EFFECT OF SEDATIVES ON *PENAEUS MONODON* FABRICIUS SEED UNDER OXYGEN PACKING FOR TRANSPORTATION

By
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THESIS

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To

My Parents

DECLARATION

I hereby declare that this thesis entitled "EFFECT OF SEDATIVES ON PENAEUS MONODON FABRICIUS SEED UNDER OXYGEN PACKING FOR TRANSPORTATION" 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.

Panangad,

1st November 1991.

JOSHI. K.

CERTIFICATE

Certified that this thesis, entitled "EFFECT OF SEDATIVES ON PENAEUS MONODON FABRICIUS SEED UNDER OXYGEN PACKING FOR TRANSPORTATION" is a record of research work done independently by Sri. Joshi, K. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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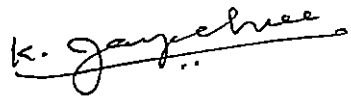
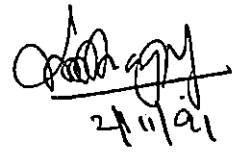
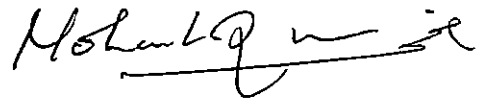
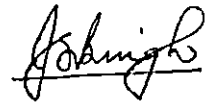
1. Dr. K. Jayasree Vadhyar , Major Advisor Associate Professor (Aquaculture)	
2. Dr. D.M. Thampy , Member Professor and Head, Department of Aquaculture	
3. Mr. C. Mohanakumaran Nair , Member Assistant Professor (Fish Breeding)	
4. Dr. I.S. Bright Singh , Member Lecturer Department of Environmental Studies, Cochin University of Science and Technology. (Formerly Assistant Professor, Microbiology, College of Fisheries, Panangad).	

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INTRODUCTION

1. INTRODUCTION

Amongst the aquatic organisms cultured at present, crustaceans, especially prawns form the most important group. On a global basis, the commercial culture of prawns has been boosted up in the past two decades by the ever increasing market demand, particularly the export market. With the adoption of scientific management and development of high conversion feeds, the south east Asian countries have already revolutionised the prawn production from aquaculture. The prawn producing countries are now competing with one another to cope with the world market for prawns to supplement their foreign exchange earnings. Subsequent to this revolutionised production, India's reputation as the highest prawn producing and exporting country till 1987 has gone down (Ferdouse, 1990). Nevertheless, prawns form the lions share of her marine product export.

Although India has an estimated total potential area of 2.6 million hectares (Silas, 1980) only about 58,430 hectares are utilized for brackishwater shrimp culture (Ferdouse, 1990), with the result that she still depends primarily on marine capture production to meet the domestic as well as export markets. Nevertheless, innovative ventures are underway in India by governmental as well as private agencies to enhance prawn production through aquaculture.

Of all the prawns commercially cultivated, the tiger prawn, Penaeus monodon Fabricius occupies the top rank in terms of production, forming 33% of the total world shrimp production from farming (Anon, 1989). By virtue of its fast growth rate (Subrahmanyam, 1973), largest size (Muthu et al., 1982), ability to tolerate wide ranges of salinity (Pantastico, 1979; Reddi et al., 1984; Singh, 1989), ability to mature in captivity and successful hatchery production of seed (Alikunhi et al., 1975; Muthu et al., 1982; Hajra et al., 1988), suitability for intensive rearing operations (Alava and Lim, 1983) and acceptance of artificial feed (Lee, 1971; Forster, 1972; Aquacop, 1977), P. monodon has gained the status of the most preferred of all the brackishwater prawns cultured presently.

In India P. monodon is available in plenty in the estuaries and lakes on the east coast, particularly in the northern parts (Alikunhi, 1980). It is scarce along the other parts of India. With the commencement of the commercial scale prawn farming in India recently, the long distance transport of prawn seed - especially the 'farmers' favourite' P. monodon - either from the natural collection centres or from the hatcheries to the farm sites has become a necessity. Though the prawn production industry has revolutionised a great deal, the present day knowledge on the seed transportation is scant.

Although considerable work has been conducted on the transport of fish seed, only very little has been done on the transport of prawn seed, as is apparent from the review of literature. Transport of live fish is a century old practice. Traditionally, fish seed were

transported in open containers such as hundies in India (Jhingran, 1975), galvenised iron cans in Taiwan (Chen, 1976), Waluh in Indonesia (Korringa, 1976) and palayok in the Philippines (Smith, 1981). Later, the transporting carriers passed through several modifications such as open or closed metal containers with facilities for aeration or filled with oxygen. The present day carriers range from the simplest polythene bags of convenient sizes filled with oxygen to much sophisticated vehicle-hauled carriers with cooling and aeration units. The polythene bags still remain the easiest and cheapest mode of transporting live fishes and prawns.

Simultaneous with these developments of the transporting carriers, research on the maintenance of quality of the packing medium during transport also progressed, thereby increasing the survival. In this direction, the application of buffers for maintaining pH (McFarland and Norris, 1958), absorbents for absorbing gases such as ammonia and carbon dioxide (Jorgenson et al., 1976, Teo et al., 1989), antibiotics and antiseptics to control the growth of microbes (Johnson, 1979) and anaesthetics for keeping the fish less active (Aitken, 1936; Nemoto, 1957; McFarland, 1960) in the packing medium were studied. With all these developments, the transport of live fishes has become almost risk-free. Eventhough it seems possible to make use of these developments in the transport of prawn also, little attempt has so far been made to check the suitability of their applications. Over and above, the prawn seed transport faces another problem of cannibalism. Cannibalism in prawn is particularly severe during the younger stages (Mammen et al., 1978).

The prawn farmers in locations far away from a hatchery or seed collection centre still encounter difficulty in bringing prawn seed safely for farming. They often meet with high percentage of mortality which may be due to various factors. This entails understocking of their farms resulting in low production and low financial output. In order to overcome this possible loss, the farmers usually buy more seed than necessary from hatcheries, presuming a percentage of mortality during transport, which they derive from their previous experiences. This practice by the prawn farmers, adversely affects the economics of prawn production because of increase in the production cost. It also creates a dearth of prawn seed for farming. Undoubtedly, transport of the seed with good survival will not only economise the culture operation but also ensure sufficient supply of the seed to more number of farmers, which in turn will encourage the prawn production and export.

In this context, the present study has been taken up to determine the effect of using suitable sedatives during transportation of P. monodon seed in oxygen packed containers, on the survival and duration of transport. The study is also aimed at finding out the effect of salinity, temperature and packing density on the survival and duration of transport of sedated and non-sedated P. monodon seed under oxygen packing.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Methods of transport of prawn seed

The transport of live aquatic organisms, perhaps started in 1870's (Norris et al., 1960). Later, when man developed interest in commercial farming of aquatic organisms, several methods of transport were developed, particularly for fishes. The same methods could be applied to other aquatic organisms including prawns. The traditional mode of transport was in open earthen and metal containers (Jhingran, 1975; Chen, 1976).

2.1.1. Open system

A scientific approach to the transport of live prawns in open containers was later developed by several scientists. Mohanty and Patra (1972) conducted an experiment on the transport of post-larvae/juvenile prawns (penaeids) in open containers for a nine hour journey by boat. They used open glass jars and cool trays. Shigueno (1975) reported the use of tanks loaded on trucks with facilities for aeration for the transport of prawn. Mammen et al., (1978) used milk can type containers for transporting Penaeus monodon seed which resulted in mortality of over 50%. Varghese (1978), while transporting P. monodon fry in open containers for a short distance, provided plastic strips overgrown with periphyton in the containers for the fry to graze upon, which resulted in better survival. At-Atlar and Kenow (1979) described a fibre glass tank of 2 m^3, which could be loaded on a truck for the transport of juveniles of P. semisulcatus.

Alikunhi et al. (1980) recommended open containers only for a duration not exceeding 4 hours for the transport of prawn seed. They tried successfully 300 litre capacity plastic pools and 200 litre capacity barrels for short distance transport. Selvaraj et al. (1980) suggested earthen pots as the best containers for long duration holding (more than 4 days) as they maintained cool temperature. Rao et al. (1986) transported P. monodon fry (18-26 mm) in clean open aluminium vessels at 1.5 ppt salinity for a period of 3 hours by road and obtained survival of 10% at a low packing density of 54 fry/l. Simon (1986) recommended open containers for transport involving short distances. Kungvankij et al. (1986) reported the use of plastic, fibre glass and canvas tanks for the transport of nauplii and post-larvae of prawns.

2.1.2. Closed system

Plastic bags as transporting containers for fishes have been in vogue since the second world war (See Martin, 1980). These have been widely used for the transport of prawns. Liao and Huang (1972) used plastic bags with 10-15 litres of sea water and inflated with oxygen for the transport of fry of P. japonicus. Plastic bags packed with 8 litres of water and 4 litres of oxygen, encased in temperature controlled cartons are used in Japan for transporting 6000 post-larvae of P.japonicus over 12 hour duration (Shigueno, 1972). De and Subrahmanyam (1975) conducted experiments on the transport of P.monodon in plastic bags of 4-5 litre capacity, with oxygen packing. They could transport 500 post-larvae (10-14 mm) with 87% survival for

a duration of 36 hours. In plastic bags of 3 litre capacity filled with 1 litre of water and oxygen under pressure, De (1977) could transport P.indicus fry with 85% survival for 20 hours with 150 seed/l. Dwivedi (1978) reported high survival of P.monodon seed when transported in insulated boxes. Hamid and Mardjono (1979), who transported P.monodon and P.merguiensis fry in polythene bags, found that bags with more than 10 litres of water damaged easily during transport. Also, low survival was obtained when a large number of shrimp fry were placed in one bag even with same density. So they recommended the use of small plastic bags for transport. Alikunhi et al. (1980) conducted a series of experiments on the transport of nauplii and post-larvae of P.indicus and P.monodon in plastic bags. They found that survival could be increased by providing live food organisms such as Artemia and Moina to the packing medium, thereby reducing cannibalism. Silas (1980) air lifted prawn seed in oxygen filled polythene bags for a period of 24 hours without considerable mortality. Selvaraj et al. (1980) conducted experiments on transport of P.indicus seed in special type of transparent polythene seed transport jars of 14 litre capacity, in which 10 litres of water and 4 litres of oxygen were filled in for transporting 500 P.indicus seed. The bags were transported over 700 Km in 4 days with reoxygenation in every 24 hours with a survival of 85%. Franklin et al. (1982) packed seed (10-20 mm) of P.indicus, P.monodon and P.semisulcatus in polythene bags with 5 litres of oxygenated water and inflated with oxygen. They could get above 90% survival during transit involving 12 hours. Singh et al. (1982) compared knotted and knotless polythene bags for the transport of

P.monodon seed. They found that bags with folds or knots were not suitable for transporting prawn seed. Krishnakumar and Pillai (1984) in their experiments on transport of P.indicus seed used flexible polythene bags with oxygen and obtained a survival of 70% with 250 seed/l. Liao (1984) reported on the transport of prawn seed in polythene or PVC bags inflated with oxygen, placed in styrofoam boxes, in the Philippines. Tenedero and Villaluz (1985) described in their manual, the procedures and techniques to be followed for short and long duration transport of prawn fry. 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 weeds or plastic strips and worms to the medium as shelter and feed respectively during long distance transport. Simon (1986) suggested the use of thick guage polythene bags of 18 litre capacity, with 5-6 litres of water and the rest oxygen for long distance transport. Alias and Siraj (1988) found that the use of habitat materials in the polythene bags during transport of M.rosenbergii seed increased survival rate by reducing cannibalism.

Alikunhi et al. (1980) reported the use of 35 litre capacity plastic jerry cans, with 17 litres of water and the rest oxygen for transport of prawn seed. They used these containers for P.indicus, P.monodon and P.semisulcatus seed and obtained good survival at ambient temperature. Selvaraj et al. (1980) also used jerry cans and found that they were suitable for keeping seed in healthy condition for long distances. Singholka (1982) devised a simple cool 'walk-in' truck with shelves for holding fish or prawn contained in inflated

plastic bags. The truck which was refrigerated using ice cubes kept the temperature low. He claimed that the device was an efficient means of transport for prawns under tropical conditions and over long road distances. Jayasree-Vadhyar et al.(1990) used reusable hard plastic jars for transport of M.rosenbergii seed.

2.2. Effect of sedatives during transportation

After the discovery of the potential use of sedating chemicals for transporting fishes by Aitken (1936), hitherto an array of these chemicals have been tried for enhancing survival of fry and adult fishes during transportation. These include thiouracil (Osborn, 1951; Sreenivasan, 1962), chloretone (Burrows, 1952), sodium amytal (Calhoun, 1953; Reese, 1953; Philips and Brockway, 1954; Saha et al., 1955; Nemoto, 1957; McFarland, 1960; Sreenivasan, 1962; Durve and Dharmaraja, 1966; Durve, 1975), MS-222 (Webb, 1958; Thompson, 1959; Martin and Scot, 1959; McFarland, 1960; Eisler and Backiel, 1960; Blahm, 1961; Meehan and Revet, 1962; Sreenivasan, 1962; Collins and Hulse, 1964; Durve, 1970; Vijayagupta and Sharma, 1974; Dick, 1975; Murai et al., 1979; Alverez-Lajonchere and Garcia-Moreno, 1982; Guest and Prentice, 1982; Dupree and Huner, 1984; Rothbard, 1988), quinaldine (Natarajan and Ranganathan, 1960; Sreenivasan, 1962; Durve, 1970; Guest and Prentice, 1982; Sado, 1985; Onyango, 1985), chloral hydrate (McFaraland, 1960; Sreenivasan, 1962; Durve and Dharmaraja, 1966; Durve, 1975; De et al., 1986; Rothbard, 1988), tertiary butyl alcohol (McFarland, 1960; Durve and Dharmaraja, 1966; Durve 1975; Alverez-Lajonchere and Garcia-Moreno, 1982; Rothbard, 1988; Mary, 1991),

tertiary amyl alcohol (McFarland, 1960; Durve, 1970; Taylor and Solomon, 1979; Alverez-Lajonchere and Garcia-Moreno, 1982; Dupree and Huner, 1984), methyl parafynol (McFarland, 1960; Rothbard, 1988), urethane (McFarland, 1960; Sreenivasan, 1962; Durve and Dharmaraja, 1966; Durve, 1975), chlorobutanol (McFarland, 1960; Durve and Dharmaraja, 1966, Durve, 1975; Hansen and Jonsson, 1988), tribromoethanol (McFarland, 1960), veronol, thiourea, methyl thiouracil, hydroxy quinaldine (Sreenivasan, 1962), sodium barbital (Durve and Dharmaraja, 1966; Durve, 1975), novocaine, barbital sodium, amorbital sodium (Kewalramani and Gogate, 1968), paraldehyde (Durve, 1970), pentobarbital sodium, phenobarbital sodium, ether (Durve, 1970), sodium bicarbonate and carbonic acid (Fish, 1943; Booke et al., 1978; Post, 1979; Mishra et al., 1983; Takeda and Hazava, 1983; Dupree and Huner, 1984; Yokoyama et al., 1989), benzocaine (Taylor and Solomon, 1979; Rothbard, 1988), etomidate, imidazole carboxylate (Guest and Prentice, 1982), propandid (Siwicki, 1984; Jeney et al., 1986), benzocaine hydrochloride (Ferriera et al., 1984), methyl pentynol (Dupree and Huner, 1984) and sodium thiopentone (Das et al., 1990).

However, only very little information on the use of sedatives on prawn during transportation has been documented hitherto. Singh et al. (1982) successfully used chloral hydrate during transportation of P.monodon seed. They tried a concentration of 400 ppm and achieved 15.6% higher survival in sedated group of prawn than in non-sedated group at a high packing density of 500/l. Obradovic (1986) tried two sedatives, namely MS-222 and halothane in freshwater cray fish Astacus astacus (not for the purpose of transportation) and found that MS-222

had no effect on cray fish. However, halothane, which is dispersed in air, was found to be effective at a concentration of 0.5 vol %. Venkataswamy et al. (1990) tried quinaldine, MS-222, and Phenoxy ethanol in Macrobrachium malcolmsonii brood stock during transportation. Chitra (1990) observed that by applying MS-222 at a concentration of 150 ppm, the metabolic rate of P.indicus seed could be reduced, but she had not studied its effect under transport conditions.

2.3. Effect of packing density of prawns in transporting containers

Packing density is very important in determining the survival of prawn during transport. Several workers demonstrated that under uniform water quality conditions, the percentage of survival varies with packing density. De and Subrahmanyam (1975) during transportation of P.monodon seed used packing densities ranging from 50 to 3000 seed/l. They concluded that 500 seed/l could be transported for a duration of 36 hours with 87% survival whereas, at higher density of 2500/l the seed could be transported only for 12 hours with similar survival (86%). De (1977) obtained a survival of 85% in P.indicus seed at 20 hours of transport, packed at a density of 150/l. But for the same survival with a density of 200/l, the duration had to be reduced to 12 hours. When the density was 750/l, the mortality was 67.8% at 12 hours. Chakraborti (1978) suggested that under normal conditions of temperature and dissolved oxygen (without oxygen packing) 180 prawn seed/l could be transported for 5 hours without any mortality, but that at increased packing density of 900 seed/l, they could be transported only for 1 hour. Mammen et al. (1978) could obtain 96% survival in P.monodon seed, when the packing density was 250/tin, whereas the

mortality was around 50% when the packing density ranged from 1500 to 2000/tin. Hamid and Mardjono (1979) in their experiments on transportation of P.merguiensis and P.monodon found that the density during transportation could be increased considerably with high survival if the duration of transport was short ($\lesssim$ 4 hours). Moreover, they observed that size of shrimp also affected the survival rate and packing density, and it was preferable to transport smaller fry of uniform size. Alikunhi et al. (1980) also reported that in transports involving short distances high packing densities could be used. Selvaraj et al. (1980) remarked that maintenance of optimum number of seed in the transporting containers was very essential because mortality rate increased as the number exceeded the optimum level. They also emphasised that the density be regulated according to the size of the seed, distance to be travelled and duration of transportation. They could transport seed at a density of 100/l with continuous oxygenation for 24 hours without any mortality, but mortalities of 3%, 15%, 75% and 100% were observed at 36, 48, 60 and 72 hours of the experiment respectively. Franklin et al. (1982) demonstrated that the mortality during transport increased with packing density. They got 100% survival, when 200 seed/l were transported for 12 hours in polythene bags of 20 litre capacity with 5 litres of water, whereas mortalities of 3% and 10% respectively were observed for packing densities of 250 and 500 seed/l for the same duration. Singh et al. (1982) observed during the transportation of P.monodon that at packing density of 250/l negligible mortality was observed till 18 hours, but at densities of 375/l and 500/l, mortality started after 12 and 10 hours respectively. Krishnakumar and Pillai (1984)

transported P.indicus seed with 70% survival for 24 hours under oxygen packing at a density of 250 PL (20 days old) per litre. When the duration was increased to 36 and 48 hours, 70% survival was obtained with 100 PL/l. They also reported the relationship between the size of prawn and packing density. When 8 day old post-larvae were used, the same survival (70%) could be obtained at packing densities of 300 and 150 PL/l for 24 and 36 hours respectively. Kungvankij et al. (1986) also established the importance of size and stage of prawn seed on the packing density. They reported that in polythene bags with 6-8 litres of water 200,000 nauplii could be transported with 80-90% survival for 4-6 hours, whereas only 3000-5000 post-larvae could be transported in the same bag. Subrahmanyam (1986) recommended a packing density of 50 seed/l for long distance transport of M.rosenbergii. At the same time for short distance involving 3-4 hours, he recommended a density of 150-800/l, even without oxygen packing. For penaeid seed transportation in plastic bags of 18 litre capacity, with 5-6 litres of water and the rest oxygen, Simon (1986) suggested a packing density of 15,000/bag for 12 hours and 10,000/bag for 48 hours, when the size of the seed was 8-10 mm. At a size of 18-20 mm, only 3000 and 500 could be packed for 12 and 48 hours of transport respectively. Alias and Siraj (1988) observed that when M.rosenbergii seed packed at 100, 200 and 300 PL/l, the density 300/l showed significantly better survival at 12 hours. However, at 24 and 36 hours, 100/l showed significantly higher survival. Jayasree-Vadhyar et al. (1990) packed M.rosenbergii seed at various densities such as 100/l, 200/l, 250/l, 300/l, 400/l and 800/l and observed that the safe duration of survival (time of initial mortality) varied as 81,35,12,12,6 and 4 hours respectively.

2.4. Water quality parameters important during transportation

2.4.1. Oxygen

2.4.1.1. Dissolved oxygen in transporting containers

Dissolved oxygen is one of the most important water quality parameters influencing the survival of prawn during transport. However, during transports under sufficient oxygen packing, usually oxygen is not depleted to the lethal levels (De, 1977). Hamid and Mardjono (1979) observed an oxygen concentration of above 11 ppm in the packing medium after 12 hours of transport even in bags packed with a high density of 400 seed/l. Singh et al. (1982) measured final oxygen concentration of 6.2, 2.7 and 3.0 ppm in bags with packing densities of 250, 375 and 500/l respectively. But, Franklin et al. (1982) noticed the dissolved oxygen concentration as low as 0.6-0.9 ppm after 12 hours of transport, which they attributed to the putrifaction of dead prawns. However, Krishnakumar and Pillai (1984) recorded the depletion of oxygen in containers with packing densities of above 200/l, when the duration of transport was longer (36 to 48 hours). They observed till total mortality occurred in long duration transport, and made clear that the mortality of post-larvae was not due to the depletion of oxygen.

2.4.1.2. Minimum level of oxygen tolerance

The minimum level of oxygen required for the survival of prawns

has been studied by several workers. Mackay (1974) observed in his experiments with Penaeus schmittii that when the dissolved oxygen was as low as 0.9 ± 1 ppm, all shrimps went into a state of lethargy, without any reflex. De and Subrahmanyam (1975) reported that P.monodon seed could survive in oxygen concentration as low as 0.6 ml/l. Chakraborti (1978) studied the oxygen consumption in P.monodon and P.indicus and found that the minimum oxygen for survival was 0.7 ppm in both species, but a concentration of 1.5 ppm oxygen was the level at which the respiration of prawn was normal, for all practical purposes. He also observed that the rate of oxygen consumption in both these species decreased with increase in time. Selvaraj et al. (1980) remarked that P.indicus seed could hardly survive in an oxygen concentration of 0.2 ml/l and that the level of dissolved oxygen for the survival of prawn seed in healthy condition was above 2.5 ml/l.

2.4.1.3. Oxygen consumption in relation to body weight

Several workers have established a relation between oxygen consumption and weight of prawn (Rao, 1958; Subrahmanyam, 1962; Kramer, 1975; Nelson et al., 1977; Stephenson and Knight, 1980; Liao and Murai, 1986; Scelzo and Zunigo, 1987). Kramer (1975) observed that sub-adult brown shrimp (P.aztecus) died at a higher mean dissolved oxygen concentration than juveniles, indicating that size might have had a positive and direct relation to the lethal dissolved oxygen levels. Studies on M.rosenbergii juveniles by Nelson et al. (1977) established a slight negative correlation between metabolic rate and

weight. But, Stephenson and Knight (1980) after their experiments with M.rosenbergii post-larvae stated that the oxygen consumption increased with weight of prawn. Licop (1984) noticed that in P.monodon post-larvae the relationship between rate of oxygen consumption and body weight was nearly linear. Studies on shrimp P.brasiliensis (Scelzo and Zunigo, 1987) also supported the view that oxygen consumption varied directly with weight.

2.4.1.4. Oxygen consumption in relation to its availability

The oxygen consumption varies with the concentration of oxygen available. Kutty (1969) reported that the metabolic rate in P.indicus declined with decrease in ambient oxygen concentration. Studies on the prawns P.semisulcatus and M.malcolmsonii and crab Paratelphusa hydrodromus by Laxminarayana and Kutty (1982) and on Penaeus schmittii by Martinez et al. (1985) supported the view that oxygen consumption decreased under hypoxic conditions. However, Liao and Murai (1986) reported that oxygen consumption in P.monodon was independent of oxygen concentration, at levels above 4 ppm, but it decreased significantly at lower levels. Taylor and Spicer (1987) also observed that oxygen consumption in Palaemon elegans, P.longirostris and P.serratus was independent of oxygen levels of a wide range.

2.4.1.5. Oxygen consumption in relation to starvation

Starvation has been reported to reduce oxygen consumption, by Kutty (1969). He observed that the oxygen consumption in Penaeus indicus and P.semisulcatus declined sharply (32% for P.indicus and 57%

for P.semisulcatus) by the second day of starvation and remained more or less constant during the subsequent days. He reported that two days-starved P.indicus and P.semisulcatus could be expected to survive for 1.7 and 2.3 times longer respectively than those not starved. Dall and Smith (1986) reported that in P.esculentus, oxygen consumption declined sharply by 24-29% during 5 days of starvation and then it levelled out. Surendranath et al. (1987) noticed a significant decrease in oxygen consumption in P.indicus starved for 15 days.

2.4.2. Salinity

2.4.2.1. Salinity tolerance limits of Penaeus monodon

P.monodon is a highly euryhaline species capable of tolerating wide ranges of salinities. But, a salinity of 20-30 ppt at temperature 21-30°C is reported to be favourable for the fry (Valencia, 1976). Pantastico (1979) reported good growth and survival of P.monodon in freshwater. During transport of P.monodon post-larvae Singh et al. (1982) noticed that a low salinity of 8 ppt resulted in good survival. Cawthrone et al. (1983) observed complete mortality of P.monodon post-larvae within 4 hours of acclimatisation to freshwater over a period of 3 days. They obtained poor growth of the prawn in all salinities below the sea water salinity (33.5 ppt). Later,Reddi et al. (1984) supported the observations of Pantastico (1979) by reporting high survival (96%) and growth of P.monodon juveniles in freshwater. However, Chakraborti et al. (1985) reported very poor survival at salinities below 2 ppt. The survival was only 68% when salinity ranged

between 2 and 5 ppt. Cheng and Liao (1986) noticed that P.monodon juveniles osmoregulated efficiently over a wide range of salinity i.e., 3-50 ppt. However, Rao et al. (1986) could obtain only 10% survival when P.monodon post-larvae were transported at a salinity of 1.5 ppt. Singh (1989) observed that the post-larvae of P.monodon could withstand an abrupt change in salinity from 30.0 to 4.0 ppt, after which heavy martalities occurred. Diwan et al. (1989) also observed that P.monodon osmoregulated well between salinities of 3 and 48 ppt. They reported that a duration of 48 hours was essential for prawns to adjust to the new medium. Zhang et al. (1989) also reported that P.monodon juvenile was able to survive and grow well over 3 ppt.

