TOXICITY OF NITRITE TO THE LARVAE OF *PENAEUS INDICUS**

P. JAYASANKAR AND M. S. MUTHU
Central Marine Fisheries Research Institute, Cochin 18.

ABSTRACT

Short-term experiments revealed that there is a progressive increase in the tolerance of the larval stages of *Penaeus indicus* to nitrite. For nauplius, protozoea and mysis the 24-h LC_{50} values were, respectively, 10.23 mg/1, 20.43 mg/1 and 33.87 mg/1 NO_2-N, 48-h LC_{50} for protozoea was found to be 15.37 mg/1 NO_2-N, while long-term experiments showed that the 9-day incipient LC_{50} was 3.29 mg/1 NO_2-N. The EC_{50}, the concentration at which 50% of the larvae did not metamorphose into postlarvae in 9 days, for nitrite was 1.8 ppm NO_2-N. "Safe level" of nitrite that should be maintained in the seawater used for larval rearing was calculated on the basis of the incipient LC_{50} value as 0.33 ppm NO_2N. More sensitive estimate of "safe level" based on EC_{50} value was 0.18 ppm NO_2-N.

INTRODUCTION

Nitrite is an intermediate product in the bacterial oxidation of ammonia to nitrite in conditioned aquaculture systems (Spotte 1970, Collins et al 1975). This nitrogen compound is highly toxic to aquatic organisms, and poses a potential threat to cultured fish and crustaceans (Russo et al 1974, Westin 1974, Brown and Mcleay 1975, Wedemeyer and Yaswtake 1978, Armstrong 1979, Mevel and Chamroux 1981). The lethel and sub-lethel effects of nitrite to juvenile and adult penaeid prawns and *Macrobrachium rosenbergii* have been reported by Wickins (1976). The toxicity of nitrite to the larvae of *Penaeus monodon* and *M. rosenbergii* was studied by Catedral et al (1977 a, b) and Armstrong et al (1976), respectively. Such studies on larval prawns are relevant to water management practices in hatcheries producing prawn seed for aquaculture purposes.

EXPERIMENT

The source of the larvae, experimental setup and the methods were described in an earlier paper (Jayasankar and Muthu 1983). Test solutions were prepared by dissolving AR grade $NaNO_2$ in seawater. Nitrite concentrations were estimated according to Strickland and Parsons (1968), using an ECIL Junior spectrophotometer.

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Prolonged-exposure experiments

Two experiments were conducted. Experiment I was started with early nauplii (NI), obtained from a wild spawner, and the larvae were reared for 10 days, up to Mysis III, in 7 test solutions having nitrite concentrations of 0.5, 1.0, 2.5, 5, 10, 30 and 50 mg/l NO_2-N. In Experiment II late-nauplius stage larvae (N V-VI). derived from pond-reared eye-ablated spawner, were reared for nine days in concentrations of 1, 2, 5, 7, and 9 mg/l NO_2-N up to post-larva I. Controls were grown in seawater to which $NaNO_2$ was not added. All concentrations including the controls were triplicated.

Short-term exposure experiments

The nauplius, protozoea and mysis larvae from a wild spawner were used in these acute toxicity experiments, that lasted for 24 or 48 h. Each larval stage was subjected to concentrations that were chosen in geometric progression. The control and test solutions were duplicated.

The environmental conditions under which these experiments were carried out were identical to those reported by Jayasankar and Muthu (1983).

Results

Long-term experiments

a) *Experiment I*: In 10.0, 30.0 and 50.0 mg/l NO_2-N all larvae died before they reached protozoea I. Survival of the larvae in various concentrations from nauplius to mysis III is shown in the Fig. 1. Many larvae in concentrations 5.0, 10.0, 30.0 and 50.0 mg/l NO_2-N showed deformed furcal setae. Better survival rate in the concentration 2.5 ppm, compared to 1.0 ppm, is due to the fact that, in the former, the larvae were introduced at the protozoea I stage. Effect of different concentrations on the survival of the larvae was found to be highly significant ($P \leq 0.01$) by Analysis of variance. The 10-day LC_{50} was estimated as 0.78 mg/l NO_2-N. Nitrite concentration of the test solution in the experimental beakers tended to increase slightly during a 24-h period (Table 1), which could be due to bacterial nitrification. Nitrite concentration in the control was not more than 0.05 ppm.

b) *Experiment II*: The brood from the eye-ablated spawner performed better than that from the wild spawner in terms of survival (Fig. 2), and the incidence of deformity was very rare. The final survivors in 7.0 and 9.0 mg/l NO_2-N appeared very weak; almost moribund. Effect of nitrite concentrations on the survival was found to be significant ($P \leq 0.05$) by Analysis of variance. The 9-day LC_{50} value was also higher viz. 3.285 ppm NO_2-N, indicating that these larvae were more tolerant to nitrite than the larvae in Experiment I.

