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Effects of Nitrite and pH on a Tropical Fish Fry, *Puntius gonionotus* (Bleeker)

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ABSTRAK

Kesan pendedahan jangka pendek dan panjang anak ikan tropikal, Banbodes gonionotus *(Bleeker) terhadap pH dan nitrite secara berasingan dan gabungan telah ditentukan dengan menggunakan biocerakinan statik dan sistem aliran terus. Nilai* LC_{50} *96-jam pH dan nitrit masing masing adalah pH 4.9 dan 7.91 mg* NO_2*-N/l. Walau bagaimanapun, nilai* LC_{50} *96-jam bagi pH adalah lebih tinggi (pH 5.39) dalam kepekatan nitrit (5 gm* NO_2*-N/l) dibandingkan dengan nilai tanpa nitrit. Pada tahap pH 5.00 100% kematian berlaku dalam 4.00 mg* NO_2*-N/l selepas pendedahan selama 48 jam. Di bawah pendedahan jangka panjang, kadar tumbesaran anak ikan menurun dengan peningkatan kepekatan nitrit. Anak ikan di dalam 2.00 mg* NO_2*-N/l (pH 7.33-7.56) menunjukkan kadar tumbesaran yang lebih rendah (*$p < 0.05$*) dibandingkan dengan kawalan, dan lebih tinggi (*$p < 0.05$*) dibandingkan dengan yang didedahkan pada tahap 4.00 mg* NO_2*-N/l (pH 7.33-7.56). Seratus peratus kematian berlaku dalam masa 30 hari pada tahap pH 5.00-7.00 apabila ikan didedahkan kepada 4.00 mg* NO_2*-N/l pada masa yang sama. Kajian menunjukkan kesan pH dan nitrit secara gabungan ke atas kemandirian dan kadar tumbesaran anak ikan adalah lebih serius dibandingkan dengan kesan setiap faktor secara berasingan.*

ABSTRACT

The effects of short term and long term exposure of a tropical fish fry, Barbodes gonionotus *(Bleeker), to pH and nitrite separately, and in combination, were evaluated using static and flow-through bioassays respectively. The 96-hour* LC_{50} *values of pH and nitrate were 4.9 and 7.91 mg/l* NO_2*-N respectively. However, the 96-hour* LC_{50} *of pH was higher (5.4 pH unit) in the presence of nitrite 5.00 mg/l* NO_2*-N) than that without nitrite. At pH 5.00, 100% mortality was found at 4.00 mg/l* NO_2*-N concentration after 48-hour exposure. Under long-term exposure, the growth rates of the fish fry decreased with increased nitrite concentrations. Fish fry grown at 2.00 mg/l* NO_2*-N had significantly lower growth rate (*$P < 0.05$*) than the control, but had a significantly higher rate (*$P < 0.05$*) than in the 4.00 mg/l* NO_2*-N (pH 7.33-7.56). One hundred per cent mortality occurred within 30 days at pH 5.00 - 7.00 when the fish were exposed to 4.00 mg/l* NO_2*-N concentration at the same time. The study demonstrated that the effects of combined pH and nitrite on the survival and growth rates of the fish fry were more serious than the effects of each factor separately.*

INTRODUCTION

Barbodes gonionotus (Bleeker), locally known as Javanese carp, is one of the most popular cultured fishes in the South-East Asian region such as Indonesia, Malaysia and Thailand. In Malaysia, it contributes about 21% of the total freshwater fish production (Department of Fisheries 1991). Thus, hatchery production of *B. gonionotus* fry is very important to supply sufficient seed to fish farmers. One of the problems preventing efficient fry production is water quality. Nitrite toxicity to fish is common in hatchery tanks and intensive culture ponds. Although nitrite accumulation is rare in natural waters with an average concentration of less than 10 µg/l (Wetzel 1983), concentrations of nitrite nitrogen in culture ponds may attain 5.00 mg/l (Boyd 1982).