2.4.2.2. Influence of salinity on oxygen consumption

Opinions on the influence of salinity on oxygen consumption in prawns in general, differ. However, reports of most of the authors apparently indicate an increase in oxygen consumption with decrease in salinity among the penaeid prawns except P.monodon (Kutty et al., 1971; Gaudy and Solane, 1981; Unnikrishnan and Laxminarayana, 1984; Dallavia 1986). In P.monodon a decrease in salinity was reported to have no effect on oxygen consumption (Gaudy and Solane, 1981; Liao and Murai, 1986). However, Licop (1984) noted the lowest rate of oxygen consumption in P.monodon kept at 20 and 30 ppt at low temperatures (15°C) as well as at 20 ppt at high temperature (30°C). In contrast to the above trend, in Metapenaeus monoceros Janakiram et al. (1989) reported a decrease in oxygen consumption when salinity was reduced from 25 ppt to 2 ppt.

Among Palaemonid prawns, a decrease in oxygen consumption with increase in salinity was reported by Nelson et al.(1977). But Stephenson and Knight (1980) and Stern et al. (1984) observed no significant change in oxygen consumption in Macrobrachium rosenbergii with increase in salinity. However, Dallavia (1987) noted an increase in oxygen consumption in Palaemonetes antennarius with increase in salinity.

2.4.2.3. Influence of salinity on ammonia excretion

The ammonia excretion in prawns is also reported to have been influenced by salinity. Bower (1978) reported that the concentration of un-ionised ammonia in water was low in higher salinities. Armstrong et al. (1981) noticed an uptake of ammonia by M.rosenbergii, when they were subjected to a hyperosmotic stress. Spaargaren et al. (1982) found high NH_4^+ - N excretion at lower salinities in Penaeus japonicus under uniform conditions. Unnikrishnan and Laxminarayana (1984) observed an increase in ammonia excretion with decrease in salinity in P.indicus. Stern et al. (1984) noticed increased ammonia excretion with increase in salinity in M.rosenbergii. Quarmby (1985) also had similar observation in Pandalus platyceros females. Taylor et al. (1987) reported an increase in ammonia excretion in Palaemon elegans when transferred to low salinity.

2.4.3. Temperature

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

It has been reported that high packing densities can be used during transport if a low temperature is maintained. Mohanty and patra (1972) could transport 200 prawns/container with 100% survival for 9 hours at low temperature, as against 100 prawns/container at ambient temperature. Hamid and Mardjono (1979) could increase both packing density and duration of transport at low temperature compared to that at room temperature, without any reduction in survival. They transported 500-600 prawns/l for 12 hours with 95% survival at 22-25°C, as against 200-300/l for 8 hours at 28-29°C with the same survival.

Low temperature during transport of prawns results in better survival, because at high temperatures the prawns undergo moulting frequently, which encourages cannibalism (Shigueno, 1975). Dwivedi (1978), Hamid and Mardjono (1979), Selvaraj et al. (1980), Harison and Lutz (1980), Alikunhi et al.(1980), Krishnakumar and Pillai (1984), Kungvankij et al.(1986) and Fan and Dayue (1988) reported higher survival of prawns during transport at low temperatures.

2.4.3.2. Effect of temperature on water quality parameters

Oxygen consumption in prawn has been reported to be lower at lower temperatures and vice versa (Nelson et al.,1977; Stephenson and

Knight, 1980; Bishop et al., 1980; Licop, 1984; Liao and Murai, 1986; Dallavia, 1987).

The combined effect of temperature and salinity on the survival of prawns has been reported (Bhatacharya and Kewalramani, 1976). Low temperature reduces the tolerance of marine and brackishwater prawns to low salinities (Zein-Eldin and Aldrich, 1965, Kuttyamma, 1981, Charmantier et al., 1988).

The toxicity of ammonia to prawns greatly depends on temperature. The concentration of un-ionised ammonia in water increases with increase in temperature (Bower, 1978). Spaargaren et al.(1982) stated that the temperature had very strong effect on the ammonia excretion by prawn. Quarmby (1985) reported that the total nitrogen excretion by juvenile prawn, Pandalus platyceros did not change with temperature, while the composition of the nitrogenous excretory products changed.

2.4.4. Nitrogenous excretions

2.4.4.1. Ammonia tolerance limits

Ammonia accounts for 40 to 90% of nitrogenous excretions in crustaceans (Parry, 1960) and is the principal end product of protein catabolism (Kinne, 1976). It is found in both un-ionised (NH_3) and ionised (NH_4^+) form. The un-ionised form is usually toxic (Armstrong et al., 1978). Wajsbrot et al.(1989) pointed out that the share of ammonia - N

in the total nitrogen excretion in Penaeus semisulcatus was 61-83%.

Catedral et al.(1977) stated that the post-larvae of P.monodon could tolerate ammonia upto 10 mg/l. Huang (1979) determined the 96-h LC_{50} value of ammonia in P.monodon juveniles weighing 0.17 g as 26.67 mg/l ammonia-N. Lai and Ting (1984) found that the 24-h, 48-h and 72-h LC_{50} values of ammonia in P.monodon juveniles weighing 0.07-0.19 g were 15.99, 11.81 and 9.88 mg/l ammonia-N respectively. Chin and Chen (1987) observed LC_{50} values for post-larvae of P. monodon after 24-h, 48-h, 72-h and 96-h as 52.11, 27.73, 17.05 and 11.51 mg/l ammonia-N repectively. They determined a safe level for rearing larval P.monodon as 1.15 mg/l ammonia-N. Chen et al.(1990) subjected P.monodon juveniles (0.26-0.51 g) to ammonia-N concentrations of 30 and 40 mg/l for 168 hours. They found thatall juveniles exposed to 30 mg/l survived even after 168 hours whereas only 36.7% of those exposed to 40 mg/l survived for the same duration. Allan et al.(1990) observed the 96-h LC_{50} value of ammonia in P.monodon juveniles as 37.4 mg/l total ammonia.

2.4.4.2. Nitrite tolerance limits

Tookwinas (1984) and Chen et al. (1986) reported nitrite-N as more toxic than ammonia-N to the larvae of P.monodon. But, Chen and Chin (1988) stated the safe level of NO_2-N for larval rearing as 1.36 mg/l NO_2-N, which is higher than the safe level reported by Chin and Chen (1987) for ammonia-N (1.15 mg/l ammonia-N). However, Chen and

chin (1988 a) in a separate study found that a mixture of ammonia and nitrite-N exerted greater toxicity on the larvae of P.monodon than high concentration of either of these singly.

2.4.4.3. Effect of ammonia during transportation

Ammonia excretion in prawns was 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 80 ppm. As the pH of the medium was on the acidic side, they concluded that ionised form of ammonia was also harmful to prawn 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 packed 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.

2.4.4.4. Ammonia excretion in relation to starvation

The effect of starvation on ammonia excretion has been studied by several workers. Nelson et al.(1979) observed low ammonia excretion in starved prawns when compared to fed prawns. On the contrary, Dall and Smith (1986) reported 46-73% increase in ammonia excretion in starved prawns.

2.4.4.5. Ammonia excretion in relation to body weight

Ammonia excretion was found to have been influenced by the body weight of prawn (Nelson et al., 1977 a). Nelson et al.(1979) reported that the effect of weight on the rate of ammonia excretion was more pronounced in fed prawns than in starved prawns. Anantharaman et al. (1981) observed maximum ammonia excretion in the prawn Macrobrachium lanchesterii weighing 828.1±61.55 mg and the value decreased for those weighing above and below this weight. Wickins (1985) pointed out that in P.monodon, the rate of ammonia excretion decreased with increase in animal size. Similar result was obtained by Mohanty et al.(1989), who found that in P.monodon the rate of release was higher in smaller prawns although the total ammonia released was directly proportional to the size of the prawn. Similarly, in P.japonicus Marangos et al.(1990) observed that the daily specific excretion by post-larvae was about five fold higher than the excretion by adults.

2.4.4.6. Ammonia excretion in relation to oxygen levels

Ammonia excretion and toxicity of ammonia are influenced by dissolved oxygen concentration. Laxminarayana and Kutty (1982) observed 2 to 5 fold increase in the ammonia quotient values in crustaceans under hypoxic conditions. Wajsbrot et al.(1990) found that the toxicity of ammonia in the juveniles of P.semisulcatus increased when the dissolved oxygen level was below 55% saturation. At 27% oxygen saturation, ammonia toxicity was doubled. Allan et al.(1990) also noticed increased

toxicity of ammonia in P.monodon juveniles under lower dissolved oxygen levels.

2.4.5. pH

pH of water is important because toxicity due to ammonia is greatly altered by pH. The concentration of un-ionised ammonia (NH_3) increases at high pH values (Bower, 1978). Armstrong et al.(1978) reported that at higher pH (8.4), toxicity resulted from copious diffusion of NH_3 into the prawn and at lower pH (6.8) toxicity was thought to result from competitive inhibition of Na^+ transport by NH_4^+. 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 of Chen and Chin (1989) and Chen and Sheu (1990) on P.monodon and P.japonicus post-larvae respectively, supported the observations 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 of 8.31.

A decrease in pH of the transport medium was reported in all experiments on the transportation of prawns (Singh et al.,1982; Krishnakumar and Pillai, 1984; Alias and Siraj, 1988). Singh et al.(1982) and Alias and Siraj (1988) reported only a slight decrease in pH, well above the acidic mark. But, Krishnakumar and Pillai (1984) observed reduction in pH towards the acidic side in higher packing densities and recorded considerable mortality at pH below 6.6.

2.4.6. Carbon dioxide

The carbon dioxide accumulation during transport was reported to bring down the pH by the dissolution of carbon dioxide, forming carbonic acid (Krishnakumar and Pillai, 1984). These workers observed almost complete mortality due to accumulation of carbon dioxide in long duration experiments. Alias and Siraj (1988) noticed significant reduction in carbon dioxide accumulation by the incorporation of habitat material in the packing medium. They noted an increase in carbon dioxide with increase in packing rate. The interaction between habitat materials and packing density was significantly different in the carbon dioxide levels.

2.4.7. Bacterial population

The multiplication of bacteria within the packing medium, aggravated by metabolic wastes and decomposing dead organisms, is considered as a causative factor for the mortality of organisms in closed system transport (Vas, 1951). Despite this fact, only very little work has been done to ascertain the effect of bacteria population during transportation. Norris et al. (1960) reviewed the use of bactericides and fungicides during transport of fishes. Several others also reported the use of antibacterial agents during transport of fish to counteract the bacterial growth (Woiwode and Fairgrieve, 1980; Amend et al., 1982; Dupree and Húner, 1984). Lio-Po et al.(1986) observed that bacterial count increased significantly in closed systems during the transport of the milk fish, Chanos chanos. Although mortalities during transports

were minimum, they observed significant post-stocking martality. Lio-Po and Durumdez-Fernandez (1986) isolated Aeromonas hydrophila and Pseudomonas like bacteria, which were causative agents for serious diseases, from the transport samples of C.chanos. Practically no information is available on the effect of bacterial population on prawns during transport.

2.5. Cannibalism during transport

Cannibalism is one of the major problems in prawns affecting survival during transport. Cannibalism is more pronounced at higher temperatures (Shigueno, 1975). Mammen et al.(1978) got only a survival of 53.5% after the transport of P.monodon fry for 18 hours, which they attributed, mainly to cannibalism. According to Hamid and Mardjono (1979) high temperature during transportation increased cannibalism. Alikunhi et al.(1980) also agreed to this view and reported that at normal temperature, when prawns were crowded without food they tended to become more cannibalistic. They observed that within 24 hours 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 prawns for avoiding cannibalism during transport. Franklin et al.(1982) acknowledged cannibalism as one of the reasons for mortality during transport. Krishnakumar and Pillai (1984) observed that the small post-larvae moulted more frequently and became more prone to cannibalism. Considering the cannibalistic nature of post-larvae Liao (1984) and Kungvankij et al.(1986) recommended nauplius as the ideal stage for transportation, a stage at which prawn hardly feeds, but depends on

its yolk for development. Subrahmanyam (1986) reported cannibalism and injury to the soft seed (moulted) as the causes of mortality. Alias and Siraj (1988) stated that the incorporation of habitat material in the transport container had some effect in reducing cannibalism and thus increasing survival of M.rosenbergii.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Experimental prawns

The experimental prawns - Penaeus monodon seed of PL_{10} stage- were brought from the Marine Products Export Development Authority's Prawn Hatchery at Vallarpadam, Cochin, to the College of Fisheries, Cochin in two consignments. They were transported in polythene bags containing 5 litres of hatchery water having a salinity of 31.5 ppt and pH 7.5. The first consignment contained 5000 PL and the second 1300 PL, which was brought a week after the first. The first and second consignments of seed were maintained in a 1 ton capacity rectangular tank and in three 80 litre capacity circular tanks respectively, during the trial period of about 3 weeks. Before transferring them into the tanks, temperature of the water in the transport bag was equalised to that of the water in the tank by floating the bag with seed in the tank water. The post-larvae were then acclimatised to a salinity of 25 ppt over a period of 10 hours, by gradually reducing the salinity. Aeration was provided to the tanks containing the post-larvae.

The post-larvae were fed with Artemia nauplii for the first 3 days and thereafter with clam meal. Twigs were provided to the tank for the post-larvae to cling on. 50-75% of the tank water was exchanged daily with water of same salinity (25 ppt). Feed remnants and excretory matter were removed from the bottom of the tank by siphoning every week.

3.2. Experimental containers and equipments

Specially designed transparent plastic jars of 600 ml capacity with screw type lid were used for the experiments (Fig.1). These jars were made air tight to withstand the pressure of oxygen inside, by winding insulation tape and Teflon (polytetrafluoroethylene) around their rim. The lid of each jar was fitted with a one way valve (the valve that is used for pneumatic tyres) after boring a hole at the centre, to fill in oxygen and facilitate reading of oxygen pressure inside the jars. The valve fitting was made air tight using appropriate rubber washers.

A Bourdon type pressure guage with a precision of 0.02 Kg/cm^2 was used for reading oxygen pressure inside the jars, simultaneously with the filling of oxygen. The initial pressure of oxygen in all the containers was kept constant by using the pressure guage.

Locally fabricated respirometers of 320 ml capacity were used to study the metabolism of the prawns. Each of the respirometers used had three openings, i.e., an inlet and an outlet for continuous circulation of water and an air vent for the escape of the displaced air while filling the apparatus. A completely closed system was easily set-up with this apparatus by using flexible tubes fitted to the openings which were closed using clips.

Fig. 1. Specially designed transparent plastic jar

3.3. Determination of the dosage of sedatives

Three sedatives i.e., Chloral hydrate, MS-222 (sandoz) and tertiary butyl alcohol were used for the experiments. They were selected on the basis of their ease of application, availability and previous reports of successful use in the transport of live fishes.

As a first step of the experiment, the lower and upper limits of each sedative were fixed by trial and error method. This was done in experimental jars kept open, each with 5 prawns (average size 11 mm/4.92 mg) in 100 ml filtered water of salinity 25 ppt, pH 7.5 and temperature 29±1°C. Four doses i.e., 300, 400, 500 and 600 ppm for chloral hydrate, 100, 200, 300 and 400 ppm for MS-222, and 0.5, 1.0, 1.5 and 2.0 ml/l for tertiary butyl alcohol were tried in this experiment. A control was maintained simultaneously without any sedative. Chloral hydrate and MS-222 were weighed in a monopan electrical balance with a precision of 0.01 mg and tertiary butyl alcohol was measured using a micropipette of 0.01 ml precision. Each treatment was duplicated. Mortality in the containers was observed at an interval of 2 hours, for a duration of 48 hours, by which time the lower and upper limits of the doses of each sedative could be fixed for subsequent trials.

The second part of the experiment, which was designed statistically in randomised block design, was done with all the three sedatives used in the above experiment in narrower doses within the

limits, in the experimental jars kept open, each with 5 prawns (average size of 12 mm/ 5.86 mg) in 100 ml filtered water of salinity 25 ppt and pH 7.5. The jars were kept closed initially for one hour after adding the sedatives, in order to prevent the escape of any gas generated on their application, which might act in sedating the prawns. Seven narrow doses of each chemical i.e., 300, 325, 350, 375, 400, 425 and 450 ppm for chloral hydrate, 100, 125, 150. 175, 200, 225 and 250 ppm for MS-222 and 0.5, 0.75, 1.0, 1.25, 1.50, 1.75 and 2.0 ml/l for tertiary butyl alcohol were tried in this experiment. A control was maintained simultaneously without any sedative. Each treatment was replicated 3 times. Observations on the mortality of the seed were made at two hour intervals for a period of 72 hours. Behavioural as well as morphological changes in the prawns on exposure to sedatives were also observed. From this study a single dose of each chemical was selected for further studies.

3.4. Effect of sedatives on metabolism of P.monodon seed

The effect of sedatives on the metabolism of the seed was studied using respirometers. The study was conducted primarily to select a suitable sedative from those experimented, based on the rate of reduction of the metabolic activities measured in terms of oxygen consumption and ammonia excretion. The dose of each sedative derived from the previous experiment (chloral hydrate-400 ppm, MS-222-175 ppm and tertiary butyl alcohol-0.75 ml/l) formed the three treatments for this study. The fourth treatment was without any sedative. The

experiment was designed statistically in completely randomised design and each treatment was replicated four times.

Filtered water of 25 ppt salinity and 7.5 pH was used in the respirometers. Five prawns (average size of 12 mm/ 5.86 mg) were kept in the closed system of the respirometers for a period of 2 hours. Initial and final water samples were collected for analysing dissolved oxygen and total ammonia. The prawns could not be given the required acclimatisation time inside the respirometers by maintaining a continuous flow of water through the system before completely closing it, because of the practical difficulty in preventing the tiny animals passing through the outlet during the water flow.

A control experiment was conducted simultaneously by using the corresponding dose of each sedative, but without prawns, with a view to studying the changes taking place in the water quality.

3.5. Determination of the effect of chloral hydrate on the oxygen-packed seed

The effect of sedative on the oxygen-packed P.monodon post-larvae for transport was determined using chloral hydrate, which was found as the most effective in reducing the metabolic activity at the selected dose of 400 ppm. The experiment was carried out in experimental jars under an oxygen pressure of 0.2 kg/cm^2. The prawns used for this study were of an average size 14 mm/11.64 mg. The experiment was conducted statistically as an asymmetrical factorial

experiment (4×2^3) in completely randomised design.

3.5.1. Different factors combined in the experiment

Packing density, sedation, salinity and temperature were the four factors combined in the experiment. Four levels were used for packing density (number of post larvae) i.e., 200/l, 400/l, 600/l and 800/l. The other three factors had two levels each. The two levels used for sedation were (1) with sedation, achieved by adding 400 ppm of chloral hydrate and (2) without any sedation. The two levels of salinity were 25 ppt and 20 ppt, and those of temperature were 29±1°C (ambient temperature) and 23±2°C. Altogether, the experiment contained 32 combinations. Each combination was duplicated. A control was maintained for each level of the three factors i.e., sedation, salinity and temperature without any prawn.

The prawn seed were sedated by applying chloral hydrate in liquid form, i.e., by dissolving it in distilled water before application and making up a solution of 40 mg/ml concentration. 1 ml of this solution was added to 100 ml of the packing medium, so as to get a final concentration of 400 ppm.

The required 25 ppt salinity water was obtained from the Cochin backwaters. 20 ppt salinity water was prepared by adding clean freshwater to filtered water of 25 ppt salinity. The water with required salinities was kept ready on the previous day of packing.

The lowered temperature of 23±2°C was obtained by keeping crushed ice around the jars placed in a tray. The temperature of the packing medium was first lowered to 23°C by keeping the water in a freezer, before pouring into the jars, and then the temperature was maintained using ice pieces. This procedure avoided the dilution of salinity of the water that would have resulted if crushed ice pieces were added directly to the packing medium.

3.5.2. Conditioning of the prawn seed

Feeding was stopped a day before packing. The feed remnants and excretory matter were completely removed and prawns were maintained in the fresh clean aerated water of 25 ppt salinity for a day prior to packing. The excretory matter was removed from the bottom of the tank by siphoning it just before counting the prawns for packing, in order to avoid its entry into the jars. The counted prawn post-larvae were kept congested at a density of 1/10 ml in beakers for nearly 3 hours before oxygen packing in the plastic jars.

3.5.3. Packing procedure

The counted prawns were transferred without water to the transport jars with 100 ml of the packing medium. Before transferring, any dead post-larvae in the beakers were replaced with live post-larvae. Immediately after transfer, the chloral hydrate solution (1 ml) was added only in those jars in which it was required, closed tightly and filled with oxygen under a pressure of 0.2 kg/cm^2, from

an oxygen cylinder. The oxygen was filled through the pressure guage which measured the oxygen pressure inside the jars. While filling oxygen, care was taken to displace the air initially present inside the jar with oxygen. To effect this, after filling oxygen it was completely released by pressing the valve. This was repeated three times to ensure complete displacement of air with oxygen.

3.5.4. Observations

Number of prawns surviving in each jar was noted at two hour intervals for a period of 24 hours, except for 600/l and 800/l. Observation on the numbers dead was not pragmatic as the dead prawns were immediately eaten by the survivors. The time of initial mortality was recorded for all packing densities. For higher packing densities of 600/l and 800/l, observations on the survivors were limited to initial 6-8 hours i.e., before significant mortality occurred, and a final observation at 24 hours, because of the difficulty in counting the live prawns at a higher density. The jars were periodically shaken to simulate the transport conditions. At the end of 24 hours each jar was opened, samples of the packing medium collected for water quality analyses and the survivors were counted.

Initial and final quality of the packing medium was analysed using standard procedures. The parameters analysed were dissolved oxygen, carbon dioxide, total ammonia, pH and bacterial population. Of the replications of each combination, one was used for analysing dissolved oxygen and the other for carbon dioxide, because the determination

of both parameters from the same might have yielded erroneous values for the second-measured parameter. At the same time, total ammonia, pH and bacterial population were noted from all the jars.

3.6. Determination of the effect of chloral hydrate at a low salinity

This experiment was aimed at determining the effect of sedative on P.monodon seed at a low salinity of 8 ppt. The required salinity water was prepared by mixing freshwater with filtered water of 25 ppt salinity. The prawns were acclimatised to 8 ppt salinity water by gradually reducing the salinity from 25 to 8 ppt through 24 hours. They were then maintained at this salinity for two days before packing. The post-larvae used for this experiment were of an average size, 17 mm/23.27 mg. The experiment was statistically designed with two treatments, each with seven replications. Chloral hydrate added at 400 ppm formed one treatment and without any sedative formed the other treatment.

The conditioning of prawns, packing procedure, taking observations and determination of water quality were done as in the previous experiment. But, bacterial population could not be measured in this experiment. The experiment was conducted at an ambient temperature of 29±1°C.

3.7. Determination of the effect of a low dosage of chloral hydrate

This experiment was intended to determine the effect of a low

dosage (300 ppm) of chloral hydrate on P.monodon seed under oxygen packing. Filtered 25 ppt water was used in this case. The prawns used were of an average size, 17 mm/23.27 mg. The experiment was statistically designed with 2 treatments and 7 replications. Chloral hydrate added at 300 ppm formed one treatment and without any sedative formed the other treatment.

Conditioning of the prawns, packing procedure, taking observations and determination of water quality were done as in the previous experiments. In this case also bacterial population could not be measured.

3.8. 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. (APHA *et al.*, 1981).
Ammonia	:	Strickland and Parsons, 1972.
pH	:	Using Universal indicator solution. (Qualigens Fine Chemicals).
Temperature	:	Using mercury bulb thermometer having a precision of 0.1°C.

Salinity : Using Salino refractometer.

Total viable bacterial population

Total viable bacterial population of the carrying medium was determined by the following standard pour plate method using plate count agar (tryptone 5 g/l, yeast extract 2.5 g/l, dextrose 1 g/l, agar 15 g/l and pH 7±0.2). Samples were serially diluted using sterile 15 ppt aged seawater. The plates were incubated at room temperature for 72 hours and the colonies were counted using an electric colony counter.

3.9. Statistical analysis

The data obtained from the experiment for fixation of dose of sedatives (mortality expressed as number of dead prawns in each container) and from the studies on metabolism of the prawn seed (oxygen consumption/g/h and ammonia excretion/g/h) were analysed by analysis of variance in the corresponding designs. The data on time of initial mortality, cumulative percentage of mortality at 24 hours, ammonia excretion and bacterial population from the factorial experiment, on the efffect of various factors under oxygen packing, were also subjected to analysis of variance (Joshi, 1987). The data on oxygen consumption and carbon dioxide excretion from the latter experiment could not be analysed by ANOVA, but explained by tabular and graphical methods. The data on bacterial population were subjected to logarithmic transformation before analysis.

All the information viz. time of initial mortality, cumulative percentage mortality at 12 and 24 hours and water quality parameters from the last two experiments (experiment at 8 ppt salinity and experiment with a dose of 300 ppm chloral hydrate) were analysed using paired 't' test (Snedecor and Cochran, 1967). In all the cases the percentage values were subjected to Arc-sine conversion before analysis.

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

RESULTS

4. RESULTS

4.1. Selected dose of sedatives

The trial and error experiment resulted in fixing the lower and upper limits of dose of each sedative. The highest dose tried at which the survival of the prawns was the same as that of the control (without sedative) was fixed as the lower limit and the lowest dose tried at which an apparently high mortality occurred was fixed as the upper limit. In control, no mortality was observed within 48 hours and hence the lower limit was the dose which produced no mortality within 48 hours. The lower and upper limits fixed by the 48 hour experiment were 300 and 450 ppm for chloral hydrate, 100 and 250 ppm for MS-222 and 0.5 and 2.0 ml/l for tertiary butyl alcohol respectively.

