Tr. J. of Zoology
22 (1998) 163-167

Research Article

The Effect of Salinity on Larval Growth and Survival of *Penaeus indicus* (Decapoda: Penaeidae)

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Received: 13.05.1997

Abstract: This study was conducted to determine optimum salinity for the larval growth, survival and development of *Penaeus indicus.* For this, PZI (protozoea 1) larvae were stocked in 2-L round bottom glass flasks in two replicates at 20, 25, 30 and 35 ppt salinities and were reared on live diets until PL (postlarvae) stages.

Highest survival (91%) and greatest growth (6.48 mm) at postlarval stages (PLI/2) were obtained with the larvae cultured at 25 ppt ($P<0.05$). The highest salinity (35 ppt) gave a significantly lower survival (69%) ($P<0.05$) but similar total length (6.05 mm) ($p> 0.05$) compared to the other salinities. Larval mortality rate at 5 ppt and 35 ppt were 1.65 and 5.04% day^{-1}. Larval growth rate of the larvae reared at all salinities ranged between 0.63 and 0.68 mm day^{-1}. The fastest larval development at the metamorphosis was achieved at 20 and 25 ppt. The larvae (PZI) at 28°C and 25 ppt metamorphosed into PLI stage within only 7 days. Hence, the optimum salinity for the larval culture of P. *indicus* originated from India lies between 20 and 25 ppt.

Key Words: *Penaeus indicus,* larvae, salinity, feeding, live feeds, micro-algae

Tuzluluğun *Penaeus indicus* (Decapoda: Penaeidae) larvalarının yaşam ve büyümeleri üzerine etkisi

Özet: Bu çalışmada, dört farklı tuzuluk düzeyinin (‰20,25,30 ve 35), *Penaeus indicus* larvalarının büyüme ve yaşama oranları üzerine etkisi araştırılmıştır. Deneme, tabanı yuvarlak cam balonlar içinde (2-L) ve iki tekerrürlü olarak yapılmıştır. Besin kesesini tüketmiş olan PZI (protozoea 1) aşamasındaki larvalar, 2 saatlik bir adaptasyondan sonra cam balonlarda, hazırlanmış olan farklı tuzluluklarda, postlarva 1 (PLI) aşamasına kadar yetiştirilmiştir.

Deneme sonunda, ‰25 tuzlulukta yetiştirilen larvaar diğer tuzluluklara göre daha yüksek bir yaşama oranı ile hızlı bir gelişme göstermiştir. Mortalite oranı, ‰25 tuzlulukta %1.65 $gün^{-1}$ iken ‰35 tuzlulukta %5.04 $gün^{-1}$ olarak gerçekleşmiştir. PLI/2'de, ‰25 tuzlulukta yetiştirilenlerin larval yaşama oranı %91, ‰35 tuzlulukta ise %69 olarak gerçekleşmiştir ($P<0.05$). ‰20 tuzluluktaki larvaların yaşama oranı %88 iken, bu oran ‰30'da %86 olarak bulunmuştur ($P>0.05$). Larvaların büyüme oranı 0.63 ile 0.68 mm $gün^{-1}$ arasında değişmiştir. Deneme neticesinde, larvaların PL1/2'de ulaştıkları total boy 6.02 ile 6.48 arasında bulunmuştur ($P>0.05$). En hızlı larval gelişim ‰ 20-25 tuzlulukta gerçekleşmiştir. ‰25 tuzlulukta ve 28°C'de, P. *indicus* larvaları, PZI aşamasından PLI aşamasına, 7 gün gibi çok kısa bir sürede ulaşmışlardır.

Anahtar Sözcükler: *Penaeus indicus,* larva, tuzluluk, yemleme, mikro-alg.

Introduction

Salinity is one of the most important enviromental factors affecting growth, survival and development of aquatic organisms. In nature, mature penaeid shrimps normally spawn at oceanic salinities where the newly hatched larvae also complete their larval development (1). Hence, the larval development of penaeid shrimps may be best achieved at normal sea water salinity.

Despite numerous investigations on postlarvae (PL), juvenile and adult penaeids (2,3,4 and 5), there is considerably little information on salinity tolerance of these shrimps during larval development. Gopalakrishnan (6) reports a complete mortality at 20-26 ppt salinity (S) for nauplius (N) and protozoeal (PZ) stages of a penaeid species, *Penaeus marginatus.* Heavy larval mortalities were also obtained *when Metapenaeus bennettae, M. macleayi and Penaeus plebejus* were reared at 10 and 50 ppt salinities (7). It is known that even within the same species, shrimps may exhibit different salinity tolerance at different geographic regions (5). Bukhari et al., (8) report that 30 ppt salinity was the best salinity for *P. indicus* larvae obtained from the spawners kept in sea water at 43 ppt S (Red Sea).

