Report to the Fisheries Research and Development Corporation

TRANSPORTATION AND STORAGE OF LIVE PENAEID PRAWNS (Project 89/92)

Bruce Goodrick and Brian Paterson

International Food Institute of Queensland
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PROJECT INFORMATION

Project title: **Transportation and storage of live penaeid prawns.**

Research organisation: International Food Institute of Queensland, Queensland Department of Primary Industries. 19 Hercules St. Hamilton 4007.

Project leader: Mr Bruce Goodrick.

Duration of project: 1989-1991

Staff involved: Dr Brian Paterson, Mrs Pamela Dingle, Mr Stephen Grauf

Cooperating agent: Moreton Bay Prawn Farm (Australasia) Pty Ltd.

OBJECTIVES

To develop an economic and commercial system of handling and processing live prawns from the point of capture to packing and distribution.

SUB-OBJECTIVES

1. To establish acceptable prepacking acclimatisation techniques for penaeid prawns by examining a range of chilling rates and final pre-packing temperatures.

2. To examine the suitability of a range of packaging and support media, including traditionally used sawdust, for the transport of live penaeid prawns for both domestic and export markets.

3. To study the physiological effects on *P. esculentus* and *P. japonicus* of a range of storage conditions, with the packaging systems examined in objective 2.

4. To initiate a training package to disseminate the methods and techniques for handling and packing of live penaeid prawns.

EXECUTIVE SUMMARY

The objective of this project was to develop a system of handling live prawns from the point of capture to their packing and subsequent distribution. The outcome of the project exceeded that objective. We established a laboratory at a prawn farm at Cleveland, near Brisbane and applied the results of our research to a commercial handling system. In 1991, this allowed our cooperating agent, Moreton Bay Prawn Farm (Australasia) Pty Ltd, to export 1.2 tonnes of live kuruma prawns, *Penaeus japonicus*, to Japan.

These commercial trials were successful. The survival of the prawns was typically in excess of 95% at auction in Tokyo and the shipments surpassed kurumas imported from other countries, fetching prices (about $65/kg) similar to those of kuruma prawns of Japanese origin. Moreton Bay Prawn Farm has gone on to grow and export another crop of kuruma prawns in 1992.

The emphasis on kuruma prawns arose after a study of the live prawn market in Japan by the project leader Mr Goodrick. This market is dominated by the kuruma prawn, which is favoured by Japanese buyers because of its bold red and white stripes. Existing techniques for transporting live prawns are based on this species. Further, it was recognised that a successful outcome of the project would be more likely if the prawn that we studied had an existing target market.

The prawns were exported using a method developed from experiments outlined in the sub-objectives of the project. These were; to establish an acceptable method of cooling the prawns, choose a suitable packaging system and to investigate the physiology of prawns in air.

A rapid cooling rate was adopted, faster than that used by kuruma prawn farmers in Japan. This allowed the prawns to be harvested, cooled and packed in time to catch flights leaving Brisbane in the morning. The shipments could then reach Narita in time to clear customs and be despatched to Tsukiji Market in Tokyo for auction on the following morning. The prawns consequently had to survive out of water for at least 30 h.

The prawns were air-freighted in cardboard cartons filled with sawdust, a type of package commonly seen in the market, however our cartons contained coolants and insulation designed to maintain a carton temperature of 10 to 15°C until auction.

This temperature range was adopted, after physiological studies, as being warm enough not to kill the prawns but not so warm as to increase their metabolic rate in air to an unsustainable level.

The remaining sub-objective of this project, to initiate a training package to transfer the results of the research, was overtaken by the rapid development of the commercial export trials. Nevertheless, a manual giving the details of the method has been incorporated as an appendix of this report. Further extension of the results will occur through publication of findings and by direct consultation with growers wishing to export live kuruma prawns.

RECOMMENDATIONS

1. A study of seasonal acclimation and temperature tolerance of kuruma prawns is required. This prawn could possibly be grown by farmers in North Queensland and exported via Cairns International airport.

2. Information is urgently needed on the biology of the kuruma prawn in Australian waters, because the culture of this species relies upon broodstock being available.

3. The acceptability of Australian grown kuruma prawns should be studied in Japan. The Australian variety is not identical to the Japanese prawn and this may restrict the market for Australian prawns.

4. Following on from recommendation no. 2, a study of colour changes during harvesting and export should be undertaken to see if the appearance of the Australian prawns is affected by their handling.

5. A study of the short term ammonia tolerance of kuruma prawns is required so that prawns can confidently be stored for longer periods in water. If prawns can be held for long periods before packing then the number of available flights and marketing options expands. For example, prawns could leave on flights later in the day and by-pass the auction system.

6. The transport and marketability other live prawns can now be considered. In particular, the brown tiger prawn *Penaeus esculentus* and the red-spot king prawn *P. longistylus*.

7. This method may prove suitable for the live transport of prawns used as aquaculture broodstock. This dry method avoids the problems arising from shipping sea-water in aircraft.

CONTENTS

I. INTRODUCTION

A market exists in Japan for live kuruma or japanese king prawns (*Penaeus japonicus* Bate). The red and white stripes of the kuruma make the prawn highly attractive to Japanese consumers. The prawns are typically prepared live and eaten as sushi or tempura, or used as prestigious gifts. The kuruma is readily identified with Japanese aquaculture and fishing industries, but it has a much wider natural distribution.

It is largely the striking pattern and red and white colour which distinguishes the kuruma from other king prawns, and it therefore resembles a tiger prawn. The brown tiger prawn *P. esculentus* Haswell is often maroon coloured in the live state and attracts the attention of Japanese buyers.

The emphasis of this research was, for this reason, initially directed toward the export of live brown tiger prawns from farms in Australia. Brown tiger prawns are more difficult and costly to grow in Australia than black tiger prawns, (*P. monodon* Fabricius) because they have a slower growth rate and higher protein requirement (in this respect they resemble the kuruma prawn). Brown tigers are rarely grown in Australia. A study of live prawn handling and marketing in Japan by one of the authors, the project leader, Mr Goodrick, however showed that a successful outcome from this project would be more likely if kuruma prawns were sent (Goodrick 1989).

Previously, the kuruma prawn had not attracted commercial interest in Australian waters. However, the stocks existed to provide broodstock for aquaculture. Plans were therefore made, in cooperation with Moreton Bay Prawn Farm (Australasia) Pty Ltd (MBPF), for a crop of kuruma prawns to be grown specifically for the live export market. This species is not only suited for live marketing by its attractive appearance, it also is remarkably docile when handled and becomes quiescent when removed from the water. Other prawn species are thought to be poorly suited to live transport, (Richards-Rajadurai 1989) and comparisons have therefore been made in this study between the black tiger prawn and kuruma prawn.

Penaeid prawns are not well adapted to survive out of water, yet remarkably, this is precisely how kuruma prawns are transported alive. The ability of this species to survive in air is astonishing to begin with (for a prawn) and it is readily improved by cooling the prawns in sea-water to between 12 and 14°C before packing them into boxes filled with chilled, dry sawdust (Shigueno 1975).

The endurance of the kuruma prawn in air remains a question of some physiological and ecological significance. This problem is not only of academic interest, since it impinges directly upon the potential for developing a live market for the brown tiger prawn. Some prawns may be "pre-adapted" to live transport by virtue of their ability to reduce their metabolic rate under natural circumstances.

Transplanting the technology directly from Japan without making any effort to adapt it to local conditions is out of the question. The flight time from Australia to Japan is quite

long making it necessary to reduce the system of handling and packing down to the important steps so that the prawns can reach the airport in time for a morning departure. This has resulted in a system that appears deceptively simple, but is far more effective for Australian conditions than those apparently used in Japan and other countries.

A major factor in the success of this project was the location of our laboratory at the prawn farm (section II), providing access to the crop and invaluable experience working at a commercial prawn farm.

The practical and physiological aspects of live prawn transport arising from our research are discussed in this report. Harvesting and transport of the prawns to the packing facility is considered in section III. This topic has much in common with existing practices for wet transport of live fish (Berka 1986), and an early version of section III has already been published (Paterson and Goodrick 1991). Sections IV and V deal with the practical and physiological aspects of cooling the prawns, and section IV will not be published elsewhere subject to approval from FRDC.

Sections VI and VII deal with the practical and physiological aspects of removing prawns from the water. Papers are being prepared from both sections. Section VIII considers the methodology and results of the commercial trials. A manual for the transport and handling of live kuruma prawns forms a substantive appendix to this report and the authors have also contributed to a study of the kuruma prawn market in Japan (Ovenden *et al* in preparation).

The successful export of kuruma prawns that resulted from this study has spurred MBPF to produce another crop of kuruma prawns in 1992. We have continued to liaise with them during the construction of their packing facility and have followed their progress with interest. However, other exporters are being sought, so that production can be increased to take advantage of this opportunity.

II. ANIMALS, PLANT AND EQUIPMENT

The kuruma prawns used in these experiments were grown at Moreton Bay Prawn Farm (MBPF), at Cleveland, near Brisbane, from an original group of spawning adults collected by the QDPI research trawler, "Gwendolyn May" off Mackay in North Queensland. Post-larvae were raised at Gold Coast Marine Hatchery in South East-Queensland and transferred to 2 ponds at MBPF for grow out in December 1989. Water depth in these ponds were 1.5 and 1.8m. The stocking density of post-larvae in the two ponds was 30 and 60 animals m^2. Due to financial constraints, MBPF was unable to import Japanese kuruma prawn feed (which is very expensive) and opted instead to feed the prawns using black tiger prawn feed (Charoen Pokaphand, CP) supplemented with trash fish, scallop guts and prawn heads. An experimental kuruma prawn feed produced by CP was used in 1990. A combination of the late stocking time and the sub-optimal diet during early growth probably contributed to the slow growth and poor yield. The prawns had to over-winter in 1990 and experiments on the kuruma prawns commenced when they resumed growth in Spring 1990.

Research work toward developing a method of exporting live kuruma prawns was conducted on two scales. Methods were developed beforehand, initially using black tiger prawns, in an experimental scale facility built at MBPF, consisting of three 1000 L capacity holding/chilling tanks set up in an unused cool room. When a suitable method was adopted for kuruma prawns, a pilot scale system was developed consisting of two 3000 L capacity tanks. All tanks were constructed from fibreglass with wall insulation of polyurethane foam.

EXPERIMENTAL SCALE

Prawns, harvested from the ponds, were stored in three insulated fibre-glass aquariums located in an air-conditioned room. Each aquarium had its own circulation plumbing, including a removable mechanical pre-filter, a pump, solenoid valves to control water flow and a biological filter (Figure 1). All of the plumbing used (including the heat exchanger) was plastic (PVC or polypropylene) and no copper was used.

Oxygenation

Aeration was achieved passively, by an adjustable venturi in the intake of the pump. For high density storage, aeration was provided by a 12v DC or a 240v AC vane pump connecting to an array of air stones in the bottom of each tank.

Water temperature control

The water temperature in each aquarium was controlled to within ±0.1°C using an electronic process controller. This achieved a level of accuracy and control that was required for research purposes but was far in excess of that needed by commercial operators. The controllers operated the solenoid valves, and cooling was initiated by diverting the flow in each system via plastic heat exchangers immersed in an ice bath in an adjacent cold room. The chilled sea-water bypassed the biological filter and returned to

the top of the filter where it could then drain into the tank proper. The controller monitored the temperature of its respective aquarium by way of a sensor suspended in the centre of that tank. The ice was supplied using an industrial ice machine (approx. 1.5 tonne day^{-1} capacity). The maximum cooling rate achieved using the plastic heat exchangers (10-12 C°/h) was far in excess of that necessary to perform the experiments. The enormous cooling capacity using established ice reserves in the form of an ice slurry far outweighed the poor conductivity of the plastic heat exchanger. It was reasoned that any seafood processor who was intending to export live prawns would already have access to ice and that this "ice-bank" system would substantially reduce the capital costs of gearing up to a commercial operation.

Temperature, cooling rate and holding time were independently programmable in each controller, allowing several variables to be manipulated, as necessary, in the experimental design. Each tank was usually regulated at 22.0±0.1°C prior to the cooling trial. This temperature was chosen initially because it was close to the average water temperature for Moreton Bay and was 10°C higher than the initial "target" temperature of 12°C.

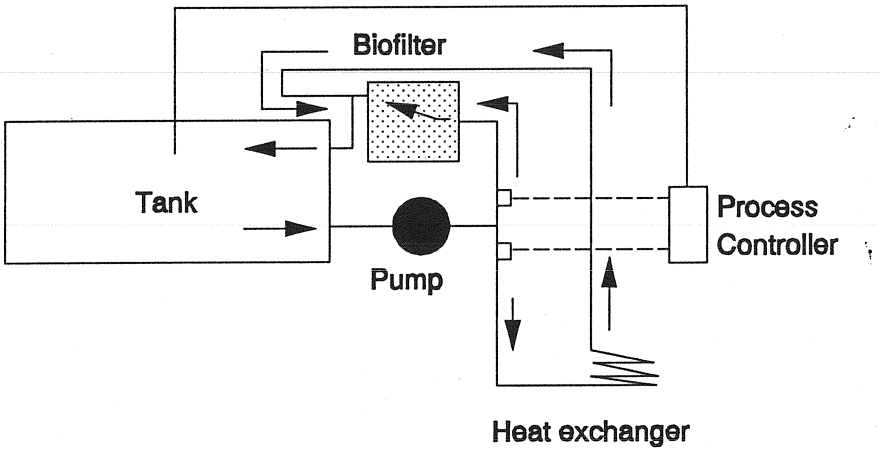

Figure 1. Diagram of experimental tank used in cooling trials.

PILOT SCALE

Harvested prawns were placed into each of two 3000 L capacity prawn bins. One bin (the cooling tank) was located in the packing room itself (a coldroom). The other bin (the storage tank) was located outside of this coldroom. Each bin replaced an experimental tank

(above) and used the same temperature controller and cooling coils. Aeration was achieved using a vane pump and an array of thirty air stones tied onto the mesh floor of the bin. The water circulation pattern was similar to that used for the experimental tanks, though no biofilter was included. Prawns were stacked inside the bins in the same wooden trays that they were harvested into (to minimise handling). Details of the equipment used are given in Appendix I.

III. HARVESTING AND STORAGE OF PRAWNS IN WATER

INTRODUCTION

A night active prawn such as the kuruma must be harvested after dusk, and it may take until as late as 10:00 pm before the harvesting operation is completed. Several hours are required because extra care must be taken when harvesting live prawns, and other factors such as too much moonlight and low pond temperature can slow the harvesting process.

In order to meet the schedules imposed by airlines, Australian prawns destined for Tokyo must be graded, packed and despatched within 7-8 h of being harvested. Shigueno (1975) however reports that Japanese farmers may take 8-12 h just to cool their prawns.

Prawns must be held at high densities in cool sea-water before packing. The need to pack prawns so soon, to meet airline and customs schedules, may mean that prawns are sent before they have recovered from being harvested. However, leaving the prawns to recover after harvest will cause an inevitable deterioration in water quality of the holding tanks.

The problems encountered in storing live prawns are broadly similar to those associated with live hauling of fish in water, a topic that has received much attention and which has been reviewed recently by Berka (1986). Fish in particular are often allowed to purge their guts of faeces before being transported.

Respiration, excretion and defecation can lead to problems with water quality and these factors must be considered when holding prawns in water prior to export. Environmental parameters (oxygen tension, pH, ammonia concentration), gut content of prawns and the survival of prawns packed at different times after harvest, have been studied here. Within the storage time being used here (< 6h), there were no problems with the water in the holding tanks, and prawns could be packed, with no ill effects, as little as 2 h after harvest.

METHODS

A more detailed description of the harvesting technique is described in the accompanying manual (Appendix I). Only details of the harvesting technique used for commercial trials are given here, as the earlier methods differ only in scale.

Kuruma prawns were harvested using a tunnel net which was opened about 1h before sunset and cleared between 19:00 and 20:00 hrs. Aeration of the basin of the tunnel net was provided by a paddlewheel. The prawns were transferred into floating wooden trays in the pond. Each tray of about 10kg of prawns was then stacked into the transport tank (an X-actics bin). The tank was filled prior to harvesting with chilled sea-water (22 to 24°C, for ponds with temperatures in excess of 28°C) drained from a holding tank.