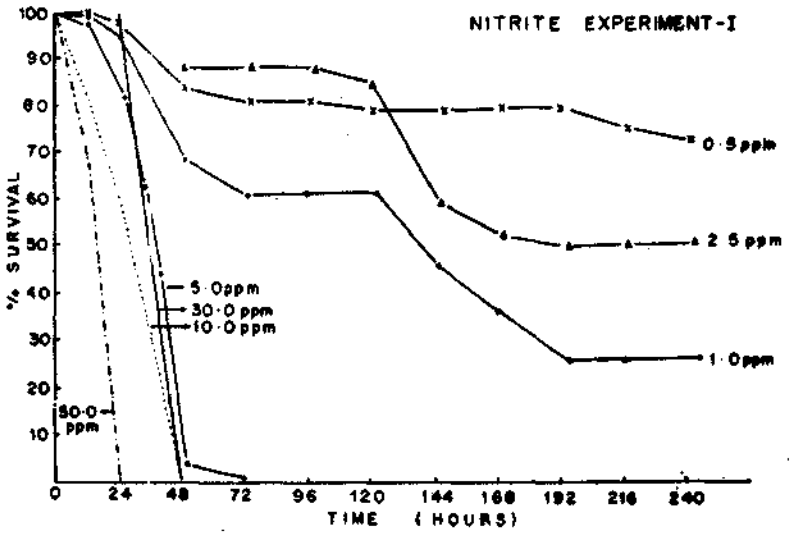

FIG. 1. Survival of *P. indicus* larvae, nauplius to mysis III, in various concentrations of nitrite during prolonged exposure (Experiment-I). (Concentration started from protozoea I).

The percentage of postlarvae among the survivors on the 9th day in each concentration of NO_2-N is,

Control	1.0 ppm	2.0 ppm	5.0 ppm	7.0 ppm	9.0 ppm
78.3	70.6	36.0	0	0	0

TABLE 1. *Nitrite concentrations at the beginning and end of 24-h period in the test solutions* (Experiment I).

Initial values		At the end of 24-h period	
Concentration (mg NO_2-N/l)	pH	Concentration (mg NO_2-N/l)	pH
Control			
0.035	8.26	0.041	7.85
Test concentrations			
0.5	8.26	0.56	8.03
1.0	8.37	1.08	8.03
2.5	8.05	2.42	8.20
5.0	7.99	4.91	7.99
10.0	8.30	10.17	7.98
30.0	7.99	36.27	8.11
50.0	7.81	49.09	8.21

Concentrations above 2·0 ppm seem to adversely affect the rate of metamorphosis. Using data from this table the EC_{50} value, or the nitrite concentration at which 50% of the larvae did not metamorphose into postlarvae in 9 days, was calculated as 1.8 mg/l NO_2-N, by probit analysis. The effect of various nitrite concentrations on the percentage of larvae that metamorphosed to postlarvae was found to be highly significant ($P \leq 0.01$) by Analysis of covariance. The general tendency of the nitrite concentrations of the test solutions in the experimental beakers to increase slightly during the 24-h period, which was noticed in the first experiment, was observed in this experiment also (Table 2).

