EFFECT OF SALINITY ON THE SURVIVAL, GROWTH AND BIOCHEMICAL COMPOSITION OF *Penaeus monodon* FABRICIUS POSTLARVAE

DISSERTATION SUBMITTED
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF FISHERIES SCIENCE IN MARICULTURE
OF THE
CENTRAL INSTITUTE OF FISHERIES EDUCATION
(DEEMED UNIVERSITY)
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JULY 1999

TO MY PARENTS AND SISTER

CERTIFICATE

Certified that the dissertation entitled **"Effect of salinity on the survival, growth and biochemical composition of the *Penaeus monodon* Fabricius postlarvae"** is a bonafide record of work done by **Mr. Binu Varghese** under our guidance at the Central Marine Fisheries Research Institute, Kochi during the tenure of his M.F.Sc.(Mariculture) programme of 1997-1999 and that it has not previously formed the basis for the award of any other degree, diploma or other similar title or for any publication.

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DECLARATION

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CONTENTS

सारांश

मोनोडॉन झींगे के पालन करनेवालों को पानी की लवणता पर सूचना देना उचित होगा। इसको ध्यान में रखते हुये मोनाडॉन झींगे के पश्चडिंभकों को लेकर यह अध्ययन चलाया गया हैं। अध्ययन में पानी की लवणता के विविध परासों में (0,10,20,30 और 35 पी.पी.टी) उनके अतिजीवितता, बढती और जैवरासायनिक संधटन पर ध्यान दिया गया। विविध लवणता में अतिजीवितता ऑकने केलिय 0,10,20,30 व 35 पी.पी.टी लवणतावाले पानी में इनका पालन किया। 35 पी.पी.टी वाले पानी में 96.67% और 10,20 व 30 पी.पी.टी वाले पानी में 97.5% जन्तू जिन्दा देखा गए। 0 पी.पी.टी वाले पानी में पाले सारे जन्तू मरगये। बढती पर चलाए निरीक्षणों से स्पष्ट हूआ कि सबसे अधिक बढती 20 पी.पी.टी. लवणतावाले पानी मे होता है। 30 पी.पी.टी, 10 पी.पी.टी. और 35 पी.पी.टी. की लवणताओं में बढती यथाक्रम धटती जाती हैं। प्रोटीन व कारबोहाइड्रेटों की मात्रा में भी पानी की विविध लवणता में अन्तर दिखाई पडी। 20 पी.पी.टी. वाले पानी में प्रोटीन का (आद्रभार में 14.45 मि.ग्रा./100 मि ग्रा.) और 30 पी.पी.टी में कारबोहाइड्रेटों का (आद्रभार में 1.01 मि.ग्रा / 100मि.ग्रा) उच्यतम मूल्य दिखाये पडे। अन्य जैवरासायनिक घटक जैसे लिपिड, नमी और क्षार में उल्लेखनीय व्यतियान नही दिखाया पडा।

ACKNOWLEDGMENTS

I wish to express my deep sense of gratitude to my major advisor, Dr. A. Laxminarayana, senior scientist, for his inspiring guidance, immense encouragement and affectionate treatment throughout the period of study. I am very much grateful to Dr. P. Laxmilatha, scientist and Dr. K.V. Somasekharan Nair, senior scientist for forming a supportive advisory committee.

Let me take this opportunity to express my sincere thanks to Dr. M. Devaraj, former director and also the Director C.M.F.R.I. for providing facilities for the successful completion of work

I wish to express my gratitude to Dr. C. Suseelan, OIC, PGPM, for the timely advises, encouragement and support provided all through my study. I extend my thanks to Sayed Koya, OIC,Narakkal for his timely help.

Thanks are due to Dr. J.P. George, Dr.A. Gopalakrishnan, Dr. N. Sridhar for their kind interests and suggestions.

The help rendered by the staff of P.G.P.M. is highly acknowledged. My sincere thanks are due to Mr. Aboobaker, Technical Officer and Shaji for providing facilities to carryout the work.

My special thanks are due to Mr. Nandakumar Rao, PNPD, Najumudeen and Jyothi SRF's for their timely suggestions and help. The help rendered by research

scholars-Abraham, Ranjith, Manoj, Satheesh and Aloysius,MFD, Sainudeen FRAD, Pavithran FRAD are also acknowledged.

I am indebted to my batchmates for their help and co-operation. My sincere thanks are also due to seniors and juniors.

I am grateful to Rosalie Shaffer, NMFS and Jeans Collins, FAO for their immediate responses to the request for references.

I would like to acknowledge my earnest thanks to the ICAR for granting the fellowship for my study.

Finally, I am very much indebted to all my teachers and well-wishers, especially my parents and sister.

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INTRODUCTION

Aquaculture has immense potential to augment finfish and shellfish production to partially meet the growing demand for animal protein. It has emerged as the only growth sector of fisheries industries. Aquaculture clearly has the potential to continue to grow rapidly and to make a further substantial contribution to employment and food security, particularly in the rural areas.

Penaeid shrimp culture has emerged as a highly profitable investment alternative. In past few decades researchers have gathered considerable information on the biology, production and culture of many of the commercially important shrimps but unfortunately not much attention has been paid to the ecophysiological aspects. In-depth studies on all biotic and abiotic factors are necessary in formulating ecofriendly aquaculture practices. This is particularly important in view of the sudden collapse of shrimp farming in 1994-95 after its rapid expansion in the early 1990's.

Among the abiotic factors, salinity plays an important role in the survival, growth, maturation, spawning and distribution of marine and estuarine biota. Salinity coupled with temperature forms one of the most important physical factors and biological effects of these factors are complex and wide ranging. Salinity can affect the functional and structural responses of invertebrates through the changes in osmotic concentration, solute proportion, dissolved gas saturation etc.

Penaeus monodon is one of the most commercially important shrimps distributed throughout the Indo-Pacific region. This species has got tremendous

importance in aquaculture because of its high survival, growth and market demand. *P. monodon* accounts for more than 50 % of the total cultured shrimp production. In India, the cultured production of tiger shrimp is 54,483 tonnes in 1997 (FAO, 1999). *P. monodon* is markedly euryhaline which can considered to be one of the most efficient osmotic and ionic regulators among the penaeids. Because of these characters, they can be cultured a wide range of salinities from marine to freshwater.

In the present study, the survival, growth and biochemical composition of *P. monodon* acclimated to different salinities has been investigated. The results of this study are of great value in the farming of this hard currency yielding shrimp, *P.* monodon.

Plate I. Harvested *Penaeus monodon* from a brackishwater pond, Kochi.

REVIEW OF LITERATURE

Salinity is one of the most important abiotic factors influencing the distribution and overall performance of many aquatic organisms including penaeid shrimps. Pearse and Gunter (1957) noted that younger animals inhabit lower salinity. In penaeids, the larval stages were completed in marine environment, postlarvae migrate to estuaries and adults return back to sea for maturation and spawning. The extent to which animal react to salinity varies between species. Several studies have been conducted to determine the effect of salinity alone or in conjunction with other abiotic factors on the commercially important penaeids such as *Penaeus setiferus* (Zein-Eldin and Griffith, 1969), *P.aztecus* (Venkataramaiah *et al.*, 1972) , *P.vannamei*, P.stylirostris , *P.californiensis*, *P.brevirostris* (Mair,1980), *P. merguensis*, *P.esculentus*, *Metapenaeus bennettae* (Dall 1981), *P.monodon* (Cawthorne *et al.*, 1983), *P. japonicus* , *P. chinenesis* (Charmantier-Daures *et al.*, 1988) *P. indicus* (Diwan and Laxminarayana, 1989) and *P. semisulcatus* (Harpaz and Karplus, 1991).

1.1. Effect of salinity on survival and growth

On studying the juveniles of *P.aztecus* and *P. duorarum* Williams (1960) has reported that survival was higher in higher salinities at low temperature. Zein-Eldin (1963) noticed the low growth was exhibited at low salinities of 2,5,10and 25 ppt. The survival was not affected by the salinity (2,5,10,25,and 40 ppt) . On studying the growth of *P.aztecus*, *P.duorarum* and *P.setiferus*, he concluded that the growth rate did not differ significantly among tested salinities. Therefore, he

concluded that the salinity *per se* does not limit growth of young shrimp. This views were contradicted by Gunter *et al.*,(1964).

Venkararamaiah *et al.*,(1974) observed highest growth rate in lower salinity (8.5 to 17 ppt) for *P.aztecus* under normal temperature. The growth of *P.indicus* postlarvae was significantly higher at 10 ppt and for juveniles it was 30 ppt (Nair and Kutty 1975), Brett (1979) was of the opinion that salinity imposes greatest additional load on the metabolic need of an animal.

Salinity below 5 ppt were stressful for many stages of most of the species of penaeids, although *P.monodon* appears to be an exception. Pantastico(1979) reported good growth and survival for *P.monodon* in freshwater. This result is in contrast with the results obtained by Cawthorne(1983), who observed that growth and survival of *P.monodon* was higher in full strength seawater than in the lower salinities. Motoh(1981) in a series of laboratory experiments on the postlarvae of *P.monodon* showed that tolerance to very low salinity with a survival rate of 64% at 0 ppt. But the juveniles exhibited the greatest tolerance with a survival rate of 100% at 0 ppt. At 52 ppt, there was mortality. These observations suggest that older stages show greater tolerance to higher salinities.

