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Short Note

Lethal Concentration of Nitrite on *Penaeus chinensis* Larvae

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Sha-Yen Cheng and Jiann-Chu Chen (1994) Lethal concentration of nitrite on *Penaeus chinensis* larvae. *Zoological Studies* **33**(3): 228-231. *Penaeus chinensis* larvae at the nauplius stage are the most susceptible, while postlarvae are the most tolerant to nitrite. The 24 hr LC50s were 23.12, 56.34, 73.61, and 67.71 mg/l nitrite-N for nauplius third substage (N3), zoea second substage (Z2), mysis second substage (M2), and postlarva twelfth substage (PL12), respectively in 30 ppt seawater at pH 8.21 $\pm$ 0.03. The "threshold time" was found to be 108 hr for PL12 on a toxicity curve approaching an asymptote. Based on the "incipient LC50" and an application factor of 0.1, the "safe level" for rearing *P. chinensis* larvae is estimated to be 1.38 mg/l nitrite-N.

Key words: *Penaeus chinensis*, Nitrite, Toxicity.

Penaeus chinensis possesses the culture attributes of cold tolerance and fast growth in subtropical regions. An attempt has been made to culture this species as a winter atlernative to *P. monodon*. A fundamental knowledge of the oxygen consumption, optimal oxygen, pH, and salinity levels were provided for *P. chinensis* by Liu (1983), Chen et al. (1991), and Chen et al. (1992). Ammonia toxicities related to *P. chinensis* larvae and juveniles has been reported by Chen et al. (1990) as well as Chen and Lin (1991). The safe and toxic levels of nitrite on *P. chinensis* larvae have yet to be documented.

Nitrite and ammonia are the most common toxicant, and main inorganic forms of nitrogen in a culture system. They may deteriorate water quality, which in turn results in a high mortality and a low growth rate for penaeid (Colt and Armstrong 1981, Chen and Chen 1992). Survival of *P. monodon* larvae which had metamorphosed from nauplius to PL12 (postlarva twelfth substage) was negatively related to average nitrite in a decaying exponential manner (Chen et al. 1986). Therefore, accumulation of nitrite and its lethal effect are of primary concern in an intensive culture system. The purpose of this study was to determine the toxicity and safe level of nitrite to *P. chinensis* larvae in the laboratory.

Materials and Methods—Fertilized eggs obtained from a single brood of fourth generation Taiwan *P. chinensis* were incubated, hatched and reared to different stages in the laboratory during the month of January 1992. The larvae used in the study were nauplius third substage (N3), zoea second substage (Z2), mysis second substage (M2), and postlarva twelfth substage (PL12).

A nitrite stock solution was made of sodium nitrite at a concentration of 1000 mg/l nitrite-N (nitrite as nitrogen), and then diluted to desired test concentrations with 30 ppt seawater (Chen and Nan 1991). The nominal concentraion of nitrite-N varied with larval development stages, ranged geometrically (factor of 2) plus 48, 96, and 352 mg/l as well as the control solution.

Short-term LC50 (median lethal concentration) toxicity tests were carried out in triplicate according to the methods of Chen and Tu (1990). Toxicity experiments to establish tolerance limits were conducted in 1 L polyethylene beaker containing 1 L of the test solution employing the static renewal method (Buikema et al. 1982). Each beaker contained 15 test larvae for N3, Z2, M2, and PL12, respectively. Temperature was maintained by water batch, 21°C for N3 and Z2, 23°C for M2, and 25°C for PL12 (Tzeng et al. 1990). All beakers were airstone aerated at 3.69 $\pm$ 0.14 (mean $\pm$ SD) ml/sec. They were fed with formulated feed powder designed and prepared for penaeid larvae by Hsin-Da Co. Ltd. (Pingtung, Taiwan). However, the nauplius larvae were not fed. Dissolved oxygen was maintained at 5.9 $\pm$ 0.2 mg/l and the pH at 8.21 $\pm$ 0.03 for all tests.

Observations were usually made at 12 hr intervals up to 24 hr for N3, up to 48 hr for Z2 and M2, and up to 120 hr for PL12. The LT50 (median lethal time) value was calculated from each time observation. Expiration was recorded when larvae became immobile and showed no response. The concentration response of test organisms is represented by nitrite-N LC50; 95% confidence limits were determined (Trevors and Lusty 1985).

Results—The LT50 value of Z2 exposed to 48 and 64 mg/l was 45.76 and 28.44 hr, respectively. Statistical analysis indicated that the LT50 value for N3, M2, and PL12 showed either linear or exponential relationships with nitrite-N (Fig. 1).

