

## Effect of Salinity and Temperature on Larval Growth and Survival of Black Tiger Shrimp *Penaeus Monodon* (Fabricius) In Laboratory Conditions

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### Abstract

The present study was conducted for the effects of temperature and salinity on the hatching success, survival and development of the larval stages of *Penaeus monodon* were investigated under five temperatures (24, 26, 28, 30 and 32°C) and four salinities (25, 30, 35 and 40 ppt). Best percentage of hatch was obtained at 33 °C and 35 ppt (87%) followed by 29 °C and 35 ppt (82%). Similarly during naupliar stages, high survival rate was obtained at 33 °C and 35 ppt (89%). Metamorphosis cycle during the protozoal stages (protozoa 1 to postlarva 1) was extended and survival rate decreased at 25 ppt. Development to PL1 was faster at 33 °C (7-8 days) as compared to 29 °C (8-10 days) regardless of the salinity levels. Maximum survival during the protozoal stages was at 35 ppt followed by 48% at 33 °C and 45% at 29°C. Results showed that salinity exerted a greater influence than temperature on the survival and development of larvae. Based on the results, the best temperature-salinity combination for larval survival and metamorphosis of *P. monodon* is 33 °C and 35 ppt. A salinity range of 30-35 ppt is ideal for larval development.

**Keywords:** *Penaeus monodon*, Larvae, Salinity, Temperature, Survival and Development.

### Introduction

*Penaeus monodon* popularly known as tiger shrimp occurs throughout the Asian countries water especially in Bay Of Bengal Sea waters. Growth and survival of *Penaeus monodon* (Fabricius) are influenced by a number of ecological factors, salinity being one of the most important of them (Chakraborti *et al.*, 1986). The successful establishment of a species in a given habitat depends on the ability of each of its developing stages to adapt to the existing environment (Charmantier, 1998). Salinity and temperature are the most important abiotic factors affecting the growth and survival of aquatic organisms (Kinne, 1963, 1964). They are reported to have dramatic effects on the hatching percentage of eggs and also on the survival of the resulting larvae (Preston, 1985). Laboratory and field studies of responses of eggs and larvae of marine organisms to the combined effects of temperature and salinity would lead to a greater understanding of the significance of these factors on survival during early larval development. Thus, defining these optimal conditions for culture of euryhaline marine species may be the fundamental for developing rearing protocol for these species. Early stages of development are the most sensitive phase in the complex life cycle of marine invertebrates and to maximise their survival larvae should be reared close to optimal conditions.

There are few reports on the growth of adult *P. monodon* in ponds of fluctuating physico-chemical conditions (Verghese *et al.*, 1975; Hideo, 1979; Manik *et al.*, 1979; Sebastian *et al.*, 1980 and Chakraborti *et al.*, 1985 and 1986 Preston, 1985; Kumulu and Jones, 1993; Parado-Esteba *et al.*, 1993; Kumulu *et al.*, 2000). The optimal environmental conditions for growth are species specific and differ between life-history stage and season (Costlow *et al.*, 1960; Bas and Spivak, 2000). During the nauplius stage, the rate of development is mainly influenced by abiotic factors, as the larvae do not feed at this stage. On reaching the protozoal stage feeding commences and the rate of development is influenced by the abundance of suitable food in addition to salinity and temperature. Salinity tolerances become broader as larval development proceeds. Protozoal stages had the lowest tolerance to changes in salinity, while mysis stages were the least affected in *Penaeus marginatus* (Gopalakrishnan, 1976) and *Metapenaeus bennettiae* (Preston, 1985). Conventionally, larval cultures of penaeid shrimp are been done in full strength seawater (Chen, 1990; Parado-Esteba, 1998; Kumulu *et al.*, 2000) though temperatures ranging from 24 to 32°C and salinities ranging from 27 to 34 ppt have been found suitable for development of penaeid larvae (Hudinaga, 1942; Cook and Murphy, 1969). It is thus

evident that proper attention should be paid to these physical properties of seawater used for rearing the larvae for large-scale aquaculture practices.