Hence, the primary aim of this study was to investigate the effect of four different salinities (20, 25, 30 and 35 ppt) on growth, survival and larval

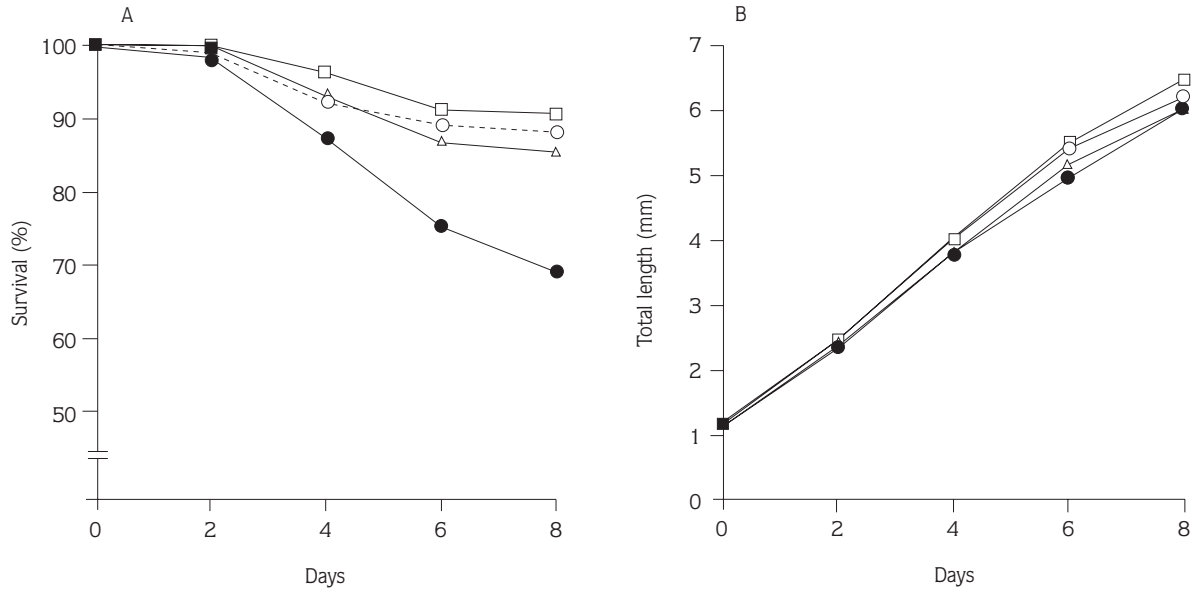

Figure 1. Survival (A) and growth (B) of *P. indicus* larvae reared at salinities of between 20 and 35 ppt from PZ1 to PL stages. ○ = 20 ppt, □ = 25 ppt, Δ = 30 ppt, ● = 35 ppt. Each symbol represents a mean (n=2) for each salinity.

development of *P. indicus* from PZI to PL stages. The results from our. *P. indicus* broodstock originated from India were compared with those of Bukhari et al., (8) who studied salinity tolerance of *P. indicus* of the Red Sea.

Materials and Methods

P. indicus larvae were obtained from broodstock, originating from India, kept in the School of Ocean Sciences, Menai Bridge, UK., in two 10 tons of circular tanks. Gravid females were spawned in 100-1 tanks in filtered (0.2 µm) and UV- irradiated sea water. Following the non-feeding nauplius stages, PZI (protozoea I) larvae were acclimatised for the experimental salinities for 2 h (9) prior to stocking in filtered and UV- irradiated sea water in 2-litre round bottom glass flasks. PZI larvae were stocked at a density of 100 individuals l^{-1} into the experimental flasks. The flasks were kept in a thermostatically controlled water bath at 28°C throughout the experiment. Live mono-specific algal cultures of two species, *Tetraselmis chui* (Butcher), *Skeletonema costatum* (Greville), grown in a semi-continuous culture as described by Walne (10), were fed to *P. indicus* larvae together at a density of 25 cells μl^{-1} of *T. chui* and 35 cells μl^{-1} of *S. costatum* throughout the experiment. Newly hatched *Artemia salina* (cysts were supplied by INVE AQUACULTURE, Belgium) at 28 °C and 34 ppt salinity were also fed to the larvae from mysis 1 (M1) until PL stages at 5 nauplii ml^{-1}.

Distilled water was used to reduce salinity of local sea water (filtered to 0.2 µm and U/V irradiated) to test salinities of 20,25 and 30 ppt. Aquarium salt "Instant Ocean" (Aquarium systems) was added into the local Sea water (33.5 ppt) to obtain 35 ppt saline water. A gentle aeration was maintained through a silicone rubber tube with a glass rod at the tip. Every day, the flasks were emptied and the larvae counted and staged according to Silas et al. (11). Total length (TL) of 10-13 larvae from each replicate were measured from the tip of their rostrum to the end of telson under a binocular microscope.

Statistical Analysis

Larval survival and growth data obtained at metamorphosis were analysed using one-way ANOVA and appropriate pairwise comparison tests (Turkey's test for equal, and Scheffé's test for unequal observations) after normality and homogeneity (Bartlett's test) of the data were checked using Minitab statistical package (12).

