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Effects of salinity on the survival of the larval stages of *Penaeus monodon* (Tiger prawn)

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Abstract

The tiger prawn, *Penaeus monodon* is cultured on a commercial basis in Sri Lanka. From the stage of spawning, through the larval stages, to the harvestable level controlled conditions are maintained especially with respect to food and quality of water. Salinity has long been considered a major factor which influences the survival and growth of penaeid shrimp during their culture cycle. In the culture of *Penaeus monodon* in Sri Lanka, full sea water (34ppt) is used throughout in the hatcheries.

This paper reports on the effects of salinity upon the survival of the larval stages of *P. monodon*. In the salinity tolerance limits test the nauplii, zoea, mysis and postlarva stages showed 0% survival at the salinity levels of 0% sw, <60% sw, <70%sw, and <70% sw respectively. These experiments clearly showed that the larval stages at *P. monodon* have a lower lethal limit for salinity. 100% surivival of nauplii, zoea, mysis and postlarva stages occurred at 40-100% sw, 90-100%sw, 100%sw respectively. Thus salinity tolerance become narrower as larval development proceeded. The salinity preferences of the larval stages were determined using a horizontal salinity gradient chamber. The nauplii stage showed equal preference for the salinity range from 50-100% sw. The zoea stage showed a preference for the 90-100% sw concentration. In the case of mysis and postlarva the preference was for the 100% sw. the salinity preference range become narrower as larval development proceeded.

Key words: salinity tolerance, salinity preference, *Penaeus monodon*

1. Introduction

The tiger prawn *Penaeus monodon* is cultured on a commercial basis in Sri Lanka. The brood stocks are collected from the sea and transported to the hatcheries for rearing. The males and females are allowed to mate. The spermatozoa produced by the male which are non-motile and packed inside spermatophores, are transferred to the thelycum of the female with the help of the petasma of the male. During spawning the males are absent. The spermatophores are also discharged simultaneously during spawning and fertilization takes place in sea water. After fertilization the eggs sink to the bottom. The eggs hatch into the nauplius stage, which metamorphoses into the zoea. The zoea passes into the mysis stage. The mysis transforms into the postlarva which passes into the juvenile phase.

From the stage of spawning, through the larval stages, to the harvestable level controlled conditions are maintained regarding food, quality of water, temperature, salinity etc. (Kurian and Sebastian, 1986). Salinity has long been considered a major factor which influence the surivival and growth of penaeid shrimp during their culture cycle. Extreme salinities can increase mortality, particularly if they are experienced without acclimation. Some species may only have an upper lethal limit for salinity, but no lower one, despite the designation of penaeid as marine shrimp (Croghan, 1958a-1958b; Mckenney and Neff 1979; Rothlisberg, 1979). Although some workers indicate that salinity *per se* is of prime importance, some others have indicated it to be of less importance. Zein - Eldin (1963) and Zein-Eldin and Aldrich (1965) in attempts to study the effects of salinity on the growth and survival of postlarvae of *Penaeus Setiferus* and *P. aztecus* in the laboratory, noted that survival was generally excellent at all salinity levels. Chakraborti *et al* (1985) found that the correlation of *Penaeus monodon* surivival with salinity was positive and significant in the case of stocking with juveniles, but was non significant in the case of stocking with postlarvae. Navas and Sebastian (1989) reported that although juveniles of *Penaeus monodon* could tolerate very low salinities down to 0.5 ppt, their growth rate was optimal beyond 4 ppt. Allan and Maguire (1992) working with juveniles of *Penaeus monodon* found no significant difference in growth between salinities of 15 and 30 ppt. Brito *et al* (2000) working on early juveniles of *Farfantepenaeus brasiliensis* found that surivival was not affected by salinities from 15 to 35 (ppt) during 96 hour exposure periods, but showed limited capacity to tolerate low salinites. Rosas *et al* (1999) found that *Penaeus setiferus* was well adapted to environments of both low and high salinites. *Penaeus vannamei* was found to increase its resistance to salinity changes with increasing postlarval development (Ogle *et al,* 1992; Samocha *et al* (1998). Lemos *et al* (2001) report that in the early post larval shrimp of *Farfantepenaeus paulensis* growth was decreased at 34 ppt. It is observed that most of the work on the effects of salinity on penaeid shrimp has been conducted using juvenile stages rather than the larval stages.