However, studies on the growth and survival of early stages of this species under different saline and temperature conditions are lacking. Lack of prior knowledge on the levels and rates at which this species can adapt to lower salinities has led in the past to unrealistic conclusions. Knowledge on these aspects has great implications in extending the culture of *P. monodon* to the saline areas. Besides, information on tolerance of early stages of this species to wide fluctuations in low salinities and lowest lethal salinity has great significance for the proper maintenance of the post larvae in the nurseries. The present study on the optimum conditions during the early stages of development will help to determine the adequate conditions for optimal production and culture of this species. Hence, the present study was conducted to examine variation in survival and development of early larval stages of *P. monodon* in response to the combined effects of temperature and salinity under laboratory conditions.

## Materials and Methods

During the present study healthy wild gravid *P. monodon* females (60-90g) were obtained from Bay of Bengal, Machilipatnam, India. The shrimps were transported to the Acharya Nagarjuna University departmental laboratory in polythene bags under oxygenated conditions and were maintained individually in 100-l tanks containing seawater of 32-33ppt salinity and temperature of 29.5°C. Spawning took place during night and viable eggs (180000eggs/female) hatched within 24-26h. Nauplii were strong-minded to a point by attracting them to a light source at the surface based on the phototactic response (Browdy, 1998) and transferred to 20-l container. Exogenous food was supplied as soon as the larvae reached the last (sixth) naupliar stages and consisted of a mixture of *Chaetoceros sp.*, (50cells/Al) and *Thalassiosira sp.*, (30 cells/Al) throughout the culture period. Algal concentration was monitored daily using a haemocytometer. Freshly hatched nauplii of *Artemia* species (10nauplii/ml) were added to the culture tanks when 50% of the Mysis transformed into Post larvae.

The combined effect of salinity and temperature on hatching rate, survival and rate of metamorphosis was determined in the laboratory under four salinities (20, 25, 30 and 35 ppt). Vertical glass troughs of 2-l capacity were used for the experiment. The required salinity was obtained either by diluting seawater with freshwater or by mixing filtered seawater with sea salt to keep variation within the

treatment salinity. The experiment followed a completely randomized design, using the larvae from a single spawn to minimize variability between experimental units (Gallardo *et al.*, 1995). Each combination of temperature-salinity was conducted in two replicates. The beakers were kept mixed and saturated with oxygen using one air stone per container and maintained at normal day-night illumination. Salinity and temperature were measured each morning using a portable refractometer (ERMA, Japan) and a mercury thermometer, respectively. All cultures were covered to prevent evaporation. Stages of development (egg, nauplii and protozoa) were treated separately and larvae were reared to the stage required for each experiment in 20-l glass beakers. Stocking densities in the experimental vessels were 100eggs/l, 100nauplii/l and 50 protozoa/l. In order to avoid abrupt changes in temperature and salinity, larvae were initially placed in 500 ml of water at ambient conditions and allowed to acclimatize to the desired experimental temperature and salinity levels over a period of 2 h. The larvae in each flask were counted and random samples of 10-15 larvae were staged (Motoh and Buri, 1979) everyday throughout the culture period. Size was not used to evaluate the effect of salinity and temperature. Some healthy larvae were found to lie on the bottom of the cultured vessel among the dead and moribund individuals. Larvae were considered dead when limb movement had ceased when nudged by a needle. Each experiment was continued until larvae at the lowest temperature had either reached the relevant stage or were dead. Time required for 50% of the larvae in a given treatment to moult to the next successive developmental stages and relative survivals of the larvae was used to infer the suitability of temperature-salinity range.

Once eggs were observed in the water column, they were removed by siphoning and collected using sieve (100Am) and transferred to experimental units maintained at different temperature-salinity combinations. Hatching success of eggs was determined after 24h. twenty 1-ml samples were collected from each experimental unit using a pipette and the hatching success was recorded as total nauplii/total eggs x100. Within 1 h after hatching, nauplii were collected from the hatching tanks, acclimatized and transferred to the experimental units. Survival was recorded after a period of 24 h. No food was supplied during the tests with nauplii. The trial was terminated after larvae reached protozoa 1. Protozoa 1 (PZ1) acclimatized to the required salinity and temperature levels were stocked into experimental units with algal culture and Larval stage index (LSI) was calculated daily using the formula:  $LSI = \sum S \times P_s$ ; where S represents the larval stage and  $P_s$  represents the proportion of larvae still alive at stage S (Lovett and Felder,

