

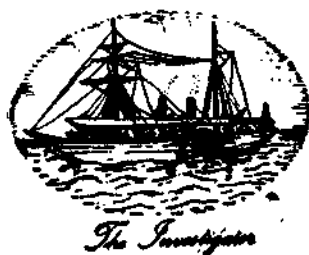
# PROCEEDINGS OF THE SYMPOSIUM ON COASTAL AQUACULTURE

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**PART 1: PRAWN CULTURE**

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## ON THE REARING OF PENAEID PRAWN LARVAE IN THE MEDIUM TREATED WITH TETRACYCLINE AND ACRIFLAVIN

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### ABSTRACT

Experiments were conducted on rearing of larvae of *Penaeus indicus* and *Metapenaeus dobsoni* in the medium treated with tetracycline and acriflavin. Two sets of experiments were carried out with tetracycline. In one set, the medium was treated at concentrations varying from 1 ppm to 5 ppm, only in the first day of experiment while in the other set, the medium was treated daily. It was observed that hatching of eggs to nauplii and subsequent larval development to consequent stages, were not affected in the experiments conducted with 1-3 ppm tetracycline treatment in the first day. However, the continuous treatment of the same and treatment with acriflavin were found to reduce the survival rate of larvae. It was also observed that the growth of the phytoplankton which form the food of developing larvae, was affected in the medium treated with acriflavin whereas tetracycline treatment did not inhibit the algal growth. The significance of the results obtained is discussed.

### INTRODUCTION

MORTALITY of larvae due to disease is one of the hurdles to be overcome for success in culture operations. Very little is known regarding the precautions to be taken for preventing the onset of infection in the course of culture. Infection and diseases are generally found to be associated with bacteria (Lightner and Lewis, 1975) and fungus (Ganaros, 1957; Lightner and Fontaine, 1973; Fisher *et al.*, 1975). The present study is carried out to understand the effect of the antibiotic tetracycline and the antifungal agent acriflavin, on survival and growth of penaeid prawn larvae.

### MATERIALS AND METHODS

Experiments were carried out on larvae of *Penaeus indicus* and *Metapenaeus dobsoni*. The concentrations of chemotherapeutic chemical tested, varied from 1 ppm to 5 ppm. Two sets of experiments were carried out to examine the potential effect of the antibiotic. In one, the medium was treated only once

on the first day of the experiment while in the other set, the treatment was continued daily. Initially 500 ml of treated medium was taken in a 2-litre beaker to rear 50 numbers of experimental larvae. As a control, larvae were reared in pure sea water. Continuous aeration was provided during the experimental period and 50 ml of *Chaetoceros* sp. (26,000 cells to 56,000 cells/ml) was given as feed every day from the last nauplius stage onwards. In addition to the medium used for larval rearing, the sea water used for phytoplankton culture in open sunlight, was also similarly treated. It was taken in 2-litre beakers and kept in open sunlight for 24 hours after treating with chemotherapeutic chemical in different concentrations. No chemical treatment was given for the control beaker. The cell concentration was measured with haemocytometer. The salinity ranged from 31.4‰ to 34.6‰, while the temperature varied from minimum value of 27.2°C to maximum of 29.3°C in the course of larval rearing experiments. However, the temperature of the medium in the open-sunlight phytoplankton culture reached upto a maximum of

37.2°C. Normal Deviate Test and Analysis of Variation by one way classification were applied for statistical analyses of the obtained results.

### RESULTS

Three trials were carried out in each experiment using different broods of larvae. In one set of experiments, larval rearing was carried out from egg stage to postlarval stage in the medium treated with antibiotic tetracycline only once in the beginning of the experiment even though the experimental duration varied from 12 to 14 days. In the second set of experiments, the antibiotic treatment to the rearing medium was given daily during the course of experiment.

In the experiment where tetracycline treatment to the medium was given only once, the average survival rate of *P. indicus* larvae from egg to postlarval stage was observed to be 13.0% in 1 ppm concentration while it was found to be 13.0%, 12.0%, 11.0%, 11.0% and 9.0% respectively in 2 ppm, 3 ppm, 4 ppm, 5 ppm and control medium. Statistical analyses revealed that the survival rate of larvae in the medium, treated with the first three antibiotic concentrations, was significantly higher than that in the control medium. However, the difference was insignificant when the survival rate of control larvae was statistically compared with that of the larvae reared respectively in 4 ppm and 5 ppm (Table 1). Similarly *M. dobsoni* larvae completed larval development with an average survival rate of 32.0%, 34.67%, 35.33%, 30.67% and 28.0% respectively in 1 ppm, 2 ppm, 3 ppm, 4 ppm and 5 ppm antibiotic media while 27.33% of control larvae attained postlarval stage. Significantly more number of postlarvae were obtained when reared in 2 ppm and 3 ppm concentration than in the control medium.

