1977

Effect of furanace on the development of larval stages of Penaeus monodon Fabricius

Gacutan, Rogelio Q.

Aquaculture Department, Southeast Asian Fisheries Development Center

Llobrera, A. T., & Gacutan, R. Q. (1977). Effect of furanace on the development of larval stages of Penaeus monodon Fabricius. SEAFDEC Aquaculture Department Quarterly Research Report, 1(1), 12–17.

http://hdl.handle.net/10862/2277

Effect of furanace on the development of larval stages of *Penaeus monodon* Fabricius

By

Rogelio Q. Gacutan and Alcestis T. Llobrera

Zoea 2 (Z_2), Mysis 1 (M_1) and Postlarva 1 (P_1) of *Penaeus monodon* artificially spawned in closed-system concrete hatchery tanks were bioassayed for their tolerance to the antibiotic furanace. The setup consisted of four 20-liter capacity plastic basins previously conditioned for 15 days with freshwater in full sunlight. During the experiment, each basin was filled with 5 liters of seawater to which was added filtered *Chaetoceros* and *Brachionus* to give densities of 5.0-7.5 $\times 10^4$ cells/mL and 10-20 individuals/mL, respectively. The following are the properties of the water used throughout the experiments: salinity, 26-32%; pH, 7.3 – 8.4; temperature, 25-30°C; dissolved oxygen, 4.5-8.4 ppm; nitrite, 0.36-0.99 ppm; and ammonia, 0.10-0.30 ppm. To each basin were added 50 healthy larvae of specific stages of *P. monodon.* After an initial acclimation of one hour in the medium, preweighed amounts of the antibiotic were added and thoroughly dissolved. The concentrations tested were 1.0, 2.0 and 3.0 ppm. One basin always served as control.

After 24 hours of exposure, the surviving population in each basin was counted. The survivors were then examined thoroughly under the microscope for unusual behavior and morphological defects brought about by the exposure. To minimize wide variations in the medium as a result of feeding and other manipulations, the systems were all prepared at 9:00 a.m. each time, and the feeds on two instances, one at 5:00 p.m. and another at 5:00 a.m.

Fifteen trials conducted with Z_2 showed survival ranges of 68% to 98% with a mean of 77.6% in the controls; 32% to 94% with a mean of 65.7% at 1 ppm, and 0% to 56% with a mean of 36.5% at 2 ppm (Table 1). There were no survivors at 3 ppm. Interpolation from the survival-dose curve gave a 24-hr LC_{50} of approximately 1.6 ppm.

P. monodon exposed to 11 ppm baths performed better with a mean survival of 69% in 8 trials against 66.5% for the controls. Statistical analysis showed no difference between the two sets of data at the .05 level. Survivals in the individual trials gave a range of 50% to 84% for the controls and 52% to 96% at 1 ppm. A significant difference was found between the control population and larvae exposed to 2 ppm wherein the range varied between 18% and 64% for a mean of 49.9%. At 3 ppm the mean survival of 34.8% was obtained from trials of 6 to 48%. The LC_{50} is placed at slightly higher than 2 ppm.

Five trials using P_1 showed progressively diminishing survival at the four concentrations of the antibiotic. The survivals were 48.8%, 83.6%, 81.85%, and 76.8% at 0, 1, 2, and 3 ppm, respectively. There were no statistical differences among the groups. No fixed LC_{50} had been arrived at although extrapolation from the curve may give a value as high as approximately 5.0 ppm.

Similar experiments were also conducted to ascertain the morphological and developmental effects of a 24-hour exposure to three different concentrations on the larvae. Singled out for study were the metamorphoses of (a) Z_1 to Z_2, (b) Z_2 to Z_3, (c) Z_3 to M_1.

The criterion consisted of finding out the proportion of a surviving population that molted to the next developmental stage and the proportion of those that remained in the initial stage.