In the second part of the experiment, seven narrower doses, within the limits mentioned above, were tried for each sedative. The treatments of all the three sedatives showed significant difference ($P < 0.01$) whereas the differences between replications were not significant ($P > 0.05$) (Table-1, 2 and 3). Pair-wise comparison for chloral hydrate showed that control and doses of 300-400 ppm formed a group with no significant difference, but 425 ppm and 450 ppm were found to be significantly different from the rest. The dose of 400 ppm was hence fixed as the dose to be tried in subsequent experiments. Pair-wise comparison for MS-222 revealed that the control and doses, 100-175 ppm showed no significant difference, but, doses higher than

175 ppm were significantly different. The dose of 175 ppm was selected for further experiments. Pair-wise comparison for tertiary butyl alcohol revealed that the control and doses, 0.5 to 1.0 ml/l formed a single group which was significantly different from the higher doses. Although the dose of 1.0 ml/l showed no significant difference from the control, as some mortality was observed at that dose, to be on the safer side, 0.75 ml/l was fixed as the dose to be tried in the subsequent experiments.

Observations on the behavioural and morphological changes in the prawns on exposure to sedatives revealed the following points.

1. The prawns became slightly sluggish, when compared to the controls, but showed no clear signs of complete lethargy.

2. The prawns which fell on their sides due to long term exposure to higher doses, hardly recovered.

3. The colour of the prawns turned to reddish orange, when the dose was higher than the safe limit. The discoloured individuals often met with mortality unless transferred to fresh medium immediately.

4. The appendages of the dead prawns quickly turned whitish.

5. The sedated prawns transferred to fresh medium were observed for 24 hours and found as active as the normal prawns.

Table 1. Analysis of variance in the cumulative mortality of P. monodon seed caused by 8 different doses of chloral hydrate at 72 hours.

Source	Degrees of Freedom	Sum of squares	Mean sum of squares	F value		
				Computed	Tabular 0.05	0.01
Treatment	7	9.8333	1.4048	5.02**	2.77	4.28
Replication	2	0.0833	0.0417	0.15	3.74	6.51
Error	14	3.9167	0.2798			
Total	23	15.8333				

** Significantly different at $P < 0.01$

Treatment means:

$\bar{x}_1$ (0 ppm) - 1

$\bar{x}_2$ (300 ppm) - 1

$\bar{x}_3$ (325 ppm) - 1

$\bar{x}_4$ (350 ppm) - 1

$\bar{x}_5$ (375 ppm) - 1

$\bar{x}_6$ (400 ppm) - 1.33

$\bar{x}_7$ (425 ppm) - 2.33

$\bar{x}_8$ (450 ppm) - 2.61

Calculated C.D. value (t 0.05) = 0.9264

Table. 2. Analysis of variance in the cumulative mortality of P.monodon seed caused by 8 different doses of MS-222 at 72 hours.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	Tabular 0.05	Tabular 0.01
Treatment	7	27.1667	3.881	8.58**	2.77	4.28
Replication	2	1	0.5	1.11	3.74	6.51
Error	14	6.3333	0.4524			
Total	23	34.5				

** Significantly different at $P < 0.01$

Treatment means

$\bar{x}_1$ (0 ppm) - 1

$\bar{x}_2$ (100 ppm) - 1

$\bar{x}_3$ (125 ppm) - 1

$\bar{x}_4$ (150 ppm) - 1

$\bar{x}_5$ (175 ppm) - 1

$\bar{x}_6$ (200 ppm) - 2.33

$\bar{x}_7$ (225 ppm) - 2.67

$\bar{x}_8$ (250 ppm) - 4.0

Calculated C.D. value (t 0.05) = 1.3262

Table 3. Analysis of variance in the cumulative mortality of P. monodon seed caused by 8 different doses of tertiary butyl alcohol at 72 hours.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	Tabular 0.05	Tabular 0.01
Treatment	7	62.6667	8.9524	7.13**	2.77	4.28
Replication	2	1.75	0.875	0.70	3.74	6.51
Error	14	17.5833	1.256			
Total	23	82				

**. Significantly different at $P < 0.01$

Treatment means

$\bar{x}_1$ (0 ml/l) - 1

$\bar{x}_2$ (0.5 ml/l) - 1

$\bar{x}_3$ (0.75 ml/l) - 1.33

$\bar{x}_4$ (1.0 ml/l) - 2.67

$\bar{x}_5$ (1.25 ml/l) - 3.67

$\bar{x}_6$ (1.5 ml/l) - 4.67

$\bar{x}_7$ (1.75 ml/l) - 4.67

$\bar{x}_8$ (2.0 ml/l) - 5.0

Calculated C.D. value (t 0.05) = 1.7961

4.2. Effect of sedatives on the metabolism of prawn seed

The metabolic activities viz., oxygen consumption and ammonia excretion of post-larvae were analysed with and without sedatives in order to select the most effective chemical. The mean ammonia-N excretion and oxygen consumption by the prawn seed with different sedatives are represented in Fig. 2 and 3 respectively. The ammonia excretion analysis gave negative values for the prawns without sedation and positive values for those treated with all the three chemicals (Table-4). This indicates an increase in ammonia excretion by the prawn, on addition of sedatives, as is clear from Fig. 3. However, oxygen consumption was found to decrease with the addition of sedatives.

Analysis of variance showed highly significant difference ($P<0.01$) in the rate of both ammonia excretion (Table-5) and oxygen consumption (Table-6) among the treatments. The pair-wise comparison for ammonia excretion/g/h revealed that the addition of tertiary butyl alcohol did not significantly increase ammonia excretion from that of control, i.e., without sedation, whereas, the addition of the other two sedatives did. However, there was no significant difference between tertiary butyl alcohol-treated prawns and those treated with chloral hydrate, in terms of ammonia excretion. The highest ammonia excretion of 2.0973 ppm/g/h ammonia-N was recorded in the MS-222-treated prawns. Oxygen consumption/g/h was the lowest in the seed sedated with chloral hydrate (1.1423 ppm/g/h), but it had not shown significant difference ($P>0.05$) from that of MS-222, whereas those of tertiary butyl alcohol and without sedation were highly significant as indicated in Table-6.

Table 4. Ammonia excretion and oxygen consumption by P.monodon seed without sedative and with three different sedatives.

Treatments	Ammonia-N excretion/g/h* (ppm)	Oxygen consumption/g/h* (ppm)
Chloral hydrate	1.1423	2.2435
MS-222	2.0973	2.906
Tertiary butyl alcohol	0.3905	5.05
Without sedative	0.4798	9.5975

* Each value is a mean of 4 replicates.

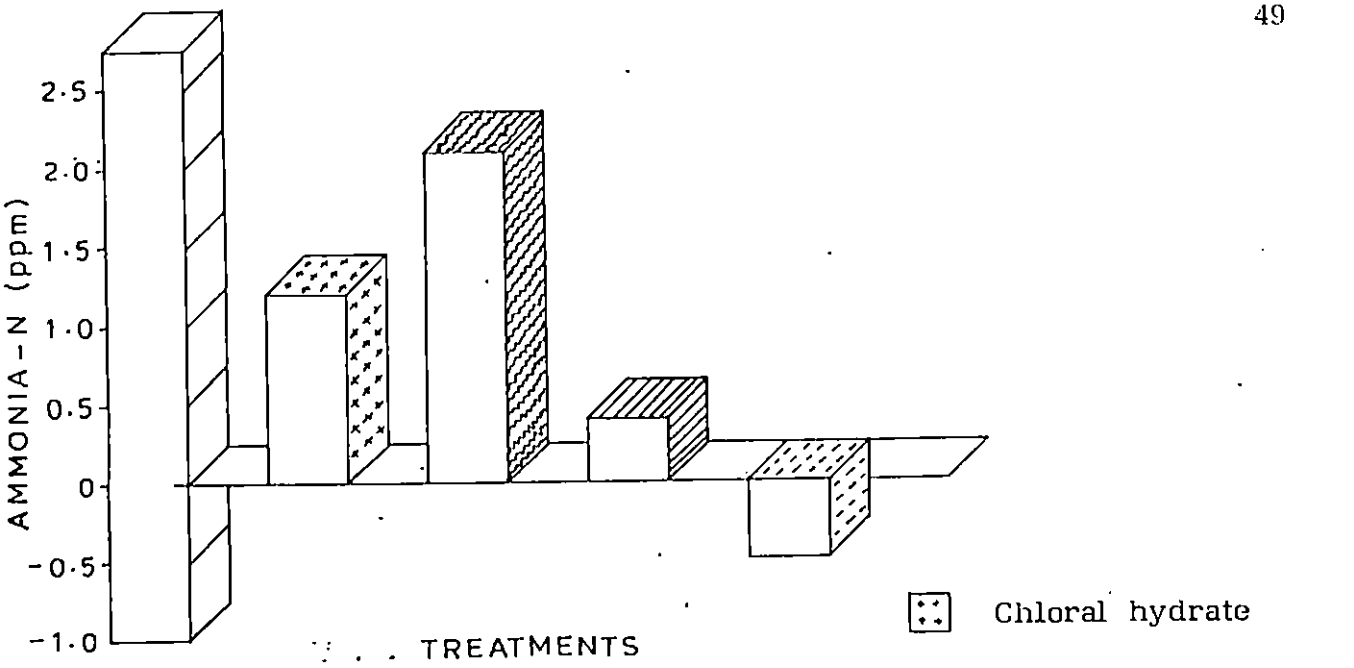

Fig.2.

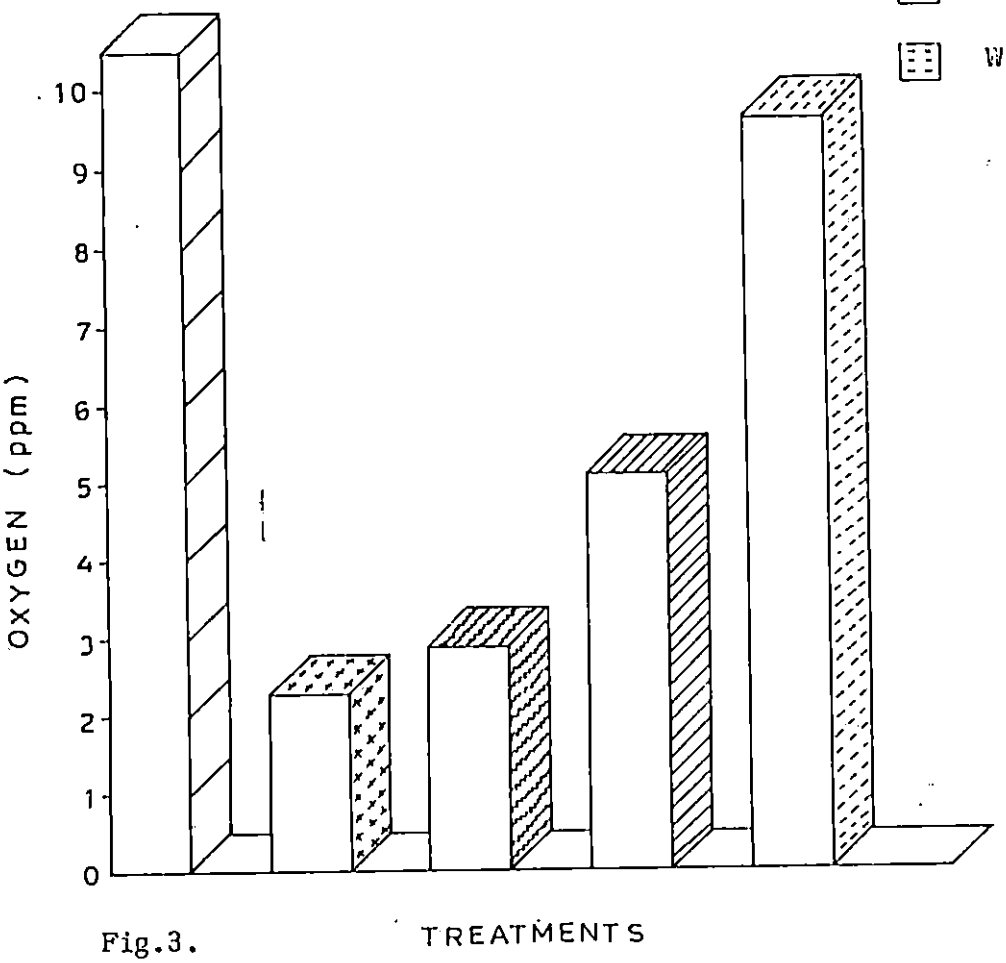

Fig.3.

Fig. 2 & 3 Rate of ammonia excretion and oxygen consumption by P. monodon seed without and with treatment of different sedatives.

Table 5. Analysis of variance in ammonia-N excretion/g/h by P.monodon seed without and with treatment of different sedatives.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	Tabular 0.05	Tabular 0.01
Treatment	3	11.9874	3.9958	11.27**	3.49	5.95
Error	12	4.255	0.3546			
Total	15	16.2424				

** Significantly different at $P < 0.01$

Treatment means

$\bar{x}_1$ - (chloral hydrate) - 2.1423

$\bar{x}_2$ - (MS-222) - 2.8473

$\bar{x}_3$ - (Tertiary butyl alcohol) - 1.3905

$\bar{x}_4$ - (without sedative) - 0.5203

Calculated C.D. value (t 0.05) = 0.9176

Table 6. Analysis of variance in the oxygen consumption/g/h by P.monodon seed without and with treatment of different sedatives.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	Tabular 0.05	Tabular 0.01
Treatment	3	132.4493	44.1498	33.82**	3.49	5.95
Error	12	15.6675	1.3056			
Total	15	148.1168				

** Significantly different at $P < 0.01$

Treatment means

$\bar{x}_1$ (Chloral hydrate) - 2.2435

$\bar{x}_2$ (MS-222) - 2.906

$\bar{x}_3$ (Tertiary butyl alcohol) - 5.05

$\bar{x}_4$ (Without sedative) - 9.5975

Calculated C.D. value (t 0.05) = 1.7605

The control experiment (without prawns) revealed that after 2 hours of addition of chloral hydrate and tertiary butyl alcohol to water, the ammonia content of water was found to have reduced by 0.07 and 0.03 ppm respectively, whereas with the addition of MS-222, the same increased by 1.74 ppm. Oxygen content of water decreased after the addition of all the three chemicals. The level of decrease was 0.083, 0.248 and 0.413 ppm for chloral hydrate, tertiary butyl alcohol and MS-222 respectively. pH of the water was reduced remarkably (from 7.5 to 5.0) by the addition of MS-222, but it was almost stable with the other two sedatives.

Chloral hydrate was selected to study its effect on the prawn seed under oxygen packing, primarily on the basis of reduction in oxygen consumption/g/h, because a valid conclusion could not be drawn from the rate of ammonia excretion.

4.3. Effect of various factors on the oxygen-packed seed

The experiment was conducted as an asymmetrical factorial experiment designed in completely randomised design. The factors viz. packing density (A), sedation (B), salinity (C) and temperature (D) significantly influenced the time of initial mortality (h), the cumulative percentage of survival and the water quality parameters.

4.3.1. Time of initial mortality

Variations in the time of initial mortality (duration of 100%

survival) at four different packing densities viz., 200/l (a_0), 400/l (a_1), 600/l (a_2) and 800/l (a_3) without and with sedation (b_0 and b_1 respectively) under two levels of salinity i.e., 25 ppt (c_0) and 20 ppt (c_1) and temperature 29±1°C (d_0) and 23±2°C (d_1) are presented in Table-7, Fig. 4 and 5. It is clear from the figures that the time of initial mortality (h) decreased with increase in packing density and temperature. By lowering the temperature from the ambient temperature of 29±1°C to 23±2°C, the duration of 100% survival could be almost doubled at all the packing densities. However, the duration was not altered significantly by the application of chloral hydrate and lowering of salinity to 20 ppt. Analysis of variance showed significant difference ($P < 0.01$) in the time of initial mortality (h) among the different packing densities and the two temperatures tried (Table-8). Pair-wise comparison by critical difference analysis revealed that all the four packing densities differed significantly ($P < 0.01$) with one another. Significant difference was shown by the interaction AD i.e., packing density-temperature ($P < 0.01$) and ABC i.e., packing density-temperature-salinity ($P < 0.05$) also (Table-8). Pair-wise comparison for AD showed that only two pairs viz., a_3d_1 (800/l-23±2°C) and a_1d_0 (400/l-29±1°C), and a_0d_0 (200/l-29±1°C) and a_1d_1 (400/l-23±2°C), formed a uniform group with no significant difference ($P \geq 0.05$). Pair-wise comparison for ABC made it clear that within the packing densities of 600/l and 800/l the interaction was insignificant ($P > 0.05$) whereas in the lower packing densities of 200/l and 400/l it was slightly significant ($P < 0.05$). The influence of all other interactions and the main effects of sedation and salinity, on the time of initial mortality

Table 7. Time of initial mortality of P.monodon seed without and with treatment of chloral hydrate under different levels of packing density, salinity and temperature

Chloral hydrate treatment	Salinity (ppt)	Temperature (°c)	Time of initial mortality(h)* at four different packing densities			
			200/l	400/l	600/l	800/l
Without treatment	25	29±1	7.5	5.0	3.5	2.5
		23±2	15.0	9.5	6.0	5.0
	20	29±1	9.5	5.0	3.5	2.5
		23±2	16.0	8.5	6.0	4.0
With treatment (400 ppm)	25	29±1	8.0	4.5	4.0	2.5
		23±2	16.0	7.5	7.0	5.0
	20	29±1	7.0	5.5	3.0	2.5
		23±2	14.0	7.5	6.0	4.0

* Each value is a mean of duplicates.

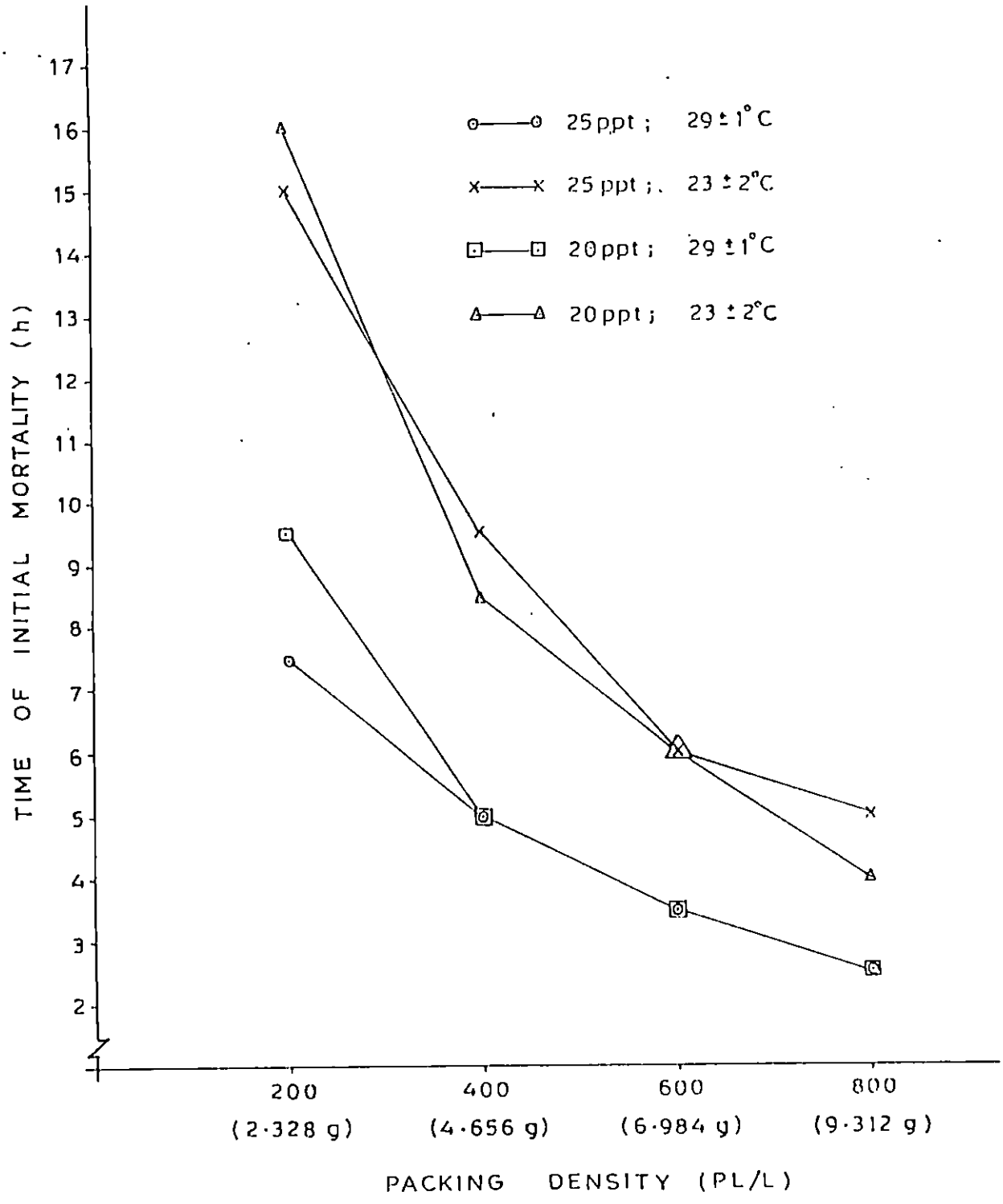

Fig. 4 Time of initial mortality of P. monodon seed without the application of chloral hydrate at four different packing densities under two different salinities and temperatures.

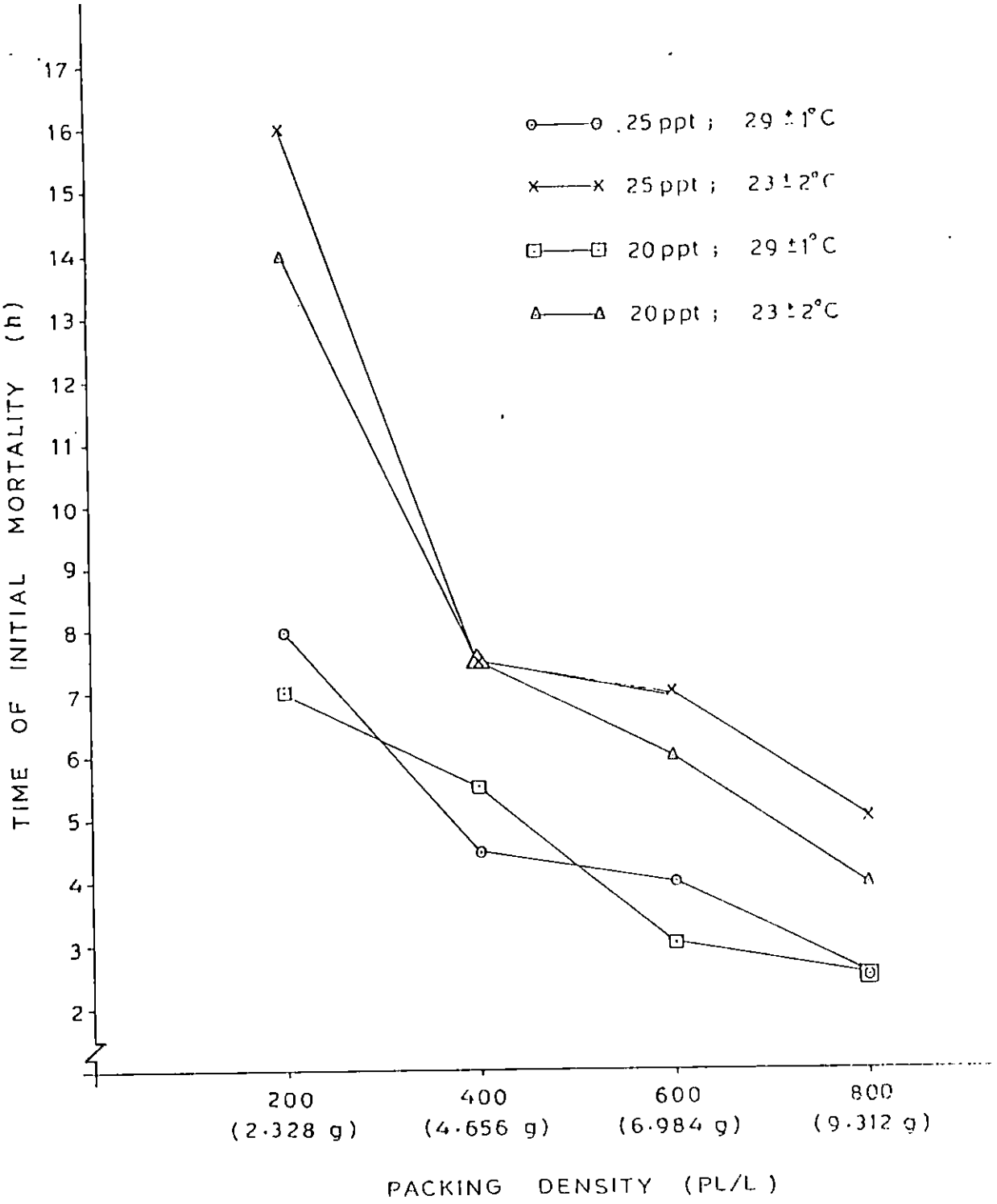

Fig. 5 Time of initial mortality of P. monodon seed with the application of chloral hydrate at four different packing densities under two different salinities and tempertures.

Table 8. Analysis of variance in the time of initial mortality of P.monodon seed without and with chloral hydrate under different levels of packing density, salinity and temperature.