Dall(1981) interprets the low salinity preference shown by the postlarvae of penaeids as a useful adaptation to their natural nursery habitats. Kuttyamma(1982) arrived at a conclusion that good growth and survival of *Metapenaeus dobsoni* postlarvae occurs in 10 and 15 ppt salinities, while juveniles showed better growth in 20 ppt salinity. On studying the effect of salinity on growth and survival of three Indian penaids viz., *P.indicus, P.semisulcatus* and *P. monodon* Raj and Raj (1982)

found that the higher survival and growth occurs in 15 and 25 ppt in all the three species out of the tested salinities of 5, 15, 25, 35 and 45 ppt.

In a 30 days experiment Huang(1983) concluded that *P.vannamei* postlarvae grew best at about 20 ppt salinity with the poorest growth at 5 and 45 ppt salinity. Deshimaru *et al.*,(1985) reported that survival and growth were lower when *P.monodon* juveniles (0.65 g) were reared at higher salinities (34 to 35 ppt) than at lower salinities (19 to 21 ppt). A correlation between salinity and pond production of *P.monodon* was reported by Chakraborti et al.,(1985). In a 20 days culture under controlled conditions with *P.monodon* postlarvae (16.1 mm) Rajyalakshmi and Chandra(1987) recorded higher survival rate at 15 ppt (82%)than at 20 ppt(74%) and 0 ppt (68%). The survival rate decreased when the rearing time was extended. These studies indicate that *P.monodon* can survive in extremely low salinity, even freshwater, for a short period under experimental conditions. They also observed a decrease in growth rate in 15, 20, and 0 ppt salinities respectively. According to Charmantier(1987) postlarvae and juveniles are more capable of withstanding lower salinities than adults.

Navas and Sebastain(1989) working on ~~studying~~ *P.monodon* observed that the growth was significantly low below 2 ppt salinity for the juveniles. In *P.merguensis* Staples and Heales (1991) obtained the greatest biomass increase at 25 ppt salinity and 28°C. Allan (1992) observed absence of significant difference in growth between salinities 15 and 30 ppt.

Kumulu and Jones(1993) found that 25 ppt salinity is optimal for the larval culture of *P.indicus* (PZ 1 to PL 1). In contrast, Bukhari et al (1994) reported that

hatchery reared *P.indicus* postlarvae (PL1- PL 60) from Red Sea acclimatised for 10 days demonstrated better survival and growth at high saline conditions (50 ppt) in comparison with salinities of 10 to 40 ppt.

In *P.vannamei* Bray et al.,(1994) found significantly higher growth at a salinity of 5 to 15 ppt salinity. Samocha (1998) concluded that there was no significant difference in salinities of 2,4 and 8 ppt and therefore it can be cultured in low saline waters with good growth and survival.

Effect of salinity on Biochemical composition

With the advancement of aquaculture, the biochemistry of cultured organisms eceived attention from many researchers. While there are several studies on the nfluence of salinity on the body composition of finfishes, informations on shrimps ıre scanty. Considerable variation in muscle protein at tested salinities was)bserved in *Mugil cephalus* by Perere and De silva(1978) who recorded highest ırotein level in 20 ppt salinity followed by 15 and 30 ppt salinities. Duchateau and 'lorkin(1961) have reported an enrichment of free amino acid content when :rayfish, *Astecus astecus*, were transferred from brackishwater to seawater ondition.

Recently enormous studies have been carried out to find out the biochemical hanges occurring during maturity stages of commercially important shrimps. But ery few studies were carried out on postlarvae and juveniles . In *P.indicus* :alyanaraman (1983) proximately estimated highest protein levels in 20 and 25 ppt alinities. Effect of salinity on Free amino acid composition have been examined for

P.japonicus by Dalla Via (1986). Fang(1992) concluded that the free amino acid (FAA) were similar in haemolymph and muscle of *P.monodon* acclimated to 30 ppt salinity indicating this as an physiologically marine adapted species. He also found that high concentration of essential free amino acid in the haemolymph of 15 ppt acclimated shrimps which suggest that these FAA could be more available for tissue to take up at this salinity. This gives better explanation for the better growth of *P.monodon* in low saline ponds.

In crustaceans, the hepatopancreas is considered to be the main storage tissue for polysaccharides(Dall and Moriarty 1983). Hall (1998) described the effect of different types of stress on blood glucose in *P.monodon.*

Gopakumar and Nair (1975) found 0.7 to 1.2 % (wet weight) lipid in the muscle tissue of five species of Indian penaeids. In *P.indicus* 3.94% (dry weight) of lipid was found in cultured specimens and this rose to about 6% when they are fed with seed oil supplemented diet (Colvin, 1976). In another study lipid content of *Metapenaeus monoceros* was found to vary between 1.20 to 3.40%. Teshima et al. (1977) found that total lipid content varied with the moult cycle from 1.04% to 1.30% (w.wt). In *P.merguensis* the lipid content was found to be 1.99%(Clarke and Wilkins 1980). Kalyanaraman (1983), in *P. indicus* observed a low level of lipid content corresponding to decreased protein level in lower salinities (5-10 ppt).

Salinity is also known to affect the moisture content. Kalyanaraman(1983) observed a higher water content in *P. indicus* at lower salinity levels of 5 and 10 ppt, which gradually decreased in higher salinities. Ferraris (1986) reported variation in tissue water content with moult stages and salinity in *P. monodon.*

Tissue water content was found to be inversely proportional to salinity of the medium (Parado-Estepa *et al.* 1987).

The information regarding the effect of salinity on postlarvae are scarce. Although *P. monodon* is the most widely farmed species, knowledge about the optimum salinity requirement at different parts of its life cycle are lacking. A thorough knowledge on this aspect will be of immense use to the shrimp farmers. Present investigation was designed to find out the salinity mediated variations on survival, growth and biochemical composition of *P. monodon* postlarvae.

MATERIALS AND METHODS

The present work was carried out at the Marine Hatchery Complex of the Central Marine Fisheries Research Institute, kochi.

1.1 COLLECTION OF SPECIMEN

The postlarvae (PL-24) of *Penaeus monodon* Fabricius were obtained from the Vallarpadom Hatchery of Marine Products Export Development Authority (M.P.E.D.A.). The postlarvae were brought to the experimental site in oxygen packed bags and are acclimated prior to releasing them into the holding tanks. The animals were maintained in the same salinity for one day with continuous aeration after which they were acclimated to the desired salinity.

1.2 EXPERIMENTAL SET UP

The experiment was carried out at Marine Hatchery Complex of C.M.F.R.I. Two storage tanks were used, one for the storage of seawater and another for freshwater. The freshwater required for the experiment was collected 2-3 days in advance and aerated vigorously till use.

The experiment was done with three replicates using plastic tubs of 50 litres capacity. In each tub, 40 *l* rearing media was provided. The tubs were kept covered with mosquito netting . The tubs were kept in elevation to facilitate the siphoning out of waste accumulated. The arrangements of tubs were in such a way that they receive similar environment. Continuous aeration was given to all tubs. No substratum was provided. Each tub was first filled with initial (23 ppt) salinity and

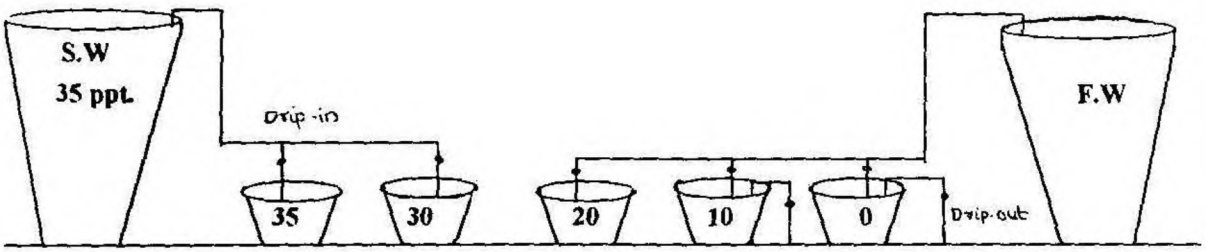
then either freshwater or seawater from storage tanks were allowed to flow into these to required level by drops using flow regulators. The initial filling was done based on the volume calculated from the formula. The desired salinity levels were prepared using the formula.

$$V = \frac{\text{Desired salinity} \times 100}{\text{Salinity of seawater}}$$

Where V is volume of seawater of known salinity to be taken and diluted with freshwater to get 1 *l* of desired salinity.

The final volume was marked in all the tubs. In low saline or freshwater media the drip-in and drip-out method was used for attaining the desired salinity.

Diagrammatic representation of the experimental set up for acclimation Fig. I



1.3. EXPERIMENTAL PROCEDURE

1.3.1. DIRECT TRANSFER TEST

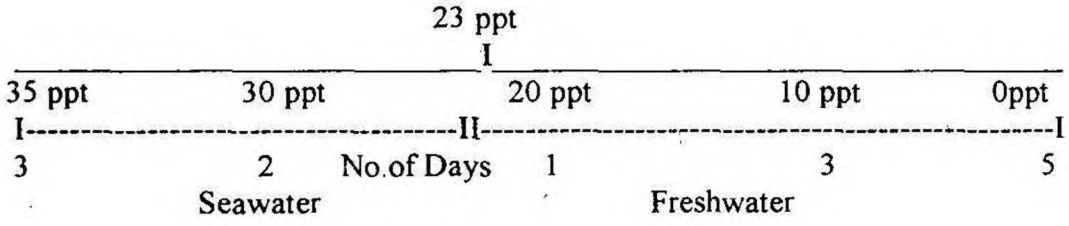
The postlarvae were abruptly exposed to the test salinities to find the tolerance of given stock. Six animals were used for this test. The test was done in duplicate. The animals from 23 ppt salinity were exposed directly to 0, 10, 20, 30 and 35 ppt salinities . All the animals introduced into freshwater died within one hour . In 10 and 35 ppt salinities the mortalities reached above 75 % in the third day

Plate II. Experimental set up

. At 20 ppt no mortality was observed.Control also did not show any mortality. The same stage continued till the termination on fifth day. The tolerance test was done to facilitate the acclimation process.

