COLD - ANAESTHETIZATION AND LIVE STORAGE OF *PENAEUS MONODON* FABRICIUS FOR TRANSPORTATION IN CHILLED SAW DUST

BY

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THESIS

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To my **Mother**
to fulfil whose dream, I strive
and my **Father**
with whose inspiration, I drive.

DECLARATION

I hereby declare that this thesis entitled COLD-ANAESTHETIZATION AND LIVE STORAGE OF *PENAEUS MONODON* FABRICIUS FOR TRANSPORTATION IN CHILLED SAW DUST 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,
30th Jan. 1997.

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CERTIFICATE

Certified that this thesis, entitled COLD-ANAESTHETIZATION AND LIVE STORAGE OF *PENAEUS MONODON* FABRICIUS FOR TRANSPORTATION IN CHILLED SAW DUST is a record of research work done independently by Sri. K.R. SALIN 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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INTRODUCTION

1. INTRODUCTION

The demand for farming shrimps on an industrial scale has been increasing in many countries of tropical and subtropical zones of the world, over the past several years. Technological advancements in the fields of captive breeding, nutrition, and disease control have all contributed to the emergence of aquaculture industry to play a positive role in augmenting the global food production. Consequent to this remarkable growth of shrimp aquaculture, considerable effort has also been made for improvement of quality of the farmed produce. As it is very obvious, the best way to maintain quality of the harvested produce is to keep them alive for marketing before being consumed. Live transportation of shrimps has thus become an important segment of the fishery trade in recent years. Live fish and shell fish, especially shrimps, enjoy a prime position as luxury items in the international market fetching very high prices, often four to ten times that of frozen products.

Live transportation technology has been perfected in Japan way back in the 1960s, where wild or cultured *Kuruma* shrimp *Penaeus japonicus,* packed in chilled saw dust are airlifted live to markets, in a state of hibernation (Shigueno,1992). Since no water is used, this method is both convenient and economical while airfreighting. Similar live shipment technologies have also been applied to a lesser extent with the black tiger shrimp, *Penaeus monodon,* and the giant freshwater prawn *Macrobrachium rosenbergii* (Subasinghe,1996). New(1995) reported that *M.rosenbergii* survives for 4 to 8 hours in chilled sawdust. The greasy back shrimp, *Metapenaeus ensis* has also been found to have live market potential in Taiwan (Liao,1992).

Aquaculture products enjoy distinct advantages over capture fisheries as far as live marketing is concerned (Csavas, 1992; Kriz, 1994; Subasinghe, 1996). Harvesting could be timed to get maximum price benefit and market access. Often the quality and uniformity of the product would improve the marketability of aquaculture products as compared to those from capture fisheries. The farmers also have the ability to modify to some extent, certain product characteristics to suit specific consumer preferences by manipulating the stock, food, feeding pattern, environment etc.

Live export of farmed shrimps is thus a new and challenging pursuit for aquaculturists. The burgeoning demand for live product in Asia, and the competitive advantages associated with farming suggest that the outlook for live shrimps is extremely positive. The high market price of live *Kuruma* shrimp coupled with poor catches in the sea, evidenced year after year stimulated the successful trend in pond cultivation. Kano(1991) reported that in 1990, about 2 tonnes/day of live *P.japonicus* arrived at Tokyo Central Wholesale Market at the peak period. Taiwan(94%) monopolized the live shrimp export market to Japan, followed by Korea(4%) and China(2%) in 1990. However, the outbreak of virus disease in Japan, Taiwan and China drastically reduced the production in these countries. During the period, 1992-'93, there was a decline of over 80% in imports of live shrimp from Taiwan. The contributions from other countries have also declined in 1993, when Korea exported 1.4%, China,1.15% and Hong Kong, 0.2% of the total market size. Japan imported 402 tonnes of live *Kuruma* shrimp in 1995, Australia being the biggest supplier (Rosenberry, 1996).

Apart from shrimps, lobsters and crabs also have a long history of airfreighting out of water. The main advantage of live storage of lobsters is that they can be main-

tained in prime condition (Beard and McGregor, 1991). Consumer demand for live lobsters continues to increase in Japan, U.K. and other European countries . Cold water lobsters may be chilled to 4^{0}C and shipped at temperatures ranging from 1-7^{0}C. Tropical species might be more sensitive to cold and might already be immobilized at temperatures as high as 14^0 C (Lisac, 1986). Wood shavings, pieces of jute sacks, straw, and *kem - pak* which is a crepe cellulose fibre blanket, are some of the materials commonly used in many countries for packing live lobsters (Kano,1991). Lobsters can be kept alive out of water for more than 24 hours, provided the humidity inside the packing containers is kept high (70-100 %). In India, Rahman and Srikrishnadas (1994) have reported live packing and transport of spiny lobsters from Tuticorin area for export to Hong Kong. The lobsters are gradually cooled down to a temperature of 12-15^0 C from the ambient temperature of 26-32^0 C by means of ice. Packing is done using pre-chilled dry saw dust, straw and pieces of gunny bags, the transport time varying from 12-30 hours.

The conditioning and packaging practices of crabs are almost similar to that of lobsters. The optimum conditions for maintaining crabs alive in air include temperatures between 16^0 C and 20^{0}C and 95 % relative humidity (Gillespie and Burke, 1991). Crabs stored at this temperature survive for about 10 days.

Critical biological criteria have to be fulfilled while shipping live crustaceans out of water (Richards-Rajadurai, 1989). The condition of the animal prior to shipping, maintenance of low storage temperature, maintenance of appropriate humidity and prevention of stress and drying through proper handling are very important. Raw shrimp has long been appreciated as a delicacy in Japan, the dish being known as *Odori ebi* meaning 'moving shrimp', prepared from fresh live shrimp (Ovenden and Kriz, 1993). Because of

the rapid autolysis that starts in crustaceans immediately after death, quality of the food prepared from them is highly dependent on their freshness. Quality conscious consumers are willing to pay a premium price for shrimps sold alive. Csavas (1992) suggested that this provides a special opportunity to aquaculturists to supply high value products to high class restaurants in the major urban centres, even by airlifting live shrimps to Japan, Hong Kong or Singapore.

There are obvious advantages to transportation of selected live aquaculture products of high value such as *P.monodon*. Different species of shrimps may require different temperatures for successful hibernation. Eventhough *P.japonicus* can withstand very low temperatures, the tropical species of shrimps may not be able to tolerate such low ranges of temperature (Schoemaker,1991). *P.monodon* is no longer a difficult species to produce, and it contributes to about 58% of the global shrimp production (Rosenberry,1996). However, the lack of knowledge on handling them live, after they are harvested right through to the point of sale to direct customers, is a major constraint to the development of live marketing of this shrimp.

The present study is therefore an attempt to examine the possibility of cold-anaesthetization and live storage of *P.monodon* in chilled saw dust, which would enable this shrimp to become a candidate species for live transportation without water.

The main objectives of the study are:

i. to find out the optimum chilling temperature and chilling rate for effective cold-anaesthetization of *P.monodon* at which the duration of live storage and the per-

centage survival are the maximum ;

ii. to determine the safe duration of live storage which will give the maximum survival rate of packed shrimp, at a given chilling rate ;

iii. to examine whether there is any difference in weight, before cold-anaesthetization, and after revitalization of the packed shrimp ;

iv. to analyse the meat quality of the cold-anaesthetized shrimp after revitalization, using sensory evaluation; and

v. to observe the behavioural changes, if any, of the shrimp as a result of cold-anaesthetization and live storage.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Enormous opportunities exist for live transportation of cultivable species of shrimps out of water, which considerably increases the final market value of the product. Attempts of live shipment without water, have so far been successful only in the case of *Kuruma* shrimp, *Penaeus japonicus* making it the most expensive shrimp in Japan. Many other crustaceans like lobsters and crabs have long been in use for live transportation out of water with well established markets. But, for the sake of brevity, this review is limited to a summary of the existing practices, and the latest findings in the out-of-water transportation of shrimps alone. An attempt has also been made to evaluate the physiological basis of cold-anaesthetization, based on the various researches done on shrimps, lobsters, crabs and cray fishes, due to an apparent paucity of information regarding the physiology of shrimps, subjected to aerial exposure in the cold.

2.1. Live Transportation Practices of *Penaeus japonicus*

2.1.1. Japan.

Earlier reports on live transportation of *P.japonicus* (Noda,1973; Shigueno,1975; 1978; Korringa, 1976; Imai,1977) have provided similar accounts of the various practices in vogue in Japan. Shigueno (1978) reported that *P.japonicus* could tolerate extremely low temperatures, and hence low temperature storage and transportation was convenient. Richards-Rajadurai(1989) reviewed the existing practices of live storage & distribution of fish and shell fish. Schoemaker(1991) also described the technology of transportation of live shrimp.

The culture shrimp are made to moult at a rapid rate by changing the quality of water, and the diet. Shrimp with hard shells at the intermoult stage are harvested for packing (Shigueno,1978). Harvesting is usually done using a pound net, pump net or electric shocker, adapted for capturing shrimps without injury (Shigueno, 1978; Pillay,1990). The harvested shrimp are immediately transferred to a net basket or a net tray. These are then placed in an aerated cold water tank which is made of concrete sandwiched with heat insulating material, and provided with coiled refrigeration piping on the inner wall (Shigueno,1992). During midsummer,the tank water is first cooled down to 8°C lower than that of the pond water. This is to prevent moulting and to acclimatize the shrimp gradually to the low temperature. This step is not necessary in winter. The tank water is then cooled down to a temperature of 12-14°C, over a period of at least 8 hours, which make the shrimp inactive, moving only their walking legs and pleopods. The cold-anaesthetized live shrimp, which are now well tamed and easily handled, are sorted according to size, and weighed in baskets in lots of 2.5-3.0 kg. After the moisture in the body is shaken off, the shrimp are packed in saw dust previously chilled at -10°C. Packing is done in a cold room, kept at 5-10°C (Shigueno,1992).

2.1.2. Taiwan.

P.japonicus is one of the most important species of shrimps cultured in Taiwan (Chen,1990; Pillay,1990). Most of the *Kuruma* shrimp cultured in Taiwan are exported alive through air freight to Japan in styrofoam boxes filled with saw dust. After harvesting and initial grading, the shrimp are kept in wire baskets and are transferred into a chilling trough in which the water temperature is lowered by 7-8°C over a period of 4 hours, depending on the pond water temperature. It means that during winter, the temperature is lowered from 18°C to 11°C, and during other periods, from 25°C to 17°C (Chen, 1990) . The harvested *Kuruma* shrimp are

then acclimatized to 10-12°C in tanks. The shrimp thus anaesthetized are transferred to a packing room maintained at 10-20°C and after another round of grading, they are packed into cardboard boxes, with chilled saw dust filled in between layers of shrimp (Liao and Chien,1990). One kg of shrimp is packed in each box. On warmer days,a 5mm thick bag of frozen wet saw dust, about the same size of the bottom of the box, is placed above the shrimp inside each box. They survive for about 30 hours in the insulated boxes (Chen,1990). It takes about 3.5 hours for the shrimp to board the airplane after leaving the chill room.

Chen(1990) also reported that Taiwan's total shrimp production plummeted in 1988, due to infection and mass mortality in *P.monodon* farms. Several *P.monodon* farmers shifted to *P.japonicus*, which has a ready market in Japan and fetches a much higher price than *P.monodon.* In 1991, Taiwan harvested 20,000 mt of *P.japonicus*, but only about 3,500 mt, were exported to Japan; and most of the harvest was sold live in local markets. Ferdouse (1992) similarly reported that more *Kuruma* shrimp was consumed locally, and less used for the export markets. However, Liao(1992) reported that almost 50% of the *P.japonicus* production in Taiwan was shipped live to Japan.

2.1.3. Australia.

Recently, Australia has succeeded in exploiting a larger share of the live shrimp market in Japan, by exporting sizeable quantities of live *P.japonicus*, employing similar technologies of handling and transportation, as in Japan (Anon,1991; Ovenden,1993; Kriz,1994). Premium prices were achieved, when the survival rates of shrimp arrived at the Japanese markets, were reliably in excess of 95%(Goodrick *et al.*,1993). The survival was highest when the shrimp travelled at temperatures between 12 and 15°C, but survival improved at 10°C as winter

approached. Since 1992, Australian shrimp farms had been exporting increasing amounts of live *P.japonicus* to Japan, and the export in 1995 was projected to be 24 mt, making it a lucrative and fascinating business (Forbes,1995). Forbes(1995) further stated that in Australia, *P.monodon* farms will doubtless be the mainstay of the industry, but with insatiable demand in Japan for live *P.japonicus,* intensive *P.japonicus* farms will also succeed.

2.1.4. Other Countries.

Apart from Taiwan and Australia, the other major countries exporting live *P.japonicus* to Japan are Korea,China and HongKong, though in small quantities (Gil Rho,1990; Kano,1991; Ferdouse,1992; Kriz,1994). Gil Rho(1990) reported that the transportation of *P.japonicus* in Korea, was more conveniently done in saw dust, than in containers of seawater. Packing is done inside a cold room of 10-15°C and for shipping the shrimp alive, the temperature inside the carton is kept at 15°C. Shrimp and saw dust are alternately layered from bottom to top (Gil Rho,1990). Ferdouse(1992) noted that the Korean Kuruma shrimp once destined for Japan, was selling at a higher price in local markets. She reported that one had to pay an incredible price for shrimp in Korea, where the per capita seafood consumption was as high as 47 kg. Ferdouse(1992) observed that HongKong was the most important non-producing shrimp exporting country. Nearly 40% of China's overall external trade passes through Hong Kong, which has made Hong Kong, the second major source of Chinese shrimp for the world market. The high value live shrimp are usually imported from Southern China. Ferdouse(1992) further reported that Singapore remained a lucrative market for high value live and fresh shrimp for the neighbouring countries.

2.2. Live Transportation of Other Shrimps

The commercial success of live shipment of farmed *P.japonicus* apparently inspired subsequent researchers to try these techniques on other species of shrimps farmed in ponds, as well as those harvested from the sea. Experiments on live storage of other species of shrimps under artificial cold-hibernation for transport have been reported by various authors.

Huq *et al.* (1991) suggested that simulated hibernation of market sized *P.monodon* could be done at about 8°C, both in summer and winter. Liao (1992) reported that an important species of shrimp cultured in Taiwan, the red tail shrimp, *P.penicillatus* did not easily survive outside water, and it was not suitable for live transport. However, the sand shrimp *Metapenaeus ensis* was hardy enough to survive for rather long periods out of water, and was easily transported live, like *P.japonicus*.Huq *et al.* (1994) conducted simulated hibernation experiments using 5 months old *P.monodon*. They found that dormant shrimp kept in chilled saw dust with 5% moisture and guarded by two outer layers of relatively colder, frozen saw dust with 10% and 15% moisture in an insulated box could be kept under simulated hibernated condition, with survival rates of 100, 86, 70 and 60.4% after 12, 18, 24 and 27 hours respectively. Juveniles of *P.monodon* and *P.indicus* were also subjected to similar cold - hibernation tests, by Huq *et al.*(1994); but cold-anaesthetized juveniles were placed on mosquito nets spread over chilled saw dust, and not directly on saw dust. Highest survival of 84.7% was obtained after 12 hours for *P.monodon* juveniles followed by 72.1% after 18 hours. *P.indicus* juveniles, however, gave very poor survival (32.2%) after 6 hours of hibernation.

Goodrick *et al.*(1995) conducted live transportation trials using commercially important

shrimps in Australia such as *P.monodon*, *P.esculentus*, *P.semisulcatus* and *P.plebijus*. Farmed *P.monodon* which were cooled at 3°C/h to 12°C, and packed in chilled saw dust survived for about 24 hours. In another experiment, Goodrick *et al.*(1995) also found that the survival of the farmed *P.monodon* was slightly better at 16°C, than at 12°C. *P.esculentus* are abundantly obtained from trawl catches in Australia, have maroon or brown stripes like *Kuruma* shrimp, and are favoured for their attractive appearance. However, they are not normally cultured in Australia, unlike the *Kuruma* shrimp which though uncommon in Australian waters, are grown in ponds and exported live to Japan.

Goodrick *et al.*(1995) suggested that taking advantage of the similarity of the brown tiger shrimp with the *Kuruma* shrimp, a live market for the former could be developed using shrimp from the capture fishery. When wild *P.esculentus* that had apparently recovered from the stress due to capture were tested, they survived for only 12-18 hours, after being cooled @ 3°C/h, and packed in chilled saw dust at 12°C. *P.semisulcatus* caught from the wild also showed a similar response. This was however, not suitable for commercial trials, since survival for at least 24 hours is required for overseas marketing from Australia. Assuming that the low survival of wild caught *P.esculentus* was due to the stress caused by trawling, a crop of brown tiger shrimp was grown in ponds and harvested using a gentler method (Goodrick *et al.*,1995). But,it was observed that farmed *P.esculentus* did not survive in saw dust for as long as *P.monodon* caught at the same farm. This result was, however, confounded by the evidence that the brown tiger shrimp at the farm were in poor condition. Goodrick *et al.*(1995) also reported that the observations while handling *P.esculentus* at 12°C indicated that this temperature may be too cold. The metabolic responses of *P.esculentus* in saw dust were broadly similar to those shown by *P.monodon;* i.e., the animal was not able to sustain itself when stored out of water for periods longer than about 12 hours at 12°C. Goodrick *et al.*(1995)

suggested that the survival of *P.esculentus* might be slightly extended by cooling to higher temperatures (about 15°C), rather than the 12°C, routinely used for shipping *Kuruma* shrimp, though there are doubts that this temperature would immobilize the brown tiger. It may be noted that as reported by Paterson(1993 a), the *Kuruma* shrimp experience no metabolic stress when held in saw dust at 12°C.

The white legged shrimp, *P. vannamei* is reported to be one of the major species of cultured shrimps in central and south America (Pillay,1990). Jiang *et al.*(1995) conducted experiments to develop a suitable live shipment technology for the white legged shrimp, without water, in South Carolina, USA. After acclimation from 27°C to 14°C, the shrimp were packed in boxes with absorbent paper, and kept in a refrigerated chamber at 14°C. The mean survival after shipment was found to be inversely related to the rate of chilling; i.e., 1-2°C/h, 3-4°C/h, and 5-6°C/h, which resulted in average survival rates of 86.4, 77.6 and 56% respectively, registering a maximum survival of 96%. The shrimp revived almost completely, when reacclimatized from 14°C to 27°C @ 3-4°C/h. It was also found that an initial increase or decrease in temperature of upto 7°C for acclimation had no adverse effect on survival.

2.3. Media for Packing Live Shrimps Without Water

2.3.1. Saw dust and Other Materials.

Saw dust seems to be the most common material used as packing medium in commercial live transport of shrimps (Shigueno, 1975 ; Imai , 1977). In Japan, coarse saw dust of Japanese Cedar tree *Cryptomeria japonica,* locally called *Sugi* is usually used (Korringa,1976). Shigueno(1978) suggested that the saw dust used should be low in resin, untreated and

pesticide-free. The saw dust is first sun dried and then kept in cold storage at-10°C in polyethylene or hemp bags. For packing, a layer of cool saw dust is then spread on the bottom of the corrugated cardboard box, lined with PVC or styrofoam, followed by a layer of shrimp, which is covered by another layer of saw dust. Three or four alternate layers of shrimp and saw dust are thus placed in the box. The full and tightly packed cartons are then sealed by cellophane tape (Shigueno,1975 ; 1978 ; 1992).

One of the characteristic features of saw dust is that it is a poor conductor of heat and since it contains minute air spaces, respiration of the shrimp, though minimum, is not obstructed (Imai,1977). Moreover, the material is light weight. However, Shigueno(1978) observed that the saw dust collected the water remaining in the gills or on the body of the shrimp during transit, which led to dehydration of the packed animals. When the weight of packed *P.japonicus* increases, the amount of filling material also increases and the problem of drying becomes severe. Hence the possibilities of using packing materials other than saw dust have been investigated.

Imai (1977) described a commercial product called *Parite* which, when moistened, remained cold for a longer time, and was about 36% lighter than saw dust. *Parite* is produced from pearl rock, which is finely powdered and rapidly heated, when the particles swell up, and minute air bubbles are formed. *Parite,* after cooling to about 5°C was used for packing at a humidity of 51%. Although the survival rate of *P.japonicus* in *parite* was better, it was reported by Imai(1977) to be more expensive than saw dust.

Schoemaker(1991) reported that substitution of sawdust with algae or sea weeds was possible, with the added advantage that algae also humidified the packed shrimp. Huq *et*

al.(1994) conducted preliminary trials to select suitable packing materials for storage under cold-hibernation of adult *P.monodon*. Saw dust of dry wood, rice husk and wheat bran were tried. Moisture contents of 5,10 and 15% were tested for each of these materials. Saw dust was found to be the most suitable insulating material, and the shrimp could be kept under artificial simulated hibernated condition for longer time, compared to wheat bran and rice husk. Jiang *et al.*(1995) used absorbent paper as the medium for packing cold-anaesthetized *P.vannamei* out of water.

2.3.2. Packing with Oxygen

The survival rate of *P.japonicus* packed in saw dust or other materials, and kept air tight in boxes, filled with oxygen or carbon dioxide had been examined by Imai(1977). Experiments were carried out using cold-anaesthetized *P.japonicus* by packing them in vinyl bags containing some sea water, and filling the bags with oxygen or carbon dioxide and then closing the bags tightly. The survival rate was found to be inversely related to the weight of the bag. Imai(1977) found that the saw dust packs were more economical, the average survival in oxygen pack being less (75%) than that in saw dust packs (93%). All the shrimp packed in carbon dioxide died within a very short time.

Jiang *et al.*(1995) reported that packing of *P.vannamei* after cold-treatment in pure oxygen markedly improved survival, when compared to that in air. Oxygen also appeared to increase resistance of the shrimp to lower temperature. Jiang *et al.* (1995) suggested that the higher survival rates achieved at low shipping temperatures in pure oxygen may be related to lower metabolic rates, reduced respiration and reduced internal acidosis.

2.4. Physiology of Cold-anaesthetization

During live transportation of the *Kuruma* shrimp, *P.japonicus*, using chilled saw dust as the transport medium, mortalities are reported to occur at various degrees (Nakamura,1994). The effect of handling of shrimp at low temperature as well as the endurance of the shrimp in air have not been well studied, unlike in the case of lobsters and crabs. There is virtually a dearth of information, except a few works, regarding the biochemical and physiological changes in the shrimp muscle, in response to falling temperature, and subsequent live exposure out of water. However, with a sound basic knowledge of the normal physiological processes in relation to low temperature responses and the known behavioural patterns, the survival of shrimps exposed to air in chilled saw dust can well be explained.

This section of the review summarizes the available information on crustacean physiology which would be of much significance in establishing a sound physiological basis for the endurance of cold-anaesthetized shrimps, stored out of water.

2.4.1. Principle of Live Storage Without Water.

The underlying principle of live storage for transportation is cold-induced hibernation/ anaesthetization. Lowering the body temperature of live fish/shell fish to the extreme limit will dramatically lower their metabolism, and the animal might go into hibernation (Schoemaker,1991). Hibernation brings many advantages; large live transportation tanks are not necessary because hibernating animals do not swim; death rates from loss of physical strength or from stress caused by vibrations, noise and light are negligible; there is no weight

loss usually and the animals produce no excrement, because they have no need to eat (Schoemaker,1991; Subasinghe, 1996).