Source x	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	Tabular 0.05	Tabular 0.01
A	3	605.19	201.73	259.37**	2.86	4.38
B	1	1.5625	1.5625	2.01	4.11	7.39
C	1	1.00	1.00	1.29	"	"
D	1	232.5625	232.5625	299.01**	"	"
AB	3	3.185	1.0617	1.37	2.86	4.38
AC	3	0.9975	0.3325	0.43	"	"
AD	3	66.185	22.0617	28.37**	"	"
BC	1	2.25	2.25	2.89	4.11	7.39
BD	1	0.0625	0.0625	0.08	"	"
CD	1	2.25	2.25	2.89	"	"
ABC	3	8.7525	2.9175	3.75*	2.86	4.38
ABD	3	2.69	0.8967	1.15	"	"
ACD	3	0.7525	0.2508	0.32	"	"
Error	36	28.0	0.7778			
Total	63	955.44				

* Significantly different at $P < 0.05$

** Significantly different at $P < 0.01$

Table 8 Contd...

Treatment^{xx}means of A

1. a_0 - 11.625
2. a_1 - 6.625
3. a_2 - 4.875
4. a_3 - 3.5

Calculated C.D. value (t 0.05) = 0.8031

Treatment^{xx} means of AD

1. a_0d_1 - 15.25
2. a_1d_1 - 8.25
3. a_0d_0 - 8.0
4. a_2d_1 - 6.25
5. a_1d_0 - 5.0
6. a_3d_1 - 4.5
7. a_2d_0 - 3.5
8. a_3d_0 - 2.5

Calculated C.D. value (t 0.05) = 0.8643

Treatment^{xx} means of ABC

1. $a_0b_0c_1$ - 12.75
2. $a_0b_1c_0$ - 12.0
3. $a_0b_0c_0$ - 11.25
4. $a_0b_1c_1$ - 10.5
5. $a_1b_0c_0$ - 7.25
6. $a_1b_0c_1$ - 6.75
7. $a_1b_1c_1$ - 6.5
8. $a_1b_1c_0$ - 6.0
9. $a_2b_1c_0$ - 5.5
10. $a_2b_0c_0$ - 4.75
11. $a_2b_0c_1$ - 4.75
12. $a_2b_1c_1$ - 4.5
13. $a_3b_0c_0$ - 3.75
14. $a_3b_1c_0$ - 3.75
15. $a_3b_0c_1$ - 3.25
16. $a_3b_1c_1$ - 3.25

Calculated C.D. value (t 0.05) = 1.2223

Table 8 Contd..

x	A	-	packing density
	B	-	sedation
	C	-	Salinity
	D	-	Temperature
xx	a_0	-	200/l
	a_1	-	400/l
	a_2	-	600/l
	a_3	-	800/l
	b_0	-	without chloral hydrate treatment
	b_1	-	with chloral hydrate treatment
	C_0	-	25 ppt salinity
	C_1	-	20 ppt salinity
	d_0	-	29±1°C
	d_1	-	23±2°C

Table 8 Concl.

Table 9. Analysis of variance in the time of initial mortality of P.monodon seed without chloral hydrate treatment under different levels of packing density, salinity and temperature.

Source[x]	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	Tabular 0.05	Tabular 0.01
A	3	337.375	112.4583	128.52**	3.24	5.29
C	1	0.125	0.125	0.14	4.49	8.53
D	1	120.125	120.125	137.29**	"	"
AC	3	5.375	1.7917	2.05	3.24	5.29
AD	3	30.375	10.125	11.57**	"	"
CD	1	1.125	1.125	1.29	4.49	8.53
ACD	3	0.375	0.125	0.14	2.91	5.29
Error	16	14	0.875			
Total	31	508.875				

** Significantly different at $P < 0.01$

Treatment[xx] means of A

1. a_0 - 12.0
2. a_1 - 7.0
3. a_2 - 4.75
4. a_3 - 3.5

Calculated C.D. value (t 0.05) = 0.9915

Table 9 Contd..

Treatment[xx] means of AD

1.	a_0d_1	-	15.5
2.	a_1d_1	-	9.0
3.	a_0d_0	-	8.5
4.	a_2d_1	-	6.0
5.	a_1d_0	-	5.0
6.	a_3d_1	-	4.5
7.	a_2d_0	-	3.5
8.	a_3d_0	-	2.5

Calculated C.D. value (t 0.05) = 1.4022

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x and xx - See foot note under Table 8.

Table 9 Concl.

was insignificant ($P > 0.05$). The analysis of variance for the exclusive data on the time of initial mortality (h) without chloral hydrate treatment also showed similar results (Table-9). However, the pair-wise comparison for interaction AD exhibits the highly significant influence of temperature on the time of intial mortality which is evident from Table-9. No significant difference was observed between a_0d_0 and a_1d_1, a_1d_0 and a_2d_1, a_1d_0 and a_3d_1, a_2d_0 and a_3d_1, and a_2d_0 and a_3d_0.

4.3.2. Percentage survival

The cumulative percentage of survival which is the most important index in evaluating the success of seed transport, has been found to have influenced by all the factors combined in the experiment. The analysis of variance for the data on cumulative percentage of mortality showed highly significant difference ($P < 0.01$) amongst the different levels of packing density, sedation and temperature (Table-11). The mean values of the cumulative percentage survival are depicted in Table-10 and Fig. 6 to 9. The pair-wise comparison for packing density revealed that no significant difference existed between the two higher packing densities of 600/l and 800/l. Eventhough the main effects of packing density, sedation and temperature altered the cumulative percentage of survival significantly only two interactions, i.e., AD (packing density-temperature) and BD (sedation-temperature) exhibited significant difference ($P < 0.05$). The pair-wise comparison for AD showed uniformity within the pairs of a_2d_1 and a_3d_1, and a_2d_0 and

Table 10. Cumulative percentage survival of P.monodon seed without and with treatment of chloral hydrate under different levels of packing density, salinity and temperature at 24 hours

Chloral hydrate treatment	Salinity (ppt)	Temperature (°C)	Cumulative percentage survival* at four different packing densities			
			200/l	400/l	600/l	800/l
Without treatment	25	29±1	65.0	61.25	59.17	58.13
		23±2	92.5	87.5	81.7	83.13
	20	29±1	72.5	61.25	55.85	51.88
		23±2	90.0	08.75	79.15	80.00
With treatment (400 ppm)	25	29±1	50.0	46.25	40.85	36.88
		23±2	72.5	71.25	56.7	56.25
	20	29±1	45.6	41.25	35.8	30.0
		23±2	77.5	71.25	50.85	53.75

* Each value is a mean of duplicates.

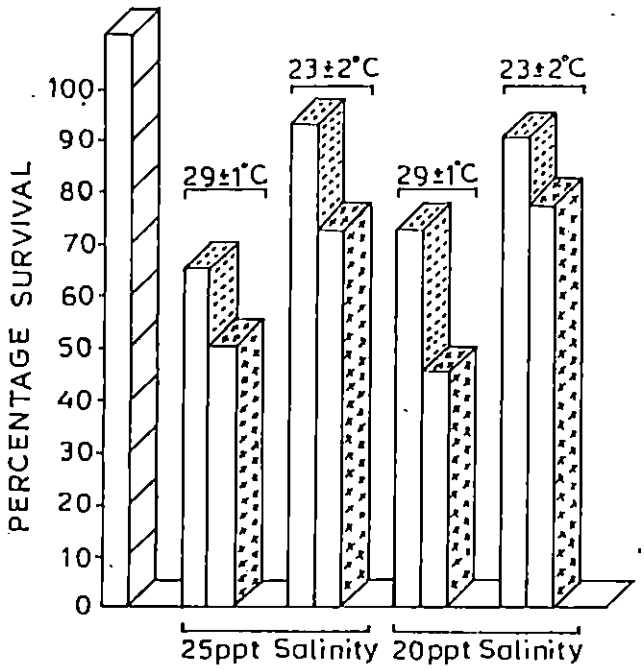

Fig. 6 200/l

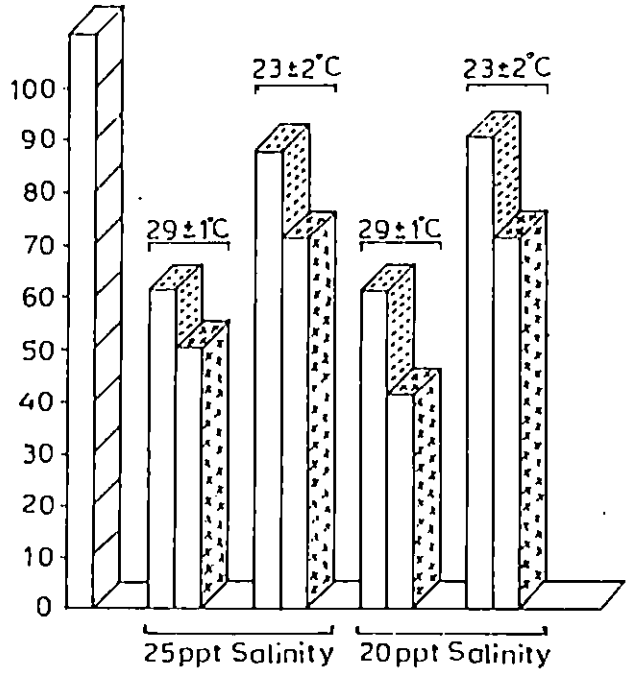

Fig. 7 400/l

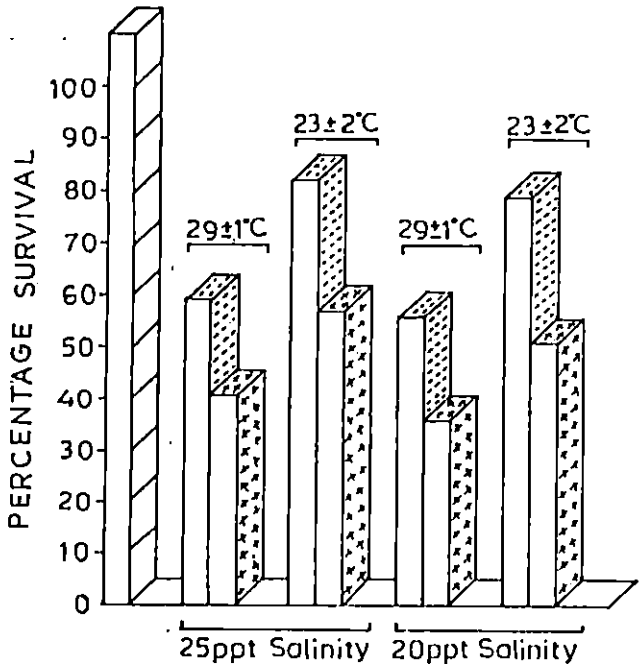

Fig. 8 600/l

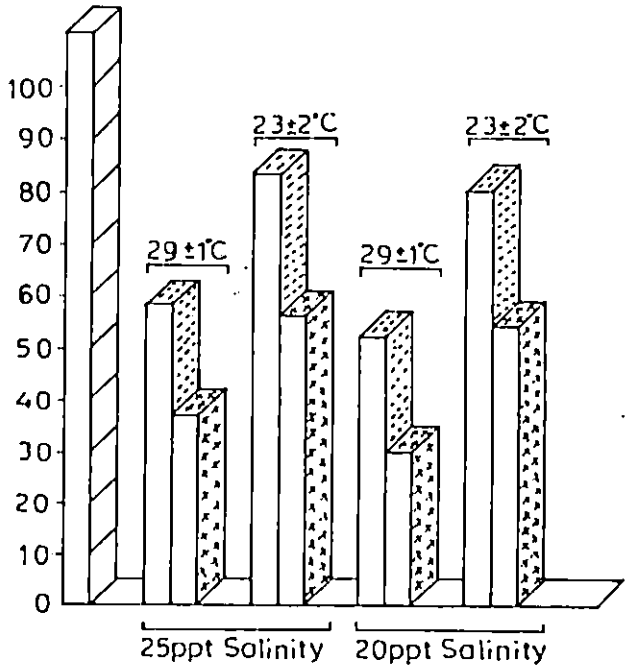

Fig. 9 800/l

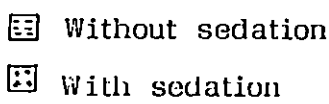

Fig. 6-9 Mean percentage survival of P. monodon seed at four different packing densities without and with the application of chloral hydrate under two different salinities and temperatures at 24 hours.

Table 11. Analysis of variance in the cumulative percentage mortality of P.monodon seed without and with chloral hydrate treatment under different levels of packing density, salinity and temperature at 24 hours.

Source [x]	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	Tabular 0.05	Tabular 0.01
A	3	947.0523	315.6841	47.6**	2.9	4.46
B	1	2750.8714	2750.8714	414.78**	4.15	7.50
C	1	27.1051	27.1051	4.09	"	"
D	1	3651.6338	3651.6338	550.60**	"	"
AB	3	26.6478	8.8826	1.34	2.9	4.46
AC	3	33.7324	11.2441	1.70	"	"
AD	3	76.8317	25.6106	3.86*	"	"
BC	1	4.259	4.259	0.64	4.15	7.50
BD	1	37.684	37.684	5.68*	"	"
CD	1	2.9886	2.9886	0.45	"	"
ABC	3	2.6552	0.8851	0.13	2.9	4.46
ABD	3	14.9953	4.9984	0.75	"	"
ACD	3	6.0779	2.0260	0.31	"	"
BCD	1	16.1305	16.1305	2.43	4.15	7.50
ABCD	3	31.5093	10.5031	1.58	2.9	4.46
Error	32	212.229	6.6322			
Total	63	7842.972				

* Significantly different at $P < 0.05$

** Significantly different at $P < 0.01$

Table 11 Contd..

Treatment^{xx} means of A

1. a_0 - 31.8443
2. a_1 - 34.8731
3. a_2 - 40.3669
4. a_3 - 41.9375

Calculated C.D. value (t 0.05) = 1.7846

Treatment^{xx} means of AD

1. a_0d_1 - 23.4988
2. a_1d_1 - 26.20
3. a_3d_1 - 33.7563
4. a_2d_1 - 34.5088
5. a_0d_0 - 40.19
6. a_1d_0 - 43.5463
7. a_2d_0 - 46.225
8. a_3d_0 - 48.4313

Calculated C.D. value (t 0.05) = 2.5238

Treatment^{xx} means of BD

1. b_0d_1 - 22.1675
2. b_1d_1 - 36.8148
3. b_0d_0 - 38.8094
4. b_1d_0 - 50.3869

Calculated C.D. value (t 0.05) = 1.7846

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x and xx See foot note under Table 8.

Table 11 Concl.

Table 12. Analysis of variance in the cumulative percentage mortality of P.monodon seed without chloral hydrate treatment under different levels of packing density, salinity and temperature at 24 hours.

Source [x]	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	F value Tabular 0.05	F value Tabular 0.01
A	3	366.9941	122.3314	28.31**	3.24	5.29
C	1	4.9376	4.9376	1.14	4.49	8.53
D	1	2215.616	2215.616	512.82**	"	"
AC	3	21.907	7.3023	1.69	3.24	5.29
AD	3	16.1562	5.3854	1.25	"	"
CD	1	2.6164	2.6164	0.61	4.49	8.53
ACD	3	26.6932	8.8977	2.06	3.24	5.29
Error	16	69.127	4.3204			
Total	31	2722.0474				

** Significantly different at $P < 0.01$

Treatment[xx] means of A

a_0 - 25.495

a_1 - 29.2593

a_2 - 33.4375

a_3 - 33.7675

Calculated C.D. value (t 0.05) = 2.2033

x and xx see foot note under Table 8.

$a_3 d_0$. Pair-wise comparison for BD showed significant difference between all the combinations. ANOVA for the exclusive data on the cumulative percentage mortality without chloral hydrate treatment, however, revealed that all the interactions were insignificant ($P > 0.05$) (Table 12.).

4.3.3. Ammonia excretion

The initial and final values of total ammonia in the oxygen-packed jars expressed as ammonia-N (NH_3-N and NH_4^+-N) are given in the Table-13. The variation in ammonia excretion by the prawns without and with sedation under various combinations is depicted in Table-14, Fig. 10 and 11. The data on ammonia excretion when subjected to analysis of variance indicated that the excretion increased significantly ($P < 0.01$) with increase in packing density, temperature as well as with sedation (Table-15). The two levels of salinity studied showed only insignificant ($P > 0.05$) difference. Pair-wise comparison for packing density showed that ammonia excretion for all the four packing densities differed significantly ($P < 0.01$) with one another. The interaction of AB and AD also exhibited significant ($P < 0.01$) variation. Pair-wise comparison for AB showed uniformity between the interactions a_0b_1 and a_1b_0, a_1b_1 and a_2b_0, and a_3b_0 and a_3b_1. However, pair-wise comparison for AD revealed that significant difference existed between all the combinations except a_2d_1 and a_1d_0.

ANOVA for the exclusive data on the ammonia excretion without sedation also exhibited significant difference ($P < 0.01$) within the

Table 13. Initial and final (at 24 hours) values of ammonia-N in the oxygen-packed jars without and with chloral hydrate treatment under different levels of packing density, salinity and temperature.

Chloral hydrate treatment	Salinity (ppt)	Temperature (°C)	Ammonia-N values* (ppm)					
			Initial values	Final values at different packing densities				
				0/l	200/l	400/l	600/l	800/l
Without treatment	25	29 ±1	0.38	2.54	12.43	14.65	18.78	22.08
		23±2	1.04	3.42	10.04	12.69	16.32	19.66
	20	29±1	0.38	2.54	12.01	14.85	19.01	24.07
		23±2	1.04	3.42	10.70	13.54	16.16	20.74
With treatment (400 ppm)	25	29±1	0.38	2.54	14.23	19.66	21.85	23.19
		23±2	1.04	3.42	13.12	15.70	18.78	19.66
	20	29±1	0.38	2.54	13.77	17.99	21.43	23.85
		23±2	1.04	3.42	14.00	16.39	17.93	19.66

* Each value is a mean of duplicates.

Table 14. Ammonia-N excretion by P.monodon seed, without and with chloral hydrate treatment under different levels of packing density, salinity and temperature , for 24 hours

Chloral hydrate treatment	Salinity (ppt)	Temperature (°C)	Ammonia-N (ppm)* excreted by P.monodon seed at four different packing densities			
			200/l	400/l	600/l	800/l
Without treatment	25	29±1	9.90	12.12	16.24	19.54
		23±2	6.63	9.28	12.91	16.25
	20	29±1	9.47	12.31	16.47	21.28
		23±2	7.28	10.13	12.75	17.33
With treatment (400 ppm)	25	29±1	11.69	17.13	19.31	20.66
		23±2	9.71	12.29	15.36	16.25
	20	29±1	11.23	15.39	18.86	21.31
		23±2	10.59	12.98	14.51	16.25

* Each value is a mean of duplicates

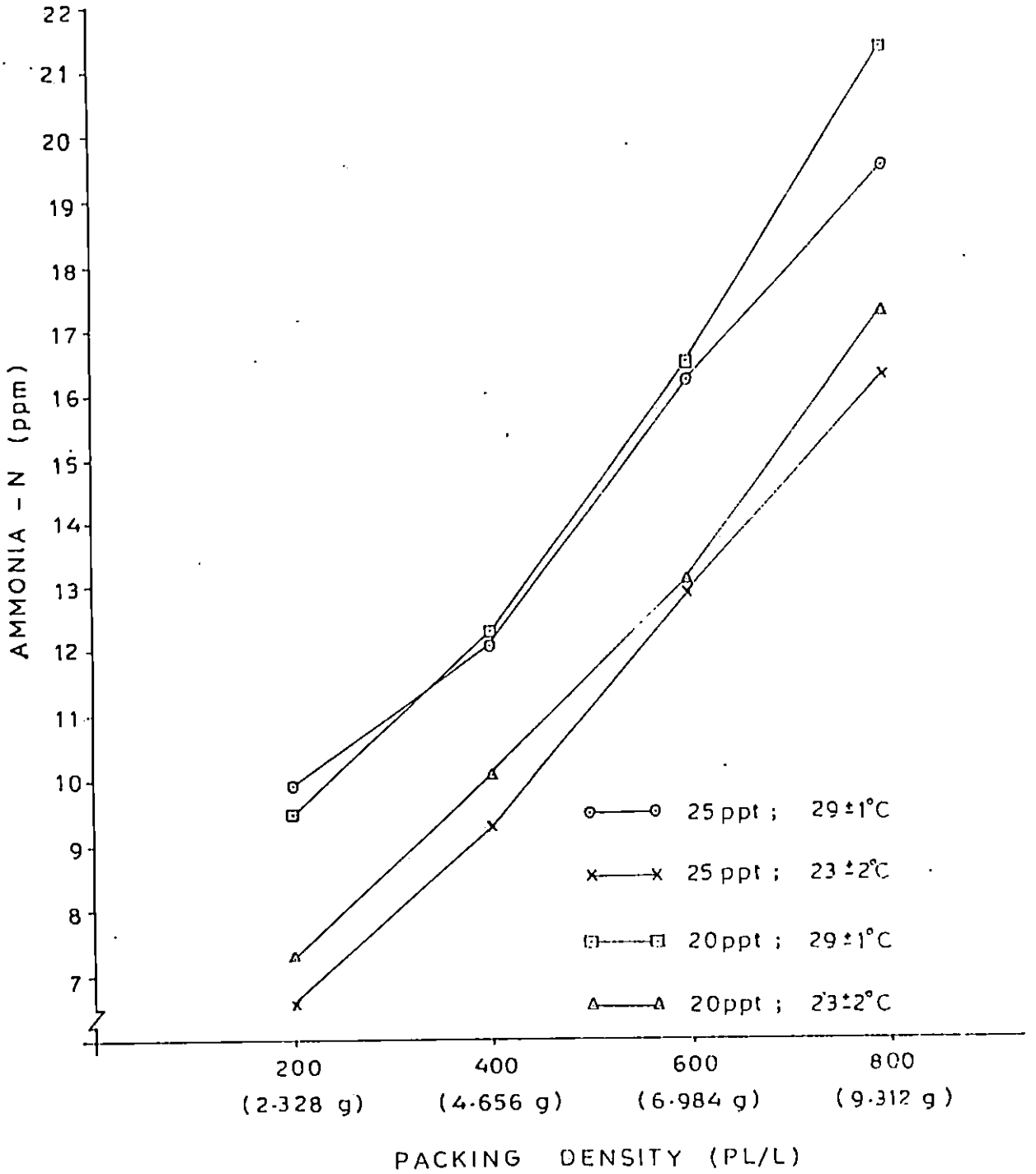

Fig. 10 Ammonia excretion by P. monodon seed without the application of chloral hydrate at four different packing densities under two different salinities and temperatures, for 24 hours.

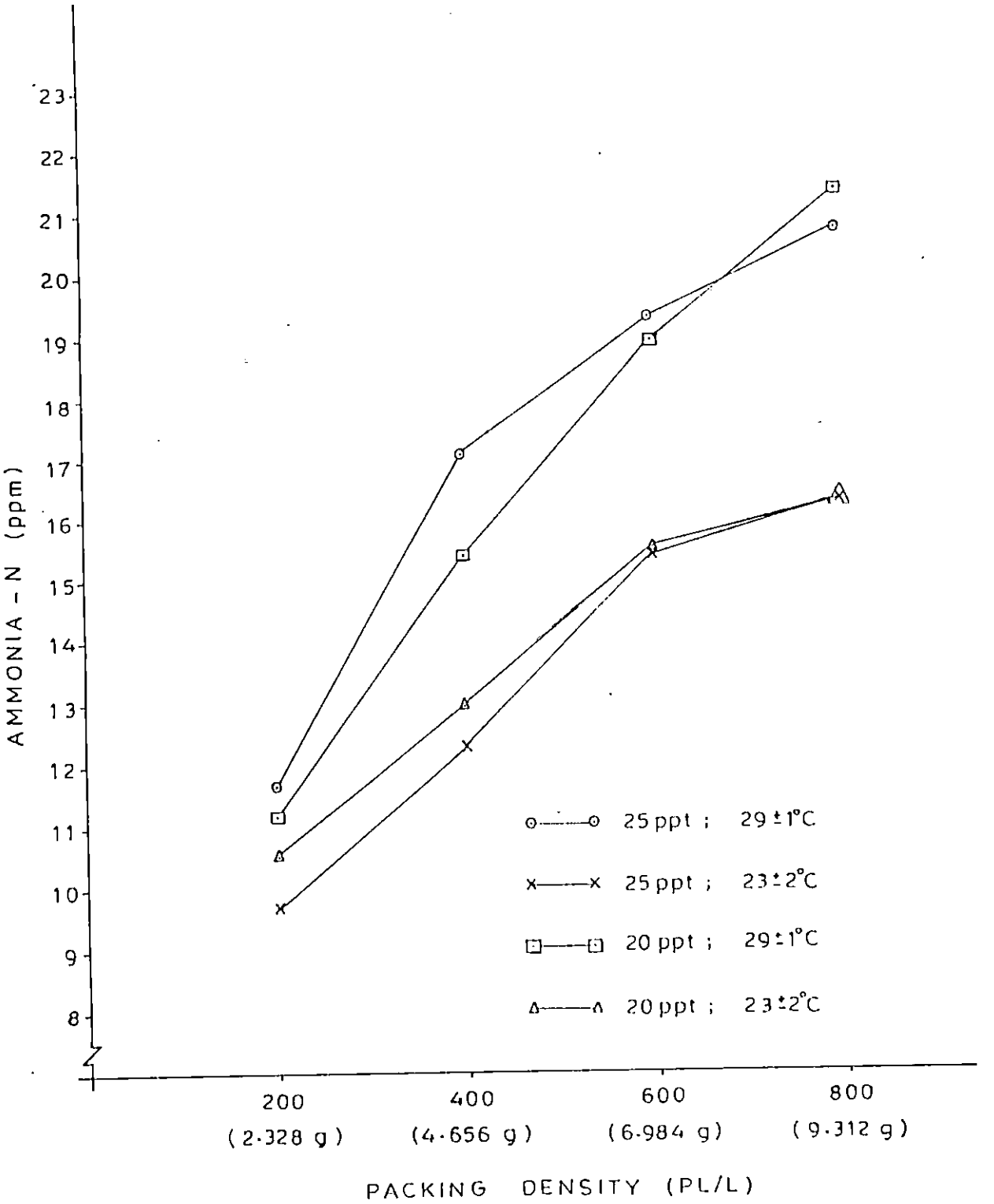

Fig. 11 Ammonia excretion by P. monodon seed with the application of chloral hydrate at four different packing densities under two different salinities and temperatures, for 24 hours.

