CONDITIONS FOR OXYGEN-PACKED TRANSPORTATION OF PENAEUS INDICUS SEED

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THESIS

Submitted in partial fulfilment of the requirement for the degree

MASTER OF FISHERIES SCIENCE

Faculty of Fisheries

Kerala Agricultural University

DEPARTMENT OF AQUACULTURE

COLLEGE OF FISHERIES

PANANGAD, COCHIN

1994

DEDICATED

TO

MY BROTHER & SISTER

DECLARATION

I hereby declare that this thesis entitled "CONDITIONS FOR OXYGEN-PACKED TRANSPORTATION OF *Penaeus indicus* SEED" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other university or society.

Cochin,

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GEETHA RANI V. MANI

CERTIFICATE

Certified that this thesis, entitled "CONDITIONS FOR OXYGEN-PACKED TRANSPORTATION OF *Penaeus indicus* SEED" is a record of research work done independently by Kumari GEETHA RANI V. MANI under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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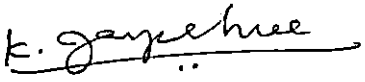
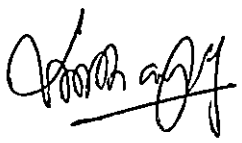
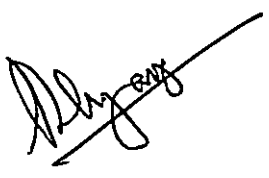
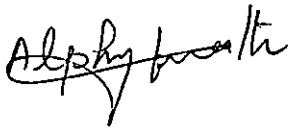
Dr. K. Jayasree Vadhyar Associate Professor, Department of Aquaculture, College of Fisheries, Panangad.	(ChairPerson)	
Dr. D. M. Thampy, Professor, Department of Aquaculture, College of Fisheries, Panangad.	(Member)	
Shri. P. S. Mrithunjayan Associate Professor, (Chemical Hydrography) Department of Fishery Hydrography, College of Fisheries, Panangad.	(Member)	
Smt. Alphi Korath Assistant Professor, (Statistics) Department of Management studies, College of Fisheries, Panangad.	(Member)	

ACKNOWLEDGEMENTS

I wish to place on record my deep sense of gratitude to all those who have contributed in one way or the other to the succesful completion of the research work and preparation of this thesis.

I am fortunate to have benefitted from the valuable guidance and constant encouragement of my advisor, Dr. K. Jayasree Vadhyar, Associate Professor, Department of Aquaculture, College of Fisheries, Panangad, during the course of the study. I owe it to her for whatever success I have had in preparing this thesis and submitting it in its present form.

I am greatly indebted to the members of the advisory committee, Dr. D. M. Thampy, Professor (Aquaculture) and Head i/c Department of Fishery Biology, Sri. P. S. Mrithyunjayan, Associate Professor, Department of Fishery Hydrography, Smt. Alphi Korath, Assistant Professor, Department of Management studies, College of Fisheries, Panangad, for their valuable advice and suggestions imparted to me throughout the study and preparation of the manuscript.

I am grateful to Dr. M. J. Sebastian, Dean i/c College of Fisheries, for providing all the necessary facilities for the successful conduct of the work.

I wish to extend my sincere thanks to Dr. Susheela Jose, Associate Professor and to all other staff of the Department of Aquaculture, for their constructive criticism which helped me in no small way in improving this work. I owe a great deal to Dr. C. J. Cherian, Associate Professor, Department of Fishery Hydrography, Dr. T. M. Jose, Associate Professor, Department of Fishery Biology, Dr. D. D. Namboodiri, Associate Professor and Head, Department of Processing Technology, Dr. P. M. Sherif, and Shri. S. Krishnakumar, Assistant Professors, Department of Processing Technology, Smt. V. Malika, Assistant Professor, Shri. T. M. Sankaran, Associate Professor and Head, Department of Management studies who spared their valuable time and allowed me to partake of their profound knowledge.

My special thanks are also due to Smt. Tessy K. Thomas, Junior Programmer, for the computerised data processing and to Dr. K. K. Varma, Associate Professor and Head, Department of Fishery Hydrography for allowing me to use the computer facility for word-processing.

The assistance rendered by the staff of the Libraries of College of Fisheries, Panangad, Central Marine

Fisheries Research Institute, Cochin, Marine Products Export Development Authority, Vallarpadam and Cochin, in the collection of all the necessary literature was invaluable.

I am grateful to the sincere help rendered by my friends and colleagues in conducting the experiments.

The fellowship awarded to me by the Kerala Agricultural University is thankfully acknowledged.

GEETHA RANI V. MANI.

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INTRODUCTION

1. INTRODUCTION

Consumption of fishery products has been increasing in tandem with the exponential growth of world population, but the growth of fishery production has been sluggish, leaving a huge gap between production and demand. This has compelled the world to explore alternative avenues for fish production. Growing selected aquatic organisms under controlled condition or aquaculture has been the answer. Obviously, the shrimps among the crustaceans which fetch high unit value requiring only short duration crop, with persistant demand and fast expanding world market, have been the best choice. The past decade has been marked by dramatic increases in the world production of shrimps that contributed almost entirely by increased productivity of aquaculture. India is a late entrant to commercial aquaculture, even though traditional culturing of shrimps has been practised in West Bengal and Kerala from a very long time. In India, much priority is given at present for the culture of shrimps in low-lying areas along the coast.

Penaeid shrimps are the most important and extensively cultured crustaceans all over the world . This is due to their great demand and high market value. They

are also ideal for intensive cultivation because of their adaptability to different culture systems, rapid growth, availability of seed through artificial propagation and positive response to supplemental feeding.

In any large scale culture operation, one of the foremost requirements is the availability of seed as and when required by the farmers. In this context, the utilisation of the available natural resources by organised seed collection from the wild assumes importance. But, the abundance and distribution of seed in the wild show fluctuation from season to season and from year to year. It is in this context that controlled breeding and hatchery production of seed of cultivable aquatic animals gain importance. Whether the seed is produced in the hatchery or collected from the wild, they are to be transported and distributed to the farm sites where they are cultured. This necessitates transportation of seed to shorter or longer duration depending on the distance from the site of seed production or collection, to the farm site. Any large scale mortality of seed during transportation incurs heavy economic loss to the enterprise. Transportation of large quantity of seed involving long transit entails several complex problems, such as maintenance of water quality, biological and physiological requirements of animals transported, optimum packing density during transportation,

conditioning them before transportation and acclimatisation soon after reaching the destination. It is in this context, that concerted effort is made through this study to investigate these vital problems, so as to ensure high survival rate and to minimise the economic loss.

Rapid growth of aquaculture demands information regarding the requirements of the cultivable species at each of their life stages and during culture, so as to maximise their survival and yield. Penaeus indicus is one of the most sought after species for farming in the brackish water and coastal aquaculture systems. The seed of the species is presently available from different hatcheries. Several devices have been evolved for the transportation of the seed to the farm site, primarily based on empirical knowledge and later on scientific lines. Although many of these methods/ devices have proved to be handy and economical, they are not free from the risk of incurring mortality. So far, only little is known about the optimum requirements for the transportation of the valuable shrimp seed. In this context, there is a vital need to specify the conditions for the shrimp seed transportation, instead of betaking the empirically based methods presently used in shrimp seed transportation.

The present study on the transport of P. indicus seed is therefore undertaken with a view to elucidating the

important environmental and physiological factors that contribute to mortality in the closed microcosm and to standardize the procedure.

Main objectives of the study are :

(1) to study the effect of different packing densities, temperatures and salinities on the oxygen consumption of P. indicus seed,

(2) to study the effect of packing density, salinity and temperature on the survival and safe duration of the oxygen-packed seed,

(3) to study the effect of introducing inert habitat material in the packing medium on the survival and safe duration of the oxygen-packed seed with a view to solving the problem of cannibalism and,

(4) to streamline the conditions for oxygen-packed transportation of P. indicus seed.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The techniques of shrimp seed transportation are based on those of fish seed transportation. Over the years several devices have been evolved for the transportation of fish seed, initially on the basis of empirical knowledge and later on scientific lines. Two methods of packing are in vogue (a) open system, comprising of open carriers, with or without aeration/ oxygenation/ water circulation and (b) closed system having sealed air-tight carriers with oxygen. Different types of carriers used in open system include earthen containers, tar-coated plaited bamboo baskets, metal containers, glass carboys, canvas containers, live-fish carrier boats, truck-mounted tanks and trucks with built-in hydro-tanks. Closed system include flexible and rigid plastic bags and containers filled with oxygen. Because of the sealed nature of this system there exists a unique microcosm with varying environmental and physiological factors that contribute to the survival and duration of the oxygen-packed seed. These factors mainly include temperature, dissolved oxygen, ammonia, carbon dioxide and pH levels in the packing medium. The present review mainly covers the closed system of shrimp seed transportation.

2.1 Shrimp seed transportation techniques.

Published information concerning the transportation of shrimp seed is limited, eventhough long distance transport of seed has become relatively common. Often, transport procedures are based on experience or "rules of thumb" passed among culturists rather than on hard data. Some of the techniques in the shrimp seed transportation are the following.

2.1.1. Plastic bags.

Containers with large bottom area were recommended by Liao and Huang (1972). They used plastic bags with 10-15 l of seawater and inflated with oxygen to transport 10,000 to 15,000 seed of P. japonicus at lowered temperatures of 15-18°C, packed within hardboard box. They found the safe duration of transport as 9.5h. In Japan, use of polythene bags with 8 l of seawater and 4 l of oxygen, packed within temperature-controlled containers for transporting P. japonicus seed were reported by Shigueno (1972). De and Subrahmanyam (1975) conducted experiments on the transport of P. monodon in plastic bags of 4-5 l capacity, with oxygen packing and could transport 500 post-larvae (10-40mm) with 87% survival for a duration of 36h. Korringa (1976) reported the use of 20% plastic bags, with 6-8 l low temperature seawater, filled with oxygen and

placed in cardboard boxes, for carrying 5,000-10,000 seed for a trip lasting for 12h in refrigerated truck. Experiments conducted by De (1977) on the transport of P. indicus seed under oxygen packing in polythene bags having a capacity of 3 l, suggested that 200 seed could be transported for a duration of 12h and 150 seed/l for 20h with 85% survival. Dwivedi (1978) reported high survival of P. monodon seed when transported in insulated boxes. Hamid and Mardjono (1979) got a low survival rate when a larger number of shrimp seed were placed in one bag without changing the density while transporting P. merguiensis and P. monodon seed. They used plastic bags with 5 l of water and an equal volume of oxygen. It was also found that plastic bag with more than 10 l water was easily damaged during transportation especially during air-lifting. Alikunhi et al. (1980) tried long distance transportation of nauplii and post-larvae of P. indicus and P. monodon in plastic bags, in which the survival was increased by providing live food organisms to combat cannibalism. Silas (1980) air-lifted shrimp seed in polythene bags for a period of 24h without considerable mortality. Polythene seed transportation jars of 14 l capacity filled with 10 l of water and the rest oxygen, were used for an experiment involving the transport of 500 post-larvae of P. indicus by Selvaraj et al. (1980). These jars were transported over 700 Km in 4 days with reoxygenation in every 24 h, with 85% survival at the end of the experiment. Franklin et al.

(1982) used transparent polythene bags of 20 l capacity filled with 5 l of oxygenated water for transportation of P. indicus, P. monodon and P. semisulcatus (10-20mm) post-larvae at different packing densities. Survival of 100% was obtained when 200 seed/5 l was transported for 18h. Singholka (1982) devised a simple cool "Walk-in" truck, with shelves for holding fishes or crustaceans packed in inflated plastic bags. The temperature in the transport truck was kept at approximately 22°C by ice cubes and battery-driven fans. Singh et al. (1982) used knotless and knotted polythene bags for the transport of P. monodon seed. They noticed that the bags with folds or knots at the bottom were not suitable for transporting shrimp seed, as the post-larvae got entangled within the folds and died. Krishnakumar and Pillai (1984) in their experiment used 4 l capacity PVC transportation bag with a firm base and double safety internal valve (to stop oxygen from leaking out). Tenedero and Villaluz (1985) described the techniques and procedure to follow for short and long duration transport of shrimp seed. They suggested the use of oxygen-packed plastic bags for short duration transport and oxygen-packed plastic bags in styrofoam boxes with ice to lower the temperature of transport water for long duration. Subrahmanyam (1986) observed a high survival of 98-100% in the transport of Macrobrachium rosenbergii seed in polythene bags with oxygen under pressure. He recommended

the addition of weed or plastic strips and worms to the medium as shelter and feed respectively during long distance transport. Simon (1986) suggested the use of thick gauge polythene bags of 18 l capacity, with 5-6 l of water and the rest oxygen for long distance transport. Kungvankij et al. (1986) reported the use of plastic containers of 20 l capacity and plastic bags of 6-8 l for the transportation of nauplii. Alias and Siraj (1988) packed M. rosenbergii post-larvae in plastic bags with different habitat materials. They found that the use of habitat materials had some effect on reducing cannibalism and increasing survival of post-larvae. Camillo (1990) described the packing procedure for transporting shrimp post-larvae in two plastic bags, with tied corners for inner bag. Chen (1990) reported the use of plastic bag with 10-15cm water of 25-30ppt salinity, filled with oxygen, for the shipment of PL_{25-30} of tiger shrimp in Taiwan. New (1990) recommended inflated plastic bags in insulated containers for journey of up to 12h at 300/l packing density and for journeys of 24-36h at 100/l in inflated plastic bags in insulated containers. Pillai et al. (1992) investigated the feasibility of transporting P. indicus nauplii in polythene packet of 15 l capacity with 5 l of water and the rest oxygen packed with 5 lakhs of larvae. Transportation within 30h showed 100% survival. Muthuraman et al. (1993) used double lined polythene bags containing 5-10 l of oxygenated, chilled water for

transportation of P. monodon nauplii at a packing density of 50,000-2,01,000 numbers/bag. These bags were kept inside water proof wax_coated master-cartons with inner thermocole linings, with ice bags in all the four corners.

2.1.2. Other types of containers.

Mohanty and Patra (1972) conducted an experiment on the transport of post-larvae of shrimps (penaeids) in open glass jar and galvanized trays with sand bottom for nine hour journey by boat. Shigueno (1975) reported the use of small canvas bags placed in a makeshift metal frame loaded on truck, aerated during riding, for holding about 5,00,000 post-larvae, $(PL_{20-30})/m^3$ for a maximum of 3h of driving and also the use of live fish-holds in fishing boats for the same. Korringa (1976) reported the use of 0.5 m^3 transportation tank, filled with water for transfer of P. japonicus seed to outdoor rearing ponds in the immediate vicinity. An account of short distance transportation of larvae of P. monodon in open container was given by Varghese (1978). Plastic strips overgrown with periphyton were provided for the seed to graze during transport which resulted in a better survival. Mammen et al. (1978) used milk can type container for transporting P. monodon seed for short distance transport and for air lifting with an average survival of 53%.

Fibreglass tanks of 2 m^3 capacity, with an opening at the top for putting the shrimps were used by At-Atlar and Kenow (1979) for P. semisulcatus transportation; these were loaded on a truck. Alikunhi et al. (1980) recommended open containers for a duration not exceeding 4h for the transport of shrimp seed. 300 l capacity plastic pools and 200 l capacity barrels were tried by them. Tenedero and Villaluz (1985) described the procedures and techniques to follow for short and long duration transport. Rao et al. (1986) transported P. monodon fry (18-26mm) in clean open aluminium vessels at 1.5ppt salinity for a period of 3h by road and obtained 100% survival at a low packing density of 54 fry/l. New (1990) recommended aerated tanks for journeys upto one hour at 750/l packing density for M. rosenbergii post-larvae. Joshi (1991) used reusable plastic jars on transportation of P. monodon post-larvae under oxygen pressure to study the effect of selected sedatives on the survival and duration of oxygen-packed seed. Jayasree-Vadhyar et al. (1992) used reusable hard transparent plastic jars with air tight screw type lids fitted with one-way valves for studying the effect of introducing different types of habitat materials on the survival and duration of safe transport of the M. rosenbergii post-larvae under oxygen packing at different packing densities. Venkataswamy et al.(1992) conducted trials to standardize the transport of M. malcomsonii juveniles and broodstock in galvanized iron tins with lids of 20 l capacity, with

double plastic bags tied at both ends. Each plastic bag was filled with 5 l of river water and oxygenated to the saturation level and the results showed that oxygen packing in hypothermal condition could help in increasing survival.

2.2. Packing density of shrimp seed in transporting containers.

Packing density is one of the major factors that govern the survival and safe duration of the oxygen-packed seed. Shigueno (1975) described the oxygenated polythene bag transportation adopted for long-distance shipping of P. japonicus. Here, the safe duration of 12h is reported at packing density of 750 seed/l under a lowered temperature of 18°C. De and Subrahmanyam (1975) during transportation of P. monodon seed tried packing densities ranging from 50 to 3000 seed/l. They concluded that 500 seed/l could be transported for a duration of 36h with 87% survival whereas at higher density of 2500 seed/l, the seed could be transported only for 12h with similar survival. Korringa (1976) suggested high packing densities of 5000-10,000 seed/ 6-8 l in 20 l plastic bags for trips lasting for 12h duration inside refrigerated truck. De (1977) conducted an experiment with packing densities ranging from 50 to 750 seed/l, 85% survival was obtained for 200 seed/l for a duration of 12h and 150 seed/l for 20h. Chakraborthi (1978)

calculated the oxygen requirement of P. monodon and P. indicus on the basis of which he recommended the packing densities of shrimp seed without oxygen packing as 900, 450, 300, 225 and 180/l at temperature, 32°C, salinity 19.6ppt, and dissolved oxygen 5.76ppm over a period of 1, 2, 3, 4, and 5h respectively. Mammen et al. (1978) could obtain 96% survival of P. monodon seed, at a packing density of 250/tin, whereas at 1500 to 2000/tin, survival was only 50%. Hamid and Mardjono (1979) obtained high survival rate (95%) for 200 to 300 seed/l packing density with oxygen for 8h. They found that the density during transportation could be increased considerably if the duration was less than 4h or if the water temperature was brought down to 22°C. Selvaraj et al. (1980) highlighted the importance of maintenance of optimum number of seed in the transporting containers for maximum survival under open conditions. Packing densities tried by them of 25 to 250 seed/l under similar open conditions indicated that 50 seed/l (20 to 30mm) was the optimum number, with 100% survival for 24h, and mortalities of 3%, 15%, 75% and 100% were observed at 36, 48, 60 and 72h respectively. Singh et al. (1982) based on their experiments suggested a packing density of 500/l P. monodon seed anaesthetised, for transportation, for a period not exceeding 26 h, and a packing density of 375/l for duration upto 30h. Singholka (1982) obtained a survival of more than 95% at a packing density of 300/l for a duration of 18 h, while transporting

Macrobrachium rosenbergii at lowered temperature. Franklin et al. (1982) demonstrated that the mortality during transport of P. indicus increased with packing density. They got 100% survival, when 200 seed were transported for 12h in polythene bags of 20 l capacity with 5 l of water, whereas, mortalities of 3% and 10% respectively were observed for packing densities of 250 and 500 seed/l for the same duration. Thirunavukkarasu (1983) recommended a packing density of 1000 to 1500 seed/l at 18°C for long distance transport and 5lakh/m^3, for short distant transportation for shrimp seed in general. Krishnakumar and Pillai (1984) transported P. indicus seed with 70% survival for 24h under oxygen packing at a density of 250 seed/l. When the duration was increased to 36 and 48 h, 70% survival was obtained with 100 seed/l. They also reported the relationship between the size of the shrimp and packing density. When 8 day old post-larvae were used, the survival 70% could be obtained at packing density of 300 and 150 post-larvae/l for 24 and 36h respectively. Tenedero and Villaluz (1985) suggested a packing density between 600 and 800 seed/l (PL_{15} to PL_{20}) and 400 to 800 seed/l (PL_{30}) for short distance transport of less than 6h in polythene bags, and 200 to 300 seed/l in hydro-tanks for penaeid shrimp seed. Subrahmanyam (1986) recommended a packing density of 50 seed/l for long distance transport of M. rosenbergii and short distance of less than 3 to 4 h, a density of 150 to

800 seed/l even without oxygen packing. Simon (1986) suggested a packing density of 15,000/ bag with 5 to 6 l of water for 12h and 10,000/bag for 48 h, when the size of the seed was 8 to 10mm. At a size of 18 to 20mm, only 3000 and 5000 could be packed per bag for 12 and 48h of transport respectively. Kungvankij et al. (1986) reported that in polythene bags with 6 to 8 l of water 2,00,000 nauplii could be transported with 80 to 90% survival for 4 to 6 h, whereas only 3000 to 5000 post-larvae could be transported in similar bag for similar duration. Fan and Dayue (1988) transported P. orientalis at a packing density of 5000 PL/l, at water temperature of 15-20°C, and salinity of 18-23ppt and obtained a survival rate of 95% for 20h duration. Alias and Siraj (1988) observed that when M. rosenbergii seed packed at 100, 200 and 300 post-larvae/l, the density, 300/l showed significantly better survival at 12h. However at 24 and 36h, packing density of 100/l was significantly higher than that of 300/l. New (1990) suggested a packing density of 750/l for journeys upto one hour, 300/l for journeys upto 12h and reduced packing density of 100/l for 24 to 36 h, in the transportation of M. rosenbergii post-larvae. He also described a weight/litre classification of packing density for juvenile and subadult prawns. Camillo (1990) recommended a packing density of 8,000 to 10,000 post-larvae of 20mm length in 15 l cooled (20-24°C) oxygenated water for a period upto 30h. Jayasree-Vadhyar et al. (1992) packed M. rosenbergii

seed at densities of 100, 200, 250, 300, 400 and 800/l and observed that the safe duration of survival (time of initial mortality) varied as 81, 35, 12, 12, 6 and 4h respectively. Pillai et al. (1992) carried out nauplii transportation trials at different packing densities ranging from 2000/l to more than 1,00,000/l for a fixed period of 24h and obtained 100% survival at 1 lakh/l. Muthuraman et al. (1993) transported tiger shrimp nauplii at a packing density between 50,000 to 2,01,000 numbers per bag in 5 to 10 l of oxygenated chilled water. The average survival rate of 63.91% was obtained for surface lifts.

2.3. Cannibalism

The shrimp post-larvae are cannibalistic in behaviour and hence do not often reach their destination in totality. According to Subrahmanyam (1973) P. monodon in laboratory containers developed cannibalistic tendencies due to insufficient space. Cannibalism is more pronounced at higher temperatures (Shigueno, 1975). Varghese et al. (1975) reported that P. monodon after moulting became soft and sluggish and fell on easy prey to other active individuals, larger shrimps often found attacking smaller ones. Such cannibalistic tendencies associated with moulting has been reported in many crustaceans. In M. rosenbergii this type of cannibalism occurs and the attack

is made on the animals undergoing moult (Segal and Roe, 1975). Peebles (1978) reported that prawns of equal size are also susceptible to cannibalism during the pre-moult and early post-moult. Mammen et al. (1978) got only a survival of 53.5% after the transport of P. monodon fry for 18h which they attributed mainly to cannibalism especially in higher packing densities. According to Hamid and Mardjono (1979), higher temperature of the water in the transporting container, tends to increase cannibalistic activity of shrimp. Alikunhi et al. (1980) also agreed to this view and reported that at normal temperature when shrimp were crowded without food they tended to become more cannibalistic. They observed that within 24 h, a post-larva which survived in the transport container consumed as much as 150% of its body weight. So they recommended the addition of live food organisms to the packing medium along with shrimps for avoiding cannibalism during transport. Franklin et al. (1982) pointed out that one of the reasons for high mortality during transport of penaeid seed was cannibalism. Krishnakumar and Pillai (1984) attributed the low survival rate of small sized P. indicus during long duration transport to cannibalism as small post-larvae moulted frequently and became prone to cannibalism. New and Singholka (1985) suggested the use of water from the holding tank to fill the transporting container to avoid moulting in "new water" and the consequent losses of seed by cannibalism. Kungvankij et al. (1986) recommended

development. Subrahmanyam (1986) reported cannibalism and injury to the soft seed (moulted) as the cause of mortality. Alias and Siraj (1988) reported that the use of habitat material in the transporting container had some effects on the survival as this provided more surface area in the bag thereby reducing cannibalism among prawn larvae. They also reported that the cannibalistic behaviour was predominant during the early hours of packing. Chen (1990) suggested that, a temperature shock in new water a few days prior to transportation of black tiger shrimp seed, induced moulting before the trip, instead of during or immediately after the trip, which minimised mortality due to cannibalism and/or handling. Jayasree-Vadhyar et al. (1992) highlighted the use of hollow habitat material in reducing cannibalism of M. rosenbergii seed under oxygen packing.

2.4. Factors influencing water quality during transportation

2.4.1. Dissolved oxygen.

2.4.1.1. Dissolved oxygen during transportation.

Dissolved oxygen is one of the most important water quality parameters that affects the survival of

shrimp seed during transport. The first few hours after loading or packing are particularly critical as the shrimp seed are excited and require a large amount of oxygen with a short time for adjustment (Johnson, 1979). De (1977) stated that depletion of dissolved oxygen was not found to be the main cause of mortality of penaeid seed under oxygen packing. Hamid and Mardjono (1979) observed an oxygen concentration range between 11.6 and 16.2ppm in the packing medium after 12h of transport of P. monodon fry. Selvaraj et al. (1980) transported shrimp seed at the rate of 100/l with continuous oxygenation with 100% survival during the initial 24 h. Singh et al. (1982) measured final oxygen concentration of 6.2, 2.7 and 3ppm in bags with packing densities of 250, 375 and 500 PL/l respectively. But, Franklin et al. (1982) noticed the dissolved oxygen concentration as low as 0.6-0.9ppm after 12h of transport, which they attributed to the putrefaction of dead shrimps. Krishnakumar and Pillai (1984) reported the depletion of oxygen in containers with packing densities above 200 PL/l, when the duration of transport was longer (36 to 48 h). They observed till total mortality occurred in long duration transport, and made clear that the mortality of post-larvae was not due to depletion of oxygen. Alias and Siraj (1988) obtained final oxygen level of 6.0 mg/l for 300 PL/l packing density even after 36 h. But, according to New (1990) the survival rate during transportation was more closely related to dissolved oxygen level than to any other

water quality parameter. Jayasree-Vadhyar *et al*. (1992) observed a comparatively higher final oxygen level for habitat material enclosed packing system than the control, for *M. rosenbergii* post-larvae.

2.4.1.2. Minimum dissolved oxygen tolerance levels.

Results from the experiment conducted by Mackay (1974) showed that at 0.9ppm ± 0.1ppm dissolved oxygen, all shrimps tested went into a type of lethargy where no reflexes could be shown. At this point all specimens were found lying on their sides. At about 1.2ppm dissolved oxygen, the majority of shrimps began swimming to the top of the water. De and Subrahmanyam (1975) reported that *P. monodon* seed could survive in oxygen concentration as low as 0.6ml/l. Chakraborthi (1978) determined the minimum dissolved oxygen concentration at which the shrimp seed could survive as 0.7ppm (for 2h), but for practical purposes 1.5ppm was taken, to avoid risk. According to Selvaraj *et al*. (1980) the level of dissolved oxygen sufficient for the survival of shrimp in healthy conditions was found to be above 2.5ml/l. Thirunavukkarasu (1983) also suggested the same level. Shylaja and Rengarajan (1993) fixed the medium tolerance limit of *P. indicus* juveniles at 20ppt salinity and 28°C as 0.45ml/l dissolved oxygen; 100% mortality was observed in 0.36ml/l.