The transport tank was oxygenated using a cylinder of medical oxygen and regulator connected to an array of air-stones. Upon arrival at the holding facility, the trays of prawns were carried from the transport tank and stacked into a holding tank (pilot scale, section II). The prawns remained in these wooden trays throughout the holding and cooling process.

Gut content of kuruma prawns after harvest

One sample of prawns was taken immediately that they arrived at the holding facility (n=10) and two subsequent groups (each of 10 prawns) after storage for 3 and 6 h in the holding tanks. These prawns were frozen by placing them on the floor of a freezer (-20°C). The amount of food material in the gut was assessed by thawing and dissecting the prawns. The contents of the foregut ("stomach") and hindgut ("intestine") were removed, weighed and then dried in an oven at 70°C for 3 days before weighing the contents again.

Oxygen tension, pH and ammonia concentration of water in the holding tanks

Quantities of prawns, ranging in size from 40 to 80 kg, were harvested and transferred in wooden trays into the 18°C holding tank for storage experiments prior to packing an export trial. Water quality in the tank was monitored using sensors for dissolved oxygen, pH and gaseous ammonia. Dissolved oxygen tension (converted to kPa) was recorded continuously using an oxygen meter connected to a portable chart recorder. Water samples were taken periodically. The pH of each sample of water was measured using a pH electrode calibrated using standard buffers. The total ammonia concentration of the sample was then assayed using an ammonia gas sensing electrode according to the method of standard addition (Gilbert and Clay 1973) ensuring that the water sample was allowed to warm to room temperature before proceeding with the analysis. The ionic background of the standards was adjusted using synthetic sea water. Both the pH and ammonia assays were performed using the same pH meter, leaving the meter calibrated to the pH electrode and reading the ammonia sensor on the mV scale only. The amount of unionised ammonia present could then be estimated, knowing the temperature of the tank, from the pH and total ammonia concentration. Tank temperature was recorded continuously (±1°C) using thermocouples and a multipoint recorder, or read from the display of the process controller.

Effect of holding time on survival

Prawns were packed within 2 to 6 h of harvesting during commercial trials (section VIII). There was no evidence from these trials that there was any variation in survival between boxes of prawns packed at different times after harvest. To confirm this observation, 4 boxes of prawns packed 2 h after harvest and 4 boxes of prawns packed 4 h after harvest were put aside and stored at 14°C. Two boxes from each packing time were opened after 29 h storage and the remaining boxes opened after 53 h.

RESULTS

Observations of harvested prawns

The pigment cells or chromatophores of the kuruma prawn changed the appearance of the prawns during harvesting and holding prior to export. The prawns were often pale yellow when harvested at night, when they looked almost like conventional king prawns. The

white and red-brown markings became pronounced when the prawns were held under artificial lights. Occasionally, the white cells of the prawns failed to develop properly, causing the prawns to retain the yellow colour. Sometimes the prawns had a blue tinge of colour, again due to failure of the white chromatophores to mask the other cells.

Gut content of kuruma prawns after harvest

Most of the kuruma prawns sampled had low (and probably residual) amounts of material in the gut, but there was no significant change in the dry weight of either foregut or hindgut contents during the 6 h holding period. Figure 2 shows the dry weight of foregut contents of the dissected kuruma prawns. Cannibalism shows up as a few unusually high dry weight values, (mostly antennal and leg fragments).

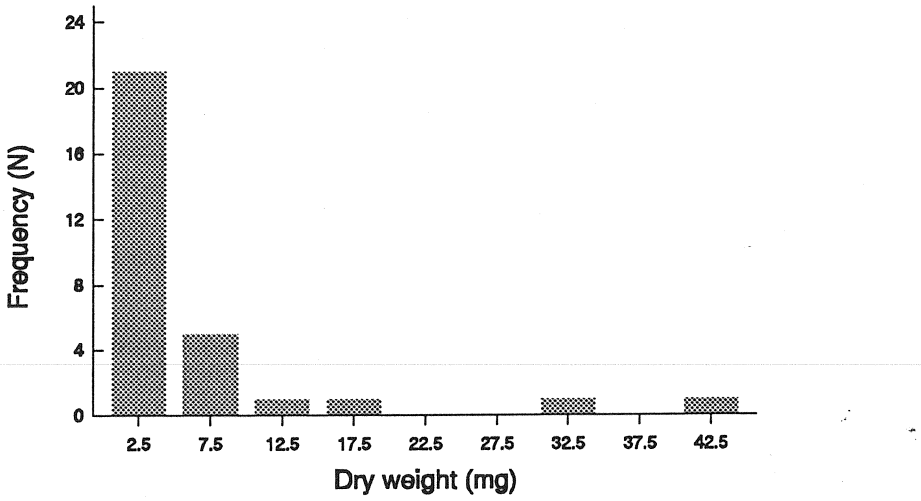

Figure 2. Dry weight of foregut (stomach) contents of 30 kuruma prawns after harvest.

Oxygen tension, pH and ammonia concentration of water in holding tanks

A number of factors influenced the concentration of gases and solutes in water in the holding tanks; the quantity of prawns involved was important. The dissolved oxygen level did not fall below 80% of full saturation (an oxygen tension of about 16 kPa) (Figure 3). The fall in oxygen tension was matched by a fall in the pH of the water, (Figure 4). Ammonia concentrations rose linearly with holding time but within 7 to 8 h did not reach levels very much above 10 ppm (e.g. Figure 4).

Holding time and survival

Trials conducted alongside commercial shipments showed negligible mortality (samples of 39-45 prawns per carton) when cartons of prawns packed 2 and 4 h after harvest were stored at 14°C for 29 h and even for 53 h.

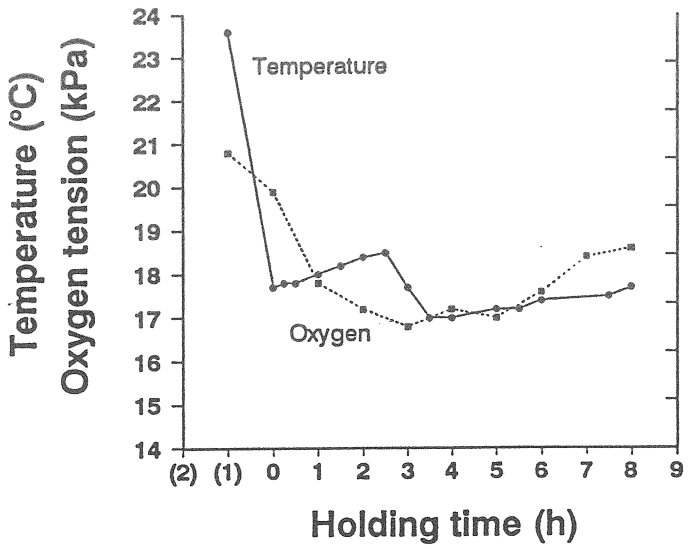

Figure 3. Changes in dissolved oxygen tension and temperature while holding 70kg of kuruma prawns in a 3000 L tank, containing water at 18°C, prior to cooling for export.

DISCUSSION

Kuruma prawns can be safely packed within a couple of hours of being harvested. To achieve this, the prawns must not be stressed by low oxygen tensions prior to packing. This was easily achieved by taking extra care in harvesting and by cooling the holding tank to between 16 to 18°C and aerating it vigorously. The build up of nitrogenous wastes was not a major problem.

Live prawns must not be fed before harvesting, because food raises an animal's metabolic rate as it digests and assimilates the meal. Fish are often starved for up to 2 days before being transported in water (Berka 1986). Fortunately, kuruma prawns starve themselves before harvest. When the prawns emerge at night from the pond bottom, their guts are virtually empty. The brown tiger prawn, which is nocturnal like the kuruma, similarly empties its gut while buried in the sea-bed by day, (Wassenberg and Hill 1987). The emerging prawns swim actively in search of food and it is this behaviour that allows them to be trapped successfully in nets.

Consequently, the prawns crowded into the net are hungry and active, and at high densities face the combined problem of low oxygen level and cannibalism. Oxygenation is provided by an aerator, which maintains a flow of water through the net. It is recommended that the nets be cleared regularly to stop too many prawns from becoming trapped.

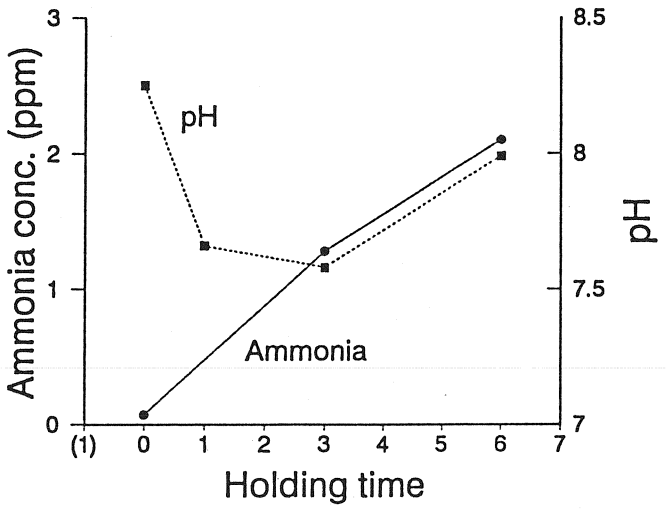

Figure 4. Changes in water pH and total ammonia concentration when holding 40 kg of kuruma prawns in a 3000 L tank, containing water at 18°C, prior to cooling and packing.

Death, and subsequent cannibalism of prawns is more likely when they are crowded in nets for long periods. Cannibalism also injures living prawns (damaging eyes, legs etc) and detracts from their appearance and value. This lowers returns to the producer. Soft, post-moult prawns are especially vulnerable, and harvesting should be avoided when many prawns are casting their shells (Shigueno 1975).

The prawns were transferred promptly from the net to the transport tank, which was well oxygenated. As a precaution, in summer, the water in the transport tank was chilled beforehand to between 4 to 6°C below the pond temperature. This reduced the metabolic rate and activity of the prawns while they were being taken to the packing facility. In late autumn and winter, when the pond temperature fell below 20°C, this pre-chilling differential was reduced.

At the packing facility, the holding tank was held at 16 to 18°C to reduce the appetite and activity of the prawns. The characteristic stripes of the kurumas became visible during storage, in response to the lights of the building. In a sense, the attractive stripes are unnatural, a response to the bright surroundings. Is it camouflage? The response appears to be pointless in a nocturnal prawn that buries deep in the sediment by day. By night, the kuruma is like a king prawn that is slightly darker than other kings.

The pigment cells or chromatophores of crustaceans are controlled by hormones (Herreid and Mooney 1984), and cases here where the white chromatophores failed to become visible possibly resulted from physiological stress. Further work is required to identify the

origin of this stress, but it presented no major obstacle to the project.

The prawns have not eaten since the previous evening so there is no immediate benefit in fasting them any more after harvest. Whyte and Carswell (1982) found that withholding food from the spot prawn *Pandalus platyceros* caused a significant fall in respiration rate after a day, and a similar time scale apparently applies for the brown tiger prawn (Dall and Smith 1986).

Fasting is sometimes used to allow animals to empty faeces from their gut before transport. However, the guts were virtually empty to begin with and there was no evidence that holding the prawns any longer would let them purge their guts completely. Nevertheless, faeces accumulated in the tank. The persistence of residual material in the gut was not surprising in an animal that was cooled down and which had no opportunity to feed prior to harvest. The residence time of food in the gut of crustaceans can be affected by feeding rate itself (Murtaugh 1984).

Storing prawns at low temperature not only reduced their appetite, it reduced their metabolic rate in general. This fact has important consequences for holding large numbers of prawns in a relatively small body of water. Cold prawns consume less oxygen and excrete less carbon dioxide and ammonia.

The need for oxygen was supplied by aerating the tanks vigorously. The oxygen partial pressure did not fall below about 16 kPa, which was well within the tolerances of this and similar prawns (Truchot and Jouve-Duhamel 1983, Dall 1986). A high aeration capacity in a closed system can also have the supplementary benefit of blowing off a certain amount of excess carbon dioxide and gaseous ammonia, (Berka 1986). The fall in oxygen tension after placing prawns in the tank was matched by a fall in the pH of the water, (Figure 4), due largely to the carbon dioxide being produced by the prawns. However, the pH did not fall below 7.0.

The total amount of ammonia present in water is not the major issue, as the gaseous form (NH_3) is the most toxic toward animals, (Trussell, 1972). The proportion of total ammonia present in the unionised, (gaseous) state can be reduced by holding the temperature down (in this case to below 18°C). Even at this low temperature, if the tank becomes significantly alkaline (pH>8.0) the levels of gaseous ammonia in the water can still become unacceptable. However, the prawns are excreting carbon dioxide, which keeps the pH low and favours the ionisation of ammonia to the ammonium ion (NH_4^+).

The threat posed by ammonia toxicity was therefore largely contained in the short term by the low pH and the low storage temperatures used. It follows that removing most of the prawns from the holding tank may actually be detrimental to the prawns remaining, as this allows the pH to rise toward 8.0. At a constant temperature, any rise in pH will increase the proportion of gaseous ammonia in solution (Berka 1986).

The recommended levels of dissolved ammonia when holding aquatic animals for long periods in aquariums are probably not applicable here. The saving grace of holding live prawns, at high density, in a tank without a biological filter, was that the time span

involved was quite short. There was no evidence that rising levels of nitrogenous wastes became critical.

Further research is required to determine the levels that ammonia and nitrite must reach before prejudicing the survival of adult prawns. For example, high ammonia concentrations in the environment (above about 10 ppm) make it difficult for prawns to excrete ammonia (Chen and Kou 1991).

This information is necessary when storing prawns for even longer periods (such as to meet later flights and bypass the auction system). However, if the tolerance of juvenile penaeid prawns to ammonia, (Chen *et al* 1990, Chen and Lei 1990) is any guide, much longer periods of exposure, to much higher concentrations of ammonia, are required than were seen in this project. Eventually, the storage period will reach a point at which it will be feasible to either flush the tank periodically with new, chilled sea-water or to use a biological filter and/or cultures of bacteria to contain the problem of ammonia build-up (by converting it to nitrite). A note of caution is required here since there appears to be a difference in the toxicity of ammonia (NH_3) and nitrite (NO_2). Mevel and Chamroux (1981) found that kuruma prawns are more sensitive to rising nitrite concentrations in aquariums than to rising ammonia concentrations.

Low temperature was used at several points in the holding process so that the prawns would not be stressed. This was done to reduce the appetite of the prawns, to reduce the uptake and excretion of gases, and, finally, to reduce the toxicity of the ammonia accumulating in the water. The live transport process itself is quite stressful, but the prawns are not yet cold enough to withstand being taken from the water for long periods. The cooling of prawns prior to packing is considered in the next section.

IV. COOLING PRAWNS BEFORE PACKING

INTRODUCTION

The kuruma prawn has a reputation for being tolerant of low temperatures (Richards-Rajadurai 1989) though published literature on the kuruma industry is vague on details of the process. Shigueno (1975) reports that in Japan, the kuruma prawn is routinely cooled to 12 to 14°C prior to being packed but does not report a preferred cooling rate or holding times or the effects of season upon the process.

Even if detailed knowledge of the methods used in Japan was available, this information may not be of any use in Australia. The nocturnal activity of the kuruma prawn places major constraints upon the timing of harvesting, and this must then be squared with the fixed timing of airline flights and customs clearance at the destination (section III). A prospective prawn exporter needs to know to what temperatures the prawns should be cooled and the fastest cooling rates that do not kill the prawns. Information on how urgently the prawns should be packed after reaching that temperature is also necessary and knowledge of the leeway in all of these variables would be invaluable to the practical operation of a live handling facility. Subsidiary questions to this involve the possible effects of storing the prawns for long periods (say up to 12 h) at moderate temperatures (17 to 18°C) after harvest, (section III), for example when harvesting before the moon rises or when meeting flights later in the day.