Table.15. Analysis of variance in the ammonia excretion by P.monodon seed without and with chloral hydrate treatment under different levels of packing density, salinity and temperature for 24 hours.

Source[x]	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	Tabular 0.05	Tabular 0.01
A	3	731.766	243.922	352.50**	2.87	4.40
B	1	70.6436	70.6436	102.09**	4.12	7.42
C	1	0.518	0.518	0.75	"	"
D	1	171.7506	171.7506	248.20**	"	"
AB	3	26.0167	8.6722	12.53**	2.87	4.40
AC	3	2.9856	0.9952	1.44	"	"
AD	3	10.9432	3.6477	5.27**	"	"
BC	1	1.8432	1.8432	2.66	4.12	7.42
BD	1	0.4361	0.4361	0.63	"	"
CD	1	0.6474	0.6474	0.94	"	"
ABC	3	0.9049	0.3016	0.44	2.87	4.40
ABD	3	4.4511	1.4837	2.14	"	"
ACD	3	4.4603	1.4868	2.15	"	"
BCD	1	0.3327	0.3327	0.48	4.12	7.42
Error	35	24.219	0.692			
Total	63	1051.6988				

** Significantly different at $P < 0.01$

Table 15 contd..

Treatment^{xx} means of A

1. a_0 - 9.56
2. a_1 - 12.7013
3. a_2 - 15.80
4. a_3 - 18.605

Calculated C.D. value (t 0.05) = 0.5764

Treatment^{xx}means of AB

1. a_0b_0 - 8.3175
2. a_0b_1 - 10.8025
3. a_1b_0 - 10.9588
4. a_1b_1 - 14.4438
5. a_2b_0 - 14.5913
6. a_2b_1 - 17.0088
7. a_3b_0 - 18.5963
8. a_3b_1 - 18.6138

Calculated C.D. value (t 0.05) = 0.8152

Treatment^{xx}means of AD

1. a_0d_1 - 8.55
2. a_0d_0 - 10.57
3. a_1d_1 - 11.6875
4. a_2d_1 - 13.8813
5. a_1d_0 - 14.2338
6. a_3d_1 - 16.515
7. a_2d_0 - 17.7188
8. a_3d_0 - 20.695

Calculated C.D. value (t 0.05) = 0.8152

---

x and xx see foot note under Table 8.

Table 15 concl.

Table 16. Analysis of variance in the ammonia excretion by P.monodon seed without chloral hydrate treatment under different levels of packing density, salinity and temperature for 24 hours.

Source[x]	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	Tabular 0.05	Tabular 0.01
A	3	479.1108	159.7036	265.81**	3.24	5.29
C	1	2.1581	2.1581	3.59	4.49	8.53
D	1	76.7252	76.7252	127.70**	"	"
AC	3	2.3786	0.7929	1.32	3.24	5.29
AD	3	1.8872	0.6291	1.05	"	"
CD	1	0.0586	0.0586	0.098	4.49	8.53
ACD	3	1.0317	0.3439	0.57	3.24	5.29
Error	16	9.613	0.6008			
Total	31	572.9631				

** Significantly different at $P < 0.01$

Treatment[xx] means of A

1. a_0 - 8.3175
2. a_1 - 10.9588
3. a_2 - 14.5913
4. a_3 - 18.5963

Calculated C.D. value (t 0.05) = 0.8216

x and xx see foot note under Table 8.

different levels of packing density and temperature, but all interactions were found insignificant ($P > 0.05$) as seen in Table 16.

4.3.4. Oxygen consumption

The initial and final dissolved oxygen values in the oxygen-packed jars are given in Table-17. The variation in oxygen consumption by the prawn seed without and with sedation under various combinations are presented in Table-18, Fig. 12 and 13. As the replicate values of dissolved oxygen were not available, the analysis of variance could not be done. From the data obtained, it is clear that the oxygen consumption increased with increase in packing density. The relation, however, is not linear. For a change in packing density from 400/l to 600/l, the increase in oxygen consumption was more than that for a change from 200/l to 400/l or from 600/l to 800/l. The chloral hydrate-treated prawns were found to consume more oxygen than the untreated prawns. However, among the control jars (without prawns) higher dissolved oxygen levels were observed in chloral hydrate-treated jars than in untreated jars, at the end of 24 hours. The effect of salinity on oxygen consumption is in conformation with the trend exhibited by the cumulative percentage of survival i.e., at lower packing densities (200/l and 400/l) the oxygen consumption was lower at 20 ppt salinity than at 25 ppt and at higher packing densities (600/l and 800/l) it was higher at 20 ppt salinity than at 25 ppt. However, the effect was not so apparent as that of sedation and temperature. At lowered temperature, considerable reduction in the oxygen consumption was observed (Fig. 12 and 13). The dissolved oxygen

Table 17. Initial and final (at 24 hours) values of dissolved oxygen in the oxygen-packed jars without and with chloral hydrate treatment under different levels of packing density, salinity and temperature

Chloral hydrate treatment	Salinity (ppt)	Temperature (°C)	Dissolved oxygen values (ppm)					
			Initial values	Final values at different packing densities				
				0/l	200/l	400/l	600/l	800/l
Without treatment	25	29±1	19.41	16.75	13.77	11.11	3.65	1.99
		23±2	19.91	17.42	15.43	12.44	6.64	4.81
	20	29±1	20.57	19.41	16.59	13.77	5.64	4.48
		23±2	20.90	18.41	16.09	13.77	7.13	5.64
With treatment (400 ppm)	25	29±1	19.41	17.09	13.44	10.78	2.49	1.00
		23±2	19.91	18.08	15.42	12.94	5.14	3.32
	20	29±1	20.57	18.75	14.76	12.44	4.31	1.99
		23±2	20.90	20.24	17.25	15.10	7.13	5.31

Table 18. Oxygen consumption by P.monodon seed, without and with chloral hydrate treatment under different levels of packing density, salinity and temperature, for 24 hours

Chloral hydrate treatment	Salinity (ppt)	Temperature (°C)	Oxygen (ppm) consumed by P.monodon seed at four different packing densities			
			200/l	400/l	600/l	800/l
Without treatment	25	29±1	2.98	5.64	13.10	14.76
		23±2	1.99	4.98	10.78	12.61
	20	29±1	2.82	5.64	13.77	14.93
		23±2	2.32	4.64	11.28	12.77
With treatment (400 ppm)	25	29±1	3.65	6.31	14.60	16.09
		23±2	2.66	5.14	12.94	14.76
	20	29±1	3.99	6.31	14.44	16.84
		23±2	2.99	5.14	13.11	14.93

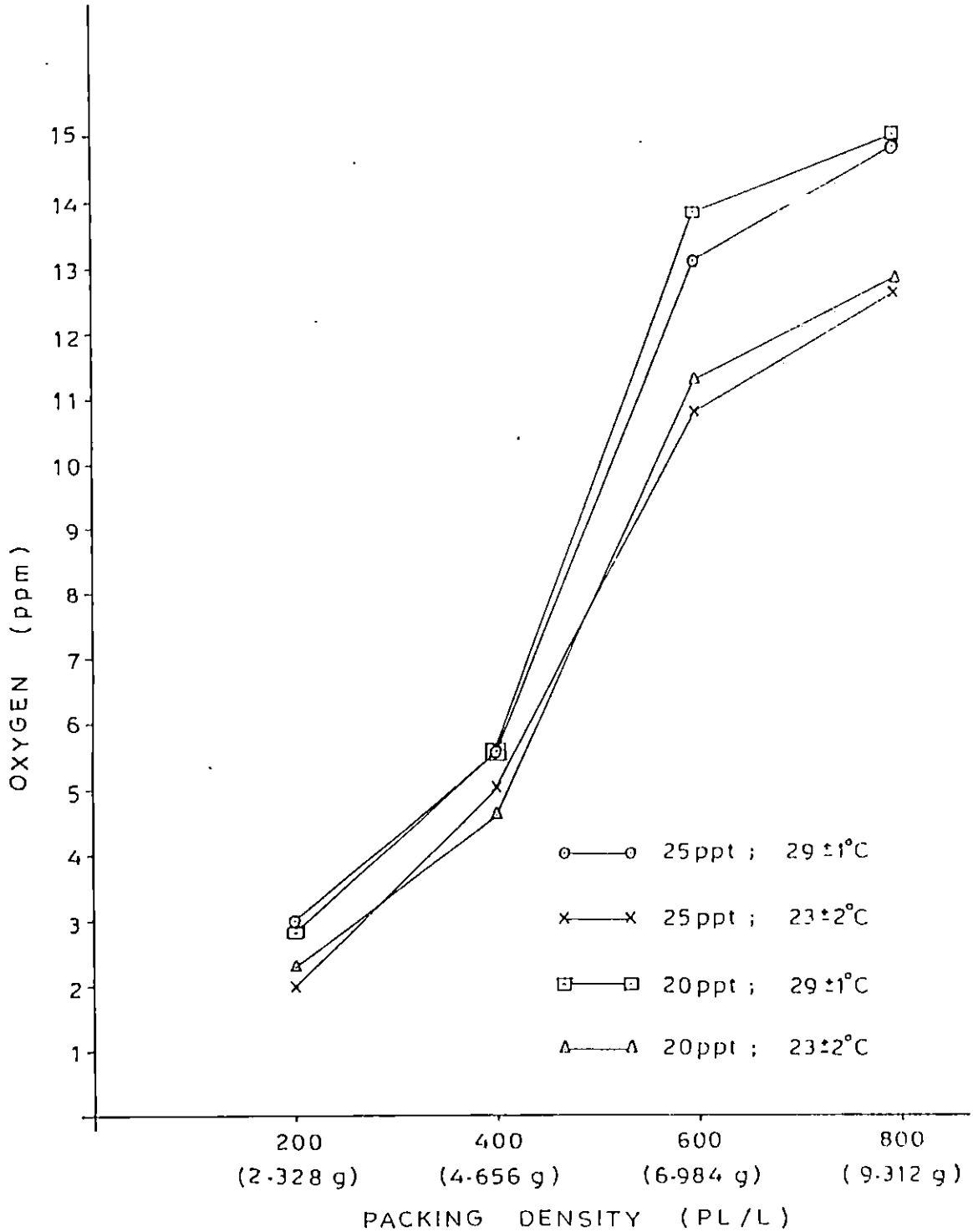

Fig. 12 Oxygen consumption by P. monodon seed without the application of chloral hydrate at four different packing densities under two different salinities and temperatures for 24 hours.

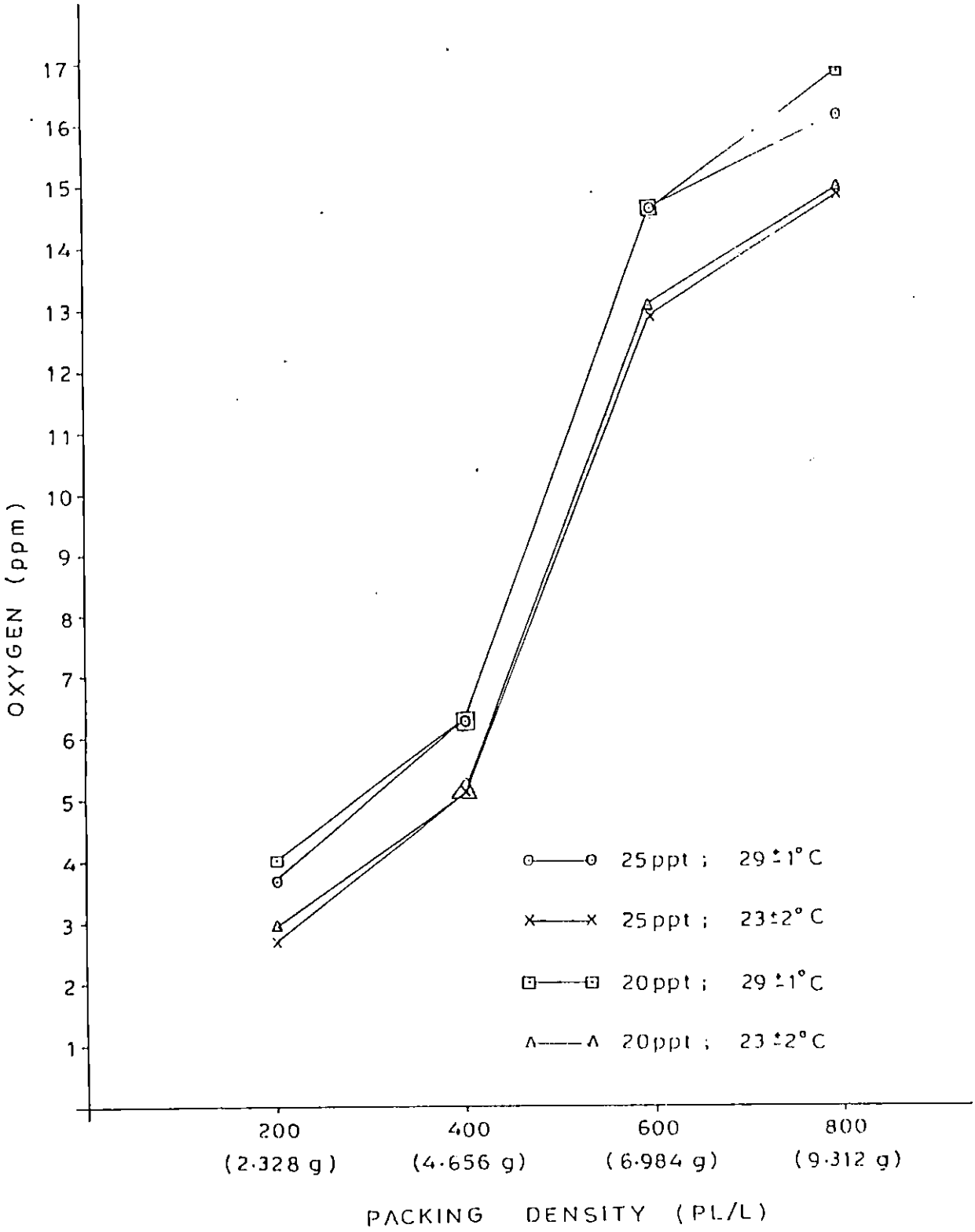

Fig. 13 Oxygen consumption by P.monodon seed with the application of chloral hydrate at four different packing densities under two different salinities and temperatures for 24 hours.

values. in the jars at the lowered temperature were not depleted considerably even at higher packing densities during the 24 hour period.

4.3.5. Carbon dioxide excretion

The initial and final values of free carbon dioxide in the oxygen-packed jars are given in Table-19. Table-20, Fig. 14 and 15 exhibit the variation in carbon dioxide excretion by post-larvae without and with sedation under various combinations. The analysis of variance could not be done as the replicate values were not available. Similar to oxygen consumption, increase in packing density and application of chloral hydrate increase the carbon dioxide excretion. Reduction of salinity from 25 to 20 ppt appears to have an insignificant effect on carbon dioxide excretion as is clear from Fig. 14 and 15. The data indicate that the final carbon dioxide values in jars at the ambient and lowered temperatures had not shown remarkable difference at packing densities of 800/l and 600/l when compared to that at 400/l, 200/l and 0/l. In controls (0/l), the carbon dioxide level was found to increase on addition of chloral hydrate.

4.3.6. pH

In none of the experimental jars the pH had gone below the neutral point, 7.0. The initial pH of the water used for packing was 7.5. In all the oxygen-packed jars, the pH ranged between 7.0 and 7.5. As the pH was measured using universal indicator solution the exact values between 7.0 and 7.5 could not be recorded.

Table 19. Initial and final (at 24 hours) values of free carbon dioxide in the oxygen-packed jars without and with chloral hydrate treatment under different levels of packing density, salinity and temperature

Chloral hydrate treatment	Salinity (ppt)	Temperature (°C)	Free carbon dioxide values (ppm)					
			Initial values	Final values at different packing densities				
				0/l	200/l	400/l	600/l	800/l
Without treatment	25	29±1	nil	0.22	3.96	7.48	12.54	14.96
		23±2	nil	0.22	3.30	6.60	12.32	15.40
	20	29±1	nil	0.22	3.96	7.48	12.76	14.96
		23±2	nil	0.22	3.52	6.60	12.98	15.40
With treatment (400 ppm)	25	29±1	nil	1.54	5.50	9.46	16.06	18.04
		23±2	nil	1.54	5.06	8.36	16.28	18.04
	20	29±1	nil	1.32	5.28	9.02	15.84	18.04
		23±2	nil	1.54	5.06	8.36	16.28	18.26

Table 20. Free carbon dioxide excreted by P.monodon seed without and with chloral hydrate treatment under different levels of packing density, salinity and temperature for 24 hours.

Chloral hydrate treatment	Salinity (ppt)	Temperature (°C)	Free carbon dioxide (ppm) excreted by P.monodon seed at four different packing denisities			
			200/l	400/l	600/l	800/l
Without treatment	25	29±1	3.74	7.26	12.32	14.74
		23±2	3.08	6.38	12.10	15.18
	20	29±1	3.74	7.26	12.54	14.74
		23±2	3.30	6.38	12.76	15.18
With treatment (400 ppm)	25	29±1	3.96	7.92	14.52	16.50
		23±2	3.52	6.82	14.74	16.50
	20	29±1	3.96	7.70	14.52	16.72
		23±2	3.52	6.82	14.52	16.72

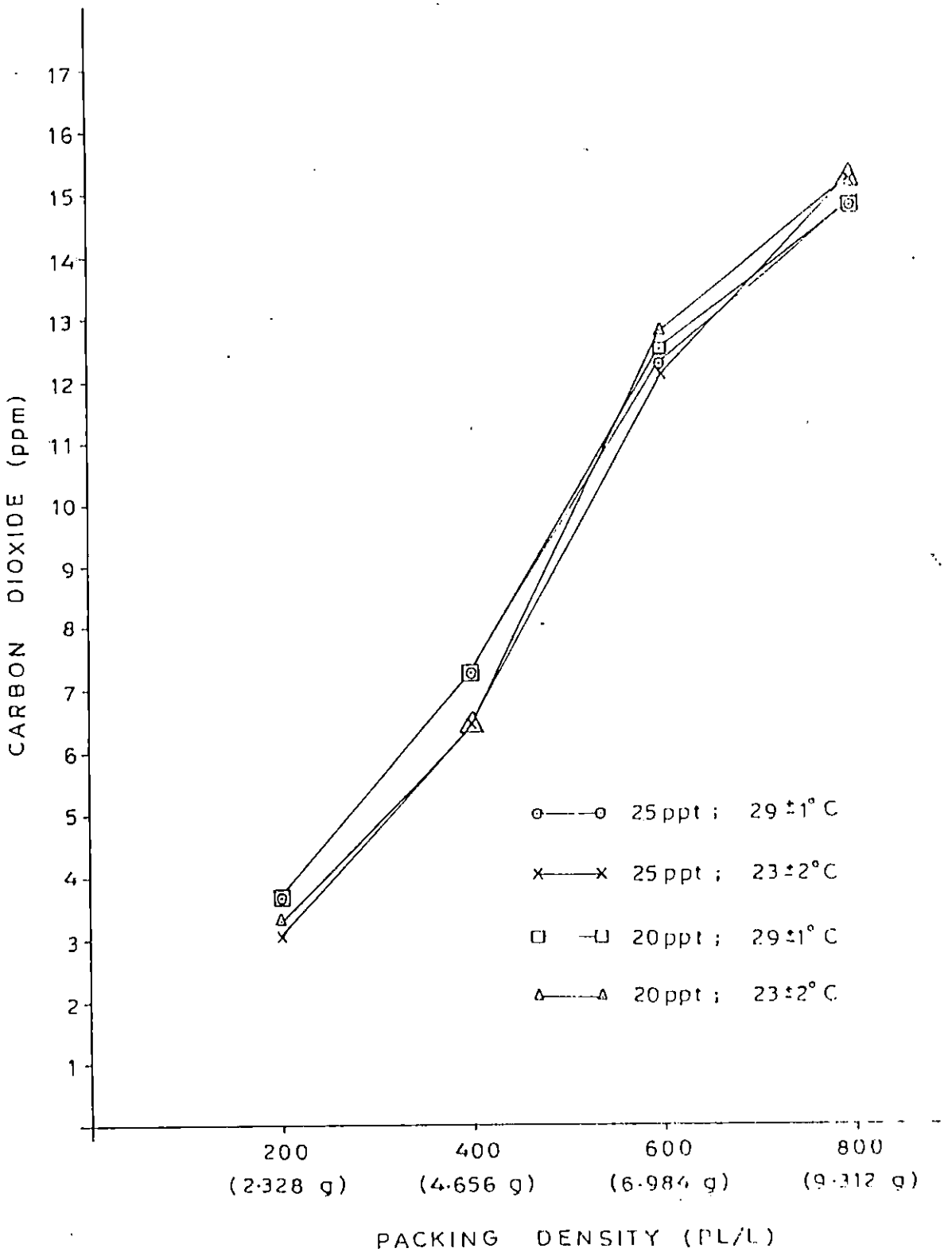

Fig. 14 Free carbon dioxide excretion by P. monodon seed without the application of chloral hydrate at four different packing densities under two different salinities and temperatures for 24 hours.

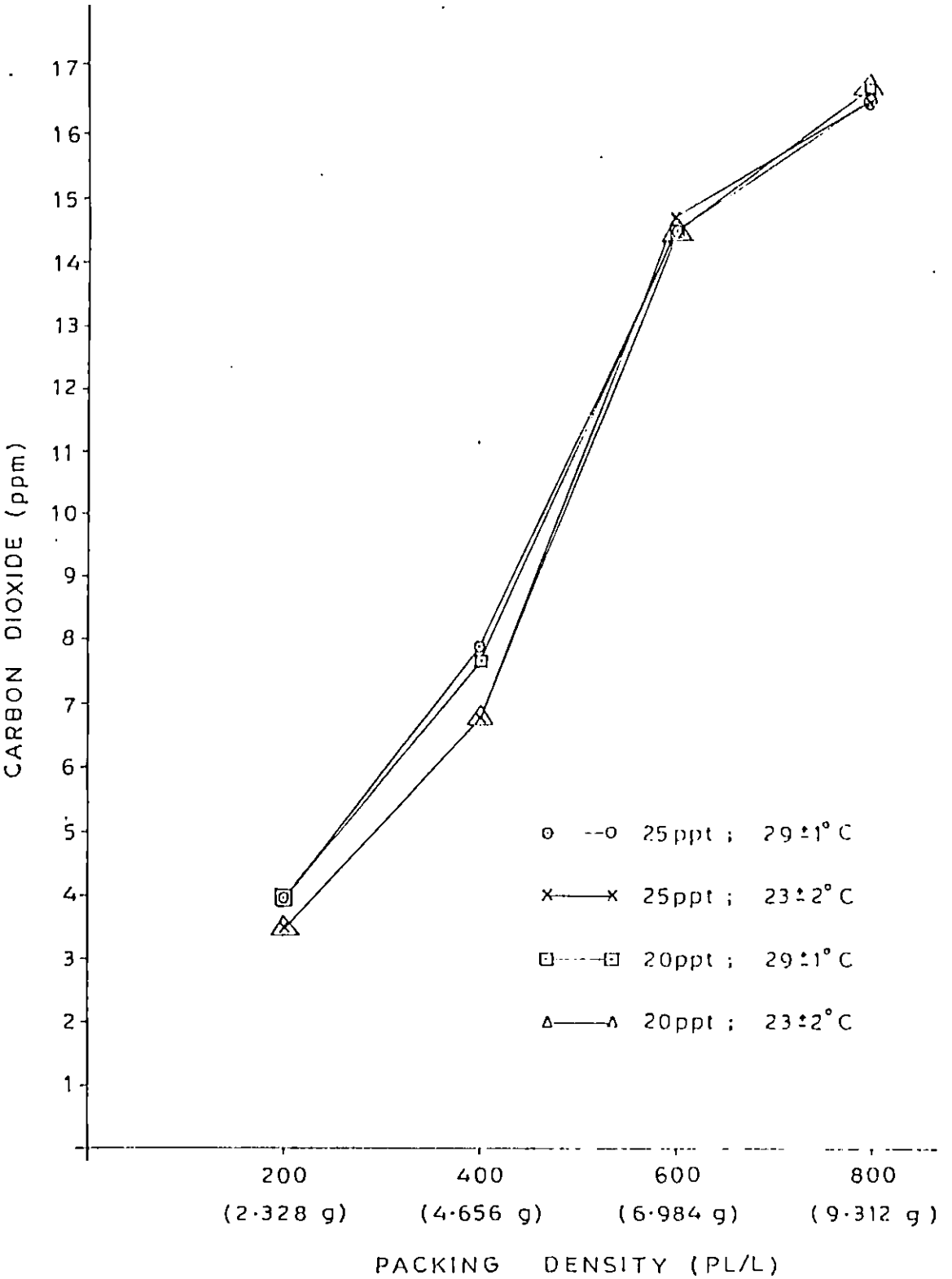

Fig. 15. Free carbon dioxide excretion by P.monodon seed with the application of chloral hydrate at four different packing densities under two different salinities and temperatures for 24 hours.

4.3.7. Bacterial population

The bacterial population in the oxygen-packed jars at the beginning and end of the experiment are presented in Table-21. As the two water samples used for the experiments i.e., one at the ambient and the other at the lowered temperature, had different initial bacterial counts, a comparison of the final bacterial population at the two temperatures became baseless. Hence, analysis of variance of the bacterial population was done separately for the ambient and lowered temperatures. The initial bacterial plate count at the ambient temperature was 5.30×10^3 /ml at both the salinities tried, whereas that at the lowered temperature was 5.58×10^4 and 4.77×10^4/ml for 25 ppt and 20 ppt salinity respectively. Because of this high initial bacterial count of the packing medium kept at the lowered temperature, more prolific multiplication took place which resulted in a higher final count than that at the ambient temperature, except at 800/l. The final bacterial count at the packing density of 800/l at the lowered temperature was lower than that at the ambient temperature.