2.4.1.3. Oxygen consumption.

Marine organisms show varying rates of oxygen consumption according to their physiological activity and ecological demand. Information on metabolic rates generally determined by measuring oxygen consumption (QO2) is of basic importance in defining the metabolic energy budget of an animal.

2.4.1.3.1. Relationship with body weight.

A number of investigators have studied oxygen consumption of shrimps in relation to body weight (Wolvekamp and Waterman 1960, Egusa 1961, Subrahmanyam 1962). Wolvekamp and Waterman (1960) reported that oxygen consumption varied from 0.67 to 1 to the power of the body weight for crustaceans. According to Egusa (1961) the resting and active oxygen uptake rates per unit weight of P. japonicus tended to drop with increase in size. Subrahmanyam (1962) established a linear relationship between body weight and oxygen consumption for P. indicus. According to Kramer (1975) the lethal dissolved oxygen concentration for brown shrimp P. aztecus varied with size. Nelson et al. (1977) got a slight negative correlation between metabolic rate and weight. Stephenson and Knight (1980) after their experiments with M. rosenbergii post-larvae stated that the oxygen consumption increased with

the weight of the prawn. Bishop et al. (1980) found that 3.7g sized shrimp consumed more oxygen than 6.7g shrimp did in different salinities. Licop (1984) noticed that the rate of oxygen consumption at various salinities were greater in younger post-larvae than in older post-larvae of P. monodon. Liao and Murai (1986) studied the effects of dissolved oxygen, temperature and salinity on the oxygen consumption of P. monodon. They found that the oxygen consumption/unit body weight (mg/g/h) tended to decrease as the body weight increased. Experiments on oxygen requirement of shrimp larvae by Pramila and Mohammed (1993) showed increased oxygen consumption with increase in size and progress in developmental stage of the larvae. With respect to respiratory rate, they also got similar results as Liao and Murai (1986). Kripa and Laxminarayana (1993) found the asphyxial level of P. indicus for smaller shrimps to be lower than that for larger ones.

2.4.1.3.2. Effect of environmental oxygen tension.

The relationship between oxygen consumption and the environmental oxygen tension has been found to be highly variable by several workers (Wolvekamp and Waterman, 1960 ; Egusa, 1961 ; Subrahmanyam, 1962 ; Kutty, 1969 ; Laxminarayana and Kutty, 1982). In most of the animals, while an increase in oxygen content is not likely to harm

the animals, in others, reduction of the same below the critical oxygen tension produces stressful effects. Subrahmanyam (1962) found that P. indicus was an oxygen conformer and that its oxygen consumption rates depend upon the partial pressure of oxygen even at saturation levels. Thus, as the ambient oxygen concentration in a closed chamber decreases from respiration, the shrimps respiratory rate also decreases. Kutty (1969) reported that the metabolic rate in P. indicus declined with decrease in ambient oxygen concentration. Studies by Liao and Huang (1975) and Chen (1985) reported that the oxygen consumption of post-larvae decreased when dissolved oxygen level fell below 3.8 to 4 mg/l. These results suggest that the oxygen regulatory system of P. monodon is well developed at early post-larval stages and the oxygen consumption is independent of dissolved oxygen, at higher dissolved oxygen levels. Studies on the shrimp P.semisulcatus and M. malcomsonii and crab Paratelphusa hydrodromus by Laxminarayana and Kutty (1982) supported the view that oxygen consumption decreased under hypoxic conditions with decrease in metabolic rate. The decrease in the rate of oxygen consumption was most significant in P. semisulcatus and less significant in P. hydrodromus. Liao and Murai (1986) reported that oxygen consumption in P. monodon was independent of oxygen concentration, at levels above 4ppm, but it decreased significantly at lower levels. Taylor and Spicer (1987) also observed that oxygen consumption in

palaemonid prawns was independent of oxygen levels of a wide range. Kripa and Laxminarayana (1993) observed a decrease in oxygen consumption rate with decrease in ambient oxygen for P. indicus.

2.4.1.3.3. Changes caused by starvation. .

In decapods the effect of starvation on oxygen consumption is in a way similar, registering an initial sharp decline and then stabilization. Kutty (1969) studied the mean rates of oxygen consumption in starved P. indicus. By the second day of starvation the metabolic rate declined sharply and further starvation did not cause any change from this level during the subsequent 5 to 10 days of starvation. He reported that P. indicus and P. semisulcatus starved for two days could be expected to survive for 1.7 and 2.3 times longer respectively than those not starved. Kulkarni and Joshi (1980) found that the oxygen consumption rate decreased with an increase in the duration of starvation. Beyond tenth day, starvation did not affect the metabolic rate of adaptation to starvation in P. japonicus. Oxygen consumption in P. esculentus fell sharply with starvation from 24 to 29% during the first 5 days and then levelled out (Dall and Smith, 1986). Surendranath et al. (1987) noticed significant decrease in the rate of oxygen consumption in P. indicus starved for 15

days indicating the minimum utilisation of reserved food material to withstand long periods of starvation.

2.4.1.3.4. Routine oxygen consumption.

Routine oxygen consumption of P. japonicus is approximately 0.2 to 0.3 mg oxygen/g/h for juveniles (Dallavia 1986). Liao and Murai (1986) found a rate of 59µmol oxygen/kg/min at 20°C and Kurmaly et al. (1989) reported a respiration rate of 55 µmol oxygen/kg/min at 15°C for P. monodon.

2.4.1.3.5. Effect of feeding and handling.

Nelson et al. (1977) found that feeding elevates QO2 in M. rosenbergii. Mean increase in QO2 after feeding was as much as 39.4%, however no significant relationship was derived between the amount ingested and the increase in the rate of metabolism. Handling increases the respiration rate of Crustacea (Winkler, 1987) apparently by increasing the activity of the animal (walking and swimming). In both P. japonicus and P. esculentus, respiration rate changes with the activity state of the shrimp (Dall, 1986).

2.4.2. Nitrogenous excretions.

Though uric acid, urea, amino N fractions have

been recorded in crustaceans, ammonia forms the major fraction (Kinne, 1976) and is found to be 9-86% of the total nitrogen excreted (Parry, 1960). Ammonia contributes as much as 72.6% of the total nitrogen excretion in P. indicus (Gerhardt, 1980). The accumulation of ammonia causes mortality of organisms reared in a closed system (Spotte, 1979). Ammonia is found in both the un-ionized (NH_3) and ionized form (NH_4^+). Both affect ammonia accumulation and cause death of shrimp (Chen and Kou, 1993). The un-ionized form is usually more toxic (Armstrong et al., 1978). The proportion of NH_3 to (NH_4^+) in water increases with increase in water temperature and pH and with decrease in salinity (Whitfield, 1974). In crustaceans the haemolymph concentrations of ammonia ranged from 2 to 18mg/l (Mangum et al., 1976 ; Armstrong et al., 1978).

2.4.2.1. Ammonia.

2.4.2.1.1. Ammonia excretion

Diffusion of ammonia from blood to water, exchange of (NH_4^+) for Na^+ and conversion to non-toxic compounds are the three routes by which fish and crustaceans lose metabolic ammonia (Campbell 1976). Diffusion of ammonia is the principal route of excretion because blood levels are normally much higher than ambient

water concentrations (Kinne 1976). Ammonia excretion rate is reported to be higher (Nelson et al., 1979) for fed than for starved prawns and is a function of body weight and the amount of feed ingested. Gerhardt, (1980) observed the ammonia excretion rates by P. indicus at 24°C to be twice that of P. esculentus at the same temperature. Body weight was found to affect ammonia excretion rate in M. lanchesterii (Anantharaman et al., 1981) which was maximum at 828.1 ± 61.55mg and decreased for those weighing above and below this weight. Under anaerobic conditions there is increase in protein degradation and nitrogen excretion in crustaceans as reported by Laxminarayana and Kutty (1982). They observed 2 to 5 fold increase in the ammonia quotient values in crustaceans under hypoxic conditions. Spaargaren et al. (1982) obtained ammonia excretion rates for P. japonicus at 25°C, which were several times higher than those for P. esculentus at the same temperature and at 10-14°C, this was two to four times higher. Nelson and Kropp (1985) reported that once shrimps were exposed to ambient water in which ammonia-N levels exceeded 10mg/l, after 30min. haemolymph ammonia became a function of ambient ammonia, suggesting the replacement of NH_3 from haemolymph to water by diffusion of NH_3 from water to haemolymph. They also determined the haemolymph ammonia-N levels of normal feeding and starved P. monodon as 12.33±2.18mg/l and 7.1±0.2mg/l respectively. Wickins (1985) pointed out that in P. monodon, the rate of ammonia excretion decreased with

increase in animal size. Dall and Smith (1986) reported 46-73% increase in ammonia-N excretion with starvation. Significant amount of ammonia excretion was observed during the first 20h after feeding in P. semisulcatus by Wajsbrot et al. (1989) with maximum concentration between 4 and 8h. Ammonia excretion rate was observed to decrease with increase in size similar to P. monodon as reported by Wickins (1985). Mohanty et al. (1989) also obtained similar results. Marangos et al.(1990) observed that the daily specific excretion by post-larvae was about five fold higher than the excretion by the adults. Allan et al. (1990) noticed increased acute toxicity in P. monodon juveniles. Similar result was observed by Wajsbrot et al.(1990). They found that the toxicity of ammonia in juveniles of P. semisulcatus increases when the dissolved oxygen level was below 55% saturation. At 27% oxygen saturation, ammonia toxicity doubled. Excretion of ammonia increased as ambient ammonia-N increased upto 1mg/l in P. chinensis, but excretion of ammonia was reduced when the level of ambient ammonia-N exceeded 10mg/l (Chen and Lin, 1992). Kripa and Laxminarayana (1993) found an increase in ammonia excretion rate with increase in body weight.

2.4.2.1.2. Ammonia tolerance limit.

Catedral et al. (1977) stated that the post-

larvae of P. monodon could tolerate ammonia upto 10mg/l. Jayashankar and Muthu (1984) calculated the incipient LC_{50} values of ammonia toxicity for wild and hatchery bred P. indicus larvae as 1.45ppm and 11.99ppm respectively. They found that the larvae from wild were more sensitive than hatchery bred broods to ammonia exposure. The safe levels of ammonia were fixed at 1.12ppm ammonia-N at pH 8.1. Chin and Chen (1987) observed LC_{50} values for post-larvae of P. monodon after 24 h, 48 h, 72h and 96h as 52.11, 27.73, 17.05 and 11.51mg/l ammonia-N respectively. Wajsbrot et al. (1990) showed that ammonia was toxic to P. semisulcatus at the 1.4mg unionized ammonia-N/l level. Chen and Kou (1993) observed that once shrimp had accumulated upto 20mg/l ammonia-N in the haemolymph they got weakened and eventually died. Those exposed to 50mg/l at pH 9 and 100mg/l at pH 8.2 were found to accumulate maximal concentration of ammonia in 1.5h and 6h respectively.

2.4.2.1.2. Changes during transportation.

Ammonia excretion in shrimps is reported to increase during transportation in closed containers (Smith and Wannamaker, 1983). Krishnakumar and Pillai (1984) found significant mortality during the transport of P. indicus when the total ammonia level reached above 80ppm. As the pH of the medium was on the acidic side, they concluded that ionised form of ammonia was also harmful to shrimp at

higher concentrations. Alias and Siraj (1988) observed that the level of ammonia-N increased with increase in packing density. They stated that the bags without habitat materials had significantly higher levels of ammonia-N than those with habitat materials. The interaction between habitat material and packing density was also significantly different in ammonia-N levels. Joshi (1991) observed an increased ammonia excretion by P. monodon during transportation, under sedation. Jayasree-Vadhyar et al. (1992) obtained similar result as that of Alias and Siraj (1988) with M. rosenbergii post-larvae in oxygen-packed jars with and without habitat material.

2.4.2.2. Nitrite

The 48h LC_{50} of nitrite to seven species of penaeid post-larvae (0.5-1.5 g) at a pH of 8 and temperature of 28°C was 170mg/l nitrite-N (Wickins, 1976). Catedral et al. (1977) reported that the toxicity levels of nitrite to P. monodon larvae varied with the larval stage. Armstrong et al. (1981) fixed the 96h LC_{50} value of nitrite to shrimps in the range of 8.5-15.5mg/l nitrite-N. Mevel and Chamroux (1981) observed that P. japonicus was sensitive to nitrite-N concentration higher than 100 µg/l. Jayashankar and Muthu (1984) found the incipient LC_{50} values of nitrite for wild and hatchery bred P. indicus

larvae as 0.78 and 3.29ppm nitrate-N respectively. Chen et al. (1986) observed that the relationship of P. monodon survival was more significant with nitrate than with ammonia. If the nitrate-N increased to 78 µg/l, a survival rate greater than 10% could not be expected. Chen and Chin (1988_a) observed the 24 h, 48 h, 72h and 96h LC_{50} values of nitrite in P. monodon post-larvae as 61.87, 33.17, 20.53 and 13.55mg/l nitrite-N. A "safe level" of nitrite was estimated at 1.36mg/l nitrite-N on the basis of LC_{50} for post-larvae.

2.4.2.3. Nitrate.

Wickins (1976) subjected shrimps (Penaeus aztecus, P. japonicus, P. occidentalis, P. orientalis and P. setiferus) to acute exposure to nitrate and they survived up to 2000mg/l for 48 h, but above this concentration mortality increased progressively.

2.4.3. Carbon dioxide.

Like other animals, aquatic animals release carbon dioxide as a respiratory waste. The carbon dioxide in the water medium will be in bound form or free form. The free form of carbon dioxide is considered as a poisonous waste product. Carbon dioxide levels reaching 20 to 30ppm in transport tanks with air supply cause severe stress

(Johnson, 1979). Carbon dioxide accumulates in the packing medium with time. When dissolved oxygen concentrations are low, the presence of high levels of carbon dioxide hinders oxygen uptake by fish (Boyd, 1982). Production of carbon dioxide from fish respiration moves water pH towards acidity with time (Amend, 1982). Even in the presence of sufficient oxygen, increased level of carbon dioxide in the medium is found to cause mortality of the seed (Thirunavukkarasu, 1983). Krishna Kumar and Pillai (1984) related mortality of P.indicus seed to reduce pH due to accumulation of carbon dioxide and increase in ammonia, during transportation at higher packing densities. They observed almost complete mortality due to accumulation of carbon dioxide in long duration experiments. Robertson et al. (1987) suggested that the containers may be opened once or twice during shipments to aerate the water, reoxygenate and reseal in order to reduce carbon dioxide accumulation during long shipment. Alias and Siraj (1988); Jayasree-Vadhyar et al., (1992) noticed significant reduction in carbon dioxide accumulation by the incorporation of habitat material in the packing medium.

2.4.4. Salinity.

2.4.4.1. Tolerance limits.

P. indicus is a highly euryhaline species

capable of tolerating wide ranges of salinities. The post-larvae could tolerate salinity as low as 7ppt when subjected to sudden exposure, when acclimated gradually they could tolerate even freshwater (Bhattacharya and Kewalramani, 1976). Kuttyamma (1980) noticed the extreme euryhaline nature of juvenile P. indicus and reported 5 to 35ppt as the best survival limit. Lakshmikanthan and Susheelan (1984) observed 5 to 40ppt salinity as the survival range for post-larvae of P. indicus, but salinity acclimation experiments revealed that the post larval stages exhibited considerable tolerance to wide salinity range on gradual acclimation to lower and upper levels and indicated that they could adopt to and thrive in a wide range of salinity, ie 0.33 to 85ppt by this process. A similar pattern was observed by Shylaja and Rengarajan (1993) with P. indicus juveniles. Their study revealed that the species can survive well in salinities between 3.9 and 40.7ppt.

2.4.4.2. Effect of salinity on oxygen consumption.

Several authors have reviewed the response of metabolic rates of a variety of crustaceans with varying salinity (Wolvekamp and Waterman, 1960; Lockwood, 1967; Kinne, 1976). Increased metabolic rates at salinities differing from the iso-osmotic point indicate the increased

energy cost due to osmoregulation (Beadle, 1931; Lofts, 1956; Rao, 1958; Venkataramiah, et al., 1974; Nelson et al. 1977). In shrimps, effect of salinity changes in respiratory rates have been shown to depend on temperature, body size, oxygen concentration as indicated by Rao, (1958) and Nelson et al.. (1977). Rao. (1958) attributed changes in oxygen consumption of shrimps in changing salinity media to variations in osmotic pressure between body fluid and seawater. Comparative data on routine metabolic rates of P. indicus at 14ppt salinity (Subrahmanyam, 1962) and at 36ppt salinity (Kutty, 1969) suggested that salinity did not influence oxygen consumption rate. Standard oxygen consumption of young juvenile shrimp (1 to 3 g) subjected to stepwise changes in ambient salinity from seawater to low saline waters (2 to 6ppt) and measured after short term (24h) salinity acclimation at each step, was lowest at salinities where shrimps such as P. indicus usually occur (10-15ppt). Here, the metabolic rates did not appear to have a direct relationship with osmotic gradient (Kutty, 1971). Kramer (1975) found out the lethal dissolved oxygen concentration for juvenile P. aztecus. According to him the lethal dissolved oxygen concentration for juvenile appears to be affected by sudden salinity changes. Venkataramiah, et al. (1977) acclimated brown shrimp to 15ppt salinity at 25°C and measured oxygen consumption rates at different levels of salinities of 2, 5, 10, 15, 25 and 36ppt. Metabolic rate increased initially, but generally tended

towards that of acclimation conditions after a day unless deviation from acclimation salinity was substantial ie. 2, 5 and 36ppt. Bishop et al. (1980) found that juvenile or subadult shrimp acclimated to salinities 10, 20, 30ppt showed no difference in oxygen consumption rates with salinity change. In P. monodon, a decrease in salinity was reported to have no effect on oxygen consumption (Gaudy and Sloane, 1981), but P. stylirostris displayed a tendency to increase respiration at lower salinities. According to Licop (1984) salinity seemed to affect the oxygen consumption of young post larvae, more than temperature. Least oxygen consumption rate was noted at salinities of 20 and 30ppt at low temperature and 20ppt at higher temperature. Unnikrishnan and Laxminarayana (1984) while experimenting on P. indicus found that the rate of oxygen consumption increased with decrease in salinity except at 25ppt and the rate of oxygen consumption was highest in 2.1ppt. Ferraris et al. (1986) reported that young P. monodon showed efficient osmoregulation over a salinity range of 5-55ppt. In this study, the oxygen consumption was also stable over the range. Liao and Murai (1986) also reported that salinity had no measurable effect on oxygen consumption in P. monodon within a range of 3 to 45ppt. Oxygen consumption increased rapidly to 300% of the initial value and stabilised at 200% after a few hours when the salinity was changed from 37 to 10ppt in P. japonicus

(Dallavia, 1986) and with the return of the salinity to 37ppt oxygen consumption returned to the original metabolic rate after 6h. Janakiram et al. (1989) reported an increase in oxygen consumption rate with increase in salinity in Metapenaeus monoceros.

2.4.4.3. Influence of salinity on ammonia excretion

Armstrong et al. (1981) subjected M. rosenbergii to hyperosmotic transfer from 0 to 24ppt salinity. Following a rapid reduction in ammonia after transfer, ammonia concentration in exposure water declined for 24h. Spaargaren et al. (1982) measured the excretion of NH_4^+-N and total organic-N of P. japonicus exposed to different salinities, and found these to be higher at lower salinities. Stern et al. (1984) reported that nitrogen excretion rates for M. rosenbergii increases with increase in salinity. Taylor et al. (1987) studied the relationship between osmoregulation and nitrogen metabolism in the intertidal prawn, Palaemon elegans and found that the animals acclimated to hyposaline condition showed pronounced increase in the rates of ammonia excretion and those exposed to hypersaline condition showed negative values, which indicated that NH_4^+ ions were being taken up by the prawn.

2.4.5. pH.

Calculations by various authors show that NH_3 fraction of ammonia, which is more toxic increases as pH rises; an increase in one pH unit elevates the NH_3 concentration tenfold (see Armstrong et al. 1978). It is proposed that sodium influx is a major factor contributing to ammonia toxicity at low pH and diffusion of NH_3 into the prawn at higher pH. They reported that 144h LC_{50} values for M. rosenbergii larvae as 0.27 mg/l NH_3-N in pH 8.34. Mohanty et al. (1989) stated that with increase in pH, the toxicity of un-ionised ammonia in P. monodon increased at any temperature level. Studies by Chen and Chin (1989) and Chen and Sheu (1990) on P. monodon and P. japonicus post-larvae respectively, supported the observation of Mohanty et al. (1989). Chen and Chin (1989) found that the post-larvae of P. monodon exposed to 60 mg/l ammonia-N and a pH of 9.1 were less tolerant than those exposed to 250 mg/l ammonia-N and pH 8.31. A decrease in pH of the transport medium was reported in all experiments on the transportation of shrimps (Singh et al. 1982; Krishnakumar and Pillai 1984; Alias and Siraj 1988; Jayasree-Vadhyar et al. 1992). According to Thirunavukkarasu (1983), pH less than 6.83 was found to be not good for shrimp seed transportation. Krishnakumar and Pillai (1984) attributed the higher mortality rate in the longer duration

transportation of 36 and 48h of higher packing densities, to reduction in pH due to accumulation of carbon dioxide. Considerable mortality was recorded when pH fell below 6.6.

2.4.6. Temperature.

Temperature is one of the most important environmental factors which determines the survival of shrimp seed during transportation. Higher temperatures demand higher oxygen consumption and waste release. Carbon dioxide and ammonia are more damaging at higher temperatures. As the temperature of the water increases, its capacity for oxygen decreases and, there is a decrease in affinity for oxygen by blood (Johnson 1979).

2.4.6.1. Temperature tolerance limits.

Bhattacharya and Kewalramani (1976) worked out the upper and lower lethal temperatures of P. indicus to be 34°C and 12°C respectively. They found that the survival of post-larvae was not affected by any change of temperature within 22°C-32°C. On the contrary, they showed excellent survival in the same range of temperature. A higher upper tolerance limit of 38°C is suggested by Selvaraj et al. (1980). According to him the lower limit of the water temperature at which the shrimp seed of P. indicus were kept during the experiment in healthy condition was 22.7°C.

The ranges of temperature tolerated by different species of penaeids, varied depending on the size of the individuals and the salinity of the medium, the larger specimen tolerating more in higher salinities and smaller ones becoming more suscepetible to death in higher salinities (Kuttyamma 1981). Sarada and Pillai (1993) found that the optimum temperature range for P. indicus post-larvae was between 30°C and 32.5°C.

2.4.6.2. Effect of temperature on packing density, duration and survival.

Mohanty and Patra (1972) could transport 200 shrimps/container with 100% survival for 9h at low temperature, as against 100 shrimps/container at ambient temperature. Low temperature during transport of shrimps results in better survival, because at high temperatures the shrimps undergo moulting frequently, which encourages cannibalism (Shigueno, 1975). Hamid and Mardjono (1979) could increase both packing density and duration of transport, at low temperatures compared to that at room temperature, without altering the survival rate. With 200-300/l packing density, they were able to transport P. merguiensis seed only for 8h with 95% survival at 28°C-25°C, the same survival was obtained even after 12h for P. monodon seed at a high packing density of 500-600/l.

Harrison and Lutz (1980) reported that survival increased with reduction in temperature during transportation of M. rosenbergii post-larvae. The metabolic activity and cannibalistic nature of the prawns are reduced by low transport temperatures and hence Singholka (1982) devised a simple cool truck for transporting juvenile fish or crustacea over long distances in tropical countries where a lowered temperature is obtained by using ice cubes and battery-driven fans.

2.4.6.3. Effect of temperature on water quality parameters.

Metabolic rate is a function of temperature for a number of crustaceans (Wolvekamp and Waterman 1960; Prosser, 1984). Within the tolerance limits, the metabolic rates of poikilothermic invertebrates increase immediately when animals are transferred from a lower to a higher temperature and reduce immediately when subjected to a temperature manipulation in the reverse direction (Kutty et al., 1971; Venkataramiah et al., 1974; Catedral et al., 1977; Nelson et al., 1977; Stephenson and Knight, 1980). Kutty (1971) observed that the ambient temperature influenced the routine oxygen consumption of very young juveniles of P. indicus. Toxicity of ammonia to shrimps greatly depends on temperature. The concentration of un-ionised ammonia in water increases with increase in

temperature (Bower, 1978). Bishop et al. (1980) observed a linear relationship between temperature and oxygen consumption rate in P. aztecus. Spaargaren et al. (1982) observed that at steady state conditions ammonia-N excretion rate by P. japonicus was strongly dependent on temperature. A higher oxygen consumption is reported at higher temperature in P. monodon post larvae by Licop (1984). The reduction in metabolic rate is expected from the decrease in temperature alone, but oxygen uptake can itself be interrupted at low temperature by the failure of the oxygen transport pigment in the blood to release its oxygen in the cold (Mauro and Mangum, 1982). Kripa and Laxminarayana (1993) studied the influence of hypoxia on metabolism and activity of P. indicus and highlighted that the rate of ammonia excretion and oxygen consumption increased with increase in temperature, and decrease with decrease in ambient oxygen.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Experimental animals

The post-larvae of P.indicus required for the experiments were obtained from Marine Products Export Development Authority's Prawn hatchery, Vallarpadom. They were transported in polythene bags filled with water of about 32ppt and oxygen, at ambient temperatures. They were maintained in a 1 ton capacity rectangular tank. The temperature of the water in the transport bags was equalised by floating them in the tank water. The bags were then opened and the post-larvae were slowly acclimated to the salinity of the tank water (25ppt) by changing them gradually to lower salinity. The seeds were then slowly released into the tank water. Aeration was provided.

Ad libitum feeding of post-larvae was done using powdered dried clam and minced prawn meat. 50% of the tank water was exchanged everyday. The remnants and excretory waste were removed, from the bottom by siphoning, once daily.

3.2. Experimental containers

Air-tight transparent plastic jars of 600ml capacity and screw type lids fitted with valves, similar to those described by Jayasree-Vadhyar *et al*. (1992) were used for the oxygen packing of the shrimp seed.

Respirometers used consisted of a cylindrical flask of 136ml capacity, fitted with a two-holed rubber stopper through which the inlet and outlet tubes were fitted. The inlet tube was fitted with a regulator for adjusting water flow by means of a latex tubing. The inlet was connected to a reservoir. For collecting final sample, the inlet was disconnected from the reservoir and formed the outlet; and the orginal outlet served as the air vent. The respirometers were placed inside a trough containing water to avoid temperature variations.

3.3. Experiment I. Determination of oxygen consumption rate by *P. indicus* seed at different salinities, temperatures and packing densities.

The experiment was carried out to study the oxygen consumption rate by *P. indicus* seed at different salinities, temperatures and packing densities, by finding out the difference in the oxygen levels of the respirometers initially and finally.

The factors under study consisted of three different levels of salinities, 20, 25 and 30ppt; four different packing densities, 200, 300, 400 and 500 PL/l at ambient temperature (30 ± 1)°C and at lowered temperature (23 ± 2)°C.

Twenty four hours prior to starting the experiment, the post-larvae (PL_{20}-average wt. 0.01g) which were maintained in the 1 ton tank, were conditioned in the following manner. They were counted and divided into three lots of roughly 800 each. The post-larvae in each lot were acclimatised to one of the three salinities mentioned above at the rate of 1ppt/h. They were then transferred to cylindro-conical tanks of 100 l capacity filled with 50 l of aerated water of the respective salinity. Feeding was not carried out in these tanks. Half the number of seed from each cylindro-conical tank were separated into two lots. Pre-cooled isosaline water was slowly added to one of these lots, until the water temperature was lowered to 23 ± 2°C.