In the culture of *Penaeus monodon* in Sri lanka, full sea water (34 ppt) is used throughout in the hatcheries. In the present study the effects of salinity upon the survival of the larval stages of *Peneus monodon* were evaluated.

2. Materials and methods

The experiments were conducted in two steps. The first step was designed to determine the salinity tolerance limits and the second was to find their salinity preference ranges. All measures were taken to maintain other environmental conditions within certain acceptable limits. Hatchery temperature was maintained at 24 to 25^0 C while the water temperature ranged from 24.5 to 25^0 C. Continuous illumination was also provided.

Larval stages of *Penaeus monodon* [Nauplii (NI - N6), Zoea (ZI-Z3), Mysis (MI - M3) and Post Larvae (PLI-PL2)] were obtained from the Hatchery of Indiwary Aqua Farm Ltd, Bangadeniya, where all the experiments were carried out.

Determination of salinity tolerance limits

Ten larvae of each larval group were considered as a sample. A series of sea water concentrations from 0% to 100% with gradual increments of 10% was made using filtered lagoon water and distilled water. 34 ppt salinity was considered as 100% sea water. Salinity was measured using a hand refractometer. Beakers of 1 litre capacity were used as experimental vessels and they were filled with 700 ml of prepared waters of different salinities, and kept under gentle aeration.

The algae, *Chaetoceros,* were used as the food for each group throughout the experiment. Required cell counts of the algae were added at selected time intervals. Before introducing the larvae, experimental vessels were tested and adjusted for the required salinity.

Batches of samples were seined from the rearing tanks in the hatchery and each group was introduced directly to experimental vessels with different saliniities. Observations were done from the 5th minute to the 5th hour of introduction. Number of larvae surviving were recorded in each sample and survival percentages calculated. Five replicates were carried out for each larval group and for each salinity level.

Determination of salinity preference range

Results of the first step (salinity tolerance limits) were used to design the second step, test for salinity preference range of each larval group.

A 35 cm x 50 cm x 7 cm glass tank partitioned into 6 equal horizontal compartments was used to determine the salinity preference range of each larval group separately. At one side of the tank, a small space was kept, allowing larvae to move to their preferred compartments of different saline water.

The compartments of the tank were filled with preaerated waters of different salinities depending on the results of the step one. The slinity range, in which high survival (90-100%) was recorded for each larval stage in the salinity tolerance limits test, was used in the salinity preference range test for the respective larval stage. Bottom compartment was filled with the highest salinity in respect to the tolerance limits of each larval group and the upper compartmets were filled with water of gradually decreasing salinities. These discontinuous salinity layers stabilized by their different densities (Fiivizzani and Spieled, 1978). Salinity of each compartment was measured and was allowed to settle for about one hour and the compartments were labelled accordingly.

Introduction of larvae to this tank was done in 3 ways. Some larvae were placed at the top compartment while some were placed at the bottom using pipettes. And some larvae were introduced to the middle of the tank. Larvae were allowed to explore over the tank for 15 minutes and observations were done at 5 minute intervals for 5 hours. Locations of larvae within the tank were recorded. Each larva was monitored 60 times during the 5 hour observation period. Five replicates were done for each group of larvae. Required cell counts of the algae Chaetoceros were added as food.

A control experiment was carried out to determine if the location of the larvae in the partitioned tank was influenced by the depth of the water. The control experiment was carried out in the same way as the salinity preference experiment, but the partitioned tank was filled with preaerated 100% sea water (34 ppt) from top to bottom.

The Chi-Square test was applied to the experimental group to determine whether the position of the larvae in the partitioned tank was influenced by salinity. The Chi-Square test was applied to the control group to determine if the position of the larvae in the partitioned tank was influenced by the depth of the water.

3. Results

Salinity tolerance limits

Salinity tolerance of nauplii stage

Table I shows the survival percentages of nauplii of *P. mondon* at various salinity levels. Survival was zero at the 0% salinity level and 100% survival was recorded from 40% to 100% salinity levels. 50% mortality was recorded at the 10% salinity level within the first 5 minutes and it dropped to 40% after the 15th minute of introduction. Nauplii showed high survival percentages within the first 5 minutes at the 20% and 30% salinity levels. However, subsequently 60% and 80% survival percentages were noted at the 20% and 30% salinity levels respectively.