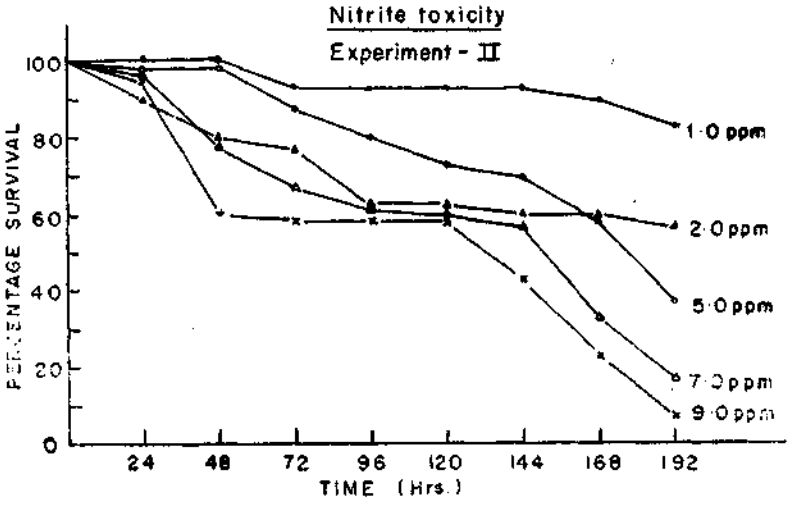

FIG. 2. Survival of *P. indicus* larvae, from nauplius to postlarva I, in various concentrations of nitrite during prolonged exposure (Experiment-II).

TABLE 2. *Nitrite concentration at the beginning and end of 24-h period in the test solutions (Experiment I).*

Initial values		At the end of 24-h period	
Concentration (mg NO_2-N/l)	pH	Concentration (mg NO_2-N/l)	pH
Control			
0.05	8.06	0.079	7.36
Test concentrations			
1.0	7.95	0.98	7.69
2.0	7.99	2.03	7.76
5.0	7.99	4.91	7.76
7.0	7.98	7.16	7.69
9.0	7.97	9.03	7.76

Acute toxicity experiments

a) *Nauplius*: Late nauplii (N V or VI) were subjected to 5 concentrations, viz., 1.0, 2.0, 4.0, 8.0 and 16.0 mg/l NO_2-N and 24 h LC_{50} was estimated as 10.23 mg/l NO_2-N. All individuals died in 16.0 mg/l NO_2-N within this time (Fig. 3).

b) *Protozoea*: Protozoea I were subjected to 5 concentrations, viz., 2.0, 4.0, 8.0, 16.0 and 32.0 mg/l NO_2-N and 24 h and 48 h LC_{50} values were estimated as 20.43 and 15.37 mg/l NO_2-N, respectively (Figs. 4 and 5).

c) *Mysis*: Mysis I were exposed to 5 concentrations, viz., 2.0, 4.0, 8.0, 16.0, 32.0 and 64.0 mg/l NO_2-N and 24 h LC_{50} was estimated as 33.87 mg/l NO_2-N (Fig. 6).

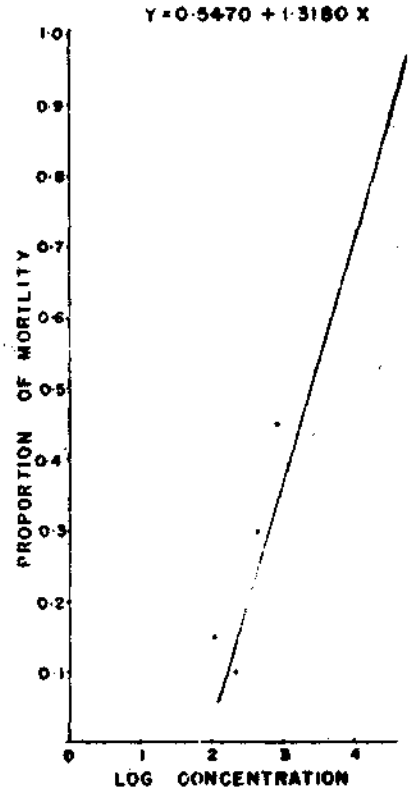

FIG. 3. Regression of mortality of nitrite concentration in 24-h acute toxicity experiment on nauplius of *P. indicus*.

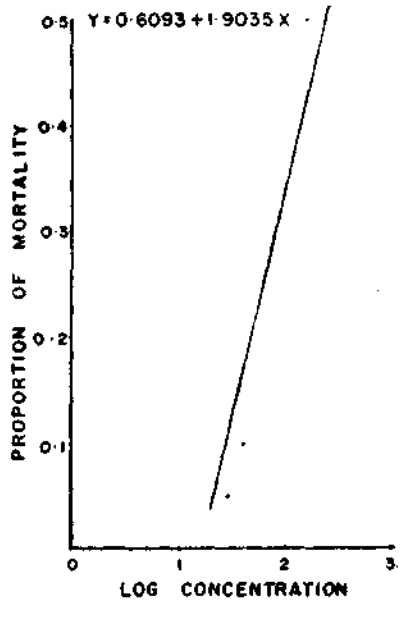

FIG. 4. Regression of mortality on nitrite concentration in 24-h acute toxicity experiment of protozoea of *P. indicus*.