The major action of nitrite in fish is oxidation of hemoglobin to methemoglobin, which is incapable of oxygen binding, thus affecting oxygen transport in blood. Fish can be adversely affected if the blood contains more than 50% methemoglobin (Bowser *et al.* 1983). The presence of nitrite in water, even at a low concentration of 15 µg/l, increases the methemoglobin concentrations in fish blood (Smith and Williams 1974; Smith and Russo 1975; Brown and McLeay 1975). Wise and Tomasso (1989) reported that plasma nitrite concentrations increased with increasing exposure time in fish exposed to both 9.1 and 5.1 mg/l nitrite-N.

Warm water fish species seem to be more tolerant to nitrite than the cold water species. Twenty-four-hour LC_{50} for rainbow trout fry was 55 µg/l NO_2-N (Smith and Williams 1974). Russo *et al.* (1974) and Brown and McLeay (1975) reported that 96-hour LC_{50} values for 12-g and 9-g rainbow trout were 190 and 230 µg/l respectively. Westin (1974), who worked with salmonid fishes, suggested that maximum NO_2-N concentration in fresh water should be 36 µg/l. On the other hand, Palachek and Tomasso (1984b) reported that the 96-hour LC_{50} for fathead minnow was 147.4 mg/l NO_2-N, and Tomasso and Carmichael (1986) found that the 96-hour LC_{50}, for guadalupe bass (*Micropterus treculi*) was 187.6 mg/l NO_2-N. Yusoff and Subasinghe (1995) reported that *B. gonionotus* fingerlings of 6-7 cm in total length were able to survive high nitrite concentrations of 20.00 mg/l NO_2-N. However, exposure to high concentration of nitrite caused sufficient stress to make fish more susceptible to a ubiquitous bacterium such as *Aeromonas hydrophila*. Other adverse effects of nitrite in fishes include reduced growth (Colt *et al.* 1981) and decreased disease resistence (Hanson and Grizzle 1985; Yusoff and Subasinghe 1995).

Nitrite toxicity is affected by other water quality parameters. Huey *et al.* (1982) reported that at low pH, bluegills (*Lepomis macrochirus*) exhibited immediate stress at NO_2- of 6.9 and higher. Fish exposed to low pH resulted in changes of gill morphology, such as hyperplasia and hypertrophy, necrosis and edema in filament epithelium (Nelson 1982, Woods 1989, Leino and McCormick 1984). McCormick *et al.* (1989) reported that these symptoms were more serious under low pH conditions and long exposure time.

Although nitrite and pH are two of the main water quality parameters in management of fish culture activities, their acute toxicity values for tropical fishes are lacking. Safe chronic exposure levels are largely unknown. Since nitrite and pH affect not only the survival, but also growth rate and disease resistance, this study was undertaken to evaluate the effects of short- and long-term exposure of Javanese carp fry to these water quality factors.

MATERIALS AND METHODS

Javanese carp fry ranging from 18-20 mm and 70-80 g, obtained from the Hatchery of the Department of Fisheries in Bukit Tinggi Selangor, were acclimatized in 500-l tanks for approximately two weeks before being used in the experiments. Fish fry were treated with 5.0 mg/l potassium permanganate and fed daily at 2-3% body weight. The feeding was stopped 24 hours before the start of the experiment.

Four experiments were carried out in the acute toxicity test for the determination of 96-hour LC_{50} for nitrite, 96-hour LC_{50} for pH, 96-hour LC_{50} of nitrite at pH 5.0, and 96-hour LC_{50} of pH at nitrite concentration of 4.00 mg/l NO_2-N. For each experiment, 25-l glass tanks containing 15 l water were used. At the beginning of the experiment, the water in the tanks was saturated with oxygen. No aeration or feeding was given during the experiment to avoid changes in the quality of the test water. Twenty fish fry were randomly selected from the holding tank and placed in each test container. Nitrite solutions were prepared using analytical grade sodium

nitrite. Desired pH levels were achieved by using sulphuric acid (H_2SO_4) or sodium hydroxide (NaOH).