Conditioning for a period of time before packing reduces stress to shellfish, and thus metabolic rate and oxygen consumption during transit. Lowered water temperature and starvation are used to condition live animals, which lowers metabolic rate, reduces the fouling of water by ammonia and carbon dioxide, thus keeping the mortality low. Immobilized shrimps could be packed densely in transport containers with almost no water during transport (Subasinghe, 1996). On arrival at the destination, the water temperature is gradually raised to ambient temperature, enabling the animal to revive from hibernation. Lowering the temperature has also been reported to improve the texture of fish flesh (Subasinghe, 1996).

2.4.2. Effect of Temperature.

Temperature is both a limiting factor, setting high and low lethal limits, and a determinant of growth through its impact on molecular activity. Under natural conditions low temperature appears to be a greater cause of shrimp mortality than high temperature. Gunter and Hildebrand (1951) recorded that large numbers of *P.aztecus* died from the cold in severe winter on the coast of the Gulf of Mexico. Ghidalia and Bourgois (1961) found that the distribution of the penaeid *Parapenaeopsis longirostris* was restricted largely to water with a temperature between 14 and 15°C. Joyce (1965) reported that when the water temperature in North Eastern Florida estuaries fell to around 8°C, *P.setiferus* became completely moribund, and about 20% of those collected were dead. At lethal low limits of temperature, metabolic processes slow down below the level required for cellular maintenance, and the shrimps die (Lester and Pante, 1992).

2.4.2.1. Lethal limits for Post-larvae.

The short term temperature tolerance of *P.aztecus* post-larvae had a broad range (Zein-Eldin and Aldrich, 1965), from 80 to 100% at temperatures of 7 to 35°C. The temperature tolerance levels, though unrelated to the sex of the individual, are modified by acclimation temperature. Acclimation to a very low temperature lowers the lower lethal limit, whereas acclimation to above normal limits raises the upper limit. Thus Aziz and Greenwood (1981) showed that the upper and lower median lethal temperature of *Metapenaeus bennettae* acclimated at 17°C were 6.4°C and 32.9°C respectively, but increased to 7.5 and 34.6°C for those acclimated at 32°C. Thermal tolerance studies (both high and low temperature) were conducted by Motoh (1981) on three age groups, viz. post-larvae, early juvenile and juvenile of *P.monodon*. All age groups could tolerate a low temperature of 10°C for a short period of time.

Huq et al.(1991) proposed the point of no return (PNR) of live shrimps, *P.monodon* and *P.indicus* which referred to the lowest temperature beyond which the shrimp would never regain consciousness even if the temperature was raised. They found that the post-larvae of *P.monodon* was more resistant to low temperature compared to juveniles and adults. The PNR for the post-larvae of *P.monodon* was found to be 3°C.

2.4.2.2. Lethal limits for juvenile and adult shrimp.

Temperature is one of the principal factors limiting shrimp culture, world wide. Liu (1983) reported that unlike other penaeids, *P.chinensis* could survive prolonged exposure to temperatures as low as 10°C and could grow well in the range of 18-25°C. The penaeid shrimps

are stenothermal with few species thriving outside a minimum water temperature of 15°C (Dall *et al.*,1990).

Experimental studies on the mortality of *P.monodon* due to extreme temperatures are scarce. Huq *et al.*(1991) found that the PNR of adult *P.monodon* (25-30g size) was around 4°C during summer, and 3°C during winter. Juvenile *P.monodon* appeared more tolerant to lower temperature compared to the adults. However, *P.indicus* juveniles were less resistant than *P.monodon* to low temperature. The PNR for *P.monodon* juveniles was 4°C and that of *P.indicus* 6°C.

Most culture species of shrimps grow best in a temperature range from 24 to 32°C. (Lester and Pante, 1992). *P.monodon* juveniles are reported to survive temperatures as low as 11°C in ponds. *Metapenaeus macleayi* juveniles are reported to tolerate about 6°C in ponds (Lester and Pante, 1992), but experimental studies in tanks have not been reported.

2.4.2.3. Behavioural changes.

Dakin (1938) observed that there was no fishery of *P.plebijus* in winter, because the shrimp remained buried. Dall (1958) noted that *M.bennettae* was less active in winter, but activity resumed in spring when temperatures rose, which suggested that temperature affected the period of time this species spent buried. Fuss and Ogren (1966) found a positive correlation between nocturnal activity of *P.duorarum* in tanks with water temperature ranging from 10 to 26°C. They showed that temperature had a differential size effect; small shrimp emerged more frequently than large ones at low temperatures. Aldrich *et al.* (1968) tested the effect of

gradually reducing temperature on the behaviour of post- larvae of *P.aztecus* and *P.setiferus*. At 12 to 17°C nearly all (94%) *P.aztecus,* but no *P.setiferus* buried. At extremely low temperature, the shrimps were probably so torpid that they were physically unable to emerge from the sand. Shrimps that failed to bury under these conditions, soon fell over and came to lie unprotected on the sediment surface. Aldrich *et al.* (1968) suggested that this was a form of hibernation which enabled the shrimps to survive cold periods until temperatures were more favourable for activity and growth.

Temperature affects the feed intake of penaeids as reported by Liao(1969) who found that food consumption by *P.japonicus* at 15°C was only 20% of that at 25°C. A strong negative correlation has been reported between temperature and intermoult period of euphausids (Fowler *et al.*,1971). Temperature also influences the type and speed of movement. *P.stylirostris* and *P.californiensis* rarely swam at temperatures below 11°C, but often swam above 25°C (Arosamena,1976).

The work of Achituv and Cook (1984) on the carid *Palaemon pacificus* provided a physiological basis for hibernation in a decapod crustacean. The influence of different temperatures, viz.10,15,20 and 25°C on the food consumption, growth, moulting rate and respiration of *P.pacificus* was studied under laboratory conditions. At higher temperatures, the food conversion was found to be temperature dependent, the rate at 25°C being twice that at 15°C. The intermoult period was 17 days at 15°C, and 11 and 10 days at 20 and 25°C respectively. Achituv and Cook (1984) showed that if *P.pacificus* was active at low temperatures their metabolic losses exceeded their ingested ration. At 15°C, the feeding rate was low, and even when the animal was at rest (basal rate), a large proportion of the ingested energy was lost in respiration. However, the respiratory losses in the active rate exceeded the

ingested ration, putting it into a negative energy balance. At high temperature, ingested energy was directed much more efficiently into growth.

Hill (1985) observed that penaeids often respond to a drop in temperature below a certain level by burying in sand and remaining there. He measured two distinctly different aspects of shrimp activity behaviour such as duration of emergence and the duration of locomotor activity, with a view to quantifying their effect in relation to temperature and light. The duration of emergence was defined as the total time spent above the surface of the substrate. They found that nearly all *P.esculentus* emerged every night at temperature above 17°C, whereas only 5% emerged at 14°C. Thus duration of nocturnal emergence is directly related to temperature, apart from light. There was also a significant relationship between the speed of walking and temperature in *P.esculentus;* it walked at around 1.3cm/s at 26°C, and at 0.3 cm/s at 16°C.

Paterson (1993 a) reported that *P.japonicus* that were cooled commercially for live export experienced a period of reflexive jumping and then fell over. They were no longer able to stand upright at temperatures below 13°C. However, shrimp disturbed at 22°C reflected activity ranging from excited swimming to 'playing possum' (*P.japonicus* will sometimes curl on its side on the tank bottom rather than fleeing from disturbance).

2.4.2.4. Temperature-Salinity interactions.

Post-larval and juvenile penaeids live in inshore habitats where extremes of temperature can occur simultaneously with low salinity. Salinity stress can affect temperature tolerance. A number of species can tolerate subnormal and supranormal temperatures better at the lower

and upper ends of their salinity ranges respectively (Kinne, 1964; 1970; Bradley, 1975). On the other hand, better survival in low and high salinities at high and low temperatures respectively has been reported in the shrimps *Palaemonetes varians, Leander serratus* (Panikkar,1940), *Penaeus duorarum* (Williams, 1960) and *P.aztecus* (Williams, 1960 ; Zen-Eldin and Aldrich, 1965).

Aziz and Greenwood (1981) estimated the temperature and salinity tolerances of juvenile *M.bennettae,* by abrupt exposure to critically high or low levels of each factor, following acclimation to 12 combinations of temperature (17, 22, 27 and 32°C) with salinity 65, 20 and 35ppt. Acclimation temperature affected both temperature and salinity tolerances, while the acclimation salinity influenced only the salinity tolerance. Irrespective of temperature and salinity acclimation levels, juvenile *M.bennettae* were able to tolerate temperatures from 8.1 to 32.9°C, and salinities from 1 to 62 ppt.

In a detailed study of the toxicity of brine effluent, Howe *et al.*(1982) reported that *P.aztecus* and *P.setiferus* differed in their interaction between temperature and salinity. As temperature rose from 20°C to 30°C, survival of *P.aztecus* declined linearly at brine doses of 350 to 750 osmolality. However, under the same conditions, *P.setiferus* showed a peak survival at 25°C, and reduced survival both at 20°C and 30°C.

2.4.2.5. Effect on Oxygen consumption.

Knowledge of the oxygen consumption at low temperatures could prove beneficial to the improvement of live transportation methods for penaeid shrimps. Several studies dealing

with the oxygen consumption of *P.monodon* have been limited to either post-larvae (Liao and Huang, 1975; Gaudy and Sloane, 1981 ; Licop, 1985) or juveniles and subadults between 1.5g and 18.0g (Ting ,1970 ; Kuwabara *et al.*,1985). Ting(1970) reported that oxygen consumption of juvenile *P.monodon* (2.53-8.3g) was higher than that of juveniles of *M.ensis* (2.67-5.0g). Cameron and Mangum (1983) reported that smaller shrimps can withstand low dissolved oxygen better than large shrimps, because of their high gill area to body volume ratio. Resting *P.esculentus* was found to be oxygen independent down to about 40% saturation (Dall, 1986). Oxygen consumption was thus independent of external oxygen tension. Dall (1986) also showed that small animals of a given species of shrimp usually have a higher metabolic rate per unit weight than large ones, with oxygen consumption/ body size giving a linear relationship on log-log axes. Similarly, Liao and Murai (1986) observed that the oxygen consumption/unit body weight tended to decrease as the body weight increased. Kurmaly *et al.* (1989) measured oxygen consumption rates of farm raised *P.monodon* from larvae to adult at temperatures between 15 and 35°C. Respiration rates varied from 0.323ml oxygen at 15°C to 1.668ml oxygen at 35°C, for the post-larvae to adults. It has been reported that shrimps of all size can regulate oxygen uptake by increasing their ventilation rate and perhaps decreasing their physiological requirement. This ability perhaps make them more independent of absolute oxygen concentrations than fish (Fast and Boyd, 1992).

Differences exist in published values of the respiration rate of different species of shrimps. Egusa (1961) reported the respiratory rate in resting shrimp *P.japonicus* (14.8-18.2g size) as 77 ml oxygen/kg/h at 22.9°C. Inactive *P.esculentus* cooled to 15°C had a low respiration rate (Dall,1986). Liao and Murai(1986) reported a respiration rate of 59μ mol oxygen/kg/min for *P.monodon* at 20°C, whereas Kurmaly *et al.*(1989) found a rate of 55μ mol oxygen/kg/ min for *P.monodon* at 15°C. Paterson (1993 a) suggested that the differences among the

results obtained for different species might be due to the differences in the circumstances of the experiments. The mean respiration rates of settled *P.japonicus* at 22°C (Paterson 1993a) was higher than the standard respiration rate of *P.esculentus* (Dall,1986). Similarly the mean respiration rate of *P.japonicus* (Paterson,1993a) at 12°C, a temperature at which the contribution of activity to respiration rate is expected to be minimized, was also higher than that of *P.esculentus* cooled to 15°C (Dall,1986). According to Paterson (1993 a) this probably occurs because *P.esculentus* is less tolerant to low temperature than *P.japonicus*. Nakamura (1994) obtained respiration rates for *P.japonicus* (26.8-33.9g size) as 100 ± 10.9ml oxygen/kg h at 20°C, which was reduced to 50 ± 4.7ml oxygen/kg h at 15°C.

Chen and Nan (1994) compared the oxygen consumption and ammonia excretion of five penaeids. Mean oxygen consumption was the highest for *P.chinensis*, followed by *P.penicillatus, P.monodon, P.japonicus* and *M.ensis* in that order.

2.4.2.6. Effect on Metabolic rate.

Knowledge of the relationship between temperature and the metabolic rate of commercially valuable cultivated animals is important for the adequate estimation of energy requirements of these organisms. The rate of metabolism of crustaceans is influenced by a variety of environmental and biological stimulii such as temperature, salinity, external oxygen concentration, light, size, sex, food, and activity (Egusa, 1961 ; McFarland and Pickins,1965; Kutty *et al.*, 1971; Leffler, 1973 ; Veerannan, 1972 ; Nelson *et al.*, 1977 ; Taylor, 1977 ; Fielder, 1979 ; Schembri ,1979 ; Kulkarni and Joshi, 1980 ; Achituv and Cook, 1984 ; Depledge, 1984 ; Houlihan *et al.*, 1984 ; Paterson, 1993 a.). In aquatic ecosystems, temperature and salinity are generally considered to be the major environmental parameters which limit the

distribution of invertebrates (Kinne, 1971). Eventhough the metabolic rates were reported to be influenced by salinity, the response of metabolic rate to temperature seemed unaltered by salinity (Nelson, *et al.*, 1977).

Bishop *et al.* (1984) listed 'routine' or 'standard' metabolic rates from the literature for seven species of penaeids. McMahon and Wilkens (1983) and Dall (1986) reported that because of lack of standardization, most of these values were appreciably higher than the standard rates obtained for *P.esculentus* under defined conditions. Dall (1986) defined routine metabolic rate as the mean 24 hour rate with the shrimp behaving as it would naturally, and the standard rate was defined as the average diurnal respiratory rate when the animal is immobile. Dall (1986) and Liao and Murai (1986) studied the effect of temperature on the metabolic rates of adult penaeids caught from the wild, over a temperature range between 20 and 30°C. Dall (1986) found that at 25°C, routine metabolic rate was only about 10% higher than the standard rate for *P.esculentus,* which is a relatively inactive penaeid.

Kurmaly *et al.* (1989) reported that the laboratory cultured post-larvae of *P. monodon* exhibited similar routine weight specific metabolic rates, to those found for wild *P.japonicus* (Egusa, 1961) and wild *P.monodon* (Dall, 1986 ; Liao and Murai, 1986). Kurmaly *et al.* (1989) also showed that the metabolic rate is least affected by temperature within the natural temperature range, to which the organism is adapted, as already reported by Wieser (1972).

2.4.3. Other Factors influencing Metabolic Rate.

2.4.3.1. Activity.

Levels of activity will obviously affect metabolic rate. Most of the penaeids are nocturnal and lie buried in the substratum during the day. Egusa (1961) measured the oxygen consumption of groups of buried *P.japonicus* and found that the rates were appreciably lower than that in emerged shrimp. Handling increases the respiration rate of crustacea (Truchot and Jouve- Duhamel, 1983 ; Winkler, 1987). Truchot and Jouve-Duhamel (1983) suggested that one of the major ways that handling affects the respiration rate of animals is by increasing their locomotor activity. In both *P.japonicus* and *P.esculentus* respiration rate changed with the activity state of the shrimp (Dall,1986). Dall (1986) found that the standard rate for immobile *P.esculentus* in the day time was similar to that obtained by Egusa (1961) for buried *P.japonicus.*

Dall *et al.(*1986) observed that *P.esculentus* normally emerged at night and spent part of the time foraging, but most of the time standing still with only small movements of its appendages. However, during this period, its metabolic rate was about 14% above the standard rate, presumably because it was digesting food.

Some crustaceans are vulnerable to disturbances during live transport in air (Taylor and Whiteley, 1989) due to handling. European lobster, *Homarus gammarus* increased its respiration rate in response to handling in air (Taylor and Whiteley, 1989; Whiteley *et al.*, 1990).

Dall *et al.*(1990) also found that in *P.esculentus* the feeding-walking behaviour raised oxygen consumption by about 45%, while swimming increased it by 130% or more. While swimming strongly, the animal was no longer oxygen independent, and thus required almost fully oxygenated water to sustain high levels of activity.

Paterson (1993 a) studied the effect of handling at low temperature on the respiration rate of *P.japonicus* which were immobilized by cooling and then handled individually when packed for live shipment out of water. The respiration rates of handled (immediately after a shrimp was placed in the respirometric chamber) and settled (after 30 minutes when the shrimp has recovered from handling) shrimp were measured at 22°C, after cooling the shrimp at a rate of 3°C/h to either 17 or 12°C. Handling the shrimp at 12°C had no effect on their respiration rate, whereas the same treatment at higher temperatures produced a significant rise in respiration rate, that became more pronounced at 22°C. At 22°C, the increased respiration rate was evident in the increased locomotor activity of the shrimp.

The respiration rates of live *Kuruma* shrimp exposed to air conditions at two different temperatures, 15 and 20°C, were examined by Nakamura (1994). All experimental shrimp were alive during the respirometric experiment without showing any active behaviour such as moving or flapping. The concentration changes of oxygen and carbon dioxide showed the evident respiratory activities of the shrimp in air condition. A difference of only 5°C yielded two fold rates of oxygen consumption. The shrimp showed significant acceleration of oxygen consumption when exposed to air. However, this acceleration was largely depressed by lowering the air temperature to 15°C.

2.4.3.2. Moult Cycle.

Moulting and reproduction are two of the endogenous factors that may make metabolic demands. Among the factors which may be responsible for the changes in the metabolic rate during moult cycle, the most important seem to be the absorption and accumulation of Calcium (Passano,1960) and the physiological changes related to the growth and renewal of tissues.

Barclay *et al.* (1983) proposed that the total oxygen demand during peak periods of moulting was largely met by metabolism of digestive gland lipids. This has been confirmed by Dall (1986) who found that the metabolic rate did not vary over most of the moulting cycle in *P.esculentus*, but increased about three days prior to moulting.

2.4.3.3. Starvation.

A decrease in the metabolic rate during starvation as a means of conserving energy is usual in animals. In starved *P.esculentus,* the 24h metabolic rate fell by about 24% after 5 days starvation, and decreased only slightly thereafter (Dall and Smith,1986). Most of this decrease occurred at night and probably resulted from a combination of decreased nocturnal activity, and cessation of digestion and absorption.

2.4.4. Structural Adaptations for Survival Out of Water .

Air and water differ greatly as respiratory media. Sea water is 1800 times more viscous (Jones,1972). Further more, at STP, a litre of dry air contains 209.3ml oxygen whereas a litre of sea water contains only 8.0ml.

Many crustaceans possess morphological and physiological features which allow them to survive periods of emersion during low tide (Newell *et al*,. 1972 ; Wallace, 1972 ; Burnett, 1988 ; DeFur, 1988). It has been demonstrated that arterial oxygen tension (PaO2) in adult *Carcinus maenus* was dramatically reduced following aerial exposure (Depledge,1982). In crabs, as the water drains from the branchial chambers, gill 'clumping' occurs. Surface tension

draws the gill lamellae together, and traps a thin layer of water between the platelets, creating a substantial 'dead space' through which oxygen must diffuse. This clumping drastically reduces the effective surface area available for gas exchange (Wallace,1972; Veerannan, 1974 ; Dejours, 1981 ; Innes *et al,*1986; De Fur *et al.,* 1988) Residual sea water in the branchial chamber may also be a significant factor with regard to impairment of gas exchange (Depledge,1982). Similarly, Depledge (1984) reported that when adult shore crabs, *C.maenus* emerged into air at the same temperature or at a lower temperature than that of sea water in which they had been held, the heart rate fell within 5-10 minutes.

Depledge (1984) presented a hypothetical model of gill function of the shore crab *C.maenus* in air, with some residual sea water in the branchial chambers. As blood passes through the gill exposed to air, normal gas exchange takes place; but in the lamellae bathed in poorly ventilated residual sea water, aeration is poor. If such a shunt operates, the dilution of well oxygenated blood with poorly oxygenated blood may explain the marked reduction of mean arterial oxygen tension in air. Evidence supporting this hypothesis has been presented by Depledge (1984) that when crabs were gently shaken and dried with blotting paper to remove the residual water, PaO2 improved dramatically. However, the oxygen uptake in juvenile *C.maenus* did not appear to be limited to the same extent by residual water as that of adults which might have resulted from better drainage of their branchial chambers and the much higher weight specific branchial chamber volumes in small crabs as shown by Depledge (1984).

However, in crabs like *Macrophthalmus* (Hawkins *et al.,* 1982) and *Cancer, Eurytium* and *Callinectes* (Burnett and McMahon,1987), with poorly developed air breathing organs, both activity and partial pressure of oxygen in the venous blood (PvO2) were reduced during air

exposure. While *Cancer* switched over to anaerobic metabolism, *Eurytium* simply reduced its overall metabolism, in order to cope with a general inability to utilize aerial oxygen (Hochachka and Dunn, 1983; Burnett and McMahon, 1987).

Maitland (1990) reported that the water retained in the lower region of the branchial chamber could provide an important source of oxygen in sematophore crab *Heloecius cardiformis* while they were active in air. These crabs were able to maintain normal activity levels without having to resort to anaerobic metabolism. No information is available regarding similar morphological adaptations, if any, in penaeid shrimps which enable them to survive prolonged exposure out of water.

2.4.5. Regulation of Acid-Base Balance.

The acid-base status of crustacean hemolymph depends on various environmental and physiological factors. Responses to these factors involve generally a primary disturbance followed by more or less complete compensatory adjustments (Truchot, 1975; Cameron, 1978 ; Dejours and Beekenkamp,1978). A proper extracellular and intracellular acid-base balance must be maintained to ensure functional integrity of many basic structures and processes, such as enzymatic function and membrane excitability (Truchot,1983).

2.4.5.1. Internal Acidosis.

The experimental evidences available suggest that lactate is the only major end product of anaerobic metabolism in crustacea (Teal and Carey, 1967; Philips *et al.*, 1977; Gade, 1984 ;

Albert and Ellington, 1985; Hill *et al.*,1989). Lobsters commercially transported live followed the typical response of aquatic crustaceans on removal into air, i.e., internal hypoxia, hypercapnia and the accumulation of lactic acid, due to anaerobic metabolism, resulted from the collapse of the gills and consequent impairment of respiratory gas exchange (Spicer *et al.*,1990). Internal acidosis due to accumulation of lactic acid has also been reported for cray fishes (Morris *et al.*, 1986; Taylor *et al.*, 1987; Wheatly *et al.*, 1996) and shrimps (Furusho **et al.**, 1988 ; Paterson, 1993 b), which were exposed to air. Furusho *et al.* (1988) investigated the changes in the levels of lactic acid and ATP related compounds in the muscle of live *P.japonicus* kept in saw dust at 15°C, in order to find an index suitable for evaluation of shrimp activity during transportation in saw dust. They found that the level of lactic acid in the muscle gradually increased after storage of 48 h in saw dust. The increase in lactic acid concentration suggested that oxygen supply to the tissues was inadequate during commercial live transport of crustaceans, causing a switch over to anaerobic metabolism, as observed in lobsters exposed to air in the laboratory (Taylor and Whiteley, 1989; Spicer *et al.*, 1990; Whiteley and Taylor, 1990).