1.3.2. ACCLIMATION

The acclimation was done by linear reduction technique. Forty postlarvae were introduced into each tub which was filled partially with the same salinity media as that of the holding tank. The postlarvae were exposed to linear decline/increase in salinity from initial to required salinities of 0, 10, 20, 30 and 35 ppt. The acclimation procedure is as mentioned below. The animals were acclimated to desired salinity over a period of 5 days at a rate of 5 ppt per day.



1.3.3. WATER QUALITY MANAGEMENT

The water quality parameters like salinity, temperature ,pH, Dissolved Oxygen were checked routinely. The necessary adjustments were done to maintain these parameters optimum for the experiment. The wastes accumulated at bottom are siphoned out every morning and evening before feeding. Daily 10-30% water exchange was carried out. Thewater exchange of near 100% was done weekly. The

water exchange was also one to avoid algal bloom at times. The bottom and sides of tubs were also cleaned weekly.

1.3.4. FEEDING

The experimental animals were fed with commercial pelletised feed (Higashi shrimp feed with 39% crude protein) . The feed was given twice daily i.e., morning and late evening. More feed was given at evening . The feeding rate was maintained optimum with prevailing environmental conditions. Normally the animals were fed *ad libitum*.

1.3.5. SAMPLING AND OBSERVATIONS

The animals were carefully handled for the weekly measurements in length and weight. They were quickly transferred to well aerated containers and brought to laboratory for measurements. Continuous aeration was given with battery operated portable aerator throughout the process. The total length and wet weight of animals were measured. Total length was measured to nearest millimeter (mm) from the rostral tip to the tip of the telson. The postlarvae were blotted dry before weighing. Weighing was done using electronic balance(Mettler PC440).

The animals were observed daily for the activity and feed intake by observing the alimentary canal. Cannibalism and moulting was also noted. The lowering to temperature decreased feeding. In freshwater, mass mortality was observed after two weeks of rearing. The cannibalism observed was very high in freshwater acclimated postlarvae. Most of the animals died were observed to be

newly moulted or under the moulting process. Survival was recorded daily during morning feeding schedule.

The survival rate, SGR and RGR are calculated using the following formulae,

$$\text{Survival (\%)} = \frac{\text{Number of animals survived}}{\text{Initial number of animals stocked}} \times 100$$

$$\text{SGR} = \frac{\text{Ln (Final wet body weight) - Ln (Initial wet body weight)}}{\text{Time (Days)}} \times 100$$

$$\text{RGR} = \frac{\text{Final wet weight - Initial wet weight}}{\text{Initial wet weight}} \times 100$$

1.4. BIOCHEMICAL ANALYSIS

Moisture, Ash, Total Proteins, Total Carbohydrates, Total Lipids in the tissue were estimated. The tissue was homogenised and weighed with an electronic balance (Mettler PC 440). A portion of tissue was used for estimation of moisture and ash. The rest were preserved in polythene containers in a Deep Freezer for further analysis.

The Optical Density of colour developed for total proteins, lipids and carbohydrates were measured using Spectrophotometer (GBC 911A) . Standard graphs were plotted with the concentration of each biochemical parameters in different dilution of the workingstandard solution , in the X-axis and Optical

Density(OD) in the Y-axis . The concentrations of different parameters in the samples were calculated (in mg%) by comparing the OD obtained for the sample with the values in the standard graph.

1.4.1. MOISTURE CONTENT

The animals were blotted to remove the adhering water. The wet weights of tissues were taken accurately and samples were gradually dehydrated to constant weight in hot air oven. The moisture content was then calculated gravimetrically as the difference in the wet weight and the dry weight in the tissue and this is expressed as percentage of wet weight.

1.4.2. ASH CONTENT

The dried samples were powdered in a mortar. The pre-weighed amount of oven dried powdered tissue samples was ignited in a silica crucible for 5 hours at 580°C in a Muffle furnace till all the organic matter was burnt out leaving carbon residue. The ignited content was weighed and the difference in weight was taken as the ash content of the tissue. The percentage ash content of the tissue was calculated as follows.

$$\text{Ash percentage} = \frac{\text{Ash weight}}{\text{Dry weight of tissue}} \times 100$$

1.4.3. TOTAL PROTEIN

The Folin-Ciocalteu Phenol method of Lowry *et al.*, (1951) was adopted for the estimation of total proteins in the tissue. The wet tissue sample weighing 50

mg were thoroughly homogenised with 1 ml of deproteinising agent (10% TCA) by keeping the tubes in ice. All samples were centrifuged for 20 minutes at 3,000 rpm. The supernatant obtained in the individual tubes was used for the estimation of total carbohydrate. The precipitate was used for the protein estimation. The precipitate was dissolved in 2 ml 1N NaOH and to 1 ml of this solution, freshly prepared 5 ml alkaline reagent was added . This was kept at room temperature for 10 minutes, after which 0.5 ml of 1N Folin-Ciocalteu reagent was added and mixed rapidly.

A standard stock solution was prepared using Bovine Serum Albumin crystals at a concentration of 25mg/5ml NaOH . Different dilutions in the range of 0.25-2.5 mg/ml were prepared from this stock solution, the alkaline reagent and Folin-Phenol reagent were added as in the case of tissue samples. A blank was prepared with 1 ml 1N NaOH and treated the same way as above.

All the test tubes were kept for 30 minutes at room temperature and the O.D of the blue colour developed was measured against the blank at 660 nm.

1.4.4. TOTAL CARBOHYDRATE

The phenol sulphuric acid method of Dubois *et al.*, (1956) was followed to estimate the total carbohydrates in the samples. To 1 ml of the supernatant obtained during protein estimation 1ml of 5% phenol was added and mixed well. One ml of concentrated Sulphuric acid was added rapidly and carefully to each tube and mixed well using a cyclomixer.

A standard stock solution was prepared using D-glucose . Different dilutions of the working solution with the concentration of glucose ranging from 10-100mg/ml were prepared and the procedure adopted for the tissue was followed. A blank solution with 2 ml of 5% phenol was prepared and the above procedure followed.

The tubes were kept for 30 minutes at 30°C and the O.D of the colour developed was measured at 490 nm.

1.4.5. TOTAL LIPIDS

The total lipids were quantitatively determined by Sulphophosphovanillin method of Barnes and Blackstock (1973). About 50 mg of tissue were homogenised well in 1 ml of chloroform : Methanol (2:1v/v) and kept overnight at 4°C for complete extraction. The mixture was then centrifuged for 15 minutes at 3,000 rpm and the supernatant was taken in dry test tubes. From that 0.5 ml of lipid extract was taken and dried *in vacuo* over silica gel in a desiccator. To the dried sample 0.5 ml of concentrated Sulphuric acid was added and shaken well. The tubes were then plugged with non-absorbent cotton wool and heated at 100°C in a boiling water bath for exactly 10 minutes. The test tubes were rapidly cooled to room temperature under running tap water. To 0.1 ml of this acid digest, 2.5 ml of phosphovanillin reagent was added and mixed well in a cyclomixer.

Stock solution was prepared afresh by dissolving 80 mg of cholesterol in 100 ml of chloroform : methanol mixture. Working solutions of different concentrations were prepared from the stock solution in the range 50-500mg/0.5 ml

and the procedure adopted for the tissue samples was followed. 0.5 ml of chloroform methanol mixture was treated as blank.

All tubes were kept at room temperature for 30 minutes . The intensity of the pinkish red colour developed was measured against blank at 520 nm.

RESULTS

1.1 SURVIVAL OF POSTLARVAE

A high survival rate was obtained in all the salinities tested, except in freshwater acclimated postlarvae. A survival rate of 97.5% was obtained in 10, 20 and 30 ppt salinities, the survival observed in 35 ppt salinity was 96.67%. In freshwater acclimated postlarvae the survival showed a decreasing trend and total mortality was observed after 20 days.

The survival rates at different salinities are given in the Table I. From the table it can be seen that the survival was almost same for the low saline and seawater media, but in freshwater the survival reduced from 93.33%, 53.33% and 10.83% after 10, 15 and 20 days respectively. Thereafter total mortality was observed. Graph I. shows the survival during the experimental period.

1.2 GROWTH OF POSTLARVAE

The growth experiment in laboratory for 30 days showed significant variations in growth. The growth rate in terms of length and weight proved to change with salinity of the medium. The growth obtained was good and comparable in laboratory conditions. The weight observed for the same length postlarvae showed variation this can be attributed to moult stages.

1.2.1 GROWTH IN WET WEIGHT

Mean weight of the postlarvae at the commencement of the experiment varied between 31.9 mg and 41.3 mg. Growth in wet weight was maximum at salinities 20

and 30 ppt salinities with the specific growth rate (SGR) of 7.31 and 7.03 respectively. It was comparatively low in 35 and 10 ppt salinities with 6.02 and 6.06 respectively. In the freshwater media the SGR obtained after 15 days was 2.60. Table II shows the difference in growth between salinities.