While the black tiger prawn, *P. monodon* is not as tolerant of dry transport as the kuruma prawn, experimental work was also carried out using the former species. The black tiger was readily available and was used to establish handling and cooling parameters that could be developed for other prawns, (particularly the brown tiger prawn, *P. esculentus*). Most of the temperature changes described in this section would never be experienced by prawns in nature and would certainly be lethal in the long term. As survival is an important index of the commercial viability of live prawn export, the effect of these variables on the survival of prawns after storage is assessed here. Using benign cooling regimes certainly appeared sensible from a physiological perspective but this was of no practical use since it did not affect survival on the market floor.

METHODS

Effect of temperature on proportion of prawns upright

The point at which kuruma prawns fall over when cooled was quantified. Approximately 60 prawns were placed on a mesh based tray in a experimental tank at 22°C and then cooled at 3C°/h until all of the prawns had fallen over. Data was recorded as the temperature at which each additional prawn fell over, because the rapid onset of this response made it impractical to record the number of prawns upright at 0.5°C intervals. The experiment was repeated on several occasions during summer and autumn in 1991.

Variables influencing the survival of prawns during storage in sawdust

In these trials, black tiger and kuruma prawns were cooled under different circumstances and packed for storage under constant conditions. After a certain period, (24 to 36 h) the boxes of prawns were opened and numbers surviving noted for each group. Prawns were counted as alive only if they moved their legs or jumped vigorously when unpacked (this is the "commercial" survival required of the method). Heart and bailer activity alone in moribund prawns was not used as an index of "survival" as experience showed that these prawns would not survive re-immersion. Prawns that were obviously soft-shelled (in post-moult or "Yawara", Shigueno 1975), and those that were weak or injured were not packed.

The prawns were placed in floating baskets or trays prior to packing. They were taken from the water as required and placed upright on a towel to drain off excess water. The prawns were then packed with dry sawdust in a cardboard "inner" carton (Appendix I). The boxes of prawns were then either stored in a refrigerator (at the prawn farm) set at 12±2°C or carried in insulated boxes on a short (40 min) journey to the IFIQ laboratories at Hamilton and stored in a coldroom at 15°C.

Cooling rate and the survival of black tiger prawns in sawdust.

The effect of cooling rate on black tigers was investigated by cooling tanks of sea-water at different rates (eg. 0.6, 1.2 and 3 C°/h) from 22°C to a single final temperature. When experiments were repeated at different final temperatures, the cooling rate assigned to each tank was varied to eliminate possible "tank" effects. All tanks were programmed to reach their final temperature at 0900 hrs the following day. Consequently, prawns cooled at faster rates remained at 22°C for longer periods beforehand. 90 prawns were packed from each treatment. Several boxes of prawns (10 prawns per box) per treatment were stored at 12°C and their survival determined after 24 h.

Cooling rate and the survival of kuruma prawns in sawdust.

Experiments on kuruma prawns were conducted by starting each of two tanks cooling at the same time (from 24°C) at 3 and 6 C°/h and packing prawns from each tank when they reached the desired temperature (12°C). Seven boxes (each containing 10 prawns) were packed from each tank. These prawns were then stored at 12°C and the survival of the prawns was measured first by opening one box from each treatment after 24 h and the remaining boxes (60 prawns per treatment) after 30 h.

Plunge cooling and the survival of kuruma prawns in sawdust.

Two groups of prawns were used. One group of prawns (N=50) was held at 20°C in a holding tank. The other prawns (N=40) were cooled from 22°C to 13.5°C at 4.8 C°/h and packed upon reaching 13.5°C. The prawns held in the meantime at 20°C were then plunged directly into the cold water and packed after 60 min. Prawns from both treatments were then stored at 17°C for 34.5 h.

The experiment was repeated in conjunction with a commercial trial (where plunge cooling was used routinely for exports). One group of prawns (N=80) were cooled from 18°C to 12°C at 4.8C°/h while a second group of prawns (N=80) was plunge cooled from

18°C to 12°C. Both groups of prawns were stored for 32h at 14.5°C after packing.

Holding kuruma prawns in water after plunge cooling.

Prawns were held in a pilot scale holding tank at 18°C and plunge cooled after spending 6 h in the tank. Groups of about 50 prawns (a 1 kg carton) were packed after they had been plunged in 14°C water for periods ranging from 0 to 60 min. The prawns were then stored at 15°C for at least 34 h and their survival at this time noted. The experiment was repeated later in the packing season.

Temperature at packing and the survival of black tigers in sawdust.

Prawns were cooled at different rates (see above) to temperatures ranging from 8 to 14°C (90 prawns at each temperature) and held at constant temperature (±0.1°C) for several hours. Holding time (0-10 h) after reaching final temperature was used as a variable in some experiments.

Temperature at packing and the survival of kuruma prawns in sawdust.

Prawns were cooled from 24°C at 3 C°/h to 14, 12, and 10°C and the prawns were then packed in sawdust boxes and stored at 12°C. 50 prawns were packed at each temperature. The survival of these prawns was assessed after 36h of storage.

Statistics

The results of each experiment were tabulated and subjected as appropriate to either Fischer Exact test, or Contingency table X^2 analysis or log likelihood ratio (refer Zar 1974) with a desired significance level of 0.05.

RESULTS

Low temperature and prawn behaviour

Observations of the effect of falling temperature on the loss of equilibrium by the prawns is shown in Figure 5. Prawns were more resistant to low temperature in winter. Prawns often jumped about before falling over, which knocked over other prawns. The reflexive jumping was quite impressive amongst large numbers of prawns. There were so many factors effecting when the prawns fell over that it was not possible to use this data to predict the temperature to which the prawns must be cooled prior to packing.

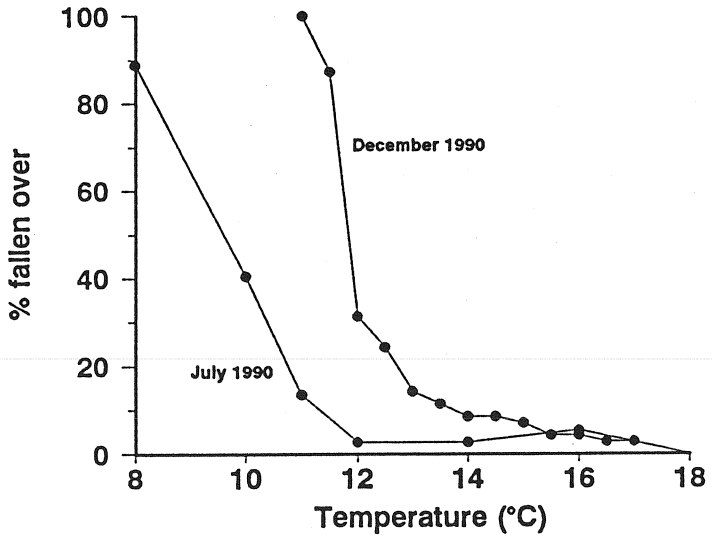

Figure 5. The proportion of kuruma prawns remaining upright at different temperatures.

Cooling rate and survival in sawdust

Pooling the survival data for black tigers held in water at 13, 12 and 11°C for 2 h and then packed showed no significant effect of cooling rate on survival. However, significant differences in survival were found when the experiment was repeated later in the growing season. The first replicate was performed by cooling the black tigers prawns to 12°C. In this experiment (Table I) the survival rate was not the same for different cooling rates ($P<0.05$). A second replicate at 11°C also showed that survival was not the same for all cooling rates ($P<0.01$) (Table II).

Cooling kuruma prawns at 3 and 6C°/h produced no significant difference in survival rate when the prawns were unpacked after 30 h at 12°C.

Plunge cooling and survival in sawdust

Kuruma prawns were cooled at 4.8C°/h to 13.5°C and compared with prawns plunged from 20 to 13.5°C. There was no significant difference between the survival of chilled versus plunged prawns after 34.5 h.

The experiment was repeated in conjunction with a commercial trial, cooling one group of prawns from 18°C to 12°C at 4.8°C/h and dunking another group of prawns from 18°C to 12°C. There was no significant difference in survival of prawns packed after these treatments at 32h.

Table I. Survival of black tiger prawns cooled at different rates to 12°C, packed and stored for 12 h.

Rate C°/h	Alive	Dead	Total	%Alive
0.6	84	16	100	84.0
1.2	87	23	110	79.1
3.0	68	32	100	68.0

Table II. Survival of black tiger prawns cooled at different rates to 11-13°C, held for 2h, packed and stored for 12h at 12°C.

Rate C°/h	Alive	Dead	Total	%Alive
0.6	102	18	120	85.0
1.2	111	6	117	94.9
3.0	92	28	120	76.7

Temperature at packing and survival in sawdust

The number of black tiger prawns alive or dead after 12h storage at 12°C was not significantly different when they were packed at 11, 12 and 13°C (pooling the data from 270 prawns at three different cooling rates).

Likewise, there was no significant effect of temperature at packing (10, 12 and 14°C) on survival of kuruma prawns stored in sawdust at 12°C. This experiment was repeated, again with no significant difference in survival between the three treatments.

Holding time after cooling and survival in sawdust

When black tiger prawns were cooled to between 11 to 13°C, at three different cooling rates, the survival of the prawns after 12h storage in sawdust at 12°C was reduced by holding them a further 4 h at the packing temperature ($P<0.01$, Table III). Further observations showed that the proportion of surviving prawns did not fall significantly when black tigers were held in water at 12°C for 2 h as against prawns packed immediately upon reaching that temperature. A later experiment showed that the proportion of surviving prawns fell when held at 11°C for 2 h before being packed ($P<0.05$, Table IV).

Table III. Survival data of black tiger prawns held at final temperature (11,12 and 13°C) after being cooling at different cooling rates. Prawns packed and stored for 12h.

Hold h	Alive	Dead	Total	%Alive
2	73	17	90	81.1
6	48	42	90	53.3
10	44	46	90	48.9

Table IV. Survival data of black tiger prawns cooled to 12°C and held up to 2h after reaching temperature. Prawns packed and stored for 12 h.

Hold h	Alive	Dead	Total	%Alive
0	121	29	150	80.6
2	118	42	160	73.8

Holding after plunge cooling and survival in sawdust

Kuruma prawns proved to be amenable to plunge cooling and two experiments showed there was no significant effect of holding time on survival (at 34.5 and 38 h respectively) of prawns packed up to 60 min after plunge cooling.

DISCUSSION

Kuruma prawns tolerated rapid changes in temperature, and the success of plunge cooling indicated that the export process worked more by shock than by acclimation, (Table V). The actual rate of cooling the prawns did not seem to matter, nor did the target temperature need to be closely monitored (Table V) though there was evidence that the black tiger prawn was less tolerant of fast cooling than the kuruma prawn.

For practical reasons, a "two tank" approach was adopted here. One tank of prawns was cooled rapidly from 18°C to below 14°C, its prawns sorted and packed and then the prawns from another tank (18°C) were plunged directly into the 11 to 14°C water. This kind of "continuous" cooling operation allowed several hundred kilograms of prawns to be cooled in each shipment.

Table V. A summary of the cooling experiments conducted in this section. Range for each variable studied is shown in brackets.

Variable (range)	Effect on survival	Species
cooling rate	no effect (>3 C°/h)	*P. japonicus*
(>0.6C°/h)	better survival (<3C°/h)	*P. monodon*
packing temperature (8-14°C)	no effect (10-14°C)	*P. japonicus*
	no effect (11-13°C)	*P. monodon*
holding after cooling	no effect up to 60 min	*P. japonicus*
	survival reduced held 2h or more	*P. monodon*

The next logical step is to have one tank dedicated as the "plunge" tank and other tanks dedicated as holding tanks. This eliminates the waiting period associated with cooling the water, and also the need for a powerful cooling system, by holding each tank at either 11 to 14°C or at 18°C.

Shigueno (1975) said that, in Japan, the prawns were cooled from a temperature not more than 8°C below the pond temperature to 12-14°C, over a period of at least 8 h. Assuming that the temperature must fall about 8°C at this time gives a cooling rate of less than 1C°/h. This low rate of cooling is said to allow the shrimp to "...become gradually acclimatised to the temperature." (Shigueno 1975). It is arguable whether this acclimation is really necessary. Prawns packed after plunge cooling typically showed a survival in excess of 95% when stored under controlled conditions for 24h. The practise of slow cooling is feasible when air-freighting prawns within Japan but it takes too long when prawns must leave on early morning flights from Australia.

Whyte and Carswell (1982) found that plunge cooling the spot prawn *Pandalus platyceros* from 12°C to 0°C caused high mortalities, giving data as mortality after 24 h in sea-water at 0°C. In the present study, the kuruma prawns were subjected to a decrease of only 4 to 5°C **and then were removed from the water.** The effect of emersion may be to

ameliorate the ion balance problems that the prawn will normally experience in extreme cold, for example Spaargaren (1973) reported that the blood osmo-concentration of the shrimp *Palaemon serratus* rose in the cold.

Plunging the prawns into cold sea-water appears to be no more stressful than cooling them gradually at between 3 and 6 C°/h. In both cases the prawns reach a point at which they enter a period of reflexive jumping: they become more active, and start flicking or jumping in the water. The "berserk" response is not mentioned by Shigueno (1975), though he reported that the tanks were covered with canvas during cooling (Appendix I). This would certainly stop jumping prawns from leaving the tank and for similar reasons it is recommended that the very top holding trays be covered with empty trays during cooling, (Appendix I).

P. platyceros showed a "severe escape response" when warmed abruptly from 0 to 12°C but not apparently when plunge cooled in the other direction (Whyte and Carswell 1982). A period of apparent panic occurred in kuruma, brown tiger and black tiger prawns below about 14°C, and Venkataramaiah *et al* (1977) noted that the brown shrimp, *Penaeus aztecus* will also "run and swim vigorously or jump out of the tanks" when stressed. Plunge cooling seems to stress the prawns at higher temperatures than would normally be the case: though quantifying this is difficult because the response is also influenced by handling and disturbance. At the end of this jumping activity, the prawns are lying on their side beating their swimming legs (swimmerets) gently, as described by Shigueno (1975). The prawns can now be packed.

The actual temperature at which they are packed at does not seem to be as important as that they are lying on their side, almost immobilised. Work with black tiger prawns shows that cooling prawns to 8 to 10°C should certainly be avoided, but it transpires that the range reported by Shigueno (1975) of between 12 and 14°C probably also applies to Australian kuruma prawns.

How long the prawns were left in the water after cooling them to between 11 and 14°C also did not appear to be critical- though work with black tiger prawns showed that survival of prawns after packing declined the longer that they remained in water at the packing temperature. Acclimation is clearly not an issue here, which is not surprising given the extreme and decidedly unnatural temperature changes being experienced.

Time must of course be allowed, after cooling, for the prawns to be graded into size categories (i.e. small, medium, medium-large, large). The grading process itself does not excite the prawns at this stage. Respiration measurements showed that handling did not increase the metabolic rate of the chilled prawns (section V) and other trials (not presented here) showed that placing the prawns into baskets before or after they were chilled had no significant effect upon survival after packing. Therefore, removing chilled prawns from the water has no detrimental effects upon them, over and above that expected when a primarily aquatic animal is taken into air.

V. HANDLING COLD PRAWNS

INTRODUCTION

The success of using "plunge cooling" to prepare prawns for export suggests that this process does not work by "acclimatising" the prawns to the cold but simply anaesthetises them, reducing their activity while they are packed.

The prawns are handled individually, a labour-intensive process, to check for any weakness or injury that could compromise their survival. It is a general principle that handling increases the respiration rate of crustacea, (e.g. Winkler 1987) apparently by increasing activity of the animal. Some crustaceans are vulnerable to disturbance during live transport in air for this reason (Taylor and Whiteley 1990). If activity is the major response of the prawn to handling, it follows that if you can anaesthetise crustacea, for example using extreme cold, you should be able to remove the effect of handling on respiration rate altogether.

This section considers the effect of handling, at different temperatures, on the respiration rate of kuruma prawns, *P. japonicus*. The prawns were so lethargic when cooled to 12°C, that handling did not increase their respiration rate.