Six reservoirs were filled with water of salinity 20, 25 and 30ppt of which three were kept at ambient temperature. The other three were maintained at lowered temperature by keeping them inside a trough filled with ice cooled water. The experimental units with uniform salinity and temperature were connected to the

corresponding reservoirs.

The shrimps were transferred to the respirometer flasks at different densities. The respirometers were stoppered using the rubber stopper and were placed inside a trough containing water of the required temperature. The water was allowed to flow through the respirometers from the corresponding reservoirs for 20 minutes. At the start of the experiment, initial water samples were collected and the circulation of water through the respirometers was cut off. After an interval of 10 minutes, final samples were collected. The size of each sample collected was 25ml. The sample was fixed immediately using Winkler solutions for analysis of dissolved oxygen. The weight of the shrimp was found at the end. A control was kept without any animals in the respirometers and the initial and final water samples were taken after the same interval, for analysis of oxygen.

3.4. Experiment II. Determination of the effect of salinity, temperature and packing density on the duration and survival of oxygen-packed *P. indicus* seed during transportation.

The experiment was done to find out the effect of salinity, temperature and packing density on the duration and survival of oxygen-packed *P. indicus* seed at

uniform oxygen pressure under three different salinities of 20, 25 and 30ppt, each at four different packing densities of 200, 300, 400 and 500 PL/1. The experiment was conducted at ambient temperature (30 $\pm$ 2°C) and at lowered temperature (23 $\pm$ 1°C) seperately.

Factorial experiment with completely randomised design was used for planning the experiment and analysis of results.

Conditioning of the seed was done as in the previous experiment. Thereafter, the post-larvae were counted and transferred into the oxygen packing jars with 100ml of water. Immediately after the transfer, the jars were closed tightly and filled with oxygen from an oxygen cylinder under a uniform pressure of 0.2Kg/cm^2 which was measured through a precision pressure gauge (Bourdon type). While filling oxygen, care was taken to displace the air initially present inside the jar with oxygen. To effect this, after filling oxygen initially, it was completely released by pressing the valve. This was repeated three times to ensure complete displacement of air with oxygen (see Jayasree-Vadhyar et al., 1992). Precooled water was filled in those oxygen-packed jars which were observed at lowered temperatures by placing them in a trough containing ice cooled water.

Plastic jars which were filled with 100ml water for each of the salinities and with oxygen at 0.2Kg/cm^2 at ambient and lowered temperatures were opened immediately after filling oxygen, to collect water samples initially.

Time of initial mortality of the oxygen-packed seed was recorded by making hourly observations. Thereafter, the number of survivors was counted at two hourly intervals until 70-80% survival occurred. The jars were periodically shaken to simulate the transport conditions. At the end of 20-30% mortality, the jars were opened, samples of packing medium were collected for water quality analysis. Initial and final quality of the packing medium was analysed using standard procedures. The parameters analysed were dissolved oxygen, free carbon dioxide, ammonia-N and pH. Of the replicates of each combination one was used for analysing dissolved oxygen and the other for free carbon dioxide, because the determination of both parameters from the same might have yielded erroneous values for the second-measured parameters. At the same time, ammonia and pH were noted from all the replicate jars.

3.5. Experiment III. Determination of the effect of habitat material on the duration and percentage survival of oxygen-packed P. indicus seed.

The conditioning of the post-larvae was done in the afore-mentioned manner to a single salinity of 25ppt. The effect of habitat material on cannibalism of the post-larvae was studied using transluscent plastic straw, at different packing densities of 200, 300, 400 and 500 PL/l. The experiment was carried out at ambient temperature 30 ± 1°C. 10-15 mm bits of transluscent plastic straw (floating) were introduced in the jars in the ratio of 1:2 (bit : post-larvae). The oxygen-packing procedure, observations on the time of initial mortality, the percentage survival with time and determination of water quality were done as described.

3.6. Determination of water quality

The following standard methods were used for analysing water quality parameters.

Dissolved oxygen	:	Winkler's method (Strickland and Parsons, 1972)
Carbon dioxide	:	Alkalimetric titration method (Strickland and Parsons, 1972)
Ammonia	:	Phenol-hypochlorite spectrophotometric method (Strickland and Parsons, 1972)
pH	:	Using Universal indicator

solution (Qualigens Fine chemicals) and potentiometric method.

Temperature : Using mercury bulb thermometer having a precision of 0.1°C.

Salinty : Using Salino refractometer periodically verified by Mohr's titration.

3.7. Statistical analysis

Data obtained from all the experiments were analysed statistically by analysis of variance. Data on oxygen consumption and carbon dioxide could not be analysed by analysis of variance but explained by tabular and graphical methods.

Pair-wise comparisons using critical difference values were made for those treatments which were found statistically significant.

RESULTS

4. RESULTS

4.1. Experiment I. Oxygen consumption rate by P.indicus seed at different salinities, temperatures and packing densities.

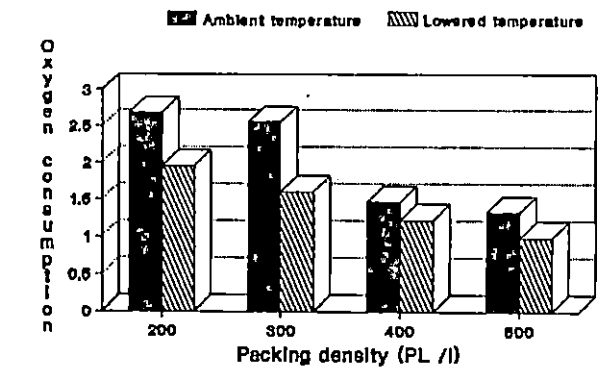
The oxygen consumption by the shrimp seed under various combinations of salinities, temperatures and packing densities are shown in table 1 and figures 1, 2 and 3. It was found to be the lowest at 25ppt salinity in all the treatment combinations except at 400PL/l. With increase in packing density, there was a decrease in oxygen consumption. Lowering of temperature from 30 ± 1°C to 23 ± 2°C reduced the oxygen consumption.

Analysis of variance showed significant difference in the oxygen consumption among the different salinities, temperatures, packing densities and for their interactions (table 2). Pair-wise comparison by critical difference analysis revealed that all the four levels of packing density, three levels of salinity and two levels of temperature differed significantly. Significant difference was shown by their interactions. Pair-wise comparisons of density and salinity (ds) interaction means showed the

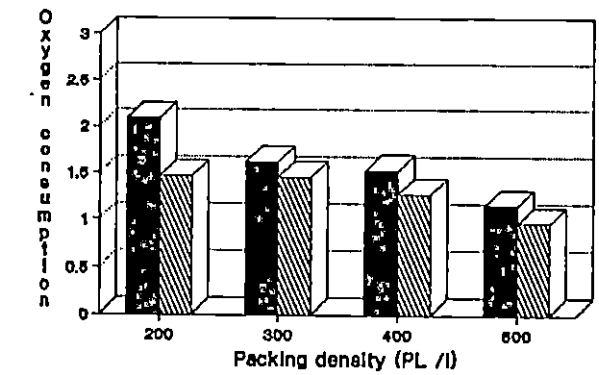
Table 1. Oxygen consumption by *P.indicus* seed under different salinities, temperatures and packing densities.

Salinity (ppt)	Temperature (°C)	Oxygen consumption (mg/g body weight/h)* at four different packing densities			
		200/l	300/l	400/l	500/l
20	30 ± 1	2.684	2.553	1.4655	1.324
	23 ± 2	1.955	1.5984	1.221	0.9808
25	30 ± 1	2.082	1.6198	1.523	1.1429
	23 ± 2	1.466	1.458	1.269	0.9524
30	30 ± 1	2.7948	2.5585	1.601	1.356
	23 ± 2	2.2866	1.6803	1.294	1.146

* Each value is a mean of duplicates.



A



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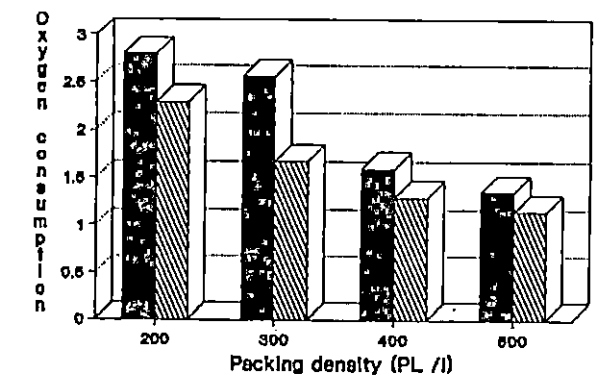
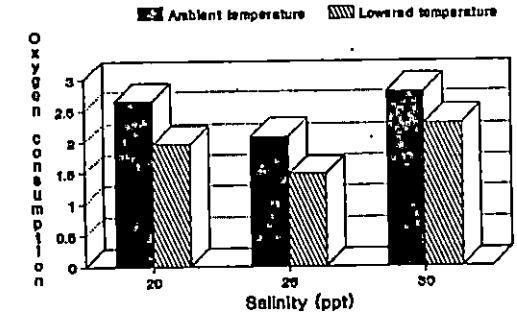
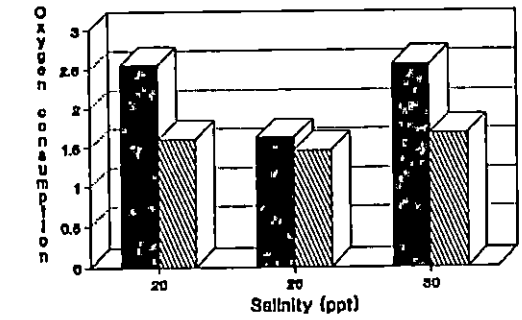


C

Fig. 1 Oxygen consumption (mg/g body wt./h) by *P. indicus* seed at 20 ppt (A), 25 ppt (B) and 30 ppt (C) under different temperatures and packing densities.



A



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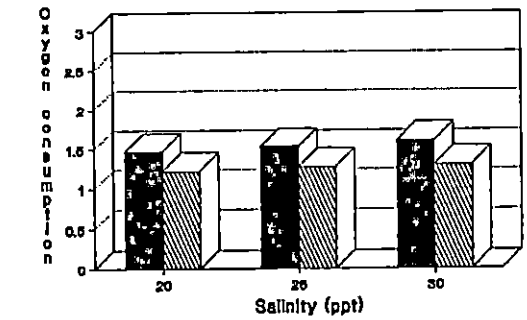

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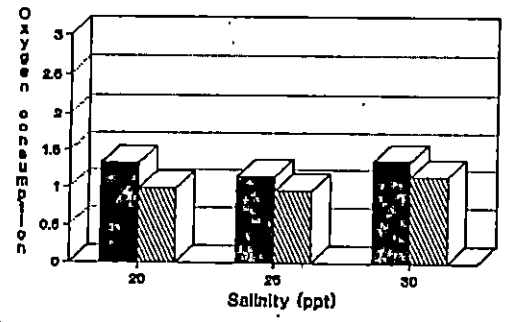
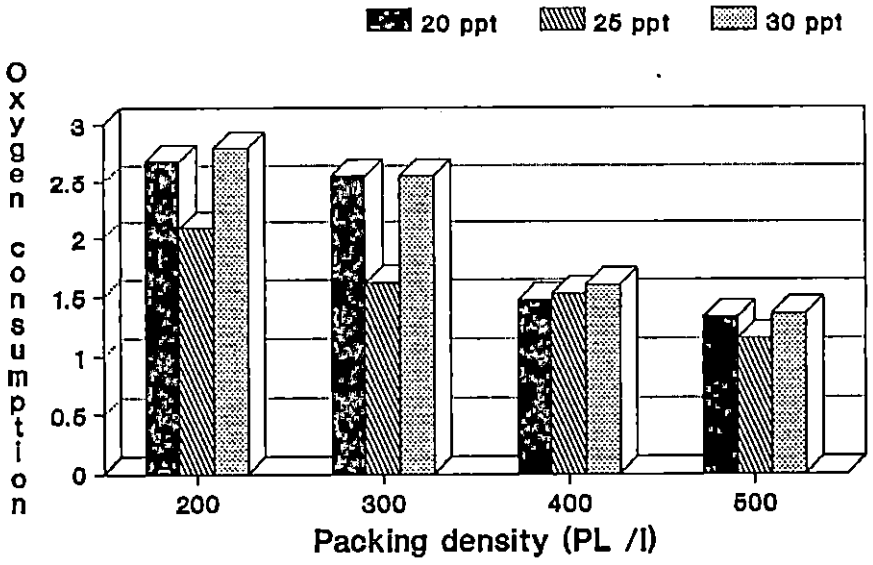
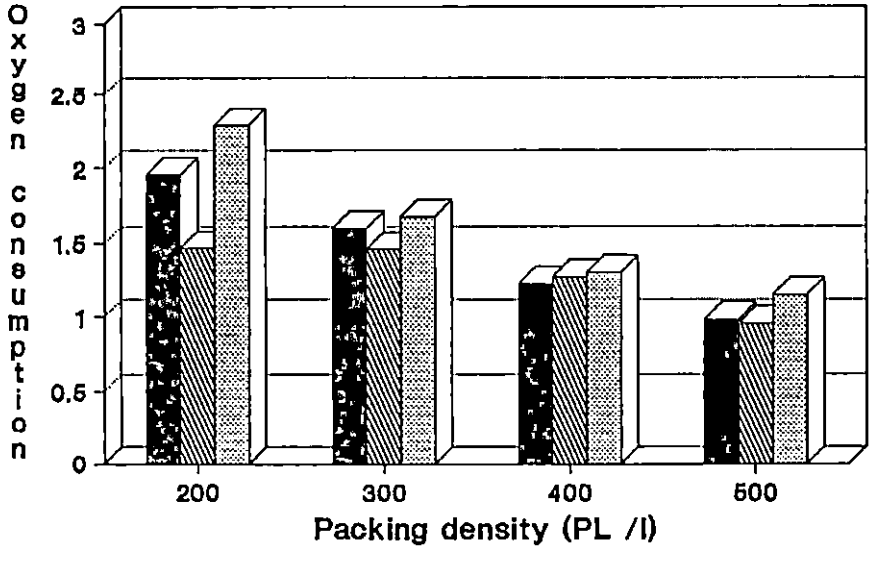


D

Fig. 2 Oxygen consumption (mg/g body wt./h) by *P. indicus* seed at 200 (A), 300 (B), 400 (C) and 500 (D) PL/l packing densities under different salinities and temperatures.



A



B

Fig. 3 Oxygen consumption (mg/g body wt./h) by *P. indicus* at ambient (A) and lowered (B) temperatures under different salinities and packing densities.

Table 2. Analysis of variance in the rate of oxygen consumption by P. indicus seed under different salinities, packing densities and temperatures.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed
d	3	8.362	2.787	315.99*
s	2	1.357	0.678	76.90*
t	1	2.426	2.426	274.97*
ds	6	0.827	0.138	15.63*
dt	3	0.445	0.148	16.80*
st	2	0.141	0.071	8.02*
dst	6	0.282	0.047	5.32*
Error	24	0.212	0.009	
Total	47	14.051		

* Significantly different at 5 % level.

Treatment means of Density

d1	d2	d3	d4
2.21175	1.911383	1.395675	1.150492

Calculated C.D. value (t 0.05) = 0.0791

Treatment means of Salinity

s3	s1	s2
1.839831	1.722769	1.439375

Calculated C.D. value (t 0.05) = 0.0685825

contd.....

Treatment means of Temperature

t1	t2
1.892121	1.442529

Calculated C.D. value (t 0.05) = 0.05599

Treatment means of DS

1. d1 s3 - 2.5407	7. d3 s3 - 1.4475
2. d1 s1 - 2.3195	8. d3 s2 - 1.396
3. d2 s3 - 2.1194	9. d3 s1 - 1.343
4. d2 s1 - 2.0757	10. d4 s3 - 1.251
5. d1 s2 - 1.774	11. d4 s1 - 1.1524
6. d2 s2 - 1.5389	12. d4 s2 - 1.04765

Calculated C.D. value (t 0.05) = 0.1384

Treatment means of DT

1. d1 t1 - 2.520	5. d3 t1 - 1.5298
2. d2 t1 - 2.2437	6. d4 t1 - 1.2743
3. d1 t2 - 1.9025	7. d3 t2 - 1.2613
4. d2 t2 - 1.5789	8. d4 t2 - 1.0264

Calculated C.D. value (t 0.05) = 0.1130498

Treatment means of ST

1. s3 t1 - 2.0775	4. s2 t1 - 1.5919
2. s1 t1 - 2.006	5. s1 t2 - 1.4388
3. s3 t2 - 1.6017	6. s2 t2 - 1.2863

Calculated C.D. value (t 0.05) = 0.0979

contd.....

Treatment means of DST

1. d1 s3 t1	2.7948	13. d1 s2 t2	1.466
2. d1 s1 t1	2.684	14. d3 s1 t1	1.4655
3. d2 s3 t1	2.5585	15. d2 s2 t2	1.458
4. d2 s1 t1	2.553	16. d4 s3 t1	1.356
5. d1 s3 t2	2.2866	17. d4 s1 t1	1.324
6. d1 s2 t1	2.082	18. d3 s3 t2	1.294
7. d1 s1 t2	1.955	19. d3 s2 t2	1.269
8. d2 s3 t2	1.6803	20. d3 s1 t2	1.221
9. d2 s2 t1	1.6198	21. d4 s3 t2	1.146
10. d3 s3 t1	1.601	22. d4 s2 t1	1.1429
11. d2 s1 t2	1.5984	23. d4 s1 t2	0.9808
12. d3 s2 t1	1.523	24. d4 s2 t2	0.9524

Calculated C.D. value (t 0.05) = 0.

d - Packing density

s - Salinity

t - Temperature

d1 - 200 PL/l

d2 - 300 PL/l

d3 - 400 PL/l

d4 - 500 PL/l

s1 - 20 ppt Salinity

s2 - 25 ppt Salinity

s3 - 30 ppt Salinity

t1 - 30 $\pm$ 1°C

t2 - 23 $\pm$ 2°C

concl.

presence of different groups. Similarly, pair-wise comparison for density and temperature (dt) showed no significant difference between d2 t2 (300PL/l-23 ± 2°C) & d3 t1 (400PL/l - 30 ± 1°C); and also between d4 t1 (500PL/ - 30 ± 1°C) & d3 t2 (400PL/l - 23 ± 2°C). Critical difference analysis of dst interaction means showed the presence of eight different groups.

4.2. Experiment II. Effect of various factors on the duration and survival of oxygen-packed *P. indicus* seed during transportation

The observations on the different duration(h) of survival at 100%, 90%, 80% down to 70% levels were taken. The analysis of variance at each of these survival levels were carried out.

4.2.1. Duration.

The duration of 100% survival referred to as safe duration of transport of oxygen-packed post-larvae at four different packing densities viz.,200PL/l, 300PL/l, 400PL/l and 500PL/l under three levels of salinity ie 20, 25 and 30ppt and temperatures of 30 ± 1°C & 23 ± 2°C are presented in table 3, figures 4, 5 and 6. The safe duration of transport of the shrimp seed showed an inverse relationship with packing density. It was shown to be the

Table 3. Duration of 100% survival* of oxygen-packed P.indicus seed under different levels of salinities, temperatures and packing densities.

Salinity (ppt)	Temperature (°C)	Duration (h)** at four different packing densities			
		200/l	300/l	400/l	500/l
20	30 ± 1	8.0	3.25	2.0	1.25
	23 ± 2	42.5	8.25	6.25	4.0
25	30 ± 1	6.5	3.5	2.0	1.25
	23 ± 2	23.0	8.75	6.5	4.25
30	30 ± 1	8.5	5.0	2.25	1.5
	23 ± 2	22.0	9.0	6.75	4.75

* Time of initial mortality

** Each value is a mean of duplicates.

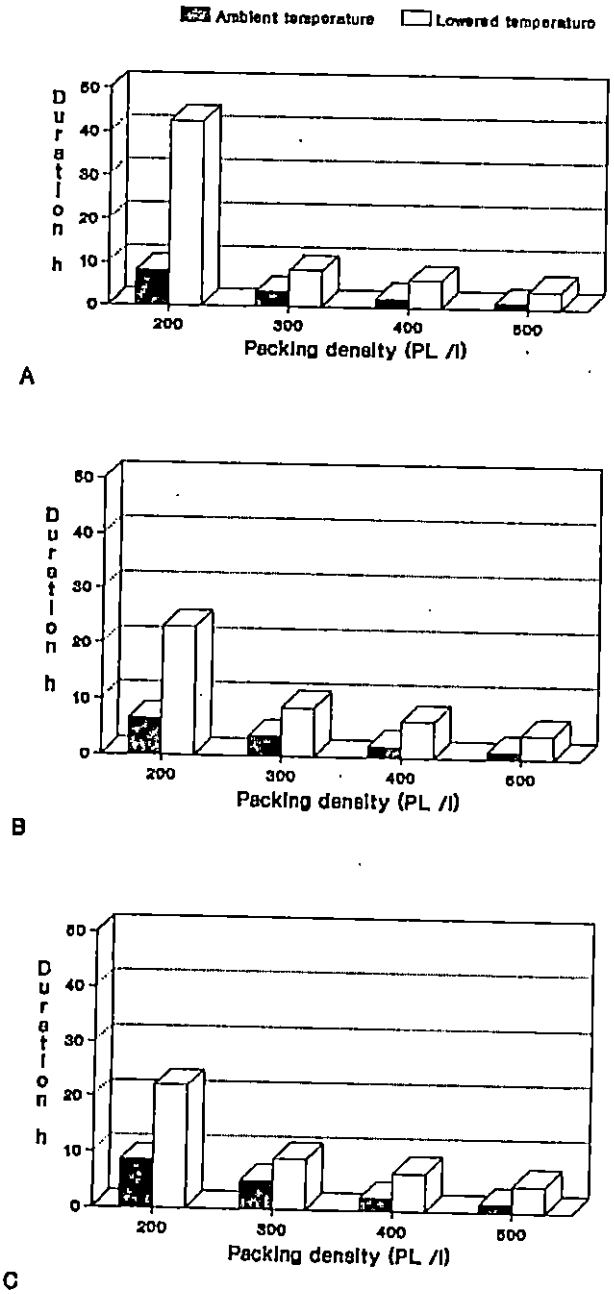


Fig. 4 Duration of 100 % survival of oxygen packed *P.indicus* seed at 20 ppt (A), 25 ppt (B) and 30 ppt (C) salinities under different temperatures and packing densities.

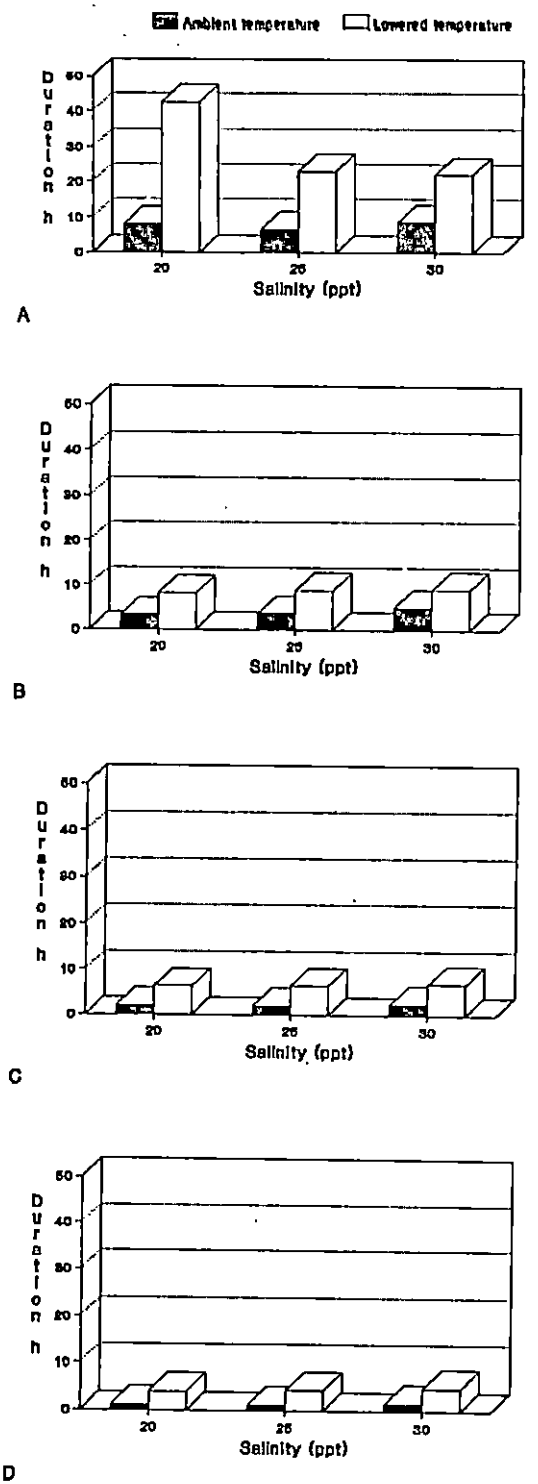
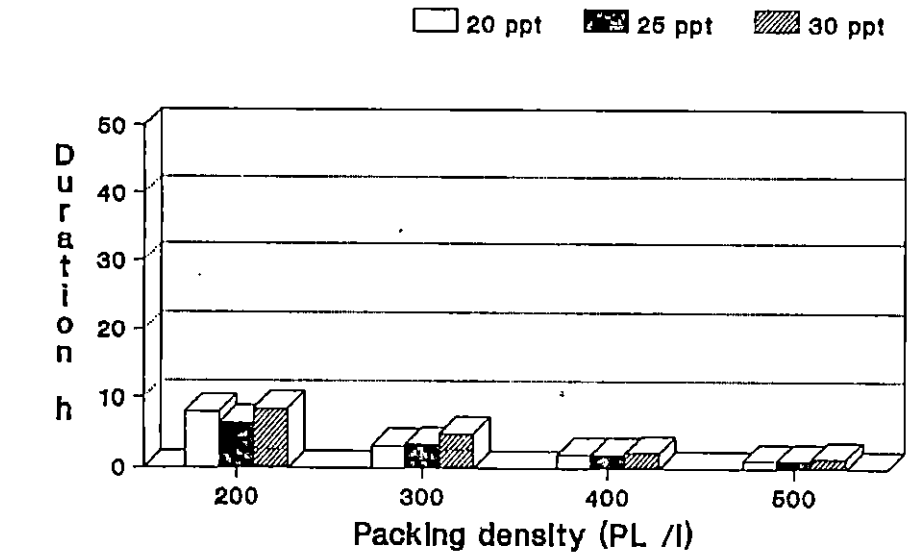
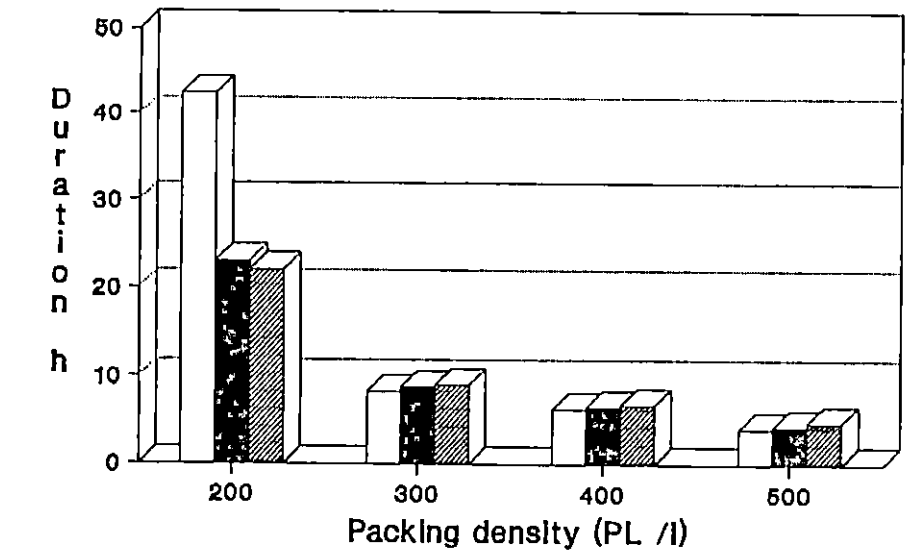


Fig. 5 Duration of 100 % survival of oxygen-packed *P.indicus* seed at 200 (A), 300 (B), 400 (C) and 500 (D) PL /l packing densities under different temperatures and salinities.



