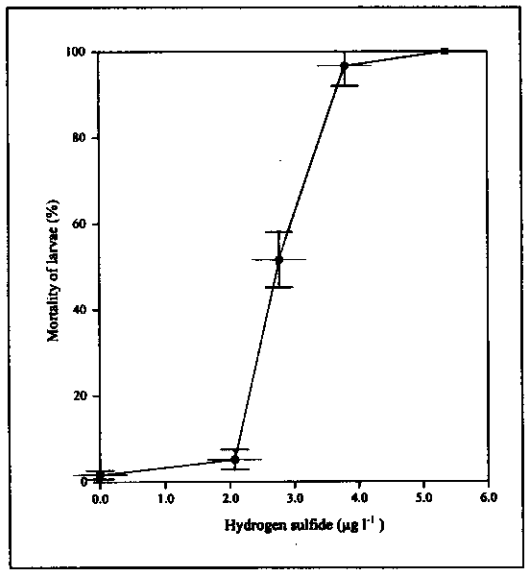


Fig. 1. Percentage of unhatched eggs after 96-hour exposure to different concentrations of H_2S.

Fig. 2. Percentage of larval mortality after 96-hour exposure to different concentrations of H_2S.



96-hr median tolerance limits (TL_{50}) value for H_2S increased from 4 µg l^{-1} H_2S at 25°C to 530 µg l^{-1} H_2S at 6.0°C in the goldfish. The 96-h LC_{50} for the fathead minnow and brown trout (*Salmo trutta*, Salmonidae) larvae was 7.0 µg l^{-1} H_2S at 24°C and 13°C, respectively (Smith and Oseid 1975; Reynolds and Haines 1980).

In the chronic flow-through study with sublethal H_2S concentrations, there was a significant reduction ($p<0.05$) in fish growth rates when the H_2S level was higher than 0.03 µg l^{-1} H_2S (1% of LC_{50}) (Fig. 3). At the highest concentration of 3.41 µg l^{-1} H_2S, fish weights increased by only about 20% after 22 d while the control fish grew by 93%. This is in agreement with Smith and Oseid (1976), who reported that growth retardation in juvenile bluegills occurred even at 2.4 µg l^{-1} H_2S.

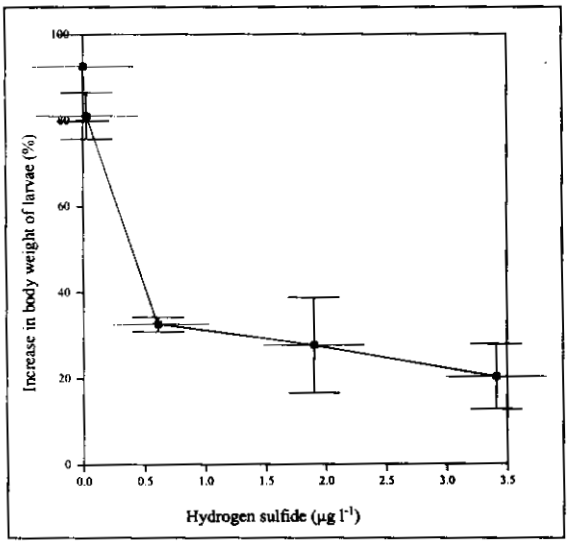


Fig. 3. Percentage increase in body weight of larvae exposed for 22 days to different concentrations of H_2S.

The no-effect level of H_2S on the growth of *P. gonionotus* in this study was about 0.03 µg l^{-1} H_2S. Smith and Oseid (1975) have examined the effects of chronic toxic levels of H_2S on the early life stages of various freshwater fish species and found that a safe level of H_2S which would ensure survival and growth of fish populations would be between 2 to 4 µg l^{-1} H_2S at 20°C. Since the no-effect level of H_2S to *P. gonionotus* is much lower than this value, any form of organic pollution in the aquatic environment would have adverse impacts on the fish population.

More than 30% of the eggs did not hatch when exposed to more than 0.6 µg l^{-1} H2S. Those that did hatch had tail deformities (Fig. 4). Similar vertebral malformations have been observed in the sheephead minnow (*Cyprinodon variegatus*, Cyprinodontidae) after a 28-day exposure to the herbicide trifluralin (Couch *et al.* 1979) and in other fish, due to chemical contaminants in general (Moore and Hixson 1977; Muramoto 1981).

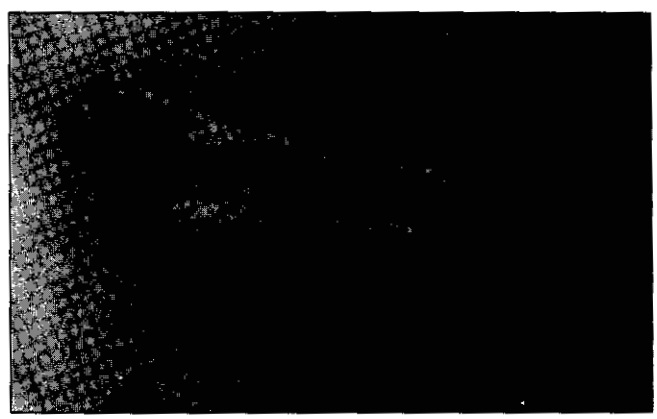


Fig. 4. Deformed tail of newly-hatched larva from *Puntius gonionotus* egg exposed for 96 h to hydrogen sulfide higher than 0.6 µg l^{-1} H_2S.

Gills of *P. gonionotus* exposed to H_2S exhibited various degrees of epithelial hyperplasia, even at the lowest concentration (0.03 μg l^{-1} H_2S). The gill lamellae of the unexposed larvae are shown in Fig. 5. Hyperplasia was generally more pronounced at the base of the secondary lamellae and the degree of hyperplastic changes increased with H_2S concentrations (Fig. 6). Slight epithelial lifting was also observed in fish exposed to H_2S. The gill lamellae of the exposed fish were congested with blood cells, indicating irritation from H_2S exposure. Epithelial hyperplasia and separation of the epithelial layer from supportive tissues usually mean disorders in gill function that affect the physiology or cause the death of fish (Eller 1975; Gardner 1975; Smart 1976). In addition, hyperplasia and separation of the epithelium are associated with asphyxiation, partial or complete loss of secretory or excretory function, impairment of oxygen-carbon dioxide exchange, and loss of plasma electrolytes or proteins (Smith and Piper 1972; Mitchell *et al.* 1978).

This study illustrates that H_2S is toxic in the early developmental stages of *P. gonionotus*, even at low concentrations of less than 2 μg l^{-1} H_2S. Smith *et al.* (1976) suggested that any detectable concentrations of hydrogen sulfide are detrimental to fish production. Since sulfide is commonly found in ponds with high accumulations of organic matter, fish farm managers must include ways to prevent or overcome anaerobic conditions in the bottom of ponds to avoid sulfide build-up to toxic levels.

Fig. 5. Normal gill lamellae of *P. gonionotus* not exposed to H_2S.

Fig. 6. Hyperplasia at the base and along the secondary gill lamellae of *P. gonionotus* larvae exposed to 3.41μg l^{-1} H_2S.

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