In the first experiment, high nitrite concentrations of 0, 30.00, 60.00, 90.00 and 120.00 mg/l NO_2-N and lower concentrations of 0, 5.00, 10.00, 15.00, 20.00 and 25.00 mg/l NO_2-N were tested. In the second experiment to determine 96-hour LC_{50} of pH, levels of 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0 and 10.0 were tested. Results from the first two experiments were used to decide pH and nitrite levels employed in the third and fourth experiments.

In the third experiment, pH levels in all treatments were held constant at 5.0 and nitrite concentrations of 4.00, 6.00 and 8.00 mg/l NO_2-N were tested. In the fourth experiment, nitrite levels were held constant at 5.00 mg/l NO_2-N, and pH levels tested were 5.0, 5.5 and 6.0. The controls for these experiments consisted of 0 mg/l NO_2-N and the pH ranged between 7.4-7.6. All experiments was done in triplicate.

Observations were made after 6, 12, 24, 48, 72 and 96 hours. The number of dead fish at each observation was noted. Death was assumed when there was no response to a light touch (Reish and Oshida 1986). During the experiments, nitrite-N and pH were monitored daily using sulphanilamide-naphthylethylene-diamine method (APHA-AWWA-WPCE 1989) and Orion pH meter model 230, respectively, and adjusted to the tested values as necessary. Water temperature and dissolved oxygen were also monitored daily using an oxygen meter equipped with a thermistor (YSI, model 57). Alkalinity (potentiometric method using pH meter) and total ammonia (phenate method according to APHA-AWWA-WPCF (1989)) were determined at the beginning and end of each experiment. Water temperature, dissolved oxygen, alkalinity and total ammonia-N were measured to ensure that no significant differences in their values occurred in all treatments (Table 1).

For the chronic toxicity test, another 3 experiments were carried out. The first and second experiments were to test the chronic effects of nitrite and pH separately. The third experiment was to test the combined chronic effects of nitrite and pH. In these experiments, a flow-through system was used. Test solutions were placed in 10-l containers set above the tanks. Solution from each container flowed into three test tanks. All tanks were aerated using air

TABLE 1
Range of water quality parameters in the acute toxicity experiments

Nitrite-N (mg/l)	Temperature (°C)	Dissolved O_2 (mg/l)	pH	Alkalinity (mg/l $CaCO_3$)	Total Ammonia-N (mg/l)
0.00	25.8 - 26.2	4.1 - 7.8	7.2 - 7.5	20.5 - 23.0	0.005 - 0.273
5.00	26.0 - 27.4	6.3 - 8.2	7.4 - 7.8	26.3 - 29.5	0.013 - 0.267
10.00	26.0 - 27.4	6.4 - 8.2	7.4 - 7.8	26.8 - 30.5	0.004 - 0.186
15.00	26.0 - 27.4	6.3 - 8.2	7.4 - 7.8	26.1 - 28.7	0.005 - 0.108
20.00	26.0 - 27.4	6.6 - 8.2	7.4 - 7.8	25.4 - 29.2	0.003 - 0.115
25.00	26.0 - 27.4	6.4 - 8.2	7.4 - 7.8	26.5 - 28.6	0.012 - 0.085
30.00	25.8 - 26.2	5.9 - 7.8	7.6 - 7.4	21.0 - 26.5	0.005 - 0.125
60.00	25.8 - 26.2	5.3 - 7.8	7.2 - 7.4	22.7 - 25.8	0.010 - 0.132
90.00	25.8 - 26.2	6.0 - 7.9	7.2 - 7.5	21.6 - 25.5	0.008 - 0.086
120.00	25.8 - 26.2	6.8 - 7.7	7.3 - 7.4	23.1 - 26.7	0.011 - 0.093
pH					
4.0	25.8 - 26.2	6.8 - 7.9	3.97 - 4.01	21.7 - 23.4	0.016 - 0.063
4.5	26.0 - 27.4	7.0 - 8.2	4.47 - 4.55	22.3 - 23.7	0.015 - 0.082
5.0	26.0 - 27.4	5.9 - 8.2	4.98 - 5.03	21.7 - 23.1	0.009 - 0.243
5.5	26.0 - 27.4	5.8 - 8.2	5.46 - 5.53	22.5 - 23.3	0.007 - 0.281
6.0	25.8 - 26.2	5.5 - 8.1	5.98 - 5.05	22.5 - 24.1	0.012 - 0.182
7.0	25.8 - 26.2	5.4 - 7.8	6.98 - 6.05	21.3 - 23.8	0.014 - 0.215
8.0	25.8 - 26.2	5.5 - 7.6	7.96 - 8.03	25.6 - 28.9	0.010 - 0.194
10.0	25.8 - 26.2	5.2 - 7.8	8.92 - 9.02	24.8 - 29.2	0.009 - 0.136