Table 1. Tolerance of *Penaeus monodon* larvae to various levels of furanace given in bath for 24 hours

Dose (ppm) / Stage	Means percentage survival				Interpolated LC_{50}
	0	1	2	3	
Zoea 2 [1/]	80.3	65.3 [5/]	36.5	0	1.6
Mysis 1 [2/]	66.5	69	49.8 [6/]	34.8	2.0
Postlarva 1 [3/]	84.8	83.6	81.5	76.8	5.0 [4/]

[1/] Based on 15 trials
[2/] Based on 8 trials
[3/] Based on 5 trials
[4/] Extrapolated value
[5/] Significantly different from control at 5% level
[6/] Significantly different from control at 5% level

The results (Table 2) indicate a delaying effect on metamorphosis. This effect increased with the concentration of furanace. On the basis of 180 specimens examined from a surviving population of Z_1 given furanace, 177 or 98.3%, progressed to Z_2. For those bathed in 1 ppm, 89 out of 127, or 70.7% metamorphosed successfully. Comparison of the above indicated a "z" value of 9.36 which was higher than the tabular value of 1.96 at .05 level of significance. It appears, therefore, that 1 ppm has a delaying effect on development. Given a dose of 2 ppm, only 6 out of 47, or 13.9%, showed successful metamorphosis.

There appeared to be a decline in the delaying effect of the antibiotic with larval age. Z_2 to Z_3 and Z_3 to M_1 developments were not affected by a dose of 1 ppm but were markedly so at 2 ppm. On the basis of 101 survivors in the control population in Z_2 to Z_3 experiments, 94 or 93.1%, progressed to the next stage. The populations bathed in 1 ppm showed successful molting in 144 out of 166 individuals examined, 86.7%. The computed "z" value for the latter was 1.6 at .05% level indicating no difference. There was substantial reduction in successful molts at 2 ppm, however.

The experiments on Z_3 to M_1 transformation had almost the same result except for a lower proportion of successful molts in controls and in those subjected to baths of 1 ppm. This might be an indication of a lower tolerance of the larvae or of the natural feeds ingested by the larvae (e.g., ***Brachionus, Chaetoceros***) to the toxic effects of the antibiotic. Out of 102 survivors from all the control populations examined, 89 or 87.2% reached the mysis stage. This was not significantly higher than the 81.8% obtained from 181 individuals from those given 1 ppm baths.

Examination of the larvae for morphological changes revealed a number of damages, deviant

Table 2. The metamorphosis of *P. monodon* larvae in two levels of furanace given as a 24-hour bath

Experiment	Levels of furanace (ppm)		
Z_1 to Z_2	0	1	2
Z_1	3	38	37
Z_2	177	89	6
P	0.983	0.701[1/]	0.139
Z_2 to Z_3			
Z_2	7	22	75
Z_3	94	144	84
P	0.931	0.867[2/]	0.528[3/]
Z_3 to M_1			
Z_3	13	33	57
M_1	89	148	87
P	0.872	0.818[2/]	0.614[4/]

[1/] Significantly different from control at .05 level.

[2/] Not significantly different from control

[3/] Significantly different

[4/] Significantly different

allometry, heterotrophy and other conditions. Observed to accompany Z_1 to Z_2 ecdysis, for example, were abnormally-shaped uropods, spread carapace, and broken setae (Table 3). The number of individuals affected did not show any statistical differences between the controls and those exposed to furanace. The Z_2 to Z_3 metamorphosis was affected most in terms of damages to the telson, the frequency of which was higher with concentration. Since the differentiation between the telson and the uropods occurs at this stage such damages may be expected.

During the Z_3-M_1 transition, the form of the carapace and the antennae, the full complement of the uropods, and the growth of the pereiopods were subjects to abnormalities. Analyses showed higher incidences of abnormalities and damages at 2 ppm than at 1 ppm.