METHODS

Prawns of either sex, and moult stage C-D_1 (Smith and Dall 1985), with a weight range of 8 to 25g, were used in experiments. Prawns were harvested and kept for at least a week in a 1000 L aquarium with a sand bed (at 22°C) prior to experiments. Experiments were carried out in a second tank (Salinity, 35 ppt, 22°C) which lacked a sand bed. These tanks were in an air-conditioned room (section II). The conditions within the sand tank fluctuated during water changes according to the salinity of the intake pond of the prawn farm, but water changes were not performed if the salinity fell below 30 ppt. The temperature of both tanks was controlled to within 0.1°C using process controllers by circulating the water through a plastic heat exchanger immersed in an ice slurry.

Measurement of oxygen uptake rate

Oxygen uptake rate was measured using an automatic closed chamber similar to that used by Dall (1986) for studies of respiration of brown tiger prawns, *P. esculentus*. The chamber normally remained submerged in one of the aquariums described above, allowing animals to be transferred between chamber and aquarium without removing the chamber or the animal from the water. Water was pumped into and out of the respiration chamber using a submersible aquarium pump and plastic tubing (3mm i.d.). The flow of water through the intake tube was controlled by a 240v AC solenoid valve (plastic body) that was operated either manually or by a 60min power timer. The power timer allowed the respirometer to cycle continually between "closed" and "open" during experimental treatments (e.g. cooling).

The respirometer chamber was made from an acrylic canister (capacity 794ml, diameter 6cm, length 27cm). The screw top lid of this canister was made watertight by using a rubber washer and the oxygen sensor was inserted through a 15mm aperture in the lid, and sealed with "O" rings. Water entered the chamber through a 2mm tap at the far end of the canister and exited from a similar tap closer to the oxygen sensor. Any exchange of water between these taps and the chamber when "closed" was negligible. The oxygen tension in the respiration chamber was monitored using an O_2 sensor and meter connected to a portable chart recorder. Water was circulated across the sensor membrane and through the chamber during the closed mode by using a magnetic stirring bar located at the sensor end of the canister. The stirring bar was motivated by a magnetic stirrer located underneath the aquarium.

Unlike the chamber used by Dall (1986), the cylindrical chamber was partitioned by an incomplete acrylic floor. Water flowed away from the stirring bar, underneath the prawn and reached the end of the chamber. The water then rose up through a gap in the floor and returned past the prawn to the sensor. A gauze screen separated the animal from the stirring bar and oxygen sensor.

Experimental design

During storage in the sand tank, the prawns were fed daily with commercial prawn food (CP) and supplemented with chopped prawn, squid and scallop waste, as available. The commercial food pellets were dispensed in the late afternoon through an automatic feeder. Uneaten food was removed on the following morning. Any prawn that did not bury during the day was suspected of being diseased and was not used for experiments (see Egusa 1961). The prawns were allowed to acclimate, without feed, to the ambient conditions in the experimental tank for at least 2 days prior to measuring oxygen uptake rate. The "daylight" illumination was provided by a time switch (12h on/ 12h off) and a gradual transition from light to dark was adopted to avoid startling the prawns.

These experiments were time-consuming, and required several months (October 1990 to May 1991) to collect data from many animals, during which time the crop was growing. Experiments in which prawns were cooled to the intermediate temperature of 17°C were begun after those involving cooling to 12°C, making it necessary to consider body size of the prawns as a variable in the subsequent analysis. Average weekly pond temperature was between 15 and 31°C during these experiments.

Effect of temperature and handling on respiration rate.

In these experiments, records were taken of oxygen tension in the closed chamber either for 30 min immediately after a prawn was placed into the chamber ("handled") from the aquarium, or for 30 min after the prawn had been allowed to recover from handling overnight in the chamber ("settled") with the respirometer continually alternating between closed and open mode.

Measurements of both categories of prawns were made at 22°C or after cooling the prawns at 3C°/h to either 17°C or 12°C. Respiration rate was calculated after correcting for changes in oxygen tension in the empty chamber. Two replicates of this experiment were completed in a week, subject to the availability of intermoult prawns.

Statistics

Weight specific respiration rate is known to be correlated with prawn weight and, as circumstances prevented prawns of similar size being used for each trial, the absolute respiration rate data (µmol O_2 min^{-1}) for the two handling treatments and the three temperatures was subjected to an ANCOVA using prawn weight as the covariate. Sample sizes within treatments (combination of handling and temperature) were made equal (a requirement of the statistical program) by adding "missing data" points to bring each treatment to a sample size of 19. A separate multiple regression analysis was used to describe the relationship between body size and respiration rate at the three temperatures.

RESULTS

The "settled" or routine weight-specific respiration rate of kuruma prawns at various temperatures is shown in Table VI. Handling raised the respiration rate of all except those prawns at 12°C (Table VI). The wide variation in respiration rate of handled prawns at 22°C shows that the data does not conform to a normal distribution. Some of the variation of the data from settled prawns was accounted for by body size. The ANCOVA of absolute respiration rate showed a highly significant effect of both temperature and handling upon respiration, but, in addition, there was a highly significant interaction between temperature and handling ($P<0.01$). Handling the prawns at 12°C had no significant effect on their respiration rate, whereas the same treatment at higher temperature produced a significant rise in respiration rate that became more pronounced at the highest temperature (Figure 6).

Table VI. Effect of handling on the respiration rate of the kuruma prawn at different temperatures, (mean±S.D., number of prawns given in brackets)

	Respiration rate µmol O_2 kg^{-1} min^{-1}	
°C	settled	handled
22	79.11±31.07 (19)	131.73±58.48 (22)
17	28.21±7.78 (13)	47.26±19.58 (13)
12	22.34±8.41 (13)	21.60±9.13 (13)

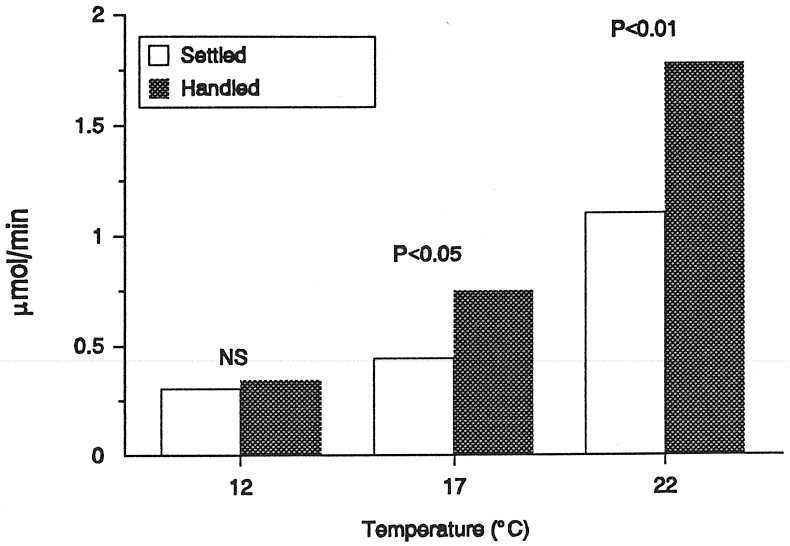

Figure 6. Aquatic respiration rate of kuruma prawns when handled at three different temperatures. Mean rate corrected to constant prawn size (15.3 g).

DISCUSSION

One of the major ways that handling affects the respiration rate of animals is by increasing their activity. In both kuruma and brown tiger prawns, respiration rate changes with the activity state of the prawn, (Truchot and Jouve-Duhamel 1983, Dall 1986) and in this study the respiration rate of prawns disturbed at 22°C reflected activity ranging from excited swimming to "playing possum". The kuruma prawn will sometimes curl up on its side on the tank bottom rather than flee from disturbance.

Therefore, preventing activity is an obvious way to reduce the metabolic stress experienced by crustaceans during live handling and transport. Chemical anaesthetics are sometimes used to reduce metabolic rate when transporting live fish (Collins 1990), but this practise is discouraged when the product is destined for human consumption. Cooling is a well understood means to reduce the metabolic rate of crustaceans during commercial transport both in and out of water. As falling temperature will increasingly incapacitate the prawn, and it follows that the contribution of activity to respiration rate will also decline. For example, Van Donk and de Wilde (1981) found that the shrimp *Crangon crangon* showed a narrowing of its "scope for activity" (the difference between active and inactive respiration rate) as the temperature fell.

Several interacting factors were probably reducing the respiration rate of kuruma prawns in the cold. A reduction in metabolic rate is expected from the decrease in temperature alone, but oxygen uptake can itself be interrupted at low temperature by the failure of the

oxygen transport pigment in the blood to release its oxygen in the cold (Mauro and Mangum 1982). Added to these physiological effects, low temperature also physically blocks the functioning of nerve and muscle in crustaceans (Blundon 1989).

Penaeids often respond to a drop in temperature by burying in sand and remaining there (Hill 1985). At extremely low temperatures, the prawns are probably so torpid that they are physically unable to emerge from the sand, and those prawns that fail to bury under these conditions soon fall over and come to lie unprotected on the sediment surface (Aldrich *et al* 1968). Kuruma prawns that are cooled commercially for live export experience a period of reflexive jumping and then fall over (section IV). They are no longer able to stand upright at temperatures below about 13°C. This response is probably subject to the seasonal acclimation of the nervous system (Blundon 1989).

Comparing the respiration data obtained in this study to that of previous workers, highlights possible differences between species of prawns. The mean respiration rate of settled kuruma prawns at 22°C obtained here was higher than the respiration rate of brown tiger prawns at 20°C of 22 µmol O_2 kg^{-1} min^{-1} (Dall 1986); the prawns studied here were not totally inactive in the respirometer, even though it was daylight. Therefore, the daylight shut-down of metabolism may be more pronounced in the latter species. When inactive brown tiger prawns are cooled to 15°C (Dall 1986), the respiration rate is lower than the respiration rate of kuruma prawns at 12°C, at a time when the contribution of activity is expected to be minimised. This probably occurs because brown tigers are less tolerant of low temperatures than kuruma prawns.

Published data for black tiger prawn, *P. monodon*, respiration also shows variation, with Kurmaly *et al* (1989) giving 55 µmol O_2 kg^{-1} min^{-1} at 15°C and Liao and Murai (1986) reporting 59 µmol O_2 kg^{-1} min^{-1} at 20°C. Therefore, differences between results obtained from other species may reflect differences in the circumstances of the experiment. This may have prevented the prawns from showing a "buried" level of respiration rate in the laboratory during daylight.

The european lobster, *Homarus gammarus* is prone to disturbance during exposure in air (Whiteley *et al* 1990). The fact that the metabolic rate of this species rises in response to disturbance in air indicates that the lobsters may not be cold enough to remove the affect of handling on activity. In practice, such intense cold is necessary only when transporting crustaceans for long periods out of water (one to three days in the case of kuruma prawns) since crustaceans need to be cooled only enough to get them to market alive.

Handling kuruma prawns in a semi-paralysed state, in which they are normally handled for export, produced no change in their respiration rate. Activity was apparently the only factor modifying the metabolic rate of the disturbed prawn. Assuming that the prawns have not been stressed during harvesting and are stored in well-aerated tanks, "cold anaesthesia" is of immediate benefit in keeping the prawns quiet while they are being packed into sawdust and, in the long term, these same temperatures keep the metabolic rate at a level that the prawn in air is easily able to sustain without incurring serious metabolic damage (section VII).

VI. PACKING PRAWNS IN SAWDUST

INTRODUCTION

Many species of aquatic crustaceans of commercial importance are transported successfully out of water, particularly crabs and lobsters (Whiteley *et al* 1990, Defur *et al* 1988, Vermeer 1987, Hill 1982). Prawns are not preadapted to surviving out of water so it is anticipated that storage temperature will be an even more crucial factor than it is in the survival of other live seafood. Other factors that may affect survival in air include moulting and reproduction and these have been considered in this study. Dehydration is undoubtedly a potential cause of death for prawns in air, but this problem can be eliminated by proper design of packaging materials. Therefore, it is probably not a major cause of mortality, particularly during the relatively short transport times involved.

"Dry" transport is cheaper and more convenient than wet transport though it has the weakness of relying upon a supply of sawdust, "wood wool" or similar material (see Appendix I). These materials are not always available (eg. Witham 1971) but it is hard to find a substitute that is as cheap and has similar properties. In addition to commercial constraints upon packaging materials, live prawns are currently transported using materials such as cardboard and sawdust. These substances have a "natural" image and pose no serious disposal problem. However, choice of sawdust is crucial because of the potential problem with disagreeable odour and taste. Fortunately, sawdust of the hoop pine (*Araucaria cunninghamii*) does not have an obvious "pine" odour and is readily available from saw mills in Queensland.

The aim of this section was to identify factors that influenced the survival of prawns in air, both with respect to intrinsic characteristics of the prawns (sex, moult stage) as well as extrinsic factors such as the type of packaging material and storage conditions.

METHODS

Storage temperature and survival in sawdust

As reported in section IV, the cartons of black tiger (*P. monodon*) and kuruma prawns (*P. japonicus*) were either stored on site in a refrigerator (12±2°C) or were transported with coolants in insulated boxes to the laboratory at Hamilton. For experiments in this section, cartons were stored in controlled temperature rooms at different temperatures. The boxes of prawns were stored for periods of up to 56 h and when the boxes were opened the prawns were allowed to warm to room temperature. A storage temperature experiment was conducted in late summer and late autumn to identify seasonal effects on temperature tolerance. Survival was measured in the same way as described in section IV.

Moult stage, sex and survival in sawdust

Living and dead prawns from a packing trial were sexed by examining the first swimmeret, (the first appendage on the "tail" or abdomen) and then moult staged

according to the scheme for the brown tiger prawn *P. esculentus* (Smith and Dall 1985) into five categories A,B,C,D_{0-1} and D_{2-4}. For the purposes of X^2 analysis of moult stage and survival, these categories were further pooled into stages A,B,C and D.

Different packing materials

The desired properties of the support media were that it was an odourless, non-toxic, and particulate material. Waste products such as sawdust and rice hulls met these criteria quite well, as long as they were not contaminated with insecticides. Hoop pine was chosen as the sawdust medium because of its lack of odour (i.e. this timber was also used for "butter boxes"). Rice hulls were obtained from CoPrice Feeds. Hoop pine sawdust was obtained from ACI Mallinson and sun-dried to a moisture content of about 14%. A standard mixture of 1:1 rice hull to sawdust (by volume) was used for most trials on black tiger prawns, except when investigating different support media. In the latter case, four media were used, rice hulls only, dry sawdust only, rice hull/dry sawdust (1:1 by volume) and wet sawdust (moisture 70% by weight). All media were stored in sealed waterproof bags in a cold room (2°C) prior to use.

Unmixed hoop pine sawdust was used for trials with kuruma prawns. Japanese sources sometimes give a specific temperature for storing the sawdust (eg. 5°C). We stored our sawdust at -20°C and brought it into the packing room as required. Dry sawdust had a lower heat capacity than the prawn so the temperature of the sawdust was irrelevant. The sawdust warmed up very quickly to the temperature of the prawns.

RESULTS

Sex and moult stage

Sorting before packaging should ideally remove stage A and B prawns (post-moult). The moult stage of living and dead prawns (ranging between C and D_{2-4}) was not significantly different from that expected from chance alone. There was no evidence that pre-moult kuruma prawns (D_{2-4}) died sooner than others. The sex ratio of dead prawns was not significantly different from that expected by chance alone.

Sawdust discolouration

A "black-spot"-like stain on sawdust around the mouth was associated with death. The proportion of surviving prawns that vomited stomach contents was not the same as the proportion of dead prawns that vomited (Fisher Exact test, $P<0.0001$), (Table VII). Living prawns were less likely to show evidence of blackened sawdust.

Packing media

Survival of black tiger prawns was independent of the 4 types of support media used.

Storage temperature

The survival of both black tiger prawns and kuruma prawns was significantly influenced by storage temperature.