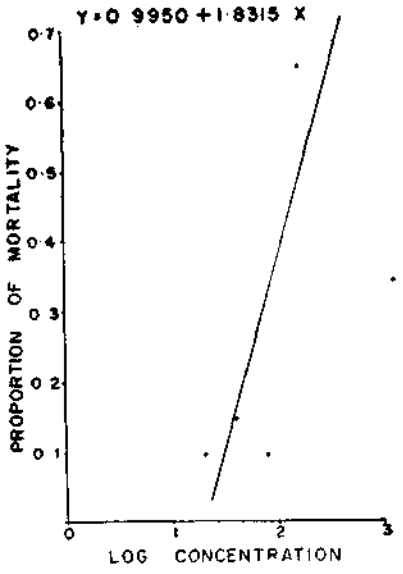

FIG. 5. Regression of mortality on nitrite concentration in 48-h acute toxicity experiment on protozoea of *P. indicus*.

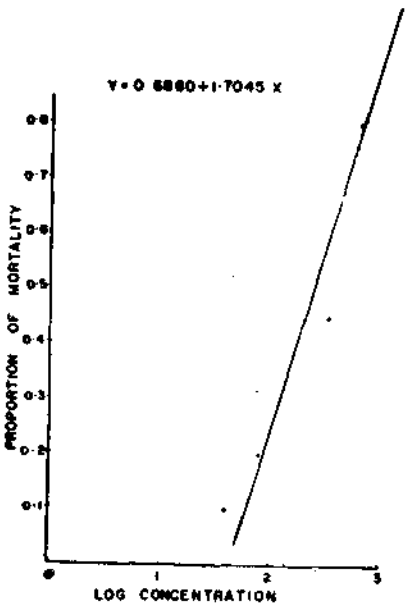

FIG. 6. Regression of mortality on nitrite concentration in 24-h acute toxicity experiment on mysis of *P. indicus*.

Discussion

The 24-h LC_{50} calculated in the acute toxicity experiments showed that, among the three larval stages, nauplius is the most sensitive stage to nitrite poisoning. Nitrite tolerance increases progressively with the later stages, the protozoea and mysis being more resistant to this toxin.

Sprague (1969) suggested that incipient LC_{50}, the level beyond which 50% of the individuals cannot live for an indefinite time, is more useful than short-term LC_{50} values in assessing the toxic action of chemicals. While Wickins (1976) has used 4-weak toxicity experiments to calculate incipient LC_{50}, Armstrong et al (1976 and 1978) have found that LC_{50} values tended to stabilize after 96 h. In the present study 9/10 day LC_{50} values have been estimated and are considered equivalent to incipient LC_{50} values. For nitrite these values were 0·78 and 3.29 ppm NO_2N, respectively for experiments I and II, much lower than the 24- and 48-h LC_{50} values calculated from the acute toxicity experiments.

It is also clear that the brood from the wild spawner (used in experiment I) was more sensitive to nitrite, since mortality and setal abnormalities were more and the rate of development slower in these larvae than those from the

eye-ablated spawner (used in experiment II). While the latter had metamorphosed to postlarva I in 9 days the former had not reached this stage even in 10 days.

Incipient LC_{50} does not indicate the sub-lethal effects of a toxicant; in this context EC_{50}, the concentration at which 50% of the population exhibit sub-lethal responses, like retardation of growth etc, is more useful. In the present study, the EC_{50} is taken as the concentration at which the proportion of larvae that metamorphosed into postlarva in 9 days is reduced to 50% of that observed in the controls. This index seems to be relevant in the context of rearing penaeid larvae in hatcheries, as it is related to the rate of development. EC_{50} value for nitrite in Experiment II was 1.8 ppm NO_2-N, which is lower than the corresponding incipient LC_{50} and gives a more sensitive estimate of the toxicity of nitrite to the larvae.