Spicer *et al.* (1990) examined the effect of aerial exposure (emersion) on the concentration of some metabolites in the blood such as L-lactate, D-glucose and other circulating sugars of a sublittoral crustacean, the Norwegian lobster *Nephrops norvegicus*. There was marked increase in the concentration of L-lactate in the blood which indicated that *N.norvegicus* relied at least partially, on anaerobic metabolism to meet its cellular energy requirements during emersion. This rate of L-lactate accumulation in the blood was similar to that measured by Bridges and Brand (1980) in *N.norvegicus* during exposure to environmental hypoxia. Spicer *et al.* (1990) observed that *N.norvegicus* was unable to survive 18h experimental emersion at 10°C. However, the survival rate increased to about 36-48h, when lobsters were

kept on ice; but the accumulation of L-lactate in the blood was not significantly different from lobsters at 10°C. There was also no apparent differences in activity of the groups noted. Spicer *et al.*(1990) thus suggested that despite lower exposure temperature and presumably lower metabolic rate the lobsters kept on ice might be more reliant on anaerobic metabolism to meet their energy demands than those maintained at 10°C, at least during the first few hours of storage.

Whiteley and Taylor(1990) observed that in contrast to the laboratory situation, the potential combined metabolic and respiratory acidosis in lobsters marketed commercially was roughly double that found under laboratory conditions, even when they were similar to the market conditions (i.e., 10°C and 14h exposure in air). However, in the case of shrimp, Goodrick *et al.*(1993) reported that the survival of *P.japonicus* air freighted from Brisbane to Japan was not significantly different from that of shrimp stored under controlled conditions in the laboratory.

In a study to investigate the longer survival of shrimps like *P.japonicus* in commercial live shipment, Paterson(1993 b) examined the metabolism of *P.japonicus* and *P.monodon* stored for upto 24 hours in dry saw dust by measuring concentrations of L-lactate, adenylate nucleotides and inosine monophosphate (IMP) in abdominal muscle. When *P.japonicus* was stored in saw dust at 12°C, adenylate energy charge (AEC) did not fall, and no lactate or IMP accumulated after 24 hours. However, AEC fell in *P.monodon* stored at a temperature of 12°C, and in *P.japonicus* stored at higher temperatures.

Jiang *et al.* (1995) analyzed the blood acid-base balance during live shipment experiments of cold-anaesthetized *P. vannamei,* in air or oxygen, at different temperatures. Blood samples

were collected from the pericardial sinus, following 3,12 and 24 h shipments at 8-10°C, 12-14°C and 16-18°C. Shipment in air or at higher temperatures resulted in declined blood pH, increased total carbon dioxide and increased lactic acid levels. At lower temperatures in oxygen the shrimp internal acidosis was reduced and hence survival of shrimp packed in oxygen improved.

2.4.5.2. Degradation of Nucleotides.

Stone (1971) and Flick and Lovell (1972) showed that the degradation sequence of ATP (Adenosine triphosphate) in the grass shrimp, *P.monodon* muscle is as follows. ATP to ADP (Adenosine diphosphate) to AMP (Adenosine monophosphate) to IMP (Inosine monophosphate) to HxR (Inosine) and to Hx (Hypoxanthine). Fatima *et al.* (1981) suggested that IMP and Hx can be considered to be good indices for assessing shrimp quality. This has been confirmed by Chen *et al.* (1990) who showed that there was marked reduction in ATP in the muscle of iced *P.monodon*.

Furusho *et al.* (1988) found that the levels of ATP were significantly decreased resulting in a rapid increase in the AMP level. They suggested that the ratio of ATP to ATP related compounds (the total nucleotides) in the muscle which is closely related to the survival rate of shrimp, could be taken as a suitable criterion to evaluate the activity of *P.japonicus* during live transportation in chilled saw dust.

Paterson(1993 b) showed that when the Adenylate Energy Charge (AEC) fell below 0.5-0.6 in *P.japonicus* and *P.monodon* stored out of water in chilled saw dust there was an increase

in muscle lactate and IMP concentrations. The results showed that high concentration of lactate and IMP in muscle tissue at a given temperature could be used to demonstrate that the shrimp had been out of water for too long.

2.4.5.3. Hemolymph buffering and electrolyte concentration.

2.4.5.3.1. Levels of Bicarbonate.

Mc Leese (1965) and Whiteley and Taylor (1990;1992) reported that high temperature (20°C) during transportation of live lobster increased the extent of internal acidosis, probably due to an increase in metabolic rate, resulting in an increased requirement for an elevation in levels of hemolymph bicarbonate buffer. Although crayfishes are hypoxic and hypercapnic in air, they compensate for accumulating acid metabolites, by elevation of buffer base, mobilizing calcium carbonate from internal stores (Taylor and Wheatly, 1981; Taylor *et al.*,1987; Wheatly *et al.*, 1996). Similar effects have also been reported in lobsters (Taylor and Whiteley, 1989; Whiteley *et al.*,1990; Whiteley and Taylor, 1990;1992.) This compensatory response together with the allosteric effects of accumulated lactate and calcium on the oxygen affinity of their respiratory pigment, hemocyanin (Morris *et al,* 1986; Taylor and Whiteley, 1989) restores oxygen transport, enabling the animals to survive in air.

Taylor and Wheatly (1981) found that aerial exposure in European crayfish *Austropotamobius pallipes* at 15°C resulted in respiratory acidosis that was compensated by metabolic bicarbonate accumulation. Taylor and Whiteley (1989) recorded the hemolymph pH values of settled, submerged lobsters at 15°C. DeSouza and Taylor (1991) induced extra

cellular acidosis experimentally by employing aerial exposure in the intermoult shore crab *Carcinus*. Extra cellular pH initially became acidotic, but was compensated by bicarbonate accumulation. Whiteley and Taylor(1992) in a study of the commercially transported live lobsters found that the hemolymph pH values of lobsters arrived at the market in good condition, were similar to those obtained by Taylor and Whiteley (1989), indicating that they were able to compensate for the potential acidosis. This was achieved by elevating hemolymph bicarbonate levels during transit, to 3 times the values found in submerged lobsters. Whiteley and Taylor (1992) also found that the lobsters shipped in poor condition showed a well developed internal acidosis due to either extremely high levels of carbon dioxide or lactate or both in the hemolymph. In many cases hemolymph bicarbonate concentrations were insufficient to buffer the marked acidosis. If hemolymph pH fell below 7.48, lobsters rarely survived after resubmersion. Wheatly *et al.*(1996) observed that the fresh water crayfish *Procambarus clarkii* tolerated 24h of aerial exposure with no apparent ill effects, but a mean loss in body weight of 10±2.1% due to loss of water. The hemolymph pH decreased significantly after 3h in air, but had recovered to submerged levels by 24 h. Wheatly *et al.* (1996) reported that this initial acidosis (recorded after 3h) was relatively small, and entirely respiratory in origin since the change in pH followed the gradient of the non-bicarbonate buffer line. This was in contrast to the higher acidosis in the European crayfish reported by Taylor and Wheatly (1981), which had a metabolic component due to lactate accumulation.

2.4.5.3.2. Levels of Circulating Calcium.

The fate of circulating calcium during emersion acidosis in the different crustacean species is variable. In air-breathing species, acidosis produced by a variety of experimental conditions had been associated with a rise in extracellular calcium (de Fur *et al.*, 1980 ; Henry *et al.*,

1981; Wood and Randall, 1981; Morris *et al.,* 1986). The rock crab *Carcinus productus,* a species that does not tolerate aerial exposure well, exhibited an increase in hemolymph calcium, during experimental emersion (de Fur *et al.,* 1980; deFur and McMahon, 1984). In the terrestrial crab, *Gecarcinus lateralis,* hypercapnic acidosis resulted in elevated circulating calcium (Henry *et al.*,1981). But the hemolymph acidosis that developed was never completely compensated, and the stoichiometry between base and Ca excess was not as predicted from calcium carbonate mobilization. In *A.pallipes* after 24 h aerial exposure at 15°C both Cl and Ca were significantly elevated by 26 and 188% respectively (Morris *et al.,*1986; Taylor *et al.,* 1987). Taylor and Whiteley (1989) also had observed an accumulation of hemolymph calcium in lobsters during live transport.

However, circulating calcium was found to remain unchanged in the shore crab *Carcinus maenus,* which is a highly amphibious species (deSouza and Taylor, 1991). But Ca levels in the epidermis ,hepatopancreas and gut increased, indicating translocation of skeletal Ca.

A comparable investigation was done by Wheatly *et al.* (1996) who reported that hemolymph Ca and Cl remained constant upto 3h of storage, but significantly decreased thereafter and recovered to submerged levels by 24 hours. This was in contrast to *A.pallipes* (Taylor *et al.,* 1987) in which case blood Ca was significantly elevated. Thus Wheatly *et al.* (1996) proposed that circulating levels of Ca increase in species not well adapted for aerial exposure, in which case calcium carbonate mobilization occurs as a last resort when other mechanisms of acid-base compensation have been exhausted.

2.4.5.3.3. Source of Bicarbonate.

For several years, researchers have postulated that dissolution of exoskeletal calcium carbonate may assist in buffering extracellular acidosis in intermoult crustaceans. As indirect evidence of this several studies have reported that hemolymph Ca rises in response to extracellular acidosis which indicated that the bicarbonate is mobilized from internal calcium carbonate stores during exposure to air, in the case of the crab *Carcinus maenus* (Truchot,1975), crayfish *A.pallipes* (Taylor and Wheatly,. 1981; Morris *et al.*,1986; Taylor *et al.*, 1987), crab *Cancer productus* (deFur and McMahon, 1984) and in lobsters (Taylor and Whiteley,1989; Whiteley and Taylor, 1990). However, Taylor and Wheatly (1981) reported that in *A.pallipes* an increase in hemolymph Ca by itself was not direct evidence of exoskeletal erosion, since the Ca could originate in the tissues. Similarly Cameron (1985) directly measured skeletal calcium carbonate erosion during compensation of hypercapnic acidosis on blue crabs, and found that shell erosion made only a minimal contribution (7%) to overall acid -base compensation, which was mediated primarily by branchial exchange. Walsh and Milligan (1989) reviewed the mechanisms by which acidic or basic equivalents are exchanged across cell membranes. Wheatly and Henry (1992) estimated that 50-90% of whole animal acid - base equivalents that accumulated during aerial exposure originated in the intracellular fluid.

Wheatly *et al.* (1996) found that in an air-breathing species like *P.clarkii,* hemolymph Ca declined after 24 h of exposure to air. They showed that *P.clarkii* was able to elevate circulating bicarbonate although the conventional branchial gas transport and ion exchange mechanisms were made non- functional, when the animal was removed from water. Wheatly *et al.* (1996) postulated that this bicarbonate originated in the exoskeleton and that the accompanying Ca was sequestered in tissues, but not in blood which resulted in a drop in the hemolymph Ca level after 24 h. It may also be possible that the accumulated HCO_3 and $CO3^{2-}$ originated in the intracellular compartment as found in other crustaceans during exposure to air (Truchot,

1975 ; Taylor and Wheatly, 1981 ,deFur and McMahon,1984; Taylor *et al.,* 1987).

2.4.5.3.4. Hemolymph Oxygen levels.

The recovery of pH during exposure to air opposes to Bohr shift and together with the accumulation of lactate and Ca^{2+} ions which have a positive modulating effect on the oxygen affinity of crustacean hemocyanin (Larimer and Riggs, 1964; Truchot, 1975 ; 1980 ; Booth *et al.,*1982; Graham *et al.,* 1983 ; Mangum, 1983; Bouchet and Truchot, 1985; Morris *et al.,* 1986) it will increase the combined oxygen content in the hemocyanin at constant $P\theta_2$. Mangum (1983) reported that increased levels of magnesium in the hemolymph could contribute to an increase in hemocyanin oxygen affinity in crustaceans. Taylor and Whiteley (1989) who quantified the effects of lactate and Ca on lobster hemolymph *in vitro,* also found an increase in oxygen affinity of lobster hemocyanin arising from accumulation of calcium. Magnesium levels, however, the did not accumulate in the hemolymph of lobster as described by Mangum (1983) during commercial transport.

Whiteley and Taylor (1990) obtained oxygen content values in the laboratory following 14h of exposure to air at 10°C. Whiteley and Taylor (1992) found that live lobsters transported commercially and arrived at the market in good condition were hypoxic and hypoxemic. Due to internal hypoxia, levels of dissolved oxygen in the hemolymph were low on arrival, so that 91% of the oxygen transported to the tissues was combined with the respiratory pigment. Whiteley and Taylor(1992) also showed that lobsters shipped in air in poor condition were severely hypoxemic with a well developed internal acidosis. Possibly the marked Bohr shift on the hemocyanin reduced its oxygen affinity to such an extent that death ensured from acute hypoxemia.

2.4.5.3.5. Effect of Moult cycle.

In crustaceans, copper content in the hemolymph can be used as a measure of respiratory pigment concentration (Redfield, 1934; Prosser, 1973) and the hemolymph copper levels very according to the moult cycle (Zuckerkandl, 1960; Djangmah, 1970; Djangmah and Grove, 1970). Hemocyanin concentration of copper has been reported to be at their highest during early pre-moult, but decreased sharply just before ecdysis (Truchot, 1978; Mangum *et al.*,1985).

During aerial exposure of shore crabs, hemolymph levels of ecdysone, initially decreased along with pH, but levels increased in target tissues and subsequently the circulating levels recovered following pH (deSouza,1992). Whiteley and Taylor (1992) reported that the live lobsters transported in poor condition were in late pre-moult (proecdysis) stage, some at more advanced stage than others. They were characterized by low circulating levels of copper. Thus Whiteley and Taylor (1992) suggested that the decline in hemocyanin concentration consequent to low circulating levels of copper could account for the severe hypoxemia noted in lobsters in poor condition on delivery. In addition, lactate levels in lobsters in late pre-moult were very low on delivery indicating that they were unable to employ anaerobic metabolism and would not benefit from the modulating effect of lactate on the hemocyanin molecule.

2.4.5.3.6. Effect of Disturbance.

Disturbance during hypoxic exposure is known to induce a rapidly developed and uncompensated acidosis in the lobster *Homarus vulgaris* (McMahon *et al .*, 1978) and *H.*

gammarus (Taylor and Whiteley, 1989). During shipments vehicle noise and vibration can cause distress in live animals, as observed in the velvet crab by Whyman *et al.* (1985). Disturbance could also disrupt the rate of oxygen consumption on aerial exposure in *H. gammarus* (Whiteley *et al.*, 1990). Whiteley and Taylor(1992) observed that lobsters transported out of water from supplier to retailers were subjected to bouts of severe disturbance during handling, sorting and packing. These disturbances could be a major factor in promoting the accumulation of acid metabolites in the hemolymph of market lobsters. However, the lobsters which arrived in good condition at the market were able to compensate for the potential acidosis (Whiteley and Taylor, 1992).

2.4.5.3.7. Changes in circulating sugars.

Marked hypercapnia has been noted in the blood of fully aquatic crustaceans like *Libinia emarginata* (Kleinholz, 1948), *Homarus americanus* (Telford, 1968), *Orconectes propinguus* and *Cambarus robustus* (Telford,1973) and *Liocarcinus puber* (Johnson and Uglow, 1985) following emersion. Telford (1968) suggested that the increase in circulating carbohydrate in the lobster *H. gammarus* might not be solely due to emersion related asphyxiation, but also due to handling stress. Transient decrease in blood sugars due solely to handling stress has also been observed in the crayfish (Telford, 1973).

Spicer *et al.* (1990) observed an increase in circulating sugars in the blood of Norwegian lobster *Nephrops norvegicus,* during experimental emersion due to stress of handling and asphyxiation. There were two peaks in the maximum concentrations of circulating carbohydrates, after 4 h and 18 h of emersion. The first peak in maximum D-glucose concentration might be due to handling stress and asphyxiation. However, in the case of

lobsters kept on ice, the first peak was much reduced and also delayed when compared to that maintained at 10°C. This might be due to the lower temperature of exposure and to the reduced activity (and presumably handling stress) of lobsters post-emersion. Thus Spicer *et al*. (1990) suggested that keeping harvested lobsters on ice greatly increased survival rate , and reduced stress, as indicated by the concentration of circulating carbohydrates. The second peak, however, was suggested solely to be due to asphyxiation and to the mobilization of energy stores as a source of fuel for anaerobic metabolism resulting in the production of L-lactate.

2.4.6. Resubmersion and Associated Changes.

The changes in the hemolymph acid-base balance and the circulating level of different metabolites after resubmergence in water have been studied in detail, in the case of lobsters exposed to air. Regarding shrimps information is still scarce on this aspect.

2.4.6.1.Hemolymph Oxygen.

Whiteley (1988) reported regulatory mechanisms across the gills and a period of hyperventilation followed by submersion of lobster *H. gammarus* which had been exposed to air. Similarly, Whiteley and Taylor (1992) noted that return to sea water enabled lobsters to replenish hemolymph oxygen levels and to adjust acid-base balance after the changes incurred during shipment in air. In lobsters in good condition on arrival at the market, submerged O_2 and PO_2 levels were restored after 1 hour on return to water. This response was however, not immediate since P_{aCO2} levels were still elevated after 30 minutes resubmersion which indicated that the lobsters were still suffering from the effects of gill collapse and retention of air in the

branchial chambers, so that after 30 minutes in water, ventilation and branchial gas and ion exchange had not been fully restored to submerged levels (Whiteley and Taylor, 1992).

2.4.6.2. Bicarbonate levels.

In aquatic crustaceans as in fish, the principal means of containing HCO_3^- is by ion exchange mechanisms over the gill epithelial cells where internal HCO_3^- is exchanged for Cl^- from the environment (Cameron, 1979; 1985). It is also possible that the HCO_3^- is enzymatically dehydrated to CO_2 for diffusion across the gill surface by carbonic anhydrase located on the basolateral membranes of the epithelial cells (Burnett and McMahon, 1985; Henry, 1986; 1987). deFur and McMahon (1984) noted that the hemolymph bicarbonate could be lost by redistribution into intracellular or exoskeletal stores but this mechanism was slower and metabolic in origin. Whiteley and Taylor (1992) reported that the bicarbonate and lactate levels in the hemolymph of lobsters in good condition took a longer period to recover than the oxygen levels.

2.4.6.3. Lactate Levels.

Butler *et al.* (1978) had shown that lobsters accumulated an oxygen debt during prolonged exposure to hypoxia. McDonald *et al.* (1979) and Booth *et al*. (1982) reported that lobsters, like other crustaceans lack the means of rapidly reoxidising lactate. There was an initial and significant increase in lactate concentration in the crayfish *A. pallipes,* observed after 30 min on recovery from exposure to air (Taylor and Wheatly, 1981) who suggested that it could be due to the redistribution of lactate ions previously sequestered in the tissues during exposure

to air. Another possible explanation of this response could be that the lactic acid production might have increased on initial resubmergence due to a high energy demand requiring a contribution from both aerobic and anaerobic metabolites, as found in molluscs following a period of anoxia (De Zwann and Putzer, 1985).

Whiteley (1988) reported that lobsters accumulated an oxygen debt during 14 h of aerial exposure at 15°C which indicated that lactate was reoxidised on resubmersion. Hemolymph lactate remained elevated even after 3 h resubmersion. Lactate levels similar to those expected in submerged lobsters were restored after 24 h resubmergence. On return to sea water, Whiteley and Taylor (1992) observed a dramatic change in acid-base status of hemolymph of lobsters, in good condition, which was adequately buffered against internal acidosis by bicarbonate. There was a significant initial increase in lactate concentration, similar to that noted by Taylor and Wheatly (1981) in crayfish. Following 24 h in holding tanks, the lobsters that arrived in good condition had fully recovered from the changes in hemolymph acid-base status, oxygen, and ion levels which occurred during exposure to air (Whiteley and Taylor, 1992).

2.4.6.4. ATP-related compounds.

Furusho *et al.* (1988) reported that the levels of ATP related compounds in the muscle of live shrimp *P.monodon* kept in saw dust at 15°C recovered to the original values within one hour after they were returned to the sea water.

2.4.6.5. Response of lobsters in poor condition.

Lewis and Haefner (1976) reported a reduction in the rate of oxygen consumption by *Callinectus sapides* during pre-moult since the ventilatory appendages were soft and ineffective. Similarly Whiteley and Taylor (1992) found that 75 % of the lobster which were considered to be in poor condition on delivery, were unable to recover from the effects of exposure to air despite return to sea water. These lobsters remained severely hypoxemic on resubmergence and were suffering either from an acute internal acidosis or were close to ecdysis and were suffering from the repercussions of the oncoming moult. Thus Whiteley and Taylor (1992) observed that in pre-moult lobsters the replenishment of O_2 levels and the elimination of CO_2 from the hemolymph on resubmersion would be seriously impaired. Lobsters that continued to accumulate an uncompensated acidosis on return to sea water died within 10 h. These lobsters were characterized by low circulating levels of copper and therefore low oxygen carrying capacities.

Despite variations in source and transport conditions between consignments, most of the intermoult lobsters were able to compensate for the incipient acidosis and related respiratory problems during transit by progressive mobilization of HCO_3 levels reflecting journey times (Whiteley and Taylor, 1992). High temperature (20°C) during shipment increased the extent of internal acidosis, probably due to an increase in metabolic rate resulting in an increased requirement for the elevation in levels of hemolymph bicarbonate buffer. Thus Whiteley and Taylor (1992) recommended that the maintenance of a relatively low temperature (5-10°C), a humid atmosphere and the absence of disturbance will help to improve the survival of lobsters, when exposed to air during commercial transport.

2.5. Sensory Evaluation.

Sensory evaluation can be a precise tool for ascertaining the quality of fish, if tests are designed properly and trained personnel are selected, the results being subjected to meaningful statistical analysis (Kramer, 1952) . Sensory evaluation is the method employed to assess the quality of food products using human perception of sight, smell, taste and touch. It is the oldest, and still the most widespread means of evaluating the acceptability and edibility of fish / shell fish (Farber, 1965). It is probable that objective quality measurements will never play an important part in fish trade, considering the complexity and variability of the process of spoilage, and the fact that quality is not just dependent on freshness (Ruite, 1965). Farber (1965) suggested that the line dividing fish that are still fresh from those with some early signs of spoilage is not well defined, and is most often subject to difference in personal opinion. It is the subjective taste panel that is used as the standard to determine the accuracy of any objective test (Gould and Peters, 1971) and is very important in determining the acceptability of all food products (Govindan, 1972). It is also one of the most reliable methods for evaluation of the freshness of raw and processed fishery products (Iyer, 1972) .

Shewan *et al.* (1953), Farber (1965) and Mammen (1966) have developed a numerical scoring system for the assessment of frozen shrimp by organoleptic evaluation. Hashimoto (1965) and Jones (1969) reported that the exact consequence of the conversion of ADP and AMP to Hypoxanthin were unknown, but that probably resulted in a loss of meaty flavour. It might also result in bitterness, as indicated by Carroll *et al.* (1968). Pedraja (1970) observed that defects like formation of malodorous substances, flavour deterioration, toughness, mushiness, juiciness, dryness and discolouration occurred in post-mortem shrimp as a result of muscle enzymes, and the interaction of the substances formed by these reactions. Montgom-

ery *et al.* (1970) and Cheuk *et al.* (1979) assessed the acceptability of ice-stored prawns and shrimps, by odour and other tests. Stone (1971) and Flick and Lovell (1972) showed the exact degradation sequence of ATP to nucleotides in the muscle of *P.monodon.*

Flick and Lovell (1972) reported that rigor was not observable in shrimp. In contrast, Lightner (1973) indicated that the loss of ATP during storage should induce rigor in shrimp. Flavour and probably texture of shrimp are influenced by post-mortem nucleotide break down (Cobb III, 1977). Myograph measurements in the laboratory have established that rigor definitely occurs in shrimp (Wiliaichon, 1976). These studies also indicated that there might be a relationship between toughening of muscle and rigor in shrimp. The low amount of rigor usually observed in shrimp may be caused by the post-mortem basic tissue pH, as reported by Cobb III (1977). Cheuk *et al.* (1979) reported that good quality of shrimp was retained upto 10 days of iced storage. Edmunds and Lillard (1979) noted that cultured shrimp were judged as good or better than wild shrimp, by sensory evaluation. Fatima *et al.* (1981) suggested that IMP and Hx can be considered to be good indices for assessing shrimp quality.