Black tiger prawns packed at 12°C in late autumn showed a significant relationship between storage temperature and survival after 24h (Figure 7) ($P<0.001$).

Table VII. Vomiting and death in sawdust of kuruma prawns.

	vomit	not vomit
Alive	12	43
Dead	21	2

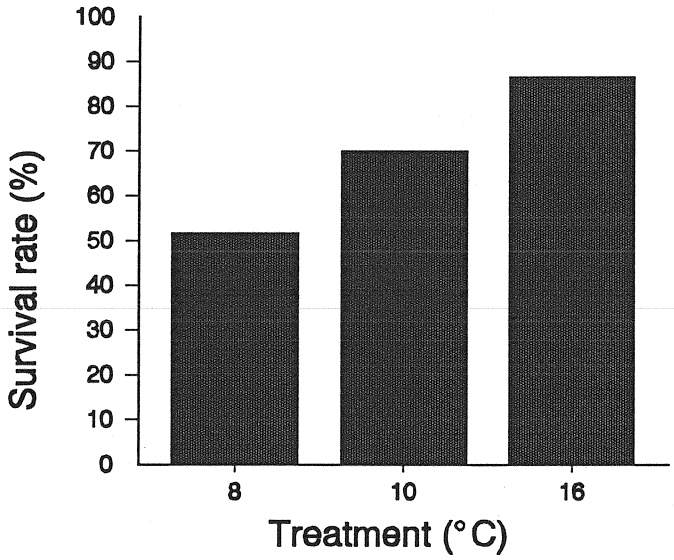

Figure 7. Survival rate of black tiger prawns packed in sawdust and stored at different temperatures for 24h.

Kuruma prawns died more rapidly at temperatures over 17°C than at cooler temperatures, but mortality also rose at 10°C.

When kuruma prawns were packed, in summer (average weekly pond temperatures of 26 to 27°C), and stored for 36h at different temperatures there was a dramatic fall in survival of prawns at 10°C (Figure 8).

Further observations on this low thermal limit were made in May 1991 on 6 kg of prawns extracted from a commercial shipment and stored at 10, 11.5 and 13°C. The average weekly pond temperatures were now about 10°C colder than in summer (i.e. 16 to 17°C). When opened after 34h there was only incidental mortality in the treatments, and a second batch opened after storage for 56h showed approximately 90% survival, and no significant difference in the number of living prawns at these temperatures.

This conflicting result at 10°C prompted a further trial in early June 1991, where prawns were again extracted from a commercial shipment and stored at 13.5, 9.8 and 5.6°C. When opened after storage for 30h, all of the prawns at 5.6°C were dead, while the prawns stored at higher temperatures were all alive. The average weekly pond temperature was now about 15°C.

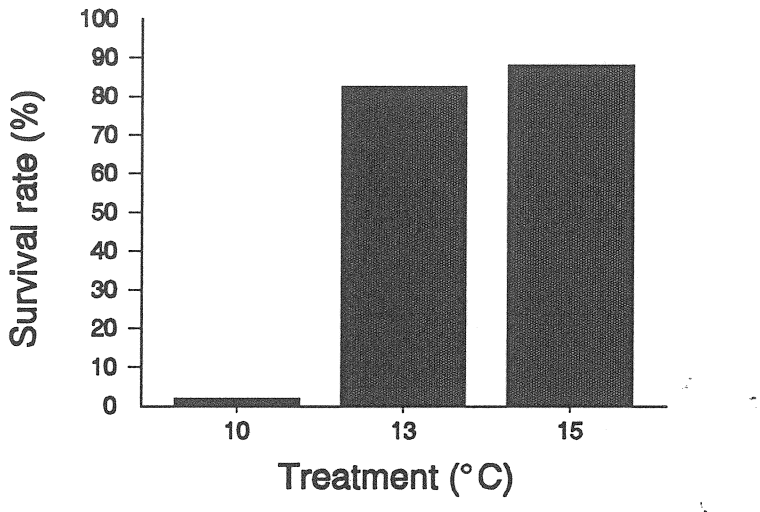

Figure 8. Survival (at 36h) of kuruma prawns stored at different temperatures in summer.

DISCUSSION

The survival of crustaceans in air can be approached from the responses of individual animals and of groups of animals. Factors intrinsic to the physiology of the animal such as injuries, moult condition and reproductive status may (because of effects on metabolism) be expected to influence survival in air. Mortality also rises at high temperature because the metabolic rate changes. This last point is considered in section VII.

Changing the support medium had no significant effect on the survival of black tiger prawns. It was also not necessary to wet the sawdust at all when packing prawns under

controlled conditions as long as the prawns were unable to crawl out of the sawdust into a "head space" in the inner carton. The prawns were probably wet enough already and any further wetting of the sawdust to ensure high humidity during actual exports must be balanced against the increase in gross weight of the package. A full understanding of the performance of different types of medium and different amounts of moisture during long periods of storage has not been reached yet and preliminary results using kuruma prawns (interrupted by the unexpectedly small crop) suggest that seasonal factors may come into play. There was however no problem with achieving "commercial" survival at auction, using existing flight schedules, so further work in this area was not considered urgent.

Of the intrinsic factors, after removing injured or moribund prawns, there was no significant effect of sex or moult stage (obviously by excluding stage A prawns) on the proportion of prawns alive when unpacked. Harvesting should not even be carried out if there is a high proportion of post-moult prawns (stage A) in the pond. These prawns die readily during harvest and are unacceptable for export. Simultaneous moulting can be brought about by judicious use of water exchanges, allowing harvesting to be synchronised around the moult-stage of the prawns. The metabolic rate of prawns and other crustaceans rises during pre-moult, but this change does not apparently cause problems with live export. This result is encouraging from a practical perspective because pre-moult prawns can only be reliably identified with the aid of a microscope- a labour intensive process even for a premium product like the kuruma prawn.

Individual prawns differ in their ability to tolerate emersion. Prawns become weak or moribund before death, and in general it seems that high levels of mortality indicate that the surviving prawns themselves will not be strong. Prior to dying, the prawns vomit up their stomach contents, as indicated by the presence of a "black-spot" stain around the mouth region (seen also when mud crabs die, Hill 1982). A mortality of about 5% persisted during these trials, at least partly due to poor grading prior to packing.

Kuruma prawns tolerated storage in air better than black tiger prawns, but with both species the temperature during storage was the variable that had the most dramatic effect upon survival. High levels of mortality resulted when kuruma prawns were stored at high (>15°C) or low temperatures (<10°C) but this affect was tempered by seasonal acclimatisation, particularly at the lower end of the range. Seasonal changes in the survival of the kuruma prawns at low temperatures are consistent with observations of their responses to cooling (section IV, Figure 5).

The survival rate of black tiger prawns improved during autumn (unpublished data). The extent that this was due to refinement of packing methods was unclear. At its best, the survival rate of black tigers in air was not as bad as we had been led to believe- and more than enough to transport broodstock using this method. The results obtained here suggested that this species survived in air best at higher temperatures (about 16°C) than are normally used to transport kuruma prawns (about 12°C)

Kuruma prawns will certainly last for more than 24h in air under controlled conditions in summer, but in this study it was not possible to perfect the export packaging for the prawns (section VIII) until well into autumn. However, the improved survival of the prawns with the approach of winter gives a marketing advantage when supplying

Australian prawns during the northern summer.

The improvement in survival of prawns in winter, and their ability to tolerate lower storage temperatures than in summer, points to a kind of acclimatisation taking place as the ponds cool below about 20°C. Two explanations can be put for the change- that the prawns become more cold tolerant in winter, or that they become more tolerant of transport stress. While both are possible, it is worth considering why the prawns survival at 10°C should improve. This may be a case of the animals "hibernating", that is, lowering their metabolic rate (reverse-acclimation) or a case of them "acclimatising", or raising their metabolic rate at this temperature.

The longevity of the kuruma prawn in air was such that attention to storage temperature alone was sufficient to ensure excellent survival within the required time span and no further intervention was necessary.

VII. METABOLISM OF PRAWNS IN SAWDUST

INTRODUCTION

When an aquatic animal is removed from the water it often experiences a dramatic reduction in its oxygen uptake rate, since its gills are adapted to operate in water, and not in air. The survival of aquatic crustacea in air is greatly improved by cooling the animals down (Whiteley and Taylor 1990, Defur *et al* 1988) so that their metabolic rate approaches this reduced level of oxygen consumption (Whiteley *et al* 1990). If the animal is unable to curtail its demand for oxygen then dramatic changes can occur in its metabolism. In order to understand these changes and the experiments conducted in this chapter, it is helpful to review the general metabolic processes occurring in a prawn (Figure 9).

Respiratory metabolism is a series of chemical reactions that generate metabolic "energy" in the form of adenosine triphosphate (ATP[1]). A major part of this process is the breakdown of carbohydrates or "sugars" to form carbon dioxide and water. However the complete breakdown or "oxidation" of sugars requires that oxygen be available to the respiring tissues. The metabolic process requiring oxygen is called the Kreb's cycle because it is a circular pathway that receives carbon compounds in the form of pyruvic acid at one point and then releases carbon dioxide and water at another. Most of the ATP synthesised from adenosine diphosphate (ADP), by respiration, arises as a result of reactions occurring in the Kreb's cycle.

In addition to the breakdown of sugars, prawns can oxidise other biological compounds, such as fats and proteins. The building blocks of proteins are amino acids that must have ammonia removed before the resulting molecules can enter various points of sugar metabolism. Ammonia excretion by prawns during storage in water was discussed in section III.

Pyruvate accumulates when oxygen is not available to "power" the Kreb's cycle and this compound is then converted to lactic acid. The accumulation of lactic acid is a classical symptom of extreme exercise (a level of metabolism exceeding the available supply of oxygen). Since ATP production falls in the absence of oxygen then it is possible for ATP consumption to outstrip its supply. Under extreme physiological stress then, ADP, and adenosine monophosphate (AMP), not only accumulate, but the latter molecule can decompose to form inosine monophosphate (IMP). This last molecule is a flavour enhancer in seafood (Komata 1990) and the postmortem breakdown of IMP to inosine and hypoxanthine is used as an index of the "freshness" of seafood (Ehira and Uchiyama 1986).

[1]Abbreviations: AEC adenylate energy charge, ΣAxP total pool of adenylate nucleotides, ATP adenosine triphosphate, ADP adenosine diphosphate, AMP adenosine monophosphate, IMP inosine monophosphate.

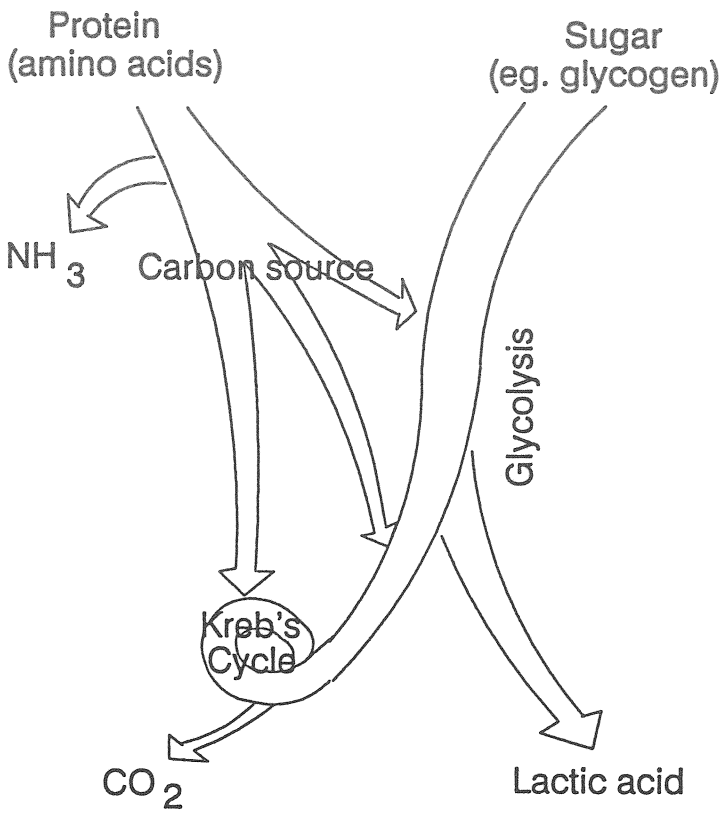

Figure 9. A general scheme of metabolism in a prawn in air. The amount of oxygen available determines the balance between production of carbon dioxide and lactic acid.

Furusho *et al* (1988) used lactate concentration and an "ATP ratio" ([ATP]/([ATP]+[ADP]+[AMP]+[IMP])) to describe changes in metabolism during emersion of live kuruma prawns, *P. japonicus*. However, this approach does not consider the possible importance of AMP deamination to form IMP. Regulation of the "adenylate energy charge" (Atkinson 1977: ([ATP]+½[ADP])/([ATP]+[ADP]+[AMP])) by AMP deamination (reducing the size of the total adenylate pool) has not been demonstrated when crustacea exercise, (Raffin and Thebault 1987) though the total adenylate pool (comprising ATP,ADP and AMP) diminishes in the shrimp *Crangon crangon* in response to pollution (Sylvestre and Le Gal 1987). Although the enzyme AMP deaminase occurs at very low levels in crustacea (Raffin *et al* 1988), IMP accumulates in the tissues of living and dead crustaceans (Suwetja *et al* 1989, Chen *et al* 1990, Nakamura and Ishikawa

1986). Thus, it seems that the deamination of AMP only occurs in crustaceans in response to unnatural levels of stress.

The metabolic changes in prawns in air at different temperatures are measured in this study to see if changes in lactate and IMP concentration can be used to explain why some prawn species survive longer in air. Contrary to expectations, anaerobic glycolysis did not dominate metabolism when the prawns were first removed from the water. Lactate and IMP only accumulated when the energy charge reached pathological levels.

METHODS

The farmed kuruma and black tiger prawns (*P. monodon*) were harvested and kept in 1000 L sea-water aquariums (22°C, Salinity 35 ppt) prior to experiments (section II). Experiments were conducted 12 to 16 h after harvest to allow the prawns to recover from handling.

Metabolism of black tiger prawns in sawdust

The metabolism of black tiger prawns was studied at 12°C so that comparisons could be made with the metabolism in air of (below) at that temperature. The prawns were cooled from 22 to 12°C at a rate of 3 C°/h, packed into cardboard boxes, 10 prawns per box, along with chilled dry sawdust and stored on rafts floating on the chilled water. The aquarium was covered with a sheet of polystyrene, 5 cm thick, and the air gap above the water aerated with an air stone in the tank to hold the air temperature between 11 and 12°C. The onset of fatigue was monitored by removing boxes after 12 and 24h and immediately freezing the abdomen of 8 to 10 live prawns, after washing them in sea-water.

Metabolism of kuruma prawns in sawdust at different temperatures

Prawns were cooled and packed into cardboard boxes as above. As this species was already known to survive well in air at 12°C, its metabolism at higher temperatures was studied in more detail because of the likelihood of commercial shipments of prawns warming during transport. Boxes of prawns were stored at 12, 17 and 22°C by floating three boxes on rafts in each of three thermostated aquariums as before.

Boxes in the 17°C treatment were removed after 12 and 24h and, each time, 10 prawns that showed signs of life were frozen immediately. Ten living prawns were also frozen after 24 h in air in the 12°C and 22°C treatments. Surplus prawns were packed in each treatment to ensure that an adequate sample of prawns would show signs of life (at least weak leg movements).

Freezing the abdominal muscle

Each black tiger prawn was quickly cut in half at the junction of carapace and abdomen and the abdomen was dropped into a bioassay bag, which was then sealed and dropped into a dry ice-acetone bath. ATP is reputed to be a highly labile molecule, (Sklar and McKee 1984), but no difficulty was found in freezing the abdomen of the black tiger prawn rapidly enough to prevent the ATP from degrading. However, with kuruma prawn, initial trials suggested that, when handled identically to black tigers, large amounts of AMP showed up in the nucleotide analysis. For this reason, whole kuruma prawns were

bagged and frozen immediately. Samples were stored at -20°C and the abdomen of whole prawns was cut off prior to extraction.