Nitrite-N LC50 values and their 95% confidence limits for *P. chinensis* N3, Z2, M2, and PL12 are shown in Figs. 2 and 3. The 24 hr LC50 values were 23.12, 56.34, 73.61, and 67.71 mg/l nitrite-N, respectively. The 48 hr LC50 values were 29.89, 25.54, and 52.14 mg/l nitrite-N, for Z2, M2, and PL12, respectively. Susceptibility to nitrite was the greatest at nauplius stage and the lowest at PL12 among the various larvae stages tested. The LC50 decreased with increasing exposure time for all stages of *P. chinensis* larvae tested. The LC50 sharply declined in 24 hr for N3, in 48 hr for Z2 (Fig. 2), in 48 hr for M2, and in 72 hr for PL12 (Fig. 3). The "threshold time" (the time at which responses cease) for PL12 was 108 hr. The "incipient LC50" (the LC50 for an exposure time at the asymptotic point on the toxicity curve) was determined to be 13.83 mg/l nitrite-N for PL12 in 30 ppt at pH of 8.21 and 25°C.

Discussion—Seven penaeids (500-1500 mg) were tested for nitrite-N toxicity in 30-34 ppt seawater at pH 8.0 and 26-28°C, the overall LC50 for 48 and 120 hr were 170 and 62 mg/l nitrite-N, respectively (Wickins 1976). However, the LC50 was not species or larval stage specific; exposure time also lacked clear definition.

Previous studies on *P. indicus* and *P. monodon* larvae indicated that the LC50 decreased with different stages of larvae and that the increase of time exposure and nitrite susceptibility decreased progressively as the larvae metamorphosed from nauplius to postlarval stage (Jayasankar and Muthu 1983, Chen and Chin 1988). The 24 hr and 48 hr LC50 values of nitrite-N on penaeids are given in Table 1. Our study indicated that *P. chinensis* tolerance to nitrite increased progressively as the nauplius larvae metamorphosed to postlarva stages. However, Chen and Tu (1990) reported that as the larval *P. japonicus* developed, they did not show a progressive tolerance to nitrite, Z2 larvae were the most tolerant and nauplius larvae the least during the first 24 hr. The extent of resistance to nitrite may vary with species and life cycle on a species basis.

Compared with the nitrite-N LC50 value for *P. indicus* larvae (Jayasankar and Muthu 1983), *P. monodon* larvae (Chen and Chin 1988), *P. japonicus* larvae (Chen and Tu 1990), and on *Metapenaeus ensis* (Chen and Nan 1991), the results obtained from the present study indicated that mysis larvae of *P. chinensis* are the least susceptible to nitrite. Furthermore, the nitrite tolerance of *P. chinensis* nauplius is almost the same as that of *P. japonicus* nauplius.

The fact that nitrite LC50 values were higher than those values of ammonia-N for *P. chinensis* N2, Z2, and M2 (Chen and Lin 1991), suggested that *P. chinensis* larvae exposed to nitrite-N were more tolerant than those exposed to ammonia-N. However, the fact that 24 hr nitrite-N LC50 values were lower than those of ammonia-N for *P. monodon* nauplius and mysis, suggested that *P. monodon* nauplius and mysis were less tolerant to nitrite than ammonia (Chin and Chen 1987, Chen and Chin 1988).

The primary lesion in fish exposed to environmental nitrite is the formation of methemoglobin when the ferrous iron in hemoglobin is oxidized to the ferric state. Methemoglobin is incapable of binding with oxygen, so exposure to nitrite results in hypoxia and cyanosis (Brown and McLeay 1975, Smith and Russo 1975). Such a conversion may occur with crustacean hemocyanin (Needham 1961).

Sprague (1969) reported that the short-term LC50 value can be very misleading, and recommended that toxicity should be described in terms of "incipient LC50". Our study indicates that the 96 hr LC50 and "incipient LC50" were 14.70 and 13.83 mg/l nitrite-N, respectively for PL12. "Incipient LC50" is an important parameter in calculating a "safe level" from "incipient LC50" by using an empirical "application factor" of 0.1 (Sprague 1971). A "safe level" was calculated to be 1.38 mg/l for nitrite-N in 30 ppt at pH 8.21 and 25°C for rearing *P. chinensis* larvae.