As it is very obvious, much of the literature on sensory evaluation of shrimps deal with the quality changes in shrimp, post-mortem. There is a lacuna in the information regarding the organoleptic quality of live shrimp, fully revitalized after cold-anaesthetization and storage out of water. Furusho *et al.* (1988) observed that the concentration of ATP, in the muscle of live *P.japonicus* kept in saw dust at 15°C, markedly decreased after storage of 48 hours, with consequent increase in the AMP level. Chen *et al.* (1990) compared the sensory scores of live shrimp *P.monodon* in oxygenated water during transportation, with that of iced shrimp. They found that the sensory score for overall acceptability decreased from 8.9 to 8.6 during a period of 26 hours. However, for the iced shrimp, it showed a decrease in acceptability scores

from 8.9 to 7.7 during the same period of iced storage. There was no significant difference in sensory quality between iced or oxygenated shrimps during the first 10 hours of storage. However, after 18 hours, the sensory quality of the oxygenated shrimp was significantly better than that of the iced shrimp. Paterson (1993 b) has shown that the levels of ATP markedly decreased in cold-anaesthetized *P.joponicus* packed in saw dust and kept for 24 hours, with subsequent increase in nucleotide degradation products.

The prices *Kuruma* shrimp fetch in Japan market is greatly affected by its physical attributes and perceived quality criteria, which include liveliness and freshness, distinctive colour bands, smell, taste, and flavour (Ovenden & Kriz, 1993). Ovenden and Kriz (1993) and Kriz (1994) observed that the *Kuruma* shrimp with prime quality have a transparent pale gray coloured flesh with distinct maroon/brown stripes, and a blue tail tinged with yellow. The bands are also very distinctive when cooked, turning from maroon to bright red, with the fleshy parts turning white. There should be no pine smell from the saw dust used for packaging, nor a fishy odour, but should have a mild to sweet flavour (Kriz, 1994). Subasinghe (1996) reported that lowering of the temperature during cold anaesthetization improves the texture of fish flesh.

Kriz (1994) also observed that the consumer image and perception of the live *Kuruma* shrimp are among the major factors influencing the market. Being seen eating *Kuruma* shrimp or rock lobster in a high class restaurant in Japan is often far more important to the customer, than physically eating the product. The more obscure and unique the product is, the more interesting and appealing it becomes. Thus Kriz (1994) suggested that as far as the live product is concerned, the emphasis is certainly not on taste, but image and perception, though this may sound strange.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. *Source of shrimps.*

Adult specimens of *P. monodon* ranging in weight from 22-25 g, were harvested from a brackish water pond of area 400 m^2 at College of Fisheries (Kerala Agricultural University) farm by cast netting. The harvested shrimp were immediately transferred to circular fibre glass tanks containing brackish water, and then acclimatized to the required salinity (see Plate I A). During the initial period of the experiment in July 1996, the salinity of the pond varied from 15-20 ppt which was later reduced to 2-4 ppt in August, by rain. The shrimp were in healthy condition in both extremes of salinity. A total number of 456 shrimps were used for the study.

3.1.2. *Conditioning and revitalization tanks.*

The selected shrimps were conditioned in oval, flat-bottomed fibre glass tanks of one tonne capacity, half-filled with brackish water of 15 ppt salinity. Revitalization of the anaesthetized shrimps was done in small circular fibre glass tanks with a capacity of 80 litres.

3.1.3. *Chilling tank.*

For reducing the water temperature to the required level, a water cooler manufactured by Voltas India Ltd., with a specified capacity of 40 litre/hour served as the chilling tank (See Plate I B*a*). The stainless steel water tank was provided with a stand pipe, leading to a drain pipe at the bottom. Refrigeration coils fitted around the chilling tank cooled the water down to a temperature of $14 \pm 1^{\circ}C$.The temperature was regulated by the thermostat switch provided in the equipment. The chilling tank unit had a covering of heat insulating material.

3.1.4. *Chilled Storage cabinet.*

A domestic refrigerator (286 litre capacity) with its thermostat switch adjusted to the cold

setting position was used for live storage of the packed shrimps at low temperature. At this position, the temperature inside the storage cabinet was maintained at 14 ± 1°C.

3.1.5. *Temperature Monitor*.

A six channel continuous freezer temperature monitor with digital display and a precision of 0.1°C designed by CIFT, Kochi and manufactured by M/s Environmental System Engineers, Kochi was used for monitoring of temperature (Plate I B*b*). Long temperature sensors of the instrument were placed in the chilling tank water, which enabled gradual reduction of water temperature to 14± 1 °C (Plate I B*c*). The temperature probes were also inserted into the packing boxes, as well as the chilled storage cabinet, to accurately monitor the temperature, both inside and outside the shrimp packs.

3.1.6. *Net Boxes*.

Two plastic file trays with perforations were joined together and made to form a closed net box for conveniently keeping the shrimp while cold-anaesthetization in the chilling tank (See Plate I C). A folded opening was made on the upper side of the box, through which the shrimp could be placed with in or out of the box. The use of these boxes allowed easy handling of the shrimp with minimum stress during cold-treatment. The dimensions of the box were 36 x 25 x 10 cm.

3.1.7. *Packing medium*.

Saw dust of dry teak wood was used as the medium for packing cold-anaesthetized shrimp at low temperature. Large particles of the saw dust were selected. The saw dust was washed several times with fresh water, pressed by hand, soaked in brackish water and then dried under the sun. One day before the experiment, the saw dust was made wet with brackish water to a moisture content of approximately 10 % and chilled in a refrigerator at 2-3°C.

PLATE I

A. Harvested *P. monodon* in circular fibre glass tank, before conditioning.

B. Chilling tank (a) assembly with temperature monitor (b), temperature probes (c) and aeration lines (d) .

C. Net box used for cold-anaesthetization of *P. monodon*.

D. Card board box with a layer of saw dust (a) and temperature probe (b) used for packing cold-anaesthetized *P.monodon*.

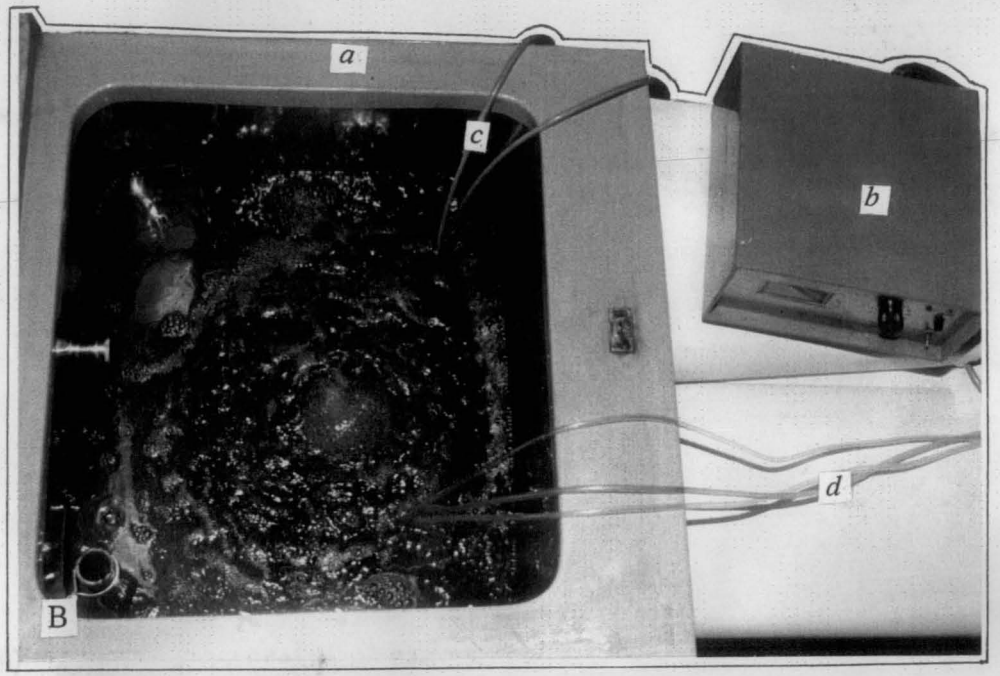
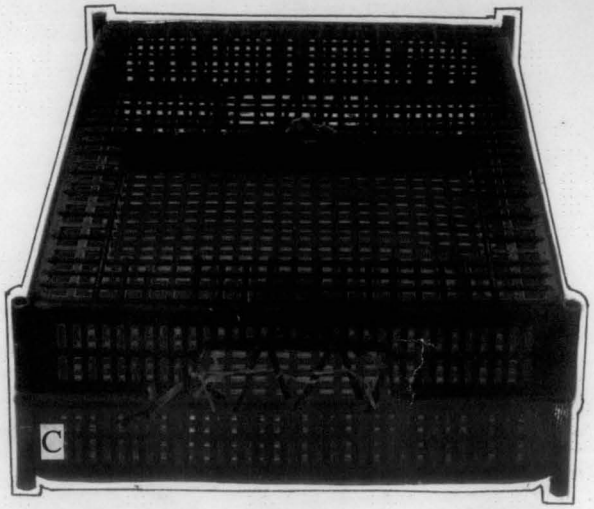
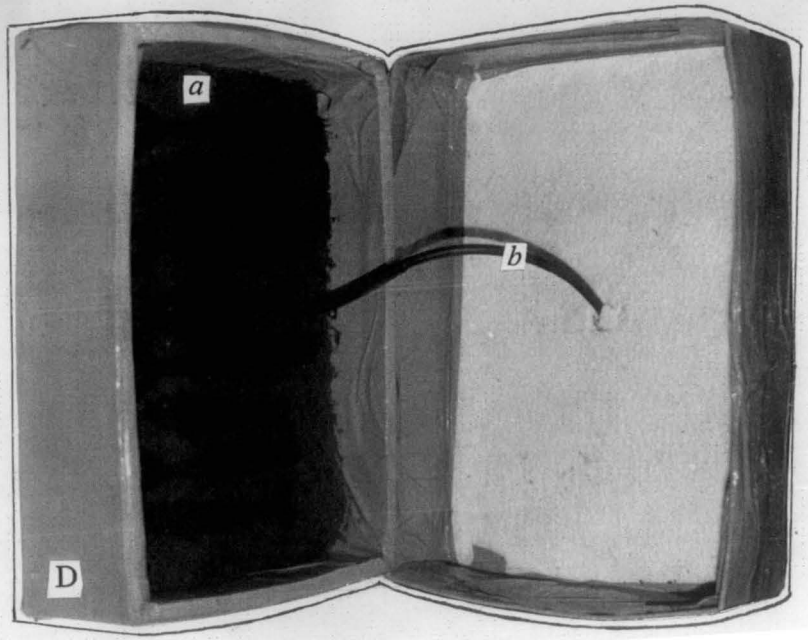


PLATE I





3.1.8. *Packing Boxes.*

Rectangular telescopic type card board boxes lined on the innerside with styrofoam sheet (12 mm thick) and plastic coated outside were used for packing cold-anaesthetized shrimp with saw dust as the packing medium. The box had dimensions of 33 x 22 x 9 cm. A small hole was made at the centre of the lid of the box to insert a temperature probe (See Plate I D).

3.1.9. *Aeration*.

Aeration was provided to the conditioning, chilling and revitalization tanks from an air-compressor through air tubes and air stones. The chilling tanks and the revitalization tanks were aerated vigorously.

3.1.10. *Weighing balance*.

A Shimadzu (Libror) electronic analytical weighing balance with a sensitivity of 0.1 mg was used for weight measurements.

3.1.11. *Salinity refractometer*.

A calibrated salinity refractometer, the accuracy of which was tested by titration was used for all salinity measurements.

3.2. Methods

3.2.1. *Conditioning*.

Healthy and active shrimps having intact appendages, and no visible signs of distress were transferred to the conditioning tank, half-filled with brackish water and provided with moderate aeration. Irrespective of the salinity of the pond, the shrimps were acclimatized to a salinity of 15 ppt, over a period of about 3 hours. The shrimps were conditioned at 15 ppt by starving for about 12 hours after which they were subjected to cold-treatment. Sufficient

numbers of shrimps were kept in this manner so that weak ones could be removed before the experiment. The pond water temperature ranged from 27 to 29 °C (Mean, 27.97 ± 1.05°C) and the tank water was precooled to about 25°C, before subsequent cooling to14±1°C.

3.2.2. *Cold - anaesthetization* .

The shrimps selected for the experiment from the conditioning tank were placed inside the plastic net boxes, and were immediately transferred to the chilling tank provided with strong aeration. In a pilot experiment the shrimps were initially cooled to a temperature of 16±1°C as previously reported by Goodrick *et al.*(1995). At this temperature, though the shrimps appeared still, they responded to prods in water, by slowly moving away from the point of stimulus. When these shrimps were taken out for packing in chilled saw dust, they leaped out amply indicating their activity state. At temperatures of 14±1°C, all the shrimps were lying on their sides, as if in a state of hibernation, with occasional weak movements of pleopods and walking legs. They could be handled easily while packing (See Plate II A) and kept live for considerable period of time. Thus a temperature of 14±1°C was chosen as the cold-anaesthetization and live storage temperature for *P.monodon* at the three cooling rates tested (section 3.2.5). The temperature of water in the tank was gradually reduced according to the selected cooling rates, to 14± 1°C. The shrimps in plastic net boxes were maintained at this temperature in water for about 15 minutes and were then taken for packing in chilled sawdust (See Plate II A).

3.2.3. *Packing and Live storage* .

At least 12 hours before the experiment, a layer of chilled saw dust (3cm thickness) was uniformly placed inside the packing boxes kept in the refrigerator until taken out for packing. At the time of packing, each anaesthetized shrimp (See Plate II B) was taken out from the plastic net box in the chilling tank and then gently placed on the layer of chilled saw dust already prepared in the packing boxes. Eight numbers of inactive shrimps were placed in each box in a single layer as shown in Plate III A. After this, another 3 cm thick layer of saw

PLATE II

A. Cold-anaesthetized *P. monodon* in the plastic net box, ready for packing.

B. *P. monodon* soon after cold-anaesthetization.

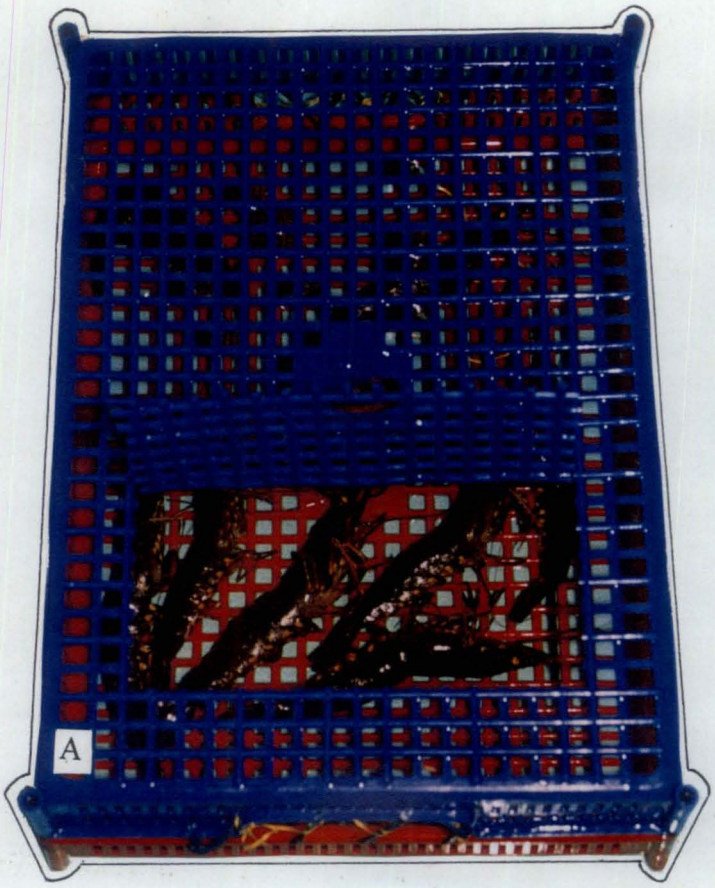


PLATE II

dust was placed over the layer of shrimps and the box closed tightly after inserting the temperature sensor properly into the box without disturbing the packed shrimps. Another sensor was kept inside the storage cabinet to determine temperature fluctuations, if any. The cabinet was closed tightly and the temperature continuously monitored. The chilling tank and the chilled storage cabinet were kept adjacent to each other in order to facilitate easy transfer of the anaesthetized shrimp without any rise in temperature. While handling the cold-anaesthetized shrimp, hand gloves were used.

3.2.4. *Revitalization of hibernated shrimp .*

After the stipulated interval, the boxes were taken out of the storage chamber, opened and the shrimp transferred to the revitalization tanks, containing chilled brackish water (at 20°C) with heavy aeration (See Plate III B). A separate bucket with chilled brackish water was also kept, in order to dip the shrimps before placing into the revitalization tank, which removed the saw dust particles adhered to the body of the shrimp. The shrimps from each box were kept in separate tanks for revitalization. The temperature of water in these tanks was then gradually raised to 28 ±1°C, within a period of 3 hours @ 2.7°C / h. The number of survived shrimps which regained active movements in each revitalization tank was recorded and the survival rate determined.

3.2.5. *Determination of the effect of cold-anaesthetization on the survival and duration of cold storage in chilled saw dust.*

Three treatments were employed for cooling the shrimp down to 14± 1°C. In the first treatment (T_1) the temperature of water in the chilling tank was gradually reduced from the tank temperature of 25°C to 14 ± 1°C over a period of 8 hours, at a cooling rate of 1.38 ± 0.16°C / h (slow cooling rate). Similarly, in the second treatment (T_2) a period of 4 hours, ie., a cooling rate of 2.76 ± 0.32°C / h (moderate cooling rate), and in the third treatment (T_3) a period of 2 hours, ie., a cooling rate of 5.52 ± 0.64°C / h (fast cooling rate) were adopted. The survival of the packed shrimp was determined at 4 hourly intervals for a period of 16 to 36 hours. The

PLATE III

Inactive *P. monodon* being placed in the packing box in head to tail pattern.

Cold-anaesthetized *P. monodon* being revitalized after live storage in a circular fibre glass tank with heavy aeration.

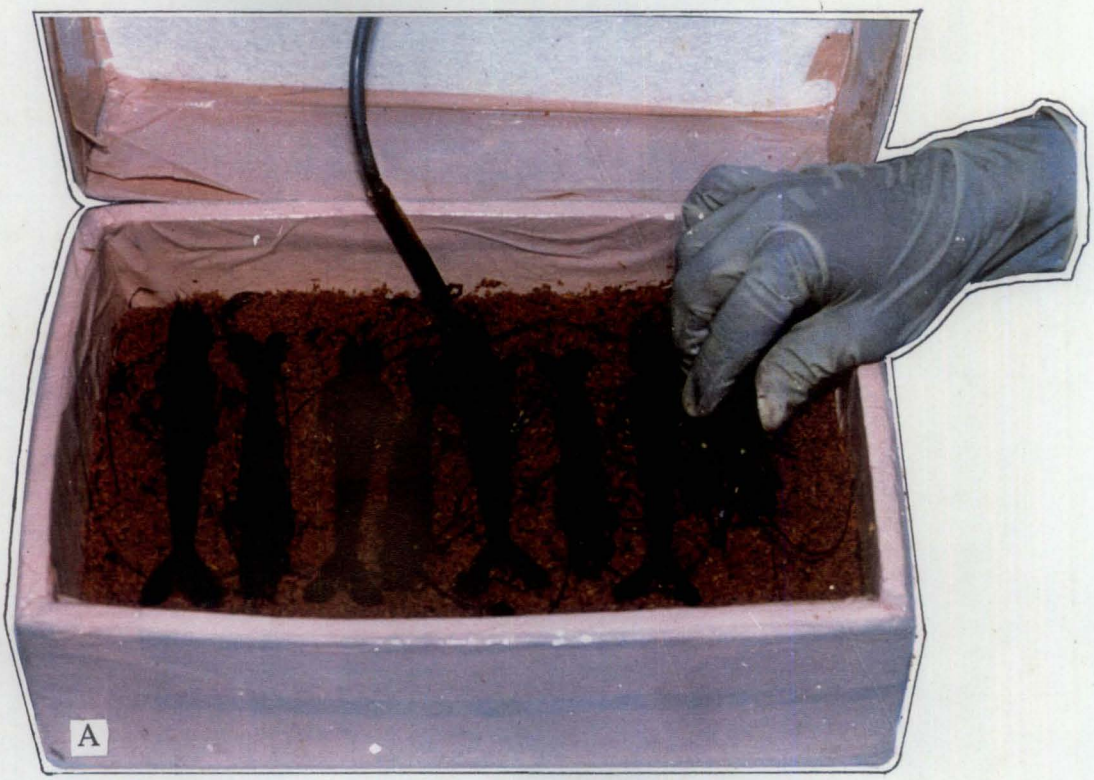
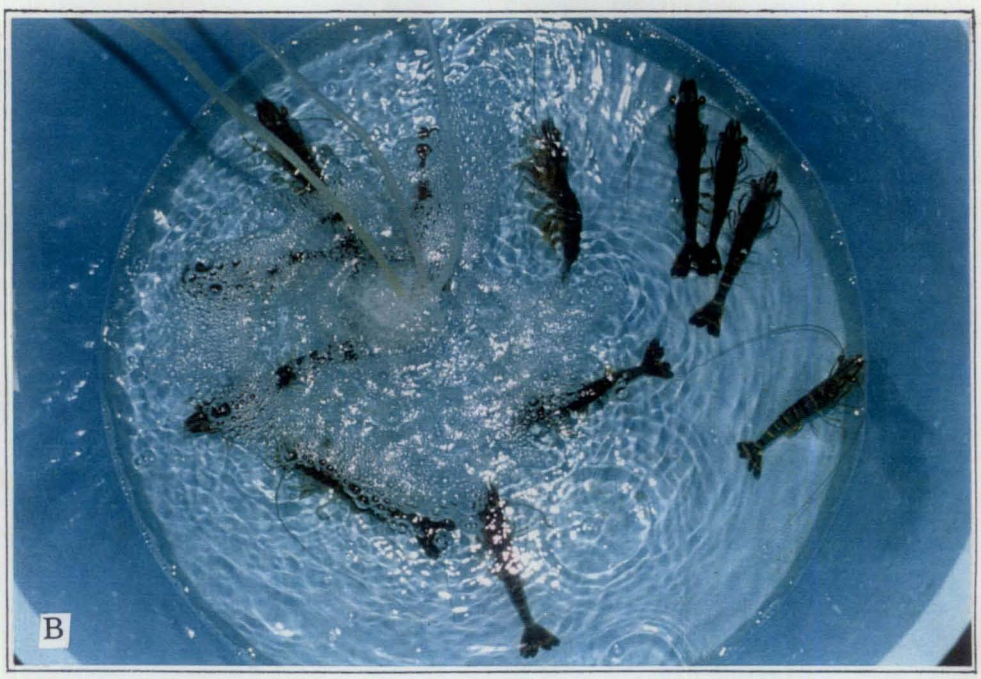


PLATE III



boxes in triplicate for each duration were kept separately for each treatment.