which was bubbled through the deionized water to remove carbon dioxide and suspended particles.

Results from the acute toxicity tests were used to determine nitrite and pH concentrations employed in the chronic toxicity trials. In the first experiment, nitrite concentrations of 0, 2.00, 4.00, 6.00 and 8.00 mg/l NO_2-N were used. Levels of pH were maintained at 7.3 - 7.6. In the second experiment, pH levels of 5.0, 6.0, 7.0, 8.0 and 9.0 were used and the test water was free of nitrite. The concentrations used in the third experiment were based on the results of the first and second experiments of the long-term exposure trial. The nitrite concentration was held constant at 4 mg/l NO_2-N and pH levels used were 5.0, 6.0, 7.0, 7.50, 8.0 and 9.0. Similar to the acute toxicity experiments, 20 fish fry were placed in each tank and each treatment was carried out in triplicate.

Fish were fed at 5% body weight twice a day at 0900 and 1700 hours. Excess feed was siphoned out every day. Fish were sampled twice to measure growth rate. Water temperature, dissolved oxygen, total ammonia, nitrite, alkalinity and pH were monitored weekly. Experiments were terminated after 30 days. at the end of the experiments, live fish were used for histology preparation and observation (Humason 1979).

Values of LC_{50} with 95% confidence level were obtained by using probit analysis (Finney 1977). One-way ANOVA was used to determine significant differences amongst various treatments in the chronic toxicity experiments.

RESULTS AND DISCUSSION

In the acute toxicity test of nitrite, all concentrations above 20.00 mg/l NO_2-N (at pH 7.4-7.8) caused 100% mortality within 96 hours (Tables 1 and 2). The fish in this study seemed to be more sensitive to nitrite than those reported by Yusoff and Subasinghe (1995), perhaps due to their smaller size. Wedemeyer and Yasutake (1978) also reported that larger steelhead trout, *Salmo gairdneri,* were more resistant to nitrite than smaller fish. However, other workers have reported that smaller fish were more tolerant to nitrite than larger fish of the same species (Russo *et al.* 1974; Perrone and Meade 1977; Palachek and Tomasso 1984a). Further studies are needed to resolve the conflicting observations of the nitrite toxicity due to fish size and species.

In this study, the value for 96-hour LC_{50} for nitrite was 7.91 mg/l NO_2-N when pH was in the neutral range (7.4-7.6). No fish survived (100% mortality) when nitrite-N concentrations were 20.00 mg/l NO_2-N and above (Table 2). However, 100% mortality occurred at a much lower nitrite concentration (4.00 mg/l NO_2-N) when the water pH level deviated (pH 5) from the natural range (Table 3). Wedemeyer and Yasutake (1978) showed that 96-hour LC_{50} for 10 g steelhead trout was 5.80 mg/l NO_2-N when pH and hardness were 7.3 and 150.0 mg/l respectively. On the other hand, Russo *et al.* (1981) showed that 96-hour LC_{50} value for 6.3 - 387 g rainbow trout, Salmo gairdneri, at pH 6.44 - 9.04 ranged from 0.11 - 1.67 mg/l NO_2-N. Colt and Tchobanoglous (1976) reported that 96-hour LC_{50} for nitrite was 43 mg/l for channel catfish. Results for nitrite acute toxicity are widely variable, depending on fish size, fish species and water chemistry. The suppression of nitrite toxicity by chloride ions has been reported for rainbow trout (Russo and Thurston 1977), coho