The Table II and Graph II clearly indicate the trend of growth in different salinities. Maximum growth in wet weight was observed in 30 ppt during first week. The 20 ppt showed maximum growth in second week, on the third week 30 ppt gave better results. The fourth week showed a maximum gain in wet weight at 20 ppt.

1.2.2 GROWTH IN TOTAL LENGTH

The mean size of postlarvae at the commencement of the experiment varied between 1.6 to 1.9 cm. The growth in total length was maximum at salinities of 30 ppt and 20 ppt with 0.597 mm and 0.565 mm respectively per day. It was comparatively low in 35 and 10 ppt salinities with 0.487 and 0.431 mm per day respectively. In freshwater acclimated ones, after 15 days, the growth in length obtained was 0.431 mm /day. The difference in growth between different salinities is depicted in Table III.

From Graph III and Table III, it can be clearly seen that the growth was different at various salinities. The 30 ppt salinity gave the maximum growth in length during first week. The second week showed better growth rate in 10 ppt

salinity, 30 ppt and 20 ppt salinities showed highest growth rates at third and fourth weeks respectively.

1.2.3. ANALYSIS OF VARIANCE FOR GROWTH

ANOVA showed a significance at 1% level for both Specific Growth Rate and Increase in total length per day. In terms of SGR, there was no significant difference between 20 and 30 ppt and also between 35 and 10 ppt. The first set is significantly superior to the second one. In case of total length there was no significant difference between 30 and 20 ppt and also between 35 and 10 ppt. But there was difference among the two groups. ANOVA was not carried out for 0 ppt because there was total mortality during the experimental period.

1.3 SALINITY AND BIOCHEMICAL COMPOSITION

The tissues were analysed for total protein, total carbohydrate, total lipids, ash and moisture. The results of analysis were presented in Table V. The first three parameters are expressed in mg/100 mg on wet weight basis and the last two on percentage wet weight basis. The mean values were taken for Table and Graph. The extent to which they vary in different salinities was tested by ANOVA. Table VI shows the ANOVA for biochemical parameters. Because of total mortality, biochemical analysis was not done in case of 0 ppt acclimated postlarvae.

1.3.1. TOTAL PROTEIN

The total protein showed a steady increase from 35 ppt down to 20 ppt, later it decreased in 10 ppt salinity. The values of total protein are given in wet weight basis and

are 7.69, 8.29, 14.45 and 12.28 respectively for salinities 35, 30, 20 and 10 ppt. Graph IV shows the relation between salinity and total protein.

ANOVA for total proteins showed that the variation in total protein was significantly high (1% level) between salinities. Between 30 ppt and 35 ppt there was no significant difference.

1.3.2. TOTAL CARBOHYDRATE

The Carbohydrate showed a significant difference. The highest Carbohydrate values were obtained at 30 ppt followed by 10 ppt with 1.01 and 0.83 respectively. The 20 and 35 ppt salinities showed a carbohydrate level of 0.76 and 0.71 mg%. Graph V shows the Carbohydrate level at different salinities tested.

ANOVA for Carbohydrate showed a significant difference at 1% level. Between 20 and 10 ppt salinities and also between 20 and 35 ppt salinities there was no significant difference.

1.3.3. TOTAL LIPID

Total lipid content showed no significant difference. The values obtained were 1.76, 1.91, 1.82 and 2.15 for 35, 30, 20 and 10 ppt salinities respectively. Graph VI shows the total lipid level at different salinities tested.

1.3.4. MOISTURE

The moisture percentage at different salinities did not show any significant variation between treatments. The highest value obtained was at 10 ppt salinity. The

Moisture percentage obtained were 77.43, 74.03, 74.11 and 74.11 respectively for 10, 20, 30 and 35 ppt salinities. Graph VII shows the moisture percentage at different salinities.

1.3.5. ASH

There was no significant difference between treatments and ash percentage. The ash percentage obtained were 5.09, 4.84, 4.84 and 3.88 for the salinities of 35, 30, 20 and 10 ppt respectively. The results obtained are depicted in Graph VIII.

TABLE I. Survival rate of *Penaeus monodon*(Pl30-PL60)at different salinities.

Days	Survival rate (%) in different salinities				
	35 ppt	30 ppt	20 ppt	10 ppt	0 ppt
1	100	100	100	100	100
5	100	99.17± 1.44	100	100	96.67± 1.44
10	97.5± 2.5	98.33± 2.89	99.17± 1.44	100	93.33± 2.89
15	97.5± 2.5	97.5± 2.5	97.5± 2.5	99.17± 1.44	53.33± 26.96
20	96.67± 1.44	97.5± 2.5	97.5± 2.5	99.17± 1.44	10.83± 9.46
25	96.67± 1.44	97.5± 2.5	97.5± 2.5	97.5	0
30	96.67± 1.44	97.5± 2.5	97.5± 2.5	97.5	0

TABLE. II Growth in wet weight of *Penaeus monodon* (PL30-PL60)at different salinities.

Salinity (ppt)	Initial weight (g)	Growth increment in successive weeks				Final weight (g)	S.G.R	R.G.R
		I	II	III	IV			
35	0.0413± 0.0012	0.0237± 0.0017	0.0647± 0.0025	0.052± 0.0027	0.0693± 0.0059	0.251± 0.0055	6.02±[b] 0.1511	513.11± 28.09
30	0.0376± 0.0023	0.0299± 0.0094	0.0473± 0.0063	0.087± 0.0098	0.0973± 0.0085	0.309± 0.0148	7.03±[a] 0.1291	729.96± 31.08
20	0.0347± 0.0014	0.0235± 0.0027	0.0648± 0.0081	0.0607± 0.0096	0.1257± 0.0116	0.309± 0.0009	7.31±[a] 0.1266	801.51± 35.09
10	0.0387± 0.0008	0.0187± 0.0013	0.0603± 0.0071	0.0573± 0.0019	0.063± 0.004	0.238± 0.004	6.06±[b] 0.0224	516.77± 4.06
0	0.0321± 0.0008	0.0181± 0.0008	.0 210± 0.0109	**	**	0.071± 0.0008	2.66±* 0.1194	123.76± 8.11

Data are means of triplicates. Different superscripts indicate significant difference using ANOVA($P<0.01$).* not included for ANOVA.
**complete mortality

TABLE III. Growth in total length of *Penaeus monodon* (PL30-PL60)at different salinities.

Salinity (ppt)	Initial length (mm)	Growth increment in successive weeks				Final length (mm)	Increase in length (mm/day)
		I	II	III	IV		
35	18.2± 0.189	3.333± 0.222	5.133± 0.239	3.133± 0.386	3.0± 0.385	32.8± 0.316	0.487± [a] 0.016
30	17.1± 0.386	4.533± 0.239	4.067± 0.407	5.133± 0.754	4.2± 0.54	35± 0.61	0.596± [b] 0.004
20	18.1± 0.239	2.867± 0.318	4.933± 0.527	4.067± 0.285	5.067± 0.574	35± 0.228	0.565± [b] 0.01
10	18.2± 0.109	2.467± 0.285	5.4± 0.718	3.8± 0.456	2.8± 0.126	32.6± 0.222	0.482± [a] 0.004
0	16.6± 0.063	2.733± 0.132	3.3± 0.239	**	**	22.6± 0.111	0.4± * 0.012

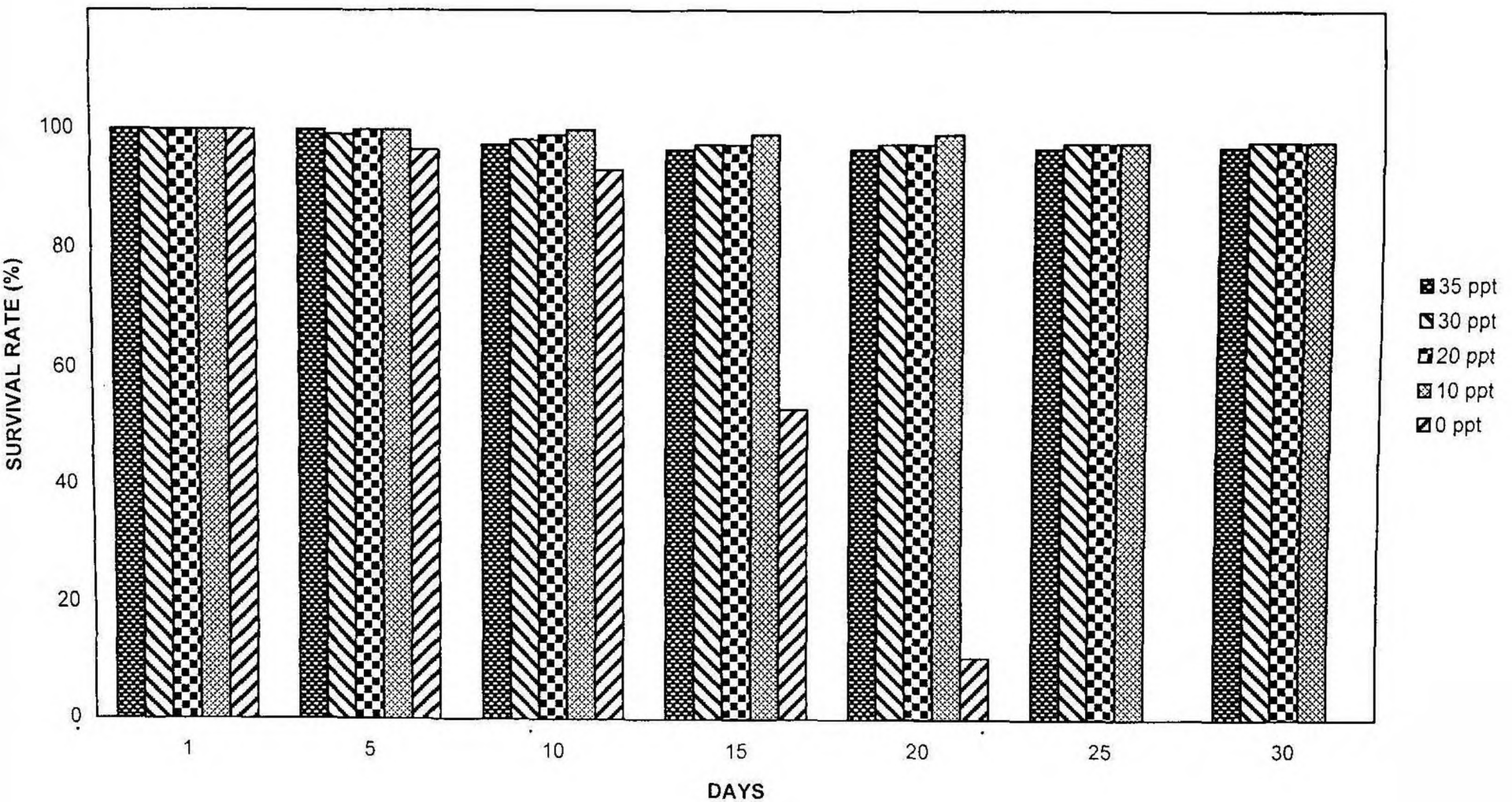
Data are means of three replicates. Different superscripts indicates significant difference using ANOVA($p<0.01$)