3.2.6. *Determination of difference in weight.*

The shrimps were weighed just before cold-anaesthetization and after 3 - 6 hours of revitalization following cold-anaesthetization and live storage. Two durations of live storage, viz., 12 and 24 hours were tested. Individual weight of shrimps was measured by gently placing each shrimp in a self sealable polyethylene bag, and taking the weight reading immediately upon placing it on the pan of the balance. After replacing the shrimp to water, the plastic bag was weighed separately and thus the initial and final weight of the shrimp determined.

3.2.7. *Sensory Evaluation*.

The sensory evaluation of the revitalized shrimps after cold storage and the untreated shrimp (control) was done by a panel of 10 judges. The shrimps which were cold-treated, and untreated (those harvested freshly from the pond) were taken separately and each lot was divided into equal numbers, to be tested in raw and cooked form. For cooking, the shrimps were beheaded, peeled and the tail was boiled in brine. The raw and cooked shrimps for cold-stored and untreated samples were then coded and presented to the taste panel.

The judges evaluated the quality of the raw/cooked samples for appearance/colour by visual feel, texture by finger feel and odour/aroma by olfaction, and flavour/taste by tongue and olfaction. All the sensory ratings were recorded in a score sheet, with the ratings based on previously assigned numerical scores. A proforma of the sensory evaluation score sheet used is enclosed in Appendix I.

For each cooling rate, samples of revitalized shrimps which survived for 12 and 24 hours of storage in chilled saw dust were drawn separately from packing boxes.

3.2.8. *Statistical Analyses.*

The experiment was conducted using Completely Randomized Design (CRD) with three replications for each cooling rate. Under this, the experiment was treated as a two factor factorial experiment, with 6 levels for the first factor (duration) and 3 levels for the second factor (cooling rate). The analysis of results was carried out using the analysis of variance technique (Snedecor and Cochran, 1967) after making angular transformations wherever found necessary. Pairwise comparison of the data was performed by 't' test and the best cooling rate was thus found out.

Probit analysis (Finney, 1971) was also done to determine the optimum duration of storage which gave the maximum survival rate for the packed shrimps. Safe duration was defined as the duration for obtaining 100% survival, and it was fixed as the hour at which initial mortality started. In the present study, since the boxes packed with cold-anaesthetized *P.monodon* were opened at set intervals of 4 hours and the survival rates determined after revitalization, the safe duration for obtaining 100% survival was established statistically using probit analysis.

For evaluating the weight difference also, asymmetrical factorial design was employed with the cooling rate at 3 levels, duration at 2 levels, and 2 levels of samples, viz., cold-treated and untreated.

Sensory evaluation of the quality attributes of shrimp such as appearance, colour, odour/ aroma and texture was conducted in asymmetrical factorial arrangement, using CRD, with 2 levels of samples, viz., raw and cooked ; 3 levels of treatments, viz., the three cooling rates ; and 2 levels of durations, viz., 12 and 24 hours for the cold-treated as well as the untreated specimens as control. Using this model, the changes in the sensory quality of the cold-hibernated shrimps were evaluated.

RESULTS

4. RESULTS

The results of the study can be conveniently grouped as follows.

i) Effect of cooling rate on percentage survival;

ii) Safe duration of live storage;

iii) Weight loss;

iv) Behavioural changes; and

v) Sensory evaluation.

4.1. Effect of cooling rate on Percentage Survival

The results obtained on the percentage survival of cold-anaesthetized *P.monodon* cooled from 25°C to 14± 1°C at three different treatments (T_1, T_2 and T_3) of cooling rates, viz., 1.38 ± 0.16°C / h (for 8 h), 2.76 ± 0.32°C / h (for 4 h) and 5.52 ± 0.64°C / h (for 2 h), and live stored for 16-36 hours are summarized in Tables 1, 2 and 3 respectively. A survival rate of 100% was obtained for the longest durations of 24, 20 and 16 hours in T_1, T_2 and T_3 respectively. The survival rates were found to decline with longer durations of storage, the lowest survival rate having obtained at 36 hours of storage in all the three treatments. The observations on survival

Table 1. **Percentage survival of packed *P.monodon* cooled from 25 °C to 14±1°C within 8 hours @ 1.38 ± 0.16°C/h, after 16 - 36 hours of live storage.**

Duration of live storage (h)	Total number of shrimp	Replications	No. of shrimp		Survival rate (%)	Total no. survived	Mean survival rate (%)
			in each pack	survived			
16	24	A	8	8	100	24	100
		B	8	8	100		
		C	8	8	100		
20	24	A	8	8	100	24	100
		B	8	8	100		
		C	8	8	100		
24	24	A	8	8	100	24	100
		B	8	8	100		
		C	8	8	100		
28	24	A	8	6	75	21	87.50
		B	8	8	100		
		C	8	7	87.5		
32	24	A	8	6	75	14	58.33
		B	8	5	62.5		
		C	8	3	37.5		
36	24	A	8	3	37.5	11	45.83
		B	8	4	50		
		C	8	4	50		

Table 2. **Percentage survival of packed *P.monodon* cooled from 25 °C to 14±1°C within 4 hours @ 2.76 ± 0.32°C/h, after 16 - 36 hours of live storage.**

Duration of live storage (h)	Total number of shrimp	Replications	No. of shrimp		Survival rate (%)	Total no. survived	Mean survival rate (%)
			in each pack	survived			
16	24	A	8	8	100	24	100
		B	8	8	100		
		C	8	8	100		
20	24	A	8	8	100	24	100
		B	8	8	100		
		C	8	8	100		
24	24	A	8	8	100	23	95.83
		B	8	7	87.50		
		C	8	8	100		
28	24	A	8	7	87.50	19	79.17
		B	8	7	87.50		
		C	8	5	62.50		
32	24	A	8	4	50	10	41.67
		B	8	4	50		
		C	8	2	25		
36	24	A	8	3	37.50	9	37.50
		B	8	2	25		
		C	8	4	50		

Table 3. **Percentage survival of packed *P.monodon* cooled from 25°C to 14±1°C within 2 hours @ 5.52 ± 0.64oC/h, after 16 - 36 hours of live storage.**

Duration of live storage (h)	Total number of shrimp	Replications	No. of shrimp		Survival rate (%)	Total no. survived	Mean survival rate (%)
			in each pack	survived			
16	24	A	8	8	100	24	100
		B	8	8	100		
		C	8	8	100		
20	24	A	8	7	87.50	22	91.67
		B	8	7	87.50		
		C	8	8	100		
24	24	A	8	6	75	21	87.50
		B	8	8	100		
		C	8	7	87.50		
28	24	A	8	6	75	17	70.83
		B	8	5	62.50		
		C	8	6	75		
36	24	A	8	1	12.50	4	16.67
		B	8	2	25		
		C	8	1	12.50		

rate at 32 hours in T_3 could not be made due to power failure during the experiment. This trial could not be repeated for want of required number of uniform sized shrimps from the culture pond. The mean survival rates of the packed shrimps under the three treatments are represented graphically in Fig.1.

The analysis of variance of the data on percentage survival (Table 4) showed that the cooling rates and durations of live storage had significant effects ($P<0.05$) on the percentage survival of packed *P.monodon.* Pairwise comparison using 't' test revealed that the first two cooling rates (T_1 and T_2) were not significantly different from each other. Regarding the durations of live storage, there was no significant difference among 16, 20 and 24 hours of storage, whereas significant difference was observed among 28, 32 and 36 hours.

4.2. Safe duration of live storage and optimum cooling rate

In order to establish the statistically valid safe duration of live storage, which would give 100% survival rate, probit analysis was performed for all the three cooling rates. Also the duration which could sustain 95% survival rate was determined for practical purposes. For the first cooling rate (T_1), the safe duration was computed to be 22.9 ± 1.09 hours (Fig.2), and that for 95% survival was 28.18 ± 0.54 hours. For the second cooling rate (T_2), the safe duration was 19.1 ± 0.4 hours, and a duration of 25.7 ± 0.54 hours would result in 95% survival (Fig. 3). These values were 14.62 ± 1.13 hours and 21.88 ± 0.71 hours, respectively (Fig.4) for the third cooling rate (T_3).

Table 4. **Analysis of variance showing the effect of different cooling rates and durations of live storage on the survival of cold-anaesthetized *P. monodon*.**

SOURCE	S.S	D.F	M.S.S	F
TREATMENT COMBINATIONS	24041.54	16		
COOLING RATES	614.39	2	307.19	4.43*
DURATIONS	22134.92	5	4426.98	63.81**
INTERACTION	1292.23	9	143.58	2.07
ERROR	2358.80	34	69.38	
TOTAL	26400.34	50		

* Significant at 5% level
** Significant at 1% level

Pairwise comparison

Critical difference = 19.98

I.	COOLING RATES	T_3	T_2	T_1
	Treatment means	192.31	202.15	217.84

II	DURATIONS	D_6	D_5	D_4	D_3	D_2	D_1
	Treatment means	103.97	135	194.13	246.2	256.2	270

* Underscored means are not significantly different.

◆ T_1 - 1.38+0.16°C /h for 8 hours

△ T_2 - 2.76+0.32°C /h for 4 hours

* T_3 - 5.52+0.64°C /h for 2 hours

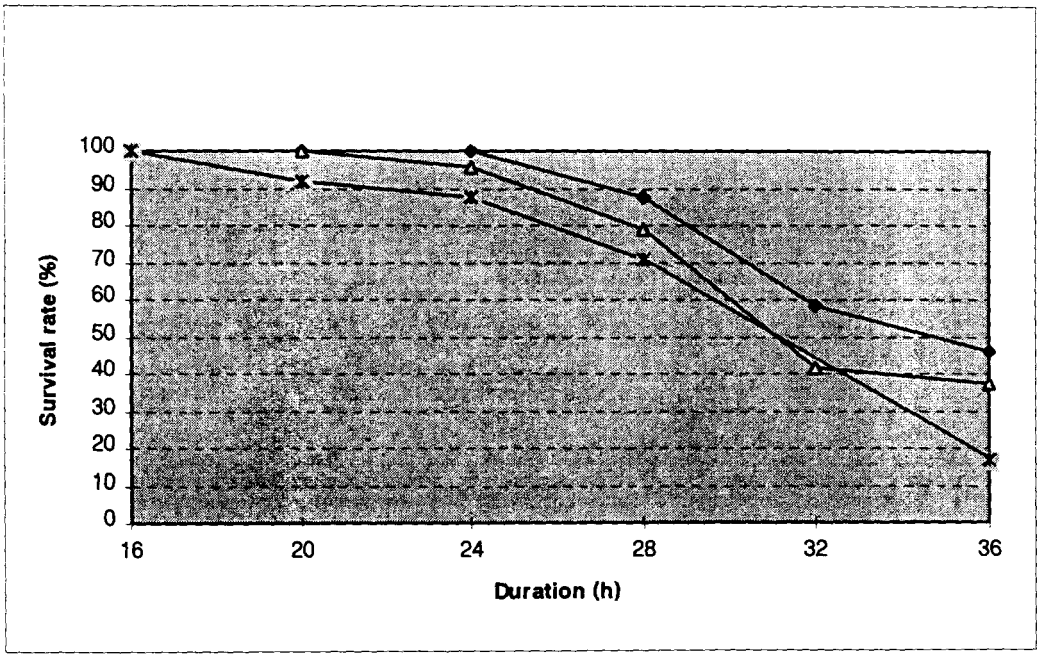
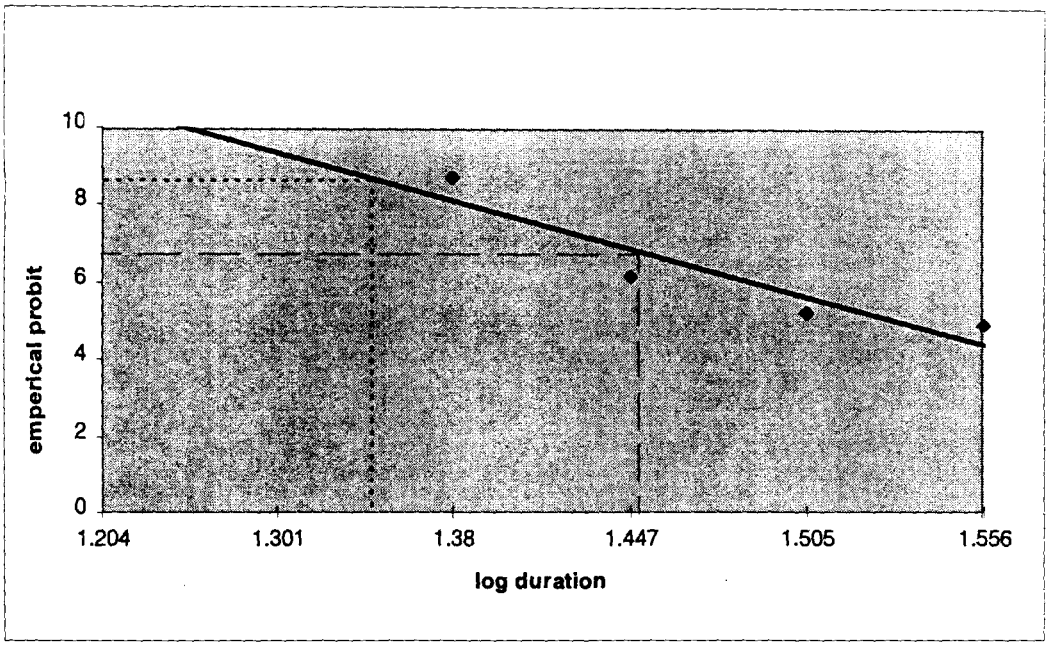
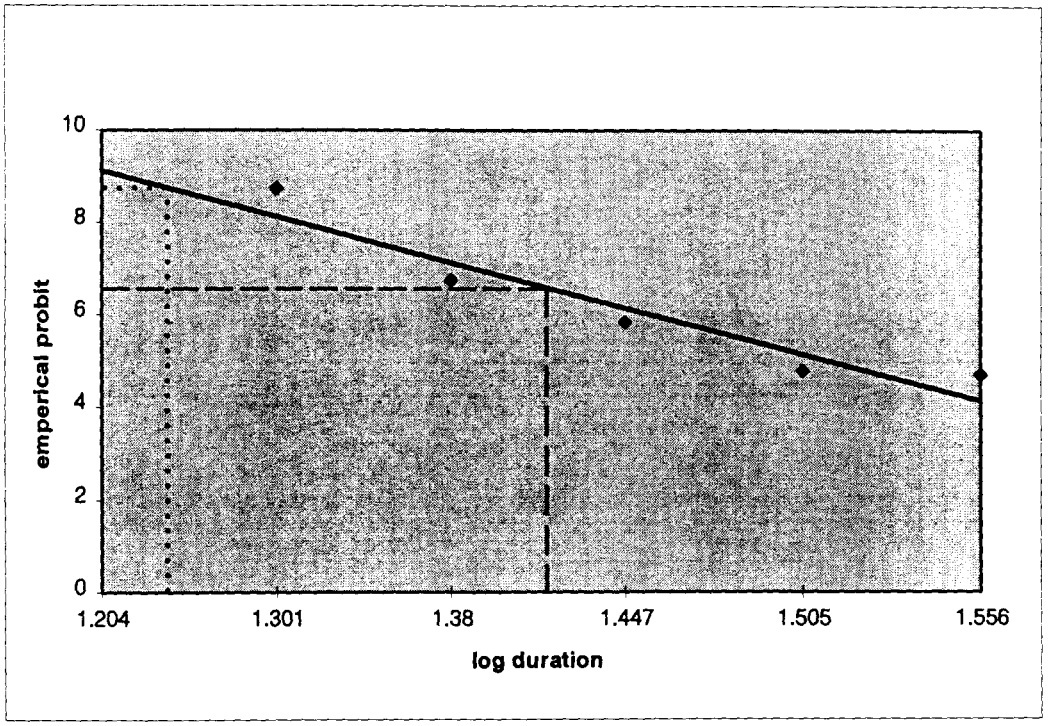


Fig.1. **Mean survival rates of packed *P.monodon* under three treatments of cooling rates after 16 - 36 h durations of live storage.**



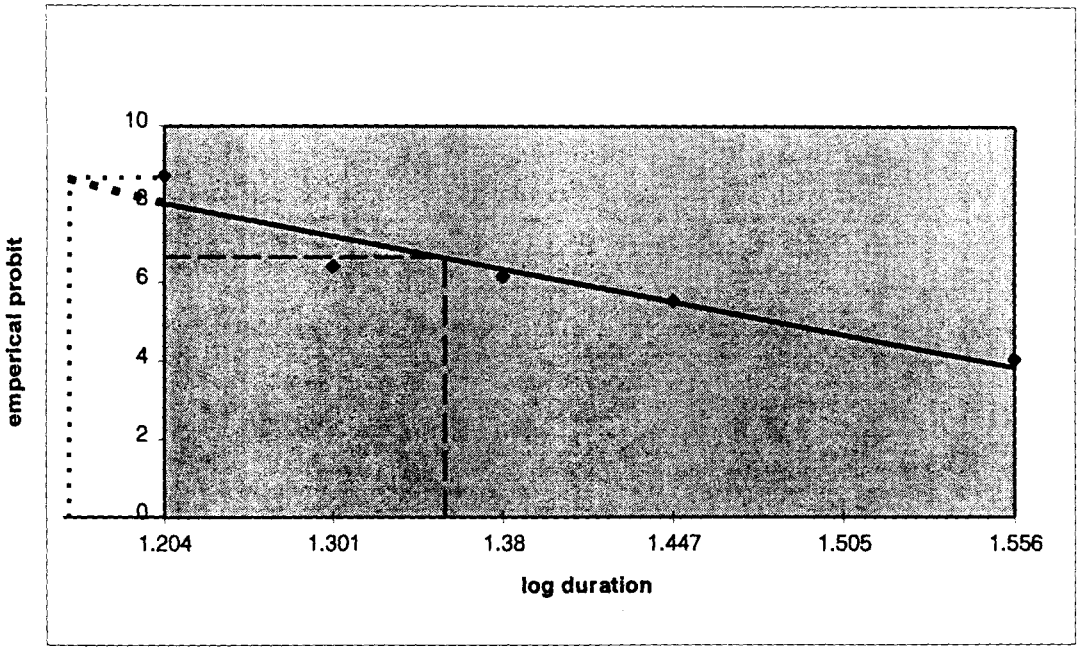
Safe duration - 22.9 ± 1.09 hours
Duration for 95% Survival - 28.18 ± 0.54 hours

Fig.2. **Safe duration (.....) and the duration for obtaining 95% survival (----) of packed *P. monodon* cold-anaesthetized @ 1.38±0.16°C /h within 8 hours, and live stored for 16 to 36 hours.**



Safe duration - 19.10 ± 0.40 hours
Duration for 95% Survival - 25.70 ± 0.54 hours

Fig.3. **Safe duration (.....) and the duration for obtaining 95% survival (---) of packed *P. monodon* cold-anaesthetized @ 2.76±0.32°C /h within 4 hours, and live stored for 16 to 36 hours.**



Safe duration - 14.62 ± 1.13 hours
Duration for 95% Survival - 21.88 ± 0.71 hours

Fig.4. **Safe duration (....) and the duration for obtaining 95% survival (---) of packed *P. monodon* cold anaesthetized @ 5.52±0.64°C /h within 2 hours, and live stored for 16 to 36 hours.**

4.3. Effect of Cold-anaesthetization and Live Storage on Weight Loss

The mean weights of *P.monodon* before cold-anaesthetization, and that of the same shrimp after revitalization, following cold-anaesthetization and live storage for 12 and 24 hours, are presented in Table 5. A decline in the mean weight of revitalized shrimp was observed in all the cooling rates and durations tested. The percentage weight loss was higher for 24 hours than 12 hours of live storage in all the treatments, except T_2. The percentage weight loss was remarkably higher for T_2 where a weight loss of 8.83% and 6.26% were obtained for 12 and 24 hours of storage, respectively. For T_1 these values obtained were 2.75% and 3.47%, and that for T_3, a weight loss of 1.49% and 2.2% were recorded for 12 and 24 hours of storage respectively.

However, the analysis of variance (Table 6) of the data on weight measurements showed that the variations were not statistically significant. These variations are depicted in Fig.5.

4.5. Behavioural Changes in Cold-anaesthetized Shrimp

The behavioural changes of *P.monodon* during cold-hibernation were closely monitored in ıe study. The observations revealed an interesting characteristic of *P. monodon* to become im-obilized and tame upon cooling, which enabled its convenient handling. As the temperature proached 14°C, they displayed some signs of stress such as convulsive flexing of the abdomen d twitching of eyestalks. Then they rolled over onto their sides and lay on the bottom with casional weak movements of pleopods and peraeopods. The shell (exoskeleton) became slightly t and glabrous upon cold-anaesthetization.

Table 5. **Mean weights before cold-treatment (A) and after revitalization (B) of *P. monodon* live stored at two different durations and cold-anaesthetized at three diferent cooling rates.**

Cooling Rate	Durations (h)	Mean Weight ± SD (g)		Wt. loss (%)
		A	B	
T_1	12	24.71 ± 4.68	24.03 ± 4.44	2.75
	24	25.15 ± 3.83	23.34 ± 4.47	3.47
T_2	12	25.15 ± 2.86	22.93 ± 4.28	8.83
	24	25.09 ± 2.86	23.52 ± 4.02	6.26
T_3	12	22.88 ± 4.34	22.54 ± 3.97	1.49
	24	23.64 ± 3.08	23.12 ± 3.07	2.20

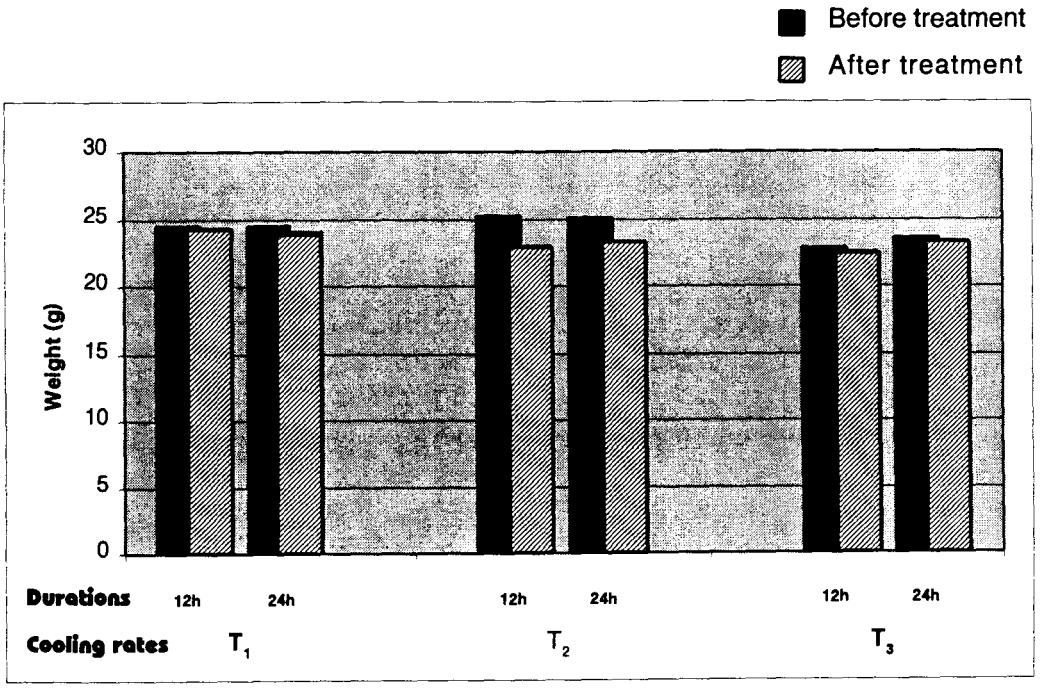


Fig. 5. **Variations in weight before cold-treatment and after revitalization of *P. monodon* live stored for two different durations and cold-anaesthetized at three different cooling rates.**

Table 6. **Analysis of variance showing the effect of different cooling rates and durations of live storage on the loss of weight due to cold-anaesthetization of *P.monodon*.**

SOURCE	S.S	D.F	M.S.S	F
TREATMENT COMBINATIONS	67.75	11		
COOLING RATES	24.73	2	12.37	0.719
DURATIONS	0.289	1	0.289	0.017
BEFORE AND AFTER TREATMENT	24.42	1	25.42	1.479
INTERACTION	18.31	7	2.62	0.152
ERROR	1444.32	84	17.19	
TOTAL	1512.07	95		

Another peculiar feature observed was that when the temperature in the packing box became relatively high (>16°C) in the first treatment (T_1), some of the shrimps were found dead with almost all the walking legs and antennae completely lost, when observed after 24 hours of storage. There were no traces of the broken appendages found in the packing medium, which perhaps were eaten by the neighbouring shrimp. These shrimps had been subjected to longer periods of starvation (22 hours) during conditioning after harvest. Since the storage temperature was higher, these results were considered only after repeating the trial at 14±1°C.