The analysis of variance for the data on bacterial population at the ambient temperature showed significant difference ($P < 0.01$) at different levels of packing density and without and with sedation (Table-22). The main effect of salinity and all interaction effects on bacterial population were insignificant ($P > 0.05$). Pair-wise comparison for packing density revealed that all the four packing densities differed in their bacterial count significantly ($P < 0.05$) with one another. At the lowered temperature the bacterial population was significantly

Table 21. Initial and final (at 24 hours) counts of bacterial population in the oxygen-packed jars without and with chloral hydrate treatment under different levels of packing density, salinity and temperature

Temperature (°C)	Salinity (ppt)	Chloral hydrate treatment	Bacterial population/ml* of packing medium					
			Initial values	Final values at different packing densities				
				0/l	200/l	400/l	600/l	800/l
29±1	25	Absent	5.3×10^3	3.67×10^4	9.41×10^4	5.195×10^5	8.4097×10^6	9.2665×10^7
		Present	5.3×10^3	2.1×10^4	6.478×10^5	6.6621×10^6	1.724×10^7	3.9664×10^8
	20	Absent	5.3×10^3	8.9×10^3	4.67×10^4	1.6059×10^6	9.417×10^6	1.0411×10^8
		Present	5.3×10^3	8.8×10^3	1.252×10^6	9.2093×10^6	1.7872×10^7	4.3136×10^8
23±2	25	Absent	5.58×10^4	1.52×10^5	2.14×10^6	5.8385×10^6	1.8129×10^7	2.7707×10^7
		Present	5.58×10^4	1.158×10^5	8.487×10^5	3.2882×10^6	1.6466×10^7	2.5895×10^8
	20	Absent	4.77×10^4	1.413×10^5	1.45×10^6	8.511×10^6	1.8216×10^7	3.5254×10^7
		Present	4.77×10^4	1.066×10^5	5.578×10^5	4.2617×10^6	1.692×10^7	2.9895×10^8

* Each value is a mean of duplicates

Table 22. Analysis of variance in the bacterial population of the packing medium without and with chloral hydrate treatment under different levels of packing density and salinity at ambient temperature of 29±1°C at 24 hours.

Source[x]	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	F value Tabular 0.05	F value Tabular 0.01
A	3	33.2954	11.098	60.16**	3.24	5.29
B	1	4.2035	4.2035	22.78**	4.49	8.53
C	1	0.1812	0.1812	0.98	"	"
AB	3	0.738	0.246	1.33	3.24	5.29
AC	3	0.2718	0.0906	0.49	"	"
BC	1	0.0115	0.0115	0.06	4.49	8.53
ABC	3	0.2823	0.0941	0.51	3.24	5.29
Error	16	2.9518	0.1845			
Total	31	41.9355				

** Significantly different at $P < 0.01$

Treatment[xx] means of A

1. a_0 - 5.3535
2. a_1 - 6.3181
3. a_2 - 7.0343
4. a_3 - 8.1464

Calculated C.D. value (t 0.05) = 0.4553

x and xx see foot note under Table 8.

Table 23. Analysis of variance in the bacterial population of the packing medium without and with chloral hydrate treatment under different levels of packing density and salinity at lowered temperature of 23±2°C at 24 hours

Source[x]	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	Tabular 0.05	Tabular 0.01
A	3	13.8482	4.6161	15.08**	3.24	5.29
B	1	0.0522	0.0522	0.17	4.49	8.53
C	1	0.0096	0.0096	0.03	"	"
AB	3	1.9042	0.6347	2.07	3.24	5.29
AC	3	0.3824	0.1275	0.42	"	"
BC	1	0.0056	0.0056	0.02	4.49	8.53
ABC	3	0.0216	0.0079	0.02	3.24	5.29
Error	16	4.899	0.3062			
Total	31	21.1227				

** Significantly different at $P < 0.01$

Treatment[xx] means of A

1. a_0 - 5.9203
2. a_1 - 6.6523
3. a_2 - 7.0543
4. a_3 - 7.7366

Calculated C.D. value (t 0.05) = 0.5866

x and xx see foot note under Table 8.

influenced ($P < 0.01$) by packing density alone (Table-23). The effect of sedation, salinity and all interactions were not significant ($P > 0.05$). Pair-wise comparison for packing density, showed that no significant difference ($P > 0.05$) existed between the bacterial count at 400/l and that at 600/l.

4.4. Effect of chloral hydrate at a low salinity

At a low salinity of 8 ppt, a dose of 400 ppm chloral hydrate appears to have some effect on increasing the survival of P.monodon seed at 24 hours but the effect was not statistically significant ($P > 0.05$). On the contrary, the time of initial mortality and survival percentage after 12 hours for the prawns treated with chloral hydrate have been found to be significantly lower ($P < 0.05$) than those for the untreated (Table-27). The time of initial mortality and survival percentages are given in Table-24, Fig. 16 and 17. The water quality analysis at the end of 24 hours showed that the ammonia excretion, oxygen consumption and carbon dioxide excretion exhibited no significant difference ($P \geq 0.05$) between the treatments (Table-27). The inital and final values of water quality parameters are given in Table-25. The ammonia excretion, oxygen consumption and carbon dioxide excretion by the prawn seed during the experimental period are represented in Table-26, Fig. 18, 19 and 20 respectively.

4.5. Effect of a low dose of chloral hydrate

Application of chloral hydrate at a dose of 300 ppm at a salinity of 25 ppt significantly reduced ($P < 0.01$) the time of initial mortality

Table 24. Time of initial mortality and cumulative percentage survival of P.monodon seed without and with chloral hydrate treatment under ambient temperature (29±1°C) and 8 ppt salinity at a packing density of 200/l (4.66 g/l)

Chloral hydrate treatment	Time of initial mortality (h)*	Cumulative percentage survival*	
		at 12 h	at 24 h
Without treatment	4.9	76.43	47.86
With treatment (400 ppm)	3.5	69.29	50.71

* Each value is a mean of 7 replicates

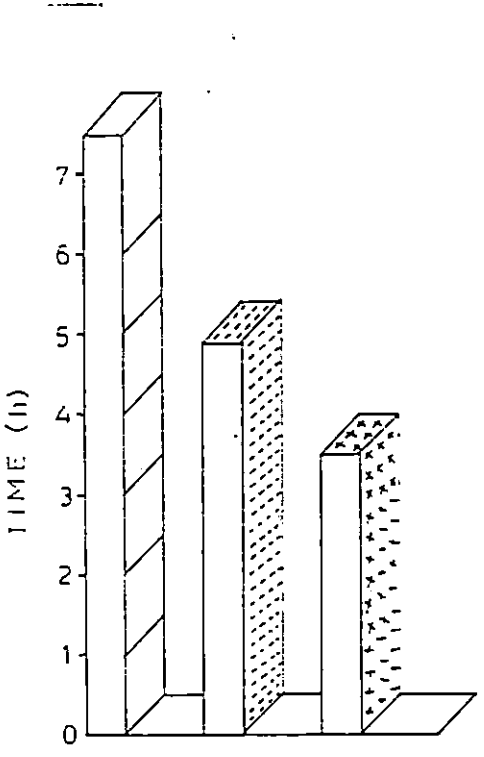

Fig. 16. TREATMENTS

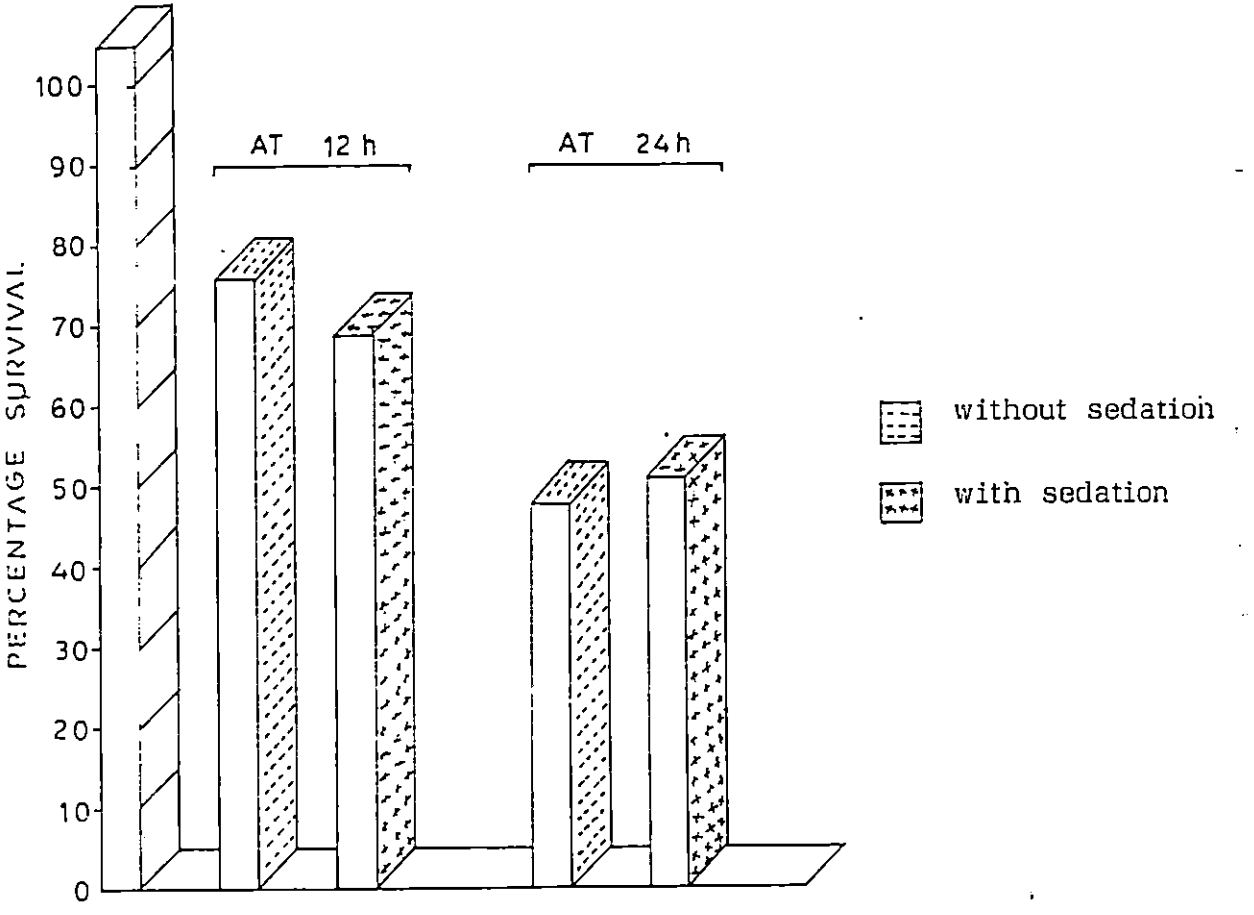

Fig. 17. TREATMENTS

Fig. 16 & 17. Time of initial mortality (h) and cumulative percentage survival of P.monodon seed without and with chloral hydrate treatment at 8 ppt salinity and ambient temperature at a packing density of 200/l (4.66 g/l)

Table 25. Initial and final water quality parameters of the packing medium in the oxygen-packed jars without and with chloral hydrate treatment under ambient temperature (29±1°C) and 8 ppt salinity at a packing density of 200/l (4.66 g/l)

	Parameters	Values of water quality parameters					
		without chloral hydrate treatment			With chloral hydrate treatment		
		Initial values	Final values		Initial values	Final values	
			0/l	200/l		0/l	200/l
+	Ammonia-N (ppm)	0.73	2.62	20.42	0.73	2.62	21.60
++	Dissolved oxygen (ppm)	18.25	17.08	4.48	18.25	17.42	4.97
+++	Free carbon dioxide (ppm)	Nil	0.22	5.06	Nil	1.54	5.28
+	pH	7.5	7.5	7.0	7.5	7.5	7.0

\+ Each value is a mean of 7 replicates

++ Each value is a mean of 3 replicates.

+++ Each value is a mean of 4 replicates.

Table 26. Ammonia-N and free carbon dioxide excretion and oxygen consumption by P.monodon seed without and with chloral hydrate treatment under ambient temperature (29±1°C) and 8 ppt salinity at a packing density of 200/l (4.66 g/l)

Parameters (ppm)	values of excretion/consumption	
	without chloral hydrate treatment	with chloral hydrate treatment
+ Ammonia-N	17.30	18.98
++ Free carbon dioxide	4.84	3.74
+++ Oxygen	12.60	12.45

\+ Each value is a mean of 7 replicates

++ Each value is a mean of 4 replicates.

+++ Each value is mean of 3 replicates.

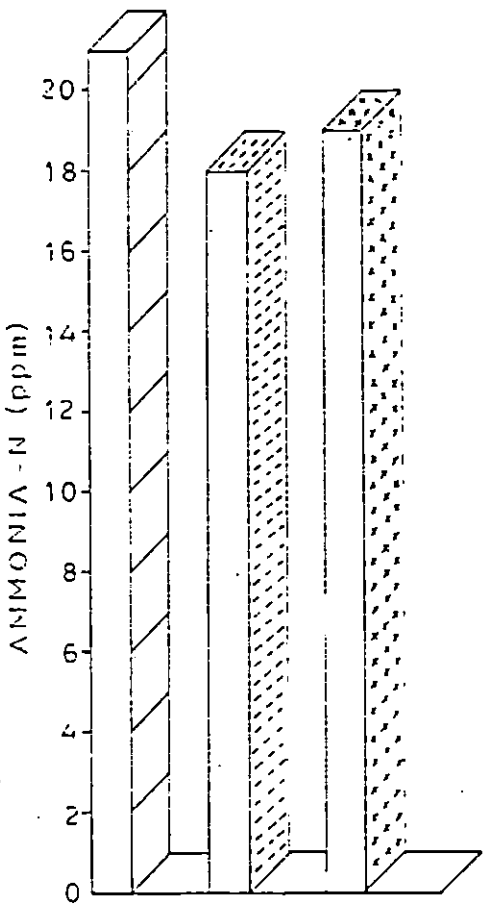

Fig.18.

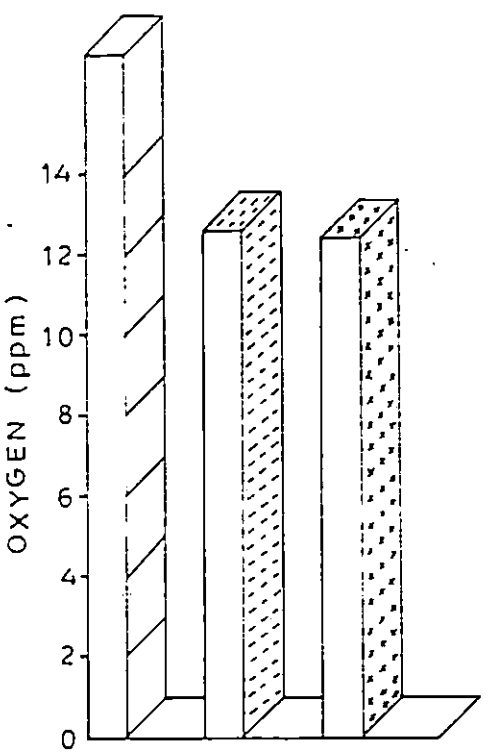

Fig.19.

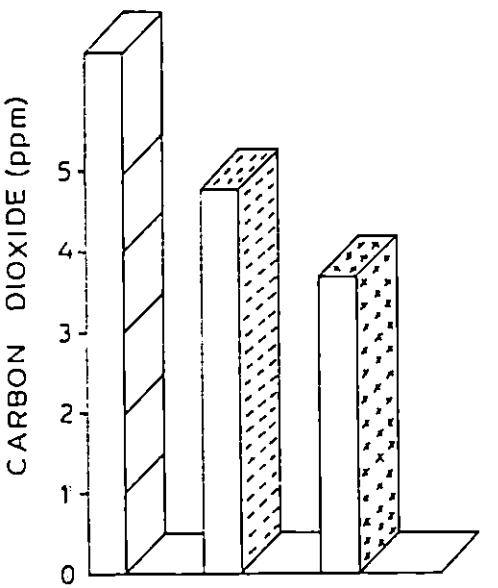

Fig.20.

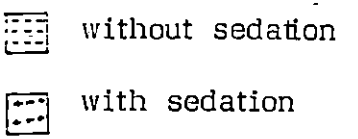

Fig. 18-20. Ammonia excretion, oxygen consumption and carbon dioxide excretion of P.monodon seed without and with chloral hydrate treatment at 8 ppt salinity and ambient temperature at a packing density of 200/l (4.66 g/l), for 24 hours

Table 27. Test of significance for comparing the important parameters of transport of P.monodon seed without and with chloral hydrate treatment under ambient temperature and 8 ppt salinity at packing density of 200/l (4.66 g/l)

Parameters	't' values		
	Computed	Tabular	
		0.05	0.01
Time of initial mortality	3.366*	2.447	3.707
Cumulative percentage mortality at 12 h	3.677*	"	"
Cumulative percentage mortality at 24 h	1.551	"	"
Ammonia-N excretion	1.734	"	"
Oxygen consumption	1.574	4.303	9.925
Carbon dioxide excretion	2.014	3.182	5.841

* Significantly different at $P < 0.05$

and. mortality percentage of the prawn seed at the end of 24 hours (Table-31). However, mortality percentage after 12 hours showed insignificant difference ($P > 0.05$) between the treatments (with and without sedation). The time of initial mortality and survival percentages are presented in the Table-28, Fig. 21 and 22. The analyses indicated that ammonia excretion, oxygen consumption and carbon dioxide excretion were significantly increased ($P < 0.01$ for ammonia excretion and $P < 0.05$ for oxygen consumption and carbon dioxide excretion) by the application of chloral hydrate at the particular dose (Table-31). The initial and final values of water quality parameters are given in Table-29. Figures 23, 24, 25 and Table-30 depict the trend of ammonia excretion, oxygen consumption and carbon dioxide excretion by the prawn seed during the 24 hour experiment.

A comparison of the effect of the two salinities, i.e., 25 ppt and 8 ppt on P.monodon seed without chloral hydrate treatment indicates that the duration of 100% survival and cumulative percentage survival at 24 hours were significantly low at 8 ppt salinity ($P < 0.05$ for duration of 100% survival and $P < 0.01$ for percentage survival at 24 hours). While the carbon dioxide excretion showed no significant difference ($P > 0.05$) between the two salinities, ammonia excretion and oxygen consumption increased significantly ($P < 0.05$) at 8 ppt salinity (Table 32).

Table 28. Time of initial mortality and cumulative percentage survival of P.monodon seed without and with chloral hydrate (300 ppm) treatment under ambient temperature (29±1°C) and 25 ppt salinity at a packing density of 200/l (4.66 g/l)

Chloral hydrate treatment	Time of initial mortality (h)*	Cumulative percentage survival* at 12 h	at 24 h
Without treatment	6.4	74.29	69.29
With treatment	4.6	72.14	48.57

* Each value is a mean of 7 replicates

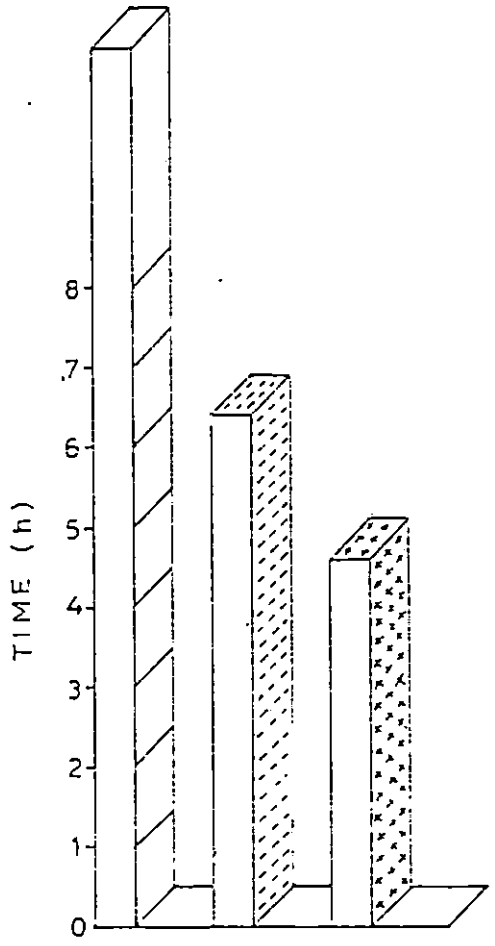

Fig.21. TREATMENTS

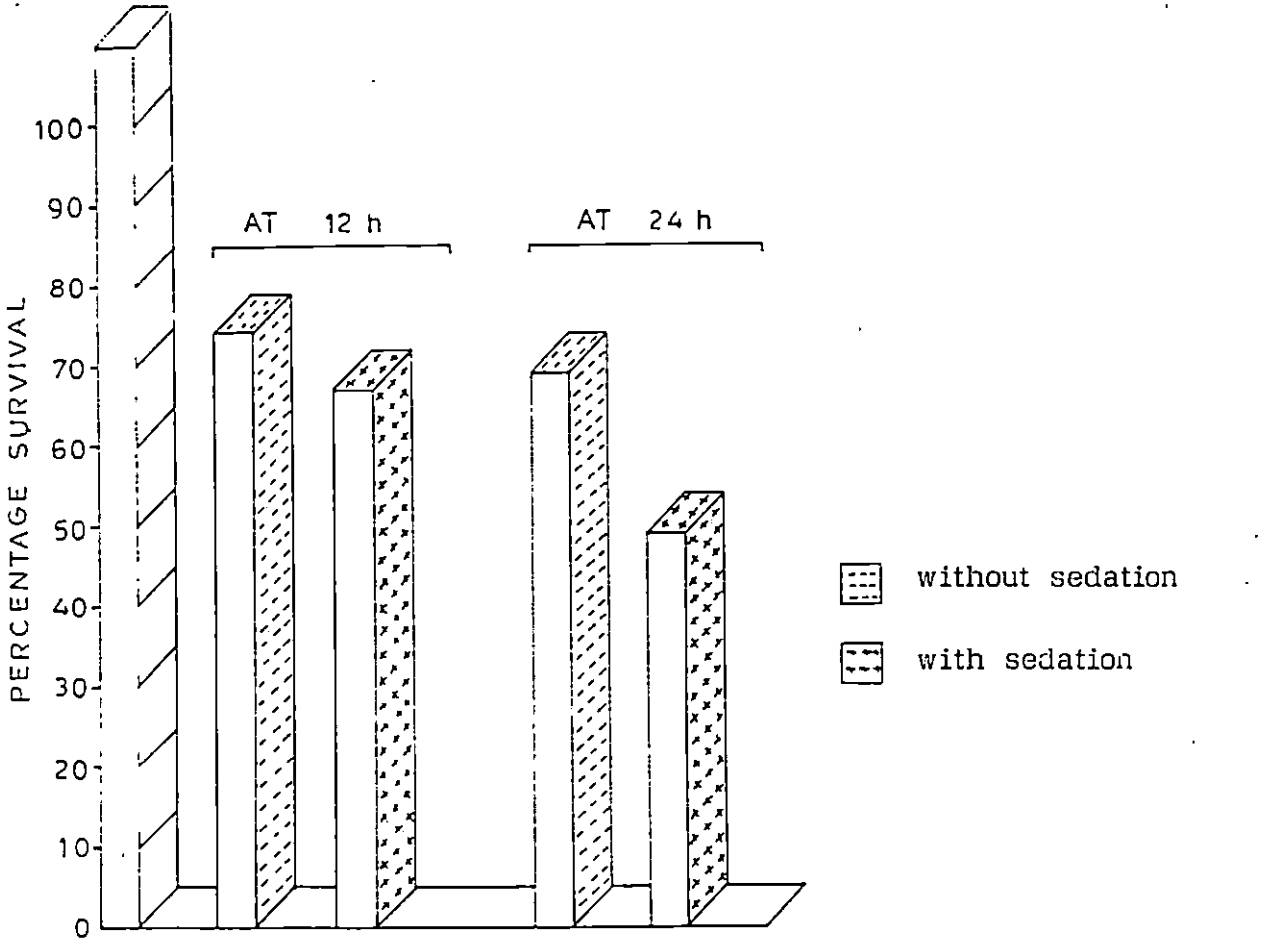

Fig.22. TREATMENTS

Fig. 21 & 22. Time of initial mortality and cumulative percentage survival of P.monodon seed without and with chloral hydrate (300 ppm) treatment at 25 ppt salinity and ambient temperature at a packing density of 200/l (4.66 g/l).

Table 29. Initial and final values of the water quality parameters of the packing medium in the oxygen-packed jars without and with chloral hydrate (300 ppm) treatment under ambient temperature (29±1°C) and 25 ppt salinity at a packing density of 200/l (4.66 g/l)

Parameters	Values of water quality parameters					
	without chloral hydrate treatment			With chloral hydrate treatment		
	Initial values	Final values		Initial values	Final values	
		0/l	200/l		0/l	200/l
+ Ammonia-N (ppm)	0.73	2.62	18.54	0.73	2.62	22.12
++ Dissolved oxygen (ppm)	17.76	16.95	9.68	17.76	16.92	6.25
+++ Free Carbon dioxide (ppm)	0.22	0.88	5.17	0.22	1.32	5.94
+ pH	7.5	7.5	7.0	7.5	7.5	7.0

+ Each value is a mean of 7 replicates.

++ Each value is a mean of 3 replicates.

+++ Each value is a mean of 4 replicates

Table 30 Ammonia-N and free carbon dioxide excretion and oxygen consumption by P.monodon seed without and with chloral hydrate (300 ppm) treatment under ambient temperature (29±1°C) and 25 ppt salinity at a packing density of 200/l (4.66 g/l)

	Parameter (ppm)	Values of excretion/consumption	
		without chloral hydrate treatment	with chloral hydrate treatment
+	Ammonia-N	15.92	19.50
++	Free carbon dioxide	4.29	4.62
+++	Oxygen	7.27	10.67

\+ Each value is a mean of 7 replicates

++ Each value is a mean of 4 replicates.

+++ Each value is a mean of 3 replicates.