A



B

Fig. 6 Duration of 100% survival of oxygen-packed *P.Indicus* seed at ambient temperature (A) and lowered temperature (B) under different salinities and packing densities.

longest at the 200PL/l packing density combination. With the change in salinities there was not much obvious change in the safe duration of transportation, except at 200PL/l. However, it could be increased by lowering the temperature from 30 ± 1°C to 23 ± 2°C. Analysis of variance showed significant difference in the safe duration of transport of oxygen-packed seed among the different packing densities, salinities and temperatures (Table 4). Pair-wise comparison revealed that the four levels of packing densities and two levels of temperature differed significantly, but salinity means of 20ppt and 30ppt belonged to the same group. Significant difference was shown by all interactions. Pair-wise comparison of density and salinity interaction (ds) as well as density and temperature interaction (dt) showed the presence of five different groups each. Critical difference analysis of salinity and temperature interaction (st), showed the presence of three different groups.

The duration of 90% survival of the oxygen-packed seed is presented in table 5, figures 7, 8 and 9. It showed a decreasing trend with increase in packing density. With change in salinities from 20 to 30ppt, no clear pattern of change could be observed. Lowering of temperature showed increase in the duration. The analysis of variance showed significant difference in the duration

Table 4. Analysis of variance in the duration of 100 % survival of oxygen-packed P. indicus seed under different levels of packing densities, salinities and temperatures.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value
d	3	1817.266	605.755	467.09*
s	2	53.531	26.766	20.64*
t	1	845.880	845.880	652.24*
ds	6	231.219	38.536	29.71*
dt	3	692.599	230.866	178.02*
st	2	64.323	32.161	24.80*
dst	6	194.760	32.460	25.03*
Error	24	31.125	1.297	
Total	47	3930.703		

* Significantly different at 5 % level.

Treatment means of Density

d1	d2	d3	d4
18.41667	6.291667	4.291667	2.875

Calculated C.D. value (t 0.05) = 0.9596

Treatment means of Salinity

s1	s3	s2
9.4375	7.46875	7.0

Calculated C.D. value (t 0.05) = 0.8310

contd.....

Treatment means of Temperature

t1	t2
12.16667	3.770833

Calculated C.D. value (t 0.05) = 0.6785

Treatment means of DS

1. d1 s1	-	25.25	7. d3 s3	-	4.5
2. d1 s3	-	15.25	8. d3 s2	-	4.25
3. d1 s2	-	14.75	9. d3 s1	-	4.125
4. d2 s3	-	7	10. d4 s3	-	3.125
5. d2 s2	-	6.125	11. d4 s2	-	2.75
6. d2 s1	-	5.75	12. d4 s1	-	2.625

Calculated C.D. value (t 0.05) = 1.662

Treatment means of DT

1. d1 t2	-	29.166	5. d4 t2	-	4.333
2. d2 t2	-	8.666	6. d2 t1	-	3.916
3. d1 t1	-	7.666	7. d3 t1	-	2.083
4. d3 t2	-	6.5	8. d4 t1	-	1.333

Calculated C.D. value (t 0.05) = 1.3571

Treatment means of ST

1. s1 t2	-	15.25	4. s3 t1	-	4.3125
2. s2 t2	-	10.625	5. s1 t1	-	3.625
3. s3 t2	-	10.625	6. s2 t1	-	3.31

Calculated C.d. value (t 0.05) = 1.1753.

contd.....

Treatment means of DST

1. d1 s1 t2 - 42.5		13. d2 s3 t1 - 5.0	
2. d1 s2 t2 - 23.0		14. d4 s3 t2 - 4.75	
3. d1 s3 t2 - 22.0		15. d4 s3 t2 - 4.25	
4. d2 s3 t2 - 9.0		16. d4 s1 t2 - 4.0	
5. d1 s2 t2 - 8.75		17. d2 s2 t1 - 3.5	
6. d1 s3 t1 - 8.5		18. d2 s1 t1 - 3.25	
7. d2 s1 t2 - 8.25		19. d3 s3 t1 - 2.25	
8. d3 s3 t2 - 8.0		20. d3 s1 t1 - 2.0	
9. d3 s3 t2 - 6.75		21. d3 s2 t1 - 2.0	
10. d3 s2 t2 - 6.5		22. d4 s3 t1 - 1.5	
11. d1 s2 t1 - 6.5		23. d4 s1 t1 - 1.25	
12. d3 s1 t2 - 6.25		24. d4 s2 t1 - 1.25	

Calculated C.D. value (t 0.05) = 2.3506049

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Table 5. Duration of 90% survival of oxygen-packed P.indicus seed under different levels of salinities, temperatures and packing densities.

Salinity (ppt)	Temperature (°C)	Duration (h)* at four different packing densities			
		200/l	300/l	400/l	500/l
20	30 ± 1	13.0	6.0	5.0	3.0
	23 ± 2	44.0	18.0	9.0	7.0
25	30 ± 1	14.5	6.5	4.0	2.5
	23 ± 2	40.5	23.0	10.25	8.0
30	30 ± 1	13.5	7.5	4.5	3.0
	23 ± 2	27.0	15.5	9.0	8.0

* Each value is a mean of duplicates.

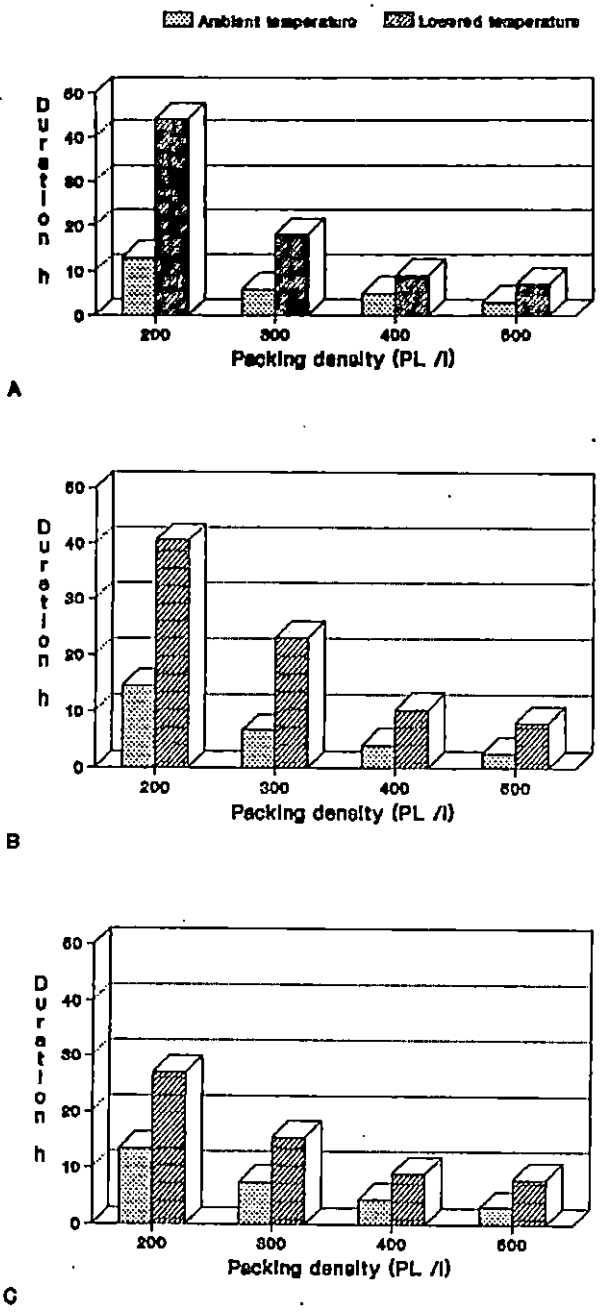


Fig. 7. Duration of 90 % survival of oxygen packed *P.Indicus* seed at 20 ppt (A), 25 ppt (B) and 30 ppt (C) salinities under different temperatures and packing densities.

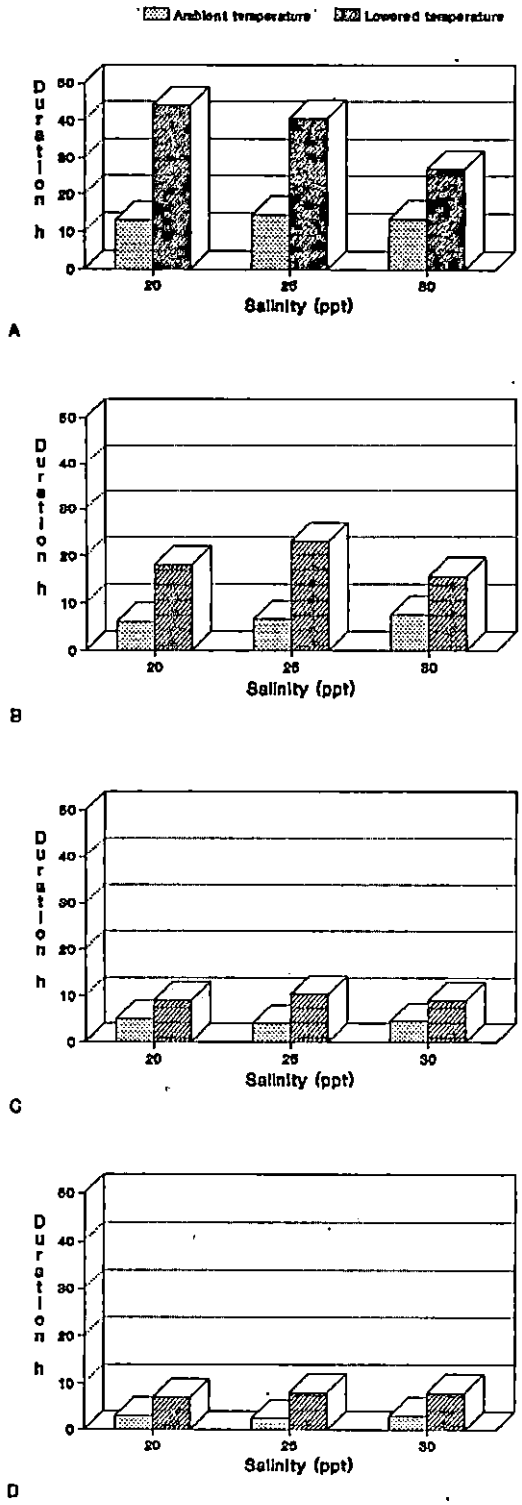
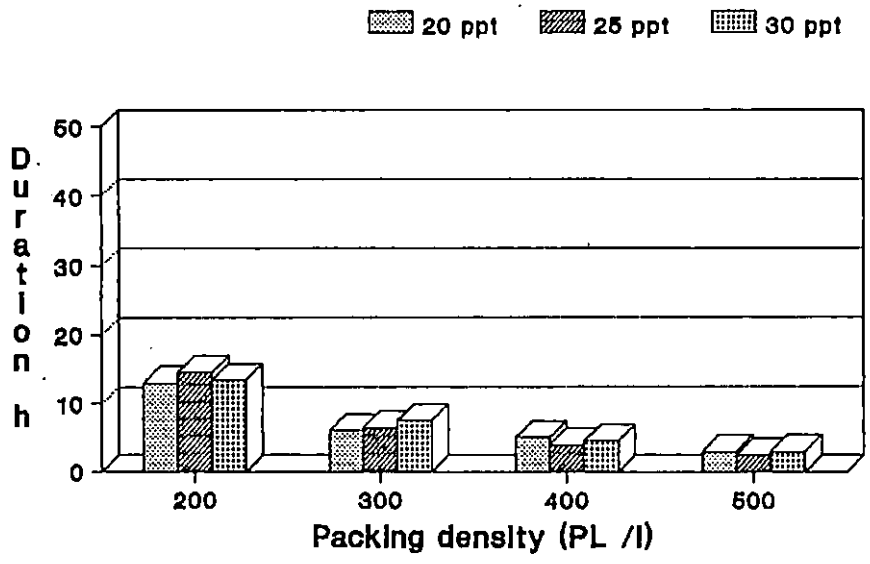
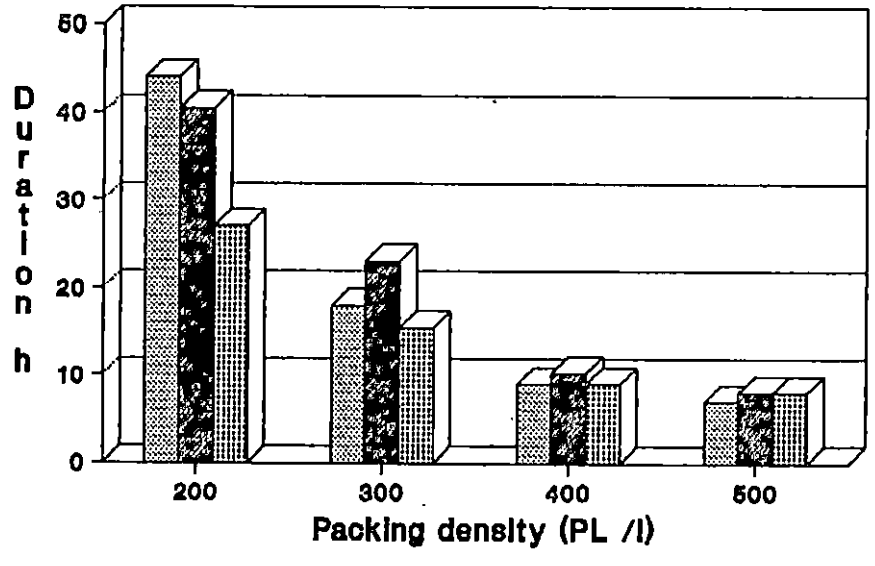


Fig. 8. Duration of 90 % survival of oxygen-packed *P.indicus* seed at 200 (A), 300 (B), 400 (C) and 500 (D) PL /l packing densities under different temperatures and salinities.



A



B

Fig. 9 Duration of 90 % survival of oxygen-packed *P.Indicus* seed at ambient temperature (A) and lowered temperature (B) under different salinities and packing densities.

among the different packing densities, salinities and temperatures (table 6). Pair-wise comparison revealed that all four levels of packing densities and two levels of temperatures differed significantly, but there was no difference between the salinity means of 20 and 25ppt. Significant difference was shown by all interactions. Pair-wise comparison of density and salinity interaction (ds) showed the presence of five different groups. The density and temperature (dt) interaction showed no difference between d2 t1 (300PL/l - 30 $\pm$ 1°C) & d4 t2 (500PL/l - 23 $\pm$ 2°C) and also between d3 t1 (400PL/l - 30 $\pm$ 1°C) & d4 t1 (500PL/l - 30 $\pm$ 1°C). But, salinity and temperature (st) interaction showed significant difference.

The duration of 80% survival of oxygen-packed seed is shown in table 7, figures 10, 11 and 12. The result is similar to 90% survival rate, showing a falling trend with increase in packing density and temperatures. Analysis of variance of the data is shown in table 8. The main effects of packing densities, salinities, temperatures and their interactions showed significant difference. Pair-wise comparison of the main effects also showed a trend similar to 90% survival rate. The interaction between density and salinity (ds) formed five different groups. Similarly, salinity and temperature (st) interaction formed three different groups.

Table 6. Analysis of variance in the duration of 90 % survival of oxygen-packed P. indicus seed under different levels of packing densities, salinities and temperatures.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value
d	3	3001.682	1000.561	406.15*
s	2	63.219	31.609	12.83*
t	1	1547.005	1547.005	627.96*
ds	6	124.240	20.707	8.41*
dt	3	696.432	232.144	94.23*
st	2	79.260	39.630	16.09*
dst	6	123.365	20.561	8.35*
Error	24	59.125	2.464	
Total	47	5694.328		

* Significantly different at 5 % level.

Treatment means of salinity

s2	s1	s3
13.65625	13.125	11

Calculated C.D. value (t 0.05) = 1.1454

Treatment means of density

d1	d2	d3	d4
25.41667	12.75	6.958333	5.25

Calculated C.D. value (t 0.05) = 1.3226

contd.....

Treatment means of temperature

t2	t1
18.27083	6.916667

Calculated C.D. value (t 0.05) = 0.9352

Treatment means of DS

1. d1 s1	-	28.5	7. d3 s2	-	7.125
2. d1 s2	-	27.5	8. d3 s1	-	7.0
3. d1 s3	-	20.25	9. d3 s3	-	6.75
4. d2 s2	-	14.75	10. d4 s3	-	5.5
5. d2 s1	-	12.0	11. d4 s2	-	5.25
6. d2 s3	-	11.5	12. d4 s1	-	5.0

Calculated C.D. value (t 0.05) = 2.2909

Treatment means of DT

1. d1 t2	-	37.166	5. d4 t2	-	7.666
2. d2 t2	-	18.83	6. d2 t1	-	6.66
3. d1 t1	-	13.666	7. d3 t1	-	4.5
4. d3 t2	-	9.416	8. d4 t1	-	2.833

Calculated C.D. value (t 0.05) = 1.87055

Treatment means of ST

1. s2 t2	-	20.43	4. s3 t1	-	7.125
2. s1 t2	-	19.5	5. s2 t1	-	6.875
3. s3 t2	-	14.87	6. s1 t1	-	6.75

Calculated C.D. value (t 0.05) = 1.6199

contd.....

Treatment means of DST

1.	d1 s1 t2 - 44.0		13.	d4 s2 t2 - 8.0	
2.	d1 s2 t2 - 40.5		14.	d4 s3 t2 - 8.0	
3.	d1 s3 t2 - 27.0		15.	d2 s3 t1 - 7.5	
4.	d2 s2 t2 - 23.0		16.	d4 s1 t2 - 7.0	
5.	d2 s1 t2 - 18.0		17.	d2 s2 t1 - 6.5	
6.	d2 s3 t2 - 15.5		18.	d2 s1 t1 - 6.0	
7.	d1 s2 t2 - 14.5		19.	d3 s1 t1 - 5.0	
8.	d1 s3 t1 - 13.5		20.	d3 s3 t1 - 4.5	
9.	d1 s1 t1 - 13.0		21.	d3 s2 t1 - 4.0	
10.	d3 s2 t2 - 10.25		22.	d4 s1 t1 - 3.0	
11.	d3 s3 t2 - 9.0		23.	d4 s3 t1 - 3.0	
12.	d3 s1 t2 - 9.0		24.	d4 s2 t1 - 2.5	

Calculated C.D. value (t 0.05) = 3.23988

concl.

Table 7. Duration of 80% survival of oxygen-packed P.indicus seed under different levels of salinities, temperatures and packing densities.

Salinity (ppt)	Temperature (°C)	Duration (h)* at four different packing densities			
		200/l	300/l	400/l	500/l
20	30 ± 1	18.5	12.0	8.0	5.0
	23 ± 2	49.5	34.5	16.0	12.0
25	30 ± 1	21.5	11.5	7.25	6.0
	23 ± 2	46.5	33.0	20.0	18.0
30	30 ± 1	18.5	15.0	8.5	5.0
	23 ± 2	36.5	27.0	16.5	13.0

* Each value is a mean of duplicates.

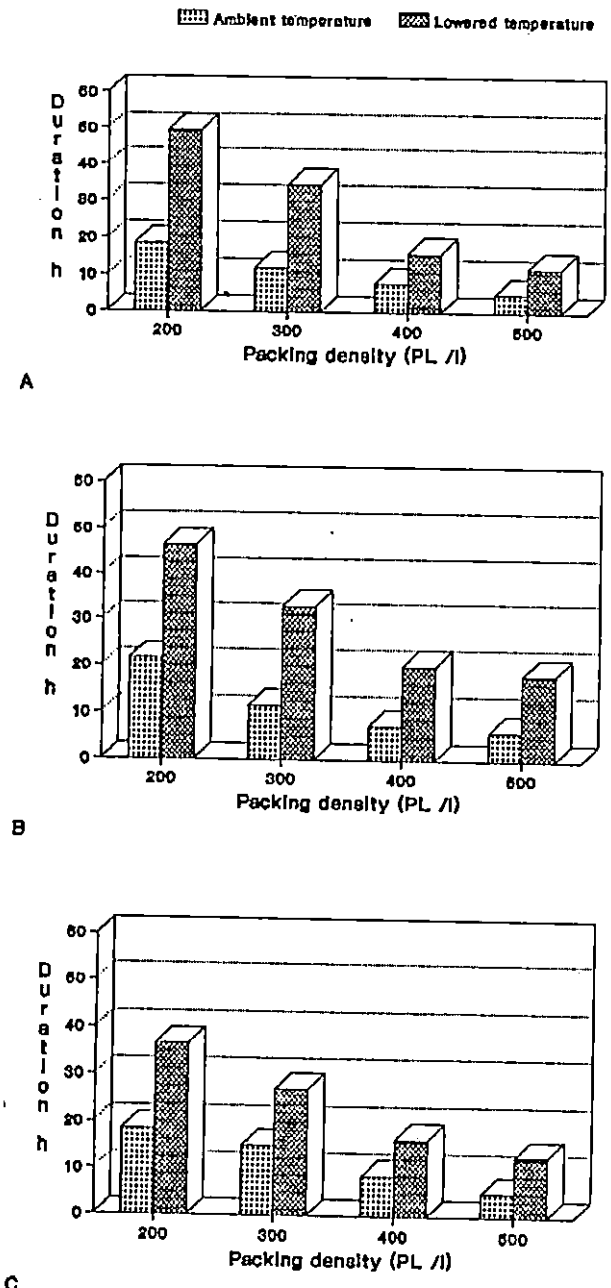


Fig.10. Duration of 80 % survival of oxygen packed *P.Indicus* seed at 20 ppt (A), 25 ppt (B) and 30 ppt (C) salinities under different temperatures and packing densities.

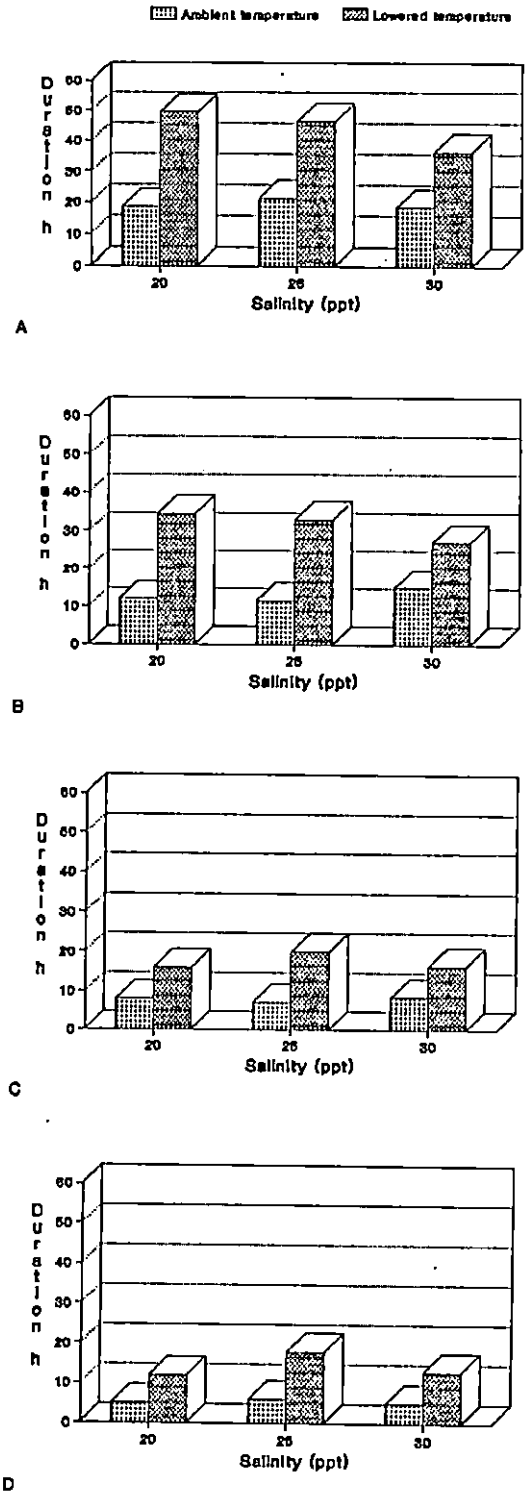
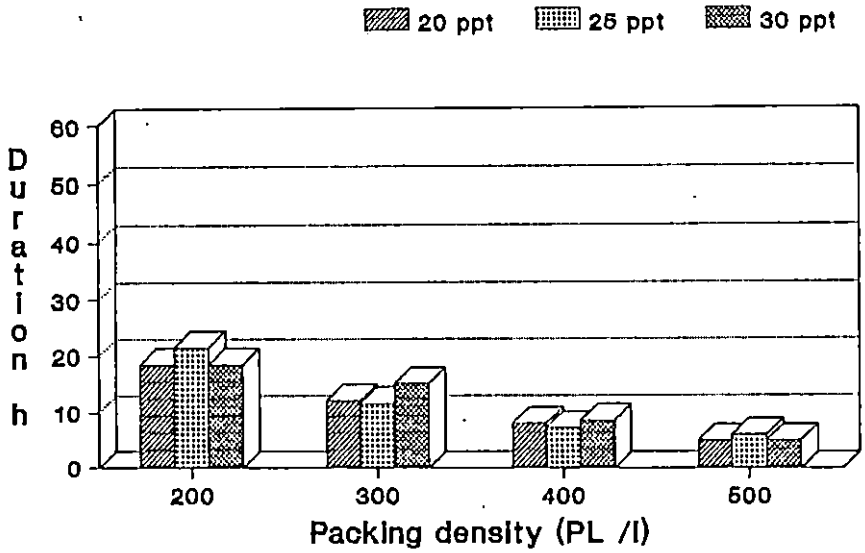
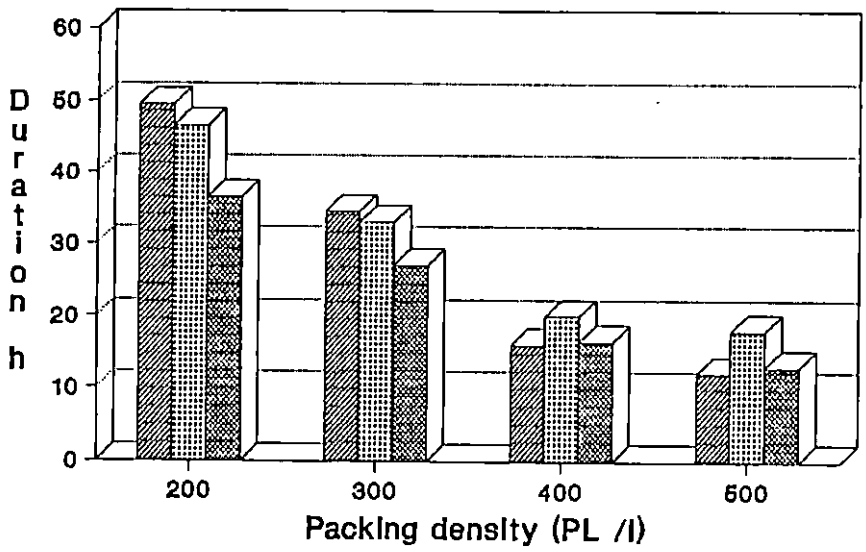


Fig.11 Duration of 80 % survival of oxygen-packed *P.indicus* seed at 200 (A), 300 (B), 400 (C) and 500 (D) PL /l packing densities under different temperatures and salinities.