During revitalization, aeration in the water was found to have a strong influence on the recovery of cold-anaesthetized shrimp. Shrimps lying near the air stones recovered faster than those lying away. The state of the shrimp while it was taken out from the box could be easily understood by the appearance of the eyes, apart from the movement of the appendages. The eyes were glowing red in live shrimps, while they appeared dull and opaque in dead ones.

Most of the revitalized shrimps, on recovery, soon became straight on belly within 30 minutes. However, some of the live shrimps were unable to become straight and continued to lie on their sides, though with active branchial movements and agitation of pleopods. They were found to swim actively at this state. A few of those shrimps which could not become straight, displayed whirling movements while still lying on sides, intermittently becoming straight, but soon falling on sides. This type of behaviour was noted in the case of the third treatment (T_3) where the shrimps were cooled within a period of 2 hours for hibernation.

The revitalized shrimps were maintained live for three days in water with strong aeration.

They readily accepted the pelletted feed given to them. Even the shrimps which were lying on their sides were alive during this period.

4.6. Sensory Evaluation

The mean scores for each quality attribute at 12 and 24 hours of storage for the cold-treated and untreated shrimps were tabulated. The raw and cooked shrimps were evaluated separately. Analysis of variance of the average scores was then done for the different attributes, for the raw and / or cooked shrimp after revitalization, taking untreated shrimp as control.

4.6.1. Appearance.

Appearance was tested only for the raw shrimp. There were marked differences in the external appearance of the cold-anaesthetized and live stored *P.monodon* after revitalization. The tips and margins of the pleopods and peraeopods turned reddish, and the entire body appeared dark brown, as if they were maintained in a dark coloured tank for a long period.

The mean scores are presented in Table 7. The analysis of variance of the mean scores (Table 8) indicated that the treated specimens were significantly different ($P<0.05$) from the control. However, the differences were not significant among the cooling rates or durations. The changes in appearance scores for the raw shrimp are shown in Fig. 6. The treated shrimp were found to have a better appearance with average scores of 9.02 compared to the average score of 8.45 for control.

Table 7. **Mean scores for the appearance of raw *P.monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

Treatments	Durations (h)	Treated	Control
		Mean Score ± S.D	Mean Score ± S.D
T_1	12	9.3 ±0.78	8.1 ±1.4
	24	9.1 ± 0.94	8.8 ±1.3
T_2	12	8.75 ± 0.75	8.45 ± 1.3
	24	9.05 ±0.85	8.25 ± 1.2
T_3	12	8.95± 0.789	8.55 ± 1.4
	24	8.95 ± 0.789	8.55 ± 1.4

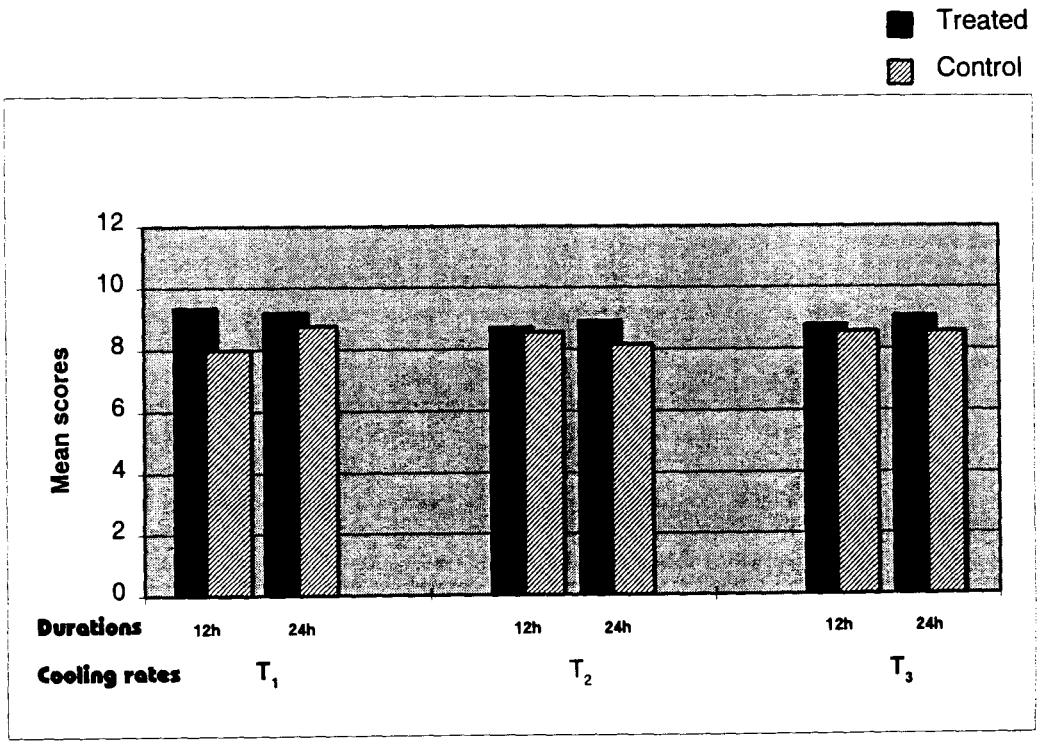


Fig. 6. **Mean appearance scores for raw *P. monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

Table 8. **Analysis of variance of the mean scores for appearance of raw *P. monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

SOURCE	S.S	D.F	M.S.S	F
TREATMENT COMBINATIONS	14.37	11		
COOLING RATES	0.82	2	0.41	0.305
DURATIONS	0.303	1	0.303	0.226
TREATED AND CONTROL	9.637	1	9.637	7.173*
INTERACTION	3.61	7	3.61	2.687
ERROR	145.1	108	1.3435	
TOTAL	159.47	119		

* Significant at 5% level.

4.6.2. Colour.

The colour of the shrimp meat was tested for the raw as well as cooked specimens after revitalization. The mean scores are given in Table 9. In all cases of the treated shrimps, colour of the raw meat was darker, that became brilliant red on cooking. The analysis of variance of the mean scores (Table 10) showed that there was significant variation ($P<0.05$) in colour between the cold-treated shrimps and the control. However, the effects of different cooling rates and durations were not appreciable. The treated specimens had better colour (mean score 8.99) when compared to the control (mean score 8.51). The variations in colour scores are presented graphically in Fig.7 and 8, for the raw and cooked shrimps, respectively.

4.6.3. Texture.

The mean texture scores assigned for raw and cooked shrimp meat are presented in Table 11, and these changes are depicted in Fig. 9 and 10, respectively for raw and cooked specimens. The analysis of variance of the mean scores (Table 11) revealed that the changes in texture of the shrimp flesh due to the treatments at two different durations were statistically insignificant.

4.6.4. Odour / Aroma.

Odour of the raw samples and aroma of the cooked samples were evaluated. The mean scores are given in Table 13. The raw shrimp meat had an algal odour, which was altogether removed while cooking. Significant difference in odour and aroma of the raw and cooked specimens was obtained from the analysis of variance (Table 14), though the variation between treated

Table 9. **Mean scores for the colour of raw and cooked *P.monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

	Treatments	Durations (h)	Treated	Control
			Mean score ± S.D.	Mean score ± S.D.
RAW	T_1	12	9.0 ± 0.82	7.5 ± 1.05
		24	9.33 ± 0.86	8.13 ± 1.23
	T_2	12	8.72 ± 0.78	8.83 ± 0.85
		24	8.3 ± 0.78	8.5 ± 0.94
	T_3	12	8.71 ± 0.70	8.43 ± 1.4
		24	9.43 ± 0.49	8.43 ± 1.4
COOKED	T_1	12	8.61 ± 1.2	8.05 ± 1.49
		24	9.67 ± 0.62	8.46 ± 1.20
	T_2	12	8.56 ± 0.79	9.0 ± 0.71
		24	9.1 ± 0.7	8.25 ± 1.2
	T_3	12	9.0 ± 1.06	9.29 ± 0.7
		24	9.43 ± 0.73	9.29 ± 0.7

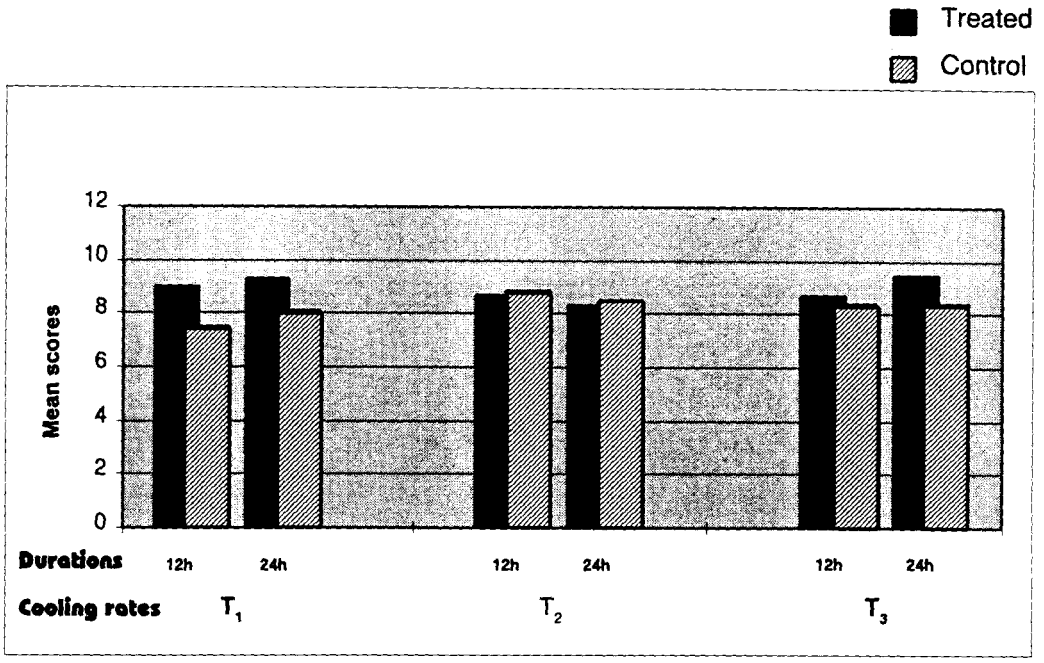


Fig. 7. **Mean colour scores for raw *P. monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

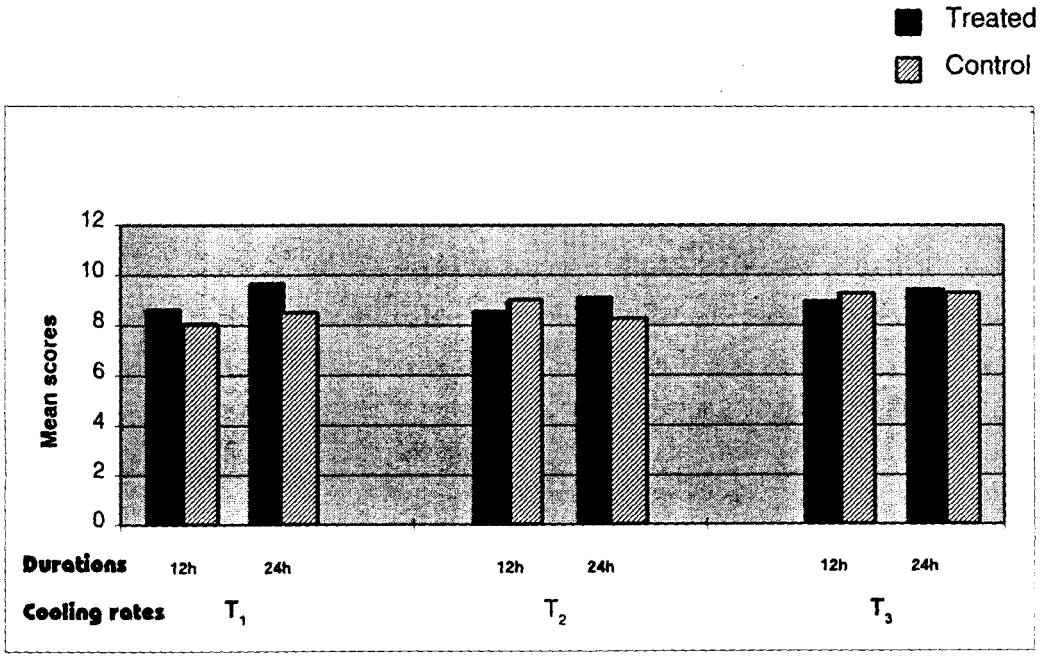


Fig. 8. **Mean colour scores for cooked *P. monodon* revitalized after live storage for two different durations,following cold-anaesthetization at three different cooling rates.**

Table 10. **Analysis of variance for the colour of raw and cooked *P.monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

SOURCE	S.S	D.F	M.S.S	F
TREATMENT COMBINATIONS	2.497	11		
TREATED AND CONTROL	1.3538	1	1.354	6.065*
COOLING RATE (A)	0.7688	2	0.384	1.722
COOKED AND RAW (B)	0.482	1	0.482	2.158
DURATIONS (C)	0.286	1	0.286	1.281
INTERACTIONS : A * B	0.149	2	0.074	0.224
A * C	0.733	2	0.366	1.641
B * C	0.024	1	0.024	0.'108
A * B * C	0.055	2	0.028	0.124
ERROR	2.4552	11	0.2232	
TOTAL	6.306	23		

* significant at 5% level.

Table 11. **Mean scores for the texture of raw and cooked *P.monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

	Treatments	Durations (h)	Treated	Control
			Mean score ± S.D.	Mean score ± S.D.
RAW	T_1	12	9.33 ± 0.94	9.56 ± 0.83
		24	9.50 ± 0.89	9.56 ± 0.99
	T_2	12	9.10 ± 0.91	9.30 ± 0.9
		24	9.70 ± 0.64	9.30 ± 0.92
	T_3	12	9.75 ± 0.66	9.25 ± 0.97
		24	9.50 ± 0.9	9.25 ± 0.97
COOKED	T_1	12	9.33 ± 0.94	9.67 ± 0.94
		24	9.83 ± 0.66	9.33 ± 0.94
	T_2	12	9.60 ± 0.68	9.10 ± 0.92
		24	9.30 ± 0.92	9.10 ± 0.94
	T_3	12	10.0	9.50 ± 0.9
		24	9.50 ± 0.87	9.50 ± 0.87

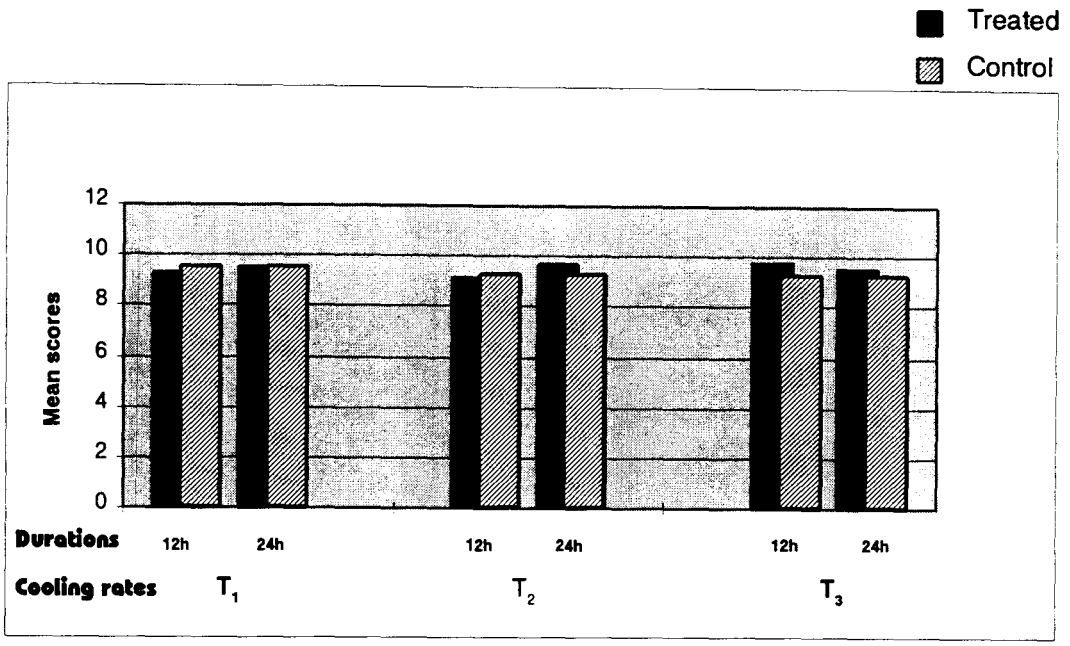


Fig. 9. **Mean texture scores for raw *P. monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

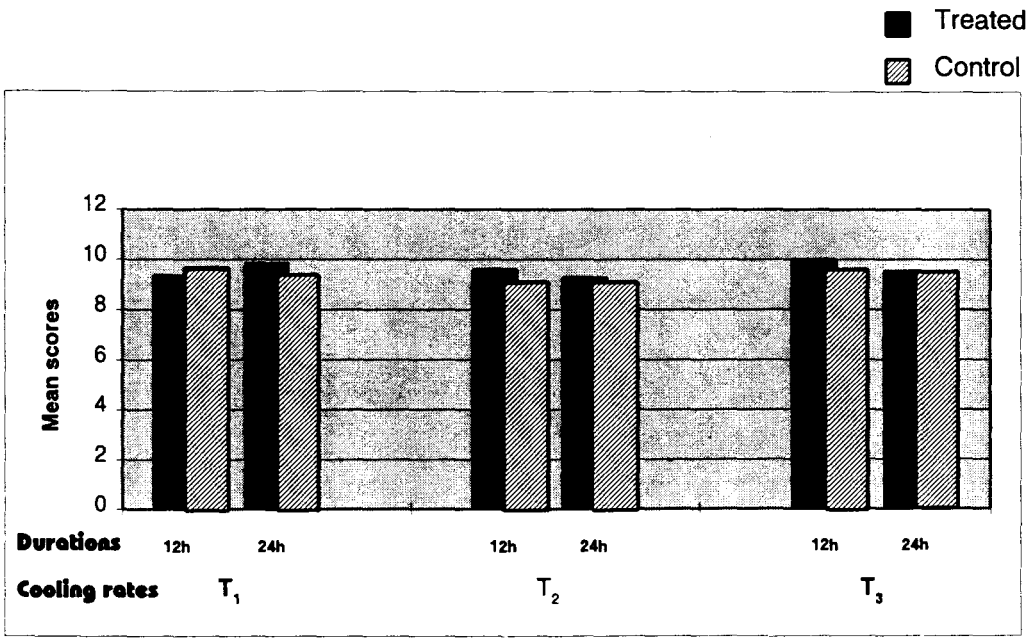


Fig. 10. **Mean texture scores for cooked *P. monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

Table 12. **Analysis of variance for the texture of raw and cooked *P.monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

SOURCE	S.S	D.F	M.S.S	F
TREATMENT COMBINATIONS	0.528	11		
TREATED AND CONTROL	0.170	1	0.170	3.302
COOLING RATES (A)	0.237	2	0.118	2.295
COOKED AND RAW (B)	0.018	1	0.018	0.355
DURATIONS (C)	0.00075	1	0.00075	0.0146
INTERACTIONS : A * B	0.069	2	0.034	0.667
A * C	0.094	2	0.047	0.916
B * C	0.056	1	0.056	1.084
A * B * C	0.053	2	0.027	0.515
ERROR	0.567	11	0.052	
TOTAL	1.265	23		

Table 13. **Mean scores for the odour / aroma of raw / cooked *P.monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

	Treatments	Durations (h)	Treated	Control
			Mean score ± S.D.	Mean score ± S.D.
RAW (ODOUR)	T_1	12	8.56 ± 0.5	7.67 ± 0.67
		24	8.83 ± 0.90	8.25 ± 0.62
	T_2	12	8.95 ± 0.79	8.65 ± 0.79
		24	8.75 ± 0.87	8.55 ± 0.65
	T_3	12	8.38 ± 0.86	8.38 ± 1.65
		24	8.5 ± 0.96	8.38 ± 1.65
Cooked (AROMA)	T_1	12	8.89 ± 0.87	8.22 ± 1.31
		24	9.0 ± 0.91	9.0 ± 0.90
	T_2	12	8.95 ± 0.82	9.0 ± 0.77
		24	8.9 ± 0.94	8.65 ± 1.1
	T_3	12	8.5 ± 0.71	9.13 ± 1.1
		24	8.88 ± 0.78	9.13 ± 1.1

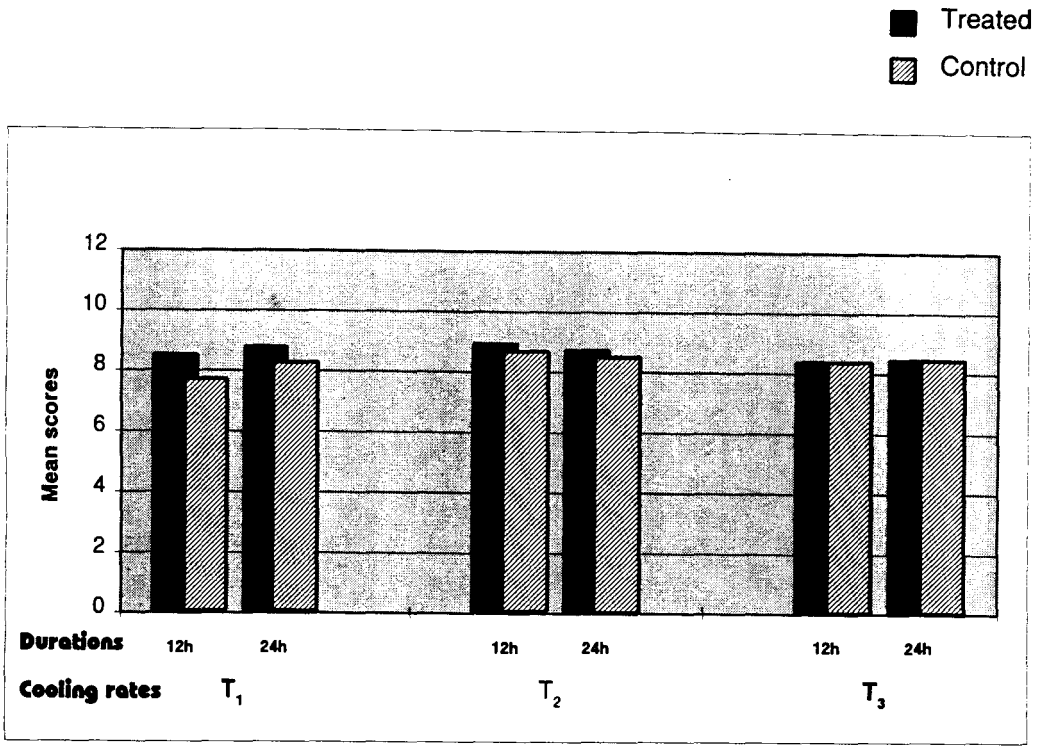


Fig. 11. Mean odour scores for raw *P. monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.