Table I - Survival percentages of nauplii stage of *Penaeus monodon* at different salinities

Elapsed Time (min)	Salinity Level (% sea water)										
	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
5	0	50	70	90	100	100	100	100	100	100	100
15	0	40	60	90	100	100	100	100	100	100	100
30	0	40	60	80	100	100	100	100	100	100	100
60	0	40	60	80	100	100	100	100	100	100	100
90	0	40	60	80	100	100	100	100	100	100	100
120	0	40	60	80	100	100	100	100	100	100	100
180	0	40	60	80	100	100	100	100	100	100	100
240	0	40	60	80	100	100	100	100	100	100	100
300	0	40	60	80	100	100	100	100	100	100	100

Salinity tolerance of zoea stage

Table II shows the survival percentages of zoea stage of *P. monodon* at various salinity levels. 100% mortalities were recorded at 0%, 10%, 20%, 30%, 40% and 50% salinity levels. Although a 20% survival percentage was observed within the first 5 minutes at the 60% salinity level, it dropped to 10% subsequently. At the 70% salinity level, early observations recorded a 60% survival which dropped to 40% subsequently. 100% survival was recorded at 90% and 100% salinity levels throughout the experimental period. Although 100% survival was recorded at the 80% salinity level during the first 5 minutes of introduction, it dropped to 70% later.

Table II - Survival percentages of zoea stage of *Penaeus monodon* at different salinities

Elapsed Time (min)	Salinity Level (% sea water)										
	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
5	0	0	0	0	0	0	20	60	100	100	100
15	0	0	0	0	0	0	10	40	70	100	100
30	0	0	0	0	0	0	10	40	70	100	100
60	0	0	0	0	0	0	10	40	70	100	100
90	0	0	0	0	0	0	10	40	70	100	100
120	0	0	0	0	0	0	10	40	70	100	100
180	0	0	0	0	0	0	10	40	70	100	100
240	0	0	0	0	0	0	10	40	70	100	100
300	0	0	0	0	0	0	10	40	70	100	100

Salinity tolerance of mysis stage

Tabele III shows the survival percentages of the mysis stage of P. monodon at various salinity levels. Survival was zero at 0%,10%, 20%, 30%, 40%, 50% and 60% salinity levels. 100% survival was recorded at the 100% salinity level only. At the 70% salinity level survival percentages were recorded as 30,20 and finally as 5 during the observed period. Survival was very low at the 80% salinity level too. It was recorded as 30% within the first 5 minutes and 20% survival was noted later. Although a 100% survival was recorded at the 90% salinity level from the 5th minute to the 30th minute of introduction, it dropped to 90% at the end of 1 hour.

Table III- Survival percentages of mysis stage of *Penaeus monodon* at different salinities.

Elapsed Time (min)	Salinity Level (% sea water)										
	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
5	0	0	0	0	0	0	0	30	30	100	100
15	0	0	0	0	0	0	0	20	20	100	100
30	0	0	0	0	0	0	0	5	20	100	100
60	0	0	0	0	0	0	0	5	20	90	100
90	0	0	0	0	0	0	0	5	20	90	100
120	0	0	0	0	0	0	0	5	20	90	100
180	0	0	0	0	0	0	0	5	20	90	100
240	0	0	0	0	0	0	0	5	20	90	100
300	0	0	0	0	0	0	0	5	20	90	100

Salinity tolerance of postlarval stage

Table IV shows the sruvival percentages of postlarvae of *P. monodon* at various salinities. 100% mortalities were recorded at 0%, 10%, 20%30%, 40% 50% and 60% salinity levels. 100% survival throughout the experimental period was observed at 100% salinity levels. 20%, 50%,90% survival was recorded respectively at 70%, 80%, and 90% salinity levels, at the end of 300 minutes of observation.

Table - IV - Survival percentges of post larvae stage of *Penaeus monodon* at different salinities.