A



B

Fig.12 Duration of 80 % survival of oxygen-packed *P.Indicus* seed at ambient temperature (A) and lowered temperature (B) under different salinities and packing densities.

Table 8. Analysis of variance in the duration of 80 % survival of oxygen-packed P. indicus seed under different levels of packing densities, salinities and temperatures.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value
d	3	3579.140	1193.047	240.36*
s	2	72.697	36.349	7.32*
t	1	2875.255	2875.255	579.27*
ds	6	84.344	14.057	2.83*
dt	3	513.933	171.311	34.51*
st	2	95.948	47.974	9.67*
dst	6	84.926	14.154	2.85*
Error	24	119.125	4.964	
Total	47	7425.369		

* Significantly different at 5 % level.

Treatment means of Salinity

s2	s1	s3
20.46875	19.4375	17.5

Calculated C.D. value (t 0.05) = 1.6258

Treatment means of Density

d1	d2	d3	d4
31.83333	22.16667	12.70833	9.833333

Calculated C.D. value (t 0.05) = 1.8773

contd.....

Treatment means of Temperature

t2	t1
11.39583	26.875

Calculated C.D. (t 0.05) = 1.3274

Treatment means of DS

1. d1 s1 - 34		7. d3 s2 - 13.625	
2. d1 s2 - 34		8. d3 s3 - 12.5	
3. d1 s3 - 27.5		9. d3 s1 - 12	
4. d2 s1 - 23.25		10. d4 s2 - 12	
5. d2 s2 - 22.25		11. d4 s3 - 9	
6. d2 s3 - 21		12. d4 s1 - 8.5	

Calculated C.D. value (t 0.05) = 3.2517

Treatment means of DT

1. d1 t2 - 44.16		5. d4 t2 - 14.33	
2. d2 t2 - 31.5		6. d2 t1 - 12.8	
3. d1 t1 - 19.5		7. d3 t1 - 7	
4. d3 t2 - 17.5		8. d4 t1 - 5.33	

Calculated C.D. value (t 0.05) = 2.655

Treatment means of ST

1. s2 t2 - 29.375		4. s3 t1 - 11.75	
2. s1 t2 - 28		5. s2 t1 - 11.56	
3. s3 t2 - 23.25		6. s1 t1 - 10.875	

Calculated C.D. value (t 0.05) = 2.922

contd.....

Treatment means of DST

1. d1 s1 t2	- 49.5	13. d3 s1 t2	- 16
2. d1 s2 t2	- 46.5	14. d2 s3 t1	- 15
3. d1 s3 t2	- 36.5	15. d4 s3 t2	- 13
4. d2 s1 t2	- 34.5	16. d2 s1 t1	- 12
5. d2 s2 t2	- 33	17. d2 s2 t1	- 11.5
6. d2 s3 t2	- 27	18. d2 s2 t1	- 11.5
7. d1 s2 t1	- 21.5	19. d3 s3 t1	- 8.5
8. d3 s2 t2	- 20	20. d3 s1 t1	- 8.0
9. d1 s1 t1	- 18.5	21. d3 s2 t1	- 7.25
10. d1 s3 t1	- 18.5	22. d4 s2 t1	- 6.0
11. d4 s2 t2	- 18	23. d4 s1 t1	- 5.0
12. d3 s3 t2	- 16.5	24. d4 s3 t1	- 5.0

Calculated C.D. value (t 0.05) = 4.5985

Table 9, figures 13, 14 and 15 shows the duration of 70% survival of the oxygen-packed shrimp seed. Analysis of variance is shown in table 10. The results showed a similarity with 90% and 80% survival rates. The analysis of variance showed significant difference for the main effects and interactions of packing density, salinity and temperature. The pair-wise comparison of the main effects showed trends similar to 90% and 80% survival rates. Critical difference analysis of density and salinity (ds) interaction means showed no significant difference between d1 s3 (200PL/l - 30ppt), d1 s1 (300PL/l - 20ppt), d2 s2 (300PL/l - 25ppt) & d2 s3 (300PL/l - 30ppt) and also between d3 s3 (400PL/l - 30ppt) & d3 s2 (400PL/l - 25ppt). Density and temperature (dt) interaction means showed the presence of two homogenous groups (1) d1 t1 (200PL/l - 30 $\pm$ 1°C) & d2 t1 (300PL/l - 30 $\pm$ 1°C) and (2) d3 t1 (400PL/l - 30 $\pm$ 1°C) & d4 t2 (500PL/l - 23 $\pm$ 2°C). But, salinity and temperature (st) interactions means showed the presence of only a single group ie., s2 t1 (25ppt - 30 $\pm$ 1°C), s3 t1 (30ppt - 30 $\pm$ 1°C) & s1 t1 (20ppt - 30 $\pm$ 1°C).

The three factor interaction (dst) was also found to be significant in all these levels of survival.

Table 9. Duration of 70% survival of oxygen-packed P.indicus seed under different levels of salinities, temperatures and packing densities.

Salinity (ppt)	Temperature (°C)	Duration (h)* at four different packing densities			
		200/l	300/l	400/l	500/l
20	30 ± 1	23.5	22.0	18.0	14.0
	23 ± 2	56.5	46.5	24.0	22.5
25	30 ± 1	25.5	24.0	19.5	13.0
	23 ± 2	51.5	43.5	27.0	20.5
30	30 ± 1	24.5	23.0	19.0	11.5
	23 ± 2	45.5	43.0	28.0	15.5

* Each value is a mean of duplicates.

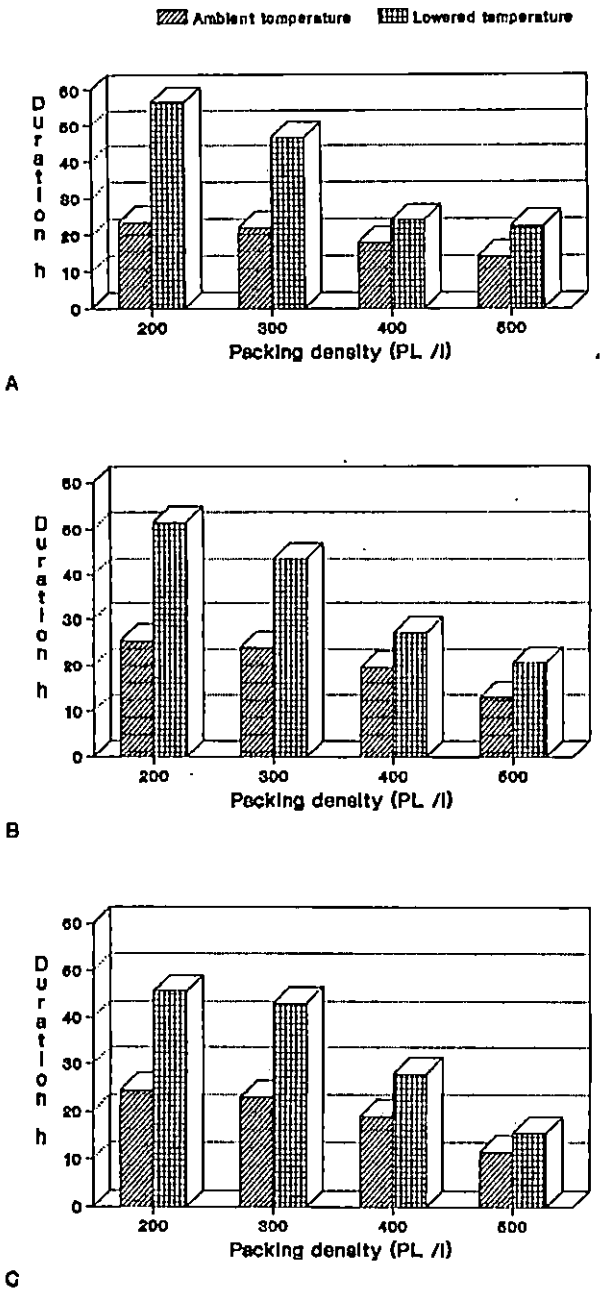


Fig.13. Duration of 70 % survival of oxygen packed *P.Indicus* seed at 20 ppt (A), 25 ppt (B) and 30 ppt (C) salinities under different temperatures and packing densities.

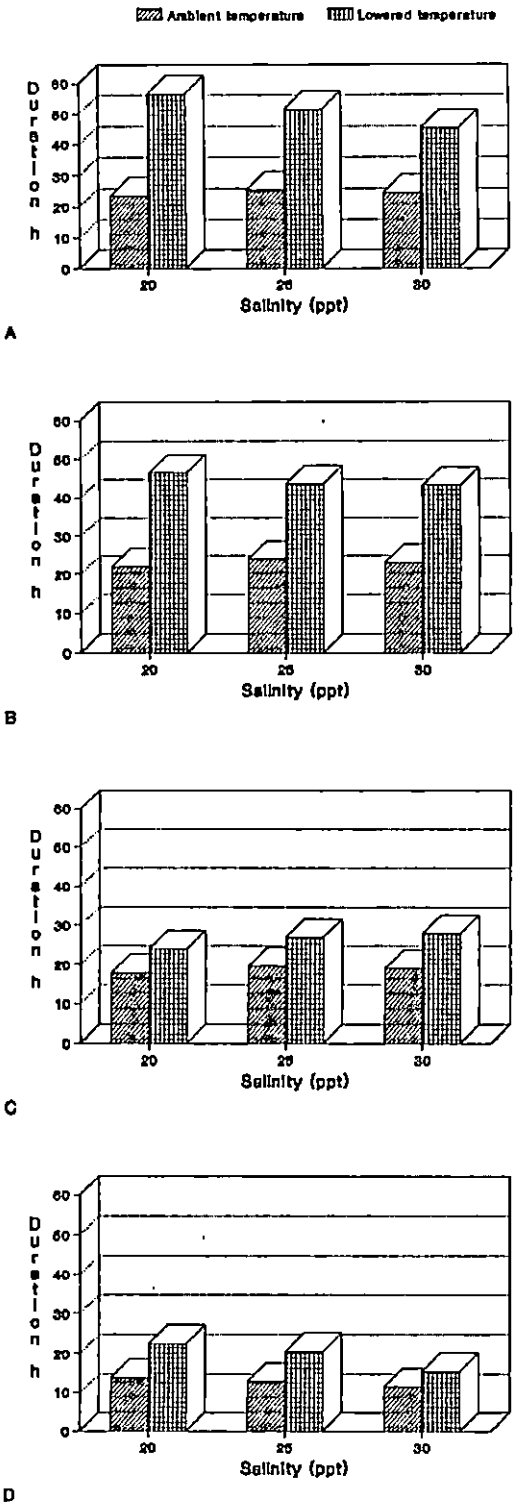
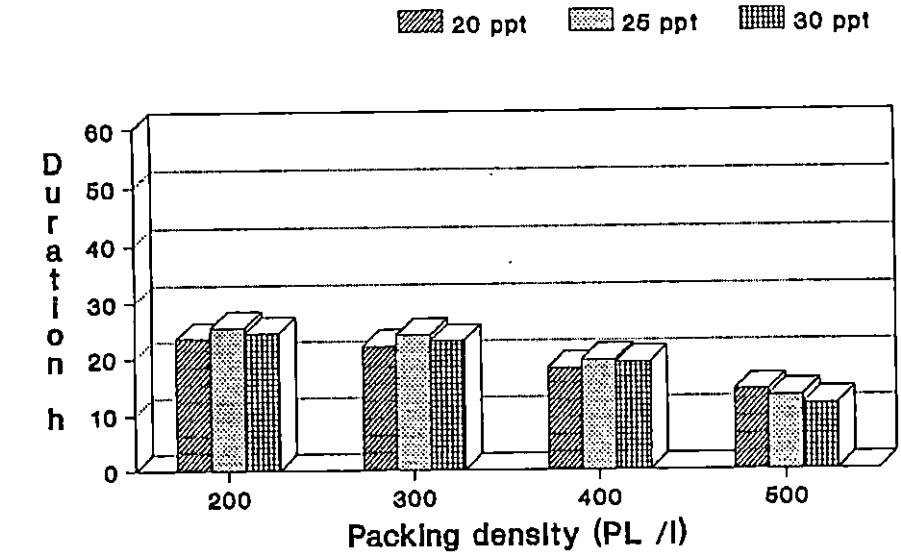
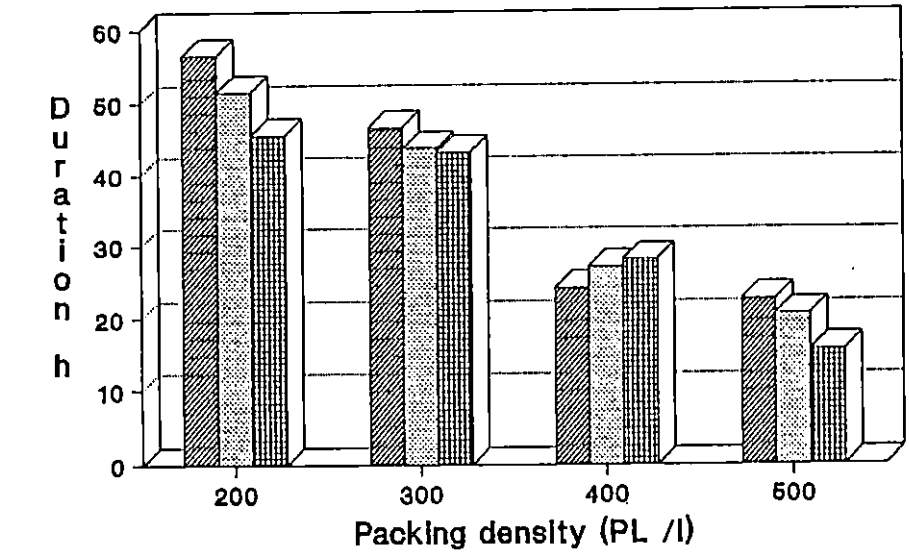


Fig.14. Duration of 70 % survival of oxygen-packed *P.indicus* seed at 200 (A), 300 (B), 400 (C) and 500 (D) PL /l packing densities under different temperatures and salinities.



A



B

Fig.15. Duration of 70 % survival of oxygen-packed *P.Indicus* seed at ambient temperature (A) and lowered temperature (B) under different salinities and packing densities.

Table 10. Analysis of variance in the duration of 70 % survival of oxygen-packed P. indicus seed under different levels of packing densities, salinities and temperatures.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value
d	3	3568.896	1189.632	641.60*
s	2	42.125	21.063	11.36*
t	1	2898.521	2898.521	1563.25*
ds	6	76.042	12.674	6.84*
dt	3	902.229	300.743	162.20*
st	2	41.542	20.771	11.20*
dst	6	61.958	10.326	5.57*
Error	24	44.500	1.854	
Total	47	7635.813		

* Significantly different at 5 % level.

Treatment means of Salinity

s1	s2	s3
28.375	28.0625	26.25

Calculated C.D. value (t 0.05) = 0.9937

Treatment means of Density

d1	d2	d3	d4
37.83333	33.66667	22.58333	16.16667

Calculated C.D. value (t 0.05) = 1.1474

contd.....

Treatment means of Temperature

t2	t1
35.33333	19.79167

Calculated C.D. (t 0.05) = 0.8113

Treatment means of DS

1. d1 s1	-	40	7. d3 s3	-	23.5
2. d1 s2	-	38.5	8. d3 s2	-	23.25
3. d1 s3	-	35	9. d3 s1	-	21
4. d2 s1	-	34.25	10. d4 s1	-	18.25
5. d2 s2	-	33.75	11. d4 s2	-	16.75
6. d2 s3	-	33	12. d4 s3	-	13.5

Calculated C.D. value (t 0.05) = 1.9872363

Treatment means of DT

1. d1 t2	-	51.16	5. d2 t1	-	23.0
2. d2 t2	-	44.3	6. d3 t1	-	18.83
3. d3 t2	-	26.33	7. d4 t2	-	19.5
4. d1 t1	-	24.5	8. d4 t1	-	12.8

Calculated C.D. value (t 0.05) = 1.6225

Treatment means of ST

1. s1 t2	-	37.375	4. s2 t1	-	20.5
2. s2 t2	-	35.625	5. s3 t1	-	19.5
3. s3 t2	-	33.0	6. s1 t1	-	19.375

Calculated C.D. value (t 0.05) = 1.4051

contd.....

Treatment means of DST

1. d1 s1 t2	- 56.5	13. d1 s1 t1	- 23.5
2. d1 s2 t2	- 51.5	14. d2 s3 t1	- 23
3. d1 s1 t2	- 46.5	15. d4 s1 t2	- 22.5
4. d1 s3 t2	- 45.5	16. d2 s1 t1	- 22
5. d2 s2 t2	- 43.5	17. d4 s2 t2	- 20.5
6. d2 s3 t2	- 43	18. d3 s2 t1	- 19.5
7. d3 s3 t2	- 28	19. d3 s3 t1	- 19.0
8. d3 s2 t2	- 27	20. d3 s1 t1	- 18.0
9. d1 s2 t1	- 25.5	21. d4 s3 t2	- 15.5
10. d1 s3 t1	- 24.5	22. d4 s1 t1	- 14.0
11. d2 s2 t1	- 24	23. d4 s2 t1	- 13.0
12. d3 s1 t2	- 24	24. d4 s3 t1	- 11.5

Calculated C.D. value (t 0.05) = 2.810

concl.

4.2.2. Cumulative percentage survival.

The cumulative percentage survival of the oxygen-packed seed under different salinities, temperatures and packing densities at set duration is summarised in table 11. The packing density showed inverse relationship with time of initial mortality and subsequent percentages of survival. The packing density of 200PL/l gave the longest duration of transport. At 12h the percentage survival was reduced to 70% in the highest packing density of 500PL/l, whereas, this was as high as 90 - 95% in the lowest packing density. At ambient temperatures, the longest duration of transport with 70% survival was observed in 25ppt salinity combinations. The safe duration of transport was observed to be 6-8h for 200PL/l, at ambient temperatures, but by lowering the temperatures from 30 $\pm$ 1°C to 23 $\pm$ 2°C, the corresponding duration was more than 20h in all salinities. At 20ppt salinity, 500PL/l showed 80% survival for 12h at lowered temperatures, whereas for the same survival and duration only 300PL/l could be packed at ambient temperatures. Similar trends were shown in other salinities.

4.2.3. Dissolved oxygen.

Analysis of variance could not be done for this

Table 11. Cumulative percentage survival of oxygen-packed P. indicus seed under different salinities, temperatures and packing densities.

Salinity (ppt)	Packing density (PL/1)	Temp. (°C)	Duration (h)*																	
			2	4	6	8	10	12	14	16	18	20	22	24	26	28	42	46	52	56
20	200	30 ± 1	100	100	100	100	95	95	85	80	80	75	75	70						
		23 ± 2	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	85	75	70
	300	30 ± 1	100	95	90	85	85	80	75	75	75	70	70							
		23 ± 2	100	100	100	100	95	95	90	90	90	85	85	85	85	85	75	70		
	400	30 ± 1	100	90	85	80	75	75	75	70	70									
		23 ± 2	100	100	100	95	85	80	80	80	75	75	70	70						
	500	30 ± 1	90	80	75	75	70	70	70											
		23 ± 2	100	100	95	85	85	80	75	75	75	70	70							
25	200	30 ± 1	100	100	100	95	95	95	90	85	85	80	75	75	70					
		23 ± 2	100	100	100	100	100	100	100	100	100	100	100	95	95	95	85	80	70	
	300	30 ± 1	100	90	85	85	80	75	75	75	75	75	70	70						
		23 ± 2	100	100	100	100	95	95	90	90	90	90	90	85	85	85	70			
	400	30 ± 1	100	90	85	80	75	75	75	75	70	70								
		23 ± 2	100	100	100	95	90	85	85	85	85	80	75	75	70					
	500	30 ± 1	95	85	80	75	75	70												
		23 ± 2	100	100	95	90	85	85	85	80	80	70								
30	200	30 ± 1	100	100	100	100	95	90	85	80	80	75	75	70						
		23 ± 2	100	100	100	100	100	100	100	100	100	100	100	95	90	85	70			
	300	30 ± 1	100	100	95	90	85	85	80	75	75	75	70							
		23 ± 2	100	100	100	100	95	90	90	85	85	85	85	80	80	75	70			
	400	30 ± 1	100	90	85	80	75	75	70	70	70									
		23 ± 2	100	100	100	95	85	85	80	80	75	75	70	70	70	70				
	500	30 ± 1	95	85	75	75	70	70												
		23 ± 2	100	100	95	90	85	80	75	70										

* Each value is a mean of duplicates.

data, as the replicate values were not available. Table 12 gives the reduction in the dissolved oxygen levels in the oxygen-packed jars under different levels of salinities, temperatures and packing densities at 70% survival. Salinity-wise, the reduction in oxygen level was the maximum in the lowest salinity, except at 400PL/l (figure 16). It is clear from the data that the fall in the dissolved oxygen is directly related to the increase in packing density (figure 17). At higher temperature considerable reduction in oxygen levels was observed (figure 18).

4.2.4. Ammonia-N .

Increase in ammonia-N levels in oxygen-packed jars under different levels of packing densities, salinities and temperatures at 70% survival is given in table 13, figures 19, 20 and 21. The analysis of variance of the data is shown in table 14. The increase in ammonia-N levels was significantly different for packing densities, salinities, and temperatures. Pair-wise comparison showed significant difference at each of the four levels of packing density, two levels of temperatures. Increase in ammonia-N levels for 25ppt and 30ppt was almost similar and formed uniform group. The pair-wise comparison of interactions of packing density and salinity (ds) showed four different groups ie, (1) d3 s2 (400PL/l - 25ppt) & d3

Table 12. Reduction in the dissolved oxygen levels in oxygen-packed jars under different levels of salinities, temperatures and packing densities at 70 % survival.

Salinity (ppt)	Temperature (°C)	Oxygen (ppm) at four different packing densities			
		200/l	300/l	400/l	500/l
20	30 ± 1	9.38	13.25	11.8	14.4
	23 ± 2	8.27	11.6	8.6	11.03
25	30 ± 1	6.9	11.18	12.6	13.2
	23 ± 2	4.09	9.4	9.5	12.9
30	30 ± 1	5.9	8.5	11.85	14.18
	23 ± 2	3.1	9.4	10.3	8.55

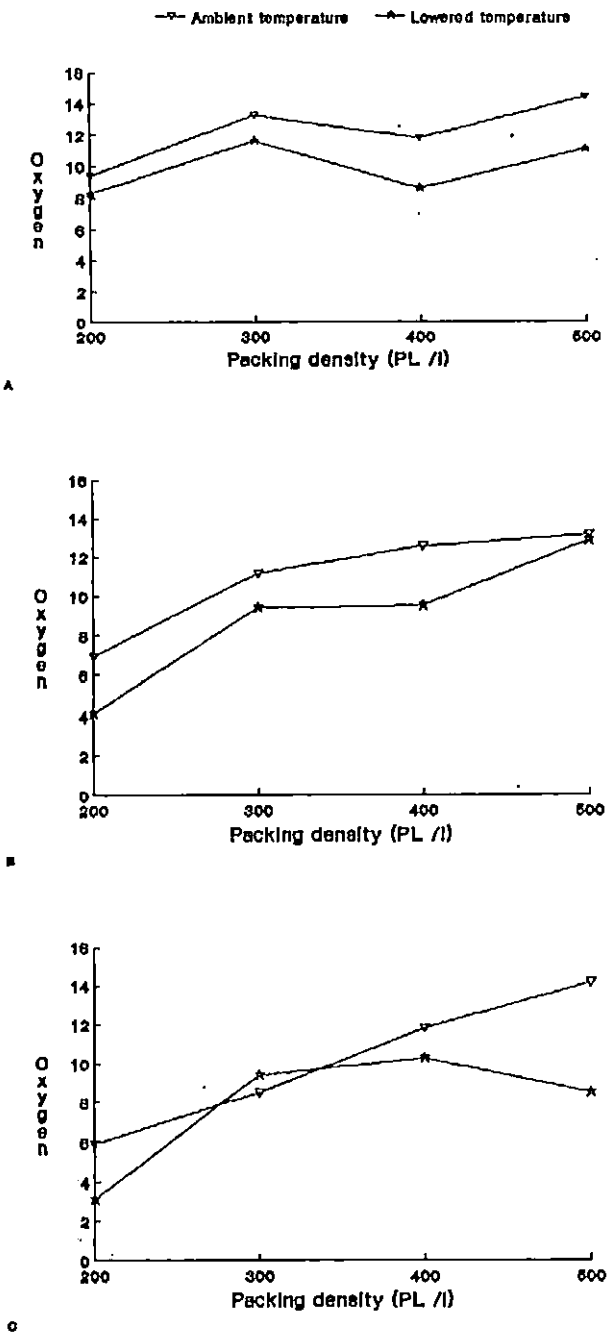


Fig. 16 Reduction in the dissolved oxygen (ppm) levels in oxygen-packed jars at 20 (A), 25 (B) & 30 (C) ppt salinities under different temperatures and packing densities.