Result

Survival of larvae reared at normal sea water was significantly lower (69%) than diluted sea water

	Salinities			
	20 ppt	25 ppt	30 ppt	35 ppt
Mortality rate (% day^{-1})	1.80	1.65	2.49	5.04
Final survival at PL1/2 (%)	88.25^{a}±1.06	90.75^{a}±1.77	85.50^{a}±0.71	69.00^{b}±1.41
Growth rate (mm day^{-1})	0.63	0.68	0.64	0.63
Final total length at PLI/1 (mm)	6.23±0.04	6.48±0.01	6.02±0.07	6.05±0.20
Postlarval stages at the end of the experiment(%)	68% PLI; 32% PL2	68% PL1; 32% PL2	91% PLI; 9 PL2	95% PL2; 5% PL2

Table 1. Mortality rate (% day^{-1}), final survival (%), growth rate (mm day^{-1}), final total length (mm), and composition of postlarval stages of *P. indicus* reared in different salinities from PZ1 to PL1/2. Treatments with different superscripts are significantly different (P<0.05) and values are means±s.d., n=2.

(P<0.05) (Fig. 1). The larvae reared at 20 and 25 ppt S had the highest larval survivals at the end of the experiment (88-91%) (see Table 1). Lowest mortality rates (1.65-1.80 % day^{-1}) were achieved at lower salinities (20 and 25 ppt) whereas the highest mortality rate (5% day^{-1}) was found for larvae reared at 35 ppt S. In fact, larval mortality rate at 35 ppt S (5.04% day^{-1}) was three times more than that of 25 ppt S. Larvae reared at 30 ppt displayed an intermediate mortality rate (2.49 % day^{-1}).

Although greatest larval growth occurred at 25 ppt (6.48 mm TL), there was no significant difference between the growth of larvae reared at salinities from 20 to 35 ppt (P> 0.05) (see Fig. 1 and Table 1). Growth rate of the arvae cultured at the experimental salinities were similar changing between 0.63 and 0.68 mm TL day^{-1}. Larvae cultured at 20 and 25 ppt had higher survivals (92-96.5%), greater total lengths (4.01-4.03 mm at M1) and faster development (29-32% M1) than larvae at 30-35 ppt which sustained 87.5-93% survival, 3.8-3.81 mm TL and slower development (4.5-18% M1) during protozoeal stages. At the termination of the experiment, the larvae at 25 pppt S exhibited 91% survival, 6.48 mm TL compared to 69% survival and 6.05 mm TL obtained from larvae at 35 ppt. At 25 ppt, on day 8, 32% of the larvae were at PL2 stage whereas at 35 ppt only 5% of the larvae were at PL2 stage (Table 1).

Discussion

Conventionally, it is accepted that penaeid larvae should grow and survive better in normal sea water salinity. Yet, The present results indicate that *P. indicus* prefers lower salinities rather than oceanic salinity during larval development. At optimum salinity, which appears to be between 20 ppt and 25 ppt, with the mixed algae (*T. chui/S. costatum)* and *Artemia* from PZ3/M1 stage onwards, over 91% larvae metamorphosed into PL1/2 stages with a final total length of 6.48 mm within only 7 days. Bukhari et al., (8), however, report that 30 ppt salinity was the best salinity for *P. indicus* larvae obtained from the spawners (originated from the Red Sea) kept in sea water at 43 ppt S. It appears that there is a difference in larval salinity tolerance between Indian and Red Sea strains of *P. indicus.* This has already been proved between the two strains at PL levels by Kumlu and Jones (5). These authors also reported maximum survival and growth at 25 ppt S during nursery stages of *P. indicus* postlarvae obtained from the Indian broodstock.

Preston (7) suggests that optimal salinity for larval growth and survival of *Metapenaeus bennettae* depends on salinity conditions where spawning take place. In the present study, although 35 ppt salinity was closest to the spawning salinity (33.5 ppt S), highest larval mortality and lowest growth were obtained at this salinity. Gopalakrishnan (6) states that none of newly hatched larvae of *P. marginatus* were able to survive further than a few hours at 20 ppt S and that the lowest salinity tolerated by the larvae (PZI to PL stages) lies between 24 and 26 ppt S. This author found the highest survival of *P. marginatus* at 33 ppt S. The complete mortality obtained by Gopalakrishnan (6) at 20-26 ppt S for nauplius and protozoeal stages could be due to abrupt salinity change to these low experimental salinities. In the current study, PZI larvae were acclimatised to experimental salinities for 2 h (9). Since, heavy larval mortalities of *M. bennettae, P. plebejus* and *M. macleayi* at 10 and 50 ppt S were reported (9), a narrower salinity range of between 20 and 35 ppt was tested on growth and survival on *P.*

indicus in the current study.

The present study demonstrates that optimal salinity for larval culture of *Penaeus indicus* from India lies between 20 and 25 ppt S. At 25 ppt salinity and 27-28 °C using a mixed algal diet of *Tetraselmis chui* (25 cells μl^{-1} and *Skeletonema costatum* (35-45 cells μl^{-1}) plus five *Artemia* ml^{-1} after PZ3/M1 stage, these larvae can be

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