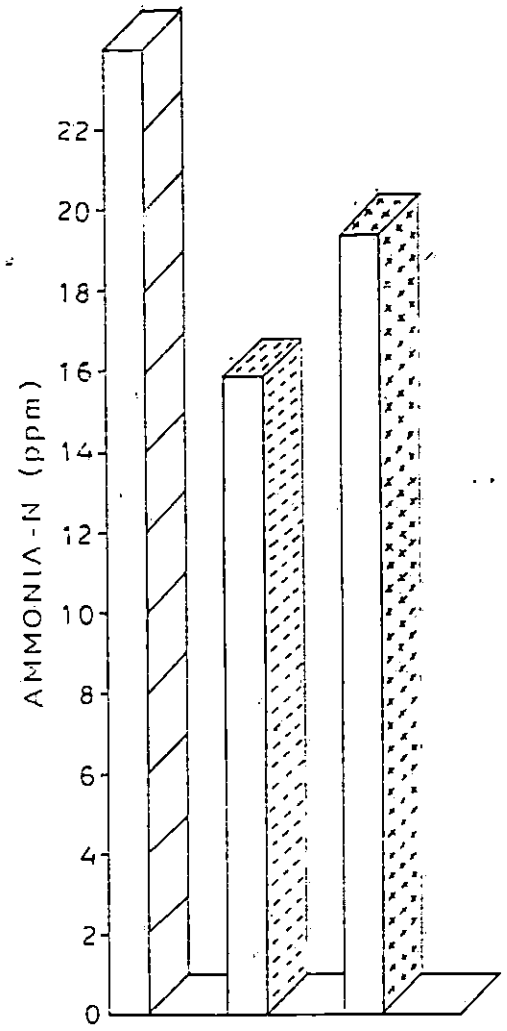

Fig.23. TREATMENTS

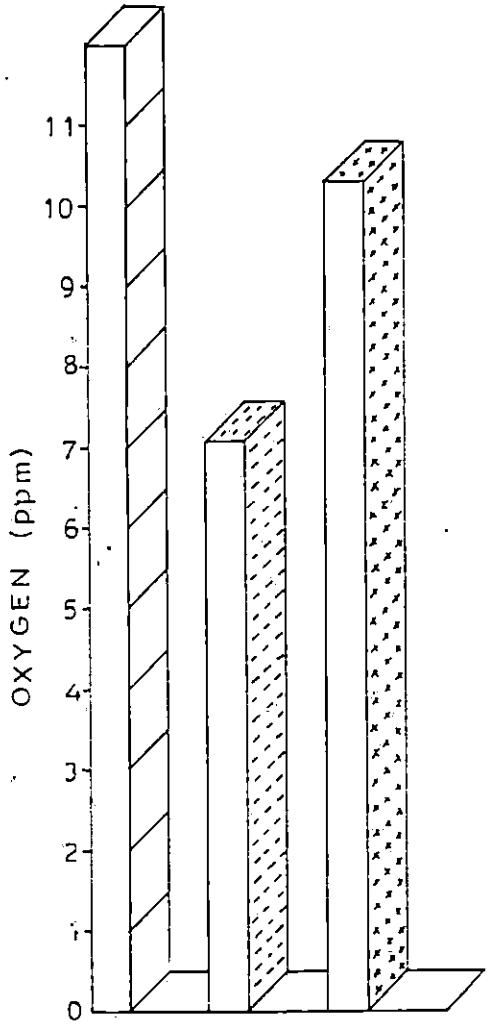

Fig.24. TREATMENTS

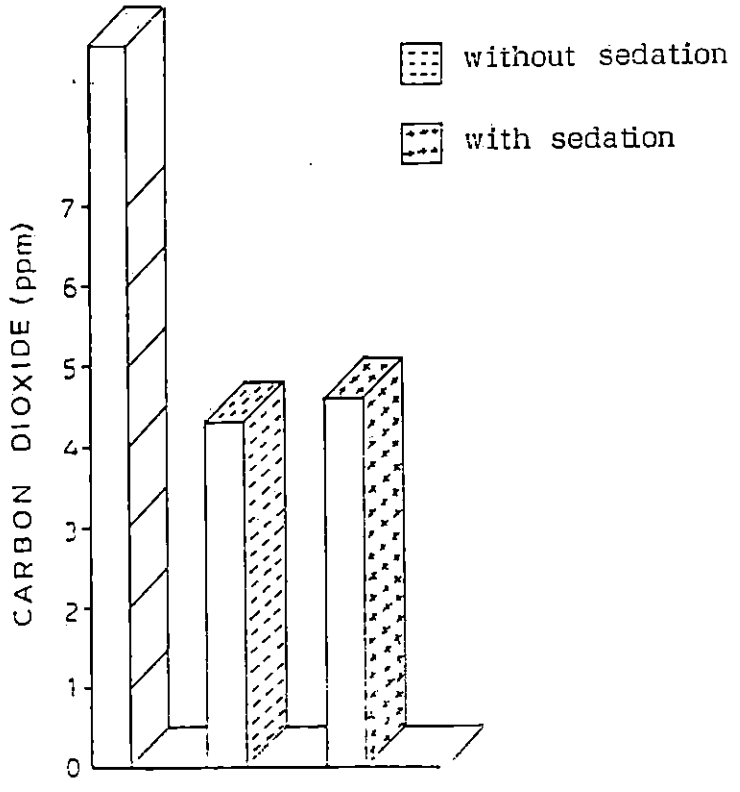

Fig.25. TREATMENTS

Fig. 23-25. Ammonia excretion, oxygen consumption and carbon dioxide excretion of P.monodon seed without and with chloral hydrate (300 ppm) treatment at 25 ppt salinity and ambient temperature at a packing density of 200/l (4.66 g/l), for 24 hours.

Table 31. Test of significance for comparing the important parameters of transport of P.monodon seed without and with chloral hydrate (300 ppm) treatment under ambient temperature (29±1°C) and 25 ppt salinity at a packing density of 200/l (4.66 g/l)

Parameters	't' values		
	Computed	Tabular	
		0.05	0.01
Time of initial mortality	5.471**	2.447	3.707
Cumulative percentage mortality at 12 h	0.455	"	"
Cumulative percentage mortality at 24 h	5.038**	"	"
Ammonia-N excretion	4.356**	"	"
Oxygen consumption	6.144*	4.303	9.925
Free carbon dioxide excretion	5.196*	3.182	5.841

* Significantly different at $P < 0.05$

** Significantly different at $P < 0.01$

Table 32. Test of significance for comparing the important parameters of transport of P.monodon seed at 25 ppt and 8 ppt salinities under ambient temperature (29±1°C) at a packing density of 200/l (4.66 g/l)

Parameters	't' values		
	Computed	Tabular	
		0.05	0.01
Time of initial mortality	3.265*	2.447	3.707
Cumulative percentage mortality at 12 h	0.761	"	"
Cumulative percentage mortality at 24 h	6.380**	"	"
Ammonia-N excretion	3.112*	"	"
Oxygen consumption	6.170*	4.303	9.925
Carbon dioxide excretion	0.617	3.182	5.841

* Significantly different at $P < 0.05$

** Significantly different at $P < 0.01$

DISCUSSION

5. DISCUSSION

5.1. Selected dose of sedatives

Use of the sedatives viz., chloral hydrate, MS-222 and tertiary butyl alcohol during transportation of fishes has been studied by several scientists. The doses prescribed by them vary with species (Webb, 1958; Thompson, 1959; Martin and Scot, 1959; McFarland, 1960; Eisler and Backiel, 1960; Blahm, 1961; Meehan and Revet, 1962; Sreenivasan, 1962; Durve, 1970; Vijayagupta and Sharma, 1974; Dick, 1975; Alverez-Lajonchere and Garcia-Moreno, 1982; De et al., 1986; Rothbard, 1988; Mary, 1991). In the case of prawns Singh et al. (1982) suggested 400 ppm of chloral hydrate for Penaeus monodon and Chitra (1990) suggested 150 ppm of MS-222 for P.indicus as the optimum doses to be applied during transportation. However, Obradovic (1986) found no effect for MS-222 in the cray fish Astacus astacus.

In the present study the selected doses of 400 ppm chloral hydrate, 175 ppm MS-222 and 0.75 ml/l tertiary butyl alcohol showed no statistically significant difference ($P > 0.05$) in mortality of the prawn from that of control after 72 hours of experiment.

5.2. Behavioural changes effected by use of sedatives on P.monodon seed

Behavioural changes as seen in the case of fishes could not be observed in the prawn P.monodon on application of any of the three sedatives, i.e., the prawns were found with some movements under light

or deep sedation, until/unless they lay on their sides almost dead. In other words, only a slight sluggishness for a few hours after exposure to the sedatives could be seen. Obradovic (1986) made similar observations in the cray fish Astacus astacus, when MS-222 was applied. They observed no effect when the concentration applied was 1:10,000 (MS-222:water) and a mild state of cessation of movement lasting for 10 minutes at a concentration of 1:1000. However, good effect was observed for halothane, an air dispersed sedative. Thus, he concluded that a water-soluble sedative might have had no effect on the cray fish. Lack of proper long term effect of sedation might be the reason for the clear negative effect of chloral hydrate on oxygen-packed P.monodon seed in the present study. The reddish orange discolouration of the prawns gives an indication of a dose at which they hardly recover, unless transferred to fresh medium.

5.3. Effect of sedatives on metabolism of P.monodon seed

All the three sedatives tested viz., chloral hydrate, MS-222 and tertiary butyl alcohol altered the basic metabolic activities viz., ammonia excretion and oxygen consumption. The ammonia excretion by the prawns without any sedative gave negative values (average $^{-}$0.4798 ppm/g/h ammonia-N) indicating a decrease in ammonia content of the water. Several authors had put forward the possibility of ammonia uptake by aquatic animals including crustaceans (Shaw, 1960; Maetz, 1973; Mangum and Towle, 1977; Armstrong et al., 1981; Taylor et al., (1987). Armstrong et al. (1981) and Taylor et al. (1987) observed ammonia uptake by Macrobrachium rosenbergii and

Palaemon elegans respectively, under hyperosmotic condition. They attributed the ammonia uptake to the exchange between Na^+ and NH_4^+ for the osmoregulation of prawn under hyperosmotic conditions as well as to the free amino acid (FAA) synthesis by prawn. Spaargaren et al. (1982) also observed acclimatory changes in amino acid pools of various tissues of Penaeus japonicus. The negative values of ammonia excretion by P.monodon without sedative in the present study might be an indication of ammonia uptake by the post-larvae. The ammonia excretion by the prawns treated with all the three sedatives, however, showed positive values (vide Table-4). Therefore, it is reasonable to assume that the capacity of the prawns to absorb ammonia from water might have been reduced by the application of sedatives, in varying degrees. Tertiary butyl alcohol, however had not reduced this capacity significantly as the prawns treated with it showed no statistically significant difference (0.3905 ppm/g/h ammonia-N) from those not treated. Moreover, one of the replicates of tertiary butyl alcohol treatment gave negative value for ammonia excretion. The highest ammonia excretion was recorded for the prawns treated with MS-222 (2.093 ppm/g/h ammonia-N).

The ammonia values of the controls (without any prawn) treated with chloral hydrate and tertiary butyl alcohol reduced by 0.07 and 0.03 ppm respectively from the initial value, after the 2 hour experiment. This might be due to the utilisation of ammonia by the bacteria present in the water. At the same time an increase in the ammonia level was observed in the control treated with MS-222. This

might be due to the interference of some compound produced on dissolution of MS-222 in water, in the estimation of ammonia.

Although there were no apparent signs of sedation in the prawns on application of the sedatives, the oxygen consumption by the prawns treated with all the three sedatives was reduced significantly ($P < 0.01$) from, that of untreated prawns. This indicates that during the initial hours of application of the sedatives, when a slight sluggishness was observed in the prawns, the oxygen consumption was reduced significantly. The lowest oxygen consumption was observed in the prawns treated with chloral hydrate (2.2435 ppm/g/h) followed by MS-222 (2.906 ppm/g/h) and tertiary butyl alcohol (5.05 ppm/g/h). The decrease in the dissolved oxygen values in the controls might have been due to the utilisation by bacteria.

Considering the changes in ammonia excretion and oxygen consumption by the prawns and the changes in the values of ammonia, dissolved oxygen and pH of the water on application of the three sedatives, chloral hydrate was selected for application to the experimental jars under oxygen packing. The prawns treated with chloral hydrate and MS-222 had not differed significantly in terms of oxygen consumption, but, the latter group of prawns showed the maximum ammonia excretion. The prawns treated with tertiary butyl alcohol showed no significant difference in ammonia excretion from those treated with chloral hydrate, but the former group of prawns showed the maximum consumption of oxygen. While MS-222 application drastically reduced the pH of the water, the application of the other

two sedatives did not change the pH. McFarland (1960) reported that MS-222 (labile) and tertiary butyl alcohol (low potency) were least desirable for transporting fishes. He recommended chloral hydrate as highly desirable because of its intermediate potency, solubility in both fresh and brackish water and compatibility with calcium content of water.

5.4. Effect of various factors on oxygen-packed seed for transportation

5.4.1. Packing density

5.4.1.1. Time of initial mortality

The time of initial mortality or the duration of 100% survival in the present study varied inversely with packing density as reported for M.rosenbergii post-larvae by Jaysree-Vadhyar et al. (1990). They reported the duration as 81 h, 35 h, 12 h, 12 h, 6 h and 4 h for packing densities of 100/l, 200/l, 250/l, 300/l, 400/l and 800/l respectively. The durations obtained for P.monodon in the present study were 7.5 h, 5.0 h, 3.5 h and 2.5 h for packing densities of 200/l (2.328 g/l), 400/l(4.656 g/l), 600/l (6.984 g/l) and 800/l (9.312 g/l) respectively at ambient temperature and 25 ppt salinity without sedation. The data reveal that at a packing density of 200/l (2.328 g/l) it is feasible to transport P.monodon seed with 100% survival for as long as 7-8 hours, at ambient temperature (29±1°C) and

salinity 25 ppt. A separate study using larger post-larvae at a packing density of 200/l (4.66 g/l) revealed the duration of 100% survival as 6.4 hours. This indicates that the number of the post-larvae is more critical than their weight in a safe prawn seed transport.

The interaction of packing density with temperature (AD) and with sedation and salinity (ABC) also influenced significantly ($P<0.01$ for AD and $P<0.05$ for ABC) the time of initial mortality. Interaction AD is discussed under the effect of temperature. The interaction ABC was insignificant amongst the combinations of 600/l and 800/l, and only a minor difference existed amongst the combinations of 200/l and 400/l. These observations reveal that sedation and salinity played only a minor role in altering the time of initial mortality.

5.4.1.2. Percentage survival

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 et al., 1978; Hamid and Mardjono, 1979; Alikunhi et al., 1980; Selvaraj et al., 1980; Franklin et al., 1982; Singh et al., 1982; Krishnakumar and Pillai, 1984; Subrahmanyam, 1986; Simon, 1986; Alias and Siraj, 1988; Jaysree-Vadhyar et al., 1990). The present study also revealed similar results. The percentage survival values at ambient temperature (29±1°C) and 25 ppt salinity without sedation were 65%, 61.25%, 59.17% and 58.13% for packing densities of 200/l (2.328 g/l), 400/l (4.656 g/l), 600/l (6.984 g/l) and 800/l (9.312 g/l) respectively at

the end of 24 hours. The experiment with larger post-larvae at a density 200/l (4.66 g/l) yielded a survival of 69.29% at 24 hours. This also corresponds to the earlier explanation that number of post-larvae is more critical than their weight in prawn seed transport. The above results conform with the findings of Hamid and Mardjono (1979), Krishnakumar and Pillai (1984) and Alias and Siraj (1988). Hamid and Mardjono (1979) reported a survival of 75% after 16 hour transport of P.monodon seed at packing densities of 200/l and 300/l at ambient temperature (28-29°C). Krishnakumar and Pillai (1984) transported P.indicus seed with 70% survival for 24 hours at a packing density of 250/l. Alias and Siraj (1988) could obtain 71.5% survival after 24 hour transport of M.rosenbergii at a density of 200/l without any habitat material. Though Alikunhi et al. (1980) suggested the addition of live food in the transport containers, Selvaraj et al.(1980) as well as Krishnakumar and Pillai (1984) did not agree to that, for fear of polluting the transport medium. In the present investigation also no live food was used.

Though the survival decreased with increase in packing density, no significant difference ($P > 0.05$) was observed between the densities of 600/l and 800/l. This means that P.monodon seed at a density of 800/l can very well be transported with more or less equal survival as that which can be obtained by using a density of 600/l. This might be due to the fact that the crowding of prawns from 600/l to 800/l does not remarkably increase their requirements and excretion, which is evident from the data on oxygen consumption and ammonia and carbon

dioxide excretion, obtained in the present study. However, it may be concluded that if P.monodon seed is to be transported for 24 hours or more at ambient temperature with a fair survival of about 80%, the packing density should be less than 200/l.

As the interaction between packing density and temperature (AD) shows a significant difference, it can be inferred that in addition to the main effects, the combined effect of packing density and temperature also changes the percentage survival. The analysis reveals that at lowered temperature of 23±2°C, the percentage survival at all the packing densities is significantly higher than the highest survival recorded at ambient temperature of 29±1°C at a packing density of 200/l. This emphasises the importance of temperature in deciding the survival. Although all other interactions are found insignificant in the anlaysis of variance, treatment means of certain interactions viz., AB, BC, CD, ABD, ABC, ACD, BCD and ABCD vary widely and are found to show significant difference in the critical difference analysis. This might be due to the linear or quadratic effect of the interactions, which might have a significant difference, though the relation is not perfectly linear (Snedecor and Cochran, 1967).

5.4.1.3. Ammonia excretion

The ammonia level in the oxygen-packed jars increased significantly ($P < 0.01$) with every 200/l increase in packing density.

The mean values of ammonia in the experimental jars at ambient temperature (29±1°C) and 25 ppt salinity without sedation were 2.54, 12.43, 14.65, 18.78 and 22.08 ppm for packing densities of 0/l, 200/l (2.328 g/l), 400/l (4.656 g/l), 600/l (6.984 g/l) and 800/l (9.312 g/l) respectively at the end of 24 hours. Smith and Wannamaker (1983), Krishnakumar and Pillai (1984) as well as Alias and Siraj (1988) reported an increase in ammonia levels with increase in packing density. Krishnakumar and Pillai (1984) observed a high mortality of P.indicus post-larvae when the ammonia (NH_3 + NH_4) level exceeded 80 ppm. They also observed that when the duration exceeded 24 hours, the ammonia excretion increased suddenly to a high level in packing densities over 200/l. In the case of M.rosenbergii transport, Alias and Siraj (1988) reported that ammonia level in the transport containers increased from 0.1 ppm to as high as 63.7 ppm ammonia-N at a packing density of 300/l, at the end of 36 hours.

Armstrong et al. (1978) reported that the total ammonia measuring above 80 ppm at a pH of 6.83 was found to be lethal to the larvae of M.rosenbergii. Chin and Chen (1987) found that the 24-h LC_{50} value for post-larvae of P.monodon was 52.11 ppm ammonia-N. In the present study the ammonia level reached only 22.08 ppm ammonia-N even at a packing density of 800/l (without sedation), which was well below the 24-h LC_{50} value reported for P.monodon post-larvae. Catedral et al. (1977) stated that the post-larvae of P.monodon could tolerate ammonia upto 10 ppm, but had not mentioned specifically whether it was in the form of NH_4Cl, ammonia, ammonia-N or NH_3-N.

However, no specific information is available on the tolerance limit of P.monodon post larvae under transport conditions, Chen and Chin (1988a) observed that a mixture of ammonia and nitrite was more toxic to P.monodon than a higher concentration of either of the two alone. Hence, although the ammonia-N levels in the packing medium at all the packing densities in the present study was less than half of the 24-h LC_{50} value reported for P.monodon post-larvae, together with the nitrite-N present, it might have contributed to the mortality. The combined effect of packing density and sedation also contributed significantly ($P < 0.01$) to the ammonia level of the packing medium, which might have been due to the stress of the prawns created by the application of chloral hydrate. This fact is obvious from the pair-wise comparison. A significant difference exists between $a_0 b_0$ (200/l - without sedation) and $a_0 b_1$ (200/l - with sedation), $a_1 b_0$ (400/l - without sedation) and $a_1 b_1$ (400/l - with sedation), and $a_2 b_0$ (600/l - without sedation) and $a_2 b_1$ (600/l - with sedation), whereas no significant difference exists between $a_0 b_1$ and $a_1 b_0$, and $a_1 b_1$ and $a_2 b_0$ i.e., the ammonia levels of the packing medium with prawns treated with chloral hydrate differ significantly from that with untreated prawns at the same packing density, but show no significant difference when compared to that with untreated prawns at immediate higher packing density. However, at 800/l, chloral hydrate application causes no significant difference in ammonia excretion.

A correlation is observed in the interaction between packing density and sedation (AB) which could not be found in that between packing density and temperature (AD). This may be due to the highly

significant main effects of packing density and temperature which act in opposite ways i.e., a higher packing density increases the ammonia excretion and lowered temperature decreases it. When ammonia excretion is compared, all the cobinations of AD except $a_2 d_1$ (600/l - 23±2°C) and $a_1 d_0$ (400/l - 29±1°C) differed significantly. However, the interaction AD was insignificant when chloral hydrate was not applied. Hence, the significant difference observed in the former case might be due to the interference of chloral hydrate.

Wickins (1985) and Mohanty et al. (1989) reported that the rate of ammonia excretion in P.monodon decreased with increase in size of prawn. Comparable results have been obtained in the present study. When larger post-larvae (average size 17mm/23.27 mg) were packed at a density of 200/l (4.66 g/l), the total ammonia excretion increased but the rate of excretion decreased. It was also noticed that although the ammonia level increased with increase in packing density, the rate of excretion decreased, irrespective of the size of the prawn.

5.4.1.4. Oxygen consumption

Usually, in the transport of prawn under oxygen packing, the dissolved oxygen does not become a limiting factor (De,1977), unless remarkable mortality and decay of dead prawns occur in the container (Franklin et al.,1982) or the duration of transport is extended considerably (Krishnakumar and Pillai, 1984). In the present study, the dissolved oxygen values at ambient temperature and 25 ppt salinity, without sedation were 16.75, 13.77, 11.11, 3.65 and 1.99 ppm for packing densities of 0/l, 200/l (2.328 g/l), 400/l (4.656 g/l),

600/l (6.984 g/l) and 800/l (9.312 g/l) respectively at the end of 24 hours.

The minimum level of dissolved oxygen required for the survival of P.monodon seed has been reported as 0.6 ppm by De and Subrahmanyam (1975) and as 0.7 ppm by Chakraborti , (1978). The latter author recommended that the concentration of dissolved oxygen should not be below 1.5 ppm for all practical purposes. In the present study, the dissolved oxygen levels in the containers was well above these limits, even at the highest packing density of 800/l. Selvaraj et al. (1980) however, suggested that the concentration of dissolved oxygen should be above 2.5 ppm for the healthy survival of the prawn seed.

In the present study the increase in oxygen consumption with increase in packing density did not exhibit a linear relationship. This was because the rate of oxygen consumption decreased when packing density was changed from 200/l to 400/l and from 600/l to 800/l, whereas it increased when the packing density was changed from 400/l to 600/l. The crowding of the prawns beyond a particular limit and the disturbance thus created by increasing the packing density from 400/l to 600/l may be the reasons for the increase in the rate of oxygen consumption. This is in agreement with the reports by Bishop et al. (1980) and Dallavia (1986). Bishop et al. (1980) observed that the disturbed shrimps consumed oxygen nearly four times faster than those at rest. However, the rate of oxygen consumption has been found to increase with increase in the size of the prawn, as reported by Stephenson and Knight (1980) for M.rosenbergii and by Scelzo and Zunigo

(1987) for P.brasiliensis.

5.4.1.5. Carbon dioxide excretion

The free carbon dioxide content after 24 hour experiment showed a direct relation with packing density. The values of free carbon dioxide at the end of 24 hours, for packing densities of 0/l, 200/l (2.328 g/l), 400/l (4.656 g/l), 600/l (6.984 g/l) and 800/l (9.312 g/l) at ambient temperature and 25 ppt salinity without sedation were 0.22, 3.96, 7.48, 12.54, and 14.96 ppm respectively. Krishnakumar and Pillai (1984) and Alias and Siraj (1988) also reported a direct relation for carbon dioxide contents with packing density. Although Krishnakumar and Pillai (1984) attributed the complete mortality of P.indicus to the decrease in pH caused by the accumulation of carbon dioxide, they had not measured the actual carbon dioxide content in the packing medium. Alias and Siraj (1988) recorded values as high as 63.5 ppm carbon dioxide at the end of 36 hours at a packing density of 300/l in M.rosenbergii transport. However, they had not specified whether they measured free carbon dioxide or total carbon dioxide.

Similar to oxygen consumption the rate of excretion of carbon dioxide has been found to increase with increase in size of prawn. Rate of change of carbon dioxide excretion also varied in an exactly similar way as that of oxygen consumption, i.e., a decrease for a shift of packing density from 200/l to 400/l and from 600/l to 800/l and an increase for packing density change from 400/l to 600/l.

5.4.1.6. pH

The pH of the water was found to have reduced from 7.5 to 7.0 at the end of the 24 hour experiment. In no case the pH was lower than 7.0. This result is comparable to the reports by Singh et al.(1982) on P.monodon transport and by Alias and Siraj (1988) on M.rosenbergii transport. However, Krishnakumar and Pillai (1984) reported pH as low as 6.6 at higher packing densities. The slight decrease in pH in the present study may be due to dissociation of carbonic acid to release bicarbonate which further dissociate to give carbonate and hydrogen ions, thus causing a reduction in pH (Alias and Siraj, 1988).

5.4.1.7. Bacterial population

The bacterial population increased significantly with every 200/l increase in packing density. The final bacterial count at the ambient temperature and 25 ppt salinity without sedation were $3.67x10^4$, $9.41x10^4$, $5.195x10^5$, $8.4097x10^6$ and $9.2665x10^7$ cells/ml for packing densities of 0/l,200/l (2.328 g/l), 400/l (4.656 g/l), 600/l (6.984 g/l) amd 800/l (9.312 g/l) respectively. The prolific multiplication of the bacteria at higher packing densities might have been favoured by the dead and decaying prawn seed, which resulted in very high bacterial counts at higher packing densities. The interaction of packing density with other factors, however, had not significantly ($P > 0.05$) altered the bacterial population.