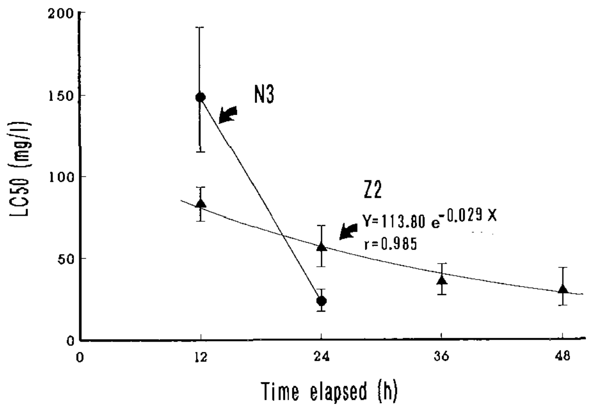

Fig. 2. Nitrite-N LC50 values (mg/l) and their 95% confidence limits for *Penaeus chinensis* nauplius third substage (N3) and zoea second substage (Z2) for different exposure periods.

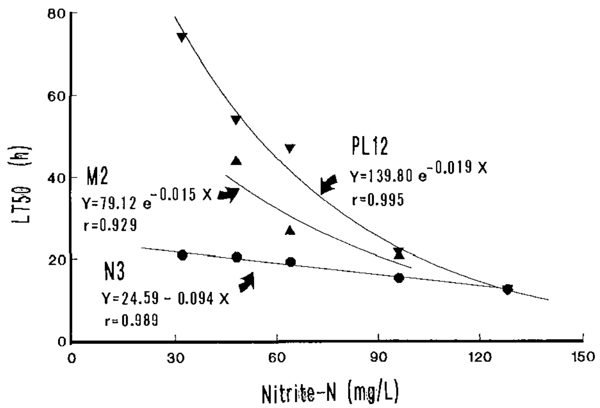

Fig. 1. The relationship between concentrations of nitrite-N and LT50 (h) for *Penaeus chinensis* nauplius third substage (N3), mysis second substage (M2), and postlarva twelfth substage (PL12).

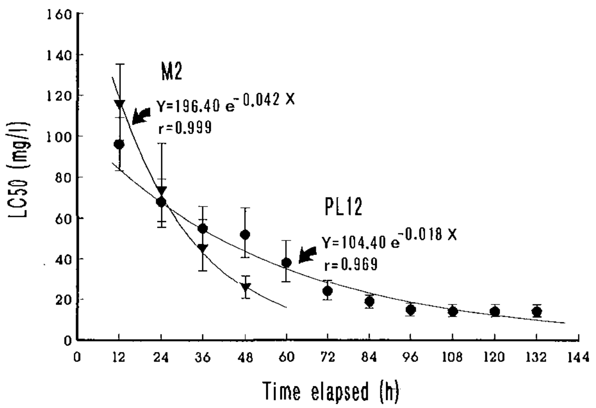

Fig. 3. Nitrite-N LC50 values (mg/l) and their 95% confidence limits for *Penaeus chinensis* mysis second substage (M2) and postlarva twelfth substage (PL12) for different exposure periods.

Table 1. The 24 and 48 hr LC50 values of nitirte-N on penaeids

Species	24 hr LC50 (mg/l)	48 hr LC50 (mg/l)	Reference
Penaeus chinensis			
N3	23.12	—	
Z2	56.34	28.89	this study
M2	73.61	25.54	
PL12	67.71	52.14	
P. japonicus			(Chen and Tu 1990)
N3	24.35	—	
Z2	141	39.42	
M2	58.91	24.08	
PL2	83.40	54.28	
PL12	87.80	57.17	
P. monodon			(Chin and Chen 1987)
N6	5.0	—	
Z1	13.20	—	
M1	20.65	8.30	
PL6	61.87	33.17	
P. indicus			(Jayasankar and Muthu 1983)
Nauplius	10.23	—	
Zoea	20.43	15.37	
Postlarva	33.87	—	
Metapenaeus ensis			(Chen and Nan 1991)
N3	31.29	—	
Z2	16.05	—	
M2	47.60	20.67	
PL1	70.06	27.10	

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亞硝酸對中國對蝦蝦苗之致死劑量

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將不同時期之中國對蝦蝦苗以靜止換水(Static renewal)方式暴露於一連串亞硝酸一氮溶液中。在所有測試之蝦苗中以無節幼蟲對亞硝酸最敏感，而以後期幼蟲之抵抗力最強。對於N3（無節幼蟲第3期）、Z2（眼幼蟲第2期）、M2（糠蝦蟲第2期）、PL12（後期幼蟲第12期）之24小時LC50分別爲23.12、56.34、73.61及67.71毫克／升（鹽度30ppt、pH 8.21±0.03）。對於Z2、M2及PL12之48小時LC50分別爲29.89、25.54及52.14毫克／升。亞硝酸對於PL12之「閾值」在108小時，對於PL12之"incipient LC50"爲13.83毫克／升亞硝酸一氮，在繁殖培育中國對蝦蝦苗，亞硝酸一氮之安全基準爲1.38毫克／升。

關鍵詞：中國對蝦, 亞硝酸, 毒性。

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