TABLE 2
Mean percentage mortality of fish fry exposed to different nitrite and pH levels separately after 96 hours

Nitrite-N concentrations (mg/l NO_2-N) (pH ranged from 7.4 - 7.6)	Mean % Mortality ± SD
0.00	5.5 ± 2.0
5.00	8.0 ± 4.0
10.00	83.0 ± 6.0
15.00	95.0 ± 4.0
20.00	100.0 ± 0.0
25.00	100.0 ± 0.0
30.00	100.0 ± 0.0
60.00	100.0 ± 0.0
90.00	100.0 ± 0.0
120.00	100.0 ± 0.0
pH Levels (Nitrite = 0.00 mg/l NO_2-N)	**Mean % Mortality ± SD**
4.0	100.0 ± 0.0
4.5	100.0 ± 0.0
5.0	8.0 ± 2.0
5.5	3.0 ± 2.0
6.0	3.0 ± 2.0
7.0	3.0 ± 2.0
8.0	7.0 ± 2.0
10.0	12.0 ± 5.0

TABLE 3
Combined effects of pH and nitrite in terms of mean percentage mortality on fish fry after 96-hour exposure

Nitrite-N Concentration (mg/L NO_2-N)	pH Levels	Mean % Mortality ±SD
5.00	5.00	100.0 ± 0.0
5.00	5.50	33.0 ± 4.0
5.00	6.00	15.0 ± 4.0
4.00	5.00	100.0 ± 0.0
6.00	5.00	100.0 ± 0.0
8.00	5.00	100.0 ± 0.0

salmon (Orcorhynchus kisutch) (Perrone and Meade 1977), steelhead trout (Wedemeyer and Yasutake 1978) and channel catfish (*Ictalurus punctatus*) (Tomasso *et al.* 1979). In the pH acute toxicity test of this study, 100% mortality occurred at pH 4.5 and below in the absence of nitrite (Table 2). However, in the presence of nitrite (> 4 mg/l NO_2-N), 100% mortality occurred at pH 5.0 (Table 3). Without nitrite, only 8% mortality was observed at pH 5.0 (Table 2). The lowest mortality (3%) occurred between pH 5.5 and 7.0 in treatments without nitrite. Thus, toxic effects of pH were more serious in the presence of nitrite ions. The 96-hour LC_{50} for pH was 4.9 in the absence of nitrite, but increased to 5.39 in 5.00 mg/l NO_2-N solution.

Similarly, as pH deviates from neutral value, the safe concentration of nitrite becomes lower. Assuming a safety factor of 0.001 of 96-hour LC_{50} (Bresch 1993), this study showed that the safe nitrite concentration was about 8 µg/l NO_2-N at neutral pH range, but lower than 4 µg/l NO_2-N at pH 5.0. Huey *et al.* (1982) reported that bluegill (*Lepomis macrochirus*) (17.3 - 7.4 g) exposed to pH 4.00 showed immediate stress at concentra-tions of 6.9 mg NO_2/l (2.1 mg/l NO_2-N). Wedemeyer and Yasutake (1978) reported that increasing pH from 6 to 8 reduced nitrite toxicity by a factor of 3 for 10 g steelhead. Huey *et al.* (1980) attributed the increased nitrite toxicity to the permeability of the uncharged nitrous acid form of nitrite predominant at low pH. Although both forms of nitrite (HNO_2 and NO_2) are known to be toxic, Huey *et al.* (1980) suggested that HNO_2 uptake is much more rapid and the sudden nitrite load converts most of the fish haemoglobin to methemoglobin, resulting in death. Bath and Eddy (1980), on the other hand, reported that acidity of the water except at extreme values (below pH 5 and above pH 10) had no significant effect on nitrite toxicity.

