Maximising Revenue within the NT Mud Crab Fishery by Enhancing Post-Harvest Survival of Mud Crabs

Sue Poole, John Mayze
Paul Exley, Carl Paulo

Project No. 2003/240

December 2008

Queensland Government
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NT CRAB FISHERMEN'S ASSOCIATION

Australian Government
Fisheries Research and Development Corporation

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2003/240	**Maximising revenue within the Northern Territory mud crab fishery by enhancing post-harvest survival of mud crabs (*Scylla serrata*)**

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OBJECTIVES:

1. Analyse available industry data (including anecdotal information from commercial operators) for correlation between high mortality rates and specific environmental or conditional factors

2. Document current mortality data in specifically designed logs to capture all possible factors

3. Establish physiological factors and stress level indicators for harvested mud crabs

4. Determine stress contributed by current post-harvest practices

5. Advance strategies for through-chain product traceability to differentiate crabs

6. Develop specific cost-effective handling procedures which minimise stress to crabs

7. Update industry of results through participation in trials

8. If appropriate, prepare a submission for amendment of the NT mud crab fishery Code of Practice

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE

The supply chain for mud crabs in Australia relies solely on live product and the major outcome of this project was increased survival of mud crabs through the chain. This was achieved through identification of stress biomarkers that were used as tools to understand which handling steps along the chain impose the greatest stress to the crabs. With this information, alternative handling practices were developed to minimise stresses experienced and consequently improve survival rates.

Current immediate post-harvest handling methods, when undertaken in line with the Code of Practice for the Northern Territory Mud Crab Fishery, can be an effective method of holding crabs. However, working closely with industry stakeholders, we were able to demonstrate the benefits of additional alternative handling practices through the supply chain. Feedback from harvesters, wholesalers and the retail sector has indicated increased survival and improved vigor of mud crabs when the alternative handling methods have been employed. Industry has reported a 50% reduction in mortalities in the processor sector and a further 10% reduction at retail level. Mud crabs reach the consuming public in premium quality, raising public confidence and perception of the commercial operators.

The information from this research provides a sound basis for commercial decisions with respect to operational procedures. It has created an ability to supply distant markets, including export markets, with greater confidence. Sustained adoption of the results from this project will also result in improved market perception of mud crab quality, leading to greater market demand and increased revenue for the industry. Increased survival of the crabs within this fishery not only improves resource sustainability, but also improves public perception of commercial activities within the mud crab fishery. Greater resource sustainability has flow on effects for the recreational sector and the indigenous community.

Mud crabs are a high value commodity and are harvested in remote coastal locations in the northern half of Australia. The fishery has a low production and there is little opportunity to increase catch volume. The maximum revenue potential from live mud crabs must be realised for the industry to remain successful. Losses can be unacceptably high, varying between 4 and 10%, with up to 35% mortality in extreme situations. A 10% loss to the Northern Territory (NT) mud crab fishery represents 60-100 tonnes of crabs with a retail value of ~$2 million/year. The supply chain from harvest to market for mud crabs from the NT fishery can be up to 15 days. The physical demand on the crabs is extreme as they are transported and distributed in air. The market requirement is that the crabs arrive in a lively and vigorous condition.

This research evaluated the handling steps along the supply chain that impose greatest stress to the crabs and identified biochemical biomarkers that are useful as indicators of stress experienced by the crab. Once the major stress factors were known, alternative handling procedures that minimised the stress impact on the crabs were developed. A range of stress indicators were measured within the haemolymph of mud crabs including glucose, lactate, urate, ammonia, total protein, pH as well as the rate of ammonia excretion from the crabs when returned to water. These biomarkers were correlated to different levels of stress experienced by the crabs. Glucose was a useful indicator, with amount present in the haemolymph directly correlating with the level of stress imposed. The amount of lactate present

was strongly influenced by changes in the metabolism of the crabs, but unlike many other crustaceans, mud crabs do not suffer acidosis. Urate and ammonia levels in the blood did not correlate directly with stress in the crabs. However, the rate of ammonia excretion after the crabs were returned to water was strongly indicative of the stress experienced by the animals.

The live mud crab supply chain was evaluated to determine the handling steps that caused most stress to the crabs. The major causes of stress include emersion (holding crabs out of water), handling disturbance and temperature changes. As mud crabs are aquatic animals, emersion results in several changes: respiratory metabolic stress because the crab is unable to obtain sufficient oxygen, accumulation of ammonia which is toxic to the crab and dehydration of the animal. When mud crabs are emersed, but held quietly, undisturbed and in a moist environment, stress levels are low. Dehydration is a significant factor as the consequent water loss from the crabs reduces the total weight of a crab which has implications for the all sectors of the industry with respect to crab revenue return.

Mud crabs are handled frequently at different points during the supply chain. Each handling event involves physical movement of the crabs and often a degree of shock, with all such disturbances adding stress to the crabs. If carried out gently and with care, the physical disturbance of the daily checking of the crabs while stored only imposes temporary stress on the crabs, from which they recover quickly. Grading and loading/unloading for transport involves greater physical movement and was found to be stressful to the crabs. A particular stress factor was exposure of the crabs to breeze which caused very high stress levels and resulted in a high proportion of mortalities.

Holding crabs at an appropriate temperature and limiting temperature change is optimal for minimising stress. Sudden variations in temperatures within the supply chain are common and the damp hessian is not always effective in moderating these changes. In this study, mud crabs were held at temperatures between 10°C and 35°C for a two day period to establish what level of stress this caused the crabs. It was concluded that mud crabs best tolerate a temperature range between 25°C and 30°C. Temperatures outside this range impose an increasing amount of stress on the animals and temperatures in the low range (≤15°C) can result in extreme stress.

Knowledge of factors that cause the most stress to mud crabs allowed the development of alternative handling practices to minimise any adverse effects. Working directly with crab harvesters, wholesalers and retailers provided excellent opportunities to select best practices and perform industry trials to demonstrate the handling modifications which benefit the industry. The major recommendation from this study is the inclusion of a recovery step within the distribution chain for live mud crab. It is important to include a purge step of 2-3 hours where the crabs are returned to aerated water to allow excretion of accumulated ammonia. The crabs can then be held in fresh seawater tanks to fully recover.

Feedback from harvesters, wholesalers and the retail sector has indicated that adopting the alternative handling methods and inclusion of a recovery step has increased survival and improved vigor of mud crabs. The industry has reported a 50% reduction in mud crab mortality rate.

KEYWORDS:
mud crab, *Scylla serrata*, stress indicators, handling stress, survival, emersion, live storage, transport, mortality

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1 Background

The mud crab (*Scylla serrata*) is the basis of a highly valuable resource for commercial, recreational and indigenous interests in the Northern Territory. The commercial wild-harvest fishery alone provided a total landed value of over $10 million in 2000/2001. With catch volume dropping significantly in recent years, the sustainability of revenue from the resource relies heavily on optimising its use.

The mud crab fishery in the Northern Territory is based predominantly in remote locations often with harsh environmental conditions and limited, if any, infrastructure. The majority of crab fishers work from dinghies and semi-permanent land-based camps where the crabs are stored prior to transport to Darwin. Crabs are caught in pots which remain set 24 hours a day and crab fishers service the pots one or more times per day depending on the tides. Individual mud crabs are checked for legal size and shell hardness, with those that are berried, undersize or recently moulted returned to the water. Crabs are tied to restrict claw movement and minimise damage either at the point of capture or after return to base camp. The live crabs are held at camp under different storage conditions according to the operator, and then generally transported once per week to wholesalers in Darwin. The basic commercial principle for holding crabs is to store them in a dark, shady, moist state out of the water. Crabs are transported to Darwin by road in trucks, usually inside crates lined with a damp hessian bag. From the more remote crab fishing areas, the transport distance can be considerable. At the wholesale/market premises the crabs are repacked in waxed cardboard or foam boxes for shipment interstate or to export markets. Crabs are held at the marketing premises for varying lengths of time according to trans-shipment timing and arrangements.

In the NT, the mud crab resource is harvested and managed sustainably, from both an ecologically and an economic perspective. This has developed through a concerted effort from both NT Fisheries Management and Research Divisions, as well as a strong commitment from the industry itself. There is continuing commitment to the robustness of the fishery by the NT Fisheries Research and the FRDC, as evidenced by the recently funded abundance and habitat study of mud crab populations (Hay *et al.* 2005).

While the resource management of this fishery is a prime example in the field, profitability within the fishery as a whole may not be reaching its full potential due to high morbidity, at times, amongst post-harvest animals.

Post-harvest product mortality is an issue in all live-market fisheries, with the severity of losses and sustainability of resource issues dictating that causes and remedies be found. A notable example, and one of similar implication to the NT mud crab fishery, is the Western Australia rock lobster industry where post-harvest mortalities caused unpredictable and enormous loss of revenue. Research conducted by Innovative Food Technologies (IFT), QDPI&F (Paterson *et al.* 2001) concluded that mortality was directly due to post-harvest stress within the animal and physiological stress indicators were investigated to provide an index for survival predictability. Additionally, alternative handling procedures were developed to minimise stress to the lobsters. Adoption of these procedures has resulted in greater post-harvest survival of the lobsters in the market.

The contribution of stress to mortalities in the post-harvest chain within the spanner crab fishery in Queensland has also been studied at IFT. Investigations showed that

reducing handling processes immediately post-capture was the critical step in minimising stress to the animal (Paterson *et al.* 1994). The successful outcomes of this research has resulted in sustainable and profitable use of the spanner crab resource and ensured a robust live-market industry.

In a similar vein, it is considered the losses caused by mortalities within the mud crab fishery can be minimised through development of specific simple post-harvest handling techniques at various points in the harvest-to-market chain. In general, crustaceans are not well equipped physiologically to survive in air and excretion of carbon dioxide is the limiting factor when the animals are out of water. The accumulation of carbon dioxide causes a symptomatic condition of acidosis in the animal because carbon dioxide dissolves readily in the blood causing the pH of the blood to drop severely. This syndrome is considered to be a major cause of mortality when crustaceans are removed from water for extended periods (Vermeer 1987; Whiteley and Taylor 1992).

In contrast to other crab species, mud crabs are known to leave the water voluntarily under certain circumstances. Their metabolic systems are apparently more adaptable to 'breathing' in air. Compared to other crustaceans, the mud crab has relatively high oxygen consumption in air and is able to handle the correspondingly high rates of carbon dioxide accumulation, along with an ability to reverse the acidosis condition (Varley and Greenaway 1992). This imparts an enormous operational advantage in successful post-harvest handling of these animals.

Certain physical factors are likely to have a bearing on the survival rates of mud crabs. For example, it is noted by the industry that mortalities are greater when seasonal temperatures are high which has been illustrated in investigative work by (Gillespie and Burke 1992). These researchers noted that at high temperatures, dehydration was the major determining factor in mortality as evidenced by weight losses recorded at death. They also found that temperature stress itself affected survival when mud crabs were at a temperature of 32^{o}C, with such animals dying relatively quickly and often regurgitating a black fluid, taken to be an indicator of stress. With mud crabs from Queensland (Hill 1982), the optimum conditions for transporting the animals are a temperature range of 16^{o}-20^{o}C and in saturated humidity. Such conditions are rare during the wet season in the northern Australia.

The physiological condition, moult stage, of the crab at capture also contributes to mortality with post-moult crabs being most susceptible. To increase the post-harvest survival of mud crabs, the contribution of harvest, holding and transport stress to animal mortality needs to be fully understood.

This project sought to do this, along with establishing stress indicator levels in mud crab. In conjunction with industry, and building on the Code of Practice for the NT Mud Crab Fishery, the project sought to further develop appropriate, practical and cost-effective handling procedures to minimise animal stress. Successful outcomes will increase returns from the resource through increased crab survival during storage and allow greater access to more distant markets. A consistent supply and quality of product therefore will realise an increase in total marketable catch.

2 Need

The mud crab fishery in the northern half of Australia is a relatively low production fishery with a high market value of product. There is little opportunity to increase catch volume and retain a sustainable fishery, consequently full revenue potential from the fishery must be realised for the industry to remain successful.

The viability of the mud crab fishery depends solely on the live seafood market with dead or 'slow' crab unable to be sold. Currently, post-harvest mortalities of animals through the supply chain are limiting the sustainable use of the mud crab resource. Losses due to mortality can be unacceptably high, varying between 4-10% at the wholesale level dependent on season and transport delays. This accounts for a loss in excess of 60 tonnes of crab annually with a value of $1 million. In extreme circumstances due to operational breakdowns, there have reportedly been post-harvest mortality rates of up to 35%. Additionally, a further 10% is frequently lost at retail level. Such losses not only negate the viability of the vertical supply chain, but also confer perceptional lack of responsibility to the sustainable use of the resource by industry members.

High mortality rates in mud crab can be minimised through development of appropriate, practical and cost-effective post-handling procedures along the harvest-to-market supply chain. This project sought to address this need.

The NT Crab Fishermen's Association has indicated the urgency for this issue to be addressed for some years and the need for the work proposed is identified in the NT Strategic Plan for Fisheries Research and Development 2002 (Draft, section 5.2 Mud crabs, Fishery Resources - optimum utilisation). The project sought to build on the existing NT Mud Crab Fisherman's Code of Practice.

3 Objectives

- Analyse available industry data (including anecdotal information from commercial operators) for correlation between high mortality rates and specific environmental or conditional factors
- Document current mortality data in specifically designed logs to capture all possible factors
- Establish physiological factors and stress level indicators for harvested mud crabs
- Determine stress contributed by current post-harvest practices
- Advance strategies for through-chain product traceability to differentiate crabs
- Develop specific cost-effective handling procedures which minimise stress to crabs
- Update industry of our research findings through participation in trials
- If appropriate, prepare submission for amendment of the NT mud crab fishery Code of Practice

4 Methods

The following methods are the standard analytical techniques used in all trials undertaken in this project.

4.1 Sampling and preparation of mud crab haemolymph

A 22G x ¾" Terumo needle attached to a 3ml Terumo syringe was inserted at the synapse where the 3rd walking leg (from the front) joins the carapace. The leg joint must be extended to stretch out the membrane and reveal a white triangular marking. The needle was inserted 5-10mm into an interstitial cavity beneath the tip of the triangular marking on the membrane of the leg joint (Plate 4.1) and angled to follow an imaginary line to the apex of the belly flap. Haemolymph (1.5ml) was withdrawn slowly to avoid collapsing the cavity.

Plate 4.1. Insertion point and syringe positioning and angle of the needle required to extract mud crab haemolymph.

This sample was immediately dispensed into two 1.5ml graduated microcentrifuge tubes (QSP cat No.509-GRD-Q) one of which was held in crushed ice for further testing lactic acid (1.0ml sample). The second tube containing 0.5ml was used to test pH immediately before being placed on crushed ice for glucose, ammonia and uric acid analysis. A haemolymph sample was directly dispensed (1-2 drops) onto the handheld refractometer and measured immediately before clots formed.

All samples were held in microcentrifuge tubes sitting in crushed ice for a minimum of 15 minutes to allow the haemolymph to clot. At this point the clot was broken up using a 3mm stainless steel rod before being spun at 10,000 RPM for 10 minutes (Beckman Coulter Microfuge 18). It was then broken up again and spun a second time at 10,000RPM for 10 minutes. The separated liquid portion of the sample was then pipetted directly into assay tubes.

4.2 pH

The pH of freshly sampled haemolymph was measured immediately by part filling a 1.5ml graduated microcentrifuge tube (QSP cat No.509-GRD-Q) with 0.5ml of haemolymph directly from the sampling syringe. This was measured within 15 seconds using a TPS hand-held pH meter (WP80) fitted with an Ionode intermediate junction pH electrode (TPS part No. 121236) calibrated at pH 4 and pH 7. This shape electrode was used because it fits the profile of the microcentrifuge tube, excluding air and only requiring 0.5ml sample size. The probe was gently inserted into the microcentrifuge tube containing the haemolymph. This forces any trapped air out until the haemolymph rises to the top of the tube ensuring the potassium junction of the glass bulb is completely covered. Results were manually read from the display once the reading had stabilized. The pH probe was cleaned with distilled water and wiped dry after each sample.

4.3 Total protein (spectrophotometric method)

Total haemolymph protein was analysed by preparing a haemolymph sample as per 4.1. A haemolymph sample (0.02ml) was analysed using a Randox assay kit TP245 as per instructions (30 minutes incubation at 20-25°C, 30 second timed intervals between samples). Fixed absorbance was read at 546nm using a Unicam Helios Alpha spectrophotometer with a 1ml quartz cuvette. Samples were read alongside a sample blank and standard (provided with the kit) and calculations were made as per the Randox kit instructions. Results were expressed as mg/ml.

4.4 Total protein (Refractive Index, RI)

The RI protein of freshly sampled haemolymph was measured immediately by placing 1-2 drops (enough haemolymph to cover the prism without air bubbles) directly from the sample syringe onto the glass prism of the hand-held refractometer (Atago model SUR-NE calibrated with distilled water). The refractometer lid was then closed before the haemolymph clotted and held towards a fluorescent strip light to assist reading the internal scale accurately. Results were manually recorded from the refractive index scale in nD units. This method was similar to that reported by (Alexander and Ingram 1980).

4.5 Total protein (conversion of refractive index)

Haemolymph was sampled as per 4.1 and RI determined as per 4.4. This RI value was then converted using the equation below, calculated from the standard curve of RI plotted against total protein derived using a spectrophotometer (Figure 4.1). The method above can be performed more quickly than the spectrophotometer method with a result obtained within one minute, (Paterson *et al.* 2001).

(RI x 4568.3018) - 6104.407 = Total protein (mg/ml)

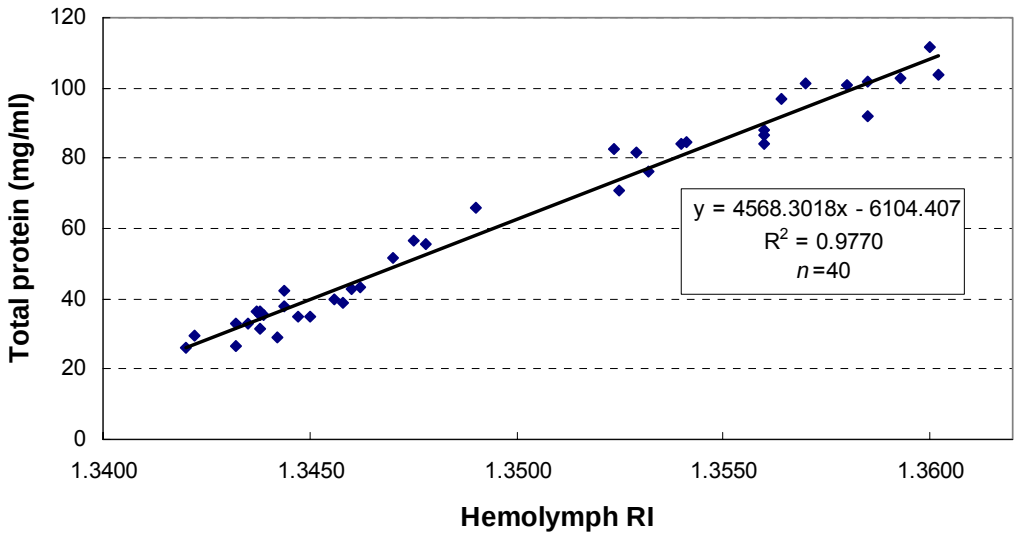

Figure 4.1 Standard curve of refractive index against total protein.

4.6 Glucose

Haemolymph glucose was analysed by preparing haemolymph sample as per 4.1. A haemolymph sample (10µl) was analysed using Randox assay kit GL2623 as per instructions, (semi-micro method, 25 minutes incubation at 22-25°C, 30 second timed intervals between samples). Fixed absorbance was read at 500nm using a Unicam Helios Alpha spectrophotometer with a 1ml quartz cuvette. Samples were read alongside a sample blank and standard (as provided with the kit) and calculations made as per Randox kit instructions. Results were expressed as mmol/L.

4.7 Ammonia

Haemolymph ammonia was analysed by preparing haemolymph sample as per 4.1. The haemolymph sample (0.1ml) was analysed using Randox assay kit AM 1054 (semi-micro procedure, 5 minutes incubation at 22-25°C, 30 second timed intervals between samples). Fixed absorbance was read at 340nm using a Unicam Helios Alpha spectrophotometer with a 1ml quartz cuvette. Glutamate dehydrogenase (0.01ml) was then added to all samples, standard (supplied with the kit) and blank before a further 5 minutes incubation at 22-25°C. Samples were read again at 340nm (30 second timed intervals) and differential calculations were made as per Randox kit instructions. Results were expressed as g/L.

4.8 Ammonia of purge water

Crabs were placed in a 200L tub containing 10L of water (sea water / fresh water depending on trial) for every 1kg of crab to be purged. An air stone provided aeration while crabs were in the tub. Water samples to be tested were taken by submersing a 100ml vial, completely filling with purge water and replacing the lid whilst under water (this excluded any air bubbles). Samples were placed on ice and tested immediately after the purge was complete.

A sample (0.1ml) of purge water was analysed for ammonia using Randox assay kit AM 1054 (semi-micro procedure, 5 minutes incubation at 22-25°C, 30 second timed intervals between samples). Fixed absorbance was read at 340nm using a Unicam Helios Alpha spectrophotometer with a 1ml quartz cuvette. Glutamate dehydrogenase (0.01ml) was then added to all samples, standard (supplied with the kit) and blank before a further 5 minutes incubation at 22-25°C. Samples were read again at 340nm (30 second timed intervals) and differential calculations made as per Randox kit instructions. Results were expressed as g/L.

4.9 Uric acid

Haemolymph uric acid was analysed by preparing haemolymph sample as per 4.1. A haemolymph sample (20µl) was analysed using Randox assay kit UA 230 as per instructions (15 minutes incubation at 22-25°C, 30 second timed intervals between samples). Fixed absorbance was read at 520nm using a Unicam Helios Alpha spectrophotometer with a 1ml quartz cuvette. Samples were read alongside a sample blank and standard (provided with the kit) and calculations were made as per Randox kit instructions for serum or plasma. Results were expressed as µmol/L.

4.10 Temperature logging

Temperature logging was tested using ibutton thermocron temperature loggers. Temperature range of the loggers is -40 to 85°C with a resolution +/- 0.5°C. Accuracy was tested against a “NATA” certified standard thermometer. These waterproof loggers were placed in direct contact with the crabs during transit and storage to assess temperature conditions. Temperature data was retrieved using USB reading fobs with eTemperature V5.10 software (onsolution.com.au).

4.11 Humidity logging

Humidity logging was tested using Tinytag ultra TGU-1500 temperature/humidity loggers range 0 – 95%/ -30°C – 50°C. Testing was carried out by placing a logger amongst the stored crab under the layer of hessian. The logger was placed within an open ended piece of PVC tube to protect from damage. Data was retrieved using a communication port adapter with Gemini GLM software v2.8 (www.hdl.com.au).

4.12 Lactic acid

Lactic acid was measured via High Performance Liquid Chromatography (HPLC) as per (Marsili *et al.* 1981) The extraction method was modified for the analysis of mud crab haemolymph by taking 0.5g of haemolymph and vortexing in a 2ml microcentrifuge tube (QSP cat No.508-GRD) for 30 seconds. The sample was centrifuged at 5,000RPM for 10 minutes (Beckman Coulter Microfuge 18). The supernatant was transferred to the HPLC vial for analysis.

5 Results and Discussion

5.1 Harvesting and handling through the live mud crab distribution chain

Project Objective

Analyse available industry data (including anecdotal information from commercial operators) for correlation between high mortality rates and specific environmental or conditional factors.

5.1.1 Harvesting/handling operations

Mud crabs captured from Northern Territory (NT) waters are all harvested and handled in a similar manner. Capture is by baited pot from estuarine mangrove habitats in isolated locations around the NT coast. The major difference from one harvest location to another is the degree of geographical isolation. Harvest location necessarily dictates the length of time the crabs are out of water (emersed) as well as transport conditions and duration before reaching Darwin for pack-out to market.

Crab pots are checked by use of a dinghy and cleared as tides permit according to location. Most commonly, this is once per day since there is a 2 to 7 metre tide change twice per day depending on locations. Once taken from the pot, the crabs' claws are usually tied and the crabs retained in the dinghy until all pots have been serviced. Commonly this takes around 2 hours. In the dinghy, the crabs are stored in a fish crate usually covered with a thick dampened hessian sack. There are different methods of tying the claws, but most operators tie as shown in Plate 5.1. This method is chosen as the mouthparts of the animal are still free and, should it accidentally be lost overboard, the animal may still be able to feed adequately to survive.

Plate 5.1. Mud crab showing claws tied.

After clearing all pots, harvesters return to camp and crabs are sorted and stored in plastic prawn crates (40kg size) and again covered with damp heavy hessian sacking (Plate 5.2). The sacking is carefully folded interlocked to retain maximum moisture for the crabs and to exclude flies, which cause a major quality problem when crabs become 'riced' (fly larvae contamination).

Plate 5.2. Mud crab stored at harvest camp.

Crab crates are stored in open-air in a damp cool position under shade, in the simplest form this is under a tree or some form of constructed shade cover. Other camps have a purpose-built shade cloth enclosure and camps at the Wearyan River and the mouth of the M^{c}Arthur River had a dedicated holding shed, one of which contained an overhead sprinkler system for spraying the crabs. The sacking is checked regularly for dampness. Information supplied from harvesters advised that during the 'dry' season (winter months: May-September) the sacking was not sprayed with water in the late afternoon as evaporation causes too low a temperature for the crabs overnight. Temperature and moisture are recognised by the harvesters as highly significant factors in holding mud crab emersed for extended periods.

5.1.2 Transport from harvest location to wholesaler

Two geographically extreme harvest locations studied in detail as the handling and transport chains for these are inherently very different. Most harvest locations fall into similar categories as the two selected (Plate 5.3):

Adelaide River – similar to Bynoe Harbour and the Finniss River
Wearyan River – similar to M^{c}Arthur River, Roper River and Blue Mud Bay

Plate 5.3. Typical mud crab locations.

Adelaide River: Crabs are held at camp for up to 6 days and taken to Darwin once a week on a closed truck. The transport time is about 1.5-2 hours duration on smooth track and road, apart from a short 5km section immediately out from the campsite that can be quite rough and boggy after rain. A rough ride is likely to add trauma for the crabs and raise stress levels. Crabs may be taken in more frequently depending on catch volumes.

Wearyan River: Crabs are held for up to 7 days, depending when captured. Once a week the crates are loaded onto an open four wheel drive vehicle and transported out to Borroloola, about 2 hours distant. This transport section includes demanding four wheel driving for the first hour out of camp, followed by corrugated roads into Borroloola. At Borroloola, the crab crates are transferred to a cool room (~16^{o}C). Time of storage at this temperature varies according to truck pick-up time co-ordination (and weather). From Borroloola to Darwin the crab crates are transported in a refrigerated truck that also does a crab pick up from King Ash Bay on the McArthur River about 1 hour north-east of Borroloola (the collection point for crab harvested from the mouth of the M^{c}Arthur River.). Time from Borroloola to Darwin is around 15 hours and is all basically smooth road. Transport is usually timed to arrive in Darwin in the early hours of a Tuesday morning to allow for pack-out to meet appropriate flights to markets. On arrival in Darwin (often 0100-0400), crabs are usually left in the truck with refrigeration switched off. Crabs remain in the truck until ~0600 when crates are transferred inside the air-conditioned facility (~22^{o}C) for grading and pack-out prior to transport to market.

5.1.3 Pack-out to live market

At the wholesaler/pack-out facility, crabs are first lightly rinsed with town supply water to remove mud and debris. This also helps rehydrate the crabs which can suffer desiccation in the refrigerated air during the trip from Borroloola. The condition of the crabs is then assessed by experienced graders (Plate 5.4) and categorised into the following grades (Table 5.1).

Plate 5.4. Grading mud crabs in Darwin.

Table 5.1. Commercial grades used for mud crabs.

Grade	Description
Grade A or B	lively/robust – often according to fullness (of meat)
One claw	lively crab but missing a claw through damage
Slow	judged by eye and antennae movement and force resistance exhibited from claws and legs upon handling
CUC	commercially unsuitable crab (soft-shell, little meat present) illegal to sell
Discard	Dead or diseased

Crabs are packed into waxed cardboard cartons which have small ventilation holes at the top of the carton. They are packed bottom end down with eyes and gills uppermost (Plate 5.5). Depending on crab size, there may be two layers within a carton and these are separated by clean paper or cardboard. Once packed, cartons are held briefly within the chilled facility ready for transport to the airport by truck or van (less than a 30 minute trip on sealed roads).

Plate 5.5. Mud crabs packed out in cartons for air-transport to market.

The mud crabs are flown out to interstate markets and may be transferred for further flights according to final a destination market. For the crabs retained in our study, cartons were flown direct from Darwin to Brisbane where they were collected and held briefly in the processing plant at 22^{o}C before assessment.

5.1.4 Importance of temperature during storage and transport stages

This project has found that change in temperature is one of the most important factors that can induce stress in mud crabs. Throughout harvest, holding and transport to the pack out phase, mud crabs are subjected to large fluctuations in temperature. For example, during the 'dry' season, diurnal temperature differences are large and can range from 30^{o}-32^{o}C to as low as 13^{o}-16^{o}C overnight.

It was noted during detailed discussions with mud crab harvesters that they were highly aware of the importance of temperature with respect to the crabs' well-being.

This was evidenced by their careful attention to keeping the crabs damp and cool during the holding period of crabs at camps prior to on-shipment. However, while aware it was important, harvesters expressed lack of knowledge of how the crabs temperature changed with ambient patterns and degree of moisture retained in the hessian etc. Hence, it was of value to monitor crab temperatures during both storage at camp and through transport stages.

Mud crabs are often at elevated temperature at the point of harvest due to necessary fishing placement of pots in near-shore waters which are subject to large tidal changes. This dictates that crabs are frequently sitting in very shallow waters at time of clearance and may have been for an hour or two. Little can be done to overcome this circumstance. Once at base camp however, the current holding method is effective in decreasing the crab temperature and holding it reasonably constant, despite fluctuations in ambient temperatures (Figure 5.1).

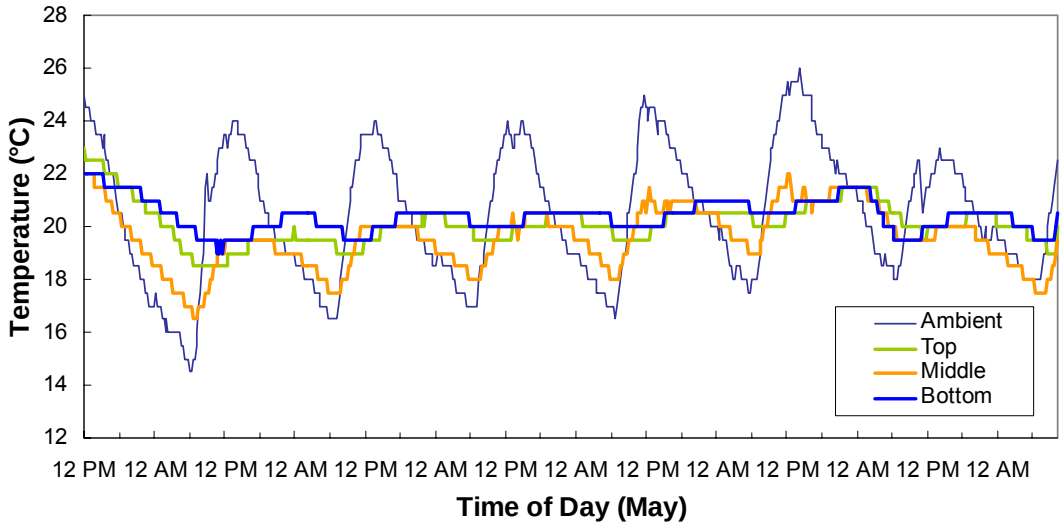

Figure 5.1. Temperature of mud crabs held in hessian and crates at camp.

5.1.4.1 Adelaide River

Temperatures of mud crabs logged during storage at Adelaide River are present in Figure 5.2. These crabs were stored in lug boxes, lined with damp hessian, in open air on the ground under tree shade. Temperature loggers were placed externally, and within the crate amongst the crabs inside the hessian at positions of bottom, middle and top of crab load.

The ambient temperature (blue line, Figure 5.2) was recorded with a logger attached to the outside of the crate so as to record the temperature of the air immediately surrounding the crate. Note that the temperature range during these late September days ranged from 23°C (early one morning) to 40°C (midday temperature), a differential of 17°C. For each 24 hour period the temperature differential, night to daytime, was at least 10°C. It is also of note that when onboard the dinghy immediately after capture (indicated on graph, “boat”, Figure 5.2), crabs are subjected to very high temperatures, 30-35°C. However, once back on land and crated within damp hessian, the mud crab temperature held fairly steadily between 23°C and 28°C regardless of the ambient temperature.

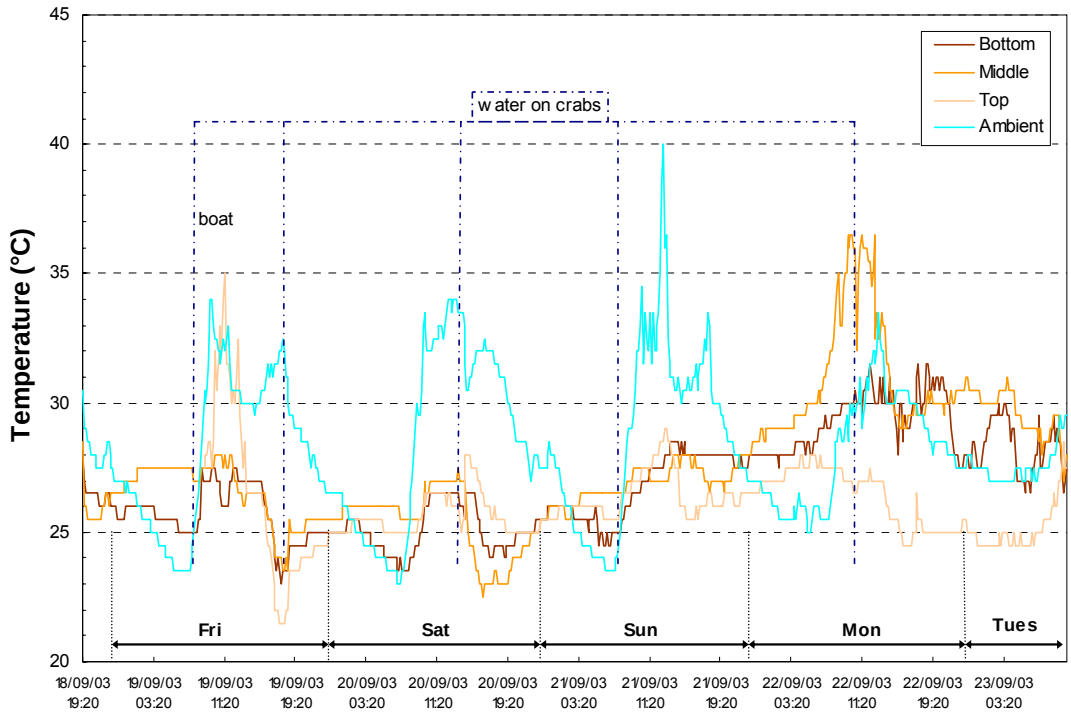

Figure 5.2. Logged temperatures of crabs held at Adelaide River in September.

Mud crabs are known to be nocturnally active and yet the logged data does not evidence greater metabolic activity during night hours (through increased temperature). This suggests that the crabs are already very quiescent after a few hours emersion.

Figure 5.2 shows that crab temperature varies according to the position of the crab within the crate as identified by temperature logged at the top, middle or bottom of the crates (data is averaged from several loggers in each position). In general, crabs in all positions follow a similar temperature change trend, with a few anomalies. Animals positioned at the top of the crate appear to be more affected by external temperature fluctuations. This could be expected as these crabs would have greatest contact to ambient and are closest to evaporation effects of the hessian sacking. The temperature of crabs positioned at the bottom of the crate appears to change in response to ground temperature, suggesting that it may be a useful practice to spray water on the ground surrounding the crates as well as the sacking covering the crabs or have the crates raised to allow air flow underneath.

The dotted dark blue lines in Figure 5.2 indicate the times when water was sprayed onto the hessian and crates. This occurred at varying times of the day which is commercial reality. The temperature of the crabs responded variously to water spray and the response seem related to time of day and the ambient temperature when they were sprayed. For example, when sprayed early afternoon when the ambient temperature was high, all crab temperatures dropped immediately in response, likely due to immediate evaporation effects. When sprayed late afternoon as ambient was dropping, the effect was not observed. Similarly, when crabs were sprayed in the morning, as ambient temperature was rising quickly, it appears that any cooling effect is over-ridden by the climb in external temperature. This is a simple handling practice that needs to be considered to determine the most effective time and frequency of spraying the crab.

Figure 5.2 also illustrates an anomaly happening with mid- and bottom-logged crab temperatures from Sunday midnight through Monday (data is expanded in Figure 5.3), where crabs in these positions have temperatures outside the general trend and appear very much hotter than ambient. For the same period, those crabs at the top of the crate seem to follow the usual trend (water spraying produces temperature reduction).

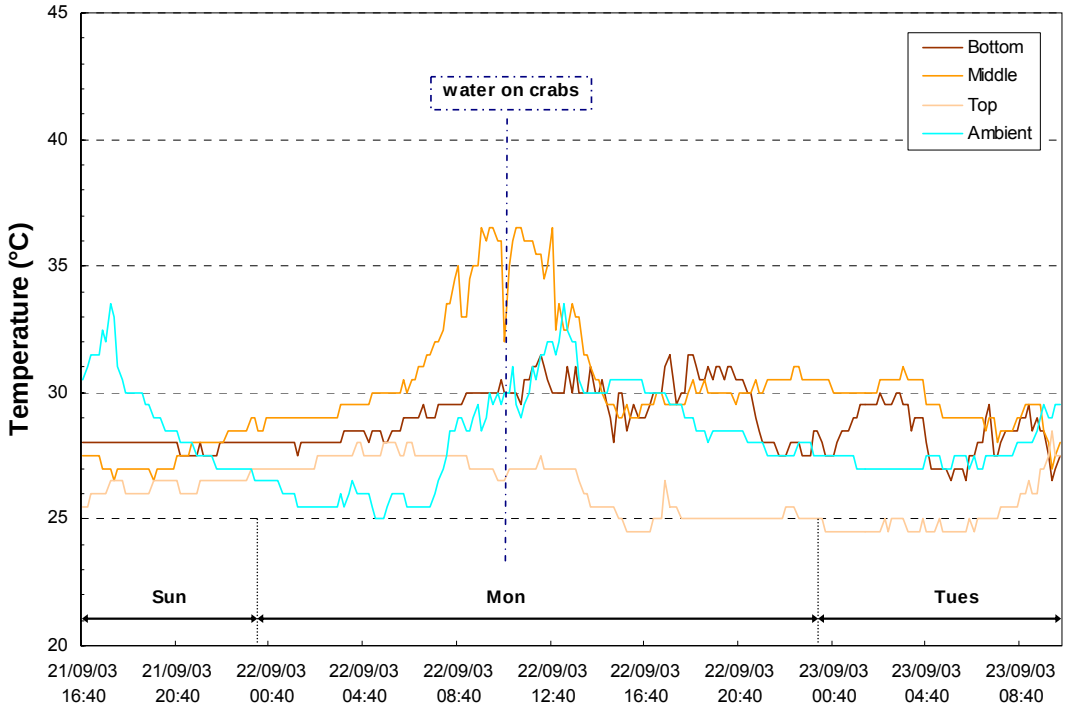

Figure 5.3. Temperatures of crab logged between Sunday and Tuesday.

From around midnight Sunday through to midday Monday the temperature of crabs positioned in the middle of the crate increases strongly. The crabs at the bottom of the crate show a similar trend but not as intense and a little delayed compared to the mid-positioned crabs. The delay in reaction may again be due to the effects of ground temperature. In contrast, the temperature of the top-positioned crab follows the 'normal' fluctuation of between 24°C and 27°C. The large increase in mid-crab temperatures is in contrast to the external temperature and begins to increase well before the day time temperature climbs. It is difficult to determine what is happening with these middle and bottom crabs from the limited data here.

However, there are a couple of suggested causes. One, the increased temperature may be a result of dehydration. The times when the crabs were re-moistened with spray varied according to camp activity demands. The time periods between spraying over the holding period were: 9 hours after harvest; then 19; 17 and 28 hours. The longest time between sprays is the last period within which the crab temperature started to rise. This could suggest that 24 hours is too long between sprays and the crabs are dehydrating. This would initially cause hyperventilation by the crabs with a consequent rise in body temperature. The second possible cause is the increased temperature may be a result of disturbance. The crabs could be reacting to noise, breeze or sorting disturbance. The onset of temperature rise occurs at around 0600 hours when the camp would be awakening and it is possible that some unexpected noise after the night-time quiet agitated the crabs resulting in rise of temperature through increased metabolic activity.

The effect of these stress factors is discussed fully in section 5.4.4 of this report.

5.1.4.2 Wearyan River

The temperature of mud crabs in three separate crates was recorded independently, during August, from the time the crabs returned to camp post-harvest through holding and transport to Darwin. Position of crabs within the crate was identified and the logged data is presented according to temperature logger position (Figure 5.4).

Differences in temperature between crabs in different positions within a crate (Plate 5.6.) are not pronounced whilst being held at camp. However, when the crab crates are transported from Borroloola to Darwin in the refrigerated truck, large temperature differences do occur depending on crab position. It is evident from these three graphs that the positioning of the crate within the total load on the truck is also important. As the temperature loggers were distributed between 3 different crates, it is clear that some crabs are colder than others in the same position in one crate compared to another crate. For example, for those crabs logged at the bottom of the crate it would seem that one of the three crates was placed directly on the floor of the truck while the other two were not, but rather stacked on other crates above the floor. Similarly, the mid-crab show different temperature responses according to crate placement. This complicates the transport temperature step but needs addressing as the temperature differences demonstrated here are large.

Plate 5.6. Mud crabs as packed in crate at crab camps.

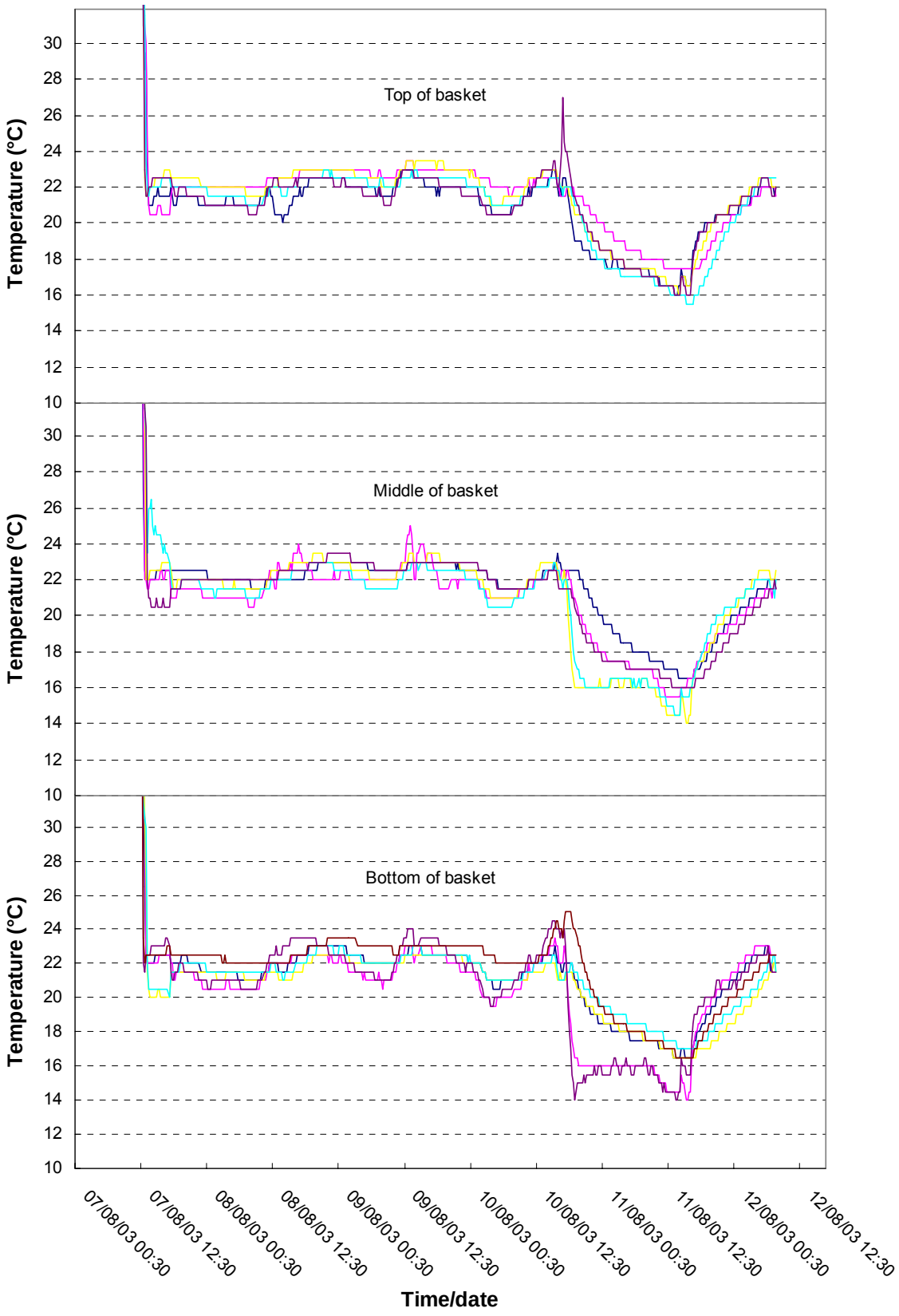

Figure 5.4. Temperatures of crabs logged from camp holding at the Wearyan River to a Darwin wholesaler facility.

The total temperature picture from harvest to wholesaler is presented in Figure 5.5 and comprises the average of all logged temperatures to illustrate the main events occurring to the mud crab during holding and transport to Darwin phases. The crabs were not logged beyond this point as they were needed commercially to supply buyers.

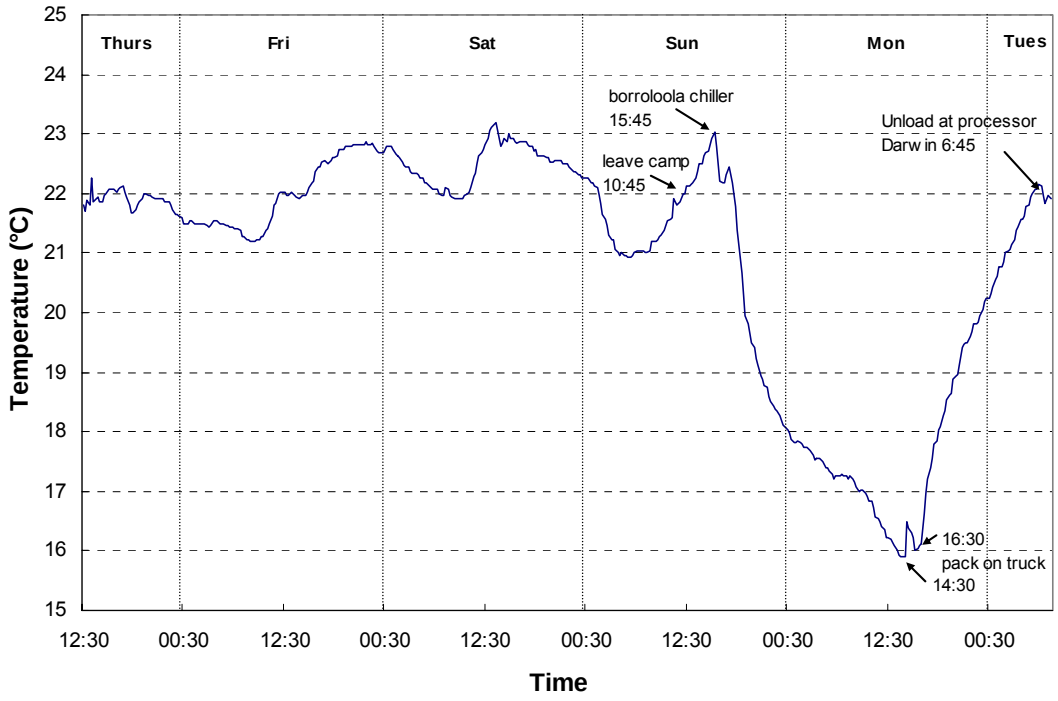

Figure 5.5. Temperatures of mud crabs through the supply chain from the Wearyan River to Darwin.

There are some points of note illustrated within the data for mud crab harvested at the Wearyan River. The crabs are held at this camp in a dedicated shade shed which is cool and well ventilated. The efficiency of this holding method is shown by the crab maintaining a temperature between 21^{o}C and 23^{o}C throughout fluctuations in ambient. Sunday afternoon the crabs are trucked out to Borroloola and the drop in temperature from corresponds to the crab being placed and held in the chill room. From the large drop and subsequent rise in temperature illustrated during Monday, it is assumed the refrigeration on the truck was not functioning from early Monday afternoon. The crabs were left in the truck after arrival in Darwin and unloaded at 0630 in the morning. At the time of grading, it was noted that the crab were in the same crates as placed in at the Wearyan River camp and there had been little movement amongst the crab since the loggers were in very similar positions to those where they were originally placed. The relative humidity in the crab crates was also logged and found to remain at full humidity (100%) during the time the crab were held at camp.

In a subsequent trip to the same Wearyan River base camp (May, dry season), a shipment of fifty crabs was temperature logged. Data was divided into the two shipment lots for ease of reading data points and because variable handling events may have occurred. The figures contain no legends but each line corresponds to the temperature of an individual crab with the thick blue line demonstrating external ambient. Figure 5.6 shows that while crabs were held at camp in the shade shed enclosure (first two and a half days), temperatures remain stable and cool despite

the fluctuations in ambient. There is a sharp temperature increase from all crabs, beyond that of ambient on Sunday morning and this timing coincides with crab disturbance in handling, sorting and re-crating the crabs for transport to Borroloola. At Borroloola the crabs were cooled down in the cool room used as a store depot. Crabs were taken out late Monday afternoon and trucked to Darwin. They were then flown down to Brisbane on the midnight flight and arrived around 0600 hours Wednesday morning.

Figure 5.7 shows a very similar pattern of temperature change during the transport chain although, these crabs were logged for a longer period at the Wearyan River base camp. Again the same cooling and re-warming trends occur during storage at Borroloola and trucking to Darwin. Relative humidity remained at 100% throughout all stages of holding and transporting the crab, including during the flight from Darwin to Brisbane.

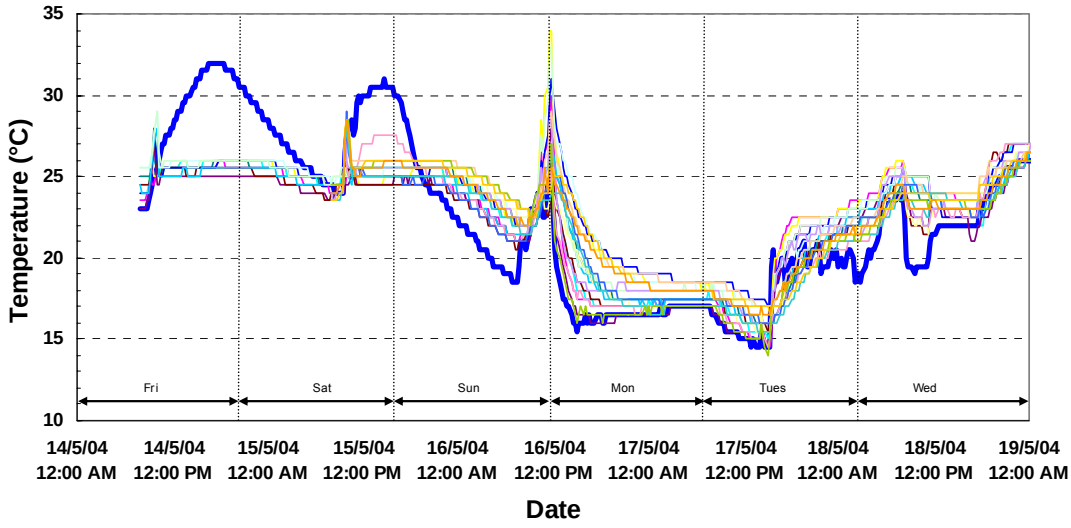

Figure 5.6. Temperature of crabs from capture through to arrival in Brisbane.

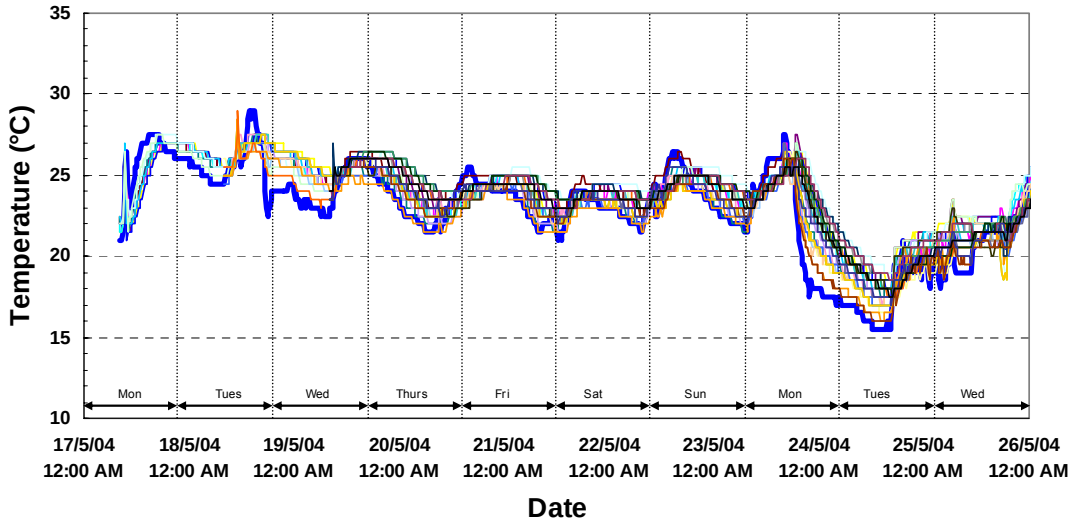

Figure 5.7. Temperature of crabs from capture through to arrival in Brisbane.

5.1.5 Summary

Information collection based on current handling and harvesting practices within the mud crab fishery in the NT indicates that:

- mud crabs are handled gently and carefully despite the lack of infra-structure restrictions existing at the isolated harvesting locations
- most harvesters claw-tie the crabs at point of clearing from the pot
- there are some instances of cleared crabs being stored untied in an underfloor area of the dinghy
- there is strong recognition amongst harvesters of the importance of keeping the crabs at a constant cool temperature and keeping them in a moist environment
- the use of thick hessian sacking was almost universal despite much acknowledgement that it was not the ideal material to store crabs in due to difficulties in cleaning the hessian between crab batches
- some operations use dedicated storage sheds made of shade cloth, but this is not universal

The information gained from monitoring mud crab temperature change during both the 'dry' and 'wet' seasons illustrates that:

- crab are subjected to large temperature changes under commercial handling practices
- the method used for holding of crabs at camp is appropriate but could be improved to further minimise temperature fluctuation
- spraying the crab sacking is important to reduce temperature
- time of day for spraying may be able to be optimised for greatest temperature benefit
- the use of dedicated storage sheds in line with the industry Code of Practice' would improve holding temperature stability

5.2 Trend analysis of commercial mud crab data

Project Objective

Document current mortality data in specifically designed logs to capture all possible factors

Mud crabs are harvested in remote locations in the Northern Territory, stored at a base camp for up to 7 days and transported to Darwin to wholesaler premises for further grading, pack out and transport to market. Storage of crabs at base camps is with the claws tied, crated and held under damp hessian at ambient. Transport from camp to Darwin is most commonly in an air-conditioned vehicle, with the exception of some crabs from Blue Mud Bay which were flown out from Groote Eylandt to Darwin. Upon arrival in Darwin, each mud crab is assessed and graded according to animal liveliness, robustness and quality. Commercial industry retains records of crab gradings and generously proffered these for analysis of mortality trends. Such an offer was appreciated because access and analysis of previous records provides an opportunity to observe whether any obvious trends exist with respect to mortality rates. If trends are evident then they could illustrate critical key factors needing to be addressed within the post-harvest survival research.

5.2.1 The Fishery

Mud crabs are harvested at remote locations around the coast of the Northern Territory, from the bottom of the Gulf of Carpentaria (McArthur River region), up the west side of the Gulf, around to the west of Darwin and down to Victoria River. The major harvest areas for mud crab in the NT are illustrated on Figure 5.8:

- Bynoe Harbour/Darwin
- Blue Mud Bay
- Roper River region
- M^{C}Arthur River region (Borroloola)

Total mud crab catch volumes over recent years are presented in Figure 5.9. Catch of mud crab steadily increased to peak at 1139 tonnes in 2001. Catch volume dropped significantly to 739 tonnes in 2002; and decreased further in the following years. The corresponding freight data indicates that nearly all mud crab harvested in the Northern Territory is transported out of Darwin. Hence the importance of understanding the stress imposed by the distribution chain.

Effort within the mud crab fishery is measured by number of pot lifts and effort has steadily increased to a current average of ~1 million pot lifts per year. Catch per unit effort (CPUE) peaked in 2001 at over 1kg/potlift, however declined in 2002 ~0.7kg/pot) and in 2003 (~0.4kg/potlift) at which level it has remained (Ward *et al.* 2008). CPUE is often used as a measure of stock abundance to assess the status of the fishery. The implication of a low or decreasing CPUE, with respect to crab quality, is that harvesters have to work harder to obtain mud crab. The low CPUE figure therefore could imply that crabs will be a longer time ‘dry’ and sitting at high temperatures, both in the water at low tides prior to pot clearing and during transport in the dinghy back to base camp. Such implications suggest a greater level of stress is imposed on the crab.

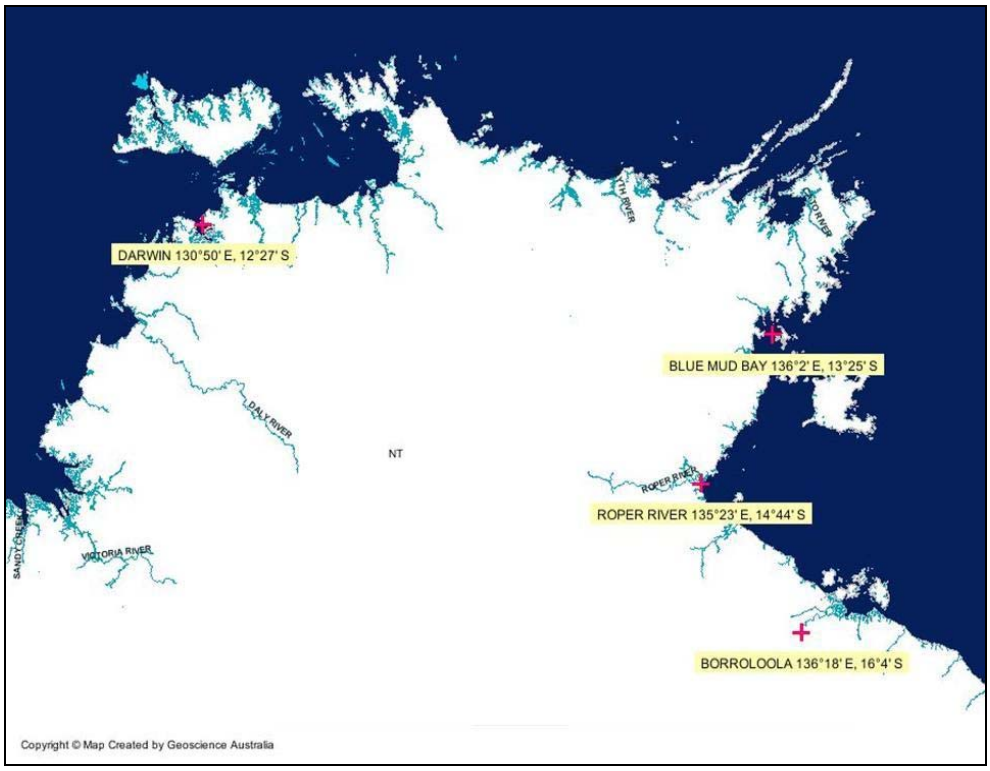

Figure 5.8. Location of major harvest areas for mud crabs in the Northern Territory showing proximity to Darwin.

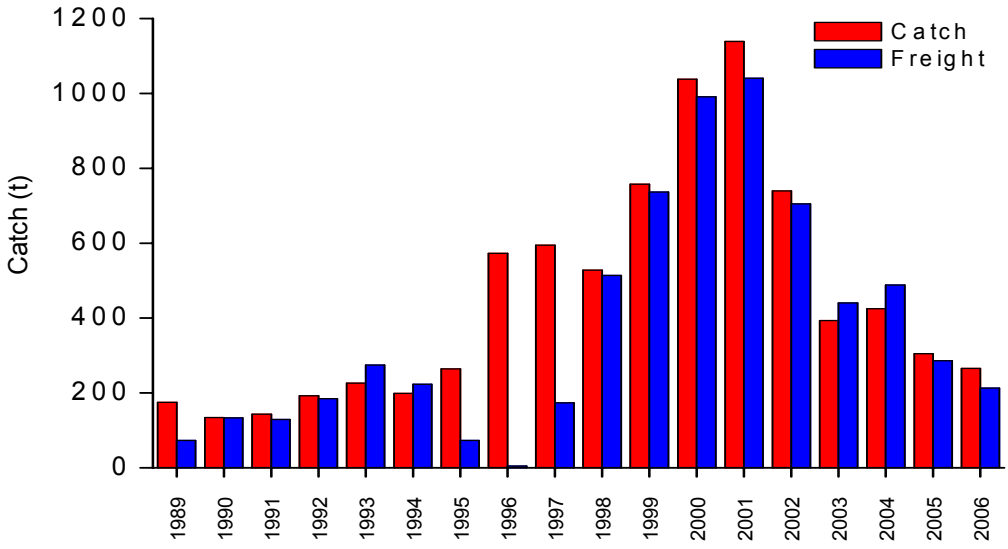

Figure 5.9. Catch and corresponding freight data for the NT Mud Crab Fishery from 1989-2005. (Reproduced from Ward *et al*, 2008 with the kind permission of Tim Ward).

5.2.2 Data source

The data analysed covers the period January 2002 to September 2005 and includes a large proportion of the mud crab catch taken within the NT fishery. The remaining effort in the fishery is harvested and marketed independently, resulting in difficulty in obtaining similar data from these operators. Further data was not readily available after 2005 owing to change in operational status of the wholesaler involved. It is understood that similar data exists within the NT Fisheries databases and so it is possible for Fisheries staff to update the following analyses to include a wider picture. The correlations investigated in this section are suggestive only and were not able to be scrutinised under rigorous statistical analysis due to the incomplete data set.

Data was obtained as individual data sets from fishers but analysed as one data set so as to preserve commercial confidentiality. Where striking differences appeared between individual data sets, commentary only is given with no depiction of actual data, again to retain confidentiality.

The data examined relevant to this study is the quality grading of the live mud crabs as applied commercially at the point of pack-out for transport to markets (Table 5.2).

Table 5.2. Commercial grading categories for less than 'A' grade mud crab.

Commercial grade	Description
Dead	crab not alive, died in transit from harvest camp
Slow	Weak, judged by antennae movement and force resistance exhibited from claws and legs upon handling
CUC	commercially unsuitable crab - crab with flexible shell, lacking muscle tissue (meat), occurs immediately post-moult
Other	poor quality, classified as 'B' grade or 'riced' (fly larvae)

The relevance of the grading categories with respect to further on-shipment is:

- Dead – not able to be marketed; the mud crab trade is a live market
- Slow – not commercially viable to send to distant markets as the crab is unlikely to survive the additional demanding transport conditions involved and is highly likely to die in transit. Of relevance is that if even one crab dies within a box of ~20-30, the consequent ammonia level increase inside the crab box will overly stress the other live crab, resulting in high probability of other consequent mortalities
- CUC – not permitted for sale under the mud crab management plan, are discarded
- Other – not viable to send to distant markets as likely to die, depending on condition may have some limited (reduced) return on local markets

The non-biological factors considered likely to impact on the crab condition and grade are:

- temperature – time of year (season); water temperature at harvest; ambient – actual temperature and the differential between minimum and maximum
- rainfall – when and how much affects salinity of harvest waters and nutrient/particulate levels

- harvest area – degree of geographical isolation and related transport difficulty and duration
- harvester practices – frequency of pot clearing; distance of fishing operations from base camp; crab claw tying practices; crab storage methods at camp
- transporters practices – time and temperatures of holding
- catch volume – excessive crab catch may dictate altered handling practices

5.2.3 Compromised crabs

For this analysis, data for crabs graded as less than robust was combined and labelled 'compromised crab', hence this category includes cumulative data for grade classifications of dead, slow, CUC and other and depicts total revenue loss for the period. Data for 2002-2005 are presented in Figure 5.10.

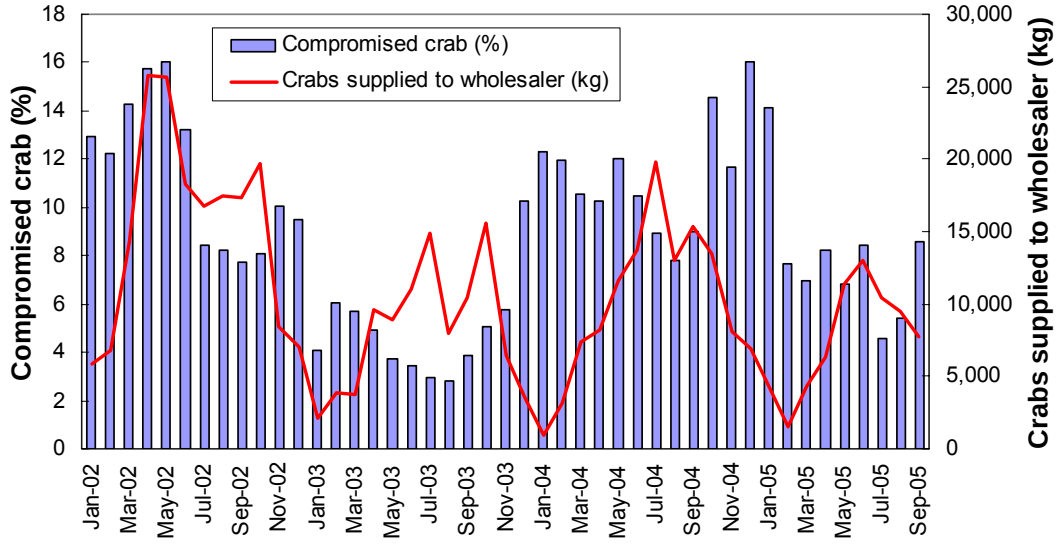

Figure 5.10. Percentage of mud crab not suitable for further distribution against the total mud crab weight supplied to wholesale sector.

The data illustrates large differences in amount of compromised crabs between years, with 2003 highlighted as an exceptional year where there was a very low percentage of crab that was compromised. A trend observed is that more crabs are compromised during the build-up and wet season months (November-May). Compromised crab levels are evidenced at 10% and above during these months implying a large revenue loss and it is likely that more crab mortality occurs further down the supply chain, increasing total losses for the industry. The higher percentage of compromised crabs during the wet season is likely to be due to warmer temperatures during this time causing additional stress to the crabs and weakening their tolerance to further transport stresses. Interestingly, percentage of compromised crabs appears to be independent of total volume, which means there is no tendency for fishers to retain unsuitable crabs during times of low catch rate.

The total percentage of compromised crab was analysed by separate grade category and data are presented separately for each year within Figure 5.11 for reason of visual clarity.

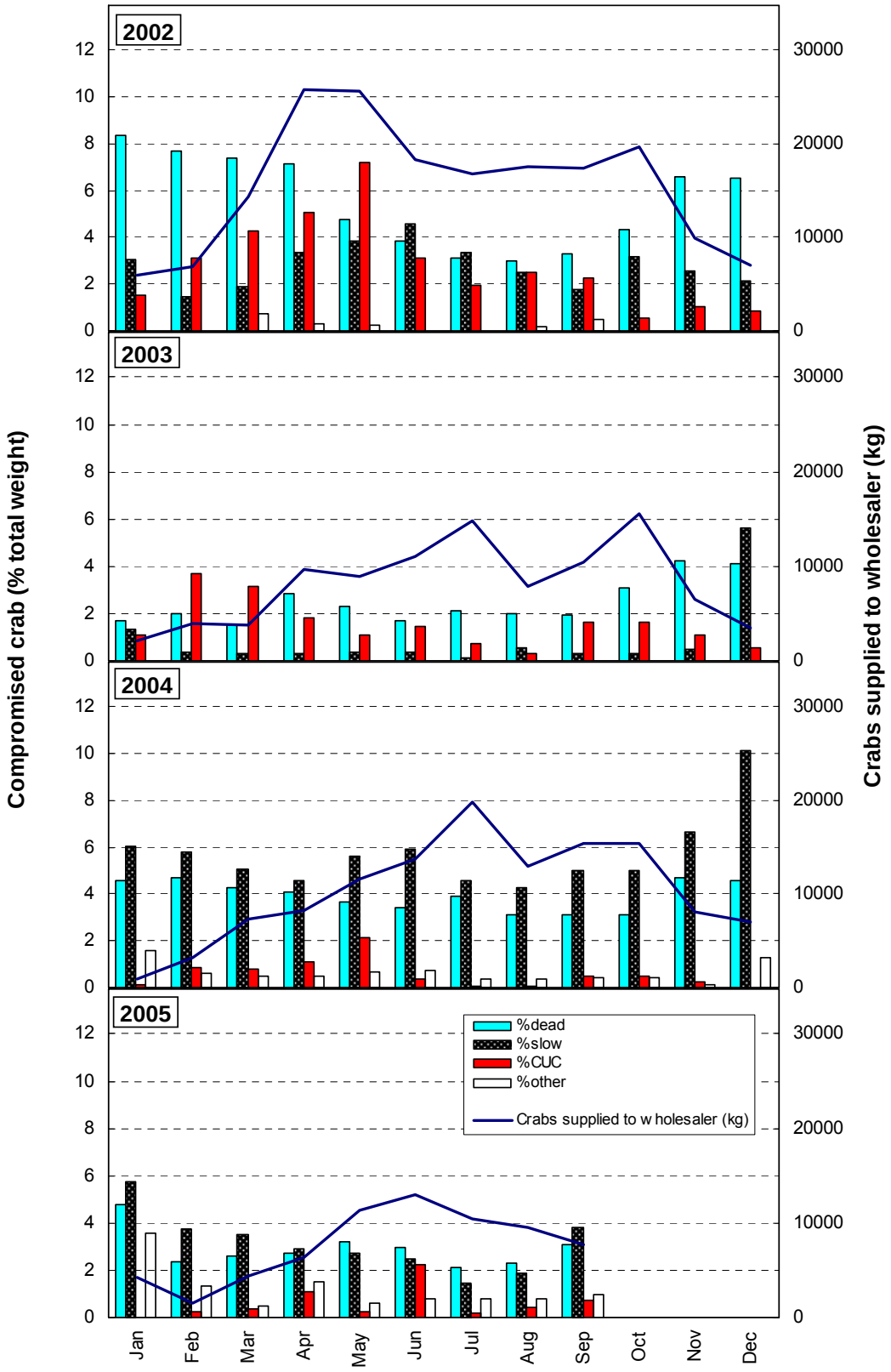

Figure 5.11. Commercial grade categories for mud crabs by month with the total crabs supplied to wholesaler illustrated.

Dead
The percentage of crab arriving dead at Darwin is relatively consistent throughout the year and low at around 3-4%, although there was a higher percentage during 2002. During 2002, there appears to be a trend of more dead crabs in the build-up and wet season months, however, this is not clearly illustrated for the following years. From this limited data, the proportion of mortalities appears unrelated to catch volume. It should be noted, this data only pertains to the first part of the distribution chain to the point of arrival at the wholesaler. It does not account for mortalities that may occur at base camp prior to transport nor further along the supply chain during distribution and holding at retail.

Slow
The proportion of crabs graded as 'slow' is variable between years and does not correlate with numbers of dead crabs. The proportion of 'slow' crab is of concern as it indicates high levels of stress in the crab and an increased risk that they will die with further handling and transport. Comparing the percentage of 'slow' crabs for 2004 and 2005 in relation to the total of crabs supplied for wholesale, a lower level is observed in 2005 while the amount supplied is similar for the two years. This suggests that proportion of 'slow' crabs occurring is independent of catch volume. Also of note from these four years data, is that there appears to be no relationship between time of year (month) and proportion of 'slow' crabs.

Other
A very low percentage of crabs fall into this category for 2002-2005, which indicates the harvesters are wrapping the damp hessian effectively and taking good care of their crabs while being held at base camps.

CUC
Commercially unsuitable crab (CUC) is based on a biological parameter directly related to the mud crab life cycle as measured by the hardness of the shell. Crabs need to moult (shed their hard outer shell) to grow and the physiologically complex moulting process requires large energy consumption. An immediately post-moult crab is considered 'empty' as the animal has taken in large quantities of water (required for splitting of the carapace) and muscle protein (meat) has been used as an energy source. It takes approximately 2-3 weeks from moulting for the crab to return to being full and robust. The degree of fullness is relevant to mortality rates and crab survival as 'non-full' crab, at whatever stage, will tolerate the impacts of stresses imposed far less efficiently than 'full' crab.

In 2001, new regulations were implemented that banned the taking and possession of CUC. It is excellent to note that levels of CUC crabs going through the wholesalers have dropped steadily since that time (Figure 5.12), providing evidence that crab harvesters are doing the correct thing and returning such crabs to the water. Through 2004 and to September 2005, there is very little CUC being harvested, which emphasizes the responsibility taken by crabbers in returning CUC to the water out of the pot.

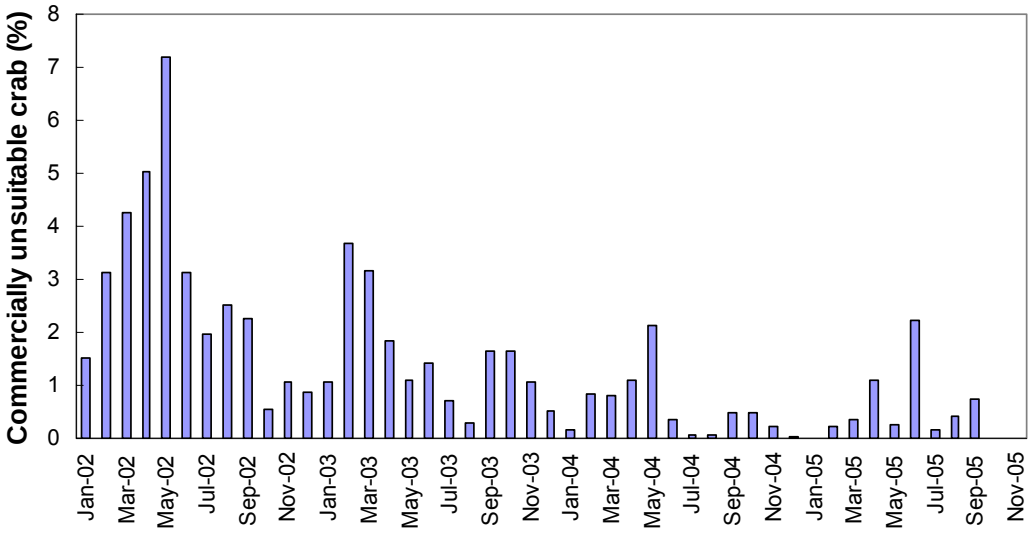

Figure 5.12. The proportion of crab graded as commercially unsuitable crab, 2002 to 2005.

In the NT fishery, both male and female crabs are allowed to be harvested, albeit with separate size limitations for each. The proportion of males and females within a catch varies according to season (Figure 5.13) but males predominate during the wet season through to the early dry (January-May). Females are predominant during the build up and very early wet season months (September - December).

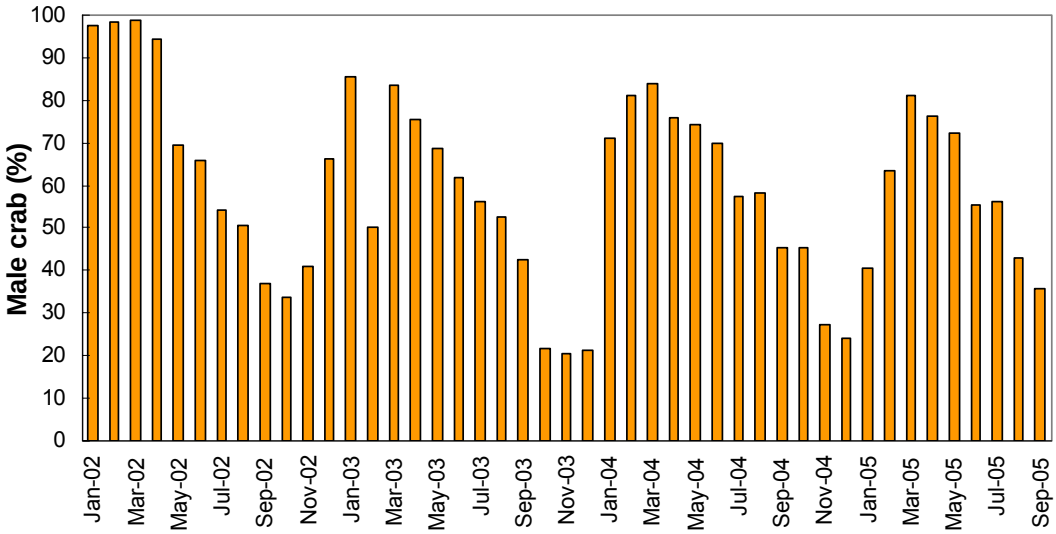

Figure 5.13. Percentage of male mud crab within the total weight of crabs supplied to the wholesale sector.

It is interesting to note that during the wet season months, the proportion of comprised crab is higher. Industry has commented that this period also corresponds strongly with newly moulted male crabs coming into the fishery. Total losses from compromised crabs appear to peak through the months of January to April and at this time the mud crab gender ratio in the catch is weighted towards males. It is possible that this is reflective of a gender difference in tolerance to harvest stresses but further data breakdown providing proportion of compromised crabs by gender

was not available. However, a greater proportion of compromised crabs occurring in these months are more likely to be directly related to high temperatures at this time.

5.2.4 Temperature and rainfall correlations with mud crab grade

As the data shows a trend of a difference in the number of compromised crab between summer and winter months, it was worth looking closer at the non-biological factors at these times, namely temperatures and rainfall. Temperature and rainfall data were obtained from the Bureau of Meteorology (BoM) stations closest to mud crab harvesting areas (Table 5.3).

Table 5.3. Data recording BoM stations nearest main harvesting areas.

Harvesting region	BoM data station
Bynoe Harbour	Darwin Airport
Blue Mud Bay	Roper River temps as no BoM stations appropriate
Roper River	Ngukurr and Flying Fox
M^{C}Arthur River	Borroloola and Central Island

5.2.4.1 Temperature

The mean monthly minimum and maximum temperatures for the different fishing regions are given in Figure 5.14. The trends for temperature are similar for all sites but several (Oenpelli, Timber Creek, Ngukurr and Borroloola) reached a higher maximum temperature during the build-up months of September-November. These areas also record the lowest minimum temperatures during the winter months and the extremes can likely be ascribed to the inland locations, except for Oenpelli.

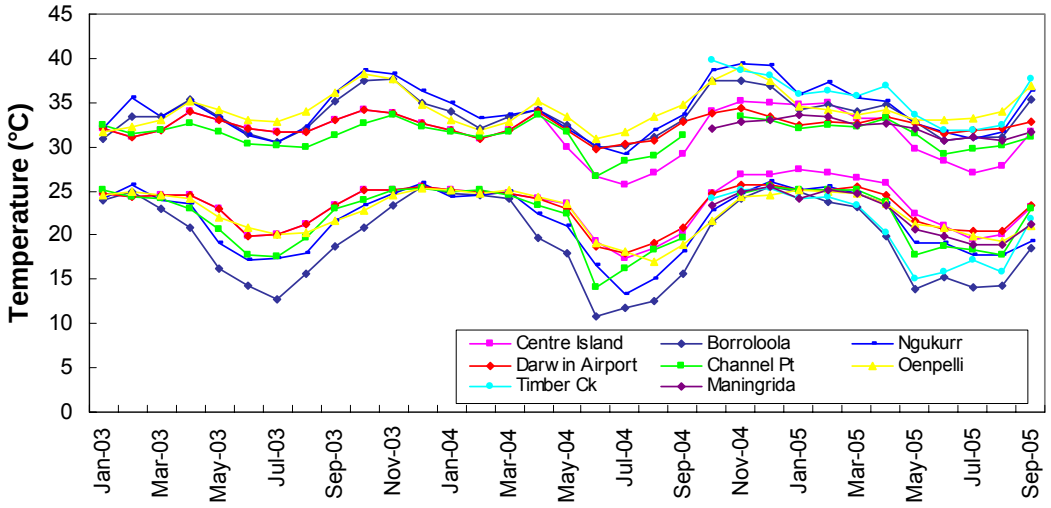

Figure 5.14. Mean monthly temperatures for main harvesting areas.

There is not a large difference in mean monthly maximum temperature between winter months (May-Oct) and summer months (Nov-Apr). During the summer months, the temperature differential (overnight minimum temp to midday maximum)

is small, around 7-8 degrees. However, there are much larger differentials during winter months (dry season) with min/max temperatures in some locations varying by over 15 degrees (most notably the Borroloola/M^{C}Arthur River region). Large temperature differentials could be expected to contribute to mud crab stress levels and yet this does not correlate to high mortality rates. The high maximums that occur in the summer months may be causing greater stress to crab through high water temperature prior to harvest, particularly if the pot is running dry or is in very shallow water at low tides. Temperature data for 2003 appeared to indicate that there was a correlation between the percentage of compromised crab and the greatest difference between minimum and maximum temperatures. However, this does not hold true for all years.

Figure 5.15 illustrates the temperature differentials (the average of all Bureau of Meteorology records from sites nearest main harvest areas) superimposed on the total level of compromised crab. It would appear that when the temperature differential is greatest, during the dry season months of May-July, there is least proportion of compromised crab.

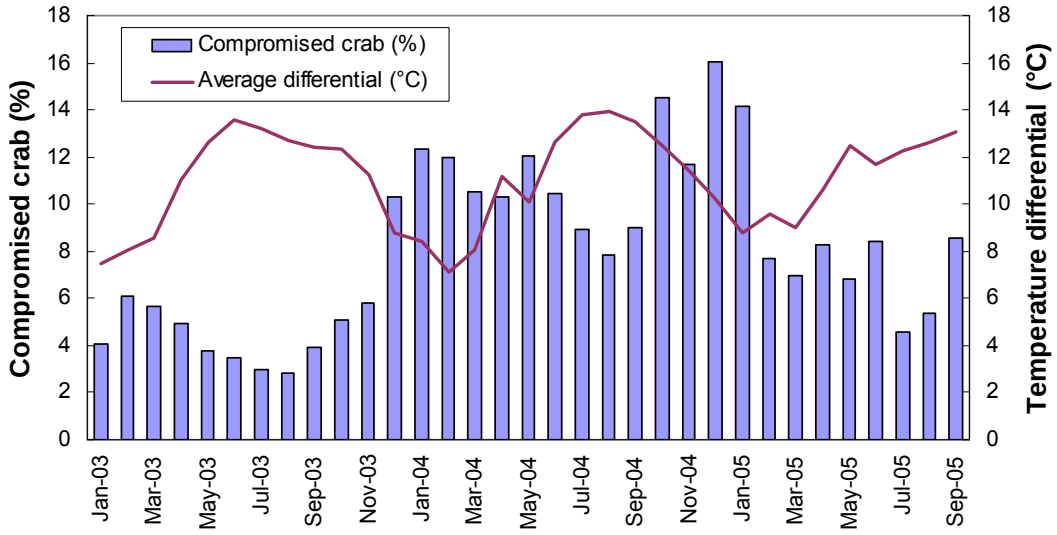

Figure 5.15. Percentage of compromised crab against temperature differential.

5.2.4.2 Rainfall

The Northern Territory has two distinct seasons: the wet and the dry. These two correlate to summer and winter months: the wet usually occurs from December to March and the dry from April to November. Popular opinion holds that the seasons are shifting with the global climate changes and the wet arrived 'late' the last couple of years. However, Figure 5.16 shows the last 10 years total monthly rainfall for Borroloola and illustrates little sign of a season shift and actually indicates earlier onset of the 'wet' season for 2001-2004.

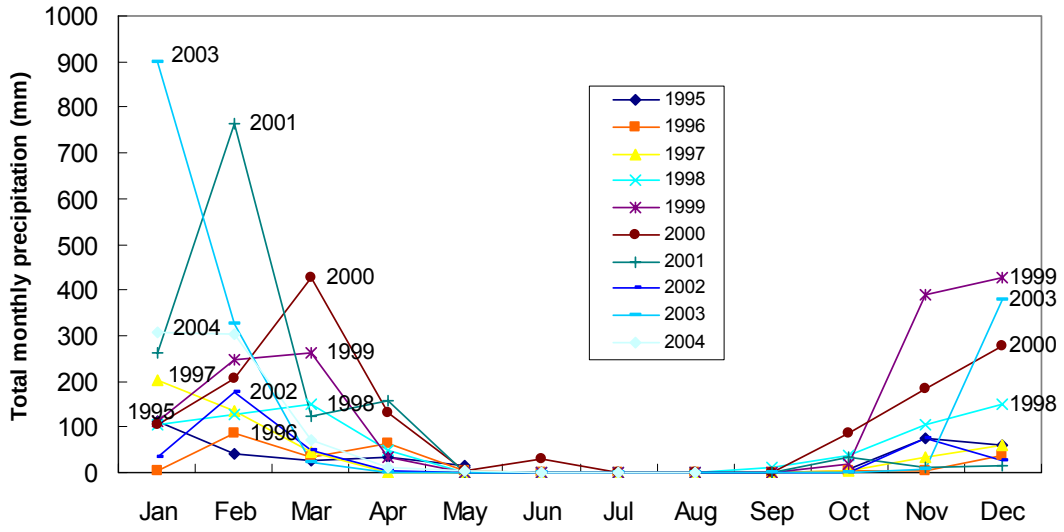

Figure 5.16. Total monthly rainfall for Borroloola, 1995-2004.

A note worth mention is the effect of rainfall on access to the crab base camps. Heavy rains during the wet can often completely isolate some locations of mud crab harvest, rendering roads into and out of camp very difficult to negotiate. This culminates in potentially longer storage times for the crab out of water and longer, physically more demanding transport sectors prior to mud crab arrival in Darwin. However, changes in the proportion of compromised crab appear to be independent of rainfall (Figure 5.17). Periods of high rainfall induce high relative humidities and this should be beneficial for the crab during storage however, this is not evident from the correlation depicted.

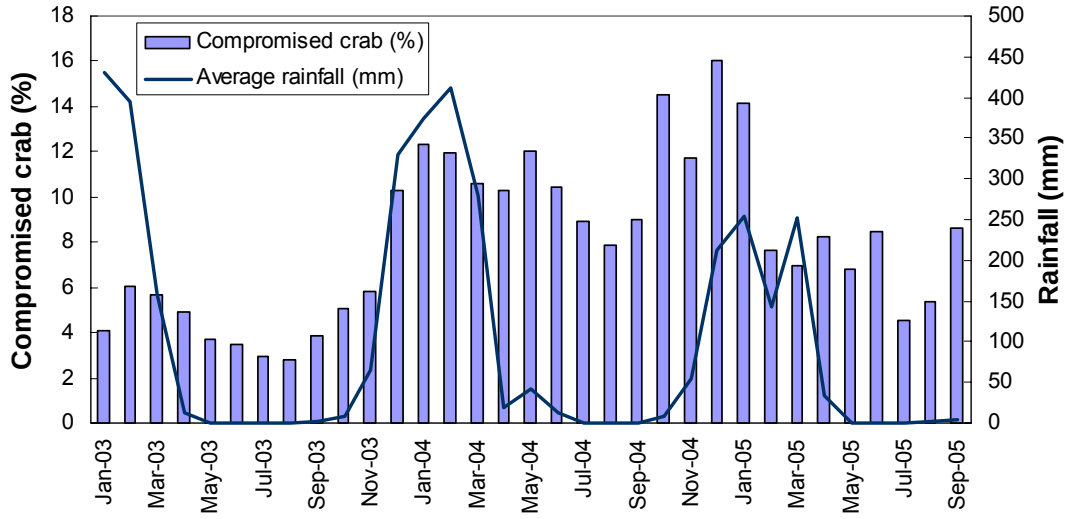

Figure 5.17. Mean monthly rainfall for all main harvesting areas against total percentage of compromised crab.

5.2.4.3 Harvesting region

There are four main areas most actively fished for mud crab in the Northern Territory fishery three of which, Blue Mud Bay, Roper River region and M^{C}Arthur River region, are in the Gulf of Carpentaria (GoC). These areas are particularly isolated and consequently dictate long transport stages in the supply of mud crab.

The amount of compromised crab from separate harvesting regions is shown in Figures 5.18-5.21. Each figure comprises three separate graphs illustrating:

(a) mean monthly rainfall
(b) mean monthly temperature
(c) total weight supplied to wholesale sector

The data evidences no commonality between regions, with numbers of compromised crab different for each region throughout the year. Additionally, on a regional basis, there is no correlative trend between proportion of compromised crab with rainfall or temperature. There are some striking differences between regions however. For example, the amount of compromised crab varies by region at different months of the year: compare compromised crab percentage during Feb-May 2004 from the Roper River region with the same months from Blue Mud Bay (Figures 5.19 and 5.20). These regions are in close proximity geographically, however the transport method from harvest camp to Darwin wholesaler is not similar and it seems that the difference is having an impact on crab stress levels. Mud crab from Roper region is trucked back to Darwin (some 800km by road) while crab from Blue Mud Bay is often taken by boat to Groote Eylandt and flown out to Darwin once a week. This latter practice is variable according to freight space availability from Groote, so sometimes the crab is transported down to the Roper River region and trucked back to Darwin.

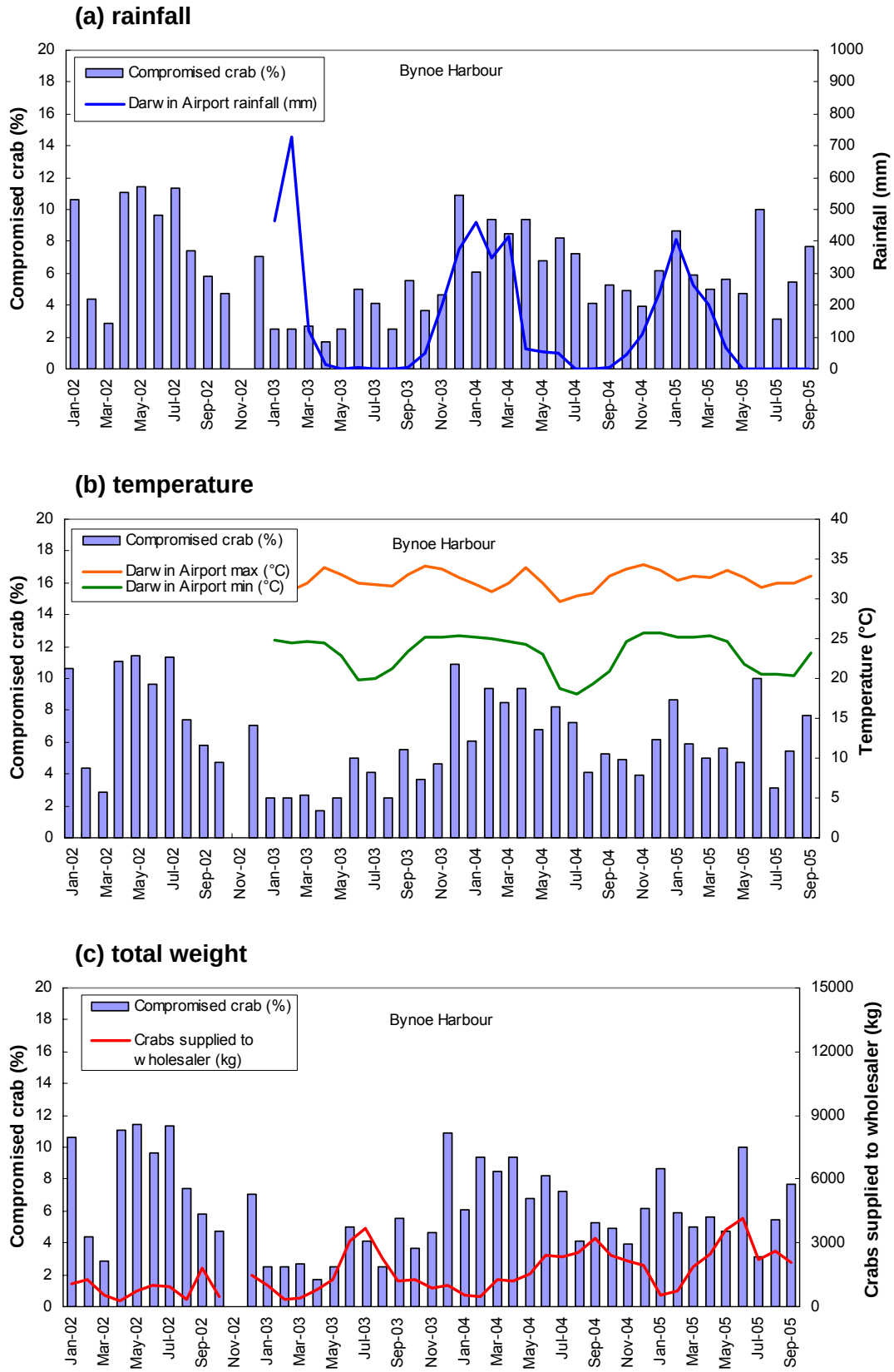

Figure 5.18. Compromised crab – Bynoe Harbour area.

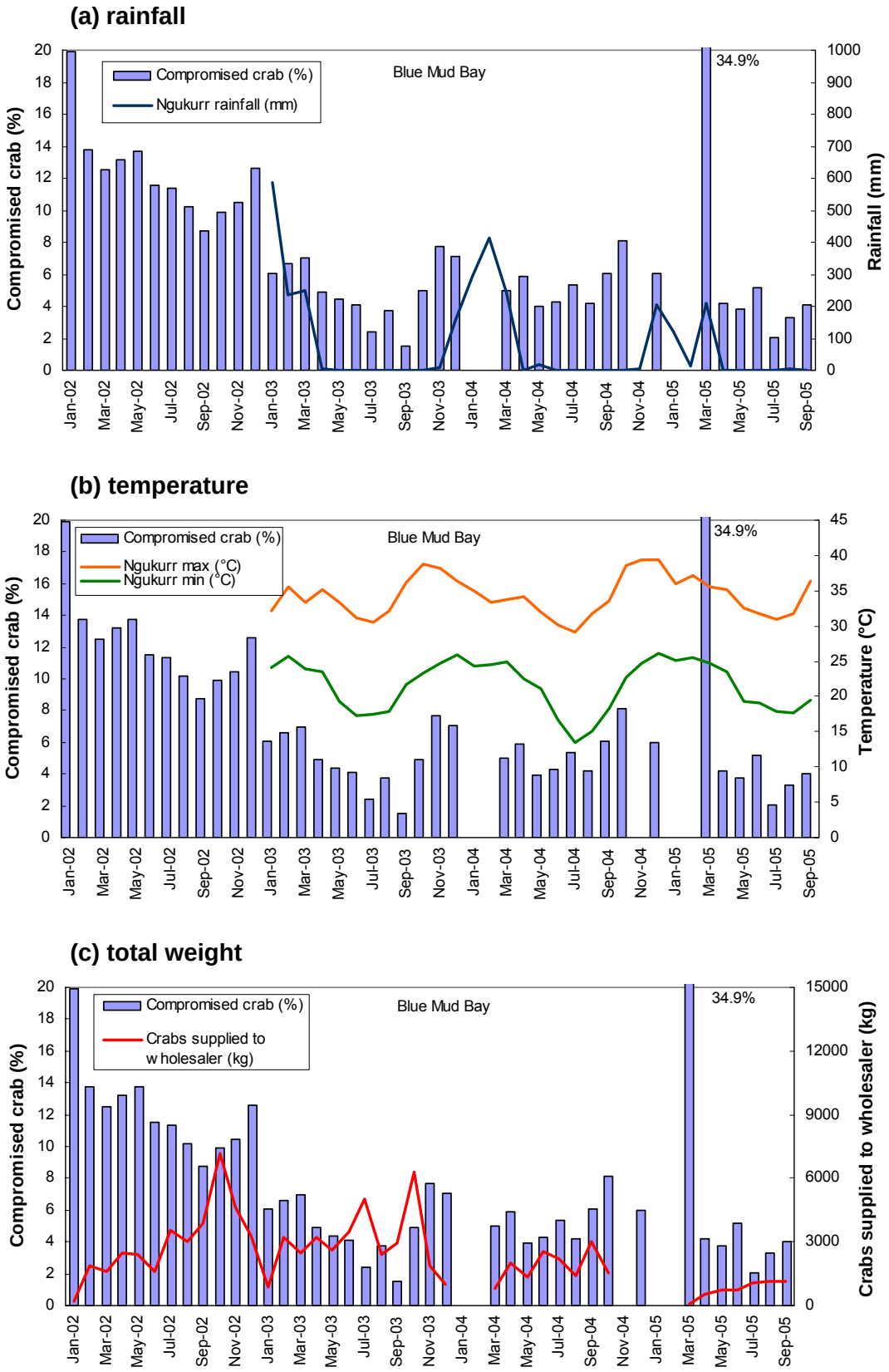

Figure 5.19. Compromised crab – Blue Mud Bay area.

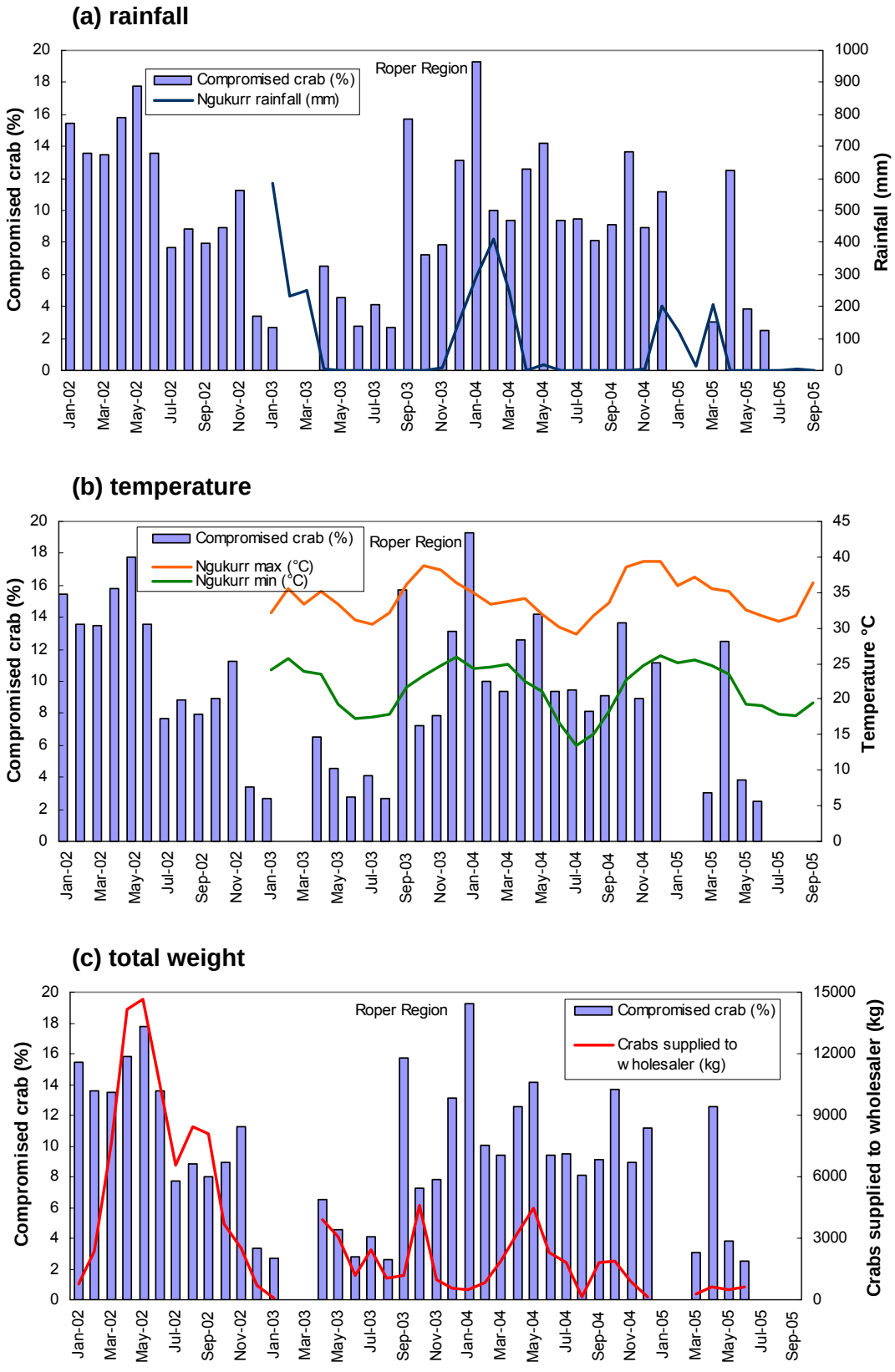

Figure 5.20. Compromised crab – Roper River area.

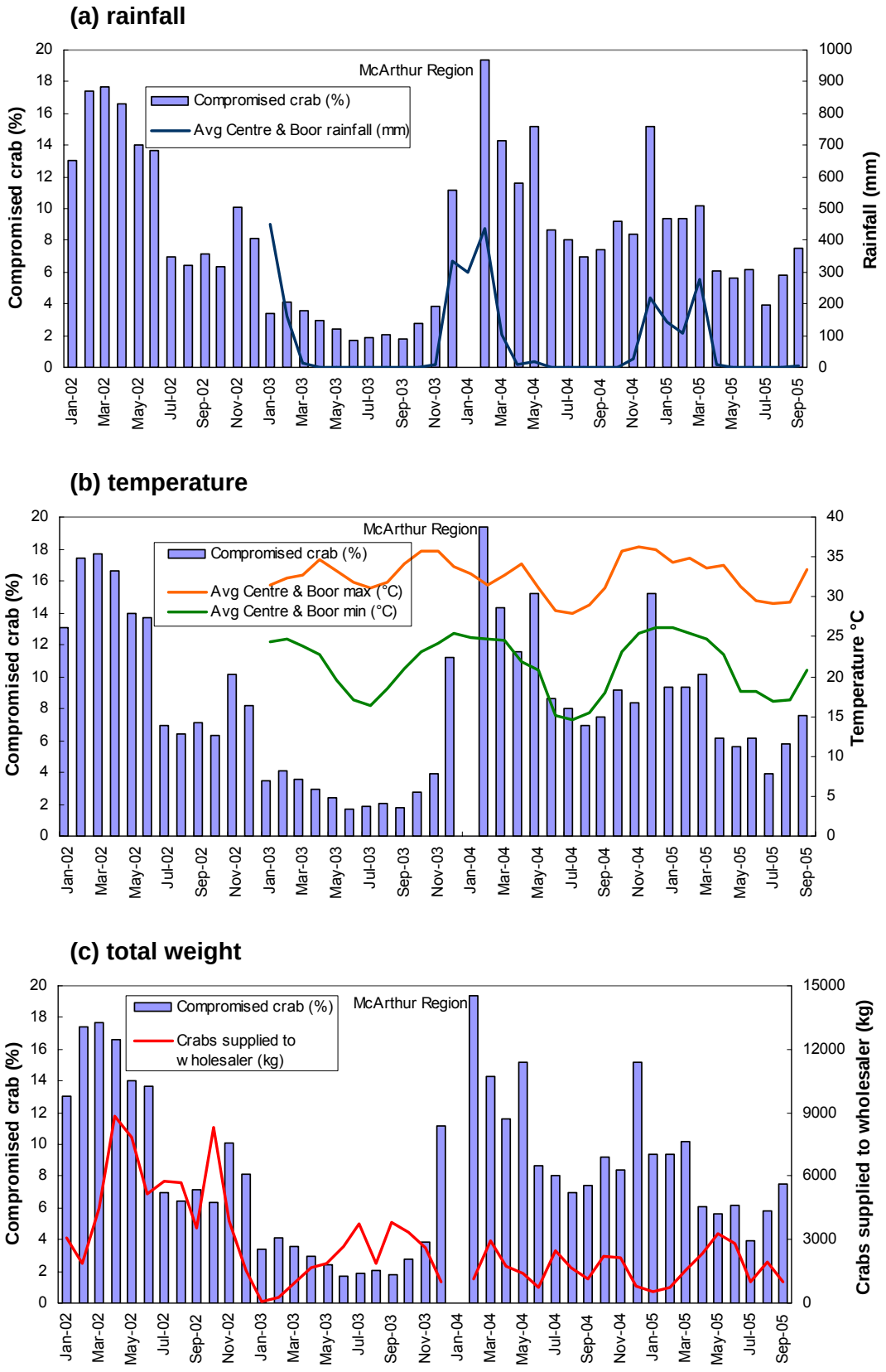

Figure 5.21. Compromised crab – M^{C}Arthur River area.

The proportion of compromised crabs totalled for the years 2002-2005 is viewed by region, it is clear that distant location and therefore long trucking distances (Roper River and M^{c}Arthur region) produce a higher proportion compromised crab (Figure 5.22). Conversely, harvesting areas close to Darwin consistently have fewer compromised crabs. Therefore, it can be concluded that the transport chain length has a strong bearing on stress levels in crabs.

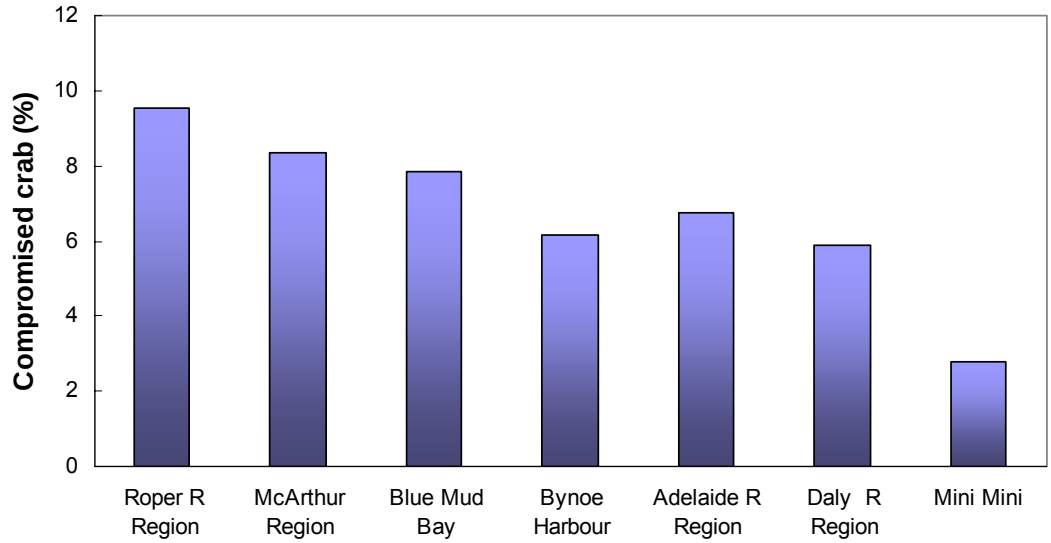

Figure 5.22. Compromised crab by major harvest region.

5.2.5 Summary

From the various correlations with different parameters, it is concluded that

- the proportion of compromised crab reaching the wholesale sector varies year to year, but averages around 10%
- the amount of compromised crabs varies seasonally
- rainfall is not related to the proportion of compromised crabs
- high temperatures do seem to affect amount of compromised crab
- large differences between minimum and maximum daily temperatures do affect compromised crab levels
- compromised crab levels are related to the length and the type of transport chain

Further collection and analysis of similar data as presented here would allow a more robust picture and understanding to be attained.

5.3 Stress indicators

Project Objective

Establish physiological factors and stress level indicators for harvested mud crabs

Stress is recognised as the main factor contributing to mortalities of crustaceans during harvest and subsequent handling through the live product distribution chains to market (de Wachter *et al.* 1997)

Stress in a mud crab is induced by numerous environmental and physiological factors. Some factors may not necessarily be lethal on their own but contribute in combination to a high overall stress level. It is likely that stresses are cumulative or synergistic.

To determine the effect of different handling and holding conditions on the stress level in mud crabs, readily measurable parameters need to be correlated directly with animal stress. The 'liveliness' index is a good 'gross' rating scale but is not sensitive enough to define increases in stress levels as they occur.

The objectives of research within this section of the project were to:

- determine appropriate and useful haemolymph biochemical indices that can be easily measured
- establish critical levels for each of the blood parameters measured
- confirm that elevated levels were directly in response to adverse external conditions and thus indicative of stress within the crab

Such information provides knowledge of applicable indices and an understanding of degree of stress in the animal imposed by one or more specific handling factors.

5.3.1 Mud crab physiology

How an individual crab responds to and/or deals with the stresses imposed during the supply chain is directly dependent on the physiological condition of the crab. 'Condition' is influenced by animal health, physical damage, phase of moulting cycle and growth phase. Clearly, a crab not fully robust and in a weakened state will have less ability to tolerate any level of stress imposed and will surrender to mortality more rapidly. Starvation during extended handling and transport chains will also contribute to lowered stress tolerance. During these periods, the crab must ultimately use its bodily energy stores, a process known as catabolism. In crabs, blood protein reserves are used first, followed by the hepatopancreas and eventually the muscle. Starvation is not likely to be a major cause of mortality in itself but rather a contributory factor reducing the stress tolerance capability of the animal.

5.3.1.1 Moulting cycle

Crustacean growth is restricted by the presence of a hard exoskeleton (shell) made of chitin and this must be shed incrementally, a process called moulting. Moulting results from complex interactions between endogenous regulation and environmental inputs or triggers to moult, the crab rapidly absorbs water, splits its old shell at the junction between carapace and under-body at the rear of the animal and backs out of it. The cuticle expands before hardening which occurs through

inorganic salts (mostly calcium) in the haemolymph and from surrounding seawater redeposited to form the hard shell (Greenaway 1983; Pratoomchat *et al.* 2002) Crabs cease feeding prior to moulting and do not recommence until after the new exoskeleton has hardened. As such, few crabs in early moult phase are caught in the fishery as the capture method relies on baited traps (pots). While the new shell is hardening and for the next few of weeks, the mud crab replenishes and rebuilds its muscle and during this period the shell may be fully hard but the crab can be 'empty' The process of moulting requires high energy expenditure by the crab and until muscle mass is fully replaced, has diminished ability to tolerate any external stresses imposed.

5.3.1.2 Vigour index

The most obvious indicator of stress is physical liveliness, general robustness and response to external stimuli. Measurement of these attributes is somewhat subjective however and relies on the experience and knowledge of the grader/assessor. Overall, the crab's alertness of movement, reaction time when approached, extent/speed of eyestalk movement and degree of force returned when exerting pressure against the crabs' limbs provide a reasonable indication of 'liveliness' (at ambient temperature).

The vigour of each animal was assessed against a four point demerit scale developed from observations of many mud crabs from different experimental trials and similar to the method described by (Spanoghe and Bourne 1997)**.** The vigour index for mud crabs is presented in Table 5.4.

Table 5.4. Vigour index for mud crabs used to assess liveliness.

Score	Term	Description
0	dead	Dead
1	very slow, weak	Walking legs give very little resistance to pressure applied, pincers non-responsive, mouth parts may be drooping, foaming from mouth
2	slow	Walking legs have some strength but not actively moving, pincers have slow movement
3	lively	Walking legs strong and active, pincers active and aggressive

The index is based on limb movement and strength or resistance against gentle applied pressure. It was noted that other moving parts of the crab such as mouthparts, eyestalks, and antennae are not a reliable or consistent indicator of liveliness nor stress.

It is important to ensure crabs are at room temperature (24^{o}C -28^{o}C) prior to assessment using the index. It was noted at the end of some distribution chains that the crabs were quite cold having likely been stored in a chill room overnight at too low a temperature. Crabs at such low temperatures are slow, unresponsive and even 'comatose'. However, if allowed to acclimatise to ambient, the vigour is

significantly improved. All assessments were always made on crabs at ambient room temperature

5.3.2 Mud crab metabolism

Crabs are aquatic animals and to remove them from this environment for extended periods is unnatural and may produce stress to respiratory and metabolic systems. However, mud crabs are among the exceptional crustacean species in that they can seemingly tolerate such treatment far more readily than many other crab species.

Crabs are poikilothermic animals and therefore the metabolic rate is largely determined by external environmental temperatures. Hence, within limits, the higher the temperature the greater the metabolic rate and the more rapidly the crab will deplete its oxygen supply. Mud crabs, similar to other crustacea, exchange respiratory gases with the environment through the gills. Oxygen is taken up from the water and the metabolic end-product carbon dioxide is excreted back to the water. When in water with low oxygen saturation level or when fully emersed (in air), mud crabs have to find a way to 'breathe' without an external supply of oxygen. They do this through anaerobic (no oxygen) respiration by switching metabolic systems to use alternative compounds to create energy. Such anaerobic glycolysis is utilised to meet the shortfall in oxygen uptake and results in accumulation of lactic acid in the haemolymph and tissues. The ultimate consequence is development of a mixed respiratory and metabolic acidosis, typical in many aquatic crustacea during air exposure (Depledge 1984; Hill *et al.* 1991; Cooper and Morris 1997; Luquet and Ansaldo 1997; Maciel *et al.* 2008). The combination of aerobic and anaerobic metabolism can be sufficient to maintain energy levels within the crab for essential existence and even allow some degree of vigour in mud crabs. However, liveliness is reduced and the crab may become comatose.

5.3.3 Biochemical indices

Under conditions of stress imposition, metabolic shifts are required to increase the oxygen affinity of the haemocyanin in the blood of the crab (Bridges 2001). Several compounds have been shown to be important for raising haemolymph oxygen affinity: pH, lactate, urate and calcium. Changes in metabolism can therefore be measured by determining changes in blood chemicals. Hence, objective indicators of stress can be obtained from measuring biochemical components in the crab haemolymph (blood) which change in direct response to changes in the metabolism of the crab. Parameters commonly measured in crustacea for this purpose are: partial pressures of oxygen and carbon dioxide, acid-base balance through pH; and total circulating protein, glucose, lactate and specific ions such as calcium, phosphate and magnesium. Excretion of ammonia can also be used as an indicator of metabolic change. As ammonia is a by-product of protein catabolism care needs to be taken with this parameter to ensure feeding history of the animal is known and considered. Uric acid is an end product of nitrogen metabolism and hence can be a marker of oxidative stress, relevant to periods of emersion (air exposure).

In this study, we restricted investigation to those parameters that could be measured readily and hence be useful as stress indicators for crabs. We did not determine gaseous partial pressures within the animals as the effect of emersion on mud crabs has been previously reported.

5.3.3.1 Glucose

Glucose is used as the major energy source in crabs by metabolism through the glycolytic pathway. Increases in circulating glucose levels have been demonstrated in the haemolymph of many crab species in response to environmental changes (Hardy *et al.* 1994; Sneddon *et al.* 1999). The glycolytic mechanisms employed by crustacea in response to various physiological stresses are essentially the same as that in higher vertebrates. Glucose is known to accumulate in the haemolymph in response to anoxia (van Aardt 1988) and often does so rapidly. (Johnson and Uglow 1985) found that circulating glucose levels in haemolymph from both green and velvet crab increased significantly to maximum levels after 4 hours of emersion with a subsequent decrease to normoxic levels after 24 hours exposure to air. It was suggested many years ago (Abramowitz *et al.* 1944; Kleinholz and Little 1949) that glycaemic responses in crustacea are regulated through a hyperglycaemic hormone and this appropriately explains the initial rise in circulating blood glucose followed by return to levels present in the animal pre-emersion within a short period. It was considered valuable to measure increase in glucose levels in mud crab haemolymph as an initial indicator of any stress imposed on the animals.

Glucose present in haemolymph of mud crabs at rest ranged from 0.02 – 1.18 mmol/L. (Figure 5.23) with a mean of 0.31 ± 0.22 mmol/L. This basal glucose level present in mud crab was similar to that reported for other commercial crab species. Of the total number of crabs 'at rest', 83% showed circulating blood glucose levels lower than 0.5 mmol/L, suggesting that blood glucose levels below this indicates an 'unstressed' crab.

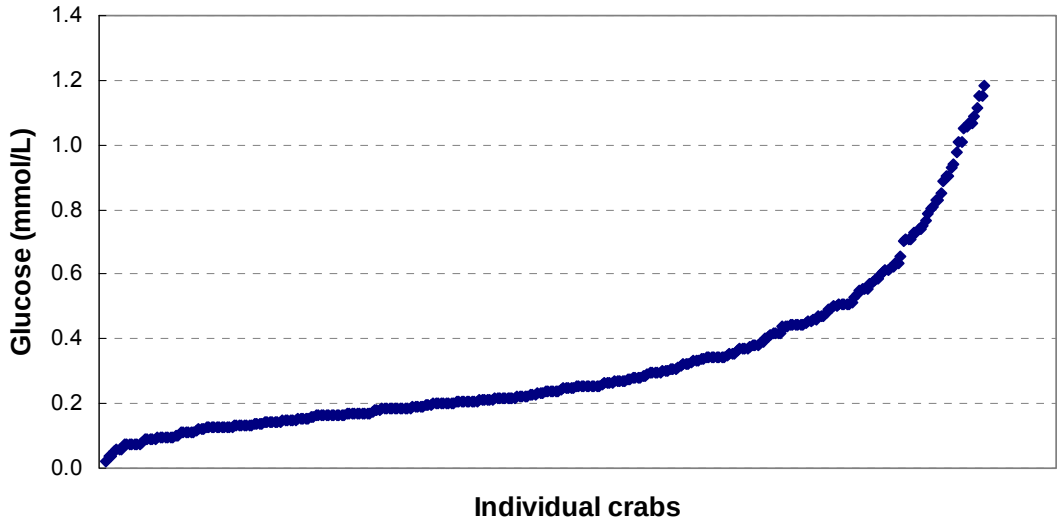

Figure 5.23. Haemolymph glucose levels in rested mud crab (*n* = 341).

Individual crabs exhibiting haemolymph glucose levels between 0.5 and 1.2 mmol/L may have already reacted to the small amount of handling stress imposed by the process of extracting the blood sample, even though this was done within 30 seconds of removing the crab from the water. Results indicated that even slight amounts of gentle handling caused elevated glucose levels. Where crabs had been handled for identification marking and then bled immediately, circulating glucose levels were between 1.5 and 2.4 mmol/L (*n* = 8).

Figure 5.24 includes the haemolymph glucose levels in crabs subjected to different levels of stress as well as rested crabs and, as expected, it was found that glucose

levels do increase in direct response to stress applied. For example, in a specific trial with extreme conditions of emersed crab (48 hours) at elevated temperatures (35°C) resulted in a blood glucose level of 6.41 mmol/L.

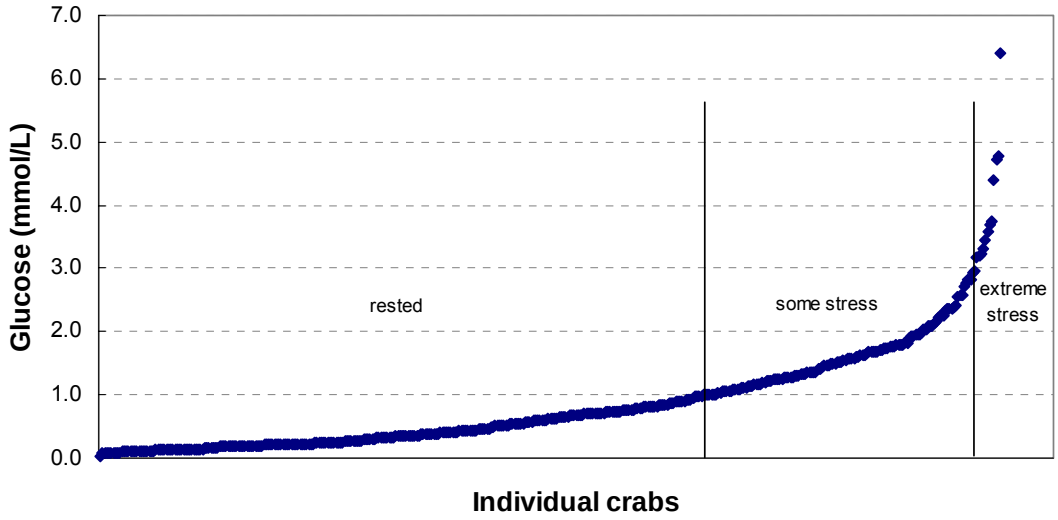

Figure 5.24. Haemolymph glucose levels in rested and stressed crabs (*n*=425).

Those crabs showing significantly elevated glucose levels in their blood, >4.0 mmol/L, had all endured various extreme handling/holding conditions, for example crabs exposed to 48 hours of steady breeze.

When correlating stress experienced by the crab with glucose levels measured in the blood, practical stress level indication cut-off levels are evidenced (Table 5.5). Generally the higher the level, the more likely mortality will occur.

Table 5.5. Haemolymph glucose levels in crabs subjected to different stress.

Haemolymph Glucose (mmol/L)	**State of crab**
<1.0	'rested' crab
1.0 – 2.0	some stress has occurred
2.0 – 3.0	high stress but will recover under resting conditions
>3.0	extreme stress experienced, high likelihood of mortality

5.3.3.2 Lactate

When a crab is exposed to conditions of low oxygen supply, it cannot metabolise energy reserves through the glycolytic pathway properly and hence conversion processes are incomplete. One of the by-products of anaerobic (no oxygen) respiration in a crab is lactic acid (lactate) which accumulates in the blood causing respiratory acidosis and changes the acid-base balance of the haemolymph (Booth *et al.* 1982; Booth *et al.* 1984; Booth and McMahon 1985). Therefore in this study, it

was relevant to assess lactate as a potential indicator of stress imposed on mud crabs.

Levels of lactic acid circulating in mud crab haemolymph when in a rested state ranged from <0.1 – 3.91 mmol/L (Figure 5.25) with a mean of 0.61 mmol/L. The lactate levels obtained from all rested crabs illustrated that 91% had levels <1.3 mmol/L.

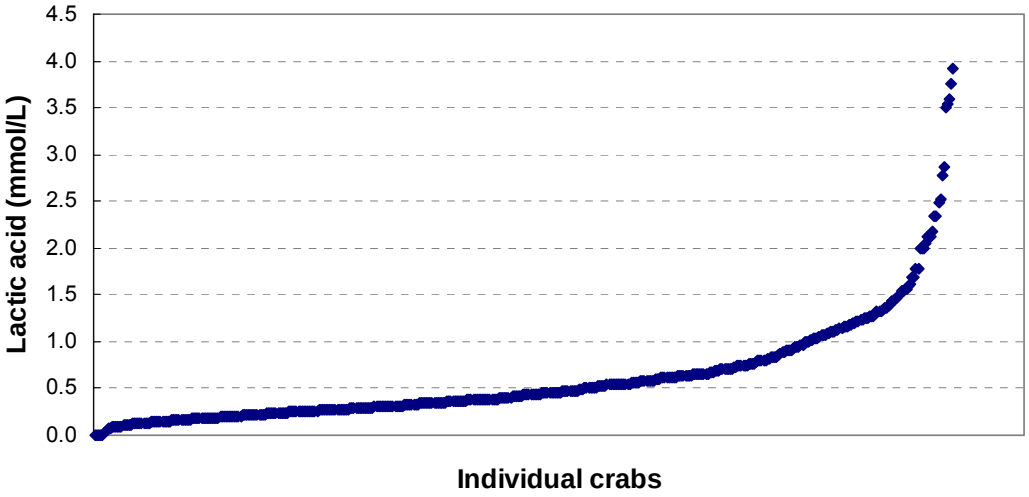

Figure 5.25. Haemolymph lactic acid levels in rested mud crabs (*n* = 462).

This data would suggest that in mud crabs, lactate levels of <1.0 mmol/L could be used to indicate rested crab, not suffering from stress. This result is similar to that found by Varley and Greenaway (1992) who demonstrated lactate levels of 1.25 ± 1.04 mmol/L for at-rest mud crab and also similar to reported levels for other species, for example *Cancer pagurus* – subtidal brown crab (Whyman *et al.* 1985; Danford *et al.* 2002).

The lactate levels in rested batches of other commercial crab species from different tidal habitats are reported as less than that for mud crab:
Carcinus maenas (green crab, intertidal): 0.14 ± 0.12mmol/L (de Wachter *et al.* 1997)
Maia squinado (spider crab - subtidal): 0.16 ± 0.01mmol/L (Durand *et al.* 2000)
Callinectes sapidus (blue crab - intertidal): 0.10 ± 0.09mmol/L (de Fur *et al.* 1990)
Liocarcinus puber (velvet crab - subtidal) 0.05 ± 0.03mmol/L (Whyman *et al.* 1985)

For the 5% of rested mud crabs that exhibited lactate levels ≥1 mmol/L, it is possible that the elevated levels were caused by the small amount of disturbance or annoyance that occurs in the holding tank during sampling and removing the crabs from the water. Although haemolymph samples are taken from a crab within 30 seconds of removal of the animal from water, blood biochemical changes may occur within this short time. Other researchers have suggested not, commenting that acute hypoxia, emersion for a very brief period, would not be expected to produce intrinsic molecular change (deFur *et* al, 1990). To react biochemically, the animal must first perceive the change in environment (de Wachter *et al*, 1997, Danford *et al*, 2002). However for mud crabs, the suggestion that a little disturbance is reflected in an almost instant biochemical response arises from evidence through experimental work to determine timeframes for responses within the haemolymph of mud crabs.

Initial haemolymph lactate levels were indicative of at-rest crabs (<1.0 mmol/L). Further sampling, immediately after the crab was gently taken fully from the water, illustrates that lactate response was rapid, increasing strongly over the first 12 minutes after emersion (Figure 5.26).

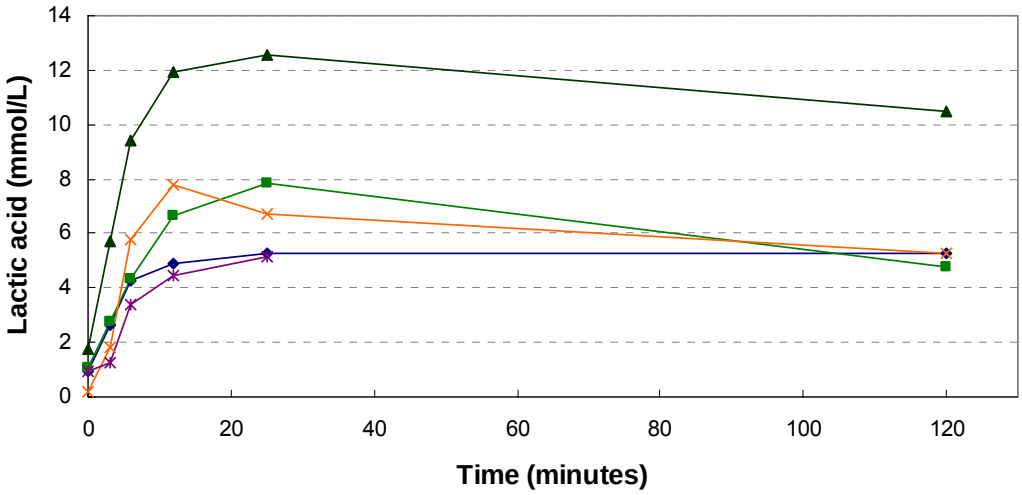

Figure 5.26. Lactic acid response in individual mud crabs immediately post-emersion.

This is new information with respect to response to environmental changes in mud crabs and has high significance for harvesting and handling practices.

Further, lactate presence in crab haemolymph was shown to change rapidly in response to stress stimuli (Table 5.6). For example, consistent disturbance (though gentle) of a crab for 15 minutes caused circulating lactate levels to rise 65-fold. When the disturbance stopped and the crab was allowed to rest, the metabolic processes instigated by stress continued their path evidenced by no reduction in lactate level after 30 minutes. Levels reduced slowly while crabs were allowed to relax, isolated in dark conditions covered in damp hessian.

Table 5.6. Circulating lactic acid in response to disturbance stress.

Treatment	Lactic acid (mmol/L)
at rest	0.17
emersed in damp hessian, 3h	7.83
‘annoyed’ for 15min	11.08
breeze for 48 hours	35.88

When subjected to any type of stress, mud crabs exhibit elevated lactate levels in their haemolymph. The lactate response of individual crab varies in magnitude, but handling disturbance definitely causes rise in blood lactate level. Lactate levels reached >10 mmol/L in those crabs subjected to severe stress (Figure 5.27) however, direct correlation of high lactate levels in the blood corresponding to extreme stress within the animal does not provide clear cut categories as glucose does.

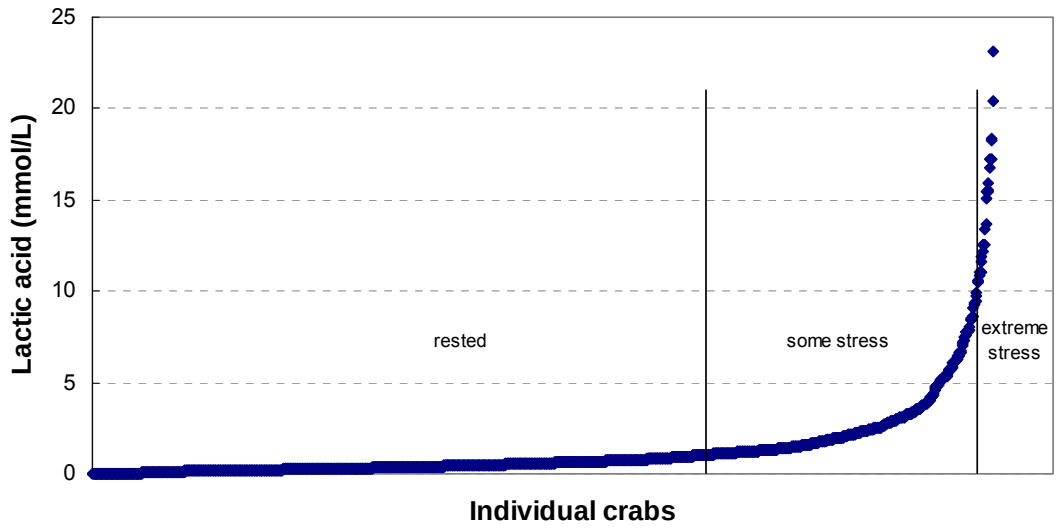

Figure 5.27. Lactic acid levels in haemolymph of crabs both rested and subjected to stress (*n* = 1415).

Correlating the degree of stress experienced by the crab to the lactate presence in the blood shows a variable picture, in part due to the individual crab's ability to tolerate stress impacts. Certainly, lactic acid levels were extremely high in crabs at the point of death: 38.0, 57.7 and 84.7 mmol/L (data not shown in Figure 5.27 for scale clarity). Levels between 10.6 and 20.4 mmol/L were attained when crabs had been subjected to severely adverse treatment and holding conditions, however where the external stress was of limited duration (<48 hours), these crabs recovered to 'resting' levels when re-immersed under favourable conditions. It is known that mud crab, *Scylla serrata* can maintain some degree of aerobic metabolism during emersion periods (Varley and Greenaway, 1992). Consequently they may be able to avoid the metabolic acidosis caused by accumulation of high levels of lactic acid. Mud crabs are well adapted to air exposure and in fact, are one of the few aquatic crabs that voluntarily leave the water when the water quality deteriorates.

It is unlikely that lactate by itself is a useful indicator of stress. This is further supported by (Truchot 1980) who showed that at concentrations between 0 and 15 mmol/L, lactate increases the affinity of haemocyanin for oxygen in two crab species. This may be a compensatory mechanism of crabs to hypoxic conditions. However, extremely high levels of lactate (>20 mmol/L) would suggest that the crab is seriously stressed and likelihood of death is acute. Lactate levels between 10 and 20 mmol/L illustrate an animal is suffering stress but careful re-immersion of the crab in a favourable aquatic environment will likely allow recovery.

5.3.3.3 Haemolymph pH

The pH of crab haemolymph is reactive in direct response to metabolic shifts affecting the acid-base balance within the animal. These shifts are engendered from changes in the physiological environment. Small changes in pH indicate major metabolic shifts within the crab and many factors can affect the measured pH including feeding, activity, environmental conditions and stress.

The physiological basal pH of mud crab haemolymph is around pH 7.5, similar to that reported by Varley and Greenaway (1992) and to that of other commercial crab species (Whyman *et al.* 1985; Lallier *et al.* 1987; de Fur *et al.* 1990)

Normal crab activity having an effect on haemolymph is evidenced by the variation of pH obtained for crabs in a rested state. pH for these mud crabs ranged from 7.23 – 7.82 with a mean of pH 7.55 ± 0.11 (Figure 5.28).

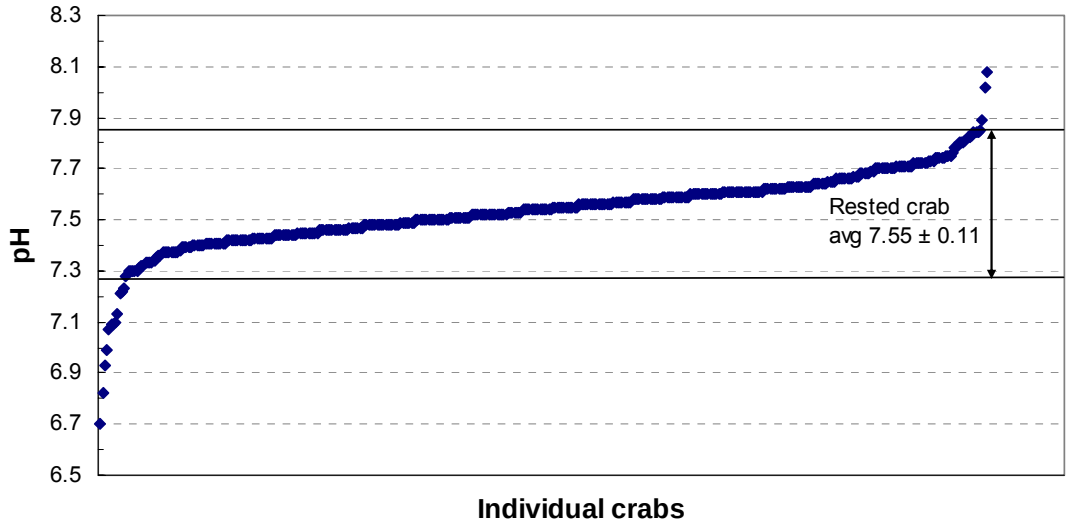

Figure 5.28. Haemolymph pH of 'rested' and stressed mud crabs (*n* = 414).

Crabs exhibiting pH values in the upper and lower extremes of Figure 5.28, had been subjected to severe stress of extreme temperature or extended emersion and hence it is deducted that very high (>7.9) or very low (<7.2) pH is indicative of a severely stressed crab that has a high likelihood of imminent death. However for some crabs subjected to similar conditions, pH remained within the 'rested' value limits. Hence, pH alone is not an effective indicator of stress levels in crab but is useful when combined with other indicator parameters.

Haemolymph pH is dictated by levels of metabolic end-product compounds present in the blood and changes in the physical state of the crab's environment. For example, when crabs are removed from water and held in air, pH decreases. When re-immersed in seawater and allowed to recover, pH rapidly returns to basal levels of ~7.65 pH units, however, inter-animal individual response is evident (Figure 5.29).

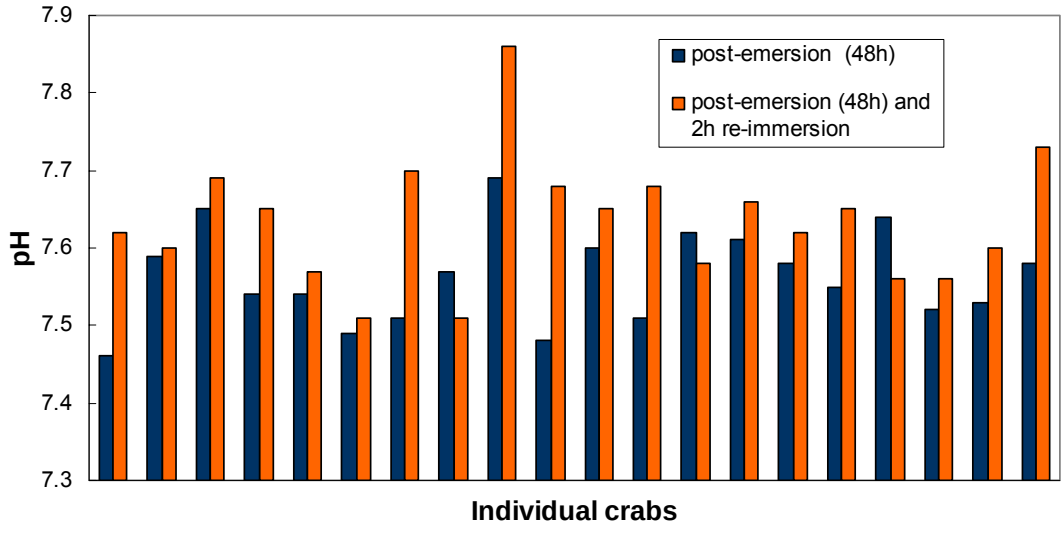

Figure 5.29. Haemolymph pH after emersion and recovery of individual crabs.

Since the pH of haemolymph directly reflects metabolic activity, certain metabolic by-products correlate well with pH changes. Figure 5.30 illustrates a correlation of circulating lactate levels with blood pH (for visual clarity, the depiction is not on a time scale but values after different periods of stress subjection. Where lactic acid levels are high, pH is low and inversely, a low lactate level reflects in a higher pH. Such a relationship being directly inverse suggests that lactate is the dominant factor determining blood pH.

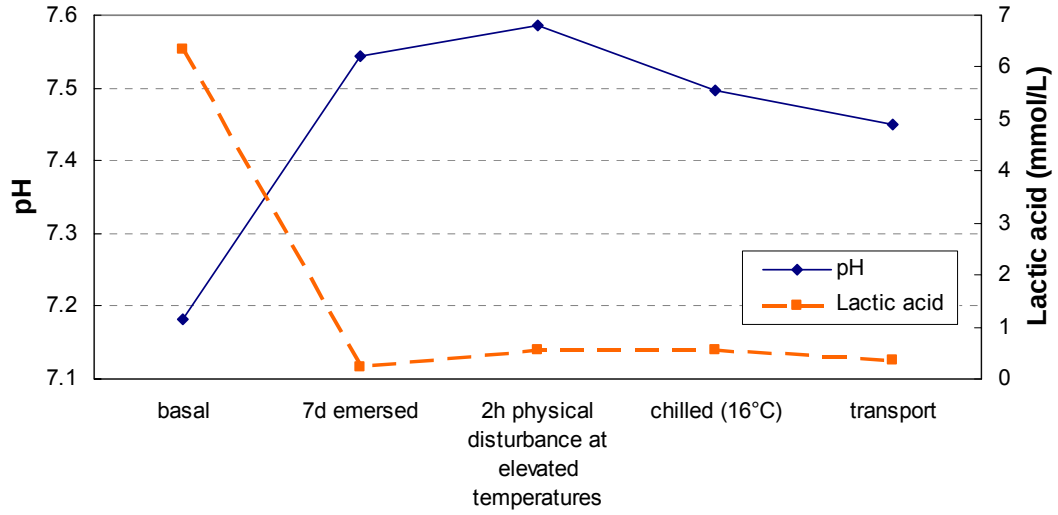

Figure 5.30. Lactate and pH change when crabs are subjected to different stresses sequentially (*n* = 8).

The reactiveness of haemolymph pH to emersion is very rapid as illustrated in the following figures depicting change in blood pH of individual crab immediately following emersion (Figure 5.31, 5.32, *n.b*. these graphs have different scales for clear depiction of data) This suggests that metabolic changes in mud crabs occur very rapidly. It also evidences the importance of the 30-second blood sampling rule, since when blood is taken from the crab after 1 minute, the pH value is lower due to stress reaction of the crab to handling during sampling and is not an accurate measure of the basal blood pH level.

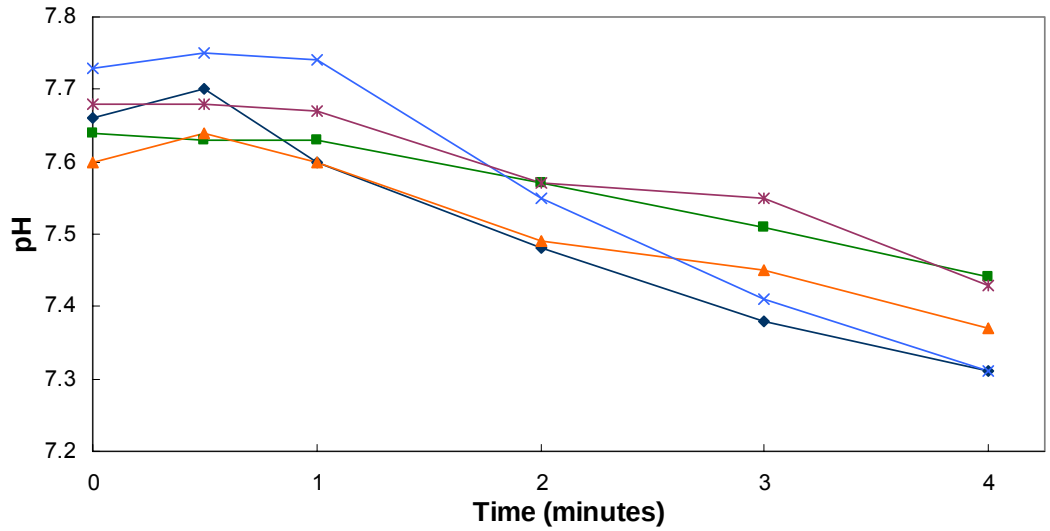

Figure 5.31. Blood pH of individual crabs over the first 5 minutes immediately post-emersion.

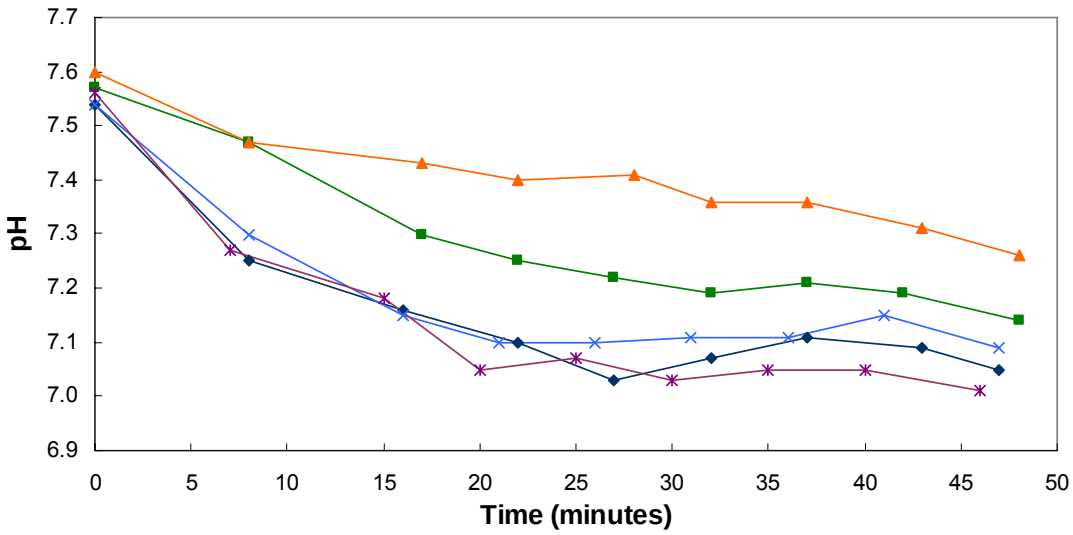

Figure 5.32. Blood pH of individual crabs during 50 minutes immediately post-emersion.

After 25 minutes, the decrease in pH slows and after 2 hours of air exposure the pH is returning towards basal levels (Figure 5.33)

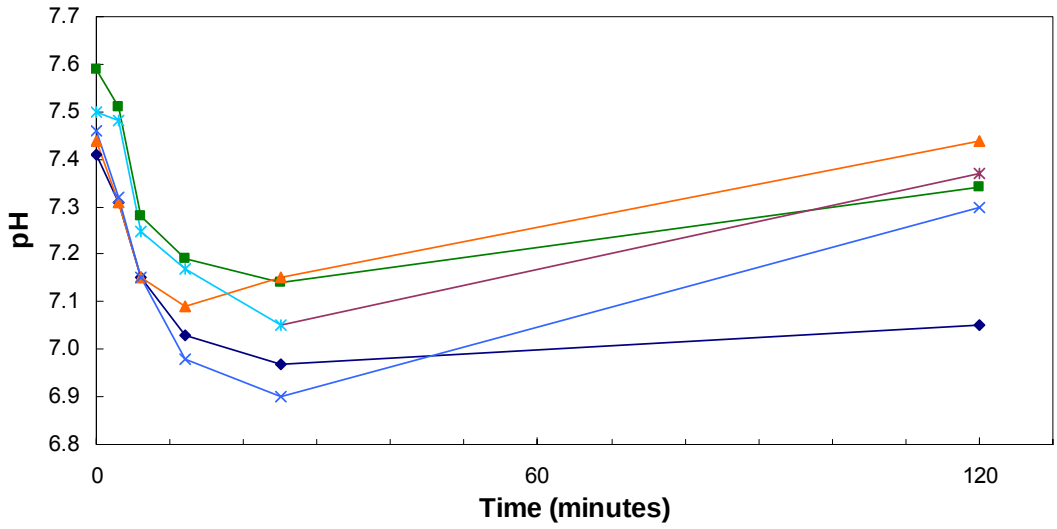

Figure 5.33. Haemolymph pH of individual crabs during 2 hours immediately post-emersion.

The pH reflects all metabolic compounds within the crab haemolymph, including carbon dioxide and ammonia (CO_2 and NH_4^+) levels. Carbon dioxide circulating in blood has an inverse relationship with pH, that is, an increase in CO_2 will cause a drop in pH (Varley and Greenway, 1992). This was not directly observed from the data obtained in this study and hence would suggest CO_2 building up during emersion is being converted by the crab through anaerobic pathways. Since the end-product of anaerobsis is lactate the mud crab should experience strong acidosis during long periods of emersion. However, we did not observe low pH values in any of our trials which provides further evidence that mud crab have compensating systems and do not suffer from acidosis.

From the information attained on change in haemolymph pH, it is concluded that pH is a useful indicator of immediate metabolic shifts in mud crabs.

5.3.3.4 Uric acid

Uric acid is an end product of nitrogen metabolism and has been suggested as a marker of oxidative stress (Becker 1993). Therefore, it could be expected that high uric acid levels would indicate stress incurred from periods of extended emersion of crabs. It is produced from biochemical reactions involving purine and pyrimidine bases, namely through conversion of xanthine to uric acid with the release of non-ionic ammonia. Although uric acid is routinely measured in human blood and tissues, due to its importance with respect to gout, its significance in crustacean haemolymph has not been widely studied. De Fur *et al* (1990) demonstrated that uric acid accumulates in decapod crustacea exposed to environmental hypoxia and Lallier *et al* (1987) described similar response in crabs exposed to elevated temperatures. While urate has been suggested to positively modulate oxygen affinity of haemocyanin in crab haemolymph (Morris *et al.* 1985; Morris *et al.* 1986), this was not the case in blue crab, *Callinectes sapidus* (Lallier and Walsh 1990).

Uric acid levels were determined in the haemolymph of mud crabs subjected to a variety of experimental conditions and stress levels. The uric acid levels measured ranged from 4.3 - 238 µmol/L (Figure 5.34). Rested crabs demonstrated basal levels anywhere within this range and, in fact, the highest values obtained were from rested crabs.

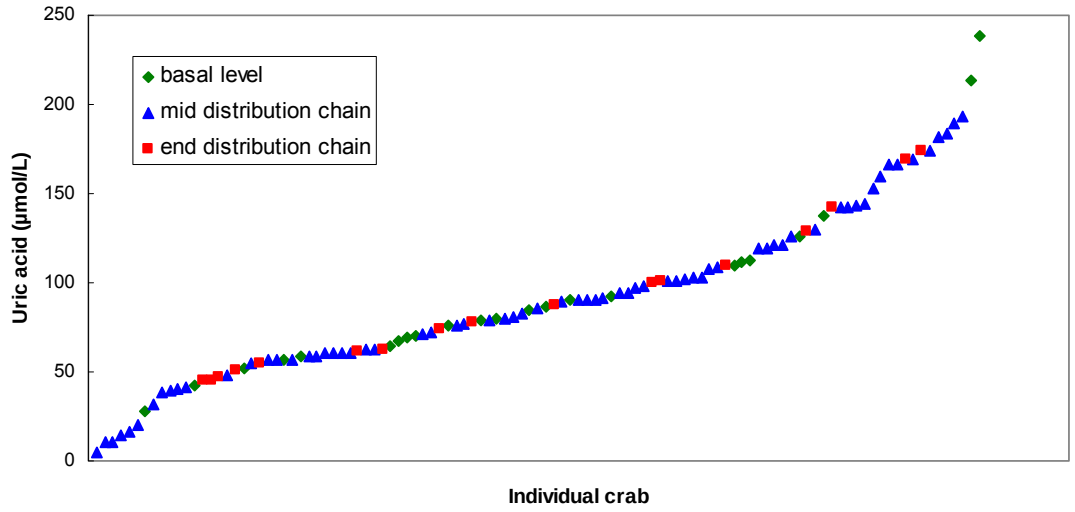

Figure 5.34. Uric acid levels in mud crab haemolymph (*n* = 110).

The average uric acid level in haemolymph of rested crab was 93.33 ± 49.48 µmol/L and this is similar to that reported for other crab species (Durand, 2000). The average level of uric acid circulating in the haemolymph of all crabs (both rested and stressed) was 91.45 ± 46.96 µmol/L. This value is not significantly different (P>0.05) from that for rested crab only and hence implies that haemolymph uric acid level is independent of the stress level experienced by the crab. This finding is contrary to that of other researchers who observed an accumulation of urate in crabs during emersion (Regnault 1992; Durand and Regnault 1998). The conclusion of independence between urate levels and stress in mud crab was confirmed within several separate trials where crabs were subjected to increasing accumulative

stress impositions and yet haemolymph uric acid varied randomly between trials with no observable pattern (Figure 5.35).

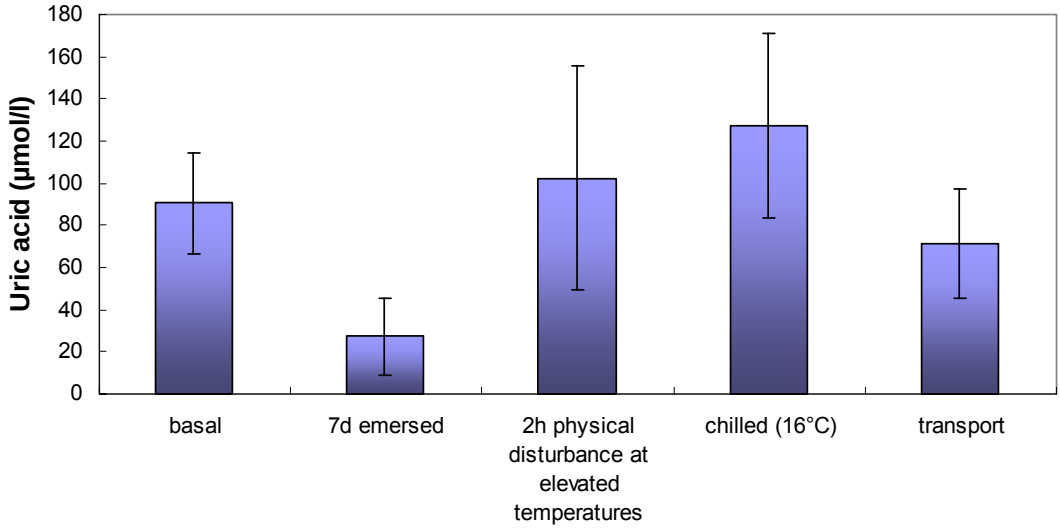

Figure 5.35. Uric acid levels from trials of imposed stress to the crabs (*n* = 8).

Nitrogen excretion is well studied in several crab species (Regnault 1987; Chen and Chia 1996; Durand *et al.* 2000; Kunzmann *et al.* 2007) and the uricolytic pathway could be a metabolic mechanism to assist the crab in excretion of excess nitrogen. However, it is thought to contribute only a small proportion of the total ammonia excreted compared to the predominant production from amino acid catabolism (Weihrauch, 2004). Many crustacean species are known to store nitrogen as uric acid (Greenaway 1991). In this study, uric acid levels in tissue samples were not determined as the focus of the research was not pathological studies but rather, live animals.

5.3.3.5 Ammonia

Ammonia is a metabolic by-product of protein breakdown or utilisation and is highly toxic to crabs. Build up of ammonia in the haemolymph is prevented by rapid gaseous exchange across the gills when crabs are in water. However, during periods of emersion this cannot occur and so an increase in circulating ammonia in the crab blood would be expected (Durand and Regnault 1998). This can be measured and correlated to levels of stress experienced by the crab.

Ammonia is the main nitrogenous by-product excreted by crustaceans. In aquatic environments, ammonia is excreted not via urine (Weihrauch *et al.* 2004) but through the gills by both passive diffusion and active sodium ion/NH_4^+ exchange (Regnault, 1987; (Weihrauch *et al.* 1999). Within crustacean haemolymph, both forms of ammonia: NH_3 and NH_4^+ exist in equilibrium, with the balance being pH dependent. For example, at a physiological pH of 7.8, the ionic form (NH_4^+) predominates comprising 98% of the total ammonia present (Weihrauch *et al.*, 2004). However, the non-ionic form (NH_3) is more diffusible through phospholipid membranes such as gills.

5.3.3.5.1 Ammonia in mud crab haemolymph

Ammonia in crab haemolymph is present as either NH_3 or NH_4^+ and exchanges between the two forms depending on the pH of the blood. Both forms are toxic to the animal as levels increase, with NH_4^+ form considered of greater toxicity than NH_3. Both forms of ammonia are usually removed by exchange across the gills in an aquatic environment but this can not occur during emersion and hence an accumulation of ammonia is expected.

Ammonia in rested mud crabs (*n* = 43) ranged from 0.66 – 21.85 mg/ml, with an average value of 7.17 ± 3.44 mg/ml. However, exactly the same range (0.66 – 21.85 mg/ml) was observed in crabs whether in resting state or after different stress factors had been imposed on them (*n* = 140). This data illustrates there is no difference between ammonia levels in stressed crab and rested crab and the expected accumulation does not appear to occur in mud crabs. In separate experiments, some trends were evident. Table 5.7 gives the ammonia levels present in crab blood through phases of handling imposing stress on the animals: from rested animals to air exposure and then during recovery.

Table 5.7. Changes in ammonia levels in crab haemolymph.

Treatment	**n**	**Ammonia** (mg/ml)	s.d.
Rested	24	7.17	1.84
Emersed - 48hr	24	7.41	1.59
Re-immersed - 2hr recovery	24	5.19	1.97
After 3 days re-immersion in seawater (and fed)	16	28.18	21.71

It is of note that haemolymph ammonia did not increase during the emersion period when it is unable to be excreted. This result was unexpected although perhaps can be explained from studies by (Weihrauch *et al.* 2002) who found evidence of active ammonia excretion in the green shore crab (*Carcinus maenas*). The authors suggest that ammonia is removed from the blood and captured intracellularly, then actively transported from the cells by specialised transport proteins. The data in Table 5.7 supports this hypothesis through no observed elevation in blood ammonia levels during emersion for 48 hours. When the crabs were recovered in water for 2 hours, the blood ammonia level drops. The high ammonia present after crabs were re-immersed for 3 days was surprising but likely due to the crabs being fed during this period and hence increased ammonia levels could arise from breakdown of protein from the diet.

The effect of feeding on ammonia levels in the haemolymph is further evidenced from the 'rested' crab ammonia levels obtained during trials. Feed was always withheld for 24 hours prior to commencement of any stress subjection trial. However, feeding prior to this influences circulating ammonia levels in the blood while crabs are in a water environment as is illustrated by the wide variation in 'rested' crab haemolymph ammonia levels (Figure 5.36). Interestingly, this data demonstrates blood ammonia increased in 6 of the 8 crabs after exposure to air for three days.

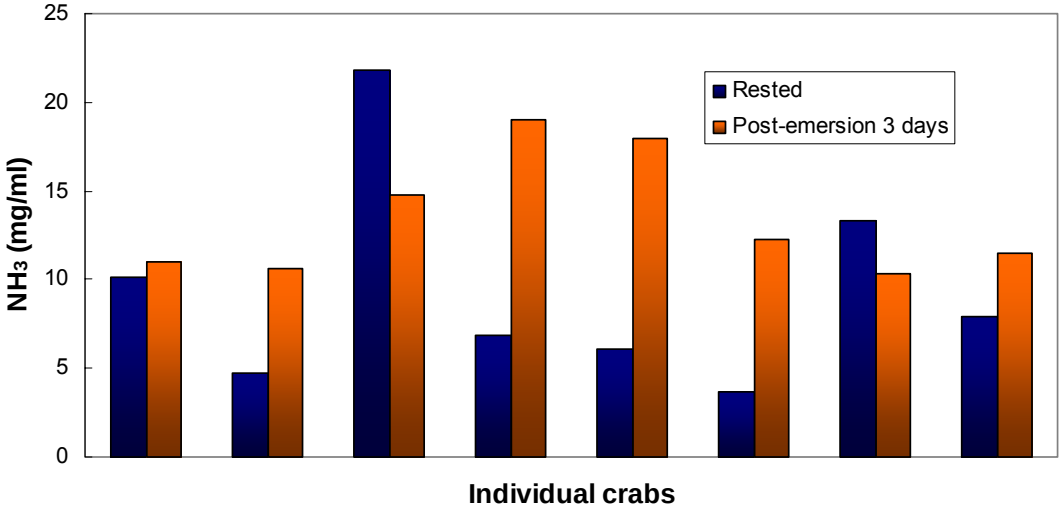

Figure 5.36. Ammonia present in crab haemolymph before and after 3 days emersion.

Emersion of aquatic crab species *Cancer pagurus* (edible crab) and *Cancer productus* (red rock crab) resulted in significantly increased production rates of haemolymph ammonia (de Fur and McMahon 1984; Regnault 1992). However, in *C.pagurus* the accumulation rate was noted to be lower than expected based on rates of accumulation when this crab was immersed, This suggests that excess nitrogen may be being stored in the crab tissues to avoid build up to toxic levels in the blood (Regnault. 1992). Many crustacean species are known to store nitrogen in solid form as uric acid (Greenaway 1991) suggesting a possible mechanism for crabs to remove excessive ammonia from their haemolymph. However, the accumulation of solid urate seems to occur directly in response to feeding rather than as a detoxification of ammonia from the blood (Linton and Greenaway 1997). In a review of ammonia excretion in crabs, (Weihrauch *et al.* 2004) provides a model for the possible vesicular sequestration of ionic ammonia, but states that this mechanism of removal of excess ammonia from crab haemolymph has not yet been considered nor demonstrated.

If ammonia is being removed from the haemolymph by encapsulation within gill cells as suggested by Weihrauch *et al* (2002), the scenario suggested by measuring haemolymph ammonia is not valid. Hence, it was considered that the rate of efflux of ammonia immediately after re-immersion would provide a more accurate picture of ammonia accumulation within crabs.

5.3.3.5.2 Excreted ammonia upon re-immersion

The rate of ammonia efflux from mud crabs re-immersed in seawater after 3 days emersion (Figure 5.37) shows a steady increase in ammonia excretion for 60 minutes. The rate of ammonia excretion appears to depend on the extent of stress the crabs are subjected to which would reflect the build up of ammonia within the animal.

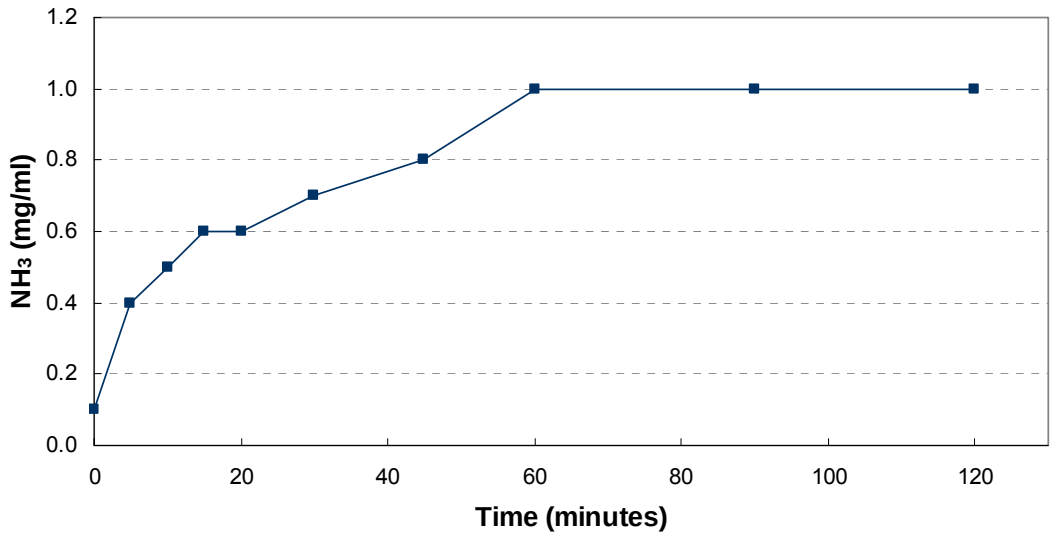

Figure 5.37. Ammonia excretion upon re-immersion after 3 days air exposure.

Danford and Uglow (2001) used ammonia efflux as an indicator of the severity and types of stress in the blue crab, *Callinectes* spp. The rate of excretion after mud crabs were exposed to different degrees of stress was illustrated during assessment of the 'commercial' transport chain for crabs from harvest to wholesaler under simulated conditions (section 5.4.3). Accumulation of ammonia was measured in the crabs by determining the excretion rate during re-immersion for 90 minutes in fresh seawater at 24°C at the end of each stage of the simulation. Results showed a definite correlation between stress imposed and rate of ammonia efflux. For example, crabs emersed for 7 days but held quietly in damp hessian excreted ammonia less rapidly upon re-immersion than those after subjection to severe physical disturbance at elevated temperatures (Figure 5.38)

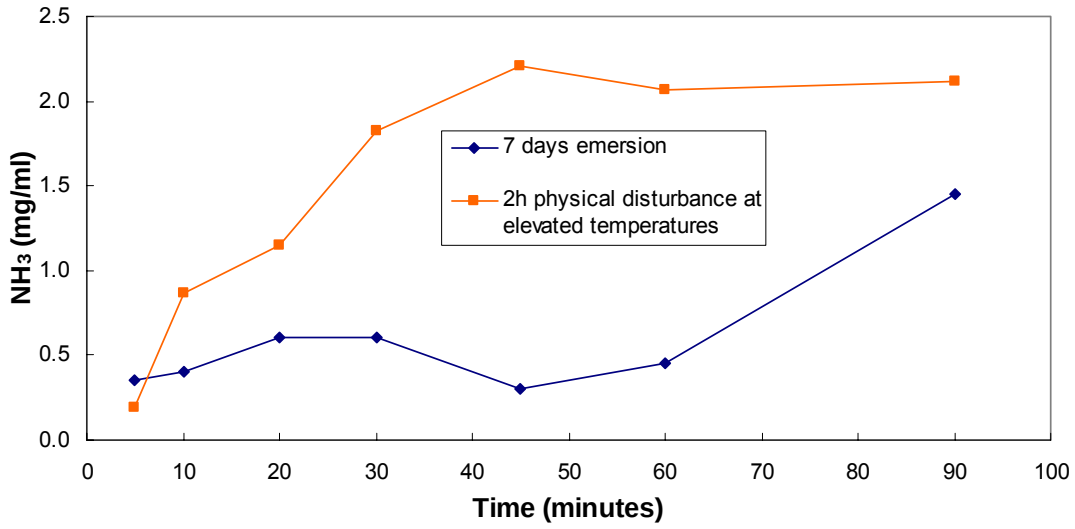

Figure 5.38. Excretion rates of ammonia by crabs subjected to different stresses.

Similar differences in rates of ammonia excretion were observed when crabs were held (tied in damp hessian) at different temperatures for 48 hours (section 5.4.4.3). The data (Figure 5.39) shows that after 2 hours re-immersion, mud crabs that had

been held at 15°, 20° and 35°C were continuing to excrete ammonia at a greater rate than those held at 25° and 30°C.

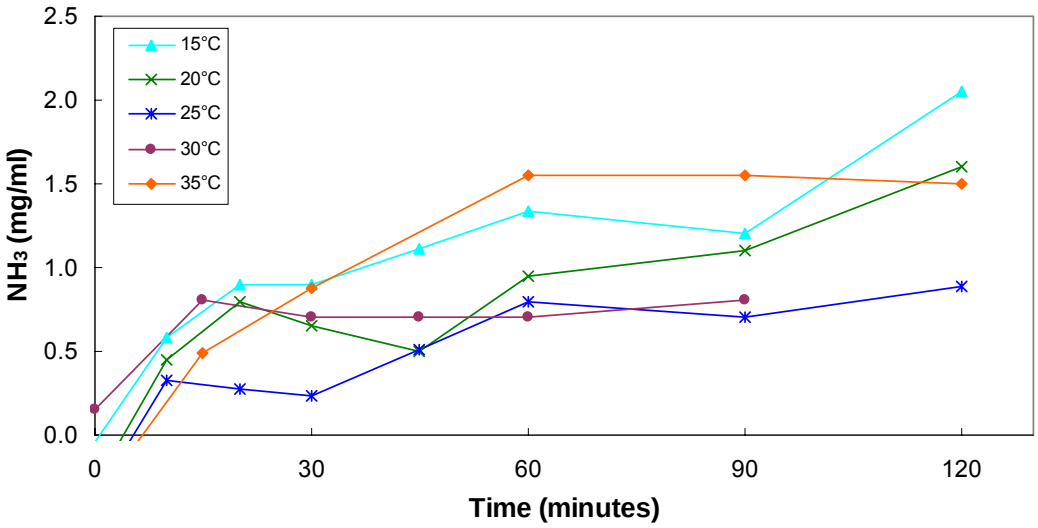

Figure 5.39. Ammonia excretion rates for crabs held at different temperatures for 48 hours prior.

The data obtained for mud crab demonstrates that ammonia excretion rate seems to be a better indicator of the stress level within the crab than ammonia presence in the haemolymph.

Ammonia excretion following re-immersion of marine crustacea has been little studied (Durand *et al*, 2000) and rates have not been documented. *Cancer pagurus* (brown or edible crab) exhibited a significant increase in ammonia excretion rate when re-immersed after a period of air exposure (Regnault 1994). During emersion the ammonia excretion rate was very low (~4% of that of the basal immersed rate). In the first five minutes of re-immersion jumped 50-fold. This excretion rate was illustrated as being transient as the ammonia excretion rate slowed with extended time back in the water. Similarly, Danford *et al.* (2002) noted that upon re-immersion of *Cancer pagurus*, all accumulated ammonia was removed from the haemolymph within 30 minutes and total ammonia returned to pre-emersion levels within 12-24 hours of re-immersion. Durand and Regnault (1998) reported different patterns of excretion in velvet crab (*Necor puber*) and green crab (*Carcinus maenas*). Both species released large amounts of ammonia within a few minutes of re-immersion but efflux was only enhanced for 3 hours in velvet crab compared to green crab that continued to excrete ammonia at an elevated rate for 24 hours. Durand and Regnault (1998) suggest that the difference in patterns of ammonia excretion (along with other biochemical measures) indicate specific processes are used to manage nitrogen overload induced by emersion (Danford and Uglow 2001) further evidenced the usefulness of ammonia excretion after re-immersion as an indicator of stress imposed on three species of *Callinectes*.

The green crab is an intertidal habitat species and is noted as able to survive prolonged air exposure (Truchot 1975) similar to the mud crab. Ammonia efflux data obtained with mud crab is also similar to that of the green crab and hence it is possible that similar mechanisms for ammonia detoxification occur in both species. Durand and Regnault (1998) suggested a possible regulation mechanism that accumulated nitrogen is stored by the formation of additional nitrogenous amino

acids within the crab's system. Upon re-immersion some trigger occurs (likely hormonal) that signals the breakdown of the excess amino acids created and subsequent release into the water environment through the usual gill exchange pathways. The same ammonia detoxifying mechanism was suggested to occur in *Carcinus maenas* but not in *Necor puber* (Durand *et al.* 1999). Within our study on mud crabs, samples of haemolymph were retained for future amino acid analysis to determine whether this regulation mechanism is indeed occurring in mud crabs as a response to nitrogen overload. Timeframe restrictions within this project and the difficulty of analysis of amino acids in the crab haemolymph due to the presence of copper which interferes with the analysis, have precluded detailed investigation of this possibility as yet.

5.3.3.6 Total protein in haemolymph

The total amount of protein circulating in the blood of a crab is, in the first instance, correlated to feeding cycles in the animal (Subhashini and Ravindranath 1982) Haemolymph protein was observed to decline significantly during periods of prolonged starvation (Uglow 1969; Busselen 1970) and this was considered to be related to either degradation of protein as an energy source or when the crab remains in water, due to haemodilution as suggested by (Dall 1974) with rock lobsters. For *Scylla serrata*, Subhashini and Ravindranath (1982) confirmed that a drop in circulating blood protein is due to protein degradation during starvation periods. The total protein level present may also be influenced by other factors such as bleeding stress after limb loss (Anbarasu and Ramalingam 1992), phase of moult cycle (Pratoomchat *et al*, 2002), environmental conditions (Kannupandi and Paulpandian 1975) and prolonged emersion (Durand *et al*, 1999).

Haemolymph protein concentration in *Scylla serrata* is reported to fluctuate according to time of day (Kannan and Ravindranath 1980; Arumugam and Ravindranath 1980`). Specifically, the researchers found that protein levels in the crab blood peaked around midday, with protein levels changing from a mean of ~53 mg/ml at 1030 hours, rising to 86 mg/ml at 1230 hours and returning to 55 mg/ml 4 hours later. Arumugam and Ravindranath (1980) did not elucidate a reason for the observation but simply concluded that this phenomenon occurs in mud crab.

It was therefore important to determine whether *S. serrata* from Australian waters demonstrated the same phenomenon. If so, and haemolymph protein is used as a stress biomarker in mud crab, the values obtained would be dependent upon the time of sampling so as to not skew stress indications. As this would affect the usefulness of total protein as an index, experiments were conducted to identify fluctuation times for haemolymph protein in *Scylla serrata* from Australian waters.

Experimental design
Experiments were designed following very similar procedures to those of Arumugam and Ravindranath (1980). Crabs were held under favourable aquatic conditions with saturated oxygen at 27^{o}C but feed was withheld for 72 hours prior to commencement of experiments. Test crabs (n = 10) were matched at each sampling time with 2 separate control crabs. A minimal blood sample was extracted and total protein assessed by refractometer measurement every 2 hours during the day and again at 24 hours. The test crabs were bled at each 2 hour sampling time but the crabs used as control samples were bled only once and then not used further in the trial. Such control samples are important to show whether any increase in blood protein level was due to stress induced by repeated extraction of blood from the test animals and/or whether an observed rise in protein level was resulting from removal

of blood and hence causing some degree of concentration of protein within the remaining haemolymph.

Haemolymph samples from individual crabs were taken every 2 hours for a total 8 hours. At each sampling time, total haemolymph protein was measured by refractive index with results given in Figure 5.40 (the experiment was replicated 4 times).

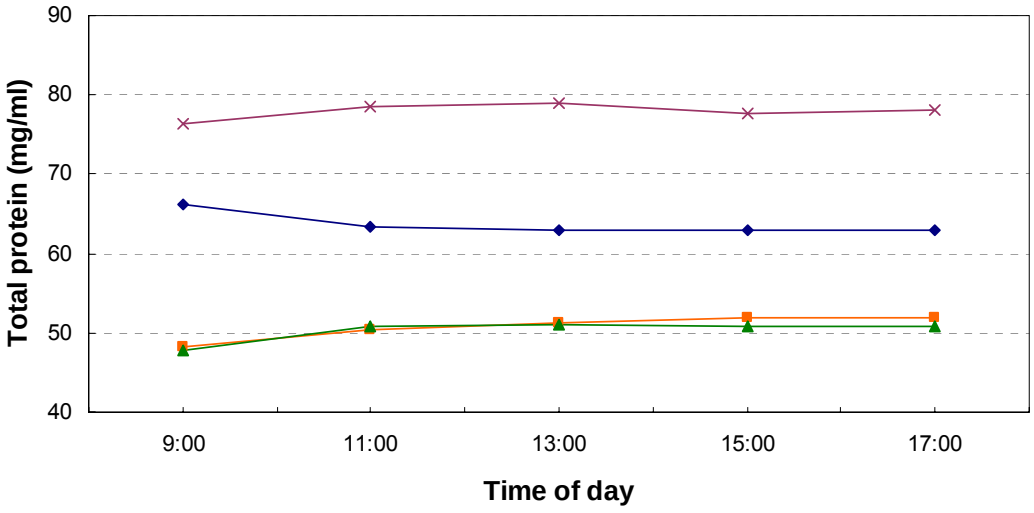

Figure 5.40. Total haemolymph serum protein in replicate trials (*n* = 10) at different times of day.

It is evident that no significant fluctuation in total protein present occurred in Australian mud crabs. This is in direct contrast to reports for Indian *Scylla serrata* but the reason for the difference is unexplained. However, on the basis of these results for Australian mud crab, the possible influence from time of day affecting protein levels in haemolymph was disregarded with respect to limiting validity of blood protein as a potential index.

The total protein present in mud crab haemolymph was measured in all experimental work (individual crabs: *n* = 439) and ranged from 29 mg/ml to 122 mg/ml. Total protein levels did not appear to vary related to amount of stress the crabs had endured (Table 5.8).

Table 5.8. Total haemolymph protein in crabs after subjection to different levels of stress.

Treatment	**Total protein** (mg/ml)
Rested	29-122
Medium stress imposed (emersion 48h)	42-113
Severe stress imposed (breeze)	38-122

The levels reported here are provided as an example but the picture holds true for any stress type the mud crabs were subjected throughout the research work. Hence, it is concluded that total protein circulating in mud crab is not a useful indicator of stress suffered by the animal.

However, in specific experimental work, total protein levels in crab haemolymph were relevant, for example under conditions of severe dehydration occurring due to prolonged emersion and exposure to breeze (section 5.4.4.2.6) and with respect to moult cycle. Total protein levels were observed throughout the moult cycle (Figure 5.41). Moult cycle dictates the fullness of crab and hence the robustness of the animal. A recently post-moult crab will not be fully robust and hence less tolerant of withstanding stresses imposed through handling and distribution transport. Relatively low protein levels for pre-moult crab can be indicative of weakness and can be predicted by haemolymph protein levels (manuscript in preparation).

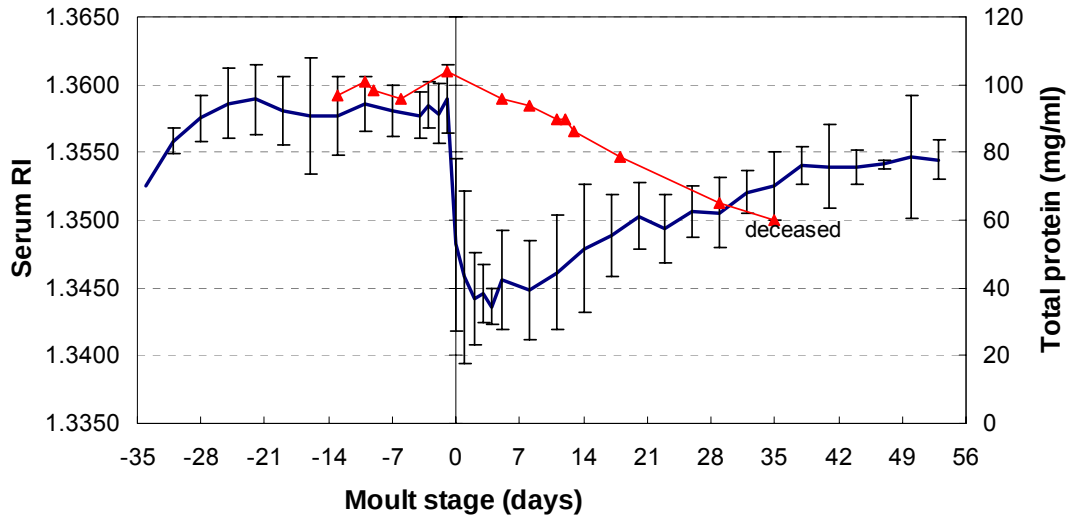

Figure 5.41. Total protein during moult cycle and a starved crab (*n* = 318).

Further discussion on this aspect of total protein in mud crabs is not given within this project report as the work was conducted additional to and outside the scope of the research project. It was undertaken as a separate project as part of the Advance in Seafood Leadership Program and will be published separately (John Mayze, 2008, pers. comm.).

5.3.3.7 Haemolymph colour

Additional to specific compounds noted above, it was observed during haemolymph sampling that the colour of the mud crab blood varied enormously from crab to individual crab. Crab haemolymph contains a pigment called haemocyanin which acts as the oxygen-carrying protein. Haemocyanin contains a copper molecule which results in a clear, blue-grey colour. This is similar to the iron within human blood haemoglobin which makes the blood red.

Throughout blood sampling over the entire research study, a complete range of haemolymph colours were observed: from blue-grey; light grey-blue; very pale grey neutral; light orange and deep orange (Plate 5.7).

From discussion with expert crustacean researchers (Dr. Richard Musgrove and Dr. Brian Paterson) such colour changes were attributed as likely being related to the moult cycle phase of the crab.

Plate 5.7. Colour range of haemolymph samples.

It is suggested that the grey-blue shades indicate early intermoult stage of the crab, progressing through accumulation of astaxanthin providing the deep orange haemolymph colour indicative of immediate pre-moult stage. Further analysis of this data, along with correlation to serum protein values, is the subject of future work and was not pursued within this study due to time constraints.

5.3.4 Summary

Metabolic changes within mud crabs are measurable through indicator parameters and these indices do correlate with stress level endured by the mud crabs. Since the biochemical indices of specific end-product compounds are inter-related, any one specific parameter measured alone does not necessarily tell the full story. Of all the haemolymph parameters measured, circulating glucose was most directly responsive for indicating level of stress imposed on the crab. However, to gain an accurate picture it is better to assess several parameters together – the most useful metabolic parameters appearing to be glucose, lactic acid and ammonia efflux.

5.4 Stress factors

Project Objective

Determine stress contributed by current post-harvest practices

5.4.1 Introduction

To achieve the greatest return to the industry, mud crabs are supplied to the market as live product. For crabs originated from the Northern Territory fishery, the physical demand on the animal is extreme as the crabs are harvested from remote locations, then transported to Darwin prior to further on-shipment to market – commonly Sydney, Melbourne and international. The duration of the supply chain from capture to receival at the retail sector can be up to 15 days. The market requirement is that the animals not only survive this period out of water but to arrive at market lively and vigorous. Amazingly, most mud crab do.

Sometimes a portion of the catch has to be rejected prior to packing for transport to market, either domestic or export and further mortalities occur at market and retail sectors. Losses range from a frequent 3% and can rise to 35% during the wet season (summer) months. A small number of the rejected crabs are likely damaged by physical injury through capture or cannibalistic aggression during transport due to close proximity with other crabs. During the wet season especially, a small number of crabs may be rendered unacceptable through fly larvae contamination often occurring in the early stages of the handling chain. However, the major portion of rejected crabs is deemed too weak (graded as ‘slow’) to survive further transport with no attributable reason obvious.

Mortality, decreased vigour (slowness) and diminished quality of crabs during transport reduces the economic value and competitiveness of the industry and restricts access to distant markets. Our ability to reduce the wastage of crabs at this point in the chain is restricted by our limited knowledge of the causes of ‘slowness’ and deaths.

The NT Mud Crab Fisherman’s Code of Practice identifies broad best practice options for the harvesting, storing and transporting of mud crab from remote locations, but more detailed knowledge is required on the effect of the handling practices on the mud crab’s physiological reactions to harvest and air-exposure (emersion).

For a crab graded as slow or weak, the likely cause is stress and the probability of mortality is high. Hence, to maximise survival of mud crab through their long transport chain to market we need to reduce stress factors and levels. Stress to a crab is induced by numerous factors, both environmental and physiological, some of which may not necessarily be lethal on their own but contribute in combination. A stress response occurs when the crab’s physiological system is pushed beyond its limits, hence causing different reactions to happen in an effort to compensate and maintain survival. It is also likely that stresses imposed will be accumulative. The foremost causes of stress arise from changes in the crab’s immediate environment and include:

1. Emersion – taking crabs out of water and holding in air
   Crabs are aquatic animals and to remove them from this environment for extended periods is unnatural causing stress to respiratory and metabolic systems. Mud crabs are among the exceptional species of the crustacea in that they can seemingly tolerate such treatment far more readily than many other crab species, up to 15 days.

2. Handling
   Mud crabs are handled frequently at different points along the supply chain. Each handling time involves physical movement of the crab and often a degree of shock. Hence, the type of handling and its severity directly influences stress levels suffered. For example, whether crabs are tied immediately upon removal from the pot, aggression level in the crowding situation within crates and 'roughness' of track/road during vehicle transport can influence the degree of physical movement/shock to the crabs.

3. Temperature change
   Mud crabs are accustomed to tropical waters and, being poikilothermic, often exhibit burrowing behaviour to regulate the temperate. Water and ambient temperatures can be excessively high during summer months (40^{o}-50^{o}C). Additionally, the transport chain dictates periods of sudden and severe temperature decrease. It is likely that it is not the physical surrounding temperature that results in stress, but the severity of the temperature change.

How an individual crab responds to and/or deals with the stresses imposed during the supply chain is directly dependent on the physiological condition of the crab. 'Condition' is influenced by animal health, physical damage, moulting cycle phase and growth/breeding phase. Clearly, a less than robust crab, in a weakened state, will have less capacity to deal with any level of stress imposed and will surrender to mortality more rapidly.

An additional stress factor through the long distribution chain is starvation. During periods of food deprivation, the crab must ultimately use its bodily reserves, a process known as catabolism. In crabs, blood protein reserves are used first followed by the hepatopancreas and eventually the muscle. Starvation is not likely to be a major cause of mortality in itself but it is rather a contributory factor reducing the stress tolerance capability of the animal.

In this section of the study, we assessed the various stresses imposed along the handling/transport chain and determined the impact of each with respect to crab condition, as measured by relevant stress indices identified in previous work (section 5.3.3).

5.4.2 Current distribution chain

By way of confirmation that crabs do suffer stress through the stages within the distribution chain, the stress levels of crabs were measured on arrival at base camp and then again on arrival at Brisbane, subsequent to typical handling and transport occurrence. Only vigour, pH, circulating total protein and lactate levels of the crabs were used as indices. Glucose was not determined due to its instability within a blood sample even when the sample was frozen in liquid nitrogen.

5.4.2.1 Seasonal differences

Harvesting areas in the Northern Territory are in tropical latitudes and therefore are subject to only two main seasons: wet – summer (November to April) and dry – winter (May to October). The main difference between seasons is not so much the maximum temperature reached but the diurnal temperature differential and the amount of rainfall, hence humidity level.

5.4.2.1.1 Total protein in crab haemolymph

Looking at differences in crabs between the seasons, total circulating protein in the haemolymph was assessed on blood samples taken immediately the crabs were brought to base-camp by refractometer measurements. Total protein values showed a slight trend towards a greater number of crabs having a higher level of protein in the wet season. The variation between each lot of 50 individual crabs for any one season was large however (Figure 5.42) and so the trend needs to be confirmed by collection of a lot more seasonal data.

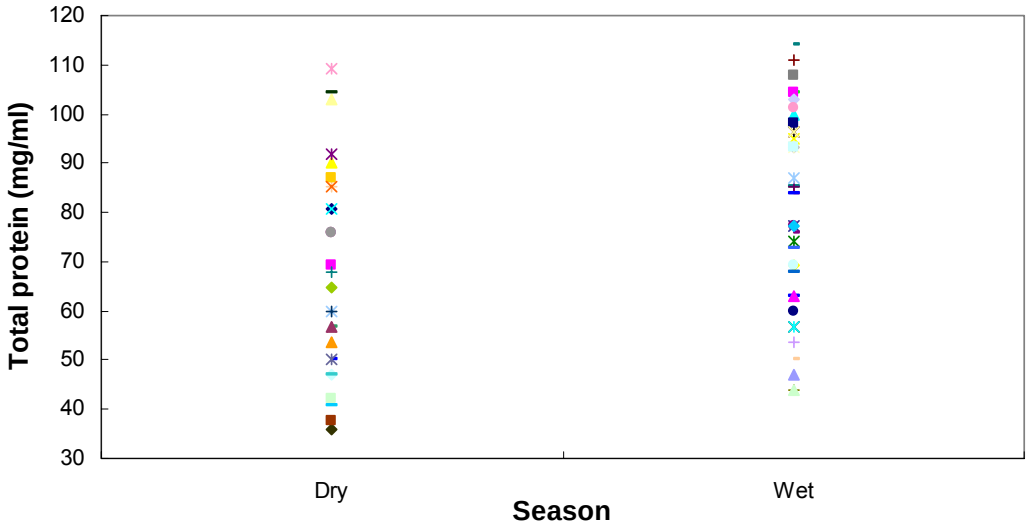

Figure 5.42. Total haemolymph protein in individual crabs harvested in different seasons.

Industry communication from experienced harvesters and wholesalers believe that there is a distinct variation in crab meat fullness related to time of year, with crabs said to be least full around May and fullest around November. As a correlation between crab meat fullness and total haemolymph protein has been established (John Mayze, 2008, pers. comm. publication in prep.) it was considered worthwhile to look at total blood protein data from all crabs obtained at different times throughout the year (Figure 5.43, no data obtained for some months due to lack of available crabs). Interestingly, total protein present in mud crab haemolymph appears to support the industry's belief that crabs are less full at specific times of year.

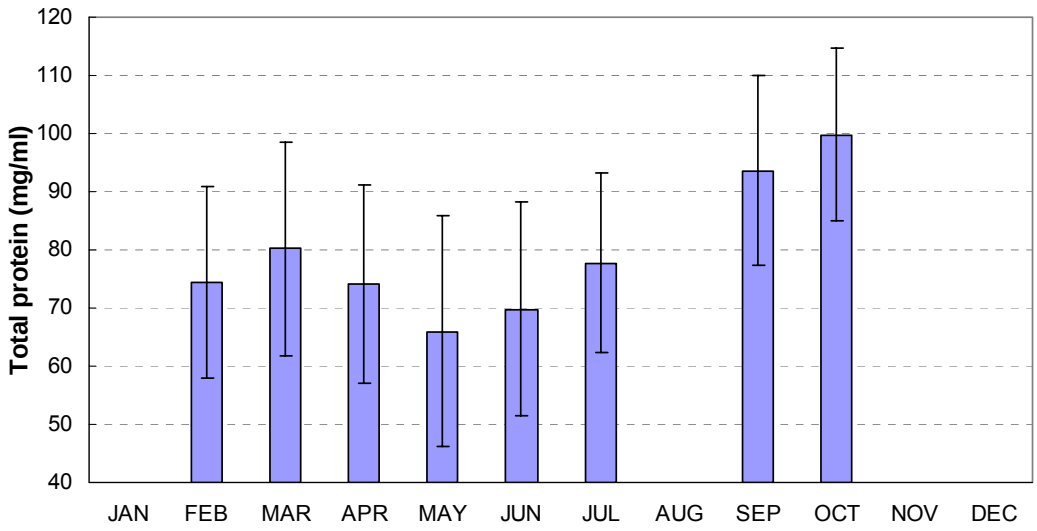

Figure 5.43. Total circulating haemolymph protein of crabs obtained at different months.

5.4.2.1.2 Haemolymph pH

The acid-base balance of haemolymph is affected by metabolic shifts and is readily assessed by blood pH value. Figure 5.44 shows the haemolymph pH from crabs harvested in wet (*n* = 41) and dry (*n* = 50, as two separate batches) seasons.

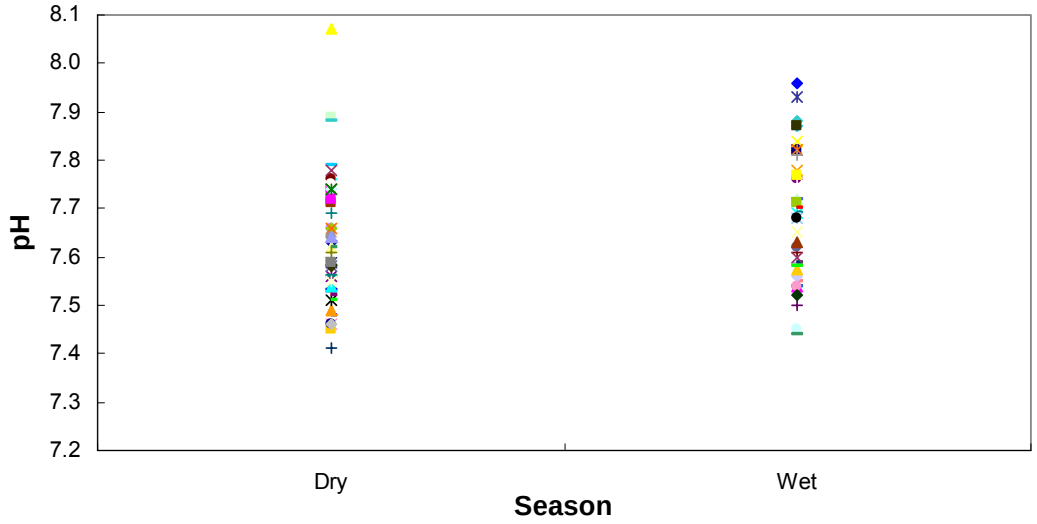

Figure 5.44. Haemolymph pH of crabs harvested in wet and dry seasons.

There is a large variation in haemolymph pH between individual crabs. Since a small shift in pH unit value represents a major change in the metabolic state of the crab. The results indicate a large variability between different metabolic responses (and perhaps the physiological condition) of individual crabs to capture, handling and transport and therefore negates conclusions being drawn from this information alone.

5.4.3 Harvest, handling and transport chain

In order to identify the specific sources of stress along the many stages within the mud crab distribution chain, it is necessary to characterize the events and influences throughout the supply chain from capture to market. Several variant chains exist within the NT mud crab fishery. It was decided to focus experimental work on one of the longest supply chains, where crab are harvested from the bottom of the Gulf of Carpentaria, the Wearyan River within the M^{c}Arthur River region and transported to Darwin for pack-out and on-shipment to southern markets.

Handling chain from the Wearyan River harvest point to market:

- at harvest, crabs are removed from the pot into the dinghy floor
- crabs are graded (under size, CUC and berried returned to water)
- crab claws are then tied and crabs placed in a hessian lined crate
- after pot-clearing, crabs are returned to base camp (~2-3 hours)
- crabs are removed from crate and sorted according to size into other crates lined with damp hessian
- crabs are held in this manner for up to 7 days during which time they are checked daily for liveliness or mortality
- a final sort and re-crating occurs before being placed on a 4WD vehicle for transport from the Wearyan River to Borroloola ~1.5 hours demanding driving over deep sand and rutted tracks
- at Borroloola, crab crates are stored in a cool room until the transporting truck arrives - up to 18 hours
- crabs are transferred to air-conditioned transport truck and driven straight through to Darwin - up to 18 hours
- at Darwin, crabs remain within the closed transport truck until early morning
- crates are transferred to air-conditioned factory
- crabs are cleaned or washed and individually graded for pack-out in waxed cartons - up to 7 hours
- cartons are transferred to van and taken to freight-forwarder at airport
- crabs are flown to markets

The airfreight sector of the transport chain can be highly variable in duration, depending on flight availability, final market destination and unexpected occurrences.

Other mud crab supply chains from remote harvest areas are similar with some specific variables that may have a significant role in stress accumulation. Crabs harvested at the mouth of the M^{c}Arthur River are transported from base camp to King Ash Bay by outboard powered 5 to 6m aluminium dinghies and picked up by air-conditioned truck prior to the Borroloola to Darwin leg. These crabs are not held chilled while awaiting truck transport. Crabs harvested from the Roper River are also held for up to 7 days at camp with a similar long transport stage out to Darwin. Some distribution involves a trucking stage from Darwin which can be 2-3 days duration.

Experimental design

The handling practices through the longest distribution chain (harvest at the Wearyan River) were simulated in Brisbane. The major stages within the chain were identified and recreated, as near as possible, using the suspected dominant factor within the stage. Naturally such simulation is not ideal, but is the only feasible way to obtain haemolymph samples throughout distribution. From previous field trips to this location, five major stages were recognised in this handling and transport chain (Table 5.9).

Table 5.9. Wearyan River supply chain stages.

Simulation phase of supply chain	Duration	Equivalent to and state of crabs
Landed	n/a	time 0, rested unstressed crabs
Camp	6 d	end of holding period at camp, claws tied, wrapped in damp hessian in crate, only slight disturbance each day checking for dead crab
4WD	2 h	arrival at Borroloola following 'rough' track transport from camp, Crabs in crates covered with tarp on back of 4WD vehicle
16°C	18 h	storage overnight in 16°C cool room Crabs still in crate
Processor	18 h	arrival at processor after refrigerated transport (1100km) Crabs still in crate

Two complete simulation trials were undertaken. All crabs in the trial were in intermoult phase. Batches of eight individual crabs were assessed for vigour and bled at the end of each simulation phase. To gain a true picture of what is happening physiologically within the crabs, ideally the same crab would have been re-sampled at each point of the simulation trial. However, as these crabs were emersed and therefore could not replace haemolymph taken from them, it was considered that sampling several different sets of crabs would avoid anomalies arising from haemolymph concentration with repetitive blood sampling.

Observations on liveliness of crabs were recorded and the following biomarkers were measured as per section 4: total protein, pH, glucose, lactate, uric acid, ammonia in the haemolymph and ammonia excreted after 90 minutes from crabs re-immersed in seawater.

Results and Discussion

The simulation trial was replicated with different batches of fresh crabs with every endeavour made to keep the simulated events as similar as possible. The temperature (Figure 5.45) and humidity (Figure 5.46) conditions are similar to those obtained from field-logged data (section 5.1).

The liveliness of the crabs was assessed at the time of taking haemolymph samples and was typical of that for emersed crab. Crabs were still and quiet while held in hessian in crates, but were highly responsive to movement disturbance. Vigour, assessed by strength of limb response when gentle pressure was applied, indicated that no crabs would be graded as 'slow' or weak. At the end of the trials, crab vigour was similar to that illustrated by crabs subjected to the usual commercial distribution handling.

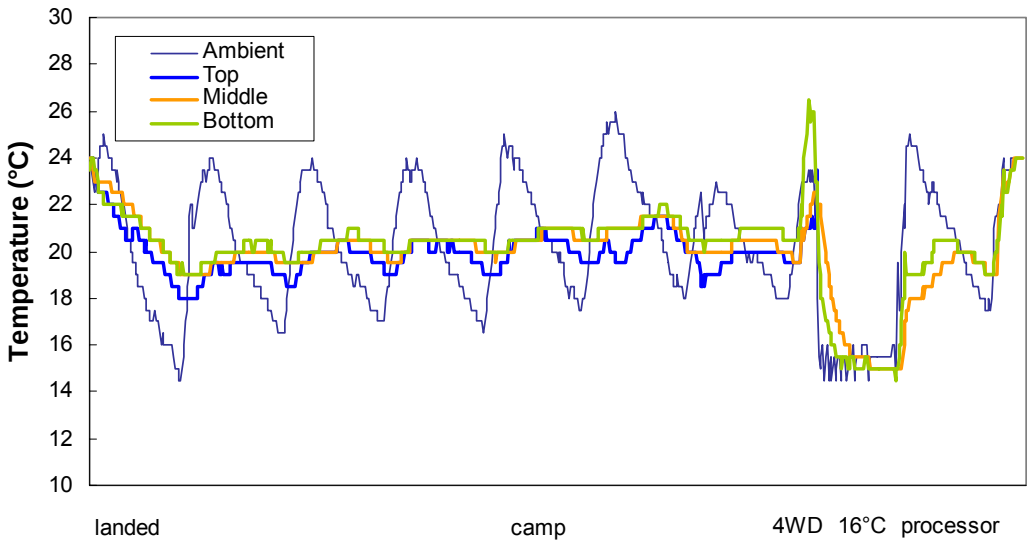

Figure 5.45. Temperature of crabs during simulation trials.

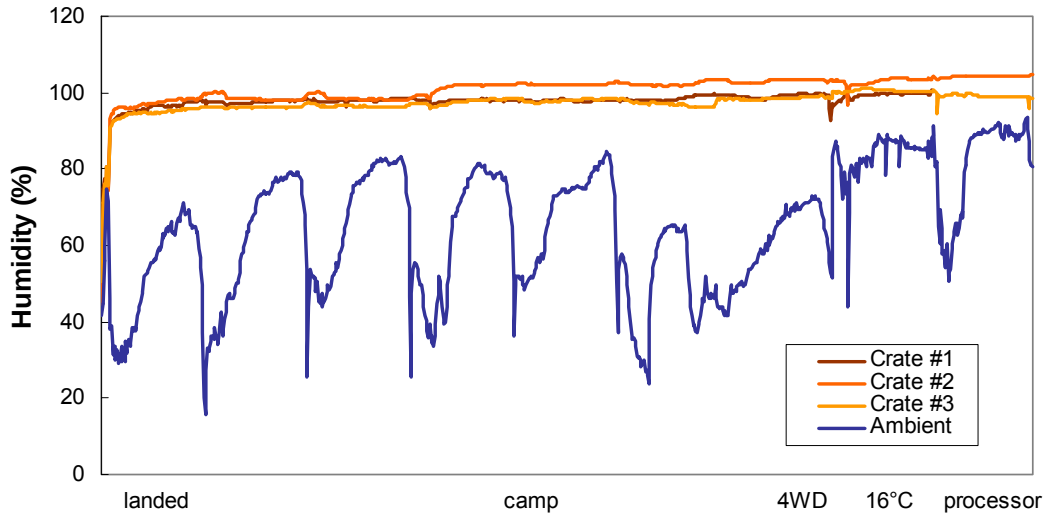

Figure 5.46. Relative humidity in middle of crab crate during simulation trials.

5.4.3.1 Haemolymph biomarkers

There was a strong correlation between the haemolymph biomarker response and the stage of the distribution chain.

Glucose

Glucose is an energy source for active metabolism in crabs and therefore responds, under hormonal control, to any external stimulus given to the crabs. Increased levels of glucose circulating in the haemolymph correspond to increased activity of the animal. Glucose levels in unstressed crabs are usually low and rise rapidly under any stress condition. Researchers working with other crustacea (brown and velvet crabs, (Johnson and Uglow 1985); rock lobsters, Paterson *et al*, 2001) consider that glucose circulating in the haemolymph correlated strongly to the stress level of the animal.

Basal glucose levels were different between trials but within the suggested range for 'rested' crabs (<1 mmol/L). Circulating glucose increased through the distribution chain stages correlating to cumulative imposed stress (Figure 5.47). However, haemolymph glucose did not reach levels indicating high stress (>3 mmol/L) at any stage.

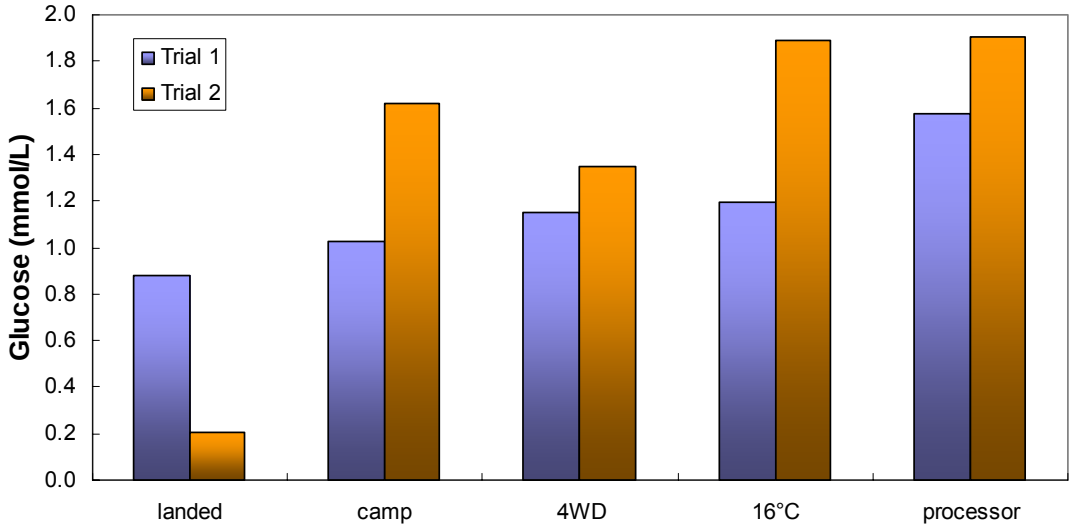

Figure 5.47. Haemolymph glucose levels during simulation trials.

Lactate

Lactate is a by-product of anaerobic metabolism and is released into the haemolymph as a response to oxygen deprivation during periods of animal activity. In crab, lactate enters the haemolymph by simple diffusion from the crab tissues. It is expected therefore that emersion for any length of time would result in elevated lactate levels in the blood. However, this was not demonstrated in simulation trials undertaken in this study (Figure 5.48). The very low lactate levels observed are unexplained, but it is possible that lactate is being produced and remaining within the muscle tissues. The high basal level in one trial may be explained by increased activity of those crabs in the holding tank prior to commencement of trial.

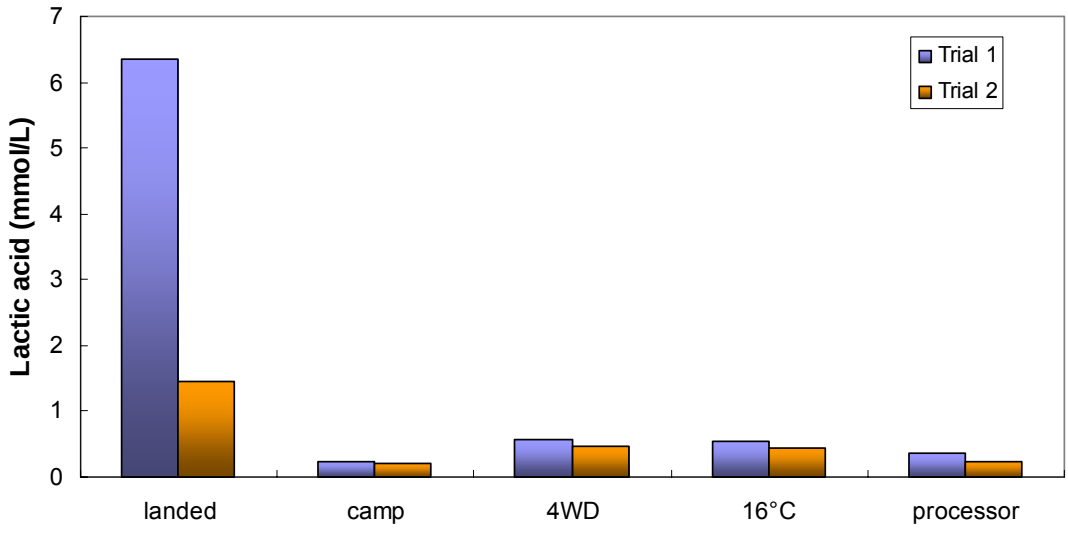

Figure 5.48. Haemolymph lactate levels during simulation trials.

pH

Haemolymph pH is influenced by levels of metabolic by-product compounds present in the blood and particularly reflects the level of lactate. This was demonstrated in both trials where lactate levels were low and pH was high.

Haemolymph pH also often reflects carbon dioxide (CO_2) levels in the blood with an inverse relationship; that is an increase in CO_2 will cause a drop in pH (greater acidity). This was not evident in the simulation trials and so suggests CO_2 building up during emersion was being converted by the crab through anaerobic pathways. However as the end-product during anaerobsis is lactate, it would be expected that lactate would increase with extended emersion but this did not occur in these trials.

Haemolymph pH measured at base camp during holding storage of crabs in damp hessian in crates (Figure 5.49) showed the pH was variable between individual crabs (*n* = 30) but the range is similar for the first 3 days.

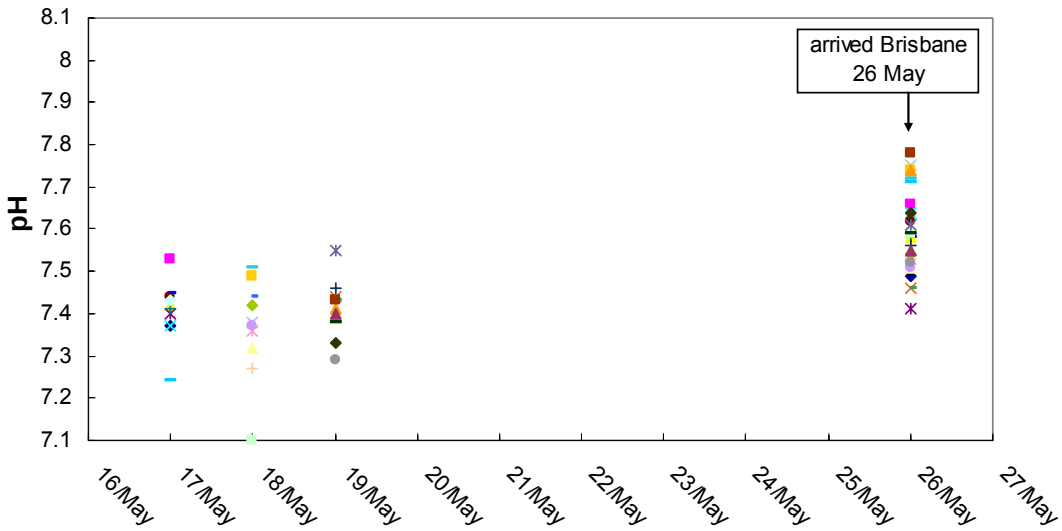

Figure 5.49. Haemolymph pH of crabs at beginning and end of distribution chain.

Of note, all crabs had a higher pH immediately after flight transport from Darwin to Brisbane. There are many handling steps between camp and destination arrival that could affect haemolymph pH: the handling at pack-out and transport to Darwin airport, storage at the airport, low temperatures during flight and so on. However, it is of particular interest that the pH after flight is high as this is in direct contrast to findings with other crustaceans, for example lobsters, which typically demonstrate an acidosis response to stress. The observed high pH does not seem to be an experimental artefact as all shipments of crabs exhibited the same response. Varley and Greenaway (1992) studying the effect of emersion on oxygen levels in *Scylla serrata* found that mean values of pH fell rapidly upon emersion but that extended emersion beyond 6 hours gave increased pH and it continued to rise over the 72 hours of the experiment. After 7 days of emersion the pH had returned to a level similar to that of immersed crab.

Uric acid

Uric acid is an end product of nitrogen metabolism and hence is a possible marker of oxidative stress. For the simulation trials carried out, there is no obvious trend of urate accumulation in the haemolymph in response to the multiple stresses imposed

on the crabs (Figure 5.50), rather the two variables appear independent of each other.

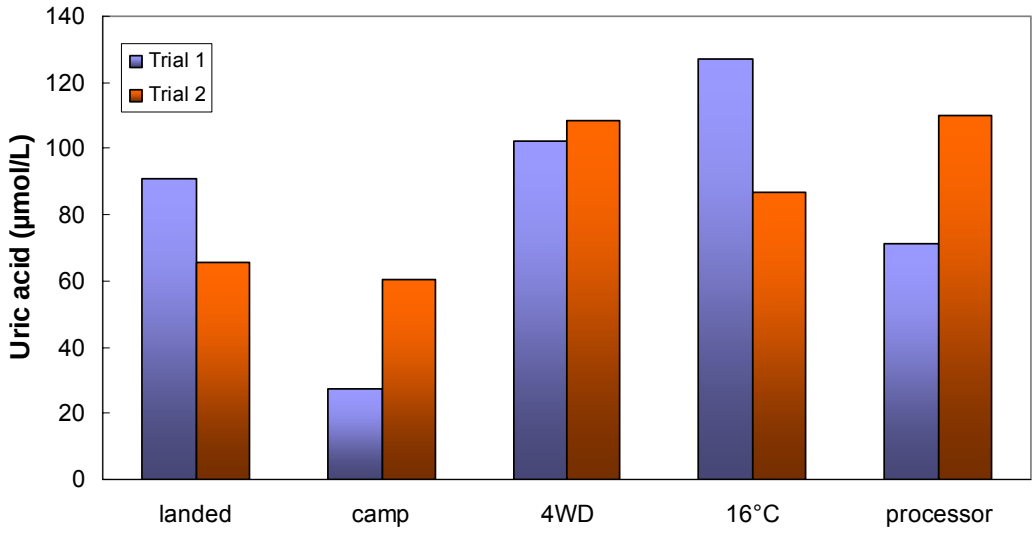

Figure 5.50. Haemolymph urate levels during simulation trials.

These results are evidence that uric acid level present in mud crab haemolymph is not a useful indicator of the stress encountered by the crab.

Ammonia

Ammonia is a metabolic by-product of protein breakdown or utilisation and is highly toxic to crabs. Build up of ammonia is prevented in the haemolymph by rapid gaseous exchange across the gills when crabs are in water. This is evidenced by the rapid increase in ammonia present in water when a purge step for crabs is carried out. However, this cannot occur when crabs are emersed.

In the first simulation trial, we measured ammonia in the blood of the crab. Variation in the ammonia blood levels between individual crab was large (indicated by error bars Figure 5.51) and hence, it is suggested that the ammonia levels in the blood did not change over the emersion period. This result was surprising since it would be expected that ammonia would build up in the crab as it cannot be excreted into the air environment. Researchers investigating respiration and metabolism in other crustacea (Durand and Regnault, 1998; Durand *et al*, 1999) have proposed that some crab species are able to cleverly remove ammonia from their haemolymph by creating extra amino acids to store the excess ammonia so as to not be toxic to the animal. It is possible that *Scylla serrata* is one of these species and haemolymph samples have been retained for further analysis, but this was beyond the scope of this project work.

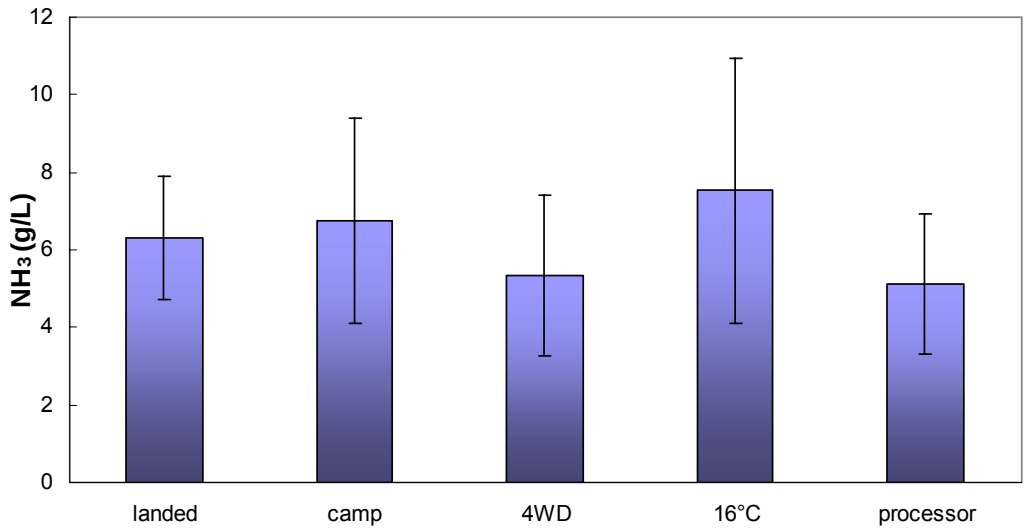

Figure 5.51. Haemolymph ammonia levels during simulation trial 1.

In the second trial, we assessed build up of ammonia in the crabs by gauging excretion level when the crabs were re-immersed for 90 minutes in fresh seawater at 24°C at the end of each stage of the simulation. Results (Figure 5.52) show a trend towards accumulation of ammonia, shown by greater excretion, as emersion is extended.

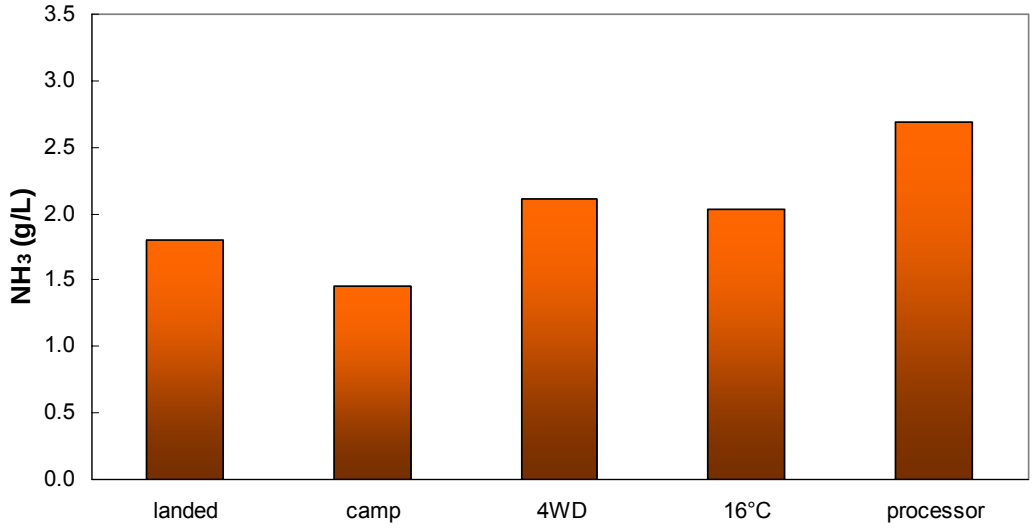

Figure 5.52. Seawater ammonia levels from excretion by crabs after 90 minutes.

Total Protein

Total protein levels between different crabs were highly variable and most likely directly related to the feeding pattern of the animal. The results (Figure 5.53) show no obvious trend of increased total protein circulating in the haemolymph as could be expected with prolonged emersion due to dehydration and loss of body fluids. Nor was there a pattern of decreased total protein present as may be expected with crab suffering feed deprivation.

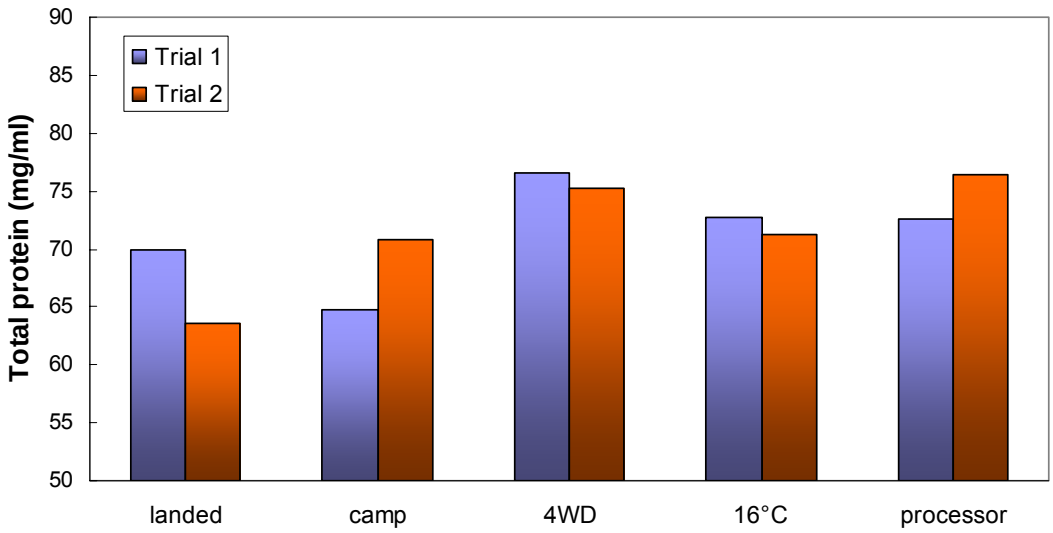

Figure 5.53. Total protein levels in haemolymph during distribution simulation trials.

Stress indicator data is presented according to duration of stage of the distribution chain and with each data value representing the mean of 8 individual crabs Figure 5.54. It is evident there is measurable response to the different handling disturbances that occur along the supply chain, however the standard deviation from the mean for the 8 individual crabs was often large for each biochemical marker, suggesting large inter-crab variation. Replicate data showed the same response trends, although there was a difference in basal levels of biochemical markers implying a difference in the 'rested' state of the batches of crabs used. This is surprising as, although different crab sets were used for each trial, the batches were obtained and acclimatised under exactly the same conditions (ideal seawater quality; same temperature; same feeding regime) for the same time period (12 days) prior to initiation of experimental work. The difference in haemolymph biochemical basal levels emphasises the importance of physiological condition of the crab with respect to individual reactions of crabs to the stresses imposed.

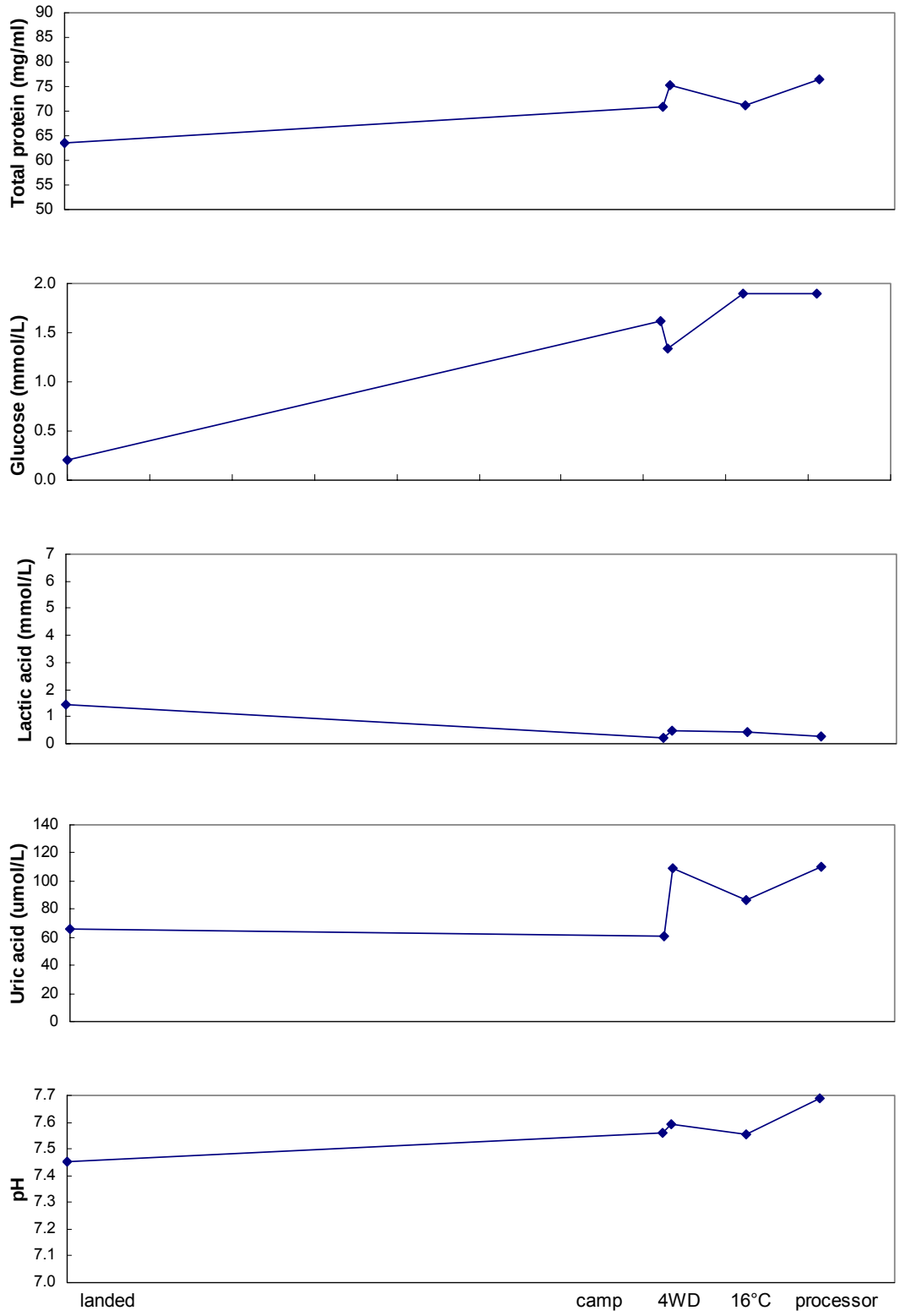

Figure 5.54. Haemolymph biomarkers in mud crabs during simulated distribution chain.

Information gained from the different stages within the distribution chain illustrates that:

Held at camp - holding crabs in storage at base camp does not result in large increases in haemolymph stress biomarkers. This is likely due to the quiet state of the crab, they are claw-tied, in dark humid conditions, at relatively constant temperature and subject to little disturbance. Hence this stage of the handling chain does not seem to cause appreciable stress to the crabs.

4WD - driving crab out of camp to Borroloola involves demanding four wheel driving terrain, deeply rutted, often soft sand and deep river crossings. The crabs are in crates stacked on a tarpaulin-covered ute tray and are subject to severe disturbance through physical shock. Additionally, the temperature increases through this phase with minimal airflow for cooling and sometimes, with exposure to direct sun. This stage appears to impose extreme stress to the crabs indicated by all haemolymph biomarkers responding sharply even though the duration of this stage was relatively short. However, from a practical perspective, alternative options for getting crab out of camp for further transport are few.

16^{o}C - stored overnight in a cool room awaiting the next transport stage. Crabs are undisturbed and held under dark conditions, however they are subjected to a sudden temperature drop and the biochemical biomarkers in the blood remain high indicating the crabs are experiencing stress.

Processor - on arrival at the processing factory (Darwin) after a long refrigerated road transport phase, the crab remain stressed but stress levels do not appear to have increased.

While the transport out from camp and chilling the crab would appear to impose the greatest stress on the crabs, it is likely that all phases of the supply chain contribute to the stress and it is cumulative within the animal. Oxygen deprivation, which occurs during emersion, is commonly accompanied by a rise in both lactate and glucose in the haemolymph and such increases would reduce the pH. This was not observed in either of these trials. Circulating glucose level did rise throughout the simulation but lactate remained very low and consequently pH remained high.

5.4.4 Major stress factors along the handling chain

5.4.4.1 Emersion

Crabs are aquatic animals and to remove them from this environment for extended periods is unnatural, hence causing stress to respiratory and metabolic systems. Mud crabs are among the exceptional of the crustacea in that they can seemingly tolerate such treatment far more readily than many other crab species. However, it was considered important to establish the effect of emersion on mud crabs especially as the periods of emersion can often be extreme within the distribution chains for these animals.

5.4.4.1.1 Crabs held emersed

Experimental design
Throughout trial investigations in this study, a batch of crabs (usually 10) were held as per standard commercial practice (claws tied, crowded together in a crate with hessian wrapping) for use as control sample crabs for each experiment. For different

trials, crabs were held for different emersion times and hence data for these crabs could be extracted and analysed together to determine the effect of different emersion periods.

Results and Discussion

Data from fifteen separate trials was considered and emersion times ranged from 0-20 days. Stress biomarker levels present in the haemolymph after different emersion times are presented in Table 5.10.

Table 5.10. Stress biomarkers in the haemolymph of crabs emersed for different periods.

Biomarker	Days emersed									
	0	2	0	5	0	7	0	14	0	20
Glucose (mmol/L)	0.15 ± 0.08	1.17 ± 0.80	0.24 ± 0.70	1.25 ± 0.57	0.20 ± 0.04	1.60 ± 0.79	0.41 ± 0.15	1.30 ± 0.30	0.24 ± 0.09	1.70 ± 0.13
Lactate (mmol/L)	0.73 ± 0.27	1.64 ± 0.79	0.69 ± 0.52	0.60 ± 0.42	1.46 ± 1.32	0.21 ± 0.20	1.36 ± 0.85	1.20 ± 0.91	nd*	nd*
pH (units)	7.60 ± 0.03	7.57 ± 0.02	7.62 ± 0.03	7.44 ± 0.08	7.18 ± 0.24	7.54 ± 0.24	7.60 ± 0.04	7.72 ± 0.13	7.60 ± 0.04	7.75 ± 0.14

*nd = not determined

Crabs emersed for 2 days under commercial storage conditions showed little stress impact in the glucose, lactate and pH biomarkers. Circulating glucose rose almost 10-fold although this increase remained within the level indicative of calm 'rested' crabs. The glucose present was also highly variable between individual crabs. After 2 days in air, haemolymph lactate and pH was unchanged. This was similar for crabs emersed for 5 days, but with an illustrated decrease in pH to greater acidity. Interestingly, this was so without a reflective increase in lactate level. With extended emersion, circulating glucose did not rise further, nor did lactate levels increase. However after 7 days held in air, crab haemolymph pH increased as storage time was extended. The increase observed with emersion is similar to that recorded after crabs have sustained extended distribution transport, as discussed with pH values obtained after arrival of crabs at the processor.

The lack of change in the metabolic markers of glucose and lactate is plausible, as when mud crabs are held under commercial conditions wrapped in damp hessian, they are calm and inactive. The significant rise in haemolymph pH however, does indicate stress experienced by the animals with extended emersion. Varley and Greenaway (1992) found that mud crabs became sluggish after 7 days emersion and there was some mortality between 3 and 7 days when the crabs were held fully dry and not in damp hessian.

The effect of emersion was very evident from excretion rates of ammonia when crabs were re-immersed into seawater. After 5 days emersion, only a small amount of ammonia was excreted (Figure 5.55). However, there was a large and rapid efflux of ammonia immediately on re-immersion after extended air-storage of the crabs (14

and 21 days). This continued at a rate corresponding to the length of previous emersion period. This observation is illustrative of the levels of accumulated ammonia during long periods of emersion of the crabs. A similar reaction was reported by Durand and Regnault (1998) for both green and velvet crabs where large amounts of ammonia were excreted within a few minutes of the crabs being re-immersed.

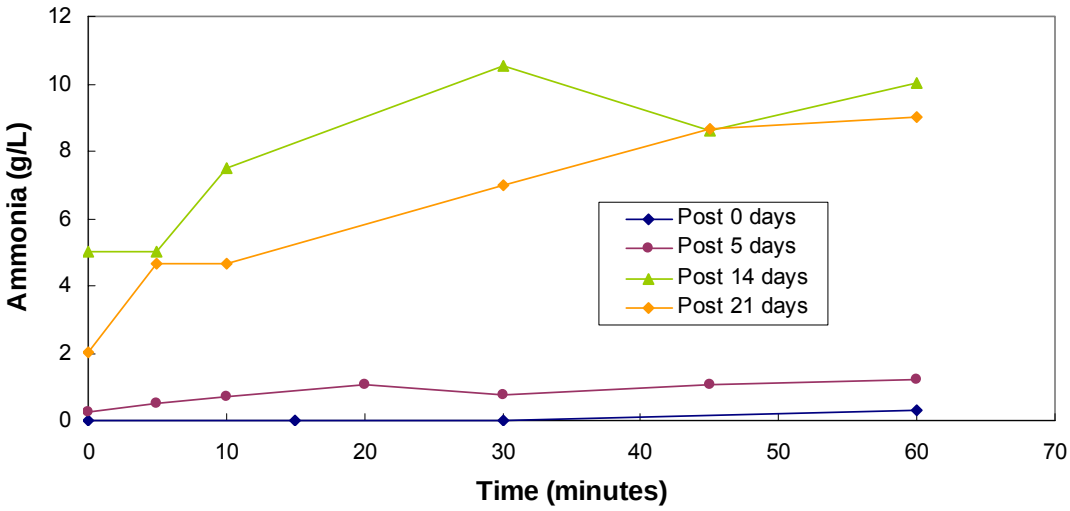

Figure 5.55. Ammonia efflux rates after different periods of emersion.

Due to the rapid build up of ammonia in the seawater used for purging, it is important to aerate the water tank to assist in the volatilisation of the ammonia. This controls the amount of ammonia dissolved in the tank water and avoids the purge water becoming laden with ammonia which is known to be toxic to crabs. Ammonia tolerance is often species specific (Rebelo *et al.* 1999; Romano and Zeng 2007a) with *Scylla serrata* demonstrating a relatively high tolerance (Romano and Zeng 2007b).

5.4.4.1.2 Dehydration

Mud crabs are aquatic animals and metabolise best when immersed in seawater, although they are happy in the intertidal region and can withstand extended periods out of water. While tolerating emersion and, seemingly quite readily for short times, it is expected that crabs will lose a certain amount of water both through simple leakage from body cavities and through respiration exchange. Water loss is important as it reduces the total weight of the crab and hence has implications for the harvester with respect to crab value. Also, it is likely to increase stress for the animal which may add cumulatively to other stress factors.

Experimental design
Crabs with claws tied were held crowded together in a crate as per commercial storage but held dry without the hessian wrap. This trial was limited to use of five crabs as it was considered that the likely outcome would be death.

Results and Discussion
This method of holding crabs was highly detrimental to crab survival as seen by the vigour index observations for the crabs (Table 5.11). Even a 24 hour storage period held completely dry was stressful to the animals with a reduction in vigour. After 2

days held dry, crabs were seriously affected and it is noted that there was 100% mortality after 4 days. It is suggested that mortalities occurred due to severe dehydration.

Table 5.11. Vigour index of crabs held dry in crate.

Individual crab	Day 0	Day 1	Day 2	Day 3	Day 4
# 115	Very lively 3	Ok 2	Ok 2	Dead 0	Dead 0
# 116	Very lively 3	Lively 2.5	Lively 2.5	Slow 1	Dead 0
# 117	Very lively 3	Ok 2	Ok 2	Dead 0	Dead 0
# 118	Very lively 3	Lively 2.5	Lively 2.5	Weak 1	Dead 0
# 119	Very lively 3	Ok 2	Ok 2	Slow 0	Dead 0

5.4.4.1.3 Weight loss in emersed crab

Trials were carried out where crabs were removed from the seawater holding tanks and held in air, with crab weight monitored regularly through the emersion period. In the absence of limb loss, the assumption is made that weight loss will be due to water loss from the crab.

Experimental design
Crabs (*n* = 10) were held emersed in individual containers with claws untied. Temperature was constant throughout the trial and air movement over the crab minimal. Relative humidity was monitored and varied a little between 72-90 % in the air immediately surrounding the crab. Crabs were weighed every 10 minutes for the first hour, then hourly up to 6 hours emersion and at 19.5 and 24 hours. Care was taken to disturb the crabs as little as possible, with the weights taken gently and swiftly.

Results and Discussion
The rate of weight loss in a typical trial is depicted in Figure 5.56 and the rapid initial weight loss during the first hour of emersion is clearly illustrated.

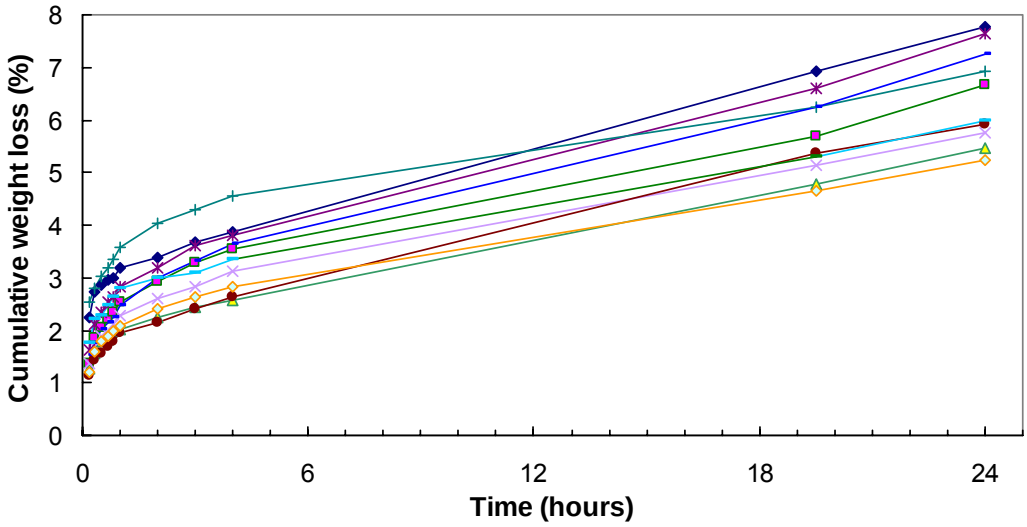

Figure 5.56. Cumulative weight loss in mud crab emersed for 24 hours.

Weight loss continues at a steady rate over time, albeit at a slower rate than occurs within the first hour of emersion. It is of note that some of these mud crabs have weight loss of almost 8%. (Gillespie and Burke 1992) reported weight losses of 8-11% in emersed mud crabs at the point of death, however this occurred after extended emersion between 6 and 10 days at different temperatures. Similar critical values (8-12%) at death of mud crabs were reported by Hill (1982).

The weight loss from mud crabs in this study compares to that from other commercially harvested crabs. Johnson and Uglow (1985) reported a lower weight loss (1.3%) after 4 hours emersion (at 75-85% relative humidity) with the green crab, *Carcinus maenas,* which has an intertidal habitat similar to that of mud crab. For the sub-littoral velvet crab, *Liocarcinus puber*, they demonstrated a 5% weight loss after the same emersion time. However, after 24 hours emersion, weight losses were similar for both crab species. The differences in rate of weight loss when crabs are first taken out of the water indicate adaptation within the intertidal species. It is highly likely that some initial weight loss is due to evaporation of surface water on the crabs. Even semi-terrestrial crabs lose weight at a rate of around 2.3% when active (Weinstein *et al.* 1994).

From a total of 43 crabs, all females, used within these trials there was no apparent correlation between the rate of weight loss and crab size. The rate of weight loss for all crabs was similar and in all trials, weight loss continued for the first 24 hours however, there was no further weight loss after this time during extended emersion.

The hypothesis that larger crabs (500-600g) will lose a greater percentage of water than small crabs (250-350g) has not been demonstrated in these trials. This was confirmed when the trial was repeated with larger crabs (>1kg) as evidenced by complete lack of correlation (r^2 = 0.0001) between crab size and water loss (Figure 5.57). This finding also concurs with that of (Jones 1982) who found percentage water loss was the same for small and large animals. This is in disagreement with (Leffler 1973) who suggests that metabolic rate of crabs is proportional to body weight) and reports from Whyman *et al* (1985) who found that the largest crab die first.

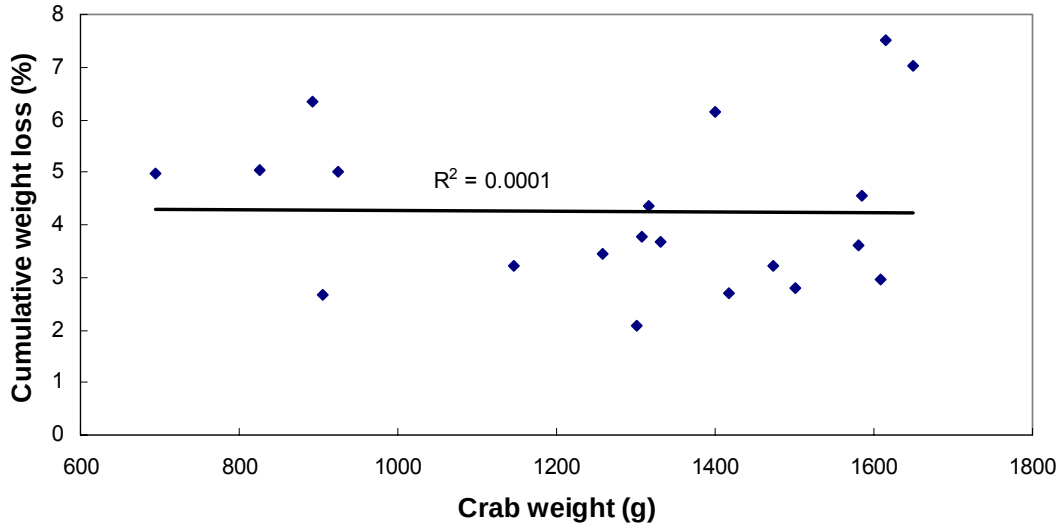

Figure 5.57. Cumulative weight loss of larger crabs (600-1600g) over 5 days.

At most stages of the commercial distribution chain crabs are not held completely dry but are packed dry into a hessian lined crates with extra hessian folded over top. The hessian sacking is doused daily to keep damp. It was important to determine the rate of water loss under these commercial holding conditions.

Experimental design
Rested crabs (*n* = 30), with claws previously tied, were placed into a crate lined with damp hessian in a similar manner to that observed from commercial harvesters. Temperature and humidity data loggers were included with the crabs. The hessian was then folded in a double layer over the top layer of crabs. Crabs were held quiet and in the absence of breeze. At each sample time, individual crabs were weighed on a calibrated Mettler scale selecting the 'live animal' mode, gently and swiftly so as to minimise disturbance to the animal.

Results and Discussion
Figure 5.58 presents the water loss during the first hour from crabs held emersed as per commercial practice and from crabs held completely dry. It is observed that initial weight loss in crab wrapped in damp hessian is more rapid than those held separately and dry. This finding was rather unexpected! Experimental factors were similar for the batches of crabs in these trials, with the obvious exception of holding conditions. Relative humidity inside the hessian sacking was actually higher (100%) than that for crabs held directly in air (70-90%). This result is contrary to those report by Hill (1982) who concluded that rate of weight loss in mud crabs was more dramatically affected by humidity than temperature.

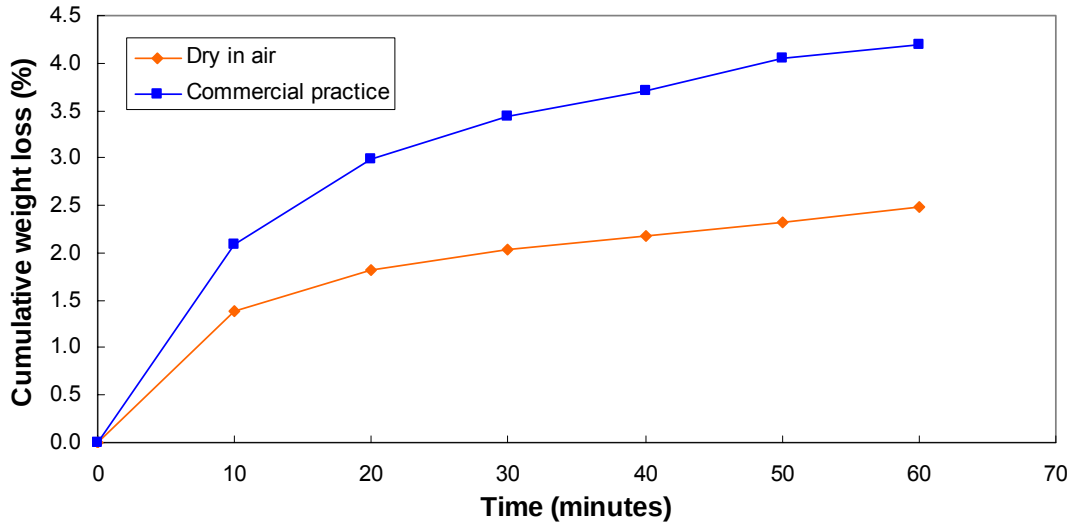

Figure 5.58. Cumulative weight loss from crabs during the first hour of emersion.

One difference in holding conditions could well be relevant. Crabs held under commercial conditions were crowded together whereas the crabs held completely dry were not. It is possible that additional stress is caused through close proximity of commercially held crabs and this is resulting in an increased metabolic rate in the animals and hence greater water loss. It was observed that water loss ceased after about 7 hours in crabs held in damp hessian, where as there was continued water loss for the full 24 hours from crabs held dry (Figure 5.59).

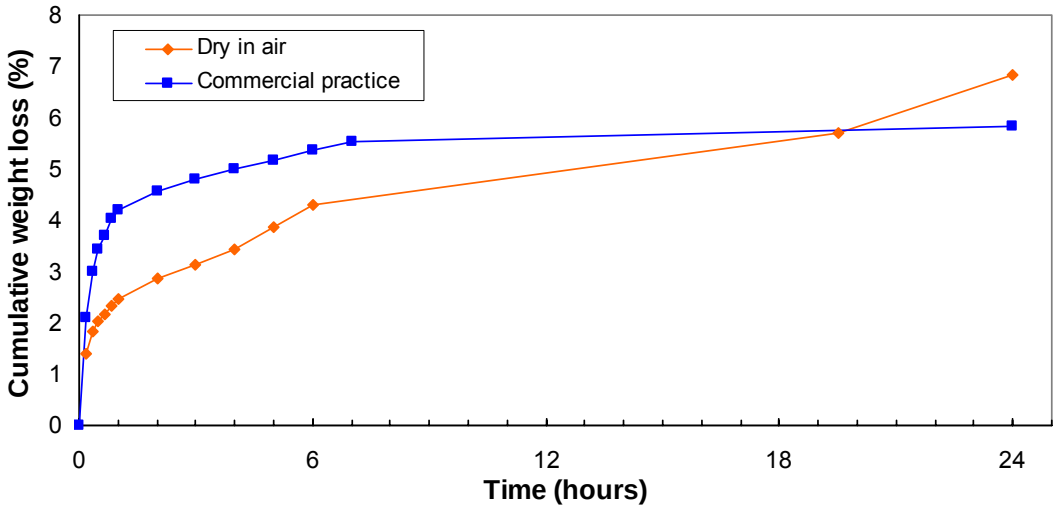

Figure 5.59. Cumulative weight loss from crabs held emersed, held under different conditions.

Crabs under both holding conditions lost a similar amount of weight after 24 hours. The conclusion from these results is that while crabs crowded together causes a rapid water loss initially, this stops after the first few hours and the humid atmosphere inside the damp hessian retains the moisture level sufficiently during further extended emersion.

5.4.4.2 Disturbance

Anecdotal opinion from experienced mud crab harvesters and wholesalers states "*just leave them alone; keep them quiet and they'll be fine*!". The handling and transport chain simulations undertaken in this study indicate that the comment has substance and that any form of disturbance, be it noise or movement, causes an instant and sometimes extreme response within the crab as measured by stress indicators.

Mud crabs are handled frequently at different points along the supply chain. Each handling event involves physical movement of the crab and often a degree of shock, potential injury and bleeding. Hence, the type of handling and its severity directly influences stress levels suffered. Handling practices including whether crabs are tied immediately upon removal from the pot; aggression level in the crowding situation within crates and 'roughness' of track/road during vehicle transport dictating the degree of physical movement/shock to the crabs.

5.4.4.2.1 Handling for sorting and grading

Mud crabs are checked every day for liveliness, mortalities and for claws remaining tied by a gentle unwrap and sort, but without removing animals fully from the crates. The check for dead crabs is particularly important as upon death mud crabs release ammonia rapidly and this immediately affects other crabs in near vicinity, often to a stress level that results in consequent mortalities of the nearby animals. Crabs receive a final sorting and grading just prior to being trucked out of camp for further transport to Darwin. This sort is more thorough than the daily checks and crabs are often completely repacked according to physical damage, sex and size.

Experimental design

Two crates of mud crabs were packed as per commercial practice, wrapped in damp hessian with each crate containing 10 crabs. Crab crates were held at a constant 25°-28°C. One crate of crabs was used as the control batch and these were left undisturbed, with a daily dousing of seawater to maintain dampness of the hessian. The other crate was subjected to sorting twice a day (morning and late afternoon) which involved unwrapping and removing all crabs to check each animal. The crabs were then gently replaced in the crate, re-wrapped and doused with seawater. The trial continued for 5 days as this time would be typical for crabs held at base camp in a commercial operation. Haemolymph samples were taken at the start and end of the trial for biomarker analysis.

Results and Discussion

No crabs died in either batch and all crabs were calm and quiet throughout the trial. Total protein levels in the haemolymph of crabs from both the control and the test batches were very slightly elevated after 5 days storage, as has been observed in other emersion trials and due to water loss from the animals concentrating the protein levels a little. The response in circulating glucose levels for all crabs was individually variable but ther was a slightly greater increase in handled crabs. The mean basal level was ~0.3 mmol/L rising by 6-fold in both lots of crabs (Table 5.12). The level of glucose present is indicative of only mild stress and the indication from these results is that the stress was caused by emersion rather than the handling and sorting.

Table 5.12. Haemolymph biomarkers in crabs subjected to daily handling or held undisturbed.

Crab treatment	Total protein (mg/ml)		Glucose (mmol/L)		Lactate (mmol/L)		pH (units)	
	0 d*	**5 d***	**0 d***	**5 d***	**0 d***	**5 d***	**0 d***	**5 d***
Handled	79.7 ± 11.3	87.3 ± 13.3	0.32 ±0.15	1.93 ±0.62	0.66 ±0.35	0.52 ±0.28	7.55 ±0.03	7.54 ±0.03
Undisturbed	73.4 ± 12.9	74.8 ± 13.2	0.24 ±0.27	1.25 ±0.57	0.71 ±0.66	0.67 ±0.52	7.57 ±0.05	7.54 ±0.06

* d = days stored

Similarly, the mean lactate levels circulating in the haemolymph were the same in both batches of crabs and remained at the basal levels of ~0.5 mmol/L, not indicative of stressed crabs. The pH of the haemolymph was variable between individual crabs but after 5 days storage, the mean blood pH for each batch of crabs was the same as the mean basal levels of 7.5 pH units.

The results of this sorting trial indicate that gentle handling for sorting purposes does not impose stress on the mud crabs and any slight stress activity that may occur during the process each day is readily recovered from. Given the importance of checking crabs for mortality due to the severe consequences of a dead animal remaining in close proximity to others, it is concluded that daily sorting is a beneficial action and does not contribute significantly to stress levels in the crabs.

5.4.4.2.2 Movement shock

Crab crates are transferred to a truck and driven out of camp to a holding room at Borroloola, ~2 hours transport stage. Often the track out of camp involves serious four wheel driving with deep ruts, soft sand or bulldust and river crossings. Therefore crabs are subject to extreme disturbance and physical shock. The effect of similar simulated movement shock was assessed.

Experimental design
Two crates of mud crabs were packed as per commercial practice, each crate containing 10 crabs. The control crabs were left undisturbed and held under the same conditions as the test crate but at a distance to ensure noise disturbance did not occur. The crate containing the test crabs was dropped from a height of 30cm every 15 minutes for 5 hours. Haemolymph stress indicators were measured at time 0 and after 5 hours.

Results and Discussion
The noise generated by each crate drop, measured at a 1m distance, was around 90 decibels, which sudden noise burst must add to the surprise (shock) for the crabs. Glucose levels rose slightly in all crabs, from a rested level of 0.2 mmol/L to 1.3 and 1.5 mmol/L in control crabs and dropped crabs respectively. It is surprising that undisturbed crabs had such a rise in circulating glucose however, when looking at data for individual crab within this set, there were two crabs with very high blood glucose levels and hence this has raised the mean. A mean value of 1.5mmol/L for dropped crabs is indicative of only some stress imposed and is well below the level implicating severe stress.

The haemolymph pH of dropped crab was severely reduced after 5 hours (Figure 5.60) with some crabs exhibiting an individual extreme decrease of 0.6 pH units.

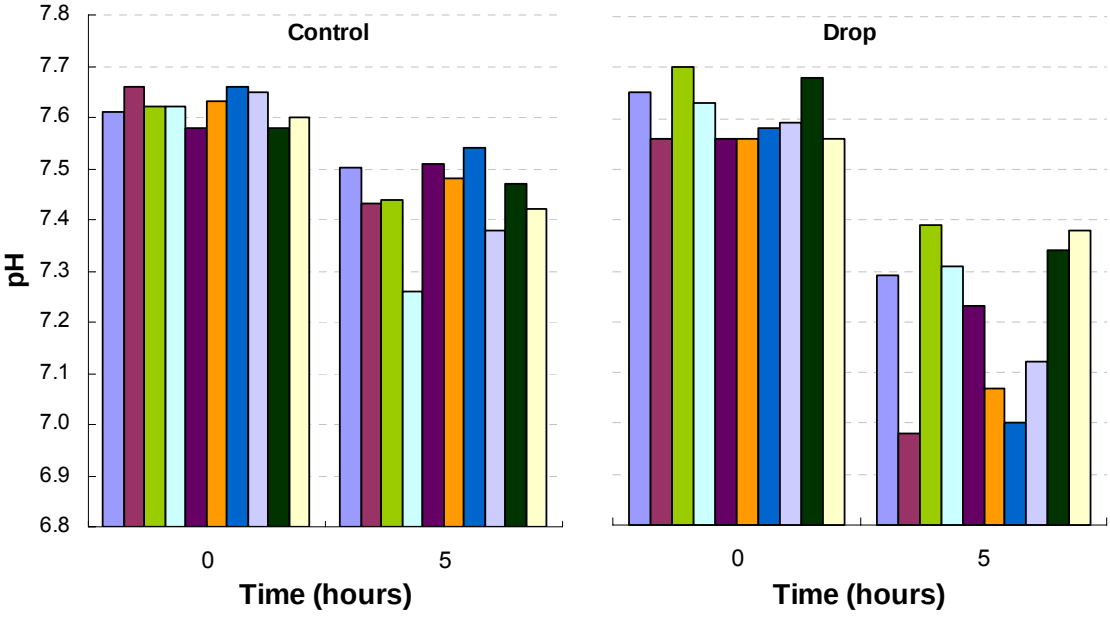

Figure 5.60. Haemolymph pH of disturbed and undisturbed crabs.

The drop in blood pH was inversely related to a strong increase in circulating blood lactate in the dropped crabs (Figure 5.61). More than half the crabs in the test batch had lactate levels indicating severe stress while those in the control group had the same lactate level after 5h as their initial level.

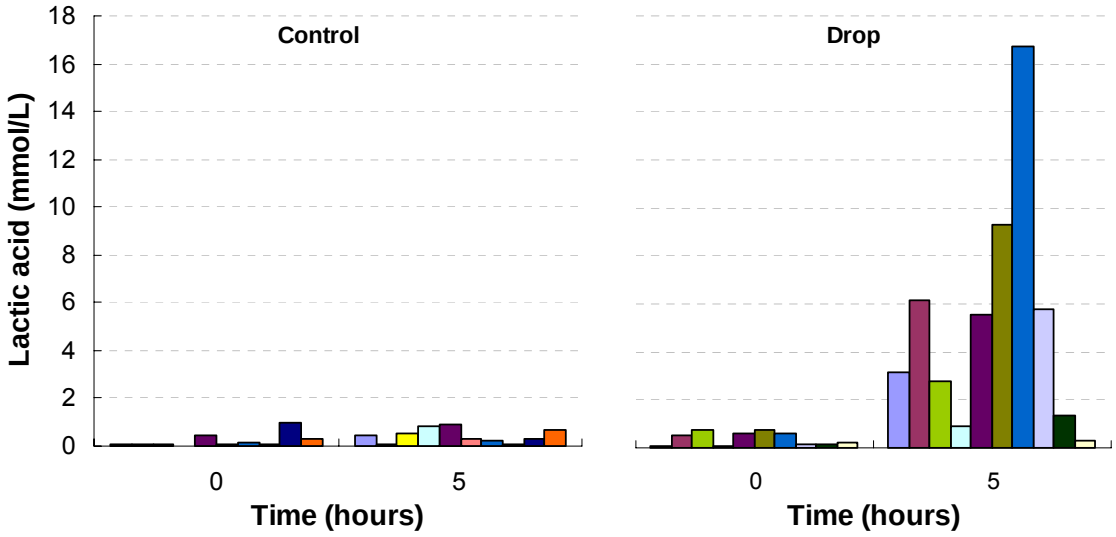

Figure 5.61. Haemolymph lactate levels in disturbed and undisturbed crabs.

At completion of the trial, all crabs were re-immersed in aerated seawater and allowed to recover. Crabs were observed for 5 days during recovery post-trial and it was noted that 50% of crabs in the dropped group died during this time, some within 2 hours, however there was no mortality in the undisturbed batch of crabs.

The results from these trials provide strong evidence that sudden and severe jolting movement causes extreme stress in mud crabs. While not too much can be done about the condition of the tracks in and out of base camp sites, care during transport stages is important. Loading and unloading of mud crabs from transport trucks is a step that can be carried out gently to minimise stress levels.

5.4.4.2.3 Animal aggression

Mud crabs are inherently aggressive in behaviour and hence close proximity of crabs to each other results in high levels of physical activity, causing increased metabolism and elevated levels of biochemical end product compounds. (Patterson *et al.* 2007) reported that the presence of other crabs in close proximity exacerbated the stress response in the edible crab (*Cancer pagarus*). Crowding also increases water loss from the crabs due to increased activity of the animals due to aggressive and defensive behaviours (section 5.4.4.2.3). Aggression is exacerbated in crabs with claws free. Crabs with claws tied remain more docile and show lower stress response. It was determined not to investigate the contribution of aggression to stress levels in mud crabs with claws untied when crowded together in a crate packed as they would be commercially. This would inherently result in severe physical damage to individuals (Plate 5.8) and likely death, if not from extreme stress, due to body fluid loss.

Plate 5.8. Haemolymph loss from mud crab.

There are documented studies of crab mortality increasing correspondingly with claw or limb breakage and severity of wound exposure (Messick and Kennedy 1990; Juanes and Smith 1995). Such situations can sometimes occur within harvesting practices where crabs are cleared from pots and stored temporarily in the underfloor area of the dinghies, to be claw-tied upon return to camp. Importantly recent legislative changes prohibit this activity and all crabs must be sorted at point of capture and non-commercial or illegal crabs returned to the water and they must also be tied before returning to camp. However, it is not known whether the unnatural state of having claws tied imposes stress on the crab by its own right.

Experimental design
Fully robust mud crabs were randomly assigned to either having the claws tied or left untied. Individual crabs were constrained in separate shallow prawn crates with lids and returned to the seawater holding tank. Haemolymph samples for biomarker analysis were taken at the start of the trial and after 5 days holding. The 30 second rule for obtaining a blood sample was maintained despite the difficulty of sampling crabs with claws untied.

Results and Discussion
Total protein in the haemolymph of both sets of crabs remained constant throughout the trial (Table 5.13), as would be expected in crabs with feed withheld but in an aquatic environment.

Table 5.13. Haemolymph biomarkers in crabs with claws tied or untied (*n*=20).

Crab treatment	Total protein (mg/ml)		Glucose (mmol/L)		Lactate (mmol/L)		pH (units)	
	0 d*	**5 d***	**0 d***	**5 d***	**0 d***	**5 d***	**0 d***	**5 d***
Claws tied	79.6 ±16.2	77.3 ±14.8	0.66 ±0.27	0.48 ±0.31	0.26 ±0.34	0.23 ±0.36	7.82 ±0.06	7.56 ±0.04
Claws untied	77.1 ±12.5	74.4 ±12.9	0.69 ±0.27	0.56 ±0.34	0.62 ±0.43	0.58 ±0.45	7.81 ±0.04	7.58 ±0.04

* d = days stored

Additionally, there was no difference observed in glucose or lactate levels, nor pH of the haemolymph between the batches of crabs after 5 days. It is concluded that the simple fact of having tied claws does not add to stress levels within mud crabs. Indeed, in an investigation into the commercial practice of declawing the edible crab, *Cancer pagurus,* and returning the animal to the sea in the UK fishery (Patterson *et al,* 2007), the stress response in the edible crabs was noted as similar to that caused by 'handling only' practices.

5.4.4.2.4 Annoyance and increased activity

Although good practice suggests (and law in the NT now requires it) that crabs should be tied as soon as removed from the pot at harvest, in some operations logistics are such that crabs remain untied for extended periods – often in the bottom of the dinghy. In such situations mud crabs show increased activity from both aggressive and defensive behaviours (Plate 5.9). The increased physical activity of the animal affects the respiration rate and hence the accumulation of metabolic biochemicals. The effect of the increased activity of crabs in this situation was assessed.

Plate 5.9. Aggressive reaction of a mud crab.

Experimental design
A batch of crabs was bled for baseline haemolymph analysis. Crabs then had their claws untied and were placed in individual open crates and were held emersed. Crabs were annoyed for 5 minutes out of every 15 minutes for a total period of 3 hours. Annoyance included moderate noise and external human activity in close proximity to the crabs. After 3 hours, the crabs were bled again for haemolymph analysis.

Results and Discussion
Figure 5.62 illustrates the rise in pH, glucose and lactate caused by annoyance to the crabs. The increase in circulating glucose is indicative of increased metabolism from greater activity of the crabs. Four of the 10 crabs had glucose levels indicative of severe stress. For the biomarker of lactate, again 4 of the 10 crabs exhibited levels in the extreme stress range with 3 of the 4 having extreme glucose levels too.

The increase in lactate levels is mirrored directly by a decrease in haemolymph pH. This observed effect directly corresponds to increased activity of the crabs as has been reported for other crab species during active exercise (Booth *et al*, 1984).

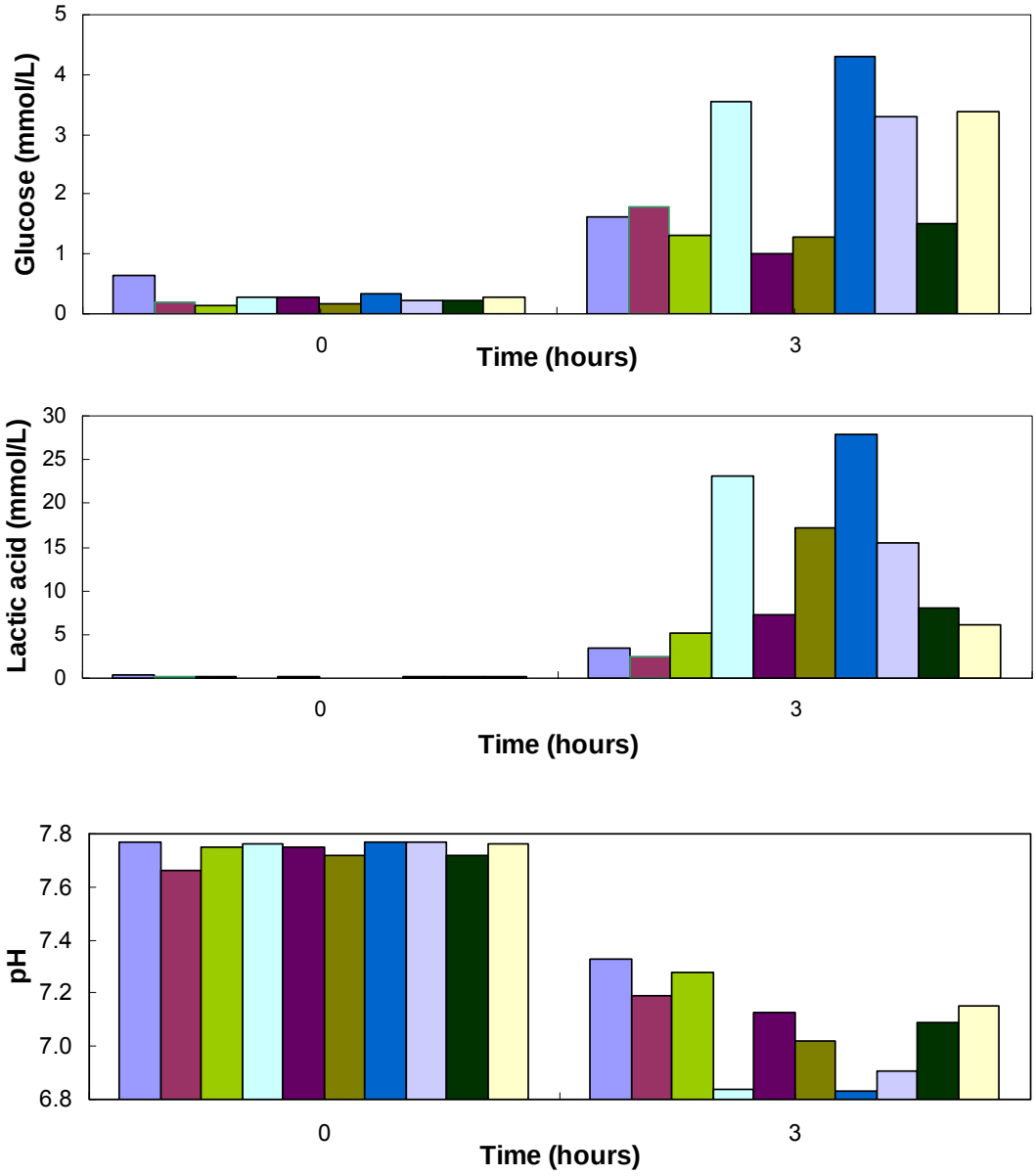

Figure 5.62. Glucose, lactate and pH levels in annoyed untied crabs (*n* = 10).

The biomarker levels exhibited by crabs annoyed in these trials are some of the highest observed under any experimentally imposed stress trials undertaken in this study. Surprisingly, all crabs recovered happily from this stress when re-immersed in aerated seawater.

The conclusion is that crabs really don't like being hassled! The extra physical activity caused by annoyance has an extreme effect biochemically, indicating severe stress imposed on the crabs.

5.4.4.2.5 Noise

The effect of exposure to noisy environments is often mentioned by industry personnel as a contributing factor in crab mortalities. Generator units in close proximity to stored crab at base-camp or in retail situations and even loud music or rowdy crabbers are reported to have detrimental effects on crabs.

Experimental design
Two 40kg plastic crates were lined with double-folded damp hessian and each packed with 10 tied mud crabs. Damp hessian coverings were carefully inter-folded over the top of the crabs to exclude draught and light and maintain humidity. The first crate of crabs was used as the control set for the experiment and was placed in a quiet room for 48 hours with background noise levels of ≤68 decibels. The second crate of crabs was exposed for 48 hours to mechanical noise created by the on/off operation of a large compressor unit from a nearby freezer. Intermittent (5min cycle) peak and baseline volumes were 95 and 70 decibels respectively with a mean volume of 86 decibels. Crates were doused with seawater each day to maintain a moist environment. Crabs were bled at time 0 and after 48 hours for analysis of haemolymph parameters.

Results and Discussion
Mean haemolymph pH and glucose levels both indicated crabs experiencing only slightly elevated stress. A drop in haemolymph pH for the crabs exposed to noise (0.06 pH unit) occurred (Table 5.14). This is not a critical drop relative to those observed for other stress factors studied where pH decreases of ≥0.5 units were noted. There was no pH change in the control crab.

Table 5.14. Haemolymph pH in crabs exposed to noise.

Crab treatment	Haemolymph pH (units)		Haemolymph glucose (mmol/L)	
	0 hours	48 hours	0 hours	48 hours
Control crabs	7.64 ±0.07	7.64 ±0.06	0.16 ±0.06	1.27 ±0.77
Crabs subjected to noise	7.66 ±0.04	7.59 ±0.09	0.15 ±0.07	1.75 ±0.64

There were elevated levels of haemolymph glucose for crabs from both treatments (Table 5.14), however the levels for the crabs exposed to noise are only indicative of slight to medium stress. This is based on previous work which demonstrated that glucose levels in crabs of <1.0 denotes unstressed crabs; levels between 1.0-3.0 indicates that stress has occurred; >3.0 indicates severe stress in the crab. No discernable difference was observed in haemolymph lactic acid levels with crab from either treatment as only an increase of 0.6 mmol/L occurred and indicates the crabs are within the 'rested' range.

Consequently, these results suggest that exposure to noise does not cause severe stress and hence is unlikely to be a major factor in crab mortalities. However, stress imposed by noise may still have a cumulative effect of all factors leading to mortalities and so should be minimised.

5.4.4.2.6 Breeze

The detrimental effect of breeze on exposed crabs has a common acceptance among experienced crab harvesters. Hence the industry practice of covering crabs with damp hessian and storing them in a draught-free position. Unfortunately, this practice is not always adopted further along the supply chain. Within wholesale and retail sectors, crabs are commonly exposed to strong air-disturbance, such as air-conditioned storage, regular opening/closing of doors and positions affected by natural breezes. The effect of breeze on stress levels in mud crabs was examined to determine the importance of this factor with respect to total cumulative stress.

Experimental design
Two lug baskets were each packed with 10 mud crabs with claws tied. The control crate was lined with double-folded damp hessian and placed in a draught-free environment. Crabs in the second crate were exposed to a constant wind equivalent to a gentle breeze of 1.4 knots (a velocity of 0.7m/s measured by a Vaneometer (Dwyer Instruments Inc.) for 48 hours by positioning an oscillating electric fan nearby. These crabs had no hessian cover. Haemolymph stress indicators were measured at time 0 and after 48 hours.

Results and Discussion
Crabs subjected to constant breeze demonstrated severe changes in all measured haemolymph stress markers, whereas control crabs maintained near constant levels for all measured markers except glucose. Decrease in haemolymph pH for wind-subjected crabs was individually variable but all crabs evidenced greater acidity of the blood, with a maximum decrease in two crabs to pH 6.8 or less (Figure 5.63). Such a drop is evidence of extreme stress as previously it was observed that a pH of <7.0 was indicative of stressed crabs (section 5.3.3.3). The mean drop in haemolymph pH in crabs subjected to breeze over 48 hours was from 7.57 to 7.19 (~0.4 pH units) indicating that breeze is a significant stress factor to mud crabs since there was no change in the pH of crabs held draught-free. With the knowledge that the haemolymph pH is very reactive to rapid metabolic changes occurring in the crabs and that the haemolymph pH of emersed crabs returns to near basal levels after 2 hours air exposure (section 5.3.3.3), the illustration of this low pH in wind-blown crabs at 48 hours exposure is proof that the constant annoyance, evaporative chilling and likely dehydration of the crabs is not allowing any compensatory metabolic shifts to occur in the crab.

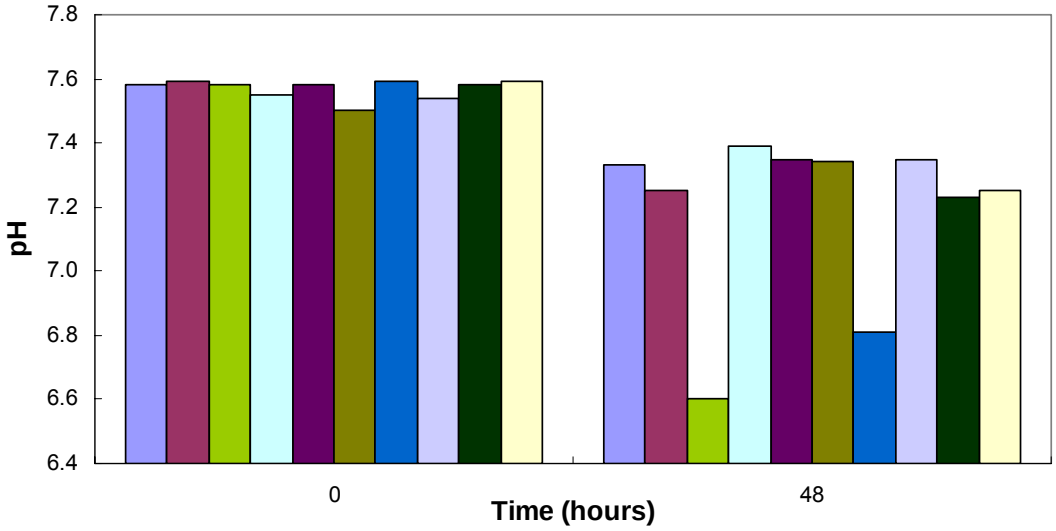

Figure 5.63. Haemolymph pH in wind-blown crabs.

Furthermore, there is no ability to restrict metabolic activity to a 'comatose' state as happens when the crabs are held quiet (as in the control batch of crabs). Conversely, haemolymph levels of circulating lactic acid, protein and glucose demonstrated pronounced increases. All wind-exposed crabs exhibited signs of extreme stress as measured by lactic acid levels which rose from a 'rested' baseline mean of 0.73 mmol/L to 4.72 mmol/L and higher (Figure 5.64). This increase is indicative of moderate stress reaction in the crabs, while those crab held quietly in a draught-free environment showed no rise in circulating lactate levels.

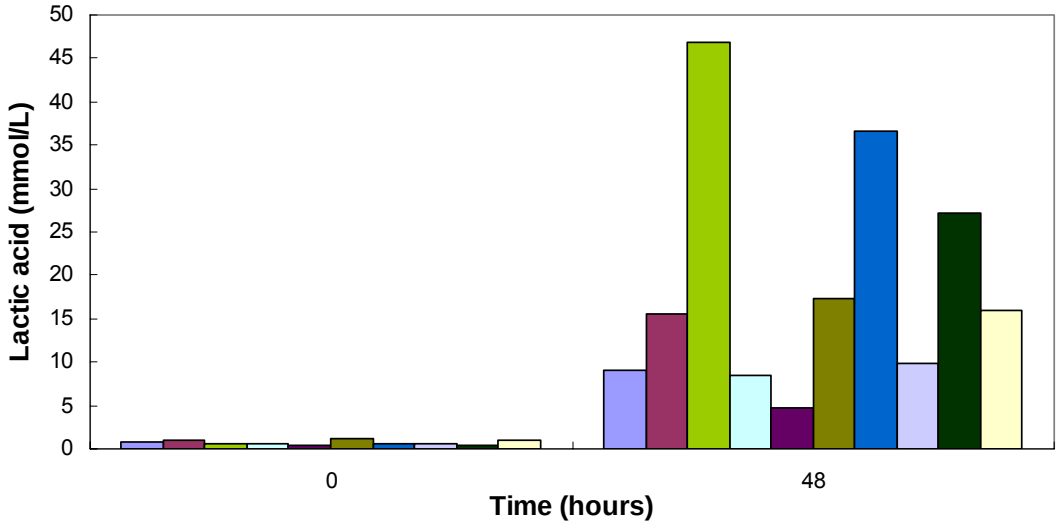

Figure 5.64. Haemolymph lactate levels in wind-blown crabs.

Stressed crabs exhibit lactic acid levels greater than 5.0 mmol/L. It was observed that multiples above this stress benchmark were observed in several crabs, with one crab demonstrating lactic acid levels over 9 times this benchmark. The mean for all wind-blown crabs was 20.0 mmol/L. A relationship between haemolymph lactate level and liveliness was also identified with the slowest, least lively crabs exhibiting the highest levels of lactic acid.

All crabs displayed increases in haemolymph glucose levels to widely different degrees, including the draught-free crabs (Figure 5.65).

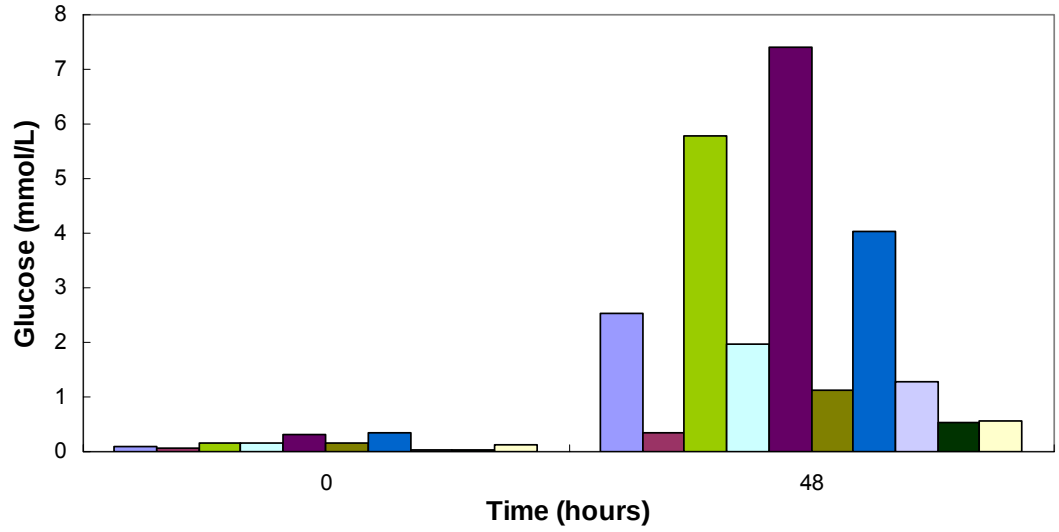

Figure 5.65. Haemolymph glucose levels in wind-blown crabs.

The draught-free crabs showed only a small increase, maintaining glucose levels above the 1mmol/L indentified for 'rested' crabs but below the 3.0 mmol/L threshold of stressed crabs. The reason for the slight increase in circulating glucose is likely to be a response to emersion of these crabs. Of the wind-blown crabs, a third were categorised as being in a state of extreme stress.

All wind-blown crabs showed slight increases in total protein levels present in the haemolymph after exposure for 48 hours. When weight loss observations (discussed below) are considered, it is concluded that this increase can most likely be attributed to the evaporation of body fluids, leading to a concentration of haemolymph total proteins. The increase in total protein levels, in particular pigmented proteins, may explain the observed change in haemolymph colour from a bluish-grey to a bluish-orange. However, this colour change could also reflect metabolic changes caused by stress. Within crab haemolymph, the oxygen carrying protein is haemocyanin which contains a copper ion and imbues a blue colouring to the crab blood. As oxygen is depleted from the haemolymph, the colour is likely to become less blue and more grey-neutral coloured and, depending on the amount of astaxanthin present (pigment of the crab shell), even show an orangey hue.

Physiological changes to weight and liveliness were also monitored. Liveliness of mud crabs was observed to decrease when subjected to constant wind over 48 hours. The reduction in liveliness was irreversible as affected crabs were unable to be revived after the trial. This observation of permanent slowness ultimately resulting in mortality emphasizes that a draught-free environment during holding mud crab emersed is critical.

Wind-blown crabs also exhibited significant weight loss compared to control crabs: an average of 12.5% compared to 3.4% respectively (Figure 5.66). This measured result highlights the potential profit loss within the supply chain of draught/breeze exposed crabs. Since crabs are sold by weight, suppliers to the crab industry will achieve lower return on crabs exposed to breeze. In the experimental trials undertaken in this study, the loss would be equivalent to 12.5% on returns.

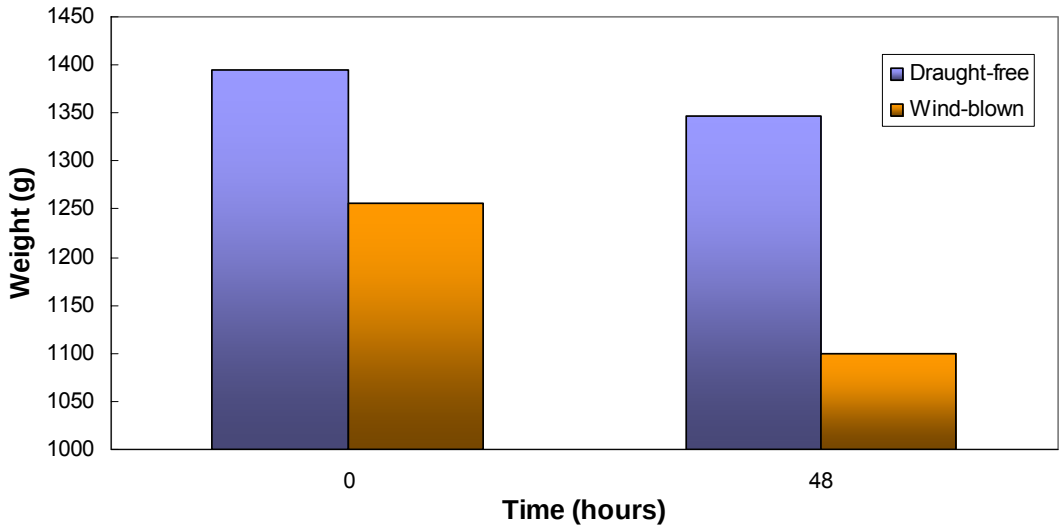

Figure 5.66. Mean weights of draught-free and wind-blown crabs (*n* = 10).

5.4.4.3 Temperature

Mud crabs are poikilothermic animals, taking on the temperature of the surrounding environment and they are without effective internal means of regulating their temperature. Hence, within limits, the higher the temperature the greater the metabolic rate and the faster the crab will suffer stress from lack of oxygen. Mud crabs are accustomed to tropical waters and often exhibit burrowing behaviour to regulate temperate. Both water and ambient temperatures can be excessively high during summer months. Additionally, the transport chain dictates periods of severe temperature drop. It is likely that it is not the actual physical temperature that results in stress but the severity of the temperature change.

5.4.4.3.1 Temperature change

In the extremes during summer months, especially at low tides, water temperature surrounding the anchored crab pot can be very high (40° - 45°C). Additionally, temperatures while the crab is held emersed are high, though this is compensated for during base camp holding periods by use of shade cloth shelters and regular dampening of the hessian wrapping in the crate. The reduction in temperature from ambient assists by reducing activity of the crabs and therefore metabolic rate. Even in the wild habitat the activity of *Scylla serrata* is greatly reduced at temperatures below 20°C (Hill 1980). During transport holding stages and repacking at the factory, the crab are subjected to sharp drops in temperature.

Holding crabs at constant temperature and minimising the temperature change is likely to be optimal for minimising stress. Current commercial practice of holding crabs in damp hessian sacking is effective for this. As shown in Figure 5.67, temperatures inside the crab packed crate remain relatively constant compared to the external day and night temperatures. This practise also helps maintain a high humidity environment around the crabs, if regular dampening of the hessian occurs.

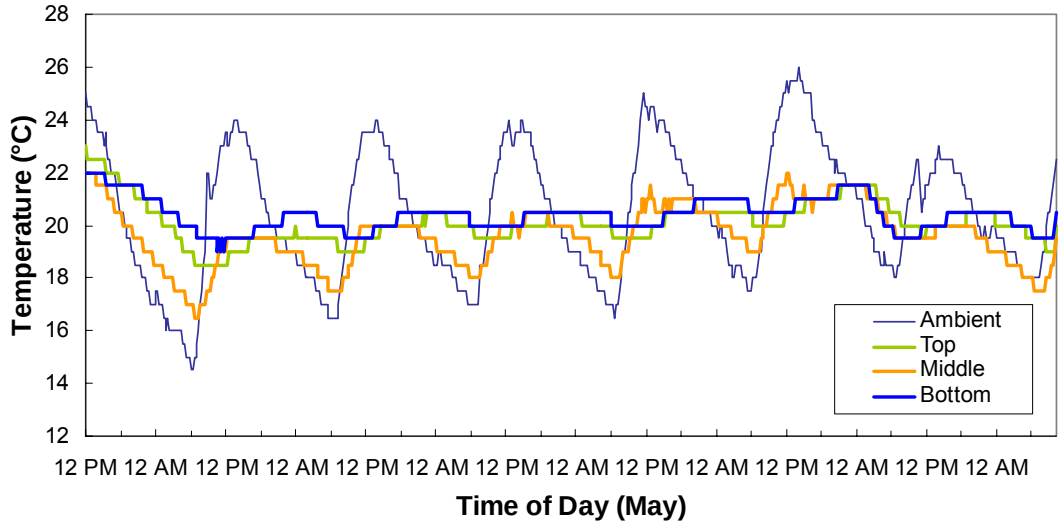

Figure 5.67. Temperature of crabs held as per commercial practice.

When held at ambient temperature under commercial practice, crabs are calm but still relatively lively and highly reactive to imposed disturbance. It is noted that during the supply chain the crabs are often subjected to sudden and severe low

temperatures and the damp hessian wrapping may not be effective in moderating sudden changes.

Observations during disturbance trials often illustrated a rise in temperature of crabs in the middle of packed crates most likely caused through increased activity of the crabs in response to the disturbance. Such temperature change is small and gradual, however where temperature change is severe or sudden, what is the effect on the crabs?

5.4.4.3.2 Effect of storage temperature on mud crabs

It is established that changes in temperature are associated with changes in heart and ventilation rates in crustaceans (Hill and Koopowitz 1975; Goodrick *et al.* 1993; Paterson 1993; Morris and Airries 1999). Recently, the mechanisms of response have been identified with, for example, neuroamines implicated in responses to acute temperature decrease in lobsters (Kuramoto and Tani 1994) but not apparent in crabs subjected to slow temperature decrease (deWachter *et al.*, 1997). Behavioural voluntary selection of a lower temperature under hypoxic conditions is reported for many crustaceans (Morris, 1999) including crabs (deWachter *et al*, 1997) and confers the advantage of lowering metabolic demand in the animal.

Without standardised holding and transportation practices within the live mud crab industry, temperature exposures to crabs can vary greatly within the supply chain and changes can often be acute. Factors such as holding conditions after harvest and during transportation, daily temperature fluctuations and exposure to sun versus shade contribute to this variation in temperature. Additionally temperature is affected by application of water douses to crab crates when maintaining a moist environment for the crabs through evaporation effects.

In this set of trials, we investigated the effect of different temperatures on mud crabs and stress levels imposed on the animals. A wide range of temperatures were studied to provide a practical temperature window that imposes least stress on crabs and therefore minimising additional cumulative stress throughout the supply chain.

Experimental design
Mud crabs (n = 10) were placed into 40kg plastic crates lined with double-layered damp hessian and an inter-folded damp hessian cover. Crates were then placed in one of six temperature controlled environments (pre-conditioned cold-rooms or incubators) at 10°C, 15°C, 20°C, 25°C, 30°C or 35°C. Crabs were held at constant temperature for 48 hours, however the crabs held at 10°C crabs were exposed for only 24 hours (see results). Monitoring of temperature was continuous throughout the trial period with crab condition assessed daily. Where relevant, the covering hessian was lightly doused with seawater to prevent drying out, with care taken to ensure that evaporative temperature effects were not introduced.

Haemolymph samples were analysed for pH, glucose, protein and lactate immediately pre- and post-incubation. After post-incubation bleeding, crabs were purged in aerated seawater (1:10, live weight (kg) : litre). Ammonia levels in the recovery water were monitored over 2 hours for 10°C to 25°C held crabs or 3 hours for 30°C and 35°C held crabs.

Crabs were subject to continued monitoring for two weeks after cessation of the trial and while in the holding tank because temperature change itself may not cause mortality but the accumulated effects of the change may become evident later.

Results and Discussion

Analysis of data identified abnormal levels for stress indicators measured in all crabs held at 20°C. Results included unusually high basal glucose and lactic acid levels and these biomarkers decreased as a consequence likely independent of the temperature effect. Review of quality control log records unfortunately revealed the mud crabs used for the 20°C trial to be 'new' and unacclimatised crabs. Therefore they were not in 'rested' state but suffering from stress imposed by the recent transport of supply, causing the high stress indices. Due to this information pertaining to this crab batch, results obtained from this trial was not included in further data analysis.

Crabs held at 10°C were only exposed for 24 hours since, at this time, these crabs showed signs of 'near death' behaviour including severe foaming at the mouth and extreme slowness. It was decided that exposure for a further 24 hours would lead to certain mortality before haemolymph samples could be extracted. Therefore, crabs were analysed after only 24 hours of temperature exposure. The data for 24 hours has been included in the graphs below. It is noted that exposure for only 24 hours still resulted in mortalities of 40% during the recovery phase. Such extreme response to low temperature is evidence that 10°C is way too low for mud crab to survive and needs to be avoided completely. A similar finding was reported by Gillespie and Burke (1992) who found that 12°C imposed temperature stress on mud crabs and limited survival. These authors stated that crabs were completely immobile and suggested that dehydration at this temperature was the main cause of stress to the animal.

Analysis of haemolymph pH illustrated different reactions within the crabs to various temperature exposures (Figure 5.68). Although storage at 15°C and 25°C lowered haemolymph pH over 48 hours, levels were still within the acceptable or normal range for rested crabs. In contrast, extreme temperatures of 10°C and 35°C dramatically increased pH indicating acute stress levels. Also noted, was the rapid rate of change in pH of extreme temperature shocked crabs, in particular a sudden drop from room temperature (25°C) to 10°C. Subjection to 30°C led to a minor increase in pH indicating a slight stress.

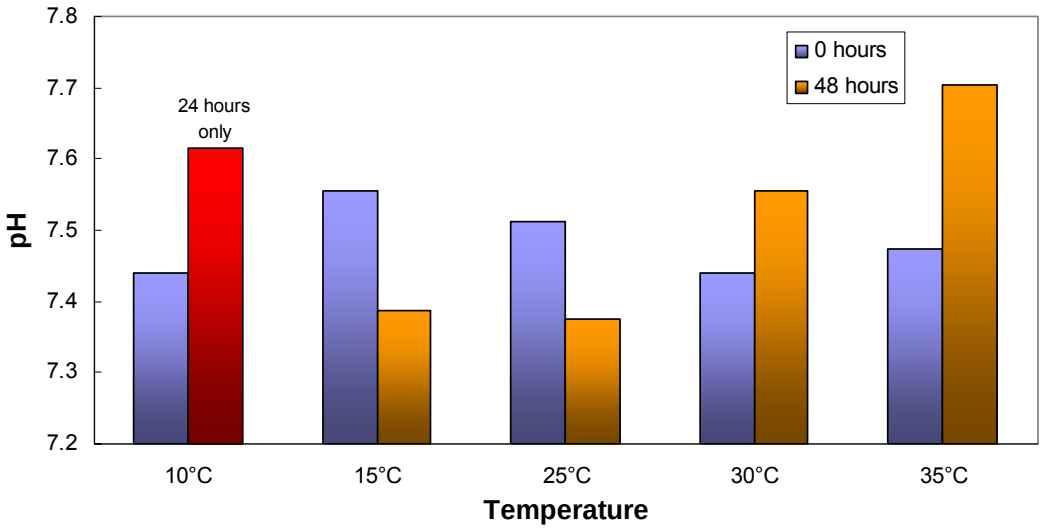

Figure 5.68. Haemolymph pH of crabs exposed to different temperatures.

Haemolymph glucose levels rose in crabs exposed to all temperatures (Figure 5.69). Mud crabs held at 25°C and 30°C maintained glucose levels within acceptably normal levels, whereas exposures at 10°C, 15°C and 35°C resulted in levels considered to be indicative of stress. Previous studies identified this stress zone to be glucose levels above 1.0 mmol/L.

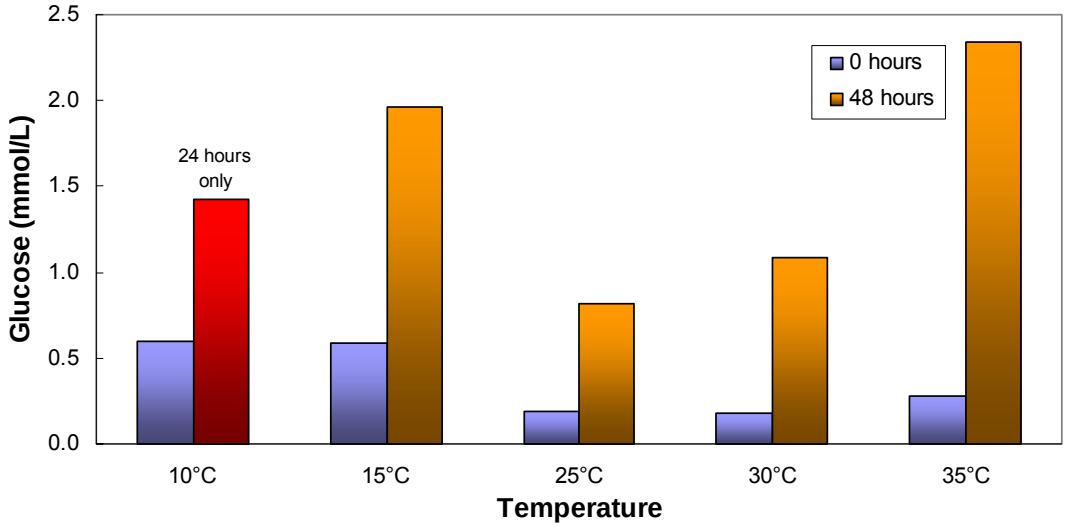

Figure 5.69. Haemolymph glucose levels of crabs exposed to different temperatures.

Replication of 30°C incubation with a separate batch of crabs verified glucose circulating at the same levels in both trials. However, replication of 35°C exposure presented contrary results. Glucose levels in the replicated trial remained low with a mean for the 10 crabs of 0.56 mmol/L, indicative of no stress imposition on the crabs. Reasons for these disparate results are unclear. It is possible that crab metabolism is operating at the limit of functionality and hence for crabs that showed stress at 35°C in one trial, there was some accumulative stress affects from unknown other factors. The variation in responses obtained for crabs subjected to this temperature is concerning as it could be expected that mud crabs harvested in tropical waters are frequently exposed to temperatures this high and higher. From the results demonstrated here it is fair to conclude that high temperatures will cause stress in mud crabs and hence should be avoided. Lactic acid levels in crabs at all temperatures appeared unaffected and remained very low (< 0.25 mmol/L).

Immersion in water is critical in a crab's ability to excrete excess ammonia through its gills. Emersed crabs accumulate ammonia due to the inability to be able to exchange it with the air environment. Previous trials in this study have established a successful method in monitoring the amount of ammonia accumulated during emersion through re-immersing crabs in fresh aerated seawater with regular water sampling and analysis. Initial work identified a purge of 2 hours was sufficient time for ammonia levels to resolve, with no further detectable increase. However, during the trials involving high temperatures, it was noted that crabs were still releasing ammonia at a steady rate after 2 hours re-immersion. Consequently, purge times were increased to 3 hours for temperature exposures of 30°C and 35°C (not shown on the figure below).

The ammonia levels for crabs held at 10°C showed little change over the 2 hours (Figure 5.70). This would suggest that mud crabs exposed to this temperature

completely 'shut-down' metabolically and were on the verge of expiration. When re-immersed, the previous 10°C temperature shock had caused irreversible damage. The inability to remove ammonia from their system would be a major contributing factor to the high mortality rates observed post-purge. It could also explain the other stress reactions exhibited in these crab, e.g. foaming and inertia.

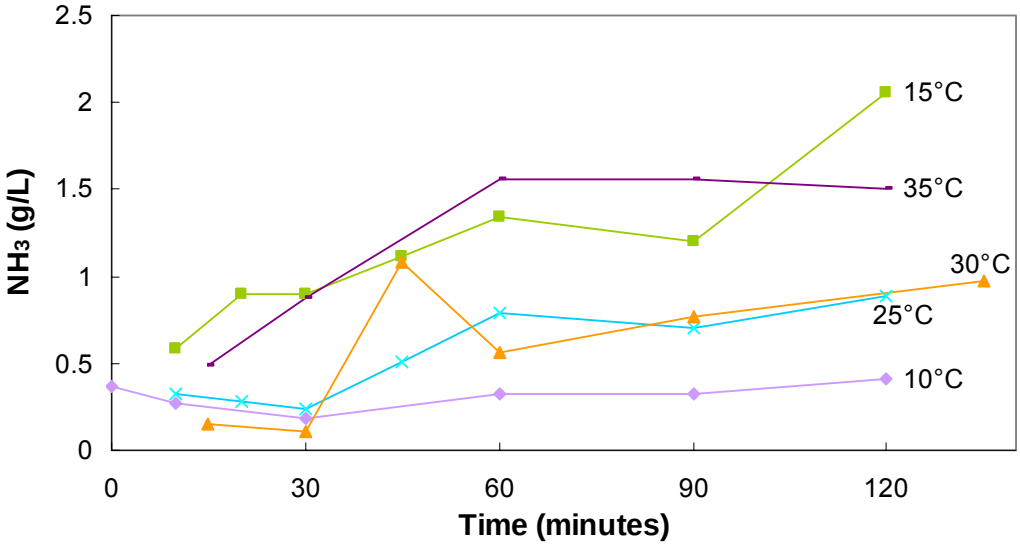

Figure 5.70. Ammonia excretion in seawater after temperature exposures.

Ammonia excretion after exposure at 25°C and 30°C appeared to be typical of crabs emersed for a similar period with ammonia excretion resolved after 2 hours for 30°C crabs. For crabs subjected to 35°C exposure, ammonia excretion was higher and occurred at a faster rate as would be expected from crabs exposed to a high stress-inducing factor. Even after 3 hours re-immersion ammonia excretion in these crabs was still increasing, evidence of very large amounts of accumulated ammonia during the temperature exposure. It is of note that exposure of mud crabs to 15°C led to ammonia excretions similar to crabs exposed at 35°C and hence this temperature is also concluded to be stressful for the crabs.

From the responses of the stress biomarkers in crab haemolymph it is concluded that mud crabs best tolerate a temperature range between 25°–30°C. Temperatures outside this range impose an increasing extent of stress on the animals and temperatures in the low range (≤15°C) can result in extreme stress. These findings imply that the temperature the crabs are stored at in Borroloola (16°C chill room) is too cold for mud crabs and is causing severe stress. However, different from many other crab species (Hopkin *et al.* 2006) mud crabs tolerate a fairly wide range of temperatures.

Other studies on the effect of temperature on mud crabs did not measure stress biomarkers but used mortality as the indicator of survival time. Gillespie and Burke (1992) reported a mean survival time of ~10 days for crabs stored emersed at 12°C, 16°C and 20°C with 95% relative humidity, but this decreased to ~6 days for crabs at 24°C, 28°C and 32°C. They further noted that 32°C was the likely upper limit of temperature tolerated by mud crabs as the animals were obviously physically stressed, regurgitated a black fluid and died sooner than crabs at other temperatures. Gillespie and Burke (1991) concluded that optimal temperatures for maintaining emersed crabs were between 16°C and 20°C, but with the additional factor of 95% relative humidity.

5.5 Modified handling procedures

Project Objective

To develop alternative handling procedures that reduce the impact on the animal by minimising stress imposed at different steps within the chain.

5.5.1 Background

The critical stress factors along the mud crab distribution chain were identified (Section 5.4) as:

- Prolonged emersion: Mud crabs are taken out of water and held in air for long periods throughout distribution. Since they are aquatic animals, holding crabs out of water results in respiratory and metabolic stress, as well as dehydration.

- Handling practices: Mud crabs are handled frequently at different points along the supply chain. Each handling involves physical movement of the crab and often a degree of shock. Hence, the type of handling and its severity directly influences the stress levels observed.

- Temperature: In the extremes of summer months, especially at low tides, water temperature surrounding the anchored crab pot can be very high (40^{o}-45^{o}C). Additionally, ambient temperatures for emersed crabs are high though this is compensated for during base camp holding periods by use of shade cloth shelters and regular dampening of the hessian wrapping in the crate. During transport stages and repacking at the wholesaler factory, the crabs are subjected to sharp drops in temperature.

Work in this part of the study focussed on ways to modify the current practices at these critical points along the chain and develop methods for minimising the stress imposed on the crabs, thereby increasing survival rate.

5.5.2 Alternative holding systems

If mud crabs were in a moist environment through the supply chain, would this reduce stress imposed by emersion during transport? Realistically, there are discrete steps within the transport chain where this can be considered, with one of the most logical being during holding at base camp awaiting transport to wholesaler. All crabbers seem aware of the benefit of holding the crabs in damp sacking to create a moist and protected environment for the crab. The sacking is checked for dampness regularly. Information supplied from harvesters advised that during the 'dry' season (winter months: May-Sept) the sacking was not sprayed with water in the late afternoon as evaporation caused too low a temperature for the crabs overnight.

Many of the most isolated harvest area camps hold crabs for up to 7 days after taking from pots, in order to collect enough crabs for cost-effective transport out of camp to Darwin. Several of these operators have considered the effects of such a long period of emersion on the crabs and have purpose-constructed shade cloth enclosures to alleviate high ambient temperatures and dehydration. Camps in the

Borroloola harvest areas (Wearyan River and the mouth of the M^{c}Arthur River) have dedicated holding sheds and one of these contained an overhead sprinkler system for spraying the crabs.

These actions illustrate that there is strong recognition among fishers that a moist environment is important to the crabs. However, there is no information about what should be done to ensure maximum survival. Communication from several harvesters queried whether 'dunking' the crabs daily would be better for them. Hence we conducted trials to determine the effect of different water applications to the crabs.

5.5.2.1 Effect of spray or dip on stress levels

Experimental design
Mud crabs were held in one of three environments:

- Commercial holding practice – tied crabs held all together in a crate with an outer liner wrap of damp hessian
- Dip – held as commercial practice with a 2 hour re-immersion once per day – the whole crate of crabs dipped in aerated seawater
- Spray – holding tank system created with constant fine spray over the crabs. Crabs were claw-tied but free to move to location of choice within a restricted space to simulate conditions of 'crowding' as would be found in commercial practice (Plate 5.10)

Plate 5.10. Crab holding conditions for spray system.

Eight individual crabs were used for each holding system and held for 5 or 14 days. Stress indicators of haemolymph protein, pH, glucose and lactate were measured. Ammonia build up at the end of the holding period was assessed by excretion rate immediately after re-immersion in fresh seawater.

Results and discussion:
For crabs held in the different systems for 5 days, there was no change in total haemolymph protein in any individual. Blood pH was about a pH unit lower for commercially held and dipped crabs indicating metabolic stress had occurred. Slightly reduced pH (0.04 pH units lower) for crabs held in the spray system demonstrates lower stress levels in these crabs compared to crabs held under commercial or dip systems. The pH of bloods was reflected in lactate levels where there was no significant difference between treatments. One of the best indicators of stress in crab is haemolymph glucose levels. Under the different holding conditions, crabs held in the spray system showed little increase in glucose levels from day 0 to day 5. However, crabs held by commercial practice and those that were dipped had

elevated glucose levels at 5 days that is likely due to metabolic responses to physical activity and aggression of the crabs (Figure 5.71). Individual data sets are shown to illustrate variable crab response, which may be related to the extent of activity for any individual.

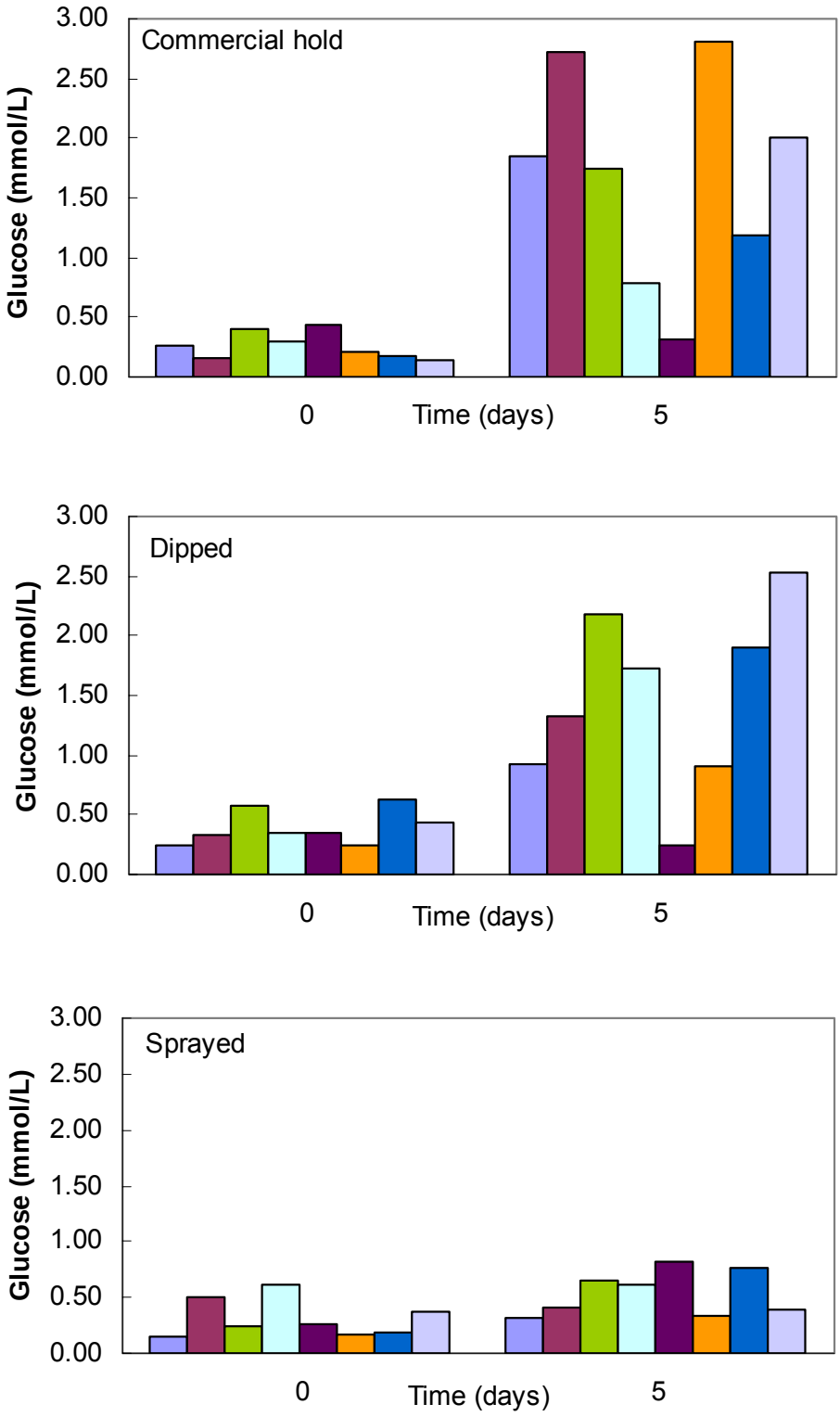

Figure 5.71. Haemolymph glucose in crabs held under different conditions for 5 days.

Both the spray-held crabs and the dipped crabs had lower rates of ammonia excretion when re-immersed after 5 days held under treatment conditions compared to crabs held under commercial practice (Figure 5.72). The baseline data included here was obtained from crabs taken directly from the main holding/recovery tank and provides ammonia excretion values of control crabs.

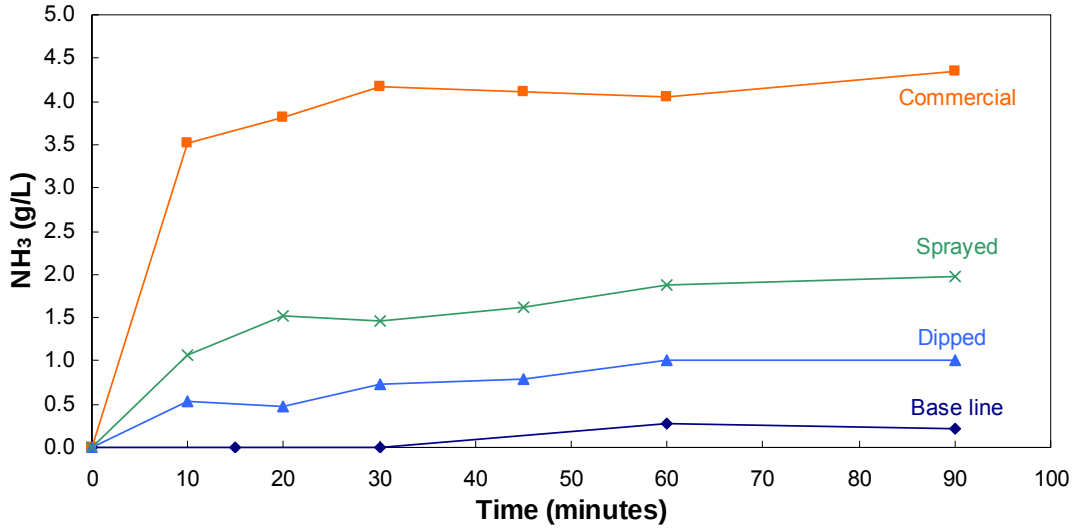

Figure 5.72. Excretion of ammonia immediately after re-immersion following 5 days held under different conditions.

A noticeable difference between the different holding systems was the temperature of the immediate environment within each system and the fluctuation throughout the holding period (Figure 5.73). In the spray system, crabs remained at a steady temperature of 26.5°C (± 0.5). The temperature of commercially held crabs was steady between 22° and 23.5°C but showed small temperature spikes when water (27°C) was added to the hessian sacking to maintain dampness (times of water addition indicated by an arrow in (Figure 5.73). The daily 2 hour dipping of crabs in aerated seawater at 27°C caused sudden peaks in temperature of the crabs with the overall practice resulting in large temperature fluctuations for the crabs. As was observed in previous investigations (section 5.4.4.3), large temperature changes contribute to stress accumulation within crabs. Temperature monitoring within these trials therefore indicate that a spray system is most effective at keeping a consistent temperature for the crabs.

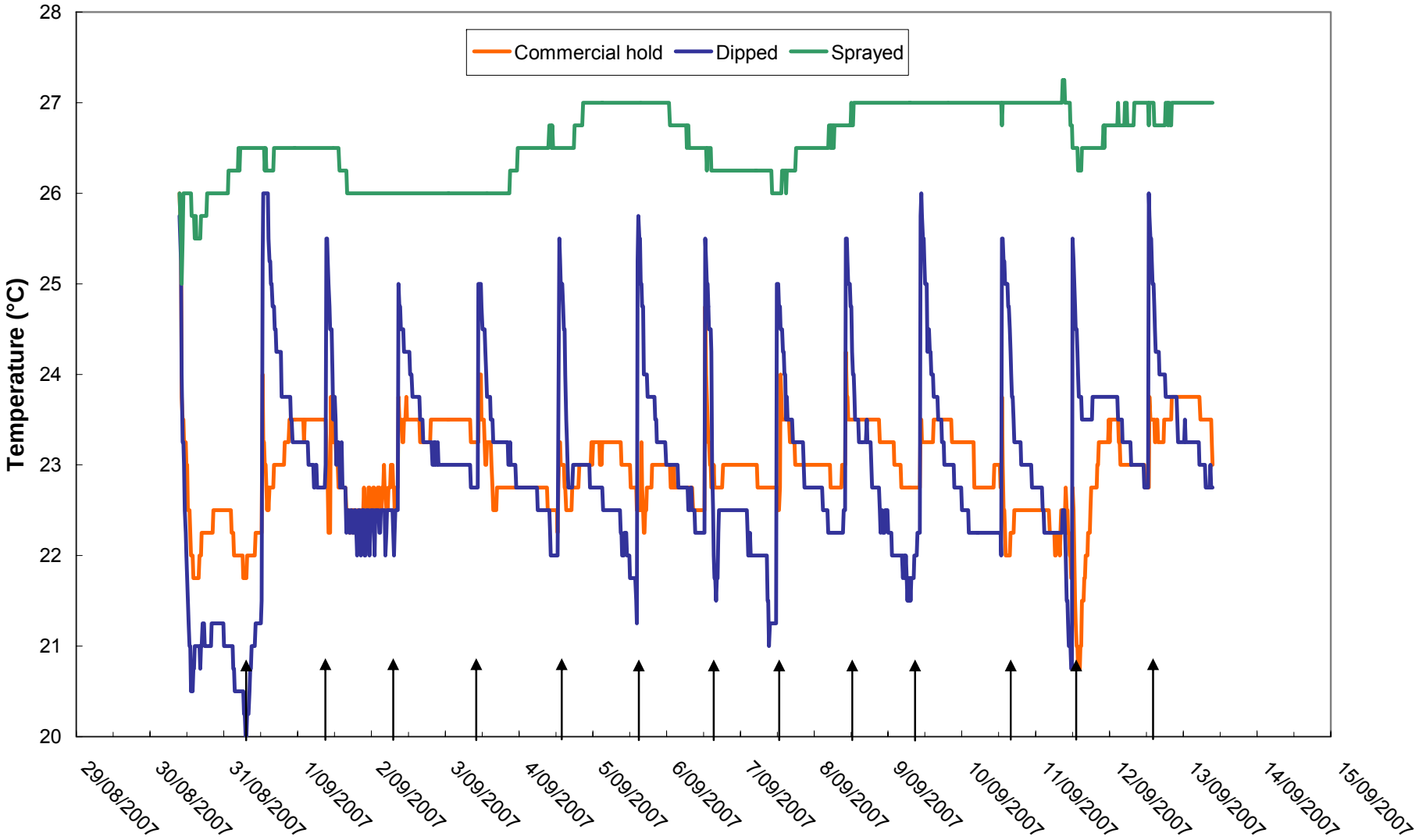

Figure 5.73. Temperature of mud crabs held under different holding conditions (times of water addition or dip indicated by arrows).

Results were similar when crabs were held for an extended period of 14 days under the same experimental conditions. Crabs that were dipped daily or held under the spray system had blood glucose levels of <1.0 mmol/L indicating a 'non-stressed' animal. There was far greater ammonia excretion from crabs held under commercial practice after 14 days compared to that from crabs held using the other systems (Figure 5.74). This would be expected for crabs held out of water for such a long period and rates given here were similar to those obtained from other trial work with emersed crabs. It is of note that both sprayed and dipped crabs had very low excretion rates of ammonia after re-immersion, implying less ammonia accumulation during the holding period.