Extraction

Prawn abdominal muscle was homogenised with 50 ml of 0.6 mol/L perchloric acid for 1 min at high speed using a Waring blender. The homogenate was filtered and immediately neutralised to pH 6.5-6.8 with 1 mol/L potassium hydroxide. After standing at 0°C for 30min, the potassium perchlorate precipitate was removed by filtration. The samples were then frozen for subsequent analysis.

ATP and lactate assay

Nucleotide analysis was performed by an HPLC method similar to that reported by Ryder (1985), with a mobile phase of 0.06M K_2HPO_4 at pH 7 containing 50mL methanol per litre and a flow rate of 2mL min^{-1}. ATP, ADP, AMP, IMP, Inosine and hypoxanthine were assayed. The "adenylate energy charge" (AEC) of each prawn was calculated from nucleotide concentrations. L-Lactic acid concentration in the same muscle extract was measured using the HPLC method of Morawski (1984). A Waters *u*-Bondapak C_{18} column was used for this assay with a mobile phase of 0.5% $(NH_4)PO_4$ solution at pH 2.8, and detection was accomplished by a UV/visible detector set at 214nm.

RESULTS

Storing black tiger prawns in sawdust (12°C)

Significant changes occurred in the lactate and nucleotide concentrations when black tigers were stored at 12°C for 24h (Table VIII). The fall in AEC from 0.98 to 0.23 reflects the loss of ATP. A significant fall in the total concentration of adenylate nucleotides (ΣA*x*P) between 12 and 24 h was matched by the significant rise in IMP concentration so that there was no change in IMP+ΣA*x*P concentration during the experiment. There was no

Table VIII. Mean lactate and nucleotide concentrations (µmol/g, ±SD) and AEC of black tiger prawns stored at 12°C.

Dry storage (h)	0	12	24
n	8	10	10
LACTATE	4.34±1.34*a*	5.82±0.94*a*	12.73±2.79*b*
ATP	7.30±1.13*a*	4.70±1.94*b*	0.91±0.87*c*
ADP	0.08±0.10*a*	0.54±0.28*b*	1.01±0.55*c*
AMP	0.08±0.07*a*	2.10±1.34*b*	4.19±1.08*c*
ΣA*x*P	7.47±1.03*a*	7.33±1.08*a*	6.11±0.92*b*
AEC	0.98±0.02*a*	0.67±0.20*b*	0.23±0.16*c*
IMP	0.12±0.09*a*	0.46±0.38*a*	1.44±0.78*b*
IMP+ΣA*x*P	7.59±1.04*a*	7.80±0.90*a*	7.55±0.91*a*

In each row, different letters indicate significant differences (P<0.05).

significant change in lactate concentration during the first 12 h but lactate concentration rose significantly thereafter. The relationship between the AEC and the concentration of lactate is shown in Figure 10.

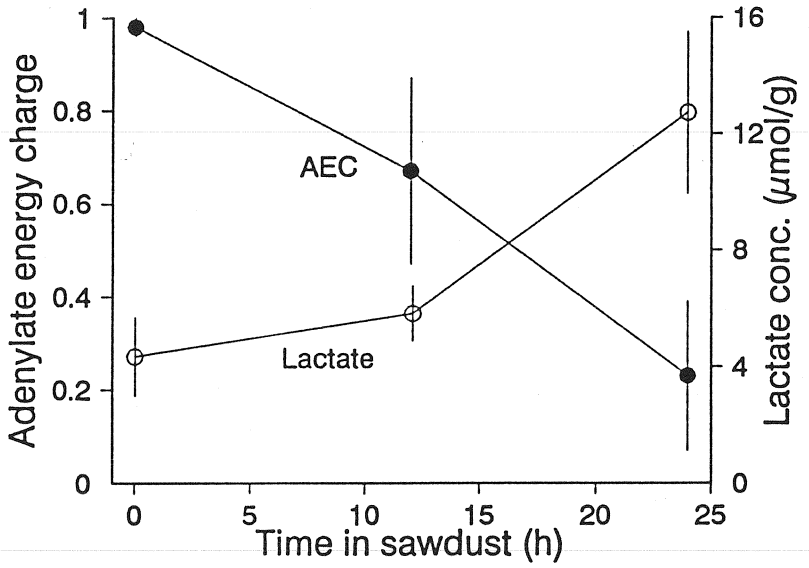

Figure 10. The role of the adenylate energy charge (AEC) in stimulating production of lactate in live black tiger prawns in sawdust at 12°C. Data given as mean±SD.

Storing kuruma prawns in sawdust at different temperatures

High temperature caused significant changes in the concentration of lactate and nucleotides in kuruma prawns stored in sawdust for 24h (Table IX) compared with the concentrations at the beginning of the experiment (0 h). The AEC remained high in prawns stored at 12°C, but was very low in prawns at 17°C and 22°C. There was no significant change in the lactate level and IMP was not detected when prawns were stored at 12°C, but lactate level and IMP concentration were both high in prawns stored at 17°C and 22°C (Table IX). Trace amounts of the IMP breakdown products, inosine and hypoxanthine, also appeared in the two warmest treatments.

A significant rise in total adenylate concentration (ΣA*x*P) occurred in prawns stored at 12°C for 24h but no change was seen in this parameter in prawns stored at 17 and 22°C. However, the calculation of ΣA*x*P in the two warmest treatments omits AMP that was degraded to IMP. The "corrected" total adenylate concentration, (IMP+ΣA*x*P) increased significantly in all treatments (Table IX).

Apart from the increase of the total adenylate concentration, the responses seen in kuruma prawns at 17°C were similar to those seen in black tiger prawns at 12°C, namely a rise in lactate and IMP concentration at low AEC values. Lactate concentration did not rise significantly during the first 12 h in air at 17°C, though small amounts of IMP had already appeared at this stage.

Table IX Mean lactate and nucleotide concentrations (µmol/g, ±SD) and AEC of kuruma prawns stored for 24h at different temperatures.

(°C)	12	12	17	17	22
(h)	0	24	12	24	24
n	10	10	10	10	10
LACTATE	3.65±0.45*a*	3.83±0.49*a*	4.11±0.38*a*	8.28±1.97*b*	8.45±3.63*b*
ATP	6.00±3.11*a*	10.25±3.10*b*	5.69±1.79*a*	0.09±0.19	0.00
ADP	0.00	0.00	0.14±0.43	0.47±0.20*a*	0.31±0.04*a*
AMP	0.18±0.46*a*	0.21±0.25*a*	1.55±1.06*b*	6.47±1.77*c*	5.94±1.10*c*
ΣA*x*P	6.18±1.06*a*	10.60±3.52*b*	7.37±0.90*a*	7.03±1.67*a*	6.25±1.11*a*
AEC	0.97±0.07*a*	0.98±0.02*a*	0.77±0.18*b*	0.10±0.16*c*	0.03±0.01*c*
IMP	0.04±0.11	0.00	0.17±0.21	3.48±1.28*a*	3.49±0.99*a*
IMP+ΣA*x*P	6.21±1.10*a*	10.60±3.52*b*	7.54±0.90*a*	10.51±1.45*b*	9.74±1.36*b*

In each row, different letters on means indicate significant differences. Means without letters were not included in tests.

DISCUSSION

The delayed accumulation of lactate and IMP when prawns were treated to simulated commercial transport shows that the prawns were not immediately stressed when removed from the water.

The concentration of lactate in the muscles of these prawns did not change during the first 12 h in air. Evidently, anaerobic glycolysis did not dominate the prawn's metabolism during this time. Lactate concentration in the blood (haemolymph) generally increases as soon as crustaceans are taken from the water (Johnson and Uglow 1985, Spicer *et al* 1990). The apparent discrepancy between this study and other studies is difficult to

explain, though the contradiction may arise because changes in blood lactate concentration may not reflect changes in lactate concentration in the tissues.

The prawns must be metabolising in such a way that lactate does not accumulate. One explanation for the delayed onset of anaerobiosis is that the prawns are initially able to consume adequate amounts of oxygen; a second is that another anaerobic pathway is initially used to produce ATP. Prawns do not tolerate low oxygen tensions in their environment so it is necessary to compare dry transport with the physiological changes that occur when a crustacean such as a prawn is stressed by exercise.

Crustaceans sometimes cannot obtain oxygen rapidly enough to meet their demand for energy during exercise (Gade 1983, England and Baldwyn 1983). The shrimp, *C. crangon* fuels its normal swimming behaviour aerobically but when flicking backwards dips into reserves of arginine phosphate to bolster its reserves of ATP (Onnen and Zebe 1983). The enzyme responsible for removing the phosphate group from arginine phosphate to produce ATP, arginine kinase, shows very high levels of activity in crustacean muscle (Gruschczyk and Kamp 1990) and fatigue sets in rapidly when arginine phosphate reserves fall too low (Onnen and Zebe 1983).

Arginine phosphate concentration was not measured in this study but it is reasonable to anticipate that these "phosphagen" reserves are available to the prawn when it is removed from the water. The concentration of arginine phosphate in the flesh of kuruma prawns was reported by Matsumoto *et al* (1991) as about 20 µmol/g, similar to concentrations reported from other crustaceans (Gade 1984, Gruschczyk and Kamp 1990).

Arginine phosphate concentration is sustained for several hours, at least partly by anaerobic glycolysis, when the crayfish *Orconectes limosis* is exposed to oxygen deficient water (Gade 1984). The rate that "phosphagen" is consumed when a prawn is in air will depend upon the contribution to ATP turnover arising from aerobic metabolism since lactate does not accumulate. If the arginine phosphate concentration decreases, then the asphyxiating prawn may react as if it were exercising in slow motion. "Phosphagen" may be consumed preferentially until fatigue occurs and at this point anaerobic glycolysis is rallied to attempt a "recovery".

Several enzymes in glycolysis are known to be sensitive to the amount of ATP present (Gade 1984, Storey 1988, England and Baldwyn 1985). This allows the rate of glycolysis to rise in times of peak demand. When oxygen supply is limiting, most, if not all, of this increase in glycolytic rate will be diverted into producing L-lactate.

The balance between ATP and AMP, an equilibrium that is an important factor in the regulation of cellular metabolism can be described by the adenylate energy charge (AEC) (Atkinson 1977). The AEC is similar to the "ATP ratio" of Furusho *et al* (1988) except that this latter index also includes IMP in the denominator. Introducing IMP into the sum confuses the issue as this molecule is actually a means of hiding AMP from the surrounding metabolism.

The AEC of 0.98 shown by resting penaeid prawns in this study is higher than values of

0.93-0.94 reported from other crustaceans (England and Baldwyn 1983, Gade 1984) and a review of historical data by Atkinson (1977) shows that the AEC is usually between 0.87 and 0.94. The higher AEC value is probably an artefact of the HPLC method, since small ADP peaks are easily masked by the ATP peak in a sample from a resting animal, making the denominator of the calculation artificially small. AEC remains high in kuruma prawns stored at 12°C for 24 h but, in this species stored at higher temperatures and in stored at 12°C, the AEC falls during storage, falling as low as 0.1-0.2 after 24 h. These levels are not normally observed in nature, since crustaceans exercised to exhaustion show AEC values of 0.5-0.7 (Onnen and Zebe 1983, Gade 1984, England and Baldwyn 1983) and it is arguable whether the prawns are even alive. Values of AEC below 0.5 are considered to signify irreversible physiological collapse (Sylvestre and Le Gal 1987), and it is significant that the rise in both lactate and IMP concentration is most pronounced when the AEC falls into this "pathological" region. High concentrations of lactate and IMP in muscle tissue are therefore evidence that a prawn of a given species has been out of water for too long at that storage temperature.

The fall in the concentration of ATP in crustacea is typically balanced by a rise in the concentration of AMP. However, in stressed black tiger prawns and kuruma prawns, AMP is deaminated to IMP. This reduction of the adenylate pool has a recognised function amongst vertebrates during exercise, since it removes the inhibitory effects of AMP on key enzymes and provides, via the "purine nucleotide cycle", a pathway for deaminating Kreb's cycle intermediates, (Greenaway 1991). However the activity of AMP deaminase in crustaceans and other invertebrates is much lower than that of vertebrates (Fijisawa and Yoshino 1987, Raffin and Thebault 1987, Suwetja *et al* 1989, Reddy and Rao 1990) and the very existence of AMP deaminase amongst these animals is sometimes questioned (e.g. Komata 1990).

Contrary to expectations, IMP accumulated in kuruma prawns while the total adenylate concentration was itself increasing, (this study, Furusho *et al* 1988). This finding is difficult to explain, as it contradicts the principle of ATP turnover, that adenylates are normally recycled rather than drawn from other sources (perhaps RNA metabolism). End-product inhibition usually prevents a "blow-out" of this kind in the adenylate pool, but prawns in air are certainly not metabolising normally. Studies of unstressed animals give the impression that the adenylate pool is immutable, (Vetter and Hodson 1982) however the pool is able to both expand and contract in response to physiological stress (Sylvestre and Le Gal 1987). Giesy *et al* (1981) found that the total adenylate pool in the shrimp *Palaemonetes paludosis* was not constant when the same population was sampled repetitively, apparently because of environmental influences (eg. storms).

The lack of a functioning purine nucleotide cycle explains why IMP does not accumulate in crustaceans under normal physiological conditions. For example, the total adenylate pool is constant when crustaceans exercise (Raffin and Thebault 1987) though Giesy *et al* (1981) reported that the total adenylate pool of *P. paludosis* decreased when the shrimps were exercised to exhaustion. These shrimps may have been stressed to a point beyond fatigue. While the AMP deaminase activity in crustacea is low, the accumulation of IMP in crustacea subjected to pollution, harvesting stress and post-mortem storage (Sylvestre and Le Gal 1987, Suwetja *et al* 1989, Furusho *et al* 1988, Ho *et al* 1986, Chen *et al* 1990,

Nakamura and Ishikawa 1986) suggest that the activity of the enzyme increases during stress.

IMP is a flavour enhancer associated with the "umami" taste of seafood (Komata 1990), making the flavour of live or fresh seafood not so much a "fresh" taste, as a morbid taste. As differences in the IMP concentration of live animals depend upon the breakdown of AMP before or during death then it seems to be possible for crustacea to be **too** fresh.

The effect of stress upon IMP concentration therefore has wide implications for seafood handling. The physiological stress associated with harvesting animals increases the IMP concentration (Fatima *et al* 1981), and processing methods applied to fresh product can also augment this parameter (Matsumoto *et al* 1991). Various methods of harvesting and handling seafood probably differ in the severity of the stress that they promote. A study is currently under way to examine the metabolism of brown tiger prawns *P. esculentus* subjected to trawling stress and the suitability of this species for live export.

To conclude, high concentrations of both L-lactate and IMP seen in prawns stored out of water can be used as an index of physiological stress, particularly when comparing different species of prawns. The kuruma prawn maintains its adenylate energy charge under conditions in which the black tiger prawn fatigues. The former species is able to reduce its metabolic rate in air to a level that it is comfortably able to sustain beyond the time limits required for commercial shipment from Australia to Japan.

VIII. EXPORTING LIVE PRAWNS TO JAPAN

INTRODUCTION

This was the first crop of kuruma prawns ever grown in Australia and MBPF had much to learn about the culture of this species. The crop took about 18 months to grow to maturity, and actually spawned in the ponds (Hansford and McGuren 1991). The prawns reached a marketable size at the end of 1990 and initial projections suggested that there would be a yield of about 6 tonnes of prawns. Plans were made to export these twice weekly during the first half of 1991.

The price of live prawns at auction in Japan is affected both their vigour and by their appearance. Mortality of over 5% is considered unacceptable. We achieved 100% survival at 24h in controlled conditions as early as November 1990 but it was not until the middle of March 1991, that a similar result was achieved in export shipments.

Controlling transit temperature was a major obstacle to successful export. The experiments in previous sections showed that temperatures of 12 to 15°C were required to maximise survival (sections VI and VII). Similar temperatures are required to inactivate the prawns before packing (Section IV). It was therefore necessary to design an export carton that would maintain this temperature during shipment.