Table 3. Morphological defects found in *P. monodon* larvae after a 24-hour exposure to 2 levels of furanace

Experiments	Center of morphological defects	Number of individuals showing morphological defects in level of furanace		
		0 ppm	1 ppm	2 ppm
Z_1 to Z_2				
	Carapace	2	0	1
	Telson	5	3	2
	Uropods	0	0	1
	Individuals examined	248	178	77
Z_2 to Z_3				
	Carapace	2	3	6
	Telson	3	6	19 [1/]
	Uropods	2	2	1
	Individuals examined	102	166	159
Z_3 to M_1				
	Carapace	18	3	35 [1/]
	Telson-Uropods	5	2	20 [1/]
	Uropods	3	3	10 [2/]
	Telson	6	5	4
	Pereiopods	9	3	16 [2/]
	Antennae [4/]	11	0	5
	Rostrum	2	1	0
	Individuals examined	102	168	144

[1/] Significantly different from both control and 1 ppm

On this basis, the optimum exposure time-dose combination that gave the highest survival rate in weak and apparently unhealthy larvae was sought. The procedure called for the set up of 9 basins as done previously. After the addition of the feeds, and acclimation, preweighed samples of furanace (1 ppm for zoea; 2 ppm for mysis) were added to eight basins. One basin had no antibiotic and served as control. The water in each of the treated basins was then drained and changed with fresh seawater containing appropriate densities of *Brachionus* and *Chaetoceros,* at 3-hr intervals until the ninth basin had been replaced. The surviving populations after 24, 48, 72 and 96 hours in each basin were then counted.

The optimum exposures for Z_2 were 6 and 21 hours based on Friedmann's two-way analysis of variance by ranks (Table 4). The results with the former batch is not so difficult to explain; that of the latter was totally unexpected. Since no quantification of the microbial populations in each setup at anytime was made, no explanation of paradoxical trend is possible. Among other things, the data showed no significant reduction in populations in all the concentrations after 24 hours; differences were apparent only after 48 hours.

In two runs with a weak and apparently diseased M_1 population, the highest mean survival after 96 hours was obtained in those exposed to furanace for 6 and 9 hours (Table 5). As with the zoea, no significant mortality was observed after 24 hours.

Table 4. Survival of weak and apparently diseased *P. monodon* Z_2, exposed for varied durations in 1 ppm furanace baths[1/]

Exposure	Mean survival after ( ) hours[2/]			
	24 hr	48 hr	72 hr	96 hr
0	33.0	14.3	9.5	4.0
3	42.0	32.3	30.0	8.3
6[3/]	40.0	33.8	26.0	19.2
19	35.2	28.3	15.3	9.3
12	36.0	26.4	12.0	9.0
15	39.2	23.8	17.8	7.3
18	40.9	22.8	14.9	8.1
21[3/]	40.4	33.7	25.2	20.8
24	37.5	26.7	12.6	5.8

[1/] Based on 4 trials

[2/] Based on an initial population of approximately 50 individuals

[3/] Optimum exposure based on Friedmann's rank analysis

Table 5. Survival of weak and potentially diseased *P. monodon* mysis exposed for varied durations in 2 ppm furanace baths [1]

Exposure	Mean survival after ( ) hours[2]			
	24 hr	48 hr	72 hr	96 hr
0	37.5	24.5	15.5	9.0
3	37.5	24.5	21.0	10.0
6 [3]	41.0	32.0	24.0	22.0
9 [3]	39.0	37.5	35.5	20.5
12	31.5	25.5	18.5	10.5
15	43.0	28.0	15.0	4.5
18	40.5	27.0	19.0	16.5
21	45.5	28.0	27.0	14.5
24	43.0	28.0	25.0	11.5

[1] Based on 4 trials

[2] Based on an initial population of approximately 50 individuals

[3] Optimum exposure based on Friedmann's rank analysis

Literature Cited

Delves-Broughton, J. 1974. Aquaculture 3:175-183.

Marking, L. L. and R. G. Piper. 1976. Progr. Fish. Cult. 38:69-72

Shimizu, M. and Y. Takase. 1967. Bull. Jap. Soc. Sci. Fish. 33:544-581.

Villaluz, D. K., A. Villaluz, B. Ladrera, M. Sheik and A. Gonzaga. 1969. Phil. J. Sci. 38:205-233.