Elapsed Time (min)	Salinity Level (% sea water)										
	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
5	0	0	0	0	0	0	0	40	70	100	100
15	0	0	0	0	0	0	0	40	50	90	100
30	0	0	0	0	0	0	0	20	50	90	100
60	0	0	0	0	0	0	0	20	50	90	100
90	0	0	0	0	0	0	0	20	50	90	100
120	0	0	0	0	0	0	0	20	50	90	100
180	0	0	0	0	0	0	0	20	50	90	100
240	0	0	0	0	0	0	0	20	50	90	100
300	0	0	0	0	0	0	0	20	50	90	100

Salinity preference range

Table V shows the results of the salinity preference range tests for the nauplii, zoea, mysis and postlarval stages. The nauplii stage showed a preference for the 50-100% sea water range and avoided the 40% sea water concentration. The zoea stage showed a preference for the 90% and 100% sea water concentrations. Mysis and postlarval stages showed a preference for the 100% sea water concentrations.

The Chi-Square test applied to the control group was not significant ($P>0.05$). Therefore it can be assumed that the location of the larvae in the partitioned tank was not influenced by the depth of the water. The Chi-Square test applied to the experimental group was significant ($P<0.05$) indicating that the location of the larvae in the partitioned tank was influenced by the salinity of the water.

Table V - Frequency distribution percentages of the nauplii, zoea, mysis and postlarvae stages of *Penaeus monodon*

Salinity level of the compartment (% sea water)	Observed Frequencies%			
	Nauplii Stage	Zoea Stage.	Mysis Stage	Postlarval Stage
40%	0	-	-	-
50%	16	-	-	-
60%	16.33	-	-	-
70%	10.33	-	-	-
80%	17.33	1.33	-	-
90%	14.33	41.33	5.66	12.66
100%	25.66	57.33	94.33	87.33

4. Discussion

In the present study, the nauplii, zoea, mysis and postlarva stages of *penaeus monodon* showed 0% survival at the salinity levels of 0% sw, <60%sw, <70% sw and <70% sw respectively. These experiments clearly show that the larval stages of *P. monodon* have a lower lethal limit for salinity. Motoh (1981) working on post larvae of *P. monodon* in the Phillipines reported a 64% survival rate at 0 ppt. He reported high survival rates at low salinities and high mortality rates at high salinities, upto 38 ppt. In a study by Deshimaru *et. al.* (1985) it was reported that survival and growth were lower when *P. monodon* juveniles (0.65g) were reared at higher salinities (34 to 35ppt) than at lower salinities (19 to 21 ppt), indicating a higher lethal limit for salinity. Charmantier *et al* (1988) studied salinity tolerance in larvae and postlarvae of *Penaeus japonicus.* They found that salinity tolerance decreased through the larval stages, and increased in post - larval stages.

In the present experiments 100% survival of nauplii, zoea, mysis and postlarva stages occurred at 40-100% sw, 90-100% sw, 100% sw and 100%sw respectively. Thus salinity tolerence became narrower as larval development proceeded. In this respect *P. monodon* differs from *P. plebejus* whose tolerance became broader as larval development proceeded as reported by Preston (1985). He reported that *P. plebejus* exhibited a peak larval survival at around 35 ppt in the nauplii stage which shifted to 30 ppt or less for the mysis stage.

In the present study the nauplii of *P. monodon* showed equal preference for the salinity range from 50-100% sw, in which 100% survival also occurred. Although 100% survival was recorded in the 40% sw concentration, they tended to avoid it in preference to the 50-100% levels. Zoea showed a preference for the 90-100% sw concentrations, in which 100% survival also occurred. In the case of mysis and postlarva the preference was for 100% sw, in which 100% survival was recorded. All the larval stages were found to avoid lower salinities and prefer higher salinities. This preference range become narrower and moved towards the full sea water level as larval development proceeded. Zein-Eldin and Renaud (1986) analyzed the influence of environmental factors on the populations of *Penaeus azetecus* and *Penaeus setiferus* along the coast of Texas. Though both species are found distributed in a wide range of salinities, *P. setiferus* preferred lower salinities and stayed near the coast to grow and reproduce, wherease *P. azetecus* preferred higher salinities, increased their buring activity in low salinities, and migrated to reproduce faraway from the coast.

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