The cargo hold of a plane is essentially uncontrolled within certain broad limits, so the obvious solution to temperature fluctuations was to insulate the carton. Coolants could then be used to keep the carton cooler than its surroundings, but this only worked if the environment was always warmer than the carton. Not all of the hold is heated. The lethal effects of extreme cold make it very risky to ship prawns in unheated sections.

In this section, the price and survival data from these exports are examined in terms of the temperature that the prawns experienced in transit. Once temperature control produced a survival rate reliably in excess of 95%, the price obtained for the prawns at auction improved considerably.

METHODS

Sample shipments of 10 to 20 kg of kuruma prawns were dispatched on direct flights to Narita or Osaka on a more or less regular basis for the first few months of 1991. Initial confusion amongst agents in Australia and Japan regarding customs clearance times in Japan led us to investigate using flights later in the day and selling prawns direct to wholesalers, thus side-stepping the auction system. However, it soon transpired that prawns could be sent to Narita, clear customs and be delivered to Tsukiji in time to be auctioned approximately 27h after leaving the farm.

Consignments of kuruma prawns were packed using the technique described in the accompanying manual (Appendix I). These shipments were then air-freighted to Tokyo (Narita) or Osaka in 10kg Nett outer cartons. Approximately 5 inner cartons from each shipment were held back from export and transferred to a cold room at IFIQ (14°C) to act as controls.

Disposable temperature recorders

In transit temperatures of cartons were monitored using 7 day disposable recorders (DTR Company) wrapped in newspaper and packed along with sawdust into dummy inner cartons. These were removed by our agents upon arrival at the markets and returned promptly by facsimile.

Survival and prices

Market reports were sent from Japan with the temperature charts and the results of the shipment (in terms of price and survival). The prices of other imported and domestic prawns auctioned alongside our own consignment were extracted from these reports for comparative purposes. As prawn size had an effect on price, this comparison was confined to prawns that were in the size range covered by our shipment.

RESULTS

Both ponds were drain harvested at the end of June 1991 after exporting only 1.2 tonne of prawns. Virtually no prawns remained, indicating that the stock estimates were overestimating the number of prawns present in the ponds.

Packaging trials

Shipments of prawns must arrive at the airport about an hour before the flight departs, to clear customs. The flights which were used during the project were JL 778 (Japan Airlines) and QF 69 (Qantas). A direct flight to Japan from Brisbane takes approximately 8 h. During the study, shipments arriving at Narita had to clear customs before 7pm, requiring that shipments arrive at the airport before 4 pm. The prawns are carried from Narita by truck to Tsukiji Market (Tokyo), where they waited overnight for the morning auction.

Average transit temperatures obtained from 5 shipments sent in February, March and April, using early carton designs, are shown in Figure 11. The first point in this figure reflects the initial temperature of the recorder (t=0) rather than the temperature of the cartons. All of these shipments were sent on indirect flights and showed extreme temperatures. One shipment was accidentally placed in a chiller truck and cooled down to 10°C at a time when this temperature was fatal (see section VI). Other shipments were too warm, and one was even off-loaded for a several hours at Guam (transit temperature of 20-23°C). Survival of these trials was 38-67%. The average weekly pond temperatures exceeded 20°C during these trials.

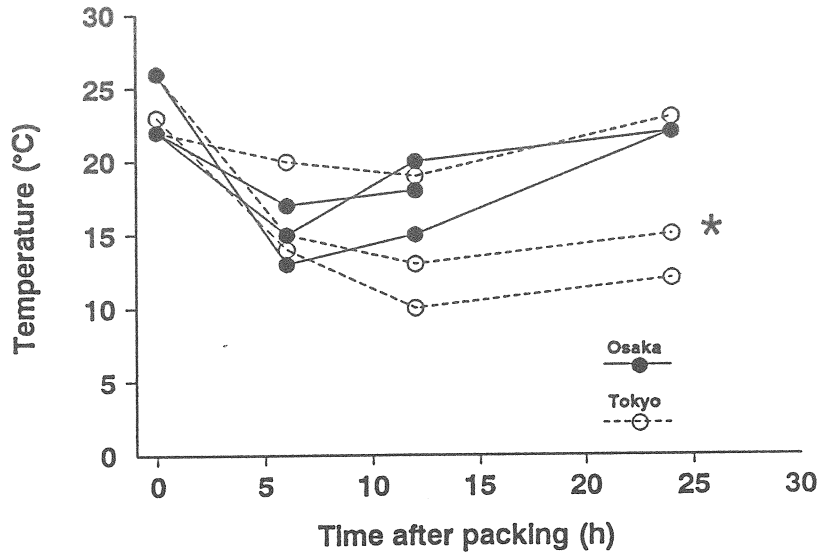

Figure 11. Transit temperatures for five preliminary shipments of live prawns using indirect flights, and for the first successful shipment (asterisk).

Export trials

Subsequent commercial trials (N=5) had none of the handling problems associated with the previous shipments. Only direct flights to Narita were used. The coolants were modified to prevent them from thawing too rapidly and a layer of polystyrene foam was added to the cartons to prevent excessive warming during transport. Details of the packaging materials and coolants are given in the manual (Appendix I, II and III). The new carton showed better characteristics, with an average temperature of 14±1.7°C after 24 h (Figure 12). Survival ranged from 89 to 97%. Average weekly pond temperatures were less than 20°C during these trials, reaching as low as 15°C in June 1991.

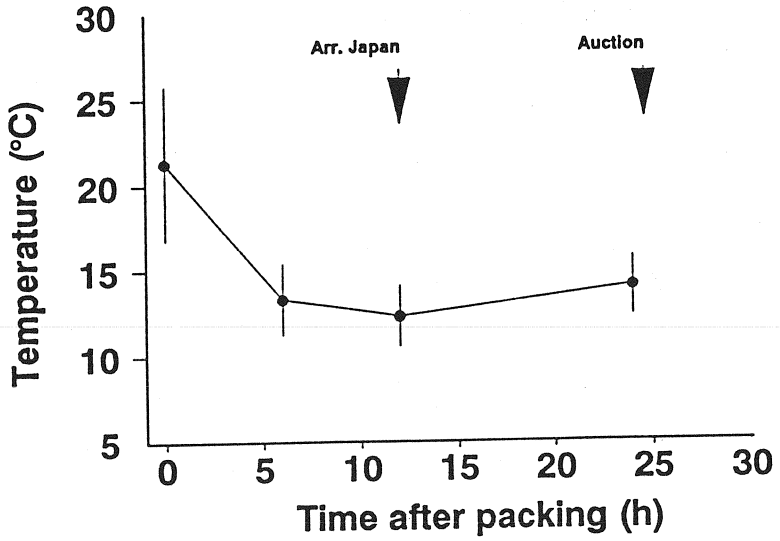

Figure 12. Transit temperatures of five export shipments using direct flights to Narita (mean±SD.).

Prices

The results of the export consignments following the first successful shipment of April 15th are given in Figure 13. The survival was 90% or better throughout this period but the price was initially depressed. The improvement in price is shown in relation to other prices in that size range in Figure 13. Prices returned for the first four shipments were similar to prices for other imported prawns. Thereafter, the price increased, entering the range covered by the domestic product. The average price for the last 6 shipments was about $65/kg.

DISCUSSION

The reliable survival obtained from the Australian product was reflected, eventually, in the price. Following the early packaging trials, survival rates in excess of 90% were achieved for almost all consignments in May and June of 1991 until all of the prawns had been harvested. The improved survival can be partly explained by the prawns acclimatising to low pond temperature in late autumn and winter. They survived out of water for much longer periods at this time of year and were more tolerant of low temperature (Section VI).

Judging by the rise in auction price, these prawns were evaluated by the market as being superior to other imported kuruma prawns and similar to domestically produced prawns of that size. The ranges of the two modes of the live prawn market are shown in Figure 13 rather than the average of the "kuruma ebi market" in order to emphasise that there remain

niches within the niche market. Lumping the entire throughput regardless of size, origin and quality into an average price is dubious statistics. What does the average price of "a motor car" mean to the manufacturers of BMW's? Obviously, if a glut of cheap prawns are dumped onto the market by other countries then the average price can fall regardless of what happens to the prices of the domestic prawns. The viability of this export industry therefore depends on the ability of the high quality mode of the market to resist the incursion of poor quality imports from other countries.

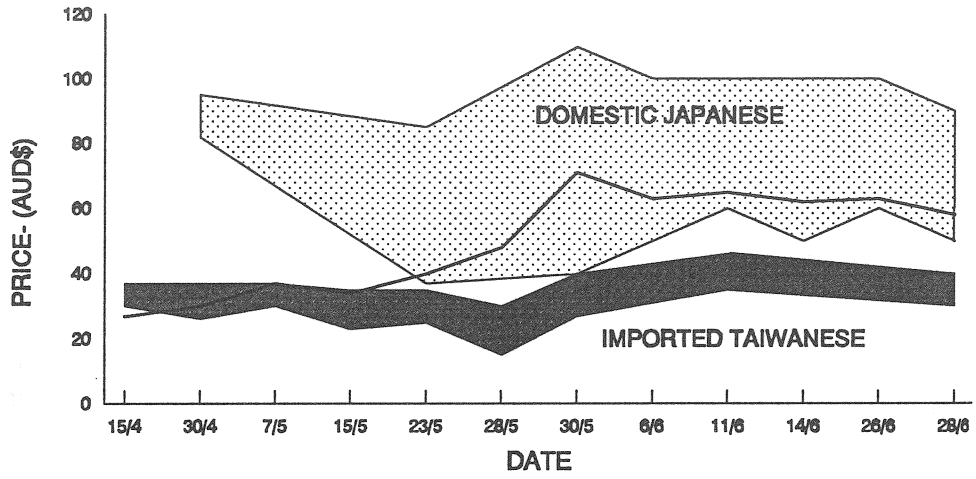

Figure 13. Change in price obtained for exports of Kuruma prawns from Australia (solid line) compared with prices for similarly sized imported and domestic prawns (shaded).

The handling problems associated with indirect flights led to use of these being abandoned. The control survival was consistently excellent. The poor survival obtained in the early trials can be blamed on transit problems. Further work needs to be carried out to confirm that survival of better than 90% is possible in February/March using direct flights and the packaging system adopted in later trials.

Quality in the live prawn market is not only determined by survival on the market floor. Colour and "muscularity" of the prawns is also important (Shigueno personal communication). "Muscularity" or fullness of the prawns appears to be a reflection of their ability to build up muscle tissue.

The movement of the Australian product into the domestic (i.e. Japanese) price range is a significant achievement considering that the crop had serious grow-out problems. It took 18 months for the prawns to reach marketable size and there were questions about the diet that they received. It is particularly important that the diet given to future crops be suitable

for the protein requirement of this species.

The colour apparently favoured in the market is sometimes said to be red, but some sources say black. The apparent contradiction reflects the fact that the colour of prawns is not fixed and depends to a great extent on diet. Some Japanese kurumas receive an expensive finishing diet with boosted levels of carotenoids, the building block of crustacean pigments (Latscha, 1990). The colour of the stripes can therefore be black, dark-red, reddish-brown, brown or grey.

We consistently received comments that our prawns were too dark in colour and were also told on more than one occasion to stop sending "soft" prawns. Moult staging showed that these allegedly "soft" prawns were in intermoult and pre-moult. The origins of these misgivings about the firmness of the shell may also be dietary.

The problem with colour was to some extent confused with differences in appearance of the Australian variety of *P. japonicus*. Contacts in Osaka noticed that these prawns were not identical to the locally grown variety (even ignoring the better colouration of the "typical" Japanese prawn).

One discrepancy seemed to be that the red-brown stripes do not reach as far down the side of the "head" or carapace as they did in the kuruma prawns, *P. japonicus*, of Japanese origin. Interestingly, Grey *et al* (1983) mention this trait as a characteristic of the striped prawn *P. canaliculatus* rather than *P. japonicus*.

The australian stocks may have arrived fortuitously, as a few individuals, in ballast water. Therefore, the limited gene pool would certainly explain their unusual appearance. On a recent visit to Tsukiji market, the authors noted that the head of the australian prawns was unremarkable: shipments of kuruma prawns from other sources vary both in colour and in the banding pattern on the head. The price achieved by australian prawns perhaps reflected the buyer's opinion of where our product belonged within the existing variation (Paterson 1991).

By concentrating on the red stripes, it is easy to forget that kuruma prawns also have white stripes. The contrast between the two colours is important, and we have received feedback that the australian prawns are not white enough in certain areas of the tail (Anton Kriz personnel communication). The explanation for the variation in colour banding is unknown though it may relate to diet or grow-out conditions (eg. water depth and ambient light levels).

Regardless of how the kuruma got into Queensland waters, our lack of knowledge about the local biology of this prawn is a potential threat. The future export of this species from Australia depends upon broodstock being available when required. Some attention should therefore be given to the management of the wild populations. Over-exploitation of the wild stocks, for example, by live export, could threaten the viability of the entire industry.

The viability of this industry also depends upon future price changes in the live prawn market as production of poor quality kuruma prawns by northern hemisphere exporters (particularly China) rises. Australian prawns must be readily distinguishable, because of

better quality and larger size from the other imports, but this will not amount to much if poor quality becomes acceptable.

The survival of live prawns was guaranteed here by controlling the temperatures that they experienced in transit to Japan. Measuring the temperatures of live prawns during real exports, backed up by laboratory studies of their survival under controlled conditions, helped to explain the survival data returned from the market floor. Ensuring high survival was obviously important but that was just the minimum requirement.

The quality of the prawns must be high enough to deserve the status and price of a premium product. Many buyers of live prawns are interested in the appearance of the product; attractively coloured and lacking injuries and defects. These requirements must be met by prawns originating from Australia. If this is done, Australian growers can then send kuruma prawns "out-of-season" to Japan and successfully mark out a place for their product in the live prawn market of that country.

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APPENDIX I: Kuruma prawn manual

Live Kuruma Prawn Handling Manual

Background

The techniques described in this manual result from extensive technological and physiological studies of several prawn species but especially the kuruma prawn *P. japonicus*. This research was undertaken by the International Food Institute of Queensland with funding from the Fishing Industry Research and Development Council and in close co-operation with the Moreton Bay Prawn Farm (Australasia) Pty Ltd.

This manual is divided into a number of sections. The first part discusses the theoretical basis of the method and what to do if something goes wrong. The remainder covers the practical steps involved from harvesting to dispatch of the consignment to market. A process flow chart and production check list are included in the appendices.

Introduction

The price obtained for live prawns in Japan depends on their quality, both in terms of appearance and liveliness when unpacked.

While the general appearance of the prawns is a consequence of diet and growout conditions, careful grading of harvested prawns is required to ensure that injured or weak prawns are not exported.

The prawns must be strong enough to withstand the preparation and dry transport stage, not only alive but also kicking. This level of activity, promoted to some degree by allowing the prawns to warm up at the market, is an indicator of their physiological condition ("health") and signifies to the buyer an ability to survive the remaining steps of the transport chain to the restaurant or sushi bar, and during subsequent holding in a display tank.

The condition of the prawns upon arrival is significantly effected by the temperatures they experience in transit. However, any problems during harvesting or prior handling will stress the prawns before they are even packed. While kuruma prawns are remarkably tough prawns this should not encourage a blase attitude toward their handling. Live prawns are a premium product and failure at any point in the process will not only kill prawns- it will tarnish the reputation of all prawns exported alive from Australia.

The method described here is proven to be able to obtain survivals of 95% or more at the time of sale at Tokyo's Tsukiji Fish Market and similar results are expected to follow from a serious application of this technique.

Theory of the Method

Kuruma prawns must be handled in such a way that any respiratory stress (eg. crowding in the net, exposure to air) is kept to a minimum. This is done by keeping the nets and holding tanks well oxygenated and by ensuring that the prawns do not remain in air while warm (>17°C) for any longer than is necessary for transfer to tanks.

Storage of the prawns is further aided by cooling the water in tanks by about 6°C below pond temperature (for example from 24°C to 18°C). This is done to reduce the activity and metabolic rate of the prawns and to encourage dissociation of toxic ammonia gas (NH_3) to the less toxic ammonium ion (NH_4^+). The principles behind the harvesting and storage of live kuruma prawns have been considered in detail elsewhere (Paterson and Goodrick 1991[2]) and the reader is referred to this source for further information.