TABLE IV. Biochemical composition of *Penaeus monodon*(PL 30-PL 60)at different salinities

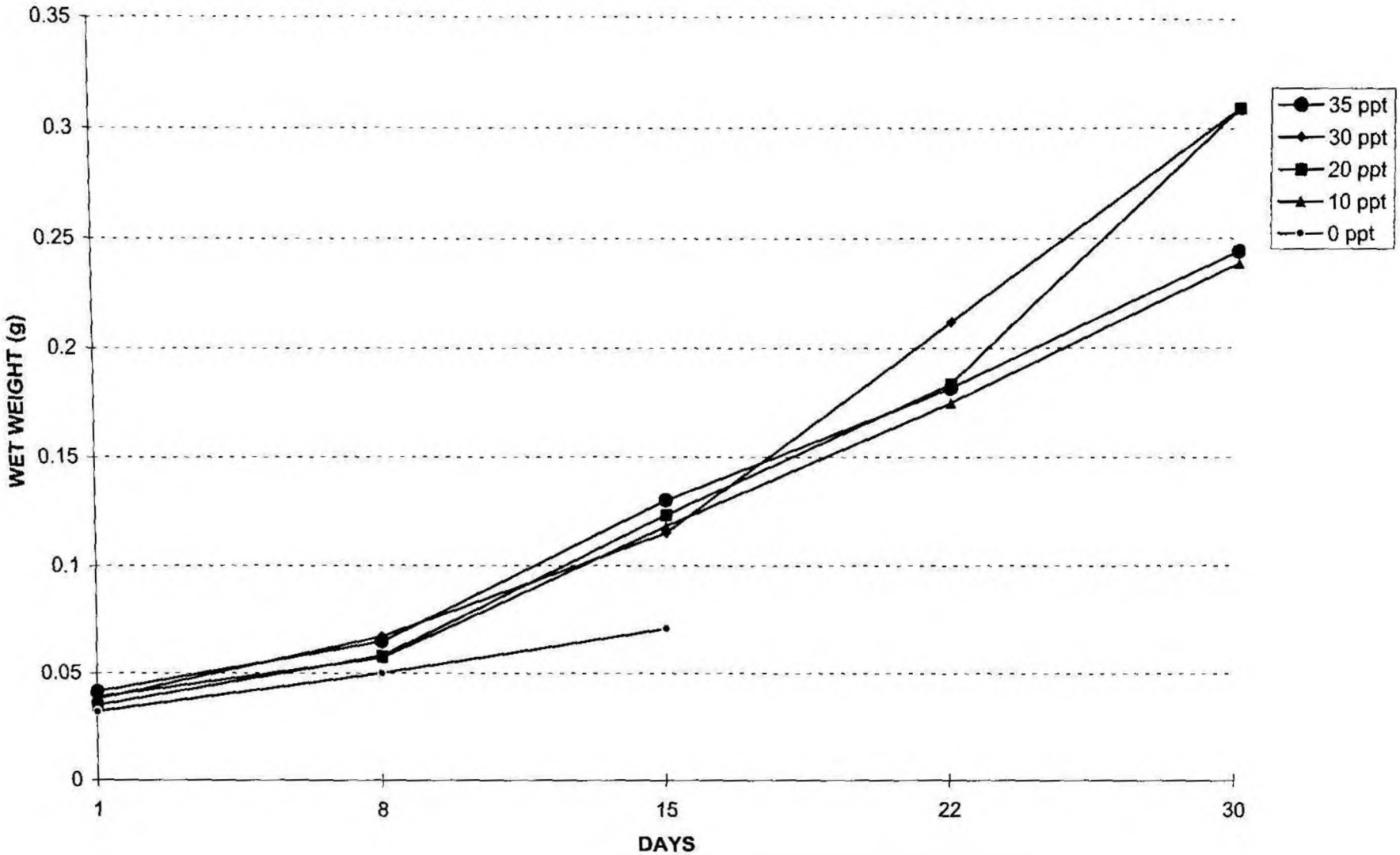
Salinity (ppt)	Moisture (%)	Ash (%)	Total Protein (mg/100mg)	Total Carbohydrate (mg/100mg)	Total Lipid (mg/100mg)
35	74.11± 1.43	5.09± 0.602	7.67± [c] 0.61	0.71± [a] 0.055	1.76± 0.4
50	74.11± 1.24	4.84± 0.609	8.29± [c] 0.24	1.01± [c] 0.058	1.91± 0.32
20	74.03± 1.11	4.84± 0.55	14.45± [a] 0.54	0.76± [a b] 0.015	1.82± 0.11
10	77.43± 3.58	3.88± 0.82	12.28± [b] 0.71	0.83± [a b] 0.015	2.15± 0.26

Data are means of three replicates. Different superscripts indicate significant difference using ANOVA ($P<0.01$)

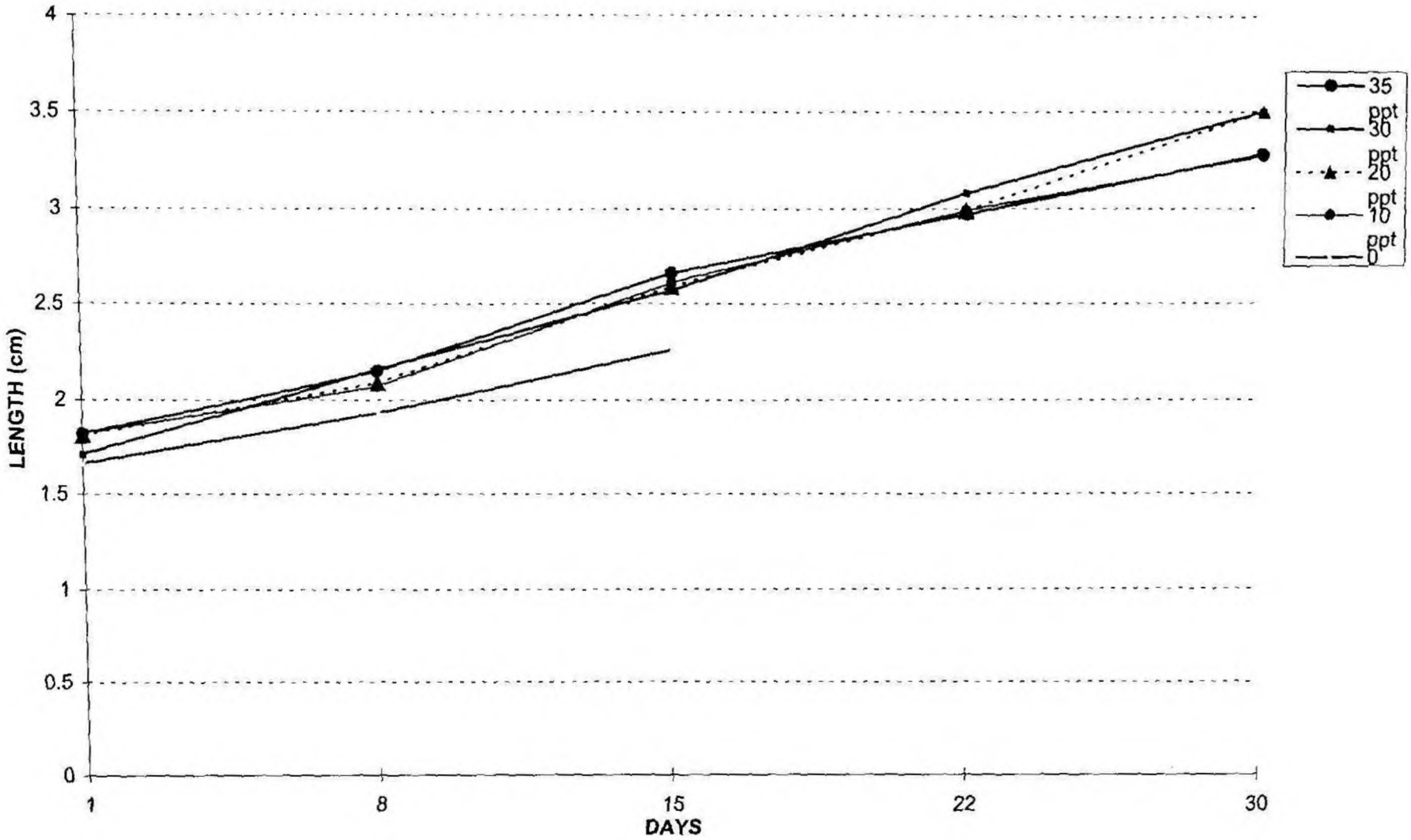
GRAPH I. SURVIVAL RATE OF *PENAEUS MONODON* (PL30- PL60) AT DIFFERENT SALINITIES