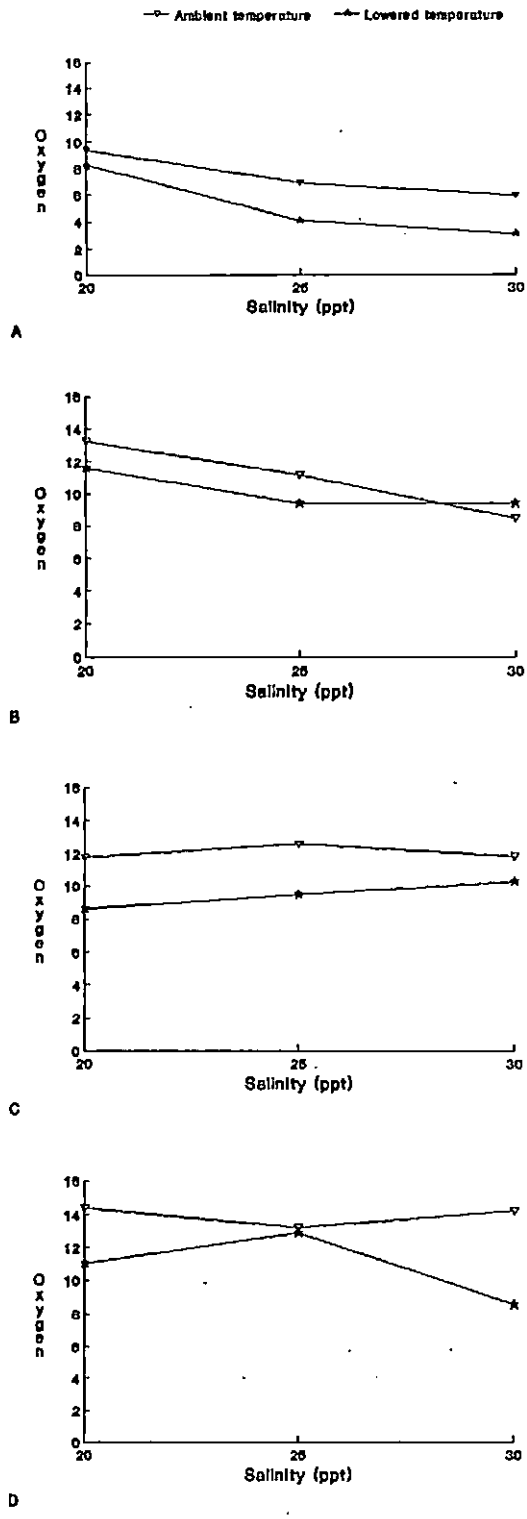
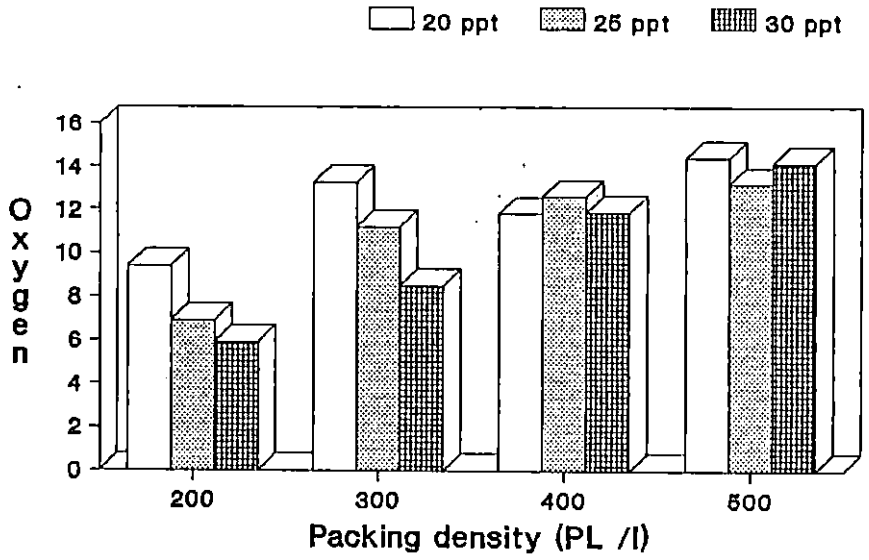
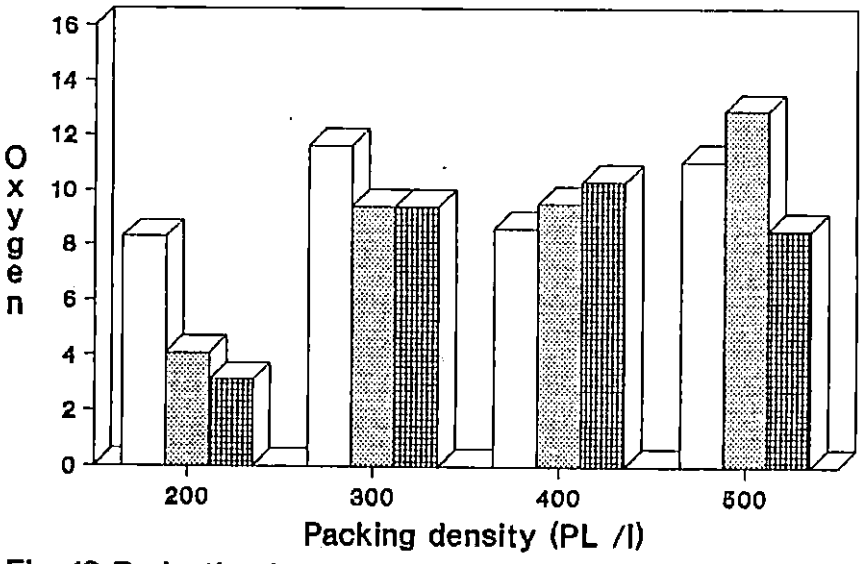


Fig. 17 Reduction in the dissolved oxygen (ppm) levels in the oxygen-packed jars at 200 (A), 300 (B), 400 (C) and 500 (D) PL/l packing densities under different levels of salinities and temperatures at 70 % survival.



A



B

Fig. 18 Reduction in the dissolved oxygen (ppm) levels in oxygen-packed jars at ambient(A) and lowered(B) temperatures under different salinities and packing densities.

Table 13. Increase in Ammonia - N levels in the oxygen-packed jars under different levels of salinities, temperatures and packing densities at 70% survival.

Salinity (ppt)	Temperature (°C)	Ammonia - N (ppm)* at four different packing densities			
		200/l	300/l	400/l	500/l
20	30 ± 1	22.16	26.96	45.04	34.28
	23 ± 2	4.57	9.41	12.13	27.16
25	30 ± 1	16.08	50.16	60.38	50.67
	23 ± 2	4.57	9.41	19.90	2.96
30	30 ± 1	13.36	46.79	60.38	56.43
	23 ± 2	2.96	9.416	14.25	6.19

* Each value is a mean of duplicates.

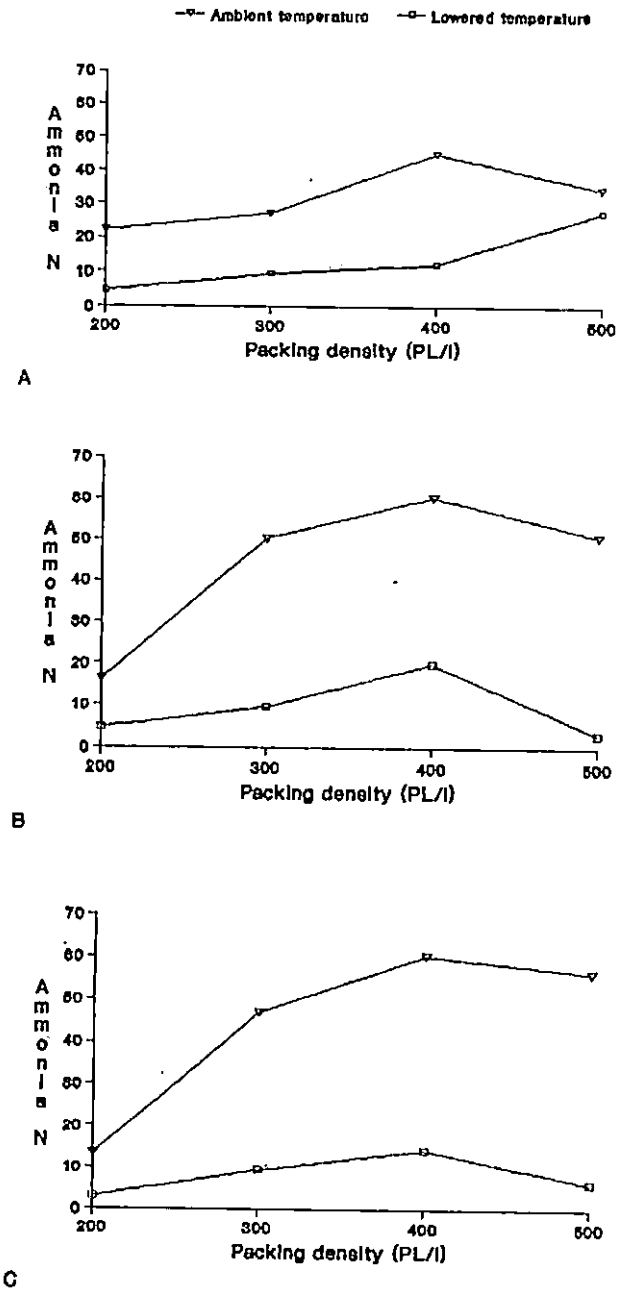


Fig. 19 Increase in the ammonia-N (ppm) levels in the oxygen-packed jars at 20 (A), 25 (B) and 30 (C) ppt salinities under different temperatures and packing densitites at 70 % survival.

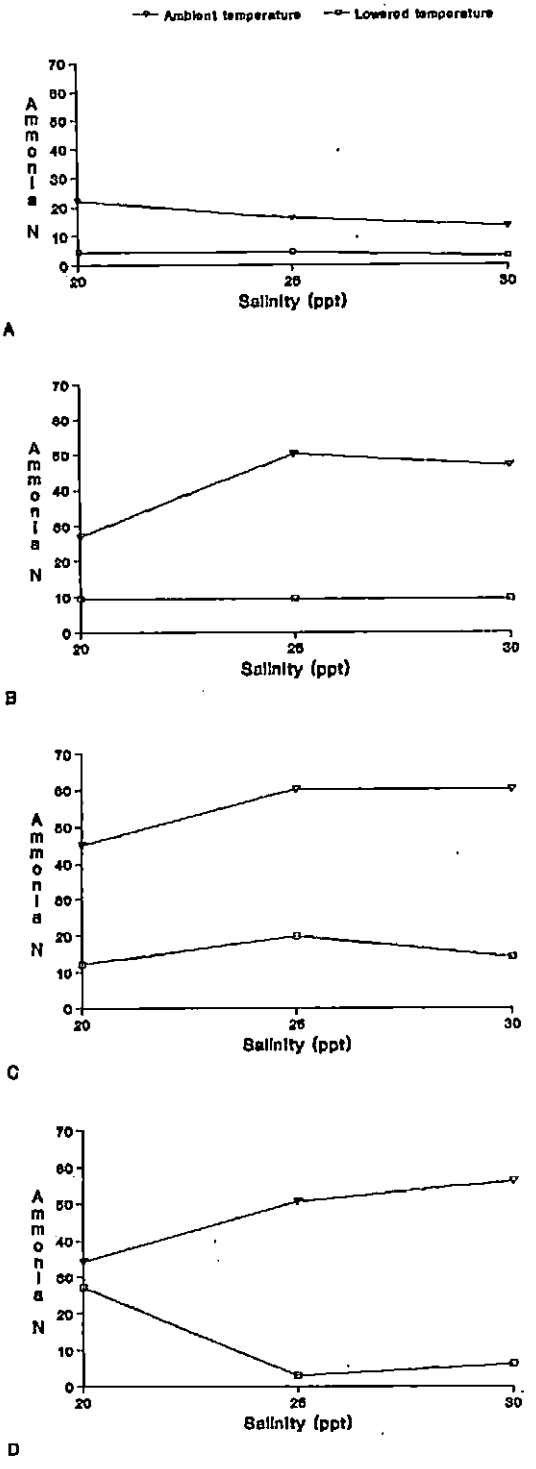
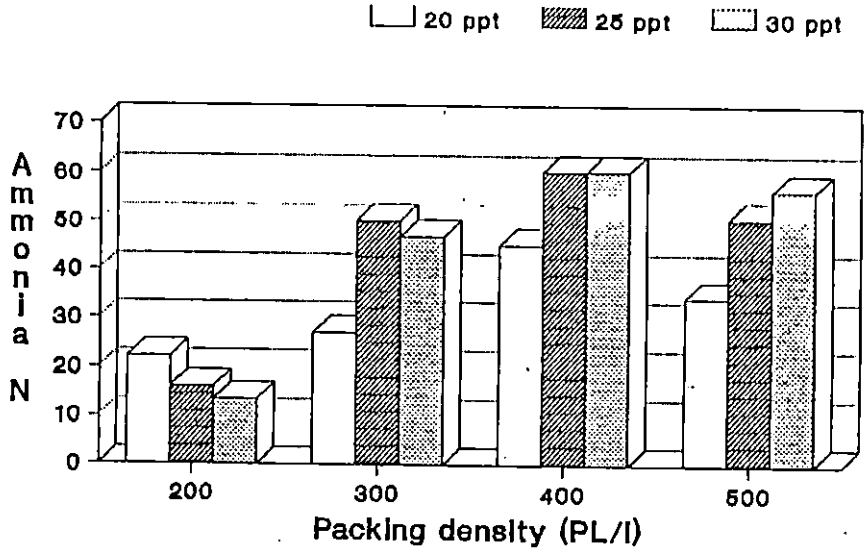
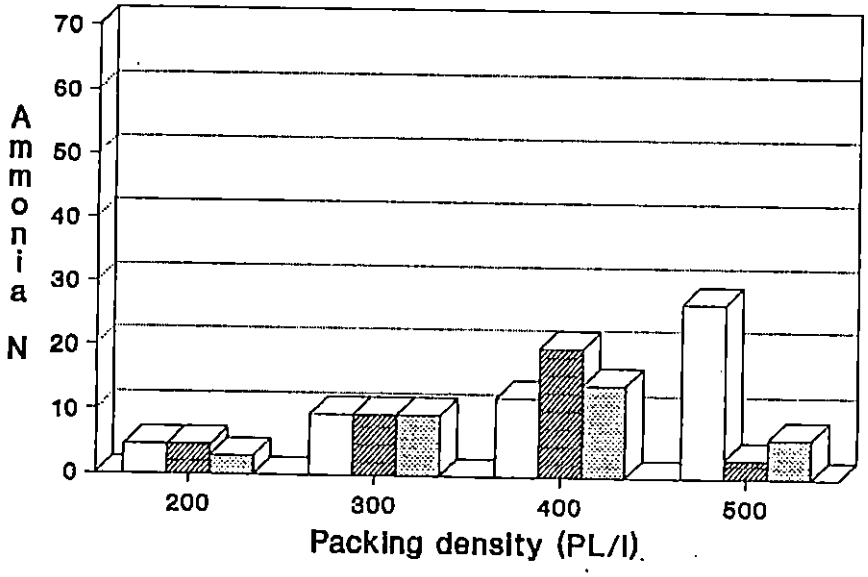


Fig. 20 Increase in the ammonia-N (ppm) levels in the oxygen-packed jars at 200 (A), 300 (B), 400 (C) and 500 (D) PL/l packing densities under different levels of salinities and temperatures at 70 % survival.



A



B

Fig. 21 Increase in the ammonia-N (ppm) levels in the oxygen-packed jars at ambient (A) and lowered (B) temperatures at different salinities and packing densities at 70 % survival.

Table 14. Analysis of variance in the increase in ammonia-N levels in the oxygen-packed jars under different levels of packing densities, salinities and temperatures at 70 % survival.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value
d	3	4017.756	1339.252	77.23*
s	2	153.674	76.837	4.43*
t	1	10795.892	10795.892	622.60*
ds	6	555.443	92.574	5.34*
dt	3	1228.525	409.508	23.62*
st	2	754.382	377.191	21.75*
dst	6	852.780	142.130	8.20*
Error	24	416.160	17.340	
Total	47	18774.611		

* Significantly different at 5 % level.

Treatment means of Salinity

s2	s3	s1
26.74856	26.22419	22.71794

Calculated C.D. value (t 0.05) = 3.0387

Treatment means of Density

d3	d4	d2	d1
35.34967	29.588	25.36133	10.62191

Calculated C.D. value (t 0.05) = 3.5088

contd.....

Treatment means of Temperature

t1	t2
40.22737	10.23308

Calculated C.D. (t 0.05) = 2.4811

Treatment means of DS

1. d3 s2	-	40.144	7. d2 s3	-	28.105
2. d3 s3	-	37.32	8. d4 s2	-	26.816
3. d4 s3	-	31.31	9. d2 s1	-	18.19
4. d4 s1	-	30.726	10. d1 s1	-	13.37
5. d2 s2	-	29.788	11. d1 s2	-	10.331
6. d3 s1	-	28.585	12. d1 s3	-	8.1635

Calculated C.D. value (t 0.05) = 6.0774

Treatment means of DT

1. d3 t1	-	55.269	5. d3 t2	-	15.43
2. d4 t1	-	47.129	6. d4 t2	-	12.105
3. d2 t1	-	41.366	7. d2 t2	-	9.416
4. d1 t1	-	17.205	8. d1 t2	-	4.038

Calculated C.D. value (t 0.05) = 4.962

Treatment means of ST

1. s2 t1	-	44.325	4. s1 t2	-	13.321
2. s3 t1	-	44.288	5. s2 t2	-	9.214
3. s1 t1	-	32.114	6. s3 t2	-	8.206

Calculated C.D. value (t 0.05) = 4.297

contd.....

Treatment means of DST

1. d3 s2 t1	- 60.384	13. d1 s2 t1	- 16.0875
2. d4 s2 t1	- 50.67	14. d3 s3 t2	- 14.256
3. d4 s3 t1	- 56.43	15. d1 s3 t1	- 13.365
4. d4 s2 t1	- 50.67	16. d3 s1 t2	- 12.13
5. d2 s2 t1	- 50.16	17. d2 s3 t2	- 9.416
6. d2 s3 t1	- 46.7955	18. d2 s1 t2	- 9.416
7. d3 s1 t1	- 45.04	19. d2 s2 t2	- 9.416
8. d4 s1 t1	- 34.288	20. d4 s3 t2	- 6.19
9. d4 s1 t2	- 27.164	21. d1 s1 t2	- 4.576
10. d2 s1 t1	- 26.9645	22. d1 s2 t2	- 4.576
11. d1 s1 t1	- 22.165	23. d4 s2 t2	- 2.962
12. d3 s2 t2	- 19.904	24. d1 s3 t2	- 2.962

Calculated C.D. value (t 0.05) = 8.5947

concl.

s3 (400PL/l - 30ppt) (2) d4 s3 (500PL/l - 30ppt), d4 s1 (500PL/l - 20ppt), d2 s2 (300PL/l - 25ppt), d3 s1 (400PL/l - 20ppt) d2 s3 (300PL/l - 30ppt) & d4 s2 (400PL - 25ppt) (3) d2 s1 (300PL/l - 20ppt) & d1 s1 (200PL/l - 20ppt) (4) d1 s2 (200PL/l - 25ppt) & d1 s3 (200PL/l - 30ppt). Similarly for packing density and temperature (dt) interaction d1 t1 (200PL/l - 30 $\pm$ 1°C) & d3 t2 (400PL/l - 23 $\pm$ 2°C) formed one group and d4 t2 (500PL/l - 23 $\pm$ 2°C) & d2 t2 (300PL/l - 23 $\pm$ 2°C), the other. Salinity and temperature interaction (st) also showed four different groups. The three factor interaction (dst) was also found to be significant.

4.2.5. Carbon dioxide.

Increase in the free carbon dioxide levels in the oxygen-packed jars under different levels of salinities, temperatures and packing densities at 70% survival rate is presented in table 15, figures 22, 23 and 24. The analysis of variance could not be done as the replicate values were not available. Carbon dioxide levels increased with increase in packing density except for 500PL/l combinations. With reduction in temperature there was a reduction in carbon dioxide level except for 200PL/l - 25ppt combination.

Table 15. Increase in free carbon dioxide levels in oxygen-packed jars under different levels of salinities, temperatures and packing densities at 70% survival.

Salinity (ppt)	Temperature (°C)	Free carbon dioxide (ppm) at four different packing densities			
		200/l	300/l	400/l	500/l
20	30 ± 1	53.224	59.13	72.93	85.73
	23 ± 2	49.28	55.19	59.13	78.84
25	30 ± 1	49.28	59.13	74.90	83.77
	23 ± 2	49.28	53.22	68.99	39.42
30	30 ± 1	55.19	68.99	76.87	82.79
	23 ± 2	49.28	59.13	59.13	39.42

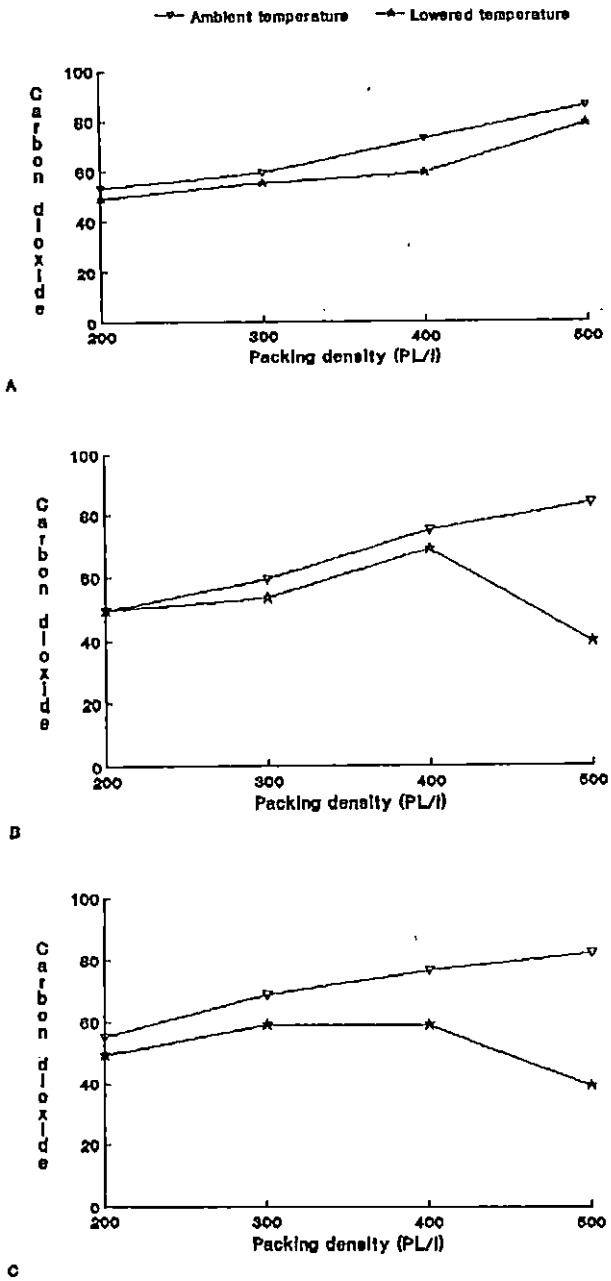


Fig. 22 Increase in the free carbon dioxide (ppm) levels in the oxygen-packed jars at 20 (A), 25 (B) and 30 (C) ppt salinities under different temperatures and packing densities at 70 % survival.

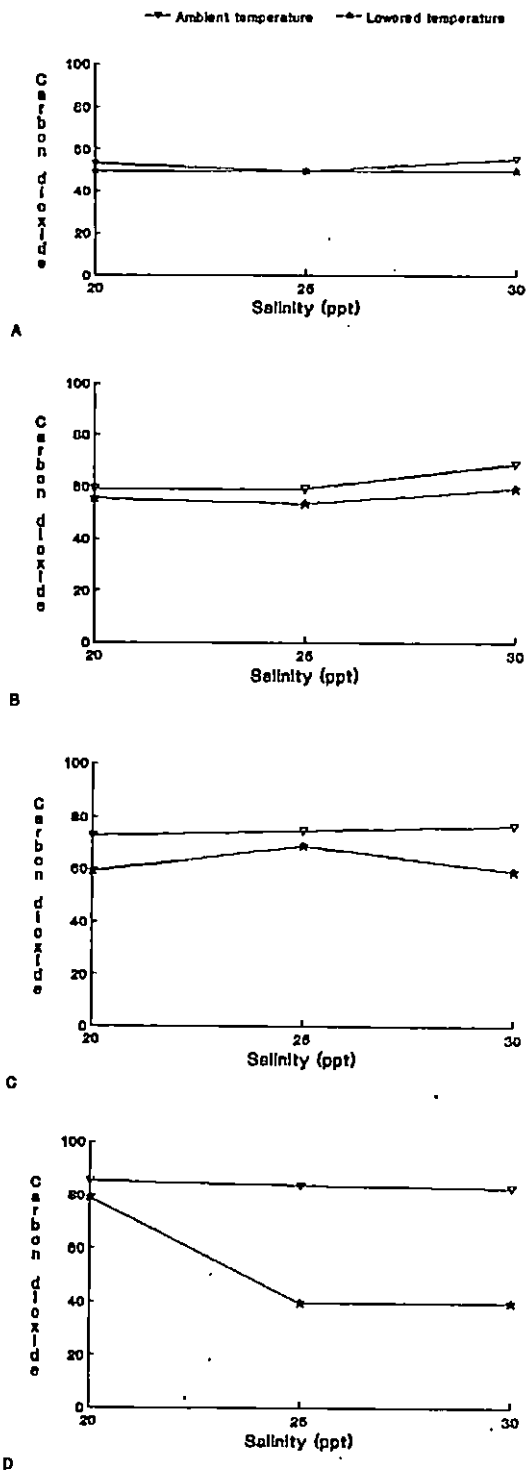
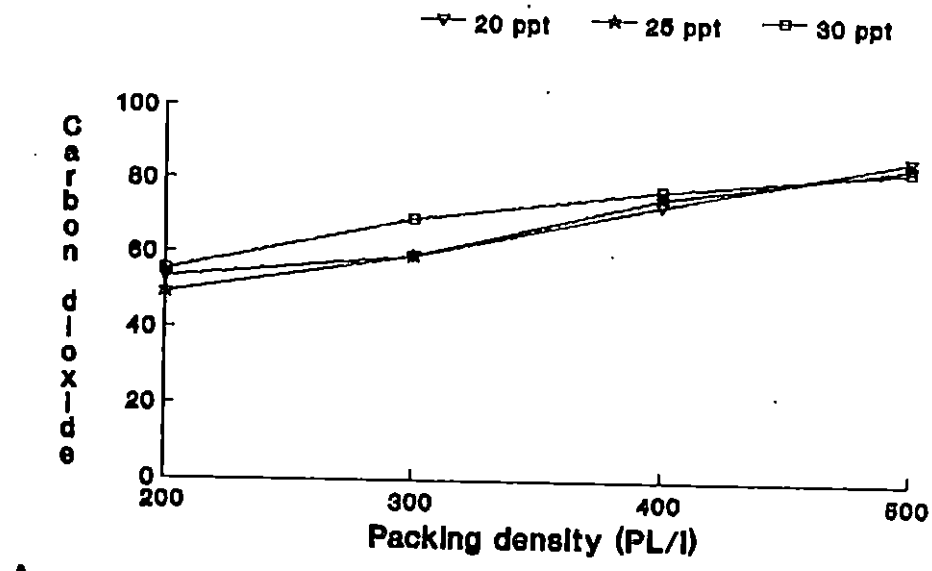
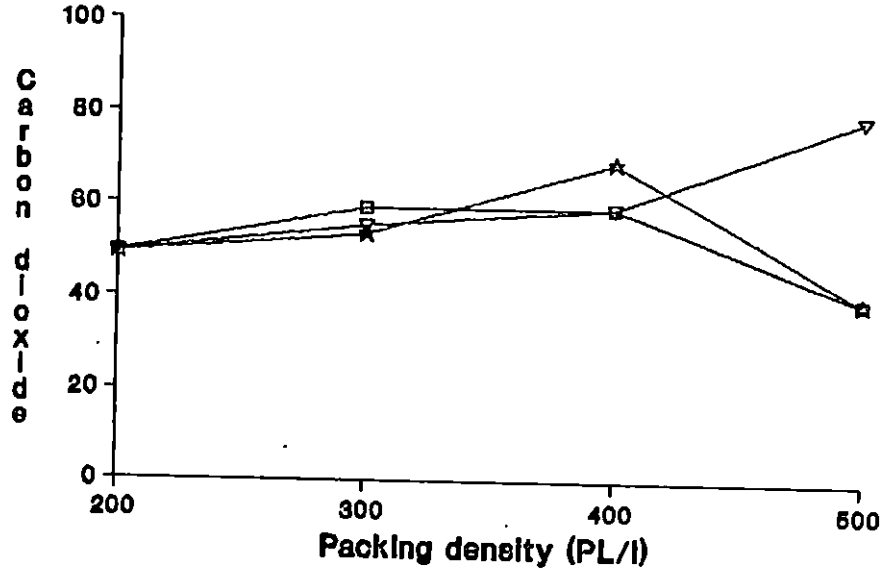


Fig. 23 Increase in the free carbon dioxide (ppm) levels in the oxygen-packed jars at 200 (A), 300 (B), 400 (C) and 500 (D) PL/l packing densities under different levels of salinities and temperatures at 70 % survival



A



B

Fig. 24 Increase in the free carbon dioxide (ppm) levels in the oxygen-packed jars at ambient (A) and lowered (B) temperatures at different salinities and packing densities at 70 % survival.