TABLE 4
Mean percentage mortality and growth rate of fish fry in chronic toxicity test at different nitrite and pH levels (separately) after 30-day exposure (Growth rates were not considered in treatments with high mortality

Nitrite-N Concentrations (mg/l NO_2-N) pH ranged from 7.33 - 7.56	Mean % Mortality ± SD	Mean Growth Rates (mg/day) ± SD
0.00	7 ± 2	8.3[a] ± 0.1
2.00	15 ± 4	7.7b ± 0.1
4.00	43 ± 2	6.1c ± 0.2
6.00	80 ± 7	–
8.00	100.0 ± 0	–
pH Levels (Nitrite = 0.00 mg/l NO_2-N)		
5.00	100.0 ± 0	–
6.00	68 ± 5	–
7.00	18 ± 5	7.6a ± 0.1
8.00	12 ± 2	8.5b ± 0.1
9.00	62 ± 2	–

Means in column with different superscripts are significantly different at $p < 0.05$

TABLE 5
Combined effects of pH and nitrite on the growth rates and mortality of fish fry after 30-day exposure (Growth rates were not considered in treatments with high mortality)

Nitrite-N Concentrations (mg/l NO_2-N)	pH	Mean % Mortality ± SD	Mean Growth Rates (mg/day) ± SD
4.00	5.0	100 ± 0 (within 48 hrs)	–
4.00	6.0	100 ± 0 (within 14 days)	–
4.00	7.0	100 ± 0 (within 30 days)	–
4.00	7.5	8 ± 2	11.3^b ± 0.1
4.00	8.0	32 ± 6	8.8^a ± 0.1
4.00	9.0	88 ± 5	–

Means in column with different sueerscripts are significantly different at $p < 0.05$

Under chronic exposure, the growth rates of the fish fry decreased with increased nitrite concentrations (Table 4). Fish fry grown at 2 mg/l NO_2-N had a significantly lower growth rate ($P < 0.05$) than the control, but had a significantly higher rate ($P < 0.05$) than in the 4.00 mg/l NO_2-N. Mortality of 100% occurred in 8 mg/l NO_2-N (in neutral pH range) after 12 days. In the absence of nitrite, 100% mortality occurred after 25 days at pH 5.0 (Table 4). Growth rates of 7.6 mg/day and 8.5 mg/day were found at pH 7.0 and 8.0 respectively. Below pH 6.0 and above pH 9.0, more than 60% mortality occurred.

Although nitrite concentration of 4.00 mg/l NO_2-N did not significantly affect fish growth when pH was 8.0, the mortality was almost three time higher in the treatment compared to the treatment without nitrite (Tables 4 and 5). In the absence of nitrite, 18% mortality occurred at pH 7.0. However, 100% mortality occurred when nitrite concentration was 4.00 mg/l NO_2-N at the same pH level. Mortality was lowest and mean growth rate was highest when pH was 7.5 although the nitrite concentration was 4.0 mg/l NO_2-N. Thus nitrite toxicity was highly influenced by pH level.

Chronic exposure to 6.0 mg/l NO_2-N for 30 days in neutral pH caused gill histological changes such as hyperplasia and oedema. Below this concentration, there was slight hyperplasia and oedema observed in the gills. Serious gill epithelial changes including hyperplasia, oedema and necrosis were observed in fish exposed to pH 6 without nitrite. At pH 7.0 and 9.0, only hyperplasia occurred. Gills appeared normal at pH 7.5 and 8.0. When nitrite concentrations were 4.00 mg/l NO_2-N, serious gill hyperplasia and necrosis were observed at pH 8.0 and 9.0. Wedemeyer and Yasutake (1978) reported that 6-month exposure of steelhead trout to 0.06 mg NO_2-N/l caused minimal gill epithelial changes with no adverse effects on survival and growth.

This study showed that both nitrite and hydrogen ions are toxic to fish, but their toxicity is enhanced by the presence of the other. Control of pH can be used to reduce nitrite toxicity as nitrite is less toxic when pH is in the neutral range.

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