The hatching rate of *P. indicus* eggs to nauplii stage when treated with 5 ppm anti-

biotic was significantly low. In the case of *M. dobsoni* eggs, significantly low hatching rates were observed in 4 ppm and 5 ppm (Table 1).

In the experiment in which antibiotic treatment was given daily during the course of experiment, hatching of *P. indicus* eggs to nauplii was similarly affected. Besides, the development and transformation to subsequent stages were observed to be prolonged. Only few of the surviving larvae reached upto mysis III stage when treated at the rate of 1 ppm per day, even after 16 experimental days.

When the treatment was at the rate of 2 ppm per day, negligible number of larvae attained only mysis I stage in 9-10 days but thereafter all the remaining larvae died when treatment was further continued. Similarly the larvae did not complete their development when daily treatment to the medium was given at the rate of 3 ppm, 4 ppm, and 5 ppm respectively. However, the control larvae successfully metamorphosed into postlarvae with a survival rate varying from 12% to 16% in 14-15 days. More or less the same pattern of results was obtained when the daily treatment was carried out in the medium used for rearing *M. dobsoni* larvae. Mortality of larvae of *P. indicus* and *M. dobsoni* was observed when the accumulation of the chemotherapeutic chemical in the medium reached concentration varying from 17 to 25 ppm.

Results of the treatment of the medium with antifungal agent, acriflavin, were not encouraging. Hatching to nauplii was adversely affected when acriflavin was applied and the larvae did not develop after protozoa I stage in all concentrations attempted even though acriflavin was applied only once in the commencement of the experiment. The larvae died generally after 4-6 days.

When the media for phytoplankton culture were treated with tetracycline the average

TABLE 1. Statistical analysis of the results obtained when the rearing of *Penaeus indicus* and *Metapenaeus dobsoni* larvae was carried out in the media treated only once in the commencement of the experiment, with tetracycline and acriflavin by applying Normal Deviate Test

Nature of Analyses	Treated Vs.	Treated Vs.	Treated Vs.	Z value		Result	
	control eggs subjected (No.)	control nauplii obtained (No.)	control postlarvae obtained (No.)	for hatch- ing	for survi- val	for hatch- ing	for survi- val
<b>EFFECT OF TETRACYCLINE :</b>							
<i>In P. indicus</i> larvae :							
1 ppm Vs Control	100 Vs 100	91 Vs 93	13 Vs 9	1.9230	2.8986	I	S
2 ppm Vs Control	100 Vs 100	93 Vs 93	13 Vs 9	..	2.8986	..	S
3 ppm Vs Control	100 Vs 100	91 Vs 93	12 Vs 9	1.9230	2.2577	I	S
4 ppm Vs Control	100 Vs 100	91 Vs 93	11 Vs 9	1.9230	1.5748	I	I
5 ppm Vs Control	100 Vs 100	90 Vs 93	11 Vs 9	2.7279	1.5748	S	I
<i>In M. dobsoni</i> larvae :							
1 ppm Vs Control	150 Vs 150	140 Vs 141	48 Vs 41	0.9767	1.9456	I	I
2 ppm Vs Control	150 Vs 150	140 Vs 141	52 Vs 41	0.9767	2.9645	I	S
3 ppm Vs Control	150 Vs 150	141 Vs 141	53 Vs 41	..	3.2147	..	S
4 ppm Vs Control	150 Vs 150	130 Vs 141	46 Vs 41	3.4024	1.4034	S	I
5 ppm Vs Control	150 Vs 150	138 Vs 141	42 Vs 41	2.6542	0.2893	S	I
<b>EFFECT OF ACRIFLAVIN :</b>							
<i>In P. indicus</i> larvae :							
1 ppm Vs Control	150 Vs 150	122 Vs 130	..	3.4326	..	S	..
2 ppm Vs Control	150 Vs 150	115 Vs 130	..	5.7803	..	S	..
3 ppm Vs Control	150 Vs 150	123 Vs 130	..	3.0523	..	S	..
4 ppm Vs Control	150 Vs 150	105 Vs 130	..	8.4848	..	S	..
5 ppm Vs Control	150 Vs 150	121 Vs 130	..	3.7939	..	S	..
<i>In M. dobsoni</i> larvae :							
1 ppm Vs Control	150 Vs 150	124 Vs 140	..	8.7234	..	S	..
2 ppm Vs Control	150 Vs 150	130 Vs 140	..	6.3931	..	S	..
3 ppm Vs Control	150 Vs 150	119 Vs 140	..	10.2489	..	S	..
4 ppm Vs Control	150 Vs 150	118 Vs 140	..	10.5165	..	S	..
5 ppm Vs Control	150 Vs 150	124 Vs 140	..	8.7234	..	S	..