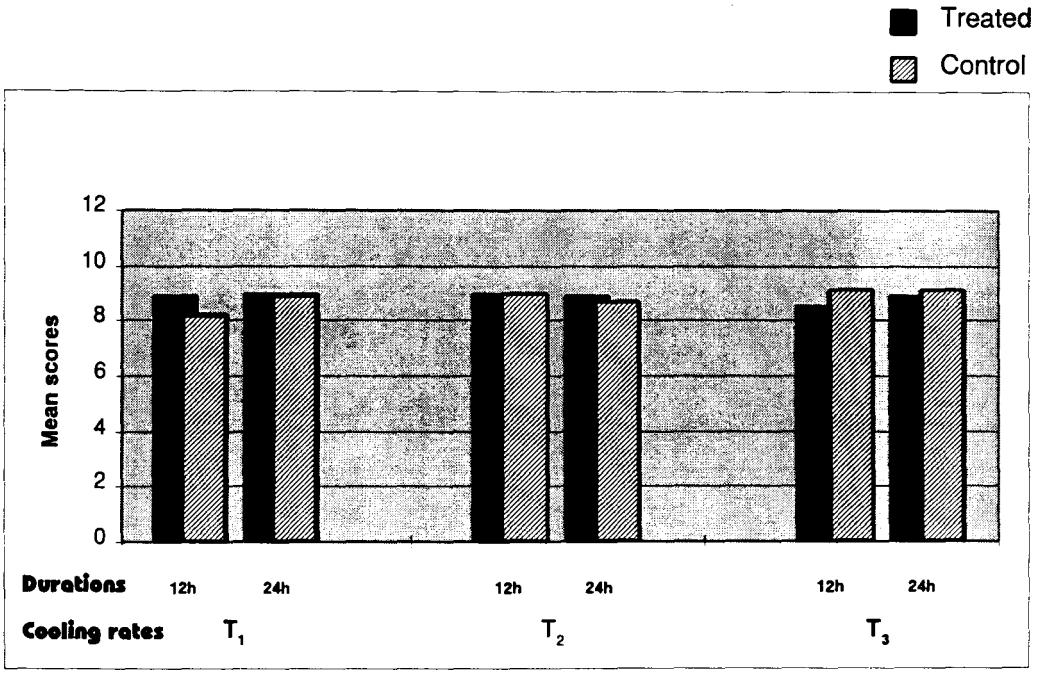


Fig. 12. Mean aroma scores for cooked *P. monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.

Table 14. **Analysis of variance for the odour / aroma of raw / cooked *P.monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

SOURCE	S.S	D.F	M.S.S	F
TREATMENT COMBINATIONS	1.677	11		
TREATED AND CONTROL	0.180	1	0.180	2.104
COOLING RATES (A)	0.247	2	0.123	1.438
COOKED AND RAW (B)	0.807	1	0.807	9.412 *
DURATIONS (C)	0.099	1	0.099	1.153
INTERACTIONS : A * B	0.143	2	0.072	0.836
A * C	0.372	2	0.186	2.171
B * C	0.0017	1	0.0017	0.0195
A * B * C	0.0082	2	0.0041	0.048
ERROR	0.943	11	0.086	
TOTAL	2.8004	23		

* Significant at 5% level.

and control, cooling rates and durations of storage were negligible. The cooked aroma was slightly better with a mean score of 8.85 than the raw odour with mean score of 8.49. The variations in odour and aroma scores of raw and cooked shrimps, are shown in Fig.11 and 12, respectively, for 12 and 24 hours of storage.

4.6.5. Flavour.

Flavour of the shrimp meat was tested only for the cooked shrimp. The mean scores are presented in Table 15. The analysis of variance of the mean scores (Table 16) showed that the cold-treated shrimps were significantly different ($P<0.05$) from the control. The comparison of the respective mean scores revealed that the flavour of the treated shrimp was better (mean 9.13) than the control (mean 8.78). The changes in flavour scores of cooked shrimp are depicted in Fig.13.

Table 15. **Mean scores for the flavour of cooked *P.monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

Treatments	Durations (h)	Treated	Control
		Mean score ± S.D.	Mean score ± S.D.
T_1	12	8.7 ± 1.2	8.1 ± 0.94
	24	9.2 ± 0.87	9.1 ± 0.83
T_2	12	9.3 ± 0.64	8.88 ± 1.08
	24	9.2 ± 0.75	9.0 ± 0.78
T_3	12	9.0 ± 0.78	8.9 ± 0.94
	24	9.4 ± 0.80	8.8 ± 0.98

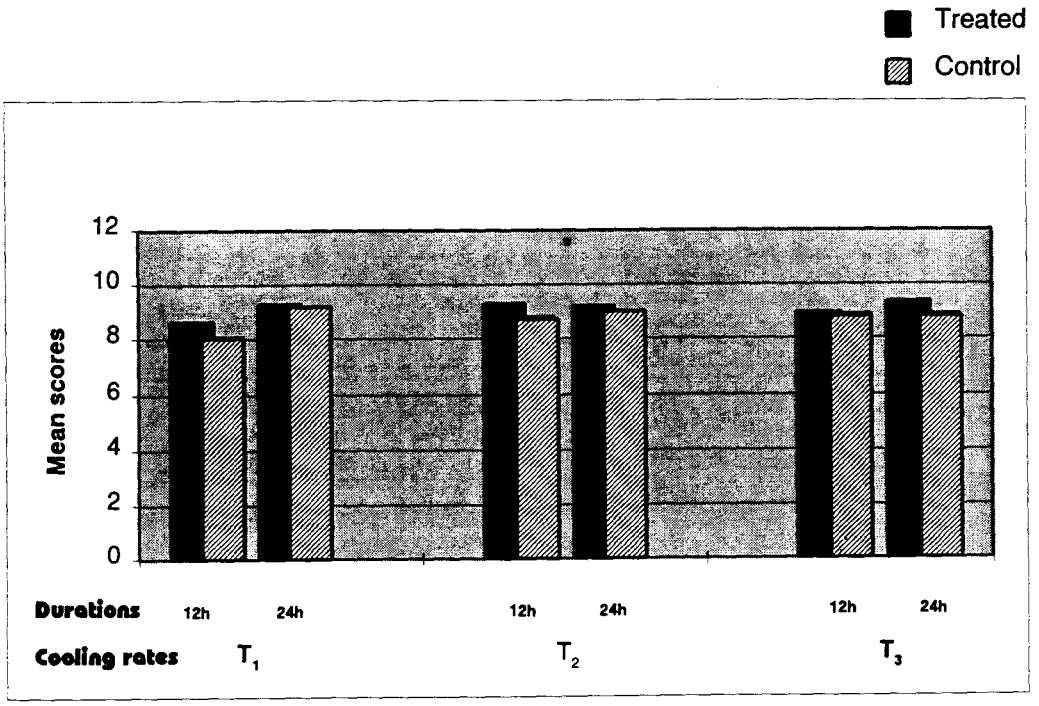


Fig. 13. **Mean flavour scores for cooked *P. monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

Table 16. **Analysis of variance for the flavour of cooked *P.monodon* revitalized after live storage for two different durations, following cold-anaesthetization at three different cooling rates.**

SOURCE	S.S	D.F	M.S.S	F
TREATMENT COMBINATIONS	13.09	11		
COOLING RATES	2.065	2	1.033	1.166
DURATIONS	3.0067	1	3.0067	3.394
TREATED AND CONTROL	3.673	1	3.673	4.146 *
INTERACTION	4.345	7	0.621	0.701
ERROR	95.7	108	0.886	
TOTAL	108.79	119		

* significant at 5% level.

DISCUSSION

5. DISCUSSION

The results obtained from the present study indicate that *P. monodon* could also be used for live storage by cold-hibernation, under conditions similar to those of *P. japonicus*. Studies on live transportation of *P. monodon* are scarce. Most of the reports describe the cold-anaesthetization and live transportation practices commonly used for the *Kuruma* shrimp *P. japonicus*. Different species of shrimps require different conditions for successful cold-hibernation and live storage . This makes a meaningful comparison of the survival rates , durations of live storage , and the rates of cooling adopted for different species, difficult . The results obtained from the present study indicate that *P. monodon* could also be used for live storage by cold-hibernation, under conditions similar to those of *P. japonicus*.

5.1. Effect of Cooling Temperature on Cold-anaesthetization and Live Storage

On the basis of a pilot study, a temperature of 14±1°C was used for cold-anaesthetization and live storage of *P. monodon* in the present investigation. This temperature was similar to that (12-14°C) suggested for live transportation of *P. japonicus* in Japan (Shigueno, 1975). However, differences exist in reports regarding the cold-hibernation and live storage temperature for *P. japonicus* in Japan. Richards- Rajadurai (1989) observed that *P. japonicus* could withstand low temperatures and that lowering the temperature during storage and transportation was convenient. In the present study, *P. monodon* has also been observed to withstand this temperature. Further, it is reported that in the case of *P. japonicus*, cold-anaesthetization begins as the temperature falls to 4°C, and the packaging temperature inside the box is kept at 4 to 10°C (Richards-

Rajadurai, 1989; Schoemaker ,1991). Goodrick *et al.* (1995) however, observed that the survival of *Kuruma* shrimp during live transport to Japan was highest when the temperature was maintained between 12 and 15°C, but improved at 10°C during winter. Shigueno (1975) also reported that *P. japonicus* could be successfully transported at 10°C during winter. Thus, it follows that the reports of Richards-Rajadurai (1989) and Shoemaker (1991), though not mentioned, apparently refer to the situation in winter, when extremely low temperatures can be used for live transportation of *P. japonicus*.

Richards-Rajadurai (1989) also reported that commercially important tropical species like *P. monodon* and *P. vannamei* may not be able to tolerate temperatures as low as that used for *P. japonicus*, and that further studies were necessary on their live transport. In the present investigation, *P. monodon* was observed to sustain well at a temperature of 14±1°C and remain alive when stored in chilled saw dust at this temperature for as long as 24 hours with 100% survival in T_1 and T_2. Eventhough, *P. monodon* is a species less tolerant to temperature compared to *P. japonicus*, in the present study it could be kept under live storage at 14±1°C which is the temperature at which *P. japonicus* are usually transported live in Japan during summer. However , additional investigations by comparing these results with simulated transport conditions are necessary, as the present study forms a sound footing upon which further investigations can be based, to standardize the cold-anaesthetization and live transportation practices of *P. monodon*. But Anon (1991) has reported that the survival of *Kuruma* shrimp airfreighted from Brisbane to Tokyo was found to have no significant difference with the survival of shrimp stored under controlled conditions.

Goodrick *et al.*(1995) had observed that farmed *P. monodon* survived better at 16°C than at 12°C during live storage under cold-hibernation. The present study does not conform to this earlier finding since 16°C was not found to be sufficient for complete cold-anaesthetization of the farmed *P. monodon* tested. They often leaped out of the saw dust packs, indicating that they were still active. A temperature similar to that used in the present study has also been reported for live transportation of other tropical decapod crustaceans. In the case of tropical lobsters, Lisac(1986) recommended a shipping temperature of 14^0 C. Rahman and Srikrishnadas (1994) suggested a temperature of 12-15 ^{0}C for effective immobilization of spiny lobsters, exported live from India. A temperature of 14 ^{0}C was used by Jiang *et al.* (1995) for experiments on cold-anaesthetization and live storage of *P. vannamei.* However, the optimum temperature for maintaining mud crabs alive in air is reported to be 16 ^{0}C (Gillespie and Burke, 1991).

5.2. Safe Duration and Optimum Cooling Rate

The concept of safe duration of transport has been previously defined in the literature of live transportation of *Macrobrachium rosenbergii* post-larvae under oxygen packing (Jayasree Vadhyar *et al.,* 1992). It is the statistically valid duration which would give 100% survival and at which initial mortality starts. In the present study, though 100 % survival rate of *P. monodon*, cold-anaesthetized at a cooling rate of 1.38 ± 0.16^0 C /h, the statistically established safe duration was 22.9 hours, using probit analysis. Similarly, survival rates of 100% were obtained for 20 and 16 hours of storage at cooling rates of 2.76 ± 0.32° C / h and 5.52 ± 0.64^0 C / h whereas the safe durations were 19.1 and 14.62 hours, respectively.

Goodrick *et al.* (1995) reported that farmed *P. monodon* survived for 24 hours when kept under live storage without water and recommended this duration for its live export. However, they did not mention the survival rate obtained during this period and hence comparison of the present result with the earlier work is difficult. Wild *P. semisulcatus* and *P. esculentus* cooled to 12^0C and packed in saw dust were reported to have survived for only 12 -18 hours (Goodrick *et al.*,1995). Farmed *P. esculentus* had also shown similar results. Therefore, they suggested that a temperature of 12^0 C might be too cold for *P. esculentus* and the survival might be extended by cooling to a slightly higher temperature of about 15^0C, though it was not established that this temperature would immobilize the shrimp. It is not extravagant to assume that tropical *P. monodon* and *P. esculentus* are less cold-tolerant than the subtropical / temperate *P. japonicus*. Apart from this, Goodrick *et al.* (1995) also observed an intrinsic weakness of *P. esculentus* compared to the endurance of *P. japonicus* in air which makes it unable to survive long enough out of water to allow routine export. However, this is not the case with *P. monodon* which can be used for live storage under cold-anaesthetization similar to *P. japonicus*, as shown by Goodrick *et al.* (1995), and the present study.

The survival rates obtained by Furusho *et al.* (1988) for cold-anaesthetized and live stored *P. japonicus* (20-30 g) at 15^0 C were slightly lower than those obtained in the present study for *P.monodon.* They reported that the survival rates of *P.japonicus* were 100% at 12 hours, 86.7 % at 24 hours, and 68.8 % at 36 hours of live storage. After 36 hours, the survival sharply declined to 6.7 % at 48 hours, and complete mortality at 60 hours. However, this temperature (15^0C) was slightly higher than the routinely used 12^0C, to ship live *Kuruma* shrimp in Japan (Goodrick *et al.*,1995). Also Paterson (1993 a) observed that handling increased the respiration rate of cold-

anaesthetized *P.japonicus* at 17^{0}C and 22 ^{0}C, but had no effect at 12 ^{0}C. Thus when packing live *Kuruma* shrimp for export, he found no evidence that handling them in water at 12 ^{0}C caused any increase in respiration rate, and hence metabolic rate. Furthermore, Nakamura (1994) reported evident respiratory activities of *P. japonicus* at 15 $^\circ$C, though this rate was only half of that at 20 ^{0} C and the shrimp did not show any active behaviour. Further investigations are necessary to determine the levels of temperature at which the respiration rate of *P.monodon* is suppressed to the bare minimum so that subsequent handling would not result in any increase in metabolic activity. Kurmaly *et al.* (1989) had pointed out the scope for research on the active rates of oxygen consumption at low temperature and on the long-term effect of low dissolved oxygen levels, since this might be beneficial to the development of live transportation methods of *P.monodon.* Because of time limitations in the present study, no attempt has been made to determine the respiration rate of *P. monodon* under different temperatures.

Pairwise comparison of the mean survival rates obtained for the treatments in the present study indicated that there are no significant differences between the cooling rates of 1.38$\pm$ 0.16^0 C and 2.76 $\pm$ 0.32^{0}C /h. Similarly, at durations of 16, 20 and 24 hours, significantly higher survival rates of the packed *P.monodon* were obtained than at 28, 32 and 36 hours of its live storage.

Hence, a cooling rate of 2.76 $\pm$0.32^{0}C for 4 hours can be considered to be the optimum for live storage of *P.monodon* in saw dust at 14$\pm$1^{0}C. This result conforms to most of the reports of live transportation of shrimps out of water. Goodrick *et al.* (1995) reported a cooling rate of 3^{0}C /h for packing *P.monodon* in chilled saw dust at 12^{0}C. A rate of 3^0 C /h was adopted by

Paterson (1993 a) to cool *P.monodon* to 20^{0}C, 17 ^{0}C or 12 ^{0}C. However, in Taiwan, a cooling rate of 2 ^{0}C /h in 4 hours is commonly used for cold-anaesthetization of *P.japonicus* (Chen ,1990). Jiang *et al.* (1995) employed three rates of cooling, viz., 1-2 ^{0} C /h, 3-4 ^{0}C /h and 5-6 ^{0}C /h for cooling *P. vannamei* down to 14 ^{0}C for live packing .

Goodrick *et al.*(1993) has reported that *P. japonicus* transported live to Japan markets fetch premium prices when the survival rates are reliably higher than 95 %, which is usually obtained for a period of 24 hours of live storage. In the present study, the optimum duration for 95% survival of the packed *P.monodon* was found to be 25.7 hours at the optimum cooling rate of 2.76 $\pm$ 0.32 ^{0}C /h. Hence at this cooling rate, the packed *P.monodon* can be safely stored live in saw dust under cold-anaesthetized condition, at 14$\pm$1 ^{0}C for a duration of 24 hours, for all practical purposes. This result is in close conformity with that obtained by Goodrick *et al. (1995)* for farmed *P.monodon.*

It is inferred from the present investigation that the choice of a particular cooling rate depends on the storage periods intended. For longer periods of storage for long distance transportation, a cooling rate of 2.76 $\pm$ 0.32^{0}C /h , for 4 hours can be adopted to cold-anaesthetize *P.monodon* . For transportation to shorter distances, when duration of live storage varies from 14 to 15 hours the faster cooling rate of 5.52 $\pm$ 0.64 ^{0}C /h for 2 hours could well be used. This method of live storage could be made suitable for domestic live transport of *P.monodon* within our country. Therefore, other than live export the present study opens up a new avenue, for live transportation of aquaculture brood stock of *P.monodon* from collection centres to the hatcheries, either as adult brood stock or as ripe spawners. However, detailed investigations regarding the

effect of such low temperature as 14±1 ^{0}C on the reproductive biology of *P.monodon* are necessary, before exploring the possibility of live transportation of broodstock of *P.monodon* without water. Further, the suitability of the present method for the cold-anaesthetization and live storage of wild collected shrimp has to be investigated.

As mentioned earlier, the cold-hibernation and live storage temperatures for a particular species are reported to vary with different seasons of the year. In winter, lower temperatures are used to immobilize shrimp than in summer. The present study was done within a period of 60 days, during which the pond water temperature ranged between 27 and 29 ^{0}C. Detailed investigations are therefore essential to find out the specific requirements, if any, for the effective immobilization of *P.monodon,* according to the temperature regime prevalent during different seasons of the year. Aziz and Greenwood (1981) had shown that the temperature tolerance levels of shrimp are modified by acclimation temperature. Acclimation to a very low temperature lowers the lower lethal limit. Since the pond water temperature was low, *P.monodon* used in the present study had already acclimatized to the low temperature and perhaps, during the cooling treatments, that would have favoured its low temperature tolerance which might be slightly higher during the hot months when the water temperature would also be higher.

In the present study, a sizeable number of shrimps were collected from the pond when the salinity had dropped to 2-4 ppt, due to heavy rain. However, this did not seem to affect survival of *P.monodon* in saw dust, since all the shrimps were acclimatized to a salinity of 15 ppt for sufficient period before cold-treatment. This observation is in agreement with that of Aziz and Greenwood (1981) who reported that while acclimation temperature affected both temperature

and salinity tolerances, acclimation salinity influenced only the salinity tolerance. According to Kinne(1970) and Bradley (1975) a number of species can tolerate subnormal and supranormal temperatures better at the lower and upper ends of their salinity ranges, respectively. Nelson(1979) also reported that though the metabolic rates were influenced by salinity, the response of metabolic rate to temperature seemed unaltered by salinity. In the present study, the sudden drop in salinity of the *P.monodon* pond was not found to have any adverse effect on the survival of *P.monodon*. However, elaborate investigations on the interaction of salinity and temperature tolerance, as a factor influencing the cold-anaesthetization temperature of *P.monodon* were not conducted.

5.3. Effect of Cold-anaesthetization and Live Storage on Weight Loss

The weight measurements taken in the present study clearly demonstrate that there is a decline in mass of *P.monodon* during live storage at a temperature of $14\pm1\ ^0C$. However, this difference in weight between the cold-treated shrimps and control was not statistically significant among the cooling rates and durations of live storage tested. Weight loss is also reported in the case of *P. japonicus,* which loses weight by about 8% while shipping from pond to market (Shigueno,1975). This is observed to be due to the draining of water remaining in the gills or on the body of the shrimp into the saw dust, during storage. In the present study, the highest loss of weight, though not statistically significant, was 8.83% at 12 hours and 6.26% at 24 hours of live storage, occurred at the optimum cooling rate of $2.76\pm0.32\ ^0C$ /h for 4 hours. However, the lowest percentage loss of weight was obtained for the fastest cooling rate, viz., $5.52 \pm 0.64\ ^0C$ / h for 2 hours. It is likely that these differences would have arisen as a result of the differences in the intervals before weight measurement during which the shrimps were kept in water for

revitalization. This is in support of the findings made by Shigueno(1975) who had observed that the weight of *P. japonicus* lost during shipping could be recovered at the market, if the shrimps were soaked in water for sufficient period.

Loss of weight during aerial exposure has been reported in the case of the cray fish *Austropotamobius pallipes* by about 10% of its body mass, in the form of water, during 24 hours of aerial exposure at 15 ^{0}C (Taylor and Wheatly,1981). Wheatly *et al.*(1996) reported that at 23 ^{0}C, the crayfish *Procambarus clarkii* had lost only 10% body water over a period of 24 hours of aerial exposure which suggested that it had reduced water permeability, possibly as a pre-adaptation for aerial exposure. In the case of shrimps, these aspects are little investigated. No information is available on other changes in the muscle of shrimp, if any, upon cold-anaesthetization and live storage, which might result in weight loss. There is ample scope for detailed investigations on these lines.