4.2.6. pH.

The pH of the packing medium was lowered from an initial value of 8.0 to 7.5-7.0 in the various treatment combinations at the end of 70% survival.

4.3. Experiment III. Effect of habitat material on the duration and survival of oxygen-packed P. indicus seed

4.3.1. Duration.

Duration of four different survival rates of the oxygen-packed P. indicus seed, with and without habitat material under different packing densities is shown in table 16. The safe duration of transport ie. 100 % survival was not altered by the introduction of the habitat material at the lower packing densities (figure 25) of 200PL/l and 300PL/l, whereas, slight increase in the safe duration was noticed at the higher packing densities by its introduction. The analysis of variance of the data showed (table 17) significant difference for the packing densities. Pair-wise comparison by critical difference analysis showed that all packing density levels were different. Table 16 and 18, figure 26 for 90% survival show similar trend as that obtained for 100% survival.

Table 16. Duration at four different survival rates of the oxygen-packed *P. indicus* seed, with (A) and without (B) habitat material, under different packing densities.

Packing density (PL/l)	Duration (h)*							
	100%		90%		80%		70%	
	A	B	A	B	A	B	A	B
200	14.0	14.17	26.8	27.16	33.34	33.5	40.5	40.66
300	6.0	6.5	15.0	15.34	21.67	23.0	33.16	32.16
400	4.5	3.66	7.5	7.17	13.33	12.33	24.66	22.5
500	2.33	2.0	5.5	4.33	12.33	10.83	15.83	13.33

* Each value is a mean of triplicates.

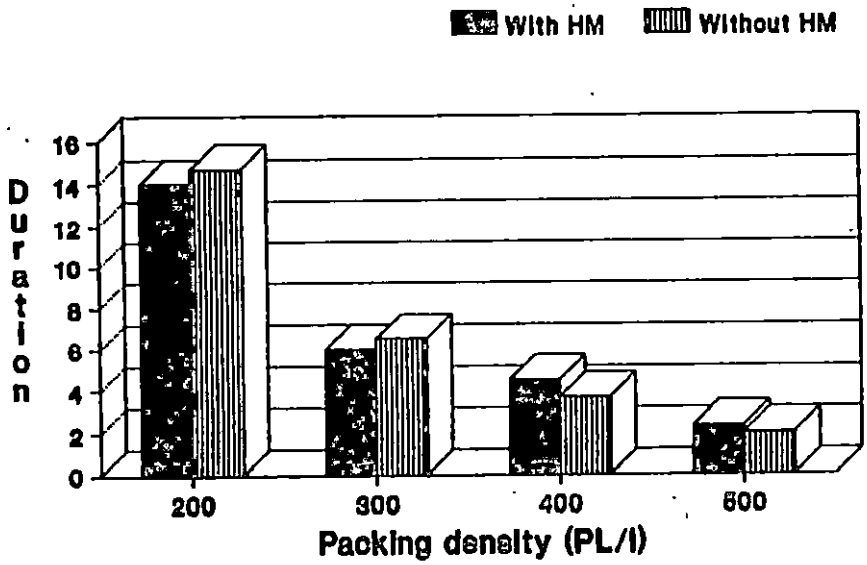


Fig. 26 Duration (h) of 100 % survival of the oxygen-packed *P. indicus* seed, with and without habitat material (HM), under different packing densities.

Table 17. Analysis of variance in the duration of 100 % survival of oxygen-packed P. indicus seed with and without habitat material under different packing densities.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value
d	3	492.61	164.20	525.46 *
h	1	0.09	0.09	0.30
dh	3	1.53	0.51	1.63
Error	16	5.00	0.31	
Total	23	499.24		

* Significantly different at 5 % level.

Treatment means of density

d1	d2	d3	d4
14.085	6.25	4.08	2.165

Calculated C.D. value (t 0.05) = 0.68148

Table 18. Analysis of variance in the duration of 90 % survival of oxygen-packed *P. indicus* seed with and without habitat material under different packing densities.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value
d	3	1780.11	593.37	876.35 *
h	1	0.26	0.26	0.39
dh	3	2.28	0.76	1.12
Error	16	10.83	0.68	
Total	23	1793.49		

* Significantly different at 5 % level.

Treatment means of density

d1	d2	d3	d4
26.98	15.17	7.335	4.915

Calculated C.D. value (t 0.05) = 1.00932

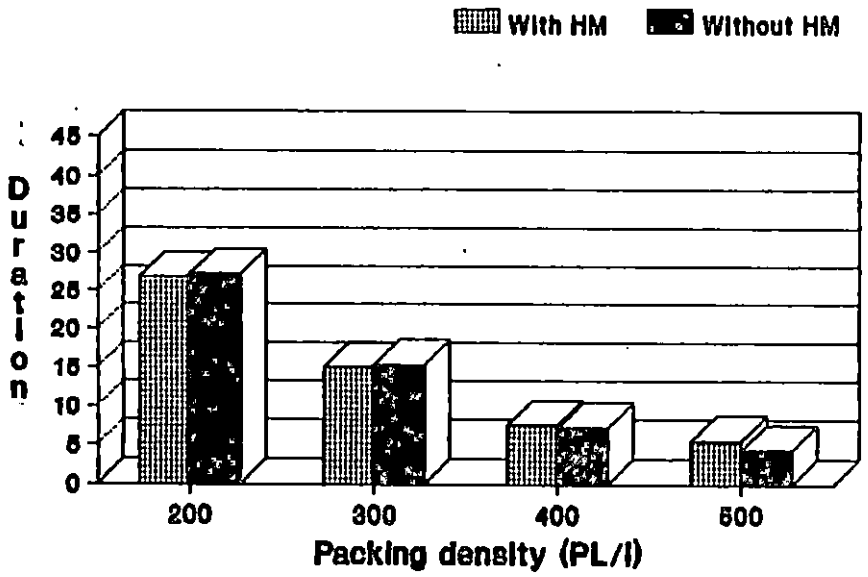


Fig. 26 Duration (h) of 90 % survival of the oxygen-packed *P. indicus* seed, with and without habitat material (HM), under different packing densities.

Similar trend could be observed at 80% survival also (see tables 16 and 19, figure 27). However, the interaction between packing density and habitat material at this level was significant. Pair-wise comparison showed that the interactions d1 h2 (200PL/l - without HM) and d1 h1 (200PL/l - with HM) formed one group and d3 h1 (400PL/l - with HM); d3 h2 (400PL/l - without HM) & d4 h1 (500PL/l - with HM) formed another group.

The duration of 70% survival rate given in table 16, figure 28 showed some difference in the higher packing density combination with the introduction of habitat material. But, the duration of 70% survival at the 200PL/l was not considerably altered with the introduction of habitat material. Analysis of variance (table 20) showed significant difference for packing density and habitat material. Pair-wise comparison of both these showed that the combinations of the levels of packing density and habitat material differed significantly.

4.3.2. Cumulative percentage survival.

The cumulative percentage survival of oxygen-packed *P. indicus* seed with and without habitat material under different packing densities at set duration is summarised in table 21. At 400PL/l and 500PL/l, the duration of 80% and 70% survival rates was comparatively

Table 19. Analysis of variance in the duration of 80 % survival of oxygen-packed P. indicus seed with and without habitat material under different packing densities.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value
d	3	1839.28	613.09	1226.19 *
h	1	0.09	0.09	0.19
dh	3	9.86	3.29	6.58 *
Error	16	8.00	0.50	
Total	23	1857.24		

* Significantly different at 5 % level.

Treatment means of density

d1	d2	d3	d4
33.42	22.335	12.83	11.58

Calculated C.D. value (t 0.05) = 0.86548

Treatment means of DH

1. d1 h2 - 33.5
2. d1 h1 - 33.34
3. d2 h2 - 23
4. d2 h1 - 21.67
5. d3 h1 - 13.33
6. d3 h2 - 12.33
7. d4 h1 - 12.33
8. d4 h2 - 10.83

Calculated C.D. value (t 0.05) = 1.22398

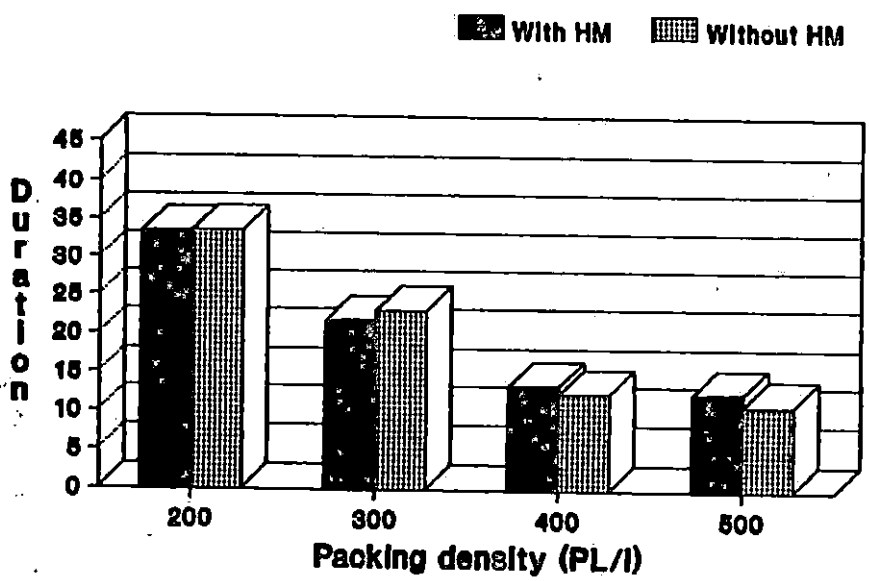


Fig. 27 Duration (h) of 80 % survival of the oxygen-packed *P. indicus* seed, with and without habitat material (HM), under different packing densities.

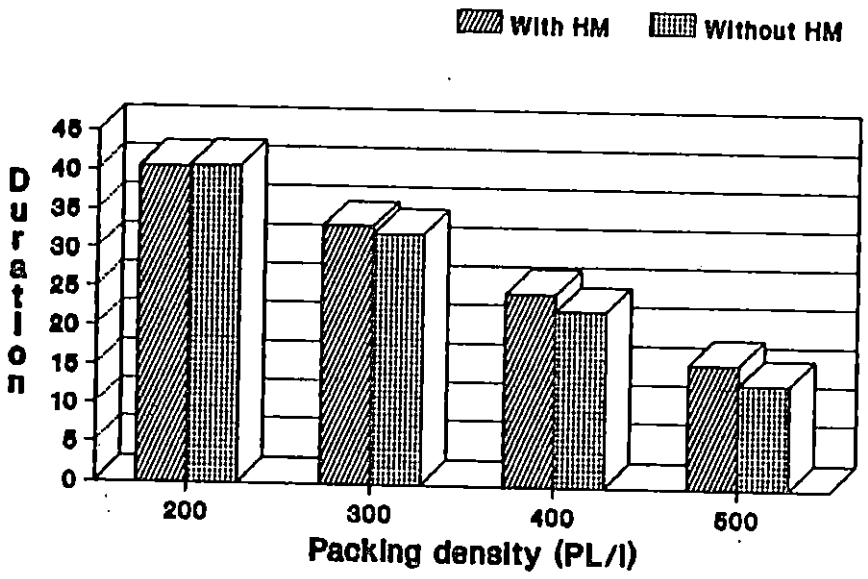


Fig. 28 Duration (h) of 70 % survival of the oxygen-packed *P. indicus* seed, with and without habitat material (HM), under different packing densities.

Table 20. Analysis of variance in the duration of 70 % survival of oxygen-packed *P. indicus* seed with and without habitat material under different packing densities.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value
d	3	2292.00	764.00	940.31 *
h	1	9.38	9.38	11.54 *
dh	3	7.46	2.49	3.06
Error	16	13.00	0.81	
Total	23	2321.83		

* Significantly different at 5 % level.

Treatment means of density

d1	d2	d3	d4
40.58	32.88	23.58	14.58

Calculated C.D. value (t 0.05) = 1.10158

Treatment means of h

h1	h2
28.5375	27.2725

Calculated C.D. value (t 0.05) = 0.77893

Table 21. Cumulative percentage survival of oxygen-packed P. indicus seed with (A) and without (B) habitat material, under different packing densities.

Packing density (PL/l)	Treatment	Duration (h)												
		2	4	6	8	10	12	14	16	22	24	32	34	40
200	A	100	100	100	100	100	100	100	95	90	90	80	75	70
	B	100	100	100	100	100	100	100	95	90	90	85	80	70
300	A	100	100	100	95	95	90	90	85	75	75	70	70	
	B	100	100	100	95	95	90	90	90	80	75	70		
400	A	100	100	95	90	85	80	80	75	75	70			
	B	100	100	95	90	85	80	75	75	70				
500	A	100	95	90	85	85	80	75	70					
	B	100	90	80	80	80	75	70						

* Each value is a mean of triplicates.

higher in the jars with habitat material than in the control.

4.3.3. Dissolved oxygen.

The reduction in dissolved oxygen given in table 22, figure 29 showed a lower oxygen requirement in the oxygen-packed jars with habitat material than in the control. With increase in packing density there was a corresponding increase in dissolved oxygen requirement in most of the cases, irrespective of the treatment.

4.3.4. Ammonia-N.

Increase in the ammonia-N levels in the oxygen-packed jars is given in table 22, figure 30. The ammonia-N level at 70% survival was slightly lower in the jars with habitat material. The ammonia-N levels showed only a slight increase with increase in packing density except at the lowest packing density (200PL/l). The analysis of variance showed significant difference for the packing densities (table 23). Pair-wise comparison by critical difference revealed that all the packing densities, other than 200PL/l formed the same homogenous group.

Table 22. Decrease in oxygen levels and increase in Ammonia-N & free carbon dioxide levels in oxygen-packed jars with (A) and without (B) habitat material, under different packing densities at 70 % survival.

Packing density (PL/l)	Habitat material treatment	Oxygen (ppm)	Ammonia-N (ppm)*	Carbon dioxide (ppm)
200	A	6.46	5.069	51.25
	B	8.66	5.86	49.28
300	A	10.50	10.035	57.16
	B	11.0	10.457	55.19
400	A	9.77	10.276	70.9
	B	14.18	11.08	72.9
500	A	14.8	10.557	61.1
	B	18.12	10.557	63.07

* Each value is a mean of triplicates.

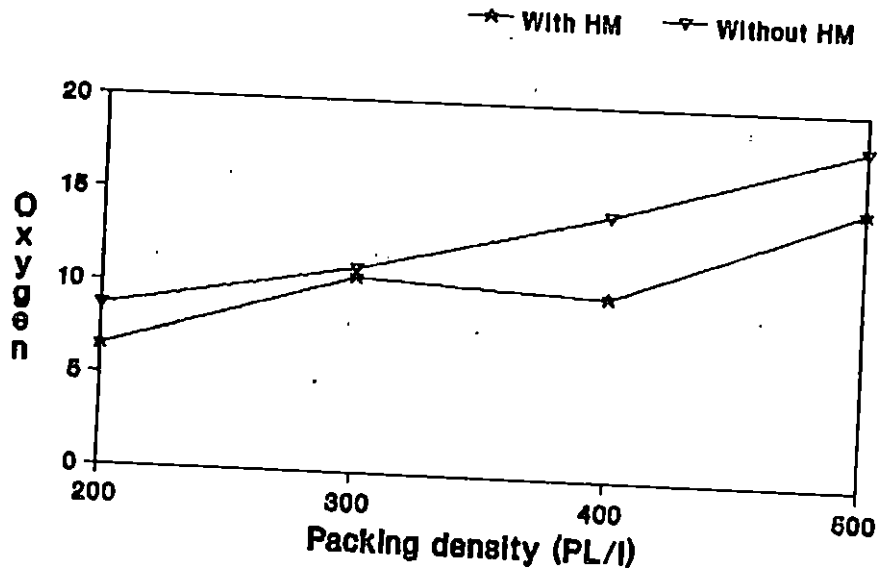


Fig. 29 Decrease in the dissolved oxygen (ppm) levels in the oxygen-packed jars, with and without habitat material (HM) under different packing densities at 70 % survival.

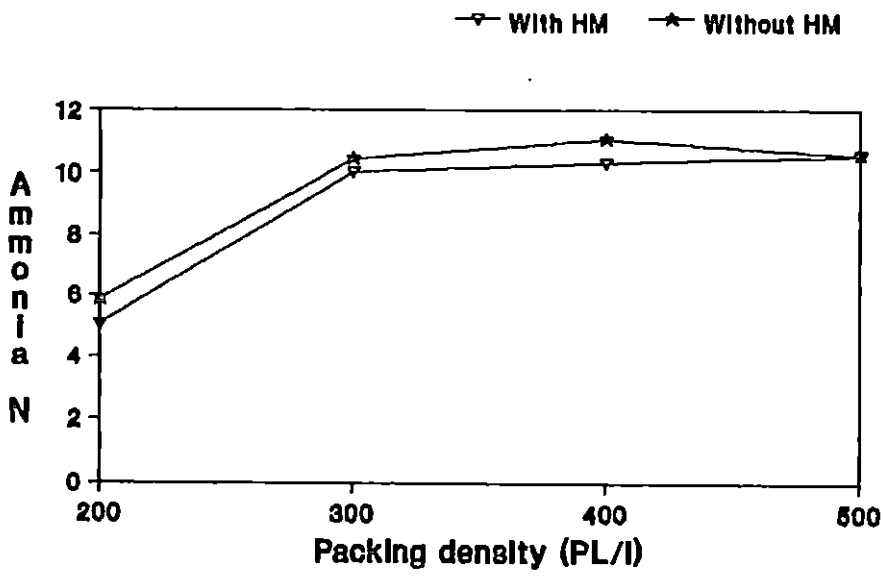


Fig. 30 Increase in the ammonia-N (ppm) levels in the oxygen-packed jars, with and without habitat material (HM), under different packing densities at 70 % survival.

Table 23. Analysis of variance in the increase in ammonia-N levels in the oxygen-packed jars with and without habitat material under different packing densities.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value
d	3	113.64	37.88	19.47 *
h	1	1.26	1.26	0.65
dh	3	0.70	0.23	0.12
Error	16	31.12	1.95	
Total	23	146.72		

* Significantly different at 5 % level.

Treatment means of density

d3	d4	d2	d1
10.688	10.557	10.246	5.4645

Calculated C.D. value (t 0.05) = 1.70919

Underscored means are not significantly different

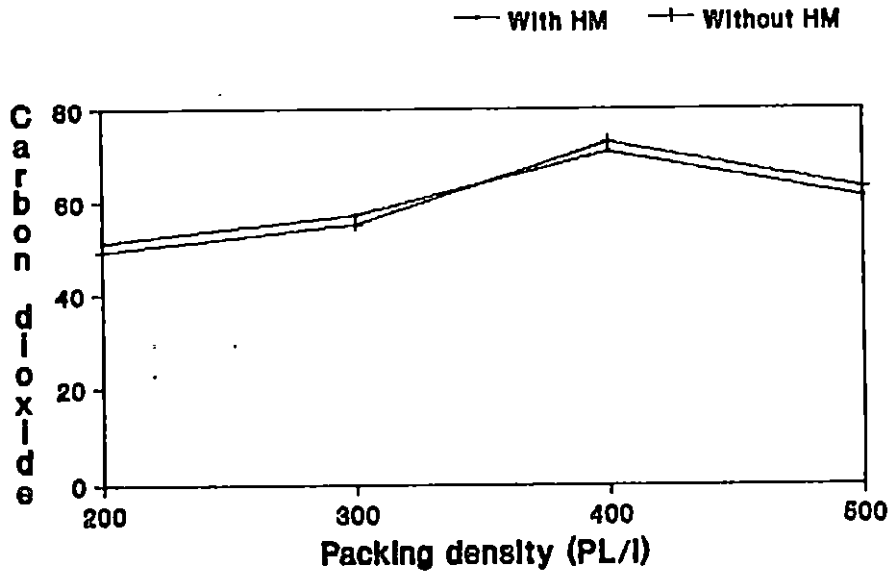


Fig. 31 Increase in the free carbon dioxide (ppm) levels in *the oxygen*-packed jars, with and without habitat material (HM) under different packing densities at 70 % survival.

4.3.5. Carbon dioxide .

Increase in the carbon dioxide levels in the oxygen-packed jars at the end of 70% survival is given in table 22, figure 31. The carbon dioxide levels in the habitat material-treated jars were lower than in those without treatment at the higher packing densities of 400PL/l and 500PL/l. Correspondingly reversed trend was observed at the lower packing densities of 200PL/l and 300PL/l.

4.3.6. pH.

The pH of the transporting medium was lowered from an initial value of 8.0 to 7.0 at the end of 70% survival rate in the various treatment combinations. The change in pH between the treatment and control was similar.

DISCUSSION

5. DISCUSSION

5.1. Experiment I. Oxygen consumption rate by *P. indicus* seed at different packing densities, salinities and temperatures.

A number of factors influence the oxygen consumption rate in the crustaceans. From the practical point of view, the most important purpose of measuring the oxygen uptake rate of a given species of aquatic animals is to determine its ability to take up oxygen under the influence of various external factors including gradations of oxygen itself.

5.1.1. Packing density.

This study was carried out at different packing densities to find out the differences, if any, in oxygen consumption rates under different degrees of crowding, in a closed system, where the dissolved oxygen in the test chamber decreases along with the oxygen consumption of the animals. Pertinent circumstances relating to the test conditions in the study, perhaps have not been reported previously. The increase in the number of shrimp/ unit

volume was found to have decreased the oxygen consumption rate significantly. The rate of oxygen consumption by the shrimp post-larvae decreased to half, with increase in packing density from 200 to 500PL/l. The final ambient oxygen level, after the same interval of time, for the lowest packing density was above 5ppm; whereas this was below 4ppm for the highest packing density. This shows that the falling rate of the ambient oxygen concentration increases with increase in packing density. With the fall in ambient oxygen level due to respiration, there is a corresponding decrease in the rate of oxygen consumption by the shrimp seed. This is in agreement with Wolvekamp and Waterman (1960), Subrahmanyam (1962), Kutty (1969), Bishop et al. (1980), Laxminarayana & Kutty (1982), Martinez et al. (1985) and Liao & Murai (1986) for studies carried out using one or two animals/ respirometer chamber.

Wolvekamp and Waterman (1960) stated that oxygen consumption in Crustacea is constant at higher oxygen levels down to critical pressure characteristic of the specimens and conditions of experiment; below this pressure a rapid linear fall proportional to external amounts of oxygen occurs to very low levels. Subrahmanyam (1962) has shown that P. indicus is an oxygen conformer and that its oxygen consumption rate depends upon the partial pressure of oxygen, even at saturation levels. Thus, as the ambient oxygen concentration in a closed chamber, which is the case

in the present work, decreases by respiration, the shrimps respiratory rate also decreases. Kutty (1969) also reported that metabolic rate in P. indicus declined with decrease in ambient oxygen concentration. Studies on the shrimp P. semisulcatus and in the prawn M. malcomsonii by Laxminarayana and Kutty (1982) and on P. schmitti by Martinez et al. (1985) supported the view that oxygen consumption decreased under hypoxic condition.

With increase in packing density there is decrease in the space availability within the respirometers. This crowding could cause some disturbance to the shrimp. Bishop et al. (1980) observed that the disturbed shrimp consumed oxygen nearly four times faster than those at rest. This increase in the rate of consumption in a closed chamber hastens the fall in ambient oxygen and subsequent oxygen consumption rate. Liao and Murai (1986) reported that oxygen consumption in P. monodon was independent of oxygen concentrations at levels above 4ppm, but was reduced by 7.4 to 11.9%, 22.2 to 35.3% and 63 to 71.4% respectively when dissolved oxygen was 3, 2 and 1ppm. However, according to Egusa (1961) the resting oxygen uptake level increases with decrease in ambient oxygen content, reaching a maximum at a certain low oxygen level, below which the rate falls rapidly. Dallavia (1986) observed a linear increase in oxygen consumption rate with

the number of animals in the respirometer chamber or per bottom area.

5.1.2. Salinity.

Marine organisms show varying rates of oxygen consumption according to their physiological activity and ecological demand. Adaptations to low salinities is highly developed in young penaeids as they are more widely distributed in estuaries than are adults. Since oxygen consumption can be expected to change with the change in demand for various activities (Kutty et al., 1971), one can expect a change in salinity of the surrounding medium reflecting on the oxygen consumption of an animal. There is growing evidence to indicate that the energy spent for osmotic regulation can be precisely judged from the oxygen consumption of an animal (Rao, 1968). With increase in the osmotic difference between the shrimp's haemolymph and its environment, oxygen consumption would be expected to increase for osmoregulators as the metabolism would increase to maintain a constant haemolymph concentration.

The oxygen consumption rates by P. indicus post-larvae at three salinities of 20, 25 and 30ppt in the present study were significantly different. Generally the least oxygen consumption rate was noted at 25ppt at lower temperatures and ambient temperatures. Studies by Kutty et

al. (1971) on P. indicus showed that salinity did not have pronounced effects on oxygen consumption if the experimental animals were acclimatised to the test salinities, which were not too extreme, wherein the 0.1 g individuals acclimatised (over 10 days) to 7, 21, or 31ppt had a metabolism independent of salinity at the concerned acclimatisation salinities. However studies by Unnikrishnan and Laxminarayana (1984) showed that the rate of oxygen consumption increased with the decrease in salinity from 34ppt to 2.1ppt, except in 25.5ppt for P. indicus seed. The present results are also in accordance with the above, information indicating that P. indicus spends least energy in 25ppt salinity.

5.1.3. Temperature.

Oxygen consumption rate is a function of temperature for a number of crustaceans. Higher temperatures promote a higher oxygen consumption. A reduction in metabolic rate is expected from the decrease in temperature alone, but oxygen uptake can itself be interrupted at low temperature by the failure of the oxygen transport pigment in the blood to release its oxygen in the cold (Mauro and Mangum, 1982). Added to these physiological effects, at low temperature, nerve and muscle functions in crustaceans become physically blocked (Blundon, 1982). In

the present study the shrimps were not totally inactive in the respirometer, for ambient and lowered temperature conditions. The respiration rate of shrimp was however significantly reduced with the lowering of temperature from 30 ± 1°C to 23 ± 2°C. The oxygen consumption at ambient temperature was 1.2 to 1.4 times that at the lowered temperature. Kutty et al. (1971) and Kripa and Laxminarayana (1993) observed an increase in rate of consumption of oxygen with increase in temperature for P. indicus. Studies by Bishop et al. (1980) in P. aztecus, Licop (1984) in P. monodon and Liao and Murai (1986) in P. monodon also observed the same pattern for oxygen consumption with variation in temperature.

Interaction of temperature with packing density (dt) and salinity (st) also influenced the rate of oxygen consumption significantly. The effect of lowered temperature became less important and fall in ambient oxygen level appeared to become increasingly important at higher packing densities. The reduction in oxygen consumption at 25ppt salinity at ambient temperature was similar to the lowered oxygen consumption at 30ppt salinity with lowering of temperature. The salinity-temperature interaction was thus found to be more influenced by temperature effect.