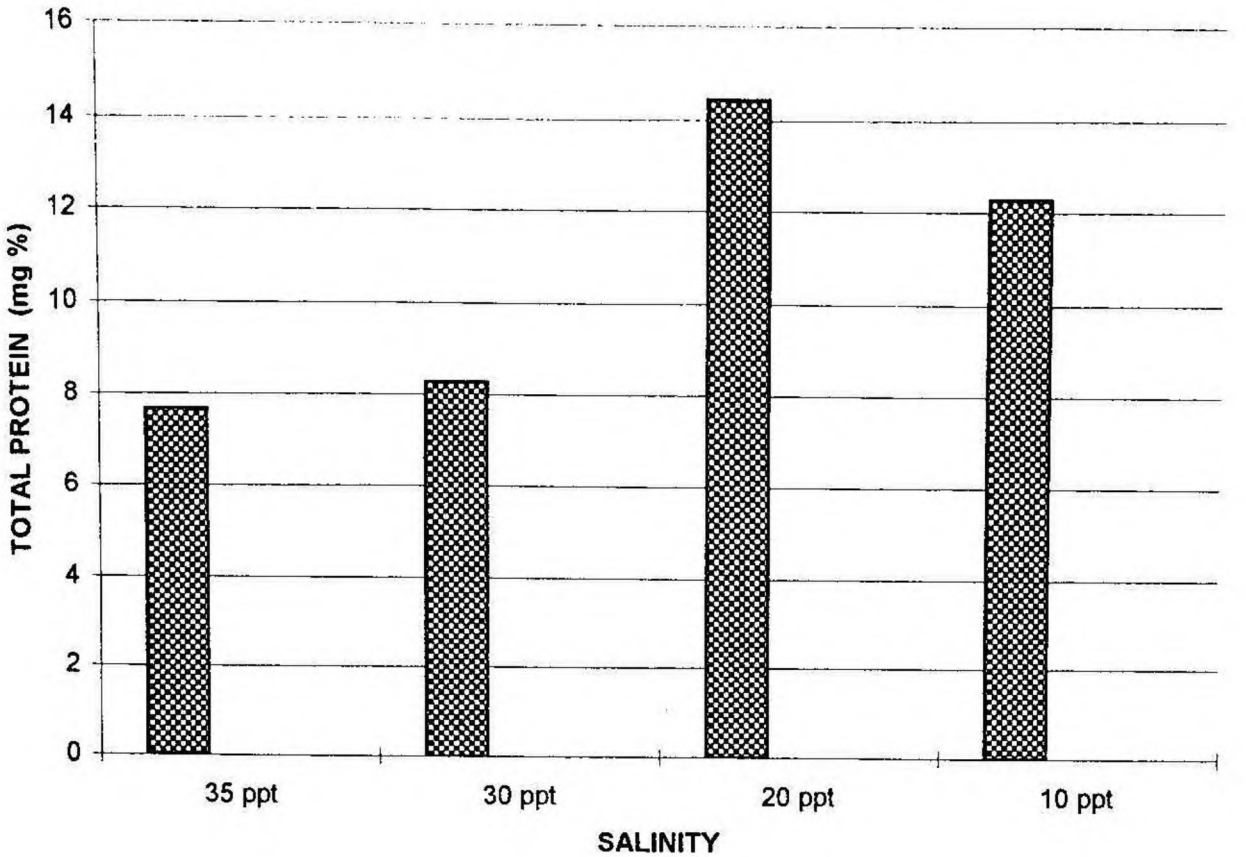
GRAPH II. Growth in wet weight of *Penaeus monodon* (PL 30 - PL 60) at different salinities



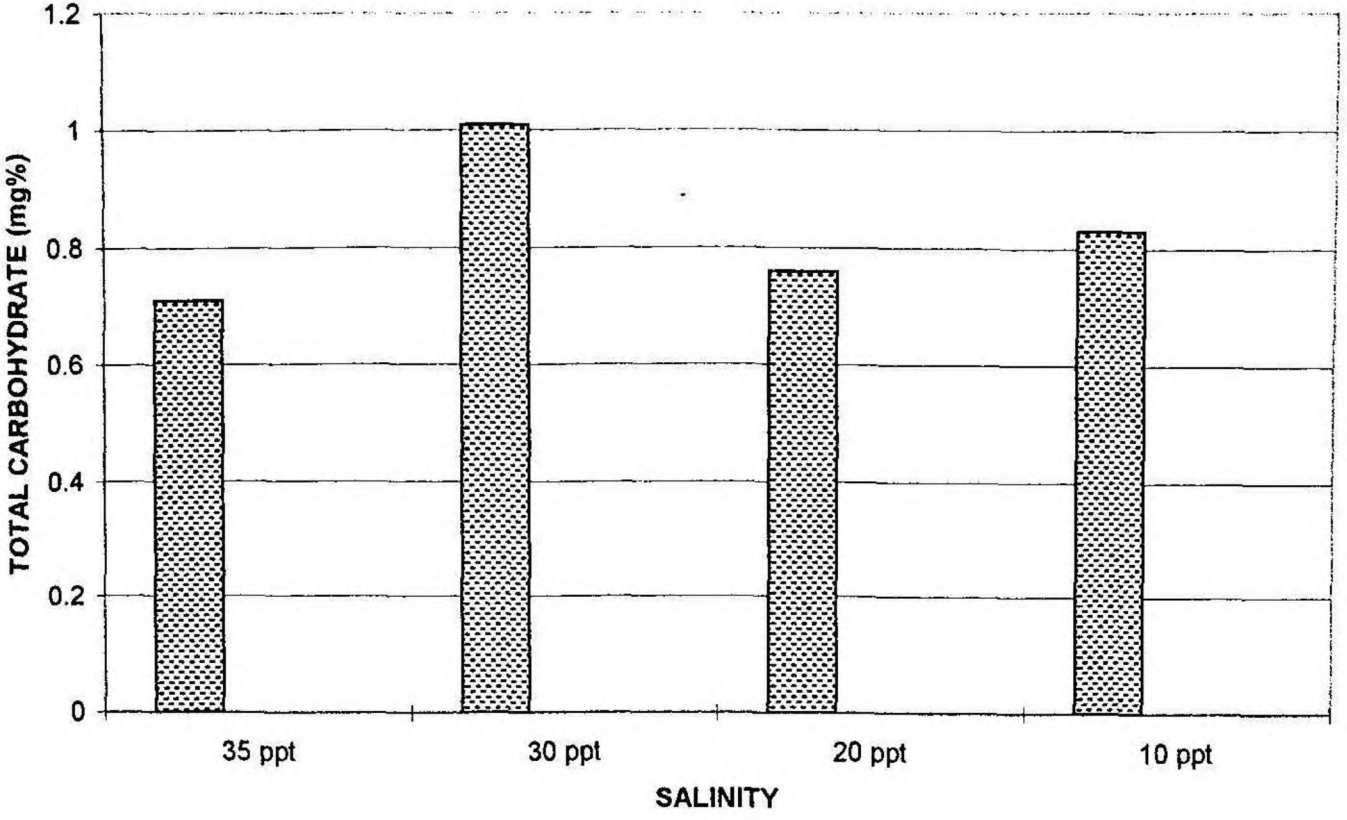
GRAPH III. Growth in lenght of *Penaeus monodon* (PL30-PL60) at different salinities.