5.4 Behavioural Changes in Cold-anaesthetized Shrimp

Abnormal behavioural responses to low temperature before being cold-anaesthetized were observed in *P. monodon*, in the present study. Low temperature obviously tend to reduce the activity of shrimps. Cooling is a well understood means of reducing the metabolic rate of shrimps during commercial transport, both in and out of water. Penaeids often respond to a drop in temperature below a certain level, by burying in sand and remaining there (Hill,1985). According to Aldrich *et al.*(1968), the shrimps are probably so torpid at extremely low temperatures that they are physically unable to emerge from the sand. Shrimps that fail to bury under these conditions,

soon fall over and lie unprotected on the sediment surface. Similar phenomenon was observed in the present study when *P.monodon* was subjected to cold-treatment. At 14 ^{0}C, the shrimps were found to have fallen on their sides, unable to make their bodies straight in the normal posture. This could be a form of hibernation which enabled the shrimp to survive cold spells, until the temperatures were more favourable for activity and growth, as reported by Aldrich *et al.*(1968). In the present study, once the temperature was raised to the optimum level, most of the shrimp returned to normal activity, including feeding.

A few of the cold-anaesthetized *P.monodon* in the present study, even when revitalized after live storage failed to retrieve to the normal state, but continued to lie on the bottom of the tank. They were alive, showing active movements of pleopods and peraeopods. At low temperature, nerve and muscle functions in crustaceans become physically blocked (Blundon,1989). In the present study, when the shrimps were cooled at the faster rate (5.52 $\pm$ 0.64 ^{0}C /h) they also showed whirling movements. Paterson (1993 a) had reported that *P. japonicus* that were cooled for live export were no longer able to stand upright at temperatures less than about 13 ^{0}C. He observed reflexive jumping of the shrimp over a period, before falling over. However, this response is probably subject to seasonal acclimatization of the nervous system (Blundon,1989). Paterson (1993 a) further observed that the benefit of cooling shrimp did not appear to be due to the effect of acclimation to the cold, but rather to a physiological shock that allowed them to be handled more conveniently. *P.monodon* also exhibited flexing of the abdomen and twitching of eyestalks in response to low temperature, in the present study, which might have arisen as a result of the effect of low temperature on the neuromuscular properties of the shrimp, as reported by Blundon (1989).

Similarly, studies on the alterations in the functions of statocysts in shrimp, which are mechanoreceptors giving inputs to equilibrium reflexes and to compensatory eye movements (Neil,1982), would be of some use to interpret these behavioural responses.

Marked changes were observed in the body colour of *P.monodon*, cold-anaesthetized and kept alive in chilled saw dust at 14±1 ^{0}C. The tips and margins of the pleopods and peraeopods turned reddish and the entire body turned dark brown. Shigueno(1975 and 1992) also reported similar changes in the body colour of cold-anaesthetized *P. japonicus*. The body colour of *P. japonicus* was reported to be darkened by a reddish tinge, especially at the walking legs, and the connecting body parts turned brilliant red (Shigueno,1992).

In crustaceans, the body pigmentation is regulated by the chromatophores. In penaeids, the number and types of chromatophores may change to match the back ground over a long period (morphological colour change); but the physiological colour change, in the degree of pigment dispersion can occur relatively rapidly (Rao,1985). Dark background adaptation usually involves expansion of melanophores, erythrophores, and xanthophores, and contraction of leucophores. Rao(1985) also reported that though these changes are largely under hormonal control, some melanophores, leucophores and erythrophores are photo/temperature sensitive. Nagabhushanam and Rao(1964) found that elevated temperatures caused contraction of melanophores as well as expansion of leucophores, in *Metapenaeus monoceros* and other species. The reverse phenomenon, whether it occurs at low temperatures, has not been investigated. However, while the morphological evidences obtained from the present study indicate that it occurs in *P.monodon* during cold-anaesthetization and live storage, physiological evidences are to be examined to substantiate this.

The eyes of the cold-anaesthetized *P.monodon* in the present study also displayed a characteristic red pigmentation, when taken out of the packing box after live storage. It is well documented that the large, prominent stalked eyes of penaeidae are their most noticeable sensory structures which suggest that their input, i.e.., light has a major influence on shrimp physiology and behaviour. Many crustaceans have the ability to change the optical properties of the eyes by light-dark adaptation (Shaw and Stowe,1982), and in such eyes, each ommatidium is sheathed by two sets of retractable dark pigments which are capable of migration. In the dark-adapted eye, the sheathing pigment concentrates and the reflective pigment disperses. Dark-adaptation in *P. monodon* takes about 90 minutes, when the ommin pigments sheathing each ommatidium retract, so that the pinkish pteridines of the middle and proximal reflective layers give the dark-adapted eye, its characteristic appearance (Nicol and Yan,1982).

Occurrence of the death of cold stored *P.monodon* in packing boxes with loss of their appendages, evades a prudent explanation. The fact that the shrimps in these boxes were purged for relatively longer periods (20-22 hours) gives some evidence to its relation with starvation. However, this phenomenon was not observed in some other boxes where the shrimps were subjected to purging for a similar period. There was however, little temperature fluctuation in the latter group. Hence, it could perhaps be assumed that a longer period of starvation coupled with a relatively less cold temperature of storage which may trigger the activity of shrimp inside the box might be the probable reasons for the cannibalistic behaviour. Incidentally in these boxes, the shrimps were found to have changed their position when the boxes were opened after storage, which indicated that they were active while they were under cold storage. It is a general principle that handling increases respiration rate in crustaceans apparently by increasing their

activity (Winkler,1987). For this reason, some crustaceans are vulnerable to disturbances during live transport in air (Taylor and Whiteley, 1989). Paterson (1993 a) had also shown that handling affected the respiration rate of *P.japonicus* by increasing their locomotor activity, at temperatures of 17 and 22°C, rather than at 12°C, at which the shrimps were anaesthetized and the contribution that handling normally made to metabolic rate was altogether removed. Liao (1969) had reported that the feeding of *P.japonicus* was 20% lesser than that at 25°C.

In the present study, the revitalized *P.monodon* after cold-anaesthetization and live storage for as long as 36 hours, could be kept alive for a period up to 3 days, during which not more than 10% of them had died. Imai (1977) reported that the revitalized shrimp survived for about 1.5 days, though 50% died after 3 days. He further observed that the quality of healthy shrimp was not affected by the shipping method. The present observations indicate that the revitalized shrimp could live for longer periods with better survival rates than that reported previously.

5.5 Survival in Relation to the Biochemical Changes in Shrimp Muscle

The survival of cold-anaesthetized *P.monodon* packed in chilled saw dust at 14 ±1°C, progressively declined with durations of live storage, in the present study. This may be interpreted in terms of the various biochemical changes that are reported to occur in the muscle of shrimps and other crustaceans during cold-anaesthetization and subsequent live storage at low temperature. Though not investigated in the present study, a discussion of the biochemical processes in the muscle of *P.monodon* possibly affecting its survival in saw dust during live storage will be worthwhile.

In crabs with poorly developed air-breathing organs, when exposed to air they switched over to anaerobic metabolism, in order to cope with a general inability to utilize aerial oxygen (Burnett and McMahon,1987). Internal hypoxia, hypercapnia and accumulation of lactic acid due to anaerobic metabolism has been reported in the case of commercially transported lobsters (Taylor *et al.*,1988; Spicer *et al.*,1990) and cray fishes exposed to air (Morris *et al.*, 1986; Taylor *et al.*, 1987; Wheatly *et al.*,1996). *P.japonicus* has also been reported to resort to anaerobic metabolism during cold-hibernation and exposure to air at 15°C (Furusho *et al.*,1988; Paterson,1993 b). The accumulation of lactic acid suggested that the oxygen supply to the tissues was inadequate during commercial live transport causing a switch over to anaerobic metabolism.

Furusho *et al.*(1988) observed that in contrast to the levels of lactic acid, the concentration of ATP in the muscle of live *P.japonicus* markedly decreased after 48 hours of storage, resulting in a rapid increase in AMP level. They proposed that the ratio of ATP to the ATP related compounds (ATP/(ATP + ADP + AMP +I MP)) is closely related to the survival rate of shrimp, and it can be a suitable criterion to evaluate its activity during transportation in saw dust. Paterson (1993 b) also showed that high concentration of lactate and IMP in the muscle tissue at a given temperature indicate that the shrimp has been out of water for too long.

The acid-base status of the blood of shrimp is drastically disrupted in response to the low temperature treatment, due to the accumulation of lactic acid and other acid metabolites. In crabs, lobsters and crayfishes, the marked fall in blood pH in aerial conditions is compensated by an increase in bicarbonate ion concentration (Whiteley and Taylor, 1990; 1992; de Souza and Taylor, 1991; Wheatly *et al.*, 1996). For several years, researchers have postulated that the exoskeletal

$CaCO_3$ may assist in buffering extracellular acidosis in intermoult crustaceans. As an indirect evidence of this, several studies have reported that hemolymph Ca rises in response to extracellular acidosis. In air-breathing species like the crayfish *P. clarkii* and amphibious crabs, circulating Ca was found to remain unchanged, but Ca levels in the epidermis, hepatopancreas and gut increased, indicating translocation of skeletal Ca (de Souza and Taylor, 1991; Wheatly *et al.*, 1996). However, the species that does not tolerate aerial exposure well, exhibited an increase in hemolymph Ca during emersion. Wheatly *et al.* (1996) proposed that circulating levels of Ca increase in species not well adapted for aerial exposure, in which case $CaCO_3$ mobilization occurs as a last resort when other mechanisms of acid-base compensation have been exhausted. This may partly explain for the slight softness of the exoskeleton of *P. monodon* following cold-anaesthetization. This may be true for a species like *P.monodon* which has no known special modifications of survival for long periods out of water.

Thus the acid-base status of the blood of *P. monodon* and the associated changes in response to low temperature during cold-anaesthetization and subsequent live storage may explain the survival of this species out of water. The internal compensatory mechanisms ameliorating these drastic changes may work well upto a certain limit, beyond which the shrimp will succumb to the cold temperature.

It is also interesting to note in this context that the serum bactericidal activity of *P. japonicus* and the Japanese spiny lobster *Panulirus japonicus* commercially transported live were found to decline with longer durations of storage (Sugita and Deguchi, 1993). They observed that

pathogenic bacteria colonize the gills and gut of the healthy crustaceans and may cause opportunistic infectious diseases when the self defence mechanisms are suppressed during transport. Sugita and Deguchi (1993) suggested that the bactericidal activity of hemolymph may become an excellent indicator to evaluate the health condition of Japanese spiny lobsters as well as shrimps. They found that the serum bactericidal activity was not significantly influenced in *P. japonicus* stored in seawater with aeration, but it decreased prominently in specimens kept in saw dust and only in air. Similar results were also observed in the Japanese spiny lobsters. Thus the reduction in serum bacterial activity of the shrimp during live storage in saw dust might have an adverse effect on its survival. Detailed examination of these changes is necessary to substantiate this phenomenon in the case of *P. monodon* during live storage.

5.6 Sensory Evaluation

Sensory evaluation of the cold-anaesthetized and live stored *P.monodon* showed that these shrimps were superior to the untreated ones in general appearance, colour, and flavour of the meat. However, there was no significant change in the texture of the meat of treated shrimp. In no case, the cooling rates or the durations of live storage tested were found to have any effect on the sensory qualities of the cold-anaesthetized and live stored shrimps. However, similar reports on the sensory evaluation of the cold-anaesthetized and live stored shrimps are not available for comparison. Most of the reports on sensory evaluation have considered only shrimp post-mortem.

In post-mortem shrimp, Hashimoto (1965) and Jones (1969) have observed that the

conversion of ADP and AMP to hypoxanthin resulted in a loss of meaty flavour. This might also result in bitterness as indicated by Carroll *et al.* (1968). However, such quality deterioration was not observed in the present study during live storage of *P. monodon.* Furusho *et al.* (1988) reported that the concentration of ATP in the muscle of live *P. japonicus* kept in saw dust at 15^{0}C markedly decreased with consequent increase in the AMP level. Paterson(1993 b) has also showed that the level of ATP decreased in cold-anaesthetized *P. japonicus* and *P. monodon* packed in saw dust and kept for 24 hours at 12 and 15^{0}C respectively.

In the present study, no difference in the meat texture of the cold-anaesthetized and live stored shrimp was observed. This result, however, differs from that of Subasinghe(1996) who reports that lowering of temperature during cold-anaesthetization improves the texture of the flesh.

SUMMARY

6. SUMMARY

The present study was aimed at standardizing the technology of cold-anaesthetization of farm raised *P. monodon* and its live storage in chilled saw dust. The methodologies used and the important results of the study are summarized below.

1. Farm raised *P. monodon* (22-25g) were used for the study. The ambient water temperature of the culture pond fluctuated between 27 and 29^{0}C. The initial salinity of the pond water ranged from 15 -20 ppt during July and later reduced to 2-4 ppt in August, due to rain. All the shrimps tested for the study were acclimatized at a uniform salinity of 15 ppt.

2. A temperature of 14$\pm$1^{0}C was found to be the optimum for effective cold-anaesthetization of *P. monodon*, on the basis of a pilot study. This was followed for cold-anaesthetization and live storage throughout the study.

3. Cold-anaesthetization of the shrimp was done in plastic net boxes kept in a refrigerated chilling tank (40 litre capacity) with thermostat. The chilling tank was provided with aeration. A six channel, digital, continuous freezer temperature monitor, with a precision of 0.1 ^{0}C was used to read the temperature accurately.

4. Three cooling rates of 1.38 $\pm$ 0.16^{0}C /h (slow cooling rate) within 8 hours, 2.76 $\pm$ 0.32^{0}C /h (moderate cooling rate) within 4 hours, and 5.52 $\pm$ 0.64^{0}C /h (fast cooling rate) within 2 hours

were tested for chilling the shrimps from 25°C to 14±1°C in the process of cold-anaesthetization. The experiment was designed in CRD with 3 replications for each cooling rate and the analysis of the results done in asymmetrical factorial arrangement.

5. The shrimps cold-anaesthetized at each cooling rate were packed separately in specially prepared card board boxes, lined inside with 12 mm thick styrofoam sheet. Coarse particles of chilled saw dust of teak wood with a moisture content of 10% were used as the packing medium. The cold-anaesthetized shrimps were packed in between two layers of the chilled saw dust, and the boxes kept inside a chilled storage cabinet. The temperature inside each box was monitored continuously.

6. Under each cooling rate, the cold-anaesthetized and live stored shrimps were observed separately at 4 hourly intervals from 16 to 36 hours, with three replications for each interval. The packed shrimps were revitalized in aerated circular fibre glass tanks of 80 litre capacity, half-filled with brackish water of 15 ppt salinity. The initial temperature in the tank was 20 °C, which was gradually warmed upto the ambient temperature of about 28 °C @ 2.7 °C /h, within a period of 3 hours.

7. Upon cold-anaesthetization, the shrimps rolled over onto their sides and remained immobilized, with bare minimum movements of pleopods and peraeopods. The survivors were counted based on the active movements of the appendages, within 3 hours, and the percentage survival at each interval and given cooling rate was computed.

8. At the slow cooling rate, 100% survival was observed after storage for a maximum duration of 24 hours. At the moderate and the fast cooling rates, the corresponding durations were 20 and 16 hours.

9. The statistically valid (using probit analysis) safe durations for obtaining 100 % survival were 22.9 ± 1.09, 19.1 ± 0.4, and 14.62 ± 1.13 hours at the slow, moderate and fast cooling rates, respectively. For practical purposes, the durations for obtaining 95% survival were computed as 28.18 ± 0.54, 25.7 ± 0.54 and 21.88 ± 0.71 hours for the slow, moderate and fast cooling rates, respectively.

10. The analysis of variance of the data on percentage survival showed significant difference ($P<0.05$) among the three cooling rates tested. However, pairwise comparison revealed that the first two cooling rates formed a homogeneous group. Hence the moderate cooling rate (2.76 ± 0.32 °C /h within 4 hours) was found to be the optimum for a safe duration of 19.1 ± 0.4 hours, as it takes only half the time for cold- anaesthetization, compared to the slow cooling rate which took 8 hours. This slow cooling rate was however, necessary for a longer duration 22.9 ± 1.09 hours. For durations of storage less than 15 hours even the fast cooling rate which took 2 hours for cold-anaesthetization was found to be sufficient.

For all practical purposes, a survival rate of 95 % could be obtained for 28, 26 and 22 hours of live storage of *P.monodon* in chilled saw dust, at the slow (1.38 ± 0.16 °C /h within 8 hours), moderate (2.76 ±0.32 °C /h within 4 hours) and fast (5.52 ± 0.64 °C /h within 2 hours) cooling rates of cold-anaesthetization and live storage at 14±1 °C .

11. The difference in weight, before cold-anaesthetization, and after revitalization of the live stored shrimp was studied at 12 and 24 hours of live storage, at the three cooling rates separately. The data were analysed using CRD in asymmetrical factorial arrangement. Although a weight loss (ranging from 1.49 to 8.83%) was observed after revitalization of the cold-anaesthetized and live stored shrimp at the three cooling rates, and at 12 and 24 hours of live storage, the analysis of variance revealed that it was not significant.

12. The effect of cold-anaesthetization and live storage of the shrimp on its appearance and meat quality was studied at the three cooling rates, after 12 and 24 hours of live storage, using sensory evaluation of the untreated shrimp (control) and those which were revitalized after live storage. The analysis of the mean scores was done in CRD using asymmetrical factorial arrangement. Marked changes were observed in the appearance of the cold-treated shrimp. The body colour turned dark brown with the tips and margins of pleopods becoming reddish. Statistical analysis showed that there was significant difference in the appearance of the treated shrimp and control. The cold-treatment improved the appearance of shrimp. However, the effect of different cooling rates and durations tested was not significant.

Meat quality of the cold-treated and untreated shrimps was analysed for the raw and cooked meat. There was significant improvement in colour and flavour of raw and cooked shrimps; but there were no changes in the texture and odour/aroma in the treated and untreated shrimp meat. The effect of different cooling rates and the durations tested on the sensory quality of shrimp meat was not significant.

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Appendix I.
SENSORY EVALUTION OF COLD-ANAESTHETIZED SHRIMP

The shrimp samples provided to you for evaluation have been subjected to cold treatment as part of a study of storing them live for transportation in chilled saw dust. Your valuable opinion will be of immense relevance in this study.

Kindly evaluate the raw & cooked tiger shrimp for six quality attributes. You may make a tick mark in the appropriate column or level, for the treated and untreated samples. Thank you.

A. APPEARANCE	R	TR	0 ..... 2 ..... 4 ..... 6 ..... 8 10
		UR	0 ..... 2 ..... 4 ..... 6 ..... 8 10
B. COLOUR	A	TR	0 ..... 2 ..... 4 ..... 6 ..... 8 10
		UR	0 ..... 2 ..... 4 ..... 6 ..... 8 10
C. ODOUR	W	TR	0 ..... 2 ..... 4 ..... 6 ..... 8 10
		UR	0 ..... 2 ..... 4 ..... 6 ..... 8 10

A. COLOUR	C O	TC	0 ..... 2 ..... 4 ..... 6 ..... 8 10
		UC	0 ..... 2 ..... 4 ..... 6 ..... 8 10
B. AROMA	O K	TC	0 ..... 2 ..... 4 ..... 6 ..... 8 10
		UC	0 ..... 2 ..... 4 ..... 6 ..... 8 10
C. FLAVOUR	E D	TC	0 ..... 2 ..... 4 ..... 6 ..... 8 10
		UC	0 ..... 2 ..... 4 ..... 6 ..... 8 10

Extremely Fresh 10
Very Fresh 8
Moderately Fresh 6
Borderline / Neutral 5
Poor / Stale odour 4
Very poor / Very Stale 2
Bad / Putrid 0

TEXTURE	SC	R	A	W	COOKED	
	ORE	TR	UR		TC	UC
Firm & Elastic	10					
Turning soft	8					
Soft, Elasticity lost	6					
Very soft	4					
Extremely soft	2					

Comments if any :

COLD - ANAESTHETIZATION AND LIVE STORAGE OF *PENAEUS MONODON* FABRICIUS FOR TRANSPORTATION IN CHILLED SAW DUST

BY
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ABSTRACT OF A THESIS

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ABSTRACT

With a view to standardizing the technology of cold-anaesthetization and live storage of adult *Penaeus monodon* in chilled saw dust, three cooling rates of 1.38±0.16 ^{0}C /h within 8 hours (slow cooling rate), 2.76 ±0.32 ^{0}C /h within 4 hours (moderate cooling rate), and 5.52 ±0.64 ^{0}C /h within 2 hours (fast cooling rate) were tested to cold-anaesthetize farm raised *P.monodon* (22-25g) at 15 ppt salinity, from 25 ^{0}C to 14± 1 ^{0}C (fixed on the basis of a pilot study) in plastic net boxes kept in a refrigerated chilling tank of 40 litre capacity, provided with aeration. The cold-anaesthetized shrimps at each cooling rate were packed separately in between two layers (about 3 cm thick) of saw dust with 10% moisture and chilled previously at 2-3 ^{0}C, in specially prepared card board boxes (33 x 22 x 9 cm) lined inside with 12 mm thick styrofoam sheet. The boxes were kept inside a chilled storage cabinet and maintained at 14 ± 1 ^{0}C for a duration of 16-36 hours, and the survival of the shrimps observed at four hourly intervals. The temperature was monitored using a six channel, digital, continuous freezer temperature monitor with a precision of 0.1 ^{0}C.

The shrimps cold-anaesthetized at each cooling rate and live stored for each duration were revitalized in aerated circular fibre glass tanks of 80 litre capacity, half-filled with brackish water of salinity 15 ppt, and temperature 20 ^{0}C which was raised @ 2.7 ^{0}C /h to the ambient temperature of 28 ^{0}C, within 3 hours. The shrimps which showed abnormal behavioural patterns by rolling over onto their sides, and remained immobilized upon cold-anaesthetization, recovered to active movements after revitalization.

Although 100% survival of the packed shrimps was obtained for maximum durations of 24, 20 and 16 hours at the slow, moderate and fast cooling rates respectively, the corresponding statistically valid safe durations for obtaining 100% survival were computed

to be 22.9 ± 1.09, 19.1 ± 0.4 and 14.62 ± 1.13 hours, using probit analysis. However, for practical purposes, the durations for obtaining 95% survival were determined as 28.18 ± 0.54, 25.7 ± 0.54 and 21.88 ± 0.71 hours for the slow, moderate and fast cooling rates, respectively. Analysis of variance of the percentage survival showed significant difference ($P< 0.05$) among the three cooling rates tested, while pairwise comparison revealed that the slow and moderate cooling rates were identical. This suggested that the moderate cooling rate which took only half the time for cold-anaesthetization of shrimp compared to the slow cooling rate can be considered the optimum, though the choice of the different cooling rates depends on the duration of storage desired.

The difference in weight before cold-anaesthetization and after revitalization of the live shrimp was studied at 12 and 24 hours of live storage at the three cooling rates, separately which indicated a loss of weight (1.49-8.83%), varying with the cooling rates and durations. However, this was not found to be statistically significant among the cooling rates and durations tested.

Sensory evaluation of the cold-treated shrimps was conducted to study the effect of cold-anaesthetization and live storage on their appearance and meat quality, at the three cooling rates after 12 and 24 hours of live storage, taking untreated shrimp as control. The body colour of the shrimps turned dark brown and the tips and margins of the pleopods and peraeopods became reddish. There was significant difference ($P<0.05$) in general appearance, and the colour and flavour of the meat between cold treated and untreated shrimps. However, the texture and odour/aroma of the raw/cooked meat remained unaffected by cold-treatment. The effect of different cooling rates and the durations tested on the sensory quality of shrimp meat was not significant.