1988; Parado-Esteva, 1998). Absolute values of 1 to 7 were assigned consecutively to the larval stages (protozoa 1 to postlarvae 1). The combined effects of both temperature and salinity on larval survival were investigated from protozoa (PZ1) to postlarvae (PL1) stages. Time required for 50% of protozoa to metamorphose to postlarvae was estimated. Data were analyzed with two-way ANOVA ( $P < 0.05$ ) to assess the effect of salinity, temperature and their interaction on hatching percentage and survival of naupliar and protozoal stages. The percentages of survival values were arcsine transformed to make the variance independent of the mean (Leung-Trujillo and Lawrence, 1987).

## Results

The hatching success of *P. monodon* eggs followed a general affinity of being lowest at the lowest salinity and temperature level (Fig.1). The percentages of hatch at different salinities tested were in general higher at 30°C than at 28°C. The highest mean hatch rate was at 30°C and 30ppt (90.5%) followed by 30°C and 35ppt (80%). The hatching percentage of eggs at different temperature-salinity combinations was principally determined by salinity. The effect of temperature was less pronounced and there was no significant interaction between salinity and temperature on the rate of embryonic development at a 5% level ( $P = 0.05$ ). Metamorphosis from the first Nauplius stage through the first protozoal stage (PZ1) was successful in all salinities at both tested temperatures, but with varying degree of survival percentages. Survival rates of more than 50% were obtained at 30 and 35ppt in tested temperatures. In general, irrespective of the salinities, percentages of survival were greater at 30°C than that at 28°C (Fig. 2). The highest survival percentage was obtained at 30°C and 30ppt (75%) followed by 28°C and 30ppt (58%). The animals kept at 20ppt were lethargic and had the lowest survival during the experimental period. From the results obtained, it was evident that the optimum temperature and salinity conditions at hatching (30°C and 35ppt) also favored naupliar metamorphosis and survival. Statistical analyses of the data by means of two-way ANOVA showed that, of the two factors studied, the effect of salinity on larval survival from nauplius to protozoa I was highly significant ( $P < 0.01$ ). Though temperature had a significant effect on Nauplius survival ( $P < 0.05$ ), temperature-salinity interaction had no effect on the same. The larvae metamorphosed to the next successive stage in all the salinities tested, but the time taken to reach postlarvae was significantly greater at 30°C (8-10 days) than at 28°C (7-9 days). The observation was continued till the larvae maintained at the lowest salinity metamorphosed to postlarvae and the time taken generally increased with

decrease in salinity. The fastest larval development until M1 (Mysis-1) took place at 30 and 35ppt at both test temperatures (28 and 30°C). At all these temperature-salinity combinations, it took 4 days for the PZ1 larvae to develop to M1 stage. Similarly, fastest development to PL was also at 30 and 35ppt where the larvae took 8 days to reach PL.

**Table1:** Survival and duration of development of *P. monodon* larvae grown at different salinity and temperature combinations from PZ1 stage to PL1 stage

Temp	Salinity	Survival		Duration	
		M1	PL1	M1	PL1
24	20	10 ±1.2	7±1.5	7	14
	25	15±2.2	12±2.8	6	12
	30	24±2.5	22±3.1	5	12
	35	22±1.5	20±4.2	5	12
26	20	15±1.5	14±2.6	6	12
	25	25±2.5	21±3.4	6	12
	30	39±3.7	34±4.5	5	11
	35	31±2.6	30±1.8	5	11
28	20	17±1.9	16±2.4	5	10
	25	42±3.6	41±3.8	5	9
	30	58±4.2	56±4.7	5	9
	35	54±2.6	50±3.4	4	8
30	20	25±3.8	20±1.1	5	10
	25	54±5.6	52±2.3	5	8
	30	75±3.9	70±5.6	4	7
	35	60±2.6	55±5.7	4	7
32	20	12±1.8	22±4.8	5	11
	25	31±5.7	27±3.5	5	9
	30	58±6.7	56±2.8	5	8
	35	38±2.7	32±3.7	4	8