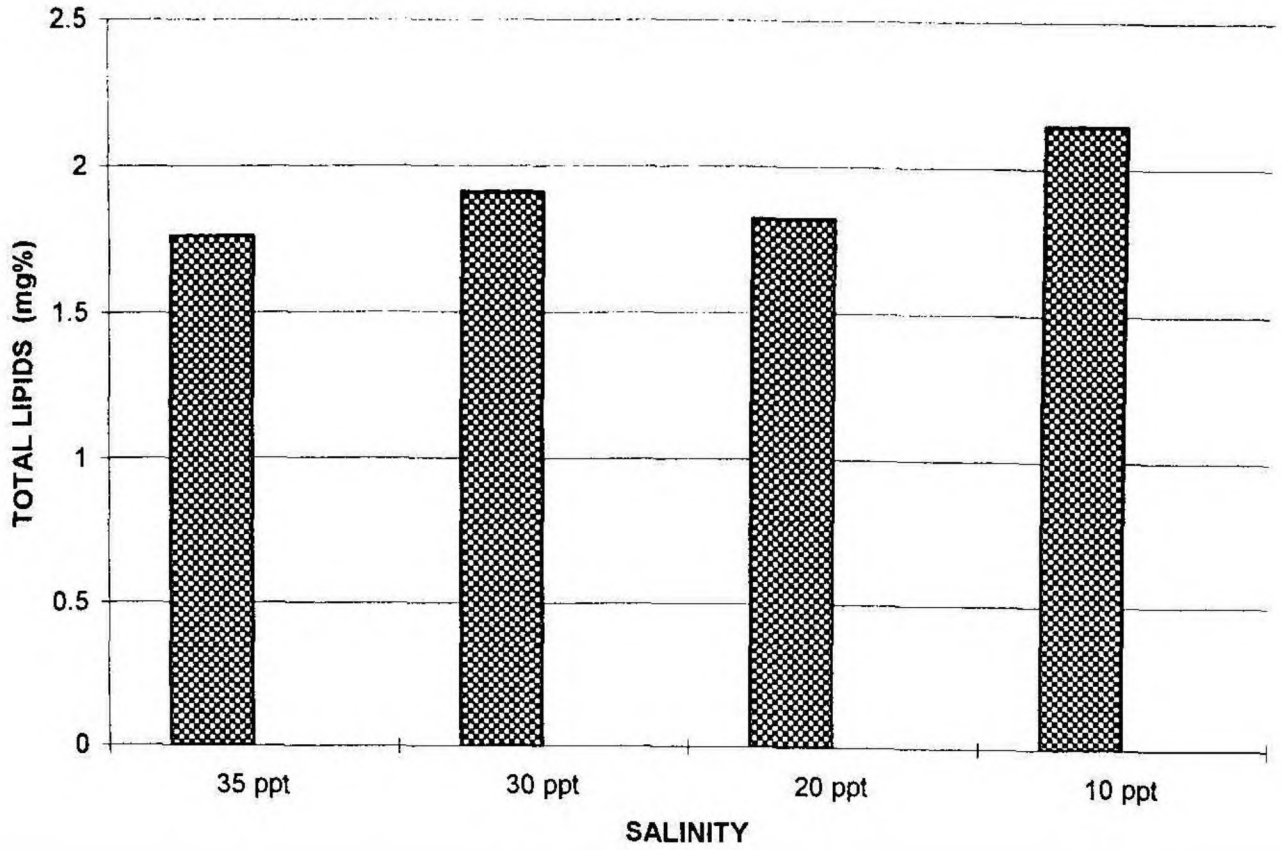
GRAPH IV. Total protein (mg%) in *Penaeus monodon* (PL30-PL60) at different salinities.



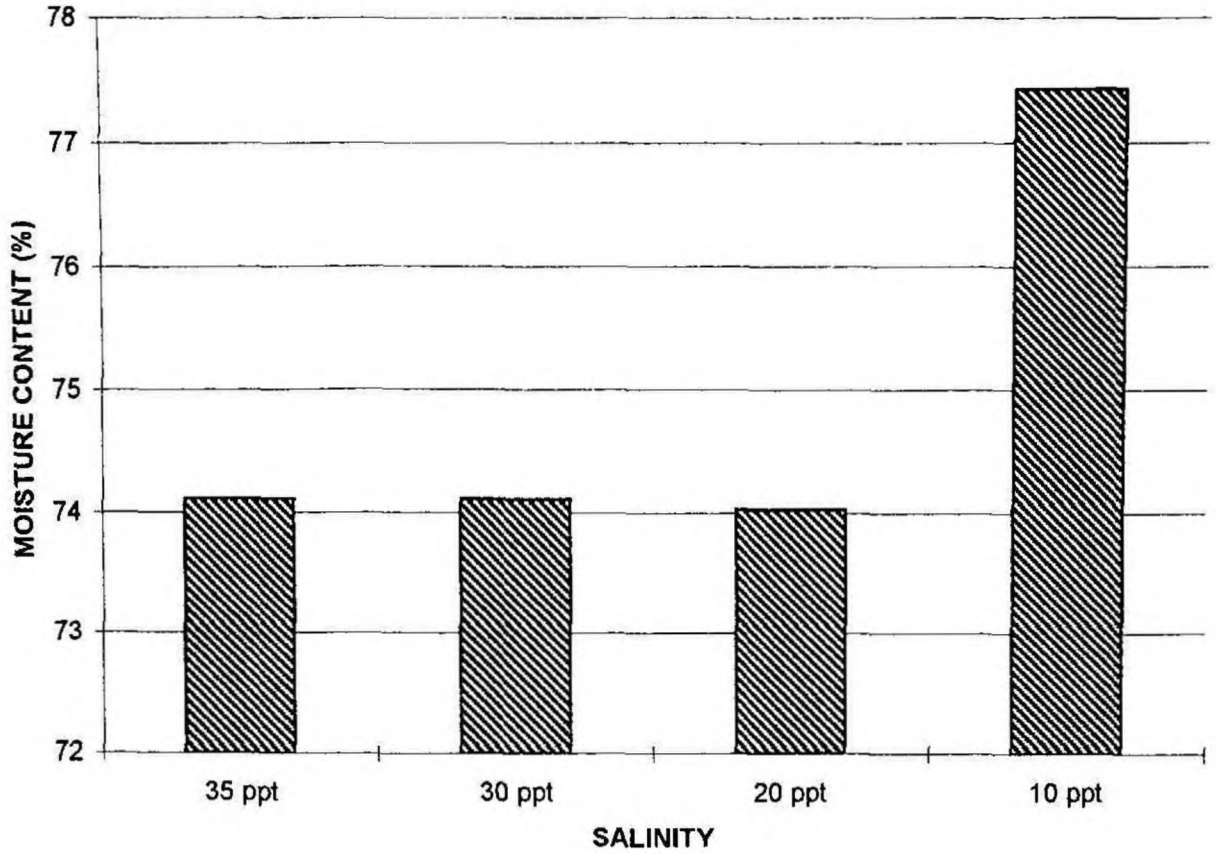
GRAPH V. Total carbohydrate in *Penaeus monodon* (PL30- PL60)at different salinities.



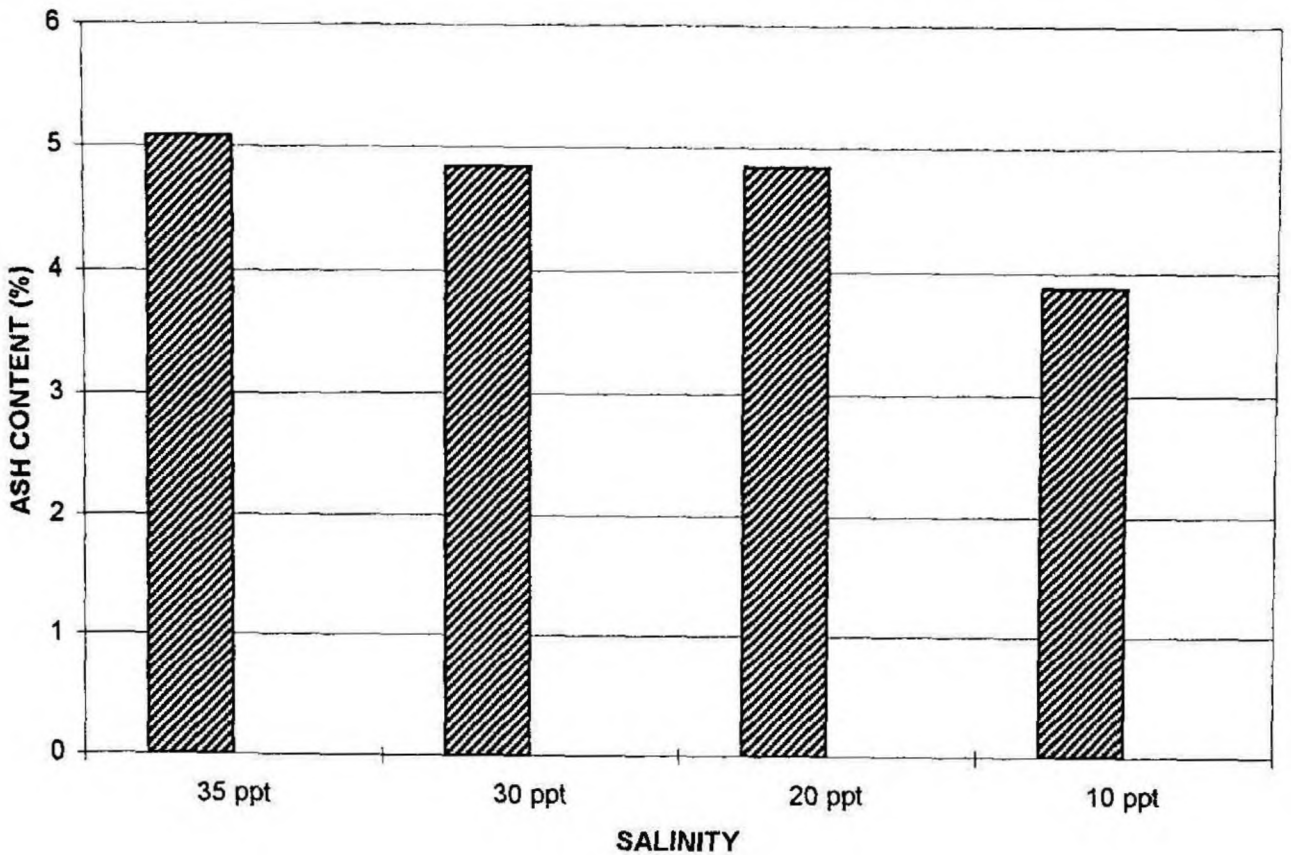
GRAPH VI. Total lipid (mg%) in *Penaeus monodon* (PL30-PL60) at different salinities.