The cooling step down from the storage temperature to the packing temperature works by further reducing the activity and metabolic rate of the prawns. The prawns become paralysed by the cold and lose their ability to sit upright after falling over. The temperature required to bring this about changes seasonally. A temperature of about 14 to 15°C is required to pack prawns in summer and early autumn, while 11 to 12°C or lower is required to render them torpid in late autumn and winter.

The idea of paralysing the prawns is to enable them to be conveniently handled and packed. In this state, the prawns come to rest on their sides with their appendages blushed a red colour and beat their swimmerets gently. Excessive cooling (<10°C) will block swimmeret activity, gill ventilation and heart rate and result in death.

The cooling rate itself is not critical. For convenience the prawns can be removed from the storage tank (between 16 and 18°C) and plunged straight into the chilling tank set 4-5 °C lower than the temperature of the storage tank (i.e. between 11 and 14°C). After allowing prawns to equilibrate for about 30min they can then be graded before packing.

The prawns will begin jumping erratically for a couple of minutes after being plunged into the colder tank and continue to do so for several minutes. The behaviour proceeds in a chain reaction and soon knocks all prawns over. At this point they are cold enough to pack. There is no evidence that holding prawns for as much as 2 hours after plunge cooling will effect their survival.

Alternatively, the storage tank can be cooled as much as 6C°/hour from the storage temperature to the packing temperature. The plunge cooling method has the advantage that it is faster and no time is lost waiting for the tank to reach packing temperature, or lost re-warming the tank to receive prawns from later harvests.

The chilled prawns are graded into various size categories and weighed (taking drip loss into account) in one kilogram lots for packing.

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[2] See section III of the report.

The object of the packing regime is to use sufficient sawdust or wood shavings to ensure that the prawns are supported in the media but not compressed nor able to crawl out of the sawdust. In a similar way, sufficient water spray is added to ensure the shavings or sawdust are moistened but not excessively wet. The top space in the carton must be filled with shavings or sawdust to prevent the prawns from crawling out of the sawdust and dehydrating.

The insulation and coolant design is used to maintain a lower temperature in the carton than the surrounding environment, hence the cartons should be shipped in heated airline holds and **never transhipped in chiller trucks (5°C)**. The heat capacity of the sawdust (removed from the freezer at -20°C) is such that the prawns themselves rapidly warm the contents of the box to their own temperature (11-15°C) and the coolants and insulation then combine to slow the rate of temperature rise within the carton. The temperature upon opening at the market should be little different from the packing temperature itself if the overall external temperature range is maintained at between 15°C and 20°C.

A small rise in temperature during the final period of storage is generally desirable. It allows the prawns to become more active when the carton is opened- a sure sign of quality. Healthy prawns jump about when unpacked, and when held in the hand, they flex their head upwards and extend their legs downwards. In weak prawns, the junction of the head and tail is limp and the legs are often pressed close to the body.

The prawns are auctioned after only 26 to 30h in sawdust but the kuruma prawn should last longer than 30h throughout the late Summer to Winter with survival approaching 60h at the end of Autumn. The longer survival time is necessary to ensure continuing survival further through the distribution chain to the end user.

Equipment and Materials

Harvest net. The type of net available for harvesting the crop may vary from farm to farm. Japanese farms use collapsible prawn traps (rather like a crab pot). These are not currently available in Australia so a "tunnel net" is described here. Any passive net system that does not cause harm to the prawns could be used. The harvest net is held in shape by "star pickets" and should be hoisted clear of the water by day so that it does not foul with foam wash from the aerator. The condition and position of the net should be noted regularly as prawns will not enter an "aged" or fouled net.

The net comprises a pair of wings and a tunnel leading to a net basin where the catch is accumulated. A paddlewheel or air-O-2 is sited between the wings and directs water flow into the tunnel mouth.

A jetty from the pond bank to the collecting basin is advisable for harvesting, as this aids transfer of the prawns to the transport tank.

A long floating boom can be used to concentrate the catch into a corner of the net basin prior to filling the prawn trays.

Prawn tray. The trays must float, be stackable and have a mesh base. A handle is provided at each end of the tray to make it easy to carry. The dimensions of the transport tank and storage tanks will determine the dimensions of the trays. The trays should be sized so that they fit comfortably into the transport tank, and lock on top of each other and do not move around when subjected to surging.

Transport tank. The transport tank should have a close fitting lid that can be locked in place and an air-release valve. The tank is filled completely during use to limit surging. An array of air stones is placed on the bottom of the tank and connected to either a cylinder of medical oxygen or to an air pump.

Storage/cooling tank. This tank contains aerated and circulated seawater. For cooling purposes the circulation is redirected through a cooling coil in an ice slurry under the control of a thermostat. Alternatively, the tank could be placed inside of a cold room and allowed to equilibrate to the room temperature (11 to 14°C). An array of air-stones in the bottom of the tank is required. Air supply via a compressor or vane pump can be used. The presence of programmable controllers and alarm thermostat allows the system to operate without constant supervision.

Packaging materials.

(a) Sawdust. The sawdust used is hoop pine, a low odour timber suitable for the transport of food products. The particle size characteristics of sawdust vary with the type of saw blade used. A circular saw produces an appropriate and consistent fragment size. Shavings are also suitable, and reduce the gross weight of the package because of the lower density. Band saws generally yield a fine sawdust, which is less desirable. **Sawdust that is mouldy or originating from contaminated or treated timber (eg. with insecticides such as copper or arsenic) should NEVER be used.** The sawdust should either be from kiln-dried timber or dried in the sun, both to reduce its heat capacity and to facilitate long term storage, then gathered into bails and stored in a freezer (which prevents entry of insects). Care should be taken to ensure that the sawdust is not contaminated from any source. Splinters can be removed from the sawdust by screening it.

(b) Cartons. Natural materials such as cardboard are favoured by the market, but only boxes of good quality should be used. Labelling on cartons must extend to contents summary as well as any agreed "trademark" adopted by users of this technique. It is not necessary in a wholesale market to use elaborately decorated cartons, which are more costly to use anyway. The dimensions of the 10kg outer carton and 1kg inner carton are included in appendix II. At the moment, most prawns arriving at Tsukiji arrive in 8kg outer cartons.

(c) Coolants tubes. Coolants are made using tubes of polyurethane tubing (5cm and 10cm flat width) which are cut into 27cm lengths and heat sealed at each end. The coolant gel was made using chlorinated tap water and a food gelling agent. The coolant tube is wrapped in a 27cm square sheet of 2mm polystyrene foam and a sheet of newspaper (appendix III) and taped or closed with a rubber band. 150ml coolants were placed between pairs of inner boxes as the outer carton was filled. Either 1 or 2 two larger coolant blocks were taped onto the underside of the polystyrene lid.

Production Checklist

Documentation

Export
Parks and Wildlife permits
Receival arrangements - faxes sent/confirmed

Airfreight bookings

bookings made

Staffing

Check staff numbers

Cooling

check pond temperature
select target temperature
check temperature sensor(s) - accuracy and location
check cooling system
check cooling program

Storage / Transport Tanks

Check all submerged fittings including air lines to avoid failure.
Fill tanks
check pump operation
check cooling
test aeration
test backup aeration
precool storage tank
fill transport tank
test transport tank aeration

Harvest

confirm staff available
Nets cleaned
Nets set
Monitor harvest rate

Transport

Check oxygen supply
Test oxygen regulator and cylinder
Backup cylinder available
Check transport vehicle
Fill tank and test
test air stone array

Processing Operations

Preparation

↓

Harvesting

↓

Transfer

↓

Storage

↓

First grading

↓

Cooling

↓

Second grading

↓

Weighing

↓

Packing

↓

Boxing

↓

Storage transfer

PREPARATION OF TANKS

Each tanks is precooled to the set temperature in time for the arrival of prawns from the harvest. Water from the storage tank is also used to fill the transport tank with chilled water. Plumbing for this filling option should be included in the design of all tanks. Take particular care that the thermostat in each tank is giving a true reading of the temperature. **Aerating the storage tank continually will ensure thorough mixing of the tank contents both before and after adding the prawns. Mixing is AN ABSOLUTE NECESSITY in tanks where the inlet and outlet are both on the bottom of the tank, (to avoid jamming the stacked trays) since the tank may only circulate the bottom water and stratify.**

Excessive cooling of a tank should be avoided as the prawns become too torpid and are difficult to grade for vigour. It is recommended that alarms be installed to alert staff to excessive low temperatures in the storage tanks before adding the prawns.

HARVESTING

The net should be set before dark and cleared every 15 to 30 minutes or semi-continuously. Depending upon the harvest rate and net size etc., the period may range from 15 minutes to 3 hours. Ideally, several nets should be used rather than relying upon a single net.

The harvesting rate will be greatly influenced by the stage of moult, the phase of the moon, its position in the sky as well as the pond water temperature. Prawns caught in the net must have an adequate supply of dissolved oxygen. A good flow of water through the net is recommended. If too many prawns are trapped in the net, then reduce the time that the net remains open prior to harvesting.

Prawns should not be exported within 3 days of moulting and should not be fed before harvesting.

The net area may be illuminated during the actual removal of prawns. The prawns are gently tipped in lots of about 10-15kg into the wooden tray. They will remain in this tray during the entire handling process until removed for size grading prior to packing.

TRANSFER

The holding/transfer tank on the transfer vehicle will be held between 6 and 8 °C below the pond temperature or approx 18 to 24 °C (depending upon time of year). The temperature decrement is not essential in late Autumn however a temperature reduction at this stage can reduce the total cooling duration. This tank will also be well aerated or oxygenated.

Turn on the oxygen or air just prior to placing prawns in the tank.

The prawns are towed in the water to the shore and then covered with a sheet such as a towel or piece of light carpet. This cover stops the prawns jumping from the tray while being carried to the transport tank. The covered trays are carried to the tank, and placed inside, ensuring that the period spent out of the water is as short as possible.

If any trays are already in the tank, the new tray must be fitted exactly on top of the tray that is already present. If this is not done, the trays will slide apart and liberate the prawns into the tank itself. Each subsequent tray pushes the stack of trays deeper into the tank until it is filled. New wooden trays may have to be soaked beforehand if they are too buoyant.

Confirm that the aeration is functioning at this stage. The lid is then clamped onto the tank, and it is driven carefully (to avoid water surging) back to the packing facilities.

STORAGE

The trays are taken from the transport tank to the primary holding tank. The trays are again, carefully fitted together as each is placed into the storage/cooling tank. Aeration, temperature and prawn condition should be monitored at this stage.

FIRST GRADING

Dead or injured prawns may be removed from the trays at this point. Sorting is achieved by temporarily turning off the aeration and methodically examining the contents of each tray for prawns that are obviously injured, discoloured or lying on their side.

COOLING

After transfer from the truck to the holding tank (which is at approximately 18°C) the prawns are examined and the tray contents evened up to approx. 8 to 10 kg/tray. After 30 to 120 mins in this first tank, the trays are covered and transferred to the second tank (at 14°C to 15°C in summer or 11 to 12°C in late autumn).

The trays should be left in the second tank for approximately 10 to 15 mins in this tank before size and quality grading commences. The prawns should not remain in the tank for more than 1 hour as a general rule.

Alternatively cooling may be performed from the first temperature (i.e 18°C) at a rate of about 3 to 4 C°/h until the prawns become torpid at approximately 11 to 14°C. During the latter stages of cooling, the prawns being sorted according to size and quality as described above.

SECOND GRADING

During this operation prawns are graded into 4 sizes which are very large (> 33g/ea), large (26g to 33g), medium (20g to 26g) and small (17g to 20g), however it may be more profitable to return these small prawns to the pond (depending on the market

circumstances). Any prawns which are soft or in any way damaged, weak or dead must be removed for disposal or returned to the pond via the primary cooling tank.

WEIGHING

Graded prawns are drained in tared baskets for at least 2 min and weighed to 1.12kg (allowing for the water on the prawns), immediately before placing in the sawdust tray for packing.

PACKING

A sheet of newspaper is firstly placed in the inner box and covered with a 5mm layer of chilled sawdust or shavings (14% to 18% moisture) at approx. 5°C to 10°C. Dry sawdust has a poor heat capacity and warms rapidly after removal from a freezer (-20°C)

The sawdust is sprayed lightly with cooled seawater at 14°C to 15°C . The prawns are finger packed in alternate head/tail configuration with care taken to avoid damage to the prawns. On the completion of each layer the prawns are sprayed lightly with water and sawdust is added to fill the spaces and create a minimum sawdust layer of approx 5mm between the prawn layers.

Note that the prawns should be moving their legs when picked up and the rear legs should be well spread. Prawns which feel soft or weak or have their legs folded close to the head should not be packed but checked for strength by gently caressing the legs in a forward direction. Rejected prawns should be put aside and replaced at the end of the pack to make up the correct weight.

After spraying the top layer of prawns the hollows are filled with sawdust or wood shavings. The paper is then folded gently on top and the box closed and taped. The contents number should be written in the appropriate position on the box and then the boxes stacked on the pallet or racks provided.

BOXING

After completion of the packaging operation the inner boxes are placed in cartons according to the number of prawns in each inner. i.e. All like counts are placed together as far as possible. The number of boxes of each count is marked on the outer packaging of each carton on the label.

The number of inners of each count is marked on the outer packaging of each carton on the label.

Special coolants as described earlier are placed between each layer of inner cartons which are stacked at right angles to the previous layer. Several coolants are placed in the top of the box depending on the in-transit time and the in-transit and storage temperatures.

Using coolants that melt quickly and cause excessive cooling and will result in high mortality caused by excessively low temperature at the earliest stages in-transit, and probably be ineffective in cooling the prawns at the critical stage after arrival in Japan (where it is spring-summer) resulting in a further increase in the mortality rate.

Once packed into the outer carton, a storage temperature of 15 to 20°C is recommended, however, slightly lower temperatures may be acceptable depending upon the time of year and the amount of coolants used.

STORAGE AND TRANSFER

The storage period at the prawn farm should be reasonably short. The product is transferred to the airport as a covered module preferably in an insulated van or truck, if available, as close as possible to the deadline but within safe limits for clearance and receival. The cartons at this stage should not be exposed to temperatures below 10°C or above 25°C for extended periods.

QUALITY ASSURANCE AND HAZARD ANALYSIS

The information provided here is a guide under which a live prawn processing and packaging operation can be based. once a system is established the individual operator must examine all critical points in the process. Operations that are of a critical nature must be identified along with the action that would be required to correct any problems arising. At the establishment stage, a program of "Total Quality Management" must be instituted.

APPENDIX II
Packaging specifications

OUTER CARTON (10 kg nett)

Internal carton dimensions (mm)
420 x 420 x 540

Internal insulating lining

Expanded polystyrene
(Individual sheet sizes, mm)

Base and inner top
420 x 420 x 20

Sides (all)
395 x 500 x 20

INNER CARTON (1 kg nett)

Internal carton dimensions (mm)
362 x 162 x 80

APPENDIX III
Coolant specifications

Coolants were made from lengths of polythene tubing filled with water. A food-grade gel was added to the water in the tube (to stop leaks in the event of puncture) and the ends of the polythene tube were then heat-sealed. The tubes were frozen and then wrapped in two layers of 2mm flexible polystyrene sheets and a sheet of newspaper.

SMALL COOLANT (150 ml)

This coolant is placed between a pair of 1 kg inner cartons in each layer within the outer carton.

Tube diameter.	50mm
Tube length.	280mm
Volume of liquid.	150ml
Styrene wrapper dimensions (mm)	2 x 300 x 250

LARGE COOLANT (450 ml)

Up to three of these coolants were taped onto the underside of the top sheet of expanded polystyrene foam (Appendix II), cooling the "head space" of the outer carton, to compensate for high external temperatures.

Tube diameter.	100mm
Tube length.	280mm
Volume of liquid.	450ml
Styrene wrapper dimensions (mm)	2 x 300 x 420