'S' denotes significant difference.

'I' denotes insignificant difference.

growth of *Chaetoceros* sp. for 24 hours was found to be  $39.33 \times 10^4$ ,  $40.4 \times 10^4$ ,  $38.93 \times 10^4$ ,  $40.4 \times 10^4$  and  $35.6 \times 10^4$  cells/ml respectively in 1 ppm, 2 ppm., 3 ppm., 4 ppm. and 5 ppm. while in the control, it was found to be  $38.0 \times 10^4$  cells/ml which differs insignificantly from that of treated ones when analysed statistically. In the medium treated with acriflavin, growth of *Chaetoceros* sp. was observed to be retarded and if at all growth was present, it was found to be negligible while average growth was  $78.87 \times 10^4$  cells/ml in 24 hours in the control.

#### DISCUSSION

It is interesting to note that significant number of *P. indicus* and *M. dobsoni* larvae attained postlarval stage in the media, treated only once in the commencement of the experiment with the antibiotic tetracycline upto 3 ppm while continuous treatment resulted in adverse effect on development.

In the case of *P. indicus* larvae, the treatment given only once in the beginning of the experiment, gives better survival rate when the dosage ranges from 1 ppm to 3 ppm. However, a dosage of 1 ppm does not significantly enhance the survival rate in *M. dobsoni* larvae but the treatment with 2 ppm and 3 ppm produces better survival rate. Hence, it may be inferred that the larvae of *P. indicus* may be more susceptible to diseases when compared to that of *M. dobsoni* as a result of which 1 ppm treatment gives significant survival in larval rearing of *P. indicus*, but not of *M. dobsoni*.

In both species, survival was poor in 4 ppm and 5 ppm which suggests that the higher concentrations are not suitable. When acriflavin was applied for fungal treatment, the larval development did not proceed beyond proto-

zoa I stage in both *P. indicus* and *M. dobsoni*. Further, treatment with acriflavin and 5 ppm tetracycline resulted in poor hatching of *P. indicus* and *M. dobsoni* eggs thereby giving a clue that toxicity of chemotherapeutic chemical may be a factor resulting in inhibition of development.

In this context, it is of interest to note the observation of Marshall and Orr (1958) who found a corresponding decrease in feeding of copepod *Calanus finmarchicus* with increasing strength of antibiotic chloromycetin when treatment was given at the rate of 10 mg and 25 mg to 50 mg/litre. The feeding of penaeid prawn larvae depends upon the concentration of phytoplankton in the rearing medium. Hence the information regarding the effect of chemotherapeutic chemical on phytofeed of prawn larvae may help in understanding the mechanisms by which toxicity results in inhibition of growth and low survival. In the present study, the growth of *Chaetoceros* sp. has been observed to be retarded when the medium for phytoplankton culture was treated with acriflavin. Hence, it may be safely concluded that toxicity of acriflavin on hatching and on larval phytofood organisms, may result in the observed larval mortality at protozoa I stage itself, which proves the unsuitability of acriflavin in penaeid prawn larval rearing even though acriflavin was found to be a suitable fungicide, to be used in the culture of juvenile lobster *Homarus gammarus* (Abrahams and Brown, 1977).

In contrast to acriflavin, the antibiotic tetracycline did not affect the phytoplankton growth in all concentrations applied. From the present study it could be concluded that treating *P. indicus* and *M. dobsoni* larvae with a single treatment of tetracycline at a concentration of 1 to 3 ppm and 2 to 3 ppm respectively would not affect their survival rate.

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