GRAPH VII. Moisture (%) in *Penaeus monodon* (PL30-PL60) at different salinities.



GRAPH VIII. Ash content(%) in *Penaeus monodon* (PL30-PL60) at different salinities.



DISCUSSION

Experiments were conducted on the influence of salinity on the survival, growth and biochemical composition of the postlarvae of *Penaeus monodon.*

1. Survival and growth of postlarvae in different salinities

In the present study, the postlarvae of. *P monodon* were acclimated to and tested in different salinities, viz, 0, 10, 20, 30 and 35 ppt. A highsurvival rate was obtained in all the saliniies tested except in freshwater acclimated postlarvae. The survival rate obtained was 97.5 % in 10, 20 and 30 ppt, in 35 ppt salinity it was 96.67 %. In freshwater acclimated postlarvae, the survival showed a decreasing trend and total mortality was observed after 20 days.

Results of the study showed that the survival rate was almost the same for the low saline and high saline media, but in freshwater the survival decreased with increase in the number of days of exposure.

Zein-Eldin(1963) observed that survival was not affected by the salinity. This view was contradicted by Gunter et al., (1964). Motoh (1981) showed that tolerance varies with salinity. He recorded a survival rate of 64 % in 0 ppt for the *P. monodon* postlarvae. Raj and Raj (1982) recorded higher survival and growth 15 to 25 ppt for *P. monodon, P. indicus and P. semisulcatus.* Rajyalakshmi and Chandra(1987) reported a high survival rate at 15 ppt than 20 and 0 ppt for *P. monodon.* The

results obtained in the present study are comparable with those obtained by other workers.

The growth experiment carried out for 30 days showed significant variations in the growth. The growth rate in terms of length and weight changed with salinity of the medium. The weight observed for the same length postlarvae showed variations which can be attributed to moult stages.

Growth rate in terms of wet weight was maximum at salinities 20 and 30 ppt with specific growth rate(SGR) of 7.31 and 7.03 respectively. It was relatively low in 35 and 10 ppt with 6.02 and 6.06 respectively. In freshwater media growth obtained after 15 days was 2.60.

In the present study, it was found that growth in terms of total length was maximum at 30 and 20 ppt salinities. It was comparatively low in 35 and 10 ppt salinities. In freshwater, growth obtained after 15 days was comparable to that obtained in 10 ppt, though subsequently there was total mortality in freshwater.

Huang (1983) recorded highest growth rate of P. vannamei postlarvae at 20 ppt salinity and the poorest growth was recorded at 5 and 45 ppt. Deshimaru et at.,(1985) obtained good growth and survival of *P. monodon* juveniles at lower salinities than at higher salinity. Rajyalakshmi and Chandra (1987) observed decreasing growth rate in 15, 20 and 0 ppt for *P. monodon*. Bukhari et al., (1994)

observed better growth and high survival in high saline conditions in *P. indicus*. The results obtained in the present study are in agreement with the results obtained by other workers in *P. monodon*.

Pantastico (1970) obtained good growth and survival of postlarval *P. monodon* in freshwater systems. In contrast Cawthorne (1983) got highest survival and growth in full strength seawater than the lower salinities. In the present study also total mortality occurred in freshwater media. One possible explanation for these contradicting results is that the substrate provided by Pantastico and the absence of substrate in Cawthorne's and present study. The mortality observed in the present study was coincided with moulting. Cannibalism was prevalent during this time in freshwater media.

Shrimps during and immediately after moulting tend to conform to environmental osmolality and chloride concentration. The osmotic and chloride concentration of *P. monodon* clearly varied with both moult stages and salinity of medium. During ecdysis, the shrimps are stressed when salinity also changes. In cultured penaeids, high mortality observed when moulting coincides with large fluctuations in salinity, suggests that moulting interacts to a large degree with salinity in inducing stress (Ferraris et al., 1987)

The shrimps moulting in extremely high(or low) salinities would require much more energy and time in normalising haemolymph osmolality. Because they would

be secreting (or absorbing) ions against a higher ionic gradient. This long time interval increases the vulnerability of shrimps to predation and cannibalism and prolongs their inability to forage for food (Ferraris et al., 1987). This explains the poor survival of *P. monodon* postlarvae in low salinities and freshwater media.

From the present study it is found that if postlarvae are acclimated to lower salinities before the monsoon, the shrimp get acclimatised to that situation and withstand further reduction in salinity. Thus it is possible to culture this species year round rather than only in high saline period.

II Effect of salinity on biochemical composition

The total protein registered a steady increase from 35 ppt down to 20 ppt later it decreased in 10 ppt. The carbohydrate showed a significant difference in different salinities. The highest carbohydrate values were obtained 30 ppt followed by 10 ppt. The total lipid showed no significant difference at different salinities.The moisture percentage at different salinities did not show any significant variation. The highest value obtained was at 10 ppt. Similarly there was no significant difference in the ash content of postlarvae acclimated, and tested at different salinities.

Changes in osmotic concentration are considered to induce drastic changes in the protein and amino acid composition in tissues. Gilles (1977) found that

haemolymph protein was affected by salinity in Chinese crab *Eriocheir sinensis*. Chen et al (1994) observed that haemolymph protein in *P. monodon* decreased with increase in salinity. Haberfield et al., (1975) observed an increase in the catabolism of amino acids that resulted in excretion of nitrogen, mainly as ammonia with decreased medium osmolarity.

In the present study, the total protein content was highest at 20 ppt. In 30 ppt also the content was highest at 20 ppt. In 30 ppt also the growth was similar to that obtained in 20 ppt but protein content was lower.

Salinity may not be an important factor in determining protein concentration in the haemolymph except in unusually high or low salinities when osmotic forces would prevail (thereby dehydrating or hydrating the haemolymph, respectively), or in conditions where excess amino acids would be utilized as osmitically active compounds to increase or decrease haemolymph osmolality (Ferraris et al., 1986).

Dalla Via (1986) examined the effect of salinity on free amino acid composition in *P. japonicus*. Studies by Fang et al.,(1992) established that the FAA were similar in haemolymph and muscle of *P. monodon* acclimated to 30 ppt. Its also found that high concentration of essential free amino acid in the haemolymph of 15 ppt acclimated shrimps indicating that these FAA could be more available for tissue take up at this salinity. This explains the better growth of *P. monodon* in low salinities.

In crustaceans, the hepatopancreas is considered to be the main storage tissue for polysaccharides (Dall and Moriarty, 1983). In the present study, the carbohydrate showed significant difference in different salinities, the highest value being in 30 ppt, studies on the carbohydrate levels in the postlarvae of penaeid shrimps in different salinities is lacking.

There are some studies on the lipid levels in the tissues of penaeid shrimps. Gopakumar and Nair(1975) found 0.7 to 1.2 % (wet weight) lipid in the muscle tissue of five species of penaeid shrimps. Kalyanaraman (1983) observed a low level of lipid content in *P. indicus* corresponding to decreased protein level in lower salinities(5-10 ppt). However in the present study, the highest level of total lipid was obtained in 30 ppt followed by 10 ppt.

The moisture content of the postlarvae is also influenced by salinity. Kalyanaraman(1983) recorded a higher water content in *P. indicus* at lower salinity levels of 5 and 10 ppt. Ferraris (1986) reported variations in tissue water content with moult stages and salinity in *P.monodon*. Parado-Estepa et al., (1987) found that tissue water content was inversely proportional to salinity of the medium. Even though there was no significant difference in the water content in different salinities the results obtained in the present study are in agreement with the results obtained by other workers in penaeid shrimps.

The ash content of the postlarvae was not significantly affected by salinity in the present experiments on *P. monodon.*

The present study indicated that *P. monodon* postlarvae grows better at 20 ppt during the first month of culture. The protein content was also highest at 20 ppt suggesting that more protein is retained in the salinity for tissue build up. In 30 ppt, even though growth was comparable to that obtained in 20 ppt, the tissue proteins level was lower at this salinity. The results of this study are very useful in farming of this species.

SUMMARY

* In the present study survival, growth and biochemical composition of *Penaeus monodon* Fabricius postlarvae acclimated to different salnities (0, 10, 20, 30 and 35 ppt) were investigated.

* The acclimation procedure used was drip-in and drip-out method.

* The survival was not affected by salinity of media except in 0 ppt(freshwater)

* A high survival rate of 96.67 %at 35 ppt and 97.5 ppt at 10, 20 and 30 ppt were obtained. 0 ppt showed total mortality.

* The growth showed signigicant variation in different salinities. The growth obtained in freshwater was low compared to those on other salinities tested. The wet weight showed maximum increment (SGR 7.31) at 20 ppt salinity. The length increment was highest at 30 ppt (0.596 mm/day).

* The biochemical composition of postlarvae grown in different salinities showed significant differences in case of total protein and total carbohydrate. Total lipid, moisture and ash didnot show any significant variation.

* The protein value was maximum (14.45 mg/100 mg w. wt.) at 20 ppt salinity. Total carbohydrate was high at 30 ppt (1.01 mg/100 mg w. wt) salinity.

* Although insignificant, total lipid value was maximum at 10 ppt (2.15 mg/100 mg w.wt), moisture also at 10 ppt (77.43 %) and ash at 35 ppt (5.09 %) salinity.

The present study provides, baseline information for the aquaculture of *P. monodon* in different salinities.

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