5.2. Experiment II. Effects of various factors on the duration and survival of oxygen-packed *P. indicus* seed during transportation.

5.2.1. Safe duration of transport.

The factors under study, ie., packing density, temperature and salinity significantly influenced the safe duration of transport.

Packing density showed an inverse relationship with the survival rate during transportation. This is in agreement with several authors (De and Subrahmanyam, 1975; De, 1977; Mammen, 1978; Selvaraj *et al.*, 1980; Singh *et al.*, 1982; Krishnakumar and Pillai, 1984; Tenedero and Villaluz, 1985; Simon, 1986; Alias and Siraj, 1988; New, 1990; Joshi, 1991; Jayasree-Vadhyar *et al.*, 1992).

The safe duration of transport for *P. indicus* seed was 6.5-8.5 h, 3.2-5 h, 2-2.5 h and 1.25-1.5 h at ambient temperature for 200PL/l(2g/l), 300PL/l(3g/l), 400PL/l(4g/l) and 500PL/l(5g/l) respectively. The range in the safe duration is due to the difference in salinity. When the temperature was lowered to 23 $\pm$ 2°C, this duration was 22-42.5 h, 8.25-9 h, 6.25-6.75 h and 4-4.75 h. This shows that by lowering the temperature from 30 $\pm$ 1°C to 23

± 2°C, the safe duration of transport can be 1.8 to 5 times longer in the latter case depending on the packing density and salinity.

The cumulative percentage survival of the oxygen-packed seed as given in table 11 shows that the longest safe duration was obtained at 200PL/l packing density at the lowered temperature of 23 ± 2°C. For the different salinities, this ranged from 22h to 42.5h. At the remaining packing densities, the data showed more or less similar trend with respect to salinity.

Hamid and Mardjono (1979) obtained 95% survival for *P. merguiensis* seed with oxygen-packing at 28-29°C for 8 h at 200-300PL/l packing density. They found that the density during transportation could be increased considerably if the duration was less than 4 h or if the water temperature was brought down to 22°C. In the present study by lowering the temperature to 23 ± 2°C the safe duration of transport of 200PL/l could be increased to more than 20h in the different salinities. Franklin *et al.* (1982) demonstrated that the mortality during transport of *P. indicus* increased with packing density; 100% survival was obtained when 200 seed/5ℓ were transported for 12 h in polythene bags at a slightly lowered temperature (27.2°C) and at 24.5ppt salinity.

5.2.2. Percentage survival.

Present study of shrimp seed transportation was carried out till the survival rate reached 70% in the oxygen-packed jars. The longest duration of 52-56 h for this survival was obtained at 200PL/l at 23 ± 2°C temperature at 20 and 25ppt salinities.

5.2.2.1. Packing density

Several authors have reported a decrease in percentage survival with increase in packing density for a fixed duration of transport (De and Subrahmanyam, 1975; De, 1977; Chakraborti, 1978; Mammen, 1978; Hamid and Mardjono, 1979; Selvaraj et al., 1980; Singh et al., 1982; Franklin et al. 1982; Krishnakumar and Pillai, 1984; Tenedero and Villaluz, 1985; Simon, 1986; Subrahmanyam, 1986; Alias and Siraj, 1988; New, 1990; Joshi, 1991; Jayasree-Vadhyar et al., 1992). In the present study, 70% survival was reached within 12 h for the highest packing density of 500PL/l, whereas for the same duration 95% survival was observed for the lowest packing density of 200PL/l at ambient temperature of 30 ± 1°C. The major cause of mortality was cannibalism. According to Subrahmanyam (1973) P. monodon in the laboratory containers developed cannibalistic tendencies due to insufficient space. Mammen et al.(1978) obtained a survival of only 53.5% after transport of P.

monodon post-larvae for 18h, which they attributed mainly to cannibalism, especially in higher packing densities. Alikunhi et al. (1980) observed that within 24h, post-larvae which survived in the transport container consumed as much as 150% of its body weight. So they recommended the addition of live food organisms to the packing medium along with shrimp for avoiding cannibalism during transport. In the present study, no feeding was done during transportation, for avoiding pollution of the transport media. For the lower packing densities, however, towards the later period of transportation, the mortality observed was not due to cannibalism. This is in agreement with the findings of Alias and Siraj (1988) for M. rosenbergii post-larvae.

The analysis of variance of the data showed that the packing densities significantly affected the survival of the shrimp seed during transportation. The interaction of packing density with temperatures and salinities also affected the survival of the shrimp seed significantly.

The critical difference analysis of the packing density and salinity (ds) interaction means, revealed that packing density apparently affected the survival more than salinity during the earlier period of transportation.

Towards the later period, the packing density - salinity interaction means differed significantly, showing that the interaction effect of these factors are significant, at this stage. The direct relationship between cannibalism and packing density has more influence on the survival soon after packing, than the interaction effect of density and salinity (ds). During the later period, the post-larvae were quite stressed, reducing their cannibalistic behaviour; at this stage, survival could be a function of other environmental and physiological factors.

The interaction between packing density and temperature (dt) showed significant difference at different percentages of survival. The analysis of data shows that at lowered temperature of 23 $\pm$ 2°C, it is possible to safely transport 300PL/l for the same duration as what is possible with 200PL/l at ambient temperature. Similarly, instead of 300PL/l at ambient temperature, 500PL/l could be transported at lowered temperature with 100% survival for the same duration. Similar trend was shown in the different percentage survival with reduction of temperature. By lowering of temperature, the number of post-larvae per unit volume for transportation can be increased as it enhances the survival. Shigueno (1975) reported a more pronounced cannibalism at higher temperatures. The higher temperature of water in transporting containers has been reported to increase cannibalistic activity of the post-larvae (Hamid &

Mardjono, 1979) because at higher temperatures the shrimps undergo moulting frequently which encourages cannibalism. Further, there is increase in metabolic activity with increase in temperature. Alikunhi (1980) also agreed to this view and reported that at normal temperature when shrimps were crowded without food they tended to become more cannibalistic.

5.2.2.2. Salinity

The main effect of salinity and its interaction with packing density (ds) and temperature (st) were found to affect the different percentages of survival of the oxygen-packed shrimp seed significantly. The critical difference analysis of the means showed that the salinities of the 20 and 25ppt formed the same homogenous group in the majority of the percentage survival rates except for the safe duration (100% survival). These salinity levels showed longest duration of transport, suggesting that salinity between 20 and 25ppt could yield better survival rates in P. indicus seed transportation. However, the salinity effect might have been modified by other factors in the transportation media. The salinity-temperature (st) interaction was also found to influence the survival of the shrimp seed during transportation. This is discussed below.

5.2.2.3. Temperature

Temperature is one of the most important environmental factors which determines the survival of shrimp seed during transportation. Higher the temperature, higher the cannibalistic tendencies, oxygen consumption, ammonia and carbon dioxide excretion. Within the temperature tolerance limits, the metabolic rates of poikilothermic invertebrates increase immediately when transferred from a lower to a higher temperature and reduce immediately in reverse direction (Kutty et al., 1971; Venkataramiah et al., 1974; Catedral et al., 1977; Nelson et al., 1977; Stephenson and Knight, 1980). All these factors together influence the survival rate of shrimp during transportation. So the reduction of temperature yielded higher percentage of survival and longer duration of transport.

The lowering of temperature from ambient levels (30 ± 1°C) to 23 ± 2°C significantly altered the survival of the shrimp seed during transportation. The duration could be increased from two to four times in the various packing densities with lowering of temperature.

The interaction of temperature and salinity was significant. However, the critical difference analysis showed homogenous grouping amongst same level of

temperature showing that the different salinity levels within each group, had similar effect. In general, the temperature seemed to affect the percentage of survival more than salinity, but for 70% survival the salinity-temperature interaction effect was significant at lowered temperature. Towards the later period of transportation, the reduction in cannibalism due to stress may be the cause of the shift from temperature to other factors as the causative factor for survival.

5.2.3. Water Quality Parameters.

The water quality parameters studied were dissolved oxygen, ammonia-N, carbon dioxide and pH at the end of 70% survival.

5.2.3.1. Dissolved oxygen.

Dissolved oxygen is one of the most important water quality parameters that affect the survival of shrimp seed during transportation. The initial dissolved oxygen in the oxygen-packed jars ranged between 29.9 and 31ppm for ambient temperature, at the different salinities. This was slightly higher for the lowered temperature, ie., 33.1ppm.

The final dissolved oxygen values at the end of

70% survival in the various treatment combinations were 22.85-23.95; 18.2-21.43; 17.33-19.7 and 15.76-17.02 ppm at ambient temperatures and 24.8-29.94; 21.43-23.64; 22.77-24.43 and 20.17-24.5ppm at lowered temperatures for 200, 300, 400 and 500PL/l for the different salinities. Usually, in the transport of shrimp under oxygen packing, the dissolved oxygen does not become a limiting factor (De, 1977), unless remarkable mortality and decay of dead shrimps occur in the container (Franklin et al., 1982) or the duration of transport is extended considerably (Krishnakumar and Pillai, 1984).

Chakraborti (1978) determined the minimum dissolved oxygen at which the shrimp seed could survive, as 0.7ppm (for 24 h), but for practical purpose he recommended 1.5ppm to avoid risk. Selvaraj et al. (1980) however, suggested that the concentration of dissolved oxygen should be above 2.5ppm for the healthy survival of shrimp seed. In the present study, the dissolved oxygen levels in the containers were well above these limits, even at the highest packing density of 500PL/l.

The reduction in dissolved oxygen (ppm) or the requirement of dissolved oxygen in the various treatment combinations, was found to vary with temperature, packing density and salinity. The dissolved oxygen requirement generally increased with increase in packing densities.

However, in those packing densities (20ppt - 400PL/l at ambient and lowered temperatures & 30ppt - 500PL/l at lowered temperature) in which the 70% survival rate occurred in a short duration of time, the corresponding oxygen requirement was lowered.

With the lowering of temperature, the oxygen requirement was remarkably reduced in most cases. In general, it was observed that the lowered temperature ensured a higher initial and final oxygen levels than the ambient temperature. As already discussed oxygen consumption rate is a function of temperature for a number of crustaceans and several authors have reported a reduction in oxygen consumption rate with lowering of temperature in crustaceans.

5.2.3.2. Ammonia-N

The packing density, salinity and temperature significantly influenced the increase in ammonia-N levels in the oxygen-packed jars at 70% survival. The ammonia-N levels increased with increase in packing densities. The final values of ammonia-N in the various packing densities increased from the lowest packing density of 200PL/l to 400PL/l. At the highest packing density of 500PL/l the ammonia-N levels were very low. This can be attributed to

the short duration of survival at this density. However, the ammonia-N level of 60.38ppm was recorded at 400PL/l at the end of 19h. Krishnakumar and Pillai (1984) observed a high mortality of P. indicus post-larvae when the ammonia ($NH_3 + NH_4^+$) level exceeded 80ppm. They also observed that when the duration exceeded 24h, the ammonia excretion increased suddenly to a high level at packing densities over 200PL/l. Alias and Siraj (1988) reported that ammonia level in the transport containers increased from 0.1ppm to as high as 63.7ppm ammonia-N at a packing density of 300PL/l, at the end of 36h. Jayasree-Vadhyar et al. (1992) observed ammonia-N values of 72.8ppm at the end of 12h in the transportation of M. rosenbergii post-larvae under oxygen packing at a density of 800PL/l.

The critical difference analysis of the salinity means showed that the increase in ammonia-N for 25ppt and 30ppt salinity combinations was not significantly different. In the higher salinities, the levels of ammonia-N were generally higher.

The ammonia-N levels in the oxygen-packed jars were significantly influenced by temperature. The temperature reduction from 30 $\pm$ 1°C to 23 $\pm$ 2°C, lowered the accumulation of ammonia-N levels by 1.2-17.11 times in the various treatment combinations at 70% survival.

The interaction of packing density, salinity and temperature significantly influenced the rise in ammonia-N levels, statistically. However, it was observed that the effects of the interactions were apparently more influenced by temperature than by the other factors.

5.2.3.3. Carbon dioxide

Increase in the free carbon dioxide levels in oxygen-packed jars at 70% survival showed direct positive relationship with packing density at ambient temperatures. The initial free carbon dioxide values were nil, whereas the final values reached as high as 86 ppm at the highest packing density of 500PL/l. Krishnakumar and Pillai (1984), Alias and Siraj (1988) and Jayasree-Vadhyar *et al.* (1992) reported a direct relation for carbon dioxide with packing density. Krishnakumar and Pillai (1984) attributed the cause of mortality of *P. indicus* seed to low pH due to accumulation of carbon dioxide. Alias and Siraj (1988) recorded carbon dioxide values as high as 67ppm at the end of 36 h at 300PL/l in *M. rosenbergii* transport. Jayasree-Vadhyar *et al.* (1992) recorded a value of 68.5 ppm for the transport of *M. rosenbergii* seed at the end of 12 h.

At ambient temperatures, the increase in free carbon dioxide was only slightly higher than that at lowered temperatures for the lower packing densities of

200PL/l and 300PL/l. In the higher packing densities, there was remarkable difference in free carbon dioxide production, with change in temperature. The lowest final level of free carbon dioxide was observed for the highest packing density of 500PL/l at lowered temperatures (39.4 ppm) for 25 and 30ppt salinities. The reduction in temperature might not have favoured the production of free carbon dioxide. However, the pH was not below 7. As long as the accumulation of free carbon dioxide does not bring down the pH of the water to acidic side, mortality due to accumulation of carbon dioxide may not take place. Further, the high levels of dissolved oxygen help in reducing the harmful effects of carbon dioxide accumulation to some level. At 30ppt salinity combination, the increase in free carbon dioxide levels were the highest among the majority of treatment combinations at 70% survival rate. For the lowered packing densities, the minimum values were observed at 25ppt.

5.2.3.4. pH

pH of the transport medium was reduced from 8 to 7.5-7.0 in the various treatment combinations. A decrease in the pH of the transport medium was reported in all experiments on the transportation of shrimps (Singh et al. 1982, Krishnakumar and Pillai 1984, Alias and Siraj 1988,

Jayasree-Vadhyar *et al.* 1992). Krishnakumar and Pillai (1984) attributed the cause of higher mortality rate in the longer duration transportation of 36 and 48 h at higher packing densities, to reduction in pH due to accumulation of carbon dioxide. Considerable mortality was recorded when pH fell below 6.6. In the present study the pH was not lower than 7.0.

5.3. Experiment III. Effect of habitat material on the duration and survival of oxygen-packed *P. indicus* seed.

The major cause of mortality observed in the experiment II was cannibalism. Hamid and Mardjono (1979) reported that higher temperature of the water in the transporting container, tended to increase cannibalistic activity of shrimp. Alikunhi *et al.* (1980) also agreed to this view and reported that at normal temperature when shrimps were crowded without food, they tended to become more cannibalistic. Franklin *et al.* (1982) and Krishnakumar and Pillai (1984) attributed the cause of low survival of *P. indicus* during long duration transport to cannibalism, as small post-larvae moulted frequently and became prone to cannibalism. Alias and Siraj (1988) and Jayasree-Vadhyar *et al.* (1992) introduced habitat material in the transporting container to reduce cannibalism in *M. rosenbergii* seed transportation.

The introduction of habitat material did not significantly influence the survival rates at 100% and 90%. However, packing density, as discussed in experiment II was found to influence at these two survival rates. The safe duration and duration of 90% survival decreased with increase in packing density.

Analysis of variance of the duration at 80% survival showed that the interaction between packing density and habitat material, significantly influenced the survival rate. However, the critical difference analysis of the interaction means showed the presence of two different groups, ie., the first group formed of 400PL/l with and without habitat material and the second formed of 200PL/l with and without habitat material & 500PL/l with habitat material combinations, showing that at 200PL/l and 400PL/l, the habitat material treatments and control had the same effect.

The introduction of habitat material resulted in significantly better survival of the shrimp seed than without habitat material, at high packing densities of 400 and 500PL/l at 70% survival duration.

The cumulative percentage survival of the post-larvae packed with and without habitat material showed that, by 14h all the post-larvae at 400PL/l in the control

reached 75% survival, whereas 5% more survival was observed in those with habitat material. At 500PL/l with habitat material, 80% survival occurred only at 12h whereas, without habitat material the corresponding duration was only 6h. Further, the 70% survival duration also was extended in the higher packing densities of 400PL/l and 500PL/l with the introduction of habitat material.

In the lower packing densities, due to the availability of sufficient space, cannibalism was less pronounced. This is in accordance with the reports by Subrahmanyam (1973). However, even at the lower packing densities, cannibalism could not be ruled out. It was observed during the early hours of packing, when the post-larvae were seen over-excited and hyperactive, but its intensity was less than that at higher packing densities. But statistical analysis showed that habitat material had little influence on the survival rates at 100% and 90%. The duration of 40h was observed at the lowest packing density both in the control and treatment. At 300PL/l, a similar trend, with slightly lower duration of 32h was observed at 70% survival.

It may be concluded that at the higher packing densities of 400 and 500PL/l, cannibalism cannot be checked with the introduction of habitat material during

early hours of packing. However, during the later period of transportation, the hollow habitat material may form a shelter especially for the over-stressed weaklings thereby increasing the duration at the higher packing densities.

5.3.1. Water quality parameters.

5.3.1.1. Dissolved oxygen

The initial value of dissolved oxygen was 28.3ppm. The final values were 21.19, 17.65, 18.44 and 13.39ppm with habitat material and 19.7, 17.33, 14.18 and 11.82ppm in the control at 200. 300. 400 and 500PL/l packing densities. The corresponding reduction in dissolved oxygen were 6.46, 10.5, 9.77 and 14.8ppm and 8.66, 11, 14.18 and 18.12 ppm with the habitat material and control respectively at 200, 300, 400 and 500PL/l packing densities. A lower final dissolved oxygen level was observed in the control, showing that the introduction of habitat material could reduce the consumption of dissolved oxygen. The final dissolved oxygen values were far above the minimum level recommended by several authors for the species, of 1.5-2.5ppm. Thus, dissolved oxygen levels as such were not found as a limiting factor; with the increase in packing density, there was a proportionate reduction in the final dissolved oxygen.

5.3.1.2. Ammonia-N levels

The ammonia-N levels in the medium were 5.069, 10.035, 10.296 and 10.557ppm in the treatments with habitat materials and 5.86, 10.457, 11.080 and 10.557ppm for the control at 200, 300, 400 and 500PL/l packing densities respectively. With the incorporation of habitat material, the accumulation of ammonia-N levels in the medium was not significantly reduced, eventhough a slight increase in the ammonia-N levels was observed in the control. The packing densities when compared by critical difference analysis revealed that the accumulation of ammonia-N was insignificant at 300, 400 and 500PL/l. But, the lowest packing density of 200PL/l formed a separate group. In this experiment, the final values were well above the limits tolerated by the species.

5.3.1.3. Carbon dioxide.

The carbon dioxide levels reached a value as high as 72.9ppm in the control. The initial carbon dioxide content in the transport medium was nil. The final levels were 51.25, 57.16, 70.9 and 61.1ppm for 200, 300, 400 and 500PL/l packing densities, in the containers with habitat material. In the control, the levels were slightly lower, being 49.28 and 55.19ppm, for 200 and 300PL/l packing densities. But in the higher packing densities the carbon

dioxide levels were higher in the control, ie, 72.9ppm and 63.07ppm for 400PL/l and 500PL/l respectively. This reduction in carbon dioxide accumulation for the higher packing density may be due to the incorporation of habitat material and its utilization by the shrimps. Alias and Siraj (1988) and Jayasree-Vadhyar et al. (1992) noticed significant reduction in carbon dioxide accumulation by the incorporation of habitat material in the packing medium. At 500PL/l, the final levels of carbon dioxide were slightly lower than that at 400PL/l at the end of 70% survival. Carbon dioxide accumulates in the packing medium with time (Johnson, 1979). The duration of 70% survival for the highest packing density was only 16h in the experiment and 14h for the control, whereas this was 24h and 22h respectively at 400PL/l packing density. This shorter duration for the higher packing density might have reduced the carbon dioxide accumulation in the oxygen-packed jars.

5.3.1.4. pH

The pH of the transporting medium was lowered from 8 to 7 at the end of 70% survival irrespective of the treatment. The slight decrease in pH in the present study may be due to dissociation of carbonic acid to release bicarbonate which may have further dissociated to give carbonate and hydrogen ions, thus causing a reduction in pH (Alias and Siraj, 1988).

SUMMARY

SUMMARY

1. The study was aimed at streamlining the conditions for oxygen-packed transportation of Penaeus indicus seed. The experiments conducted were (1) to determine the oxygen consumption by the shrimp seed at different packing densities, temperatures and salinities. (2) to determine the effect of these factors on the duration and survival of shrimp seed under oxygen-packing and, (3) to the study the effect of introducing inert habitat material on their duration and survival of shrimp seed under oxygen-packing.

2. In the first experiment, the effect of packing densities (200, 300, 400 and 500PL/1), salinities (20, 25 and 30ppt) and temperatures (30 ± 1°C and 23 ± 2°C) on the oxygen consumption rate of P. indicus seed was studied in closed type respirometers. It was an asymmetrical factorial experiment, with two replications, in completely randomised design.

3. The second experiment to study the effect of different packing densities (200, 300, 400 and 500PL/1) salinities (20, 25 and 30 ppt) and temperatures (30 ± 1°C and 23 ± 2°C) on the duration and survival of oxygen-packed

P. indicus seed during transportation was conducted in specially designed hard plastic containers of 600ml capacity, fitted with one way valves for packing oxygen under uniform pressure. It was also an assymetrical factorial experiment, in completely randomised design. The experiment was closely observed to record duration of 100%, 90%, 80% and 70% survival. The initial and final water quality parameters were analysed.

4. In the final experiment the effect of introducing inert habitat material in the packing medium on the duration and survival of the oxygen-packed seed was studied in the afore-mentioned containers. It was conducted at ambient temperatures (30 $\pm$ 1°C) and at a salinity of 25ppt selected from the previous two experiments.

5. The energy spent for osmotic regulation could be precisely judged from the oxygen consumption of the shrimp seed. The studies in respirometers showed that the least oxygen consumption rate was at 25ppt salinity in the various treatment combinations, indicating that P. indicus spends least energy at this salinity. The lowering of temperature resulted in reduced oxygen consumption rate.

6. With increase in packing density, there was a decrease in oxygen consumption rate. The rate of oxygen consumption decreased to half, with increase in packing

density from 200 to 500PL/l. The falling rate of ambient oxygen concentration increased with the number of shrimps/unit volume. Crowding increased the rate of oxygen consumption in the respirometers and thus hastened the fall in ambient oxygen resulting in lowered oxygen consumption rate subsequently.

7. Packing density showed inverse relationship with the safe duration of transport with 100% survival, and also with subsequent durations (90, 80 and 70% survival). The study showed that at packing densities of 200, 300, 400 and 500PL/l, the post-larvae could be transported with 100% survival upto 6.5-8.5h, 3.2-5h, 2-2.5h and 1.25-1.5h at ambient temperature of 30 $\pm$ 1°C respectively. The percentage survival at the end of 12h, was reduced to 70%, at 500PL/l whereas, this was 95% at 200PL/l, at ambient temperature. Analysis of variance showed that packing density significantly affected the percentage of survival. The major cause of mortality with increase in packing density was cannibalism.

8. The lowering of temperature from the ambient levels of 30 $\pm$ 1°C to 23 $\pm$ 2°C significantly increased the safe duration and subsequent durations of transport. The safe duration of transport with 100% survival was increased to 22-42.5h, 8.25-9h, 6.25-6.75h and 4-4.75h for packing

densities of 200, 300, 400 and 500PL/l respectively at the lowered temperatures. The percentage survival at 12h, was 80-85% for the highest packing density of 500PL/l and 100% for 200PL/l at the lowered temperatures. By lowering the temperature it was possible to transport 200PL/l over 20h, with 100% survival, whereas the same could be transported only for 6-8h at ambient temperatures. The lowering of temperature reduced cannibalism and physical activity of the oxygen-packed seed.

9. Among the salinity levels of 20, 25 and 30ppt, the longest duration of transport was observed for the former two salinities in the majority of the survival rates. The statistical analysis showed no significant difference between 20 and 25ppt levels. So a salinity range of 20-25ppt apparently yields better survival rates in P. indicus seed transportation.

10. The study showed that in addition to the main effects, the interaction effects of the factors analysed had significant effect on the survival of the shrimp seed. The interaction effect of lowered temperature with packing density and/or salinity reduced the negative effect of the latter two factors.

11. The reduction in dissolved oxygen levels in the oxygen-packed jars was well above the lethal limits

even at the highest packing density of 500PL/1 at 70% survival. The decrease in dissolved oxygen levels, increase in ammonia-N & free carbon dioxide and change in pH, differed with the duration of packing, temperature and packing density.

12. The incorporation of habitat material did not significantly alter the safe duration of transport in the various packing densities of 200, 300, 400 and 500PL/1. However, this incorporation resulted in longer duration at the later period of transportation (at 70% survival) only at the higher packing densities of 400 and 500PL/1.

13. The reduction in dissolved oxygen levels was slightly higher in the jars without habitat material. The increase in ammonia-N was not significantly reduced with the incorporation of habitat material. The accumulation of free carbon dioxide was slightly lowered with the introduction of habitat material at 400PL/1 and 500PL/1.

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7. REFERENCES

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ABSTRACT

The effect of four levels of packing density, (200PL/l, 300PL/l, 400PL/l and 500PL/l), three levels of salinity (20ppt, 25ppt and 30ppt) and two levels of temperature (30 ± 1°C and 23 ± 2°C) on the oxygen consumption rate of Penaeus indicus post-larvae (PL_{20}) was investigated in a closed type respirometer. The dissolved oxygen in the test chamber decreased with the oxygen consumption of the animals. Oxygen consumption was found to vary with ambient oxygen levels at the different packing densities. Among the three salinities, and two temperatures, the lowest rate of oxygen consumption was recorded at 25ppt and 23 ± 2°C.

The effect of these factors on the duration and survival of transportation of the shrimp seed in specially designed hard plastic containers fitted with facilities for oxygen packing under uniform pressure (0.2 Kg/cm^2) showed that oxygen packing in hypothermal conditions could help in increasing duration and survival. Salinity of 20-25ppt was found to give longer duration of survival. With increase in packing density, there was considerable reduction in the duration and survival of transportation of the seed.

Cannibalism was observed as the major cause of

mortality and it could be reduced by lowering of temperature. 200PL/l could be transported with 100% survival within 6.5-8.5h at ambient temperature of 30 ± 1°C under the afore-mentioned type of oxygen-packing. By lowering the temperature to 23 ± 2°C it was possible to safely transport with 100% survival the same numbers for more than 20h. Corresponding duration at 500PL/l with 100% survival was 1-1.5h at ambient temperature and 4-5h at lowered temperature.

To reduce cannibalism at ambient temperatures, hollow plastic transluscent habitat material was incorporated into the oxygen-packed jars. This experiment was conducted at 25ppt salinity at different packing densities of 200PL/l, 300PL/l, 400PL/l and 500PL/l. Relatively longer duration and higher survival was observed only at higher packing densities of 400PL/l and 500PL/l with the introduction of the habitat material.

Water quality parameters in the experimental jars were analysed initially and finally at 70% survival rate. The reduction in dissolved oxygen levels in the oxygen-packed jars was well above the lethal limits even at the highest packing density of 500PL/l at 70% survival. The decrease in dissolved oxygen levels and increase in ammonia-N and free carbon dioxide, differed with the duration of packing, temperature and packing density.