Catedral et al (1977a, b), working the larvae of *Penaeus monodon*, have reported 60% survival in 5, 15 and 20-50 ppm of nitrite, respectively for protozoea, mysis and postlarva (P3). Since these authors have not made it clear whether nitrite concentrations refer to $NaNO_2$ or NO_2-N, their values cannot be directly compared with that obtained in the present study. But their results are in agreement with the present observation that the tolerance to nitrite increases with the ontogenic development of the larvae. Wickins (1976) has reported 48-h LC_{50} of NO_2-N to be 170 ppm for juvenile penaeids and the present data indicate that all the larval stages of *P. indicus* are more sensitive than the juveniles to nitrite poisoning. Wickins (1976) calculated the incipient LC_{50} of NO -N, for juvenile *P. indicus* and juvenile *Macrobrachium rosenbergii* as 62.0 ppm and 15.4 ppm, respectively, in contrast to the incipient LC_{50} of 3.29 mg/1 NO_2-N, estimated for the larvae of the same species in the present study. The 3-week EC_{50} (i.e. the concentration at which the growth was reduced to 50% of that of the controls) for juvenile penaeids was calculated by Wickins (1976) as 6.4 ppm NO_2-N, which is higher than the EC_{50} of 1.8 ppm NO_2-N calculated for the larvae of *P. indicus* during the present work.

Adults of *Penaeus japonicus* have been reported to be very sensitive to nitrite toxicity; 32% mortality occurred at 0.61 mg/1 NO_2-N (Mevel and Chamroux 1981).

24-h LC_{50} and incipient LC_{50} for larval *M. rosenbergii* have been estimated as 500 and 3 ppm NO2-N, respectively, by Armstrong et al (1976). Although the 24-h LC_{50} of NO_2-N for *M. rosenbergii* larvae was very high compared to that of *P. indicus* larvae, the incipient LC_{50} in both the species is almost identical. Armstrong et al (1976) also found sub-lethal effects of nitrite (retardation of growth of the larvae) at 1.8 ppm which is identical with the EC_{50} of 1.8 ppm NO_2-N observed during the present study.

The crustaceans, in general, appear to be more tolerant to nitrite in the ambient medium than fin fishes, especially the salmonid species. The 24-h LC_{50} of NO_2 is 0.55 ppm and 0.50 ppm for the yearlings of *Salmo gairdneri* and *Onchorhyncus tshawyscha*, respectively, (Smith and Williams 1977), while the 12-h $LC_{58.3}$ for yearling coho salmon is 3.8 ppm NO_2-N. Even for the adults of *Salmo gairdneri* the 96-h LC_{50} ranges between 0.10 and 0.23 ppm NO_2-N (Russo et al 1974, Brown and Mcleay 1975). All these values indicate that *Penaeus indicus* larvae are much more tolerant than fishes to nitrite poisoning. However, molluscs appear to be more tolerant than crustaceans to nitrite toxicity. Epifanio and Srna (1975) have reported the 96-h TLm for juvenile *Mercenaria mercenaria* and *Crassostrea virginica* as 1133 ppm and 798 ppm, respectively.

The higher sensitivity of finfishes to nitrite poisoning is attributed to the fact that nitrite oxidises the blood pigment haemoglobin to methaemoglobin, which cannot bind oxygen for transport, whereas haemocyanin, the blood pigment in prawns, can still bind oxygen in the presence of nitrite (Needham 1961, Conant et al 1933). Further, marine fish and shellfish are said to be more resistant to this toxin than freshwater species, since the marine forms are better equipped to exclude unwanted ions from their blood (Wickins 1976). It is also likely that the better tolerance exhibited by protozoea and mysis stages, compared to nauplius, is related to the ontogenic development of the ionic and osmoregulatory mechanisms, which may enable the animals to cope with the stress caused by nitrite.

Since good water quality is crucial to the success of hatchery rearing of penaeid prawn larvae, an attempt is made here to discuss the "safe level" of one of the nitrogenous toxicants, namely nitrite. The "safe level" recommended by Sprague (1971) is 0.1 ('application factor') of incipient LC_{50} and, according to this calculation, 0.33 ppm NO_2-N is the "safe level" for rearing *P. indicus* larvae. However, since EC_{50} has been suggested to be a more sensitive parameter than incipient LC_{50}, 0.1 of the EC_{50} value can be a more useful index of "safe level", which in the present study is 0.18 ppm NO_2-N. During the present experiments nitrite levels in the control seawater did not exceed 0.08 ppm NO_2-N, which is well within the safe limit.

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