Apparently, the bacterial count had not influenced the percentage survival of the prawn seed in the experiment, because the net bacterial count at the ambient temperature was almost equal or less than that at the lowered temperature (except at 800/l), where the percentage survival was much higher than that at the ambient temperature. However, the possibility of a combined effect of the bacterial population along with other factors viz., higher ammonia and carbon dioxide as well as lower oxygen and pH at the ambient temperature than at the lowered temperature, which exert considerable stress to the prawn seed, ultimately resulting in a high mortality at the ambient temperature, cannot be ruled out. Turner and Bower (1982) reported that incorporation of nitrifying bacteria attached to a solid substrate during transportation appeared to have been effective in preventing the accumulation of ammonia and decrease in pH. Hence, of the high bacterial population found in the present study, if a significant number were nitrifying bacteria, they might have bettered the packing medium by preventing the accumulation of ammonia and decrease in pH.

5.4.2. Sedation

The application of the selected dose of chloral hydrate has evidently shown a negative effect on the survival of P.monodon seed under oxygen packing and on the water quality parameters of the packing medium. This is contradictory to the earlier report by Singh et al.(1982).

5.4.2.1. Time of initial mortality

The time of initial mortality appeared unaffected by the application of chloral hydrate. The duration of 100% survival of the sedated P.monodon seed at the ambient temperature and 25 ppt salinity were 8 h, 4.5 h, 4 h and 2.5 h for packing densities of 200/l (2.328 g/l), 400/l (4.656 g/l), 600/l (6.984 g/l) and 800/l (9.312 g/l) respectively, which had not varied significantly from the corresponding values without sedation (vide Table-7). This might be due to the fact that the sedation effect of chloral hydrate persisted only during the initial hours of application, when the application might not have become a stress to the prawn seed. However, the time of initial mortality was significantly reduced when chloral hydrate was applied at a low dose (300 ppm) on larger sized seed. This may be due to the fact that 300 ppm of chloral hydrate is not just sufficient to sedate the prawn seed, but creates an added stress on the seed and thus shortening the duration of 100% survival. However, 400 ppm of chloral hydrate applied to the prawns at 8 ppt salinity reduced the time of initial mortality which might be due to the added stress created by the low salinity.

Though the effect of sedation on the time of initial mortality was insignificant, the combined effect of sedation with packing density and low salinity shortened the time. This has been already discussed in detail under packing density.

5.4.2.2 Percentage survival

The cumulative percentage survival at the end of 24 hours was considerably reduced by the application of chloral hydrate. The percentage survival values of sedated prawns at the ambient temperature and 25 ppt salinity were 50%, 46.25%, 40.85% and 36.88% for packing densities of 200/l (2.328 g/l), 400/l (4.656 g/l), 600/l (6.984 g/l) and 800/l (9.312 g/l) respectively. The decrease in percentage survival on application of chloral hydrate from that of untreated prawns were 15%, 15%, 18.32% and 21.35% for packing densities of 200/l, 400/l, 600/l and 800/l respectively. Singh et al.(1982) reported an increase in percentage survival by 0.9% and 15.6% for packing densities of 250/l and 500/l respectively after 30 hours of transport with the application of chloral hydrate at 400 ppm. The maximum survival obtained by them for sedated prawns at a density of 250/l was 98.7% at 24 hours, but, that at a density of 500/l had not been mentioned by them. They conducted all their experiments at a low salinity of 8 ppt. In the present study, the percentage survival obtained at a salinity of 20 ppt was less than that obtained at 25 ppt salinity, the values being 45%, 41.25%, 35.8% and 30% for packing densities of 200/l, 400/l, 600/l and 800/l respectively. At 8 ppt salinity, the survival was found to have been enhanced by 2.85% on application of chloral hydrate, at a packing density of 200/l (4.66 g/l). However, it could be seen that the survival of sedated prawn seed at 8 ppt salinity (50.71%) was much less than that of untreated prawn seed at 25 ppt salinity (69.29%). It is reasonable to conclude from the above observation that by reducing the salinity to 8 ppt and then applying a sedative such as chloral

hydrate, the survival of P.monodon seed cannot be enhanced.

A lower dosage of chloral hydrate (300 ppm) applied at a packing density of 200/l (4.66 g/l) also reduced the survival significantly ($P < 0.01$) at the end of 24 hours. Although this low dosage of chloral hydrate significantly shortened the duration of 100% survival and also the percentage survival at 24 hours, it did not significantly reduce the percentage survival at 12 hours. The possibility here is that 300 ppm chloral hydrate might have effected a slight sedation only after a few hours of exposure, and this effect might have lasted for about 12 hours.

The low percentage survival obtained for the prawn seed treated with chloral hydrate at 400 ppm may be due to the losing of the slight sedation effected by chloral hydrate after a few initial hours, as observed by Obradovic (1986) for the cray fish Astacus astacus with MS-222. Further exposure of the prawn seed to the water containing chloral hydrate and the progressive metabolic wastes, might have exerted considerable stress to them causing a high mortality. This explanation is supported by the fact that the time of initial mortality of the seed has not been affected by the application of chloral hydrate, when the prawns are considered to be under slight sedation. A combination of 20 ppt salinity with the application of chloral hydrate might have further added to the stress, thereby increasing the mortality.

5.4.2.3. Ammonia excretion

Ammonia excretion by the prawns was found to have increased significantly ($P < 0.01$) by the application of chloral hydrate. However, the ammonia level of the packing medium had not crossed the 24-h LC_{50} limits even at the highest packing density of 800/l. The ammonia values of the transport jars with sedated prawns at ambient temperature and 25 ppt salinity were 2.54. 14.23, 19.66, 21.85 and 23.19 ppm ammonia-N for packing densities of 0/l, 200/l (2.328 g/l), 400/l (4.656 g/l), 600/l (6.984 g/l) and 800/l (9.312 g/l) respectively, at the end of 24 hours. The high excretion by chloral hydrate-treated prawns might be due to the stress as explained earlier.

Although a higher ammonia level was recorded in the jars with chloral hydrate-treated prawns than in those with untreated prawns, the control jars (without any prawn) showed no difference in the ammonia level. This observation makes it clear that the prawns treated with chloral hydrate excreted more ammonia than the untreated prawns.

A low dosage of chloral hydrate (300 ppm) applied on large sized post-larvae also increased the ammonia excretion significantly ($P < 0.01$). The trend in the rate of ammonia excretion with change in size and packing density remained unaffected by the application of chloral hydrate.

5.4.2.4. Oxygen consumption

Although in the preliminary studies chloral hydrate treatment was found to have reduced the oxygen consumption by P.monodon post-larvae, its application under oxygen packed transport conditions resulted in increased oxygen consumption. This observation also supports the earlier explanation that the prawns might have lost the sedation effect after a few hours of application of chloral hydrate and remained under considerable stress during the rest of the period. However, it is to be emphasised here that, the oxygen consumed during the course of the experiment might not have been used exclusively for the respiration of prawns, but partly by the bacterial activity on the dead prawns.

The dissolved oxygen values of the transport jars in which chloral hydrate was applied under the ambient temperature and 25 ppt salinity were 17.09, 13.44, 10.78, 2.49 and 1.00 ppm for packing densities of 0/l, 200/l (2.328 g/l), 400/l (4.656 g/l), 600/l (6.984 g/l) and 800/l (9.312 g/l) respectively, at the end of 24 hours. Thus it could be seen that, even with the increased consumption of oxygen by the chloral hydrate-treated prawns, only at a packing density of 800/l the dissolved oxygen concentration fell below the limit (1.5 ppm) prescribed by Chakraborti (1978) for all practical purposes. Application of a low dose of chloral hydrate (300 ppm) also resulted in a higher oxygen consumption than without treatment. The trend in the rate of oxygen consumption with change in size of prawn and packing density was not altered by the application of chloral hydrate. Singh et al. (1982) reported the final dissolved oxygen values in the

containers as 6.2, 2.7 and 3.0 ppm for packing densities of 250/l, 375/l, and 500/l respectively, but, they had not mentioned whether the values were from chloral hydrate treated or untreated containers.

In spite of the above fact, the dissolved oxygen concentration in all the jars treated with chloral hydrate (without any prawn) was higher than that in the untreated jars (without any prawn) at the end of the experiment. This means that the utilisation of dissolved oxygen by bacteria was minimised by the addition of chloral hydrate.

5.4.2.5. Carbon dioxide excretion

The carbon dioxide excretion and oxygen consumption always exhibited an inverse relationship. The application of chloral hydrate increased the carbon dioxide excretion remarkably. The final free carbon dioxide values in the oxygen-packed jars treated with chloral hydrate, at the ambient temperature and 25 ppt salinity were 1.54, 5.50, 9.46, 16.06 and 18.04 ppm for packing densities of 0/l, 200/l (2.328 g/l), 400/l (4.656 g/l), 600/l (6.984 g/l) and 800/l (9.312 g/l) respectively. Although Singh et al. (1982) reported the final carbon dioxide values of P.monodon seed transport as 6.0, 36.0 and 54.0 ppm for packing densities of 250/l, 375/l and 500/l respectively, they had neither specified the period after which they measured it, nor did they mention whether it was free carbon dioxide or total carbon dioxide measured, whether the values were for chloral hydrate treated or untreated prawns. Hence, a comparison of the present data on carbon dioxide excretion after chloral hydrate treatment is baseless.

5.4.2.6. pH

The final pH of the packing medium appeared unaffected by the application of chloral hydrate, because the pH had not varied notably between treated and untreated packing medium.

5.4.2.7. Bacterial population

The bacterial population of the packing medium containing the chloral hydrate-treated prawns, increased significantly ($P < 0.01$) at the ambient temperature, but, the increase was not significant ($P > 0.05$) at the lowered temperature. This differential trend of the bacterial count might be due to the considerably higher number of dead and decaying prawns at the ambient temperature than at the lowered temperature. A high temperature and the presence of dead and decaying organic matter form a highly favourable environment for the flourishing of bacteria. At the ambient temperature, on addition of chloral hydrate the bacterial count was as high as 0.4 billion cells/ml at a packing density of 800/l (vide Table-21).

5.4.3. Salinity

Several views have been put forward regarding the salinity tolerance of P.monodon. Pantastico (1979) and Reddi et al. (1984) reported that the species could tolerate freshwater . Chakraborti et al. (1985), Cheng and Liao (1986), Singh (1989), Diwan et al. (1989) and Zhang et al. (1989) observed that a salinity below 3 ppt was

highly lethal to the prawn. Valencia (1976) suggested that a salinity between 20 and 30 ppt was favourable for fry. However, the fact that P.monodon can tolerate a wide range of salinity, may not necessarily mean that the whole range be quite suitable for transportation. In the present study, low salinities of 20 ppt and 8 ppt were found to have reduced the survival percentage from that at 25 ppt. Although the time of initial mortality and percentage survival at 24 hours were not significantly ($P > 0.05$) affected by the reduction of salinity from 25 to 20 ppt, they were significantly reduced by the reduction of salinity to 8 ppt, despite the acclimatisation given to them for 2 days at 8 ppt salinity as suggested by Diwan et al. (1989). Bower (1978) reported that the un-ionised form of ammonia was low at higher salinities. It may be concluded that a high salinity of 25 ppt is better than the lower salinities for the transportation of P.monodon seed.

Although higher values have been observed for ammonia excretion, oxygen consumption, carbon dioxide excretion, pH and bacterial population at 20 ppt salinity than at 25 ppt, these parameters show no remarkable difference at these two salinities. However, significantly higher ammonia excretion and oxygen consumption have been noticed at 8 ppt salinity than at 25 ppt. Nevertheless, low salinities of 20 and 8 ppt ensured high initial dissolved oxygen levels in all the oxygen-packed jars including controls (without any prawn), which may be due to the higher dissolution of oxygen in water at low salinities.

5.4.4. Temperature

The lowered temperature of 23±2°C favoured significantly all the important parameters of successful transportation viz., time of initial mortality or duration of 100% survival, percentage survival and water quality parameters.

5.4.4.1. Time of initial mortality

The duration of 100% survival of P.monodon seed under all the combinations has been found to have almost doubled with the lowering of temperature from 29±1°C to 23±2°C. The time of initial mortality at the lowered temperature, 25 ppt salinity and without sedation were 15 h, 9.5 h, 6 h and 5 h for packing densities of 200/l (2.328 g/l) 400/l (4.656 g/l), 600/l (6.984 g/l) and 800/l (9.312 g/l) respectively. It may be safely concluded that a risk-free transport of P.monodon seed (with 100% survival) involving a journey lasting for about 15 hours can be implemented at a density of 200/l and that lasting for about 5 hours at a density of 800/l, provided the temperature is lowered to 23±2°C.

Analysis of the interaction between temperature and packing density exhibits a significant effect of the lowered temperature in enhancing the duration of 100% survival. No significant difference could be observed between a_3d_1 (800/l - 23±2°C) and a_1d_0 (400/l - 29±1°C) in terms of the time of initial mortality. At each of the packing densities when chloral hydrate was not applied, the time of initial mortality

at .29±1°C was not significantly different from that of the immediate higher packing density at 23±2°C.

5.4.4.2. Percentage survival

Highly significant ($P < 0.01$) increase in percentage survival could be noticed at the lowered temperature, from that at the ambient temperature. The percentage survival values at the lowered temperature, 25 ppt salinity and without sedation were 92.5%, 87.5%, 81.7% and 83.13% for packing densities of 200/l, 400/l, 600/l and 800/l respectively, at the end of 24 hours, i.e., the survival increased by 27.5%, 26.25, 22.53% and 25% for packing densities of 200/l, 400/l, 600/l and 800/l respectively. Hamid and Mardjono (1979) obtained a survival of 95% for packing densities of 300/l and 400/l and 96% for packing densities of 500/l and 600/l after 12 hours transport with P.monodon seed (10 mm) at a temperature of 22-25°C. They also reported 95% survival at 24 hours of transport in insulated boxes maintaining the temperature at 24-28°C at a packing density of 1000/l. On the basis of the present study, it is possible to recommend a packing density of 200/l (2.328 g/l) with over 90% survival and a packing density of 800/l (9.312 g/l) with 80% survival for 24 hours of transport of P.monodon post-larvae at a temperature of 23±2°C. The high survival at the lowered temperature may be due to the suppression of all the metabolic activities of prawns and thereby resulting in better conditions within the transporting containers.

A combination of either sedation effected by chloral hydrate or low salinity, or both together with lowered temperature, however, had a negative effect on the survival (vide Table-10). Zein-Eldin and Aldrich (1965) Kuttyamma (1981) and Charmantier et al. (1988) reported that low temperature reduces the tolerance of marine, and brackishwater prawns to low salinities.

The interactions of AD (Packing density-temperature) and BD (Sedation-temperature) emphasise the betterness of lowered temperature in enhancing the survival. Interaction AD is already discussed under packing density. The analysis of the interaction BD showed that the negative effect of chloral hydrate treatment was shortened by the positive effect of the lowered temperature. The survival of chloral hydrate-treated prawns at the lowered temperature was significantly higher than that of chloral hydrate-treated and untreated prawns at the ambient temperature.

5.4.4.3. Ammonia excretion

The ammonia excretion by prawns increases with increase in temperature (Spaargaren et al., 1982). The concentration of un-ionised ammonia, which is more toxic to the organisms also increases at higher temperatures. (Bower, 1978). The present study revealed that the ammonia excretion by P.monodon was significantly reduced ($P < 0.01$) by lowering the temperature. The ammonia level in each of the packing densities tried was far below the 24-h LC_{50} limit for P.monodon post-larvae. The final ammonia values at the lowered temperature, 25 ppt

salinity and without sedation were 3.42, 10.04, 12.69, 16.22 and 19.66 ppm ammonia-N for packing densities of 0/l, 200/l, 400/l, 600/l and 800/l respectively.

5.4.4.4. Oxygen consumption

The oxygen consumption by P.monodon seed was remarkably reduced by lowering the temperature. This observation corresponds to the reports by Nelson et al. (1977), Stephenson and Knight (1980), Bishop et al. (1980), Licop (1984), Liao and Murai (1986) and Dallavia (1987). The dissolved oxygen concentration at all the packing densities tried was above the limit (2.5 ppm) suggested by Selvaraj et al. (1980) for healthy survival of the prawn seed. The final dissolved oxygen values at the lowered temperature, 25 ppt salinity and without sedation were 17.42, 15.43, 12.44, 6.64 and 4.81 ppm for packing densities of 0/l, 200/l, 400/l, 600/l and 800/l respectively. Thus, it is clear that the lowered temperature ensures a high initial and final oxygen level in the packing medium. A high dissolved oxygen concentration was reported to lower the ammonia excretion by prawns (Laxminarayana and Kutty, 1982) as well as the toxicity of ammonia to prawns (Wajsbrot et al., 1990; Allan et al., 1990). Hence, a lowered temperature assures not only a high dissolved oxygen concentration but also a low toxicity of ammonia .

5.4.4.5. Carbon dioxide excretion

Regardless of the lowering of temperature, a high free carbon

dioxide concentration could be observed at all the packing densities. The free carbon dioxide values at the lowered temperature were slightly lower at packing densities of 200/l and 400/l, but slightly higher at packing densities of 600/l and 800/l than the corresponding values at the ambient temperature (vide Table-19). This might be due to the high survival of the prawn seed at the lowered temperature and not that the lowered temperature did not favour the reduction of carbon dioxide excretion. A higher number of surviving prawns at the lowered temperature than at the ambient temperature might have accumulated the free carbon dioxide to high levels. However, as long as the accumulation of free carbon dioxide does not bring down the pH of the water to the acidic side (< 7.0), mortality due to accumulation of carbon dioxide may not take place. Chakraborti (1978) reported that the accumulation of carbon dioxide on prolonged experimentation brought down the rate of oxygen consumption by P.monodon and P.indicus seed.

5.4.4.6. Bacterial population

The effect of temperature on the bacterial population could not be analysed statistically as the two water samples used at the ambient and the lowered temperature for experimental purpose had considerably different initial bacterial counts. However, from the data obtained, it is clear that the lowered temperature remarkably reduced the flourishing of bacteria in the containers. While the bacterial count at a packing density of 800/l was as high as 100 million cells/ml at the ambient temperature without sedation, it was only 30 million cells/ml

at the lowered temperature, despite the fact that initial plate count at the lowered temperature was considerably higher than that at the ambient temperature. The occurence of more number of dead and decaying prawns at the higher packing densities than at the lower, might have favoured the flourishing of bacteria at the higher packing densities and the ambient temperature.

It is obvious that the oxygen consumption, ammonia excretion, carbon dioxide excretion and bacterial population are considerably reduced, apparently by the lesser activities of the prawn seed caused by lowering the temperature from 29±1°C to 23±2°C. Because of the better water quality conditions in the oxygen-packed containers at lowered temperatures, much better survival and duration can be assured in the transportation of P.monodon seed than at the ambient temperatures of tropical conditions.

SUMMARY

6. SUMMARY

1. The objectives of the study were - (i) to select a suitable sedative at a safe dose with a view to enhancing the survival of P.monodon seed during transportation, (ii) to study the effect of the selected sedative on P.monodon seed under oxygen packing and (iii) to study the effect of different packing densities, salinities and temperatures on sedated and non-sedated P.monodon seed under oxygen packing.

2. The appropriate doses of the three sedatives tried initially were selected on the basis of the survival of P.monodon seed for a period of 72 hours, by conducting an experiment in open containers. From these sedatives one was selected on the basis of the rate of reduction in metabolic activities, by conducting an experiment in respirometers.The above two experiments were designed in randomised block design and completely randomised design respectively.

3. The experiment on the effect of sedative application at different packing densities, salinities and temperatures under oxygen packing was conducted in specially designed hard plastic containers of 600 ml capacity, fitted with one way valves to make them suitable for packing oxygen under uniform pressure. The experiment was conducted as an asymmetrical factorial experiment with four levels of packing density (200/l, 400/l, 600/l and 800/l) and two levels of sedation (without and with sedation), salinity (25 ppt and 20 ppt) and temperature (29±1°C and 23±2°C), designed in completely randomised

design. The experiment was conducted for 24 hours and water quality parameters were measured at the end of the experiment.

4. The preliminary studies in open containers resulted in the selection of doses of 400 ppm chloral hydrate, 175 ppm MS-222 and 0.75 ml/l tertiary butyl alcohol for the application under oxygen packing, as these doses caused no mortality of the prawn seed within 72 hours of exposure.

5. Unlike in the case of fishes, apparent signs of sedation could not be observed in P.monodon seed on application of any of the three sedatives studied. Only a slight sluggishness lasting for a few hours initially could be observed. Overdoses caused reddish orange discolouration of the prawn.

6. The study of the effect of the three sedatives on the metabolism of the prawn seed resulted in the selection of chloral hydrate for application under oxygen packing, because the prawn seed treated with chloral hydrate showed the lowest oxygen consumption (¼ of that of control) during the study period of 2 hours. Moreover, chloral hydrate did not alter the pH of the water, a quality which it had in common with tertiary butyl alcohol.

7. The application of chloral hydrate under oxygen packing reduced the percentage survival of P.monodon seed significantly. The oxygen-packed containers with chloral hydrate-treated prawns showed higher

values of ammonia, carbon dioxide and bacterial population and lower values of dissolved oxygen than of the containers with untreated prawns, at 24 hours. The time of initial mortality or duration of 100% survival of the prawn seed was however, not altered by the application of chloral hydrate.

8. The packing density showed inverse relation with the time of initial mortality and percentage survival and direct relation with oxygen consumption, and ammonia and carbon dioxide excretion and bacterial population. The study showed that at packing densities of 200/l, 400/l, 600/l and 800/l, the P.monodon seed could be transported without mortality upto 7.5 h, 5.0 h, 3.5 h and 2.5 h respectively at ambient temperature of 29±1°C under oxygen packing. The percentage survival at 24 hours reduced to as low as 58.13% at a packing density of 800/l. However, the water quality parameters did not fall to the lethal levels even at the highest packing density of 800/l. The study also showed that the number of post-larvae was more critical than their weight in deciding the percentage survival during transport.

9. The rate of ammonia excretion decreased with increase in size of the prawn seed and packing density. The rate of oxygen consumption and carbon dioxide excretion increased with increase in the size of prawn, but, varied differently with different levels of packing density.

10. The lowering of salinity from 25 ppt to 20 ppt did not significantly alter any of the parameters viz., time of initial mortality, percentage survival at 24 hours, oxygen consumption, ammonia and

carbon dioxide excretion and bacterial population. However, the lowering of salinity from 25 ppt to 8 ppt altered all the above parameters adversely. This leads to the conclusion that a high salinity of 25 ppt is better than a lower salinity for transportation of P.monodon seed.

11. The lowering of temperature from the ambient level of 29±1°C to 23±2°C significantly increased the time of initial mortality and percentage survival at 24 hours of P.monodon seed at all the packing densities tried. The study revealed that at the lowered temperatures the safe durations of transport (duration of 100% survival) were increased to 15 h, 9.5 h, 6 h and 5 h for packing densities of 200/l, 400/l, 600/l and 800/l respectively. The percentage survival at 24 hours was as high as 83.13% at 800/l and 92.5% at 200/l at the lowered temperature. The lowering of temperature helped in reducing the oxygen consumption and ammonia and carbon dioxide excretion by the prawn seed as well as the multiplication of bacteria in the packing medium.

12. 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 prawn seed and the water quality parameters. The interaction between packing density and sedation worsened the water quality. However, the interaction of lowered temperature with packing density and/or sedation reduced the negative effect of the latter factors. The interaction of 25 and 20 ppt salinities with other factors was insignificant.

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7. REFERENCES

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EFFECT OF SEDATIVES ON *PENAEUS MONODON* FABRICIUS SEED UNDER OXYGEN PACKING FOR TRANSPORTATION

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ABSTRACT OF A THESIS

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ABSTRACT

The study was performed with the objective of selecting a sedative at a suitable dose and testing its effect along with other factors viz., packing density, salinity and temperature on the P.monodon seed under oxygen-packed transport conditions. The selection of a sedative at an appropriate dose, from those tried viz., chloral hydrate, MS-222 and tertiary butyl alcohol, was made by conducting statistically designed experiments on the survival of the treated and untreated prawn seed for 72 hours in open containers and on the metabolic activities of the treated and untreated prawn seed for 2 hours. The experiment to find out the effect of the selected sedative was carried out in specially designed hard plastic containers fitted with facilities for packing oxygen under uniform pressure. The experiment was conducted as an asymmetrical factorial experiment with 4 levels of packing density (200/l, 400/l, 600/l and 800/l) and 2 levels of sedation (without and with sedation), salinity (25 ppt and 20 ppt) and temperature (29±1°C and 23±2°C).

Chloral hydrate was selected at a dose of 400 ppm for application on P.monodon post-larvae under oxygen-packed conditions. The application of chloral hydrate on the prawn seed under oxygen packing at the selected dose evidently showed a negative effect. A lower dose (300 ppm) than the selected dose, studied separately, also showed similar results under oxygen packing. An increase in packing density

decreased the time of initial mortality and percentage survival. At packing densities of 200/l, 400/l, 600/l and 800/l, the safe durations of transport (duration of 100% survival) were 7.5 h, 5 h, 3.5 h and 2.5 h respectively at ambient temperature of 29±1°C. In P.monodon seed transport the number of seed has been found as more important than their weight in deciding the survival. A high salinity of 25 ppt was found better than a lower salinity for P.monodon seed transport. Lowering of temperature of the packing medium, rather than applying sedatives or lowering of salinity, has been found as a suitable method for increasing the survival during oxygen-packed transport of P.monodon seed. At the lowered temperature of 23±2°C significantly longer duration of 100% survival (15 h at 200/l and 5 h at 800/l) and better percentage survival at 24 hours (92.5% at 200/l and 83.13% at 800/l) than at the ambient temperature could be observed.