The slowest larval development occurred at 24°C and 26ppt (12-14 days). The survival rates at different salinities were more or less same at 28 and 30°C temperatures (Table.1). At the termination of the experiment, the best survival from PZ1-PL1 was registered at 30°C and 30ppt with an average of 70% followed by 28°C and 30ppt (56%). In contrast, at the lowest salinity level (20ppt), only 7% of the larvae metamorphosed to PL stage at 24°C and 22% at 32°C. Statistical analyses of the survival data by means of two-way ANOVA showed that salinity exerted a significantly higher influence on the survival at different stages of *P. monodon*. LSI was determined daily. No mortality was observed during counting and transfer of larvae. On the eighth day, the experiment conducted at 30°C was terminated and the highest LSI value was observed at 30ppt followed by 32 ppt. On termination of the experiments maintained at 30°C on the tenth day, LSI values were more or less the same at 30 and 35ppt. At 25 and 30ppt, the increase in LSI over days was higher at 30°C compared to 28°C and 30°C for a period of 4 days after which a higher rate of LSI change over days was obtained at 32°C. Testing the equality of

regression lines showed that rate of change of LSI over days at 28 and 32ppt was statistically insignificant while at 30 and 35ppt, the difference was significant at 5% level. Based on larval survival and progression of LSI, a salinity range of 30-35ppt appears ideal for the larval development of *P. monodon*.

## Discussion

The completion of the life cycle of *P. monodon* in captivity was related to the ability of the species to tolerate variations in salinity and temperature. The pattern of larval development of *P. monodon* followed closely that of other species of the genus. Roberts (1971) reported each species to have a unique range of salinities suitable for embryonic development and hatching, which bears no a priori relationship to the tolerance of adults or larvae. Dissimilarities in the response of larvae of *P. monodon* were observed at both the tested temperatures. Hatching success and survival of naupliar stages were maximum at 30°C and 30ppt followed by 28°C and 32 ppt. Other tested salinities retarded hatching success and naupliar survival, though the potency of the effect was different at the two tested temperatures. The results are in coincide with that reported by Nisa and Ahmed (2000) where a salinity of 32-35 ppt was reported as optimal for hatching success and naupliar survival of *P. monodon* and *P. merguensis*. In contrast, highest mean hatching rates in *P. monodon* were obtained at the temperature-salinity combination of 23°C and 33ppt while survival rate of nauplius to first protozoal stage was highest at 28 °C and 33 ppt followed by 33°C and 33 ppt and 23°C and 33 ppt (Reyes, 1985). Similar results are also obtained by Palomeima and Dickie, (1966); Verghese., (1975); Manik *et al.*, (1979); Sundararajan, *et al.*, (1979); Sebastian *et al.*, (1980); Chakrabarthy *et al.*, (1985), (1986); Ferraris *et al.*, (1987); Sherlyzacharia and Kakati, (2004). Studies conducted on penaeid species like *P. kerathurus* (Klaoudatos, 1978), *P. plebejus* and *Metapenaeus macleayi* (Preston, 1985), *M. ensis* (Chu and So, 1987), *P. penicillatus*, *M. affinis* and *Parapenaeopsis stylifera* (Nisa and Ahmed, 2000) and *P. monodon* (Parado-Estepa *et al.*, 1993) showed that the best temperature and salinity range for hatching and naupliar survival is between 28-32 °C and 30-35 ppt. In each species, tolerance to different temperatures and salinities was least during the development of the protozoa stages and greatest during the development of mysis stages (Gopalakrishnan, 1976; Preston, 1985). Earlier studies on rearing larvae of penaeid shrimp under laboratory conditions revealed similar results of high mortality rate during protozoal stage, particularly at the first and third stages in *Penaeus japonicus* (Hudinaga, 1942), *P. marginatus* (Gopalakrishnan, 1976) and *M. bennettiae* (Preston, 1985). Similarly in the present study,

mortality was high during the development of protozoa stages than at any other stage of development. Salinity tolerances became broader as larval development proceeds. In comparison with protozoa stage, there was a marked change in the response of mysis stage to the combined effect of temperature and salinity with an increased tolerance to differences in temperature and salinity as revealed by the high MSI values. Similar results of marked increase in salinity tolerance on reaching mysis stage have been reported for many penaeid shrimp which spawn offshore like *P. marginatus* (Gopalakrishnan, 1976), *P. merguensis* (Prasad *et al.*, 1988; Nisa and Ahmed, 2000), *P. plebejus* and *M. macleayi* (Preston, 1985). The relative difference of the mysis stages to a range of salinities at both tested temperatures suggested a fundamental change in the mechanisms determining the limits of tolerance of larvae.

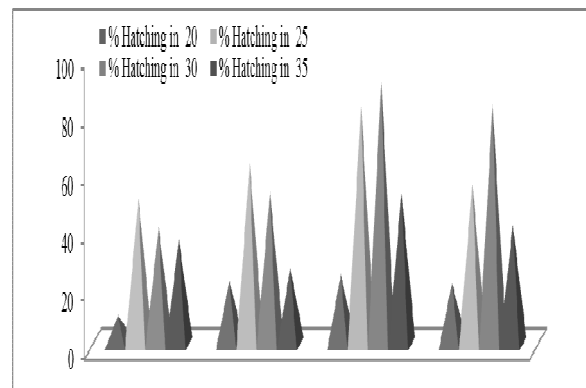


Fig 1: Percentage of hatching of *P. monodon* at various salinities and temperatures

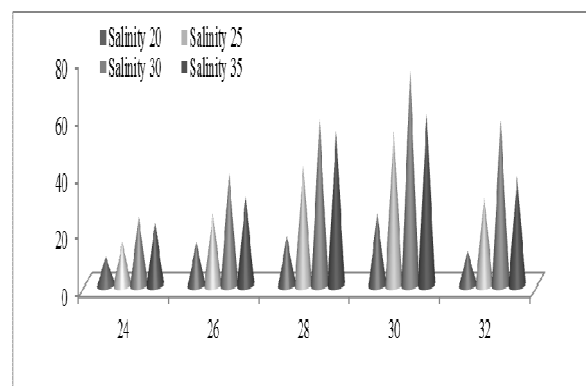
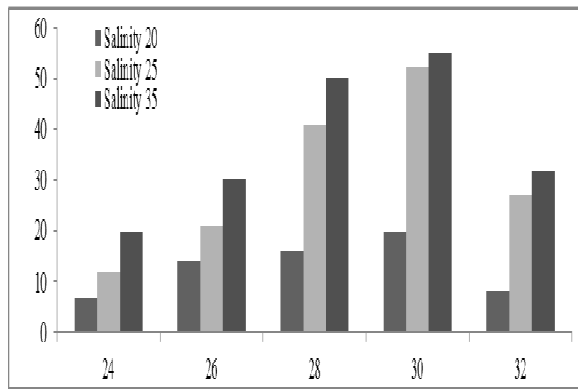


Fig 2: Survival of nauplii of *P. monodon* at various salinities and temperatures



**Fig 3:** Survival of post larvae of *P. monodon* at various salinities and temperatures

The results show that salinity had a greater influence on survival than temperature during larval development. Among the eight combinations of salinity-temperature conditions levels, the highest larval survival of post larvae was obtained at 30°C and 30ppt (70%) followed by 28°C and 30ppt (56%). Survival was considerably low at 20ppt at all tested temperatures. Although temperature did not seem to affect survival for the present study, it is known to influence Penaeid growth and therefore the length of time that shrimp spend in each larval stage. Parado-Esteva (1998) showed high temperature (30-32°C) to significantly increase the growth and metamorphosis rate during the protozoal stages and mysis stages as compared to remaining temperatures. Development to PL1 was faster at 30°C (7-8 days) as compared to 28°C (8-10 days) regardless of the salinity levels confirming the suggestion that high temperature to a certain point increases the moulting frequency and larval growth of penaeid shrimp (Staples and Heales, 1991; O'Brien, 1994; Parado-Esteva, 1998). Based on the results, the best temperature-salinity combination for larval survival and metamorphosis of *P. monodon* was 33°C and 35 ppt while a salinity range of 30-35 ppt appears ideal for larval development. This environmental range is based on the results considering survival as the most important indices and LSI as a factor to define the optimum level with greater precision (Tobias-Quinitio and Villegas, 1982; Alfonso *et al.*, 1988). The present study thus confirms that at least through its early larval stages, *P. monodon* needs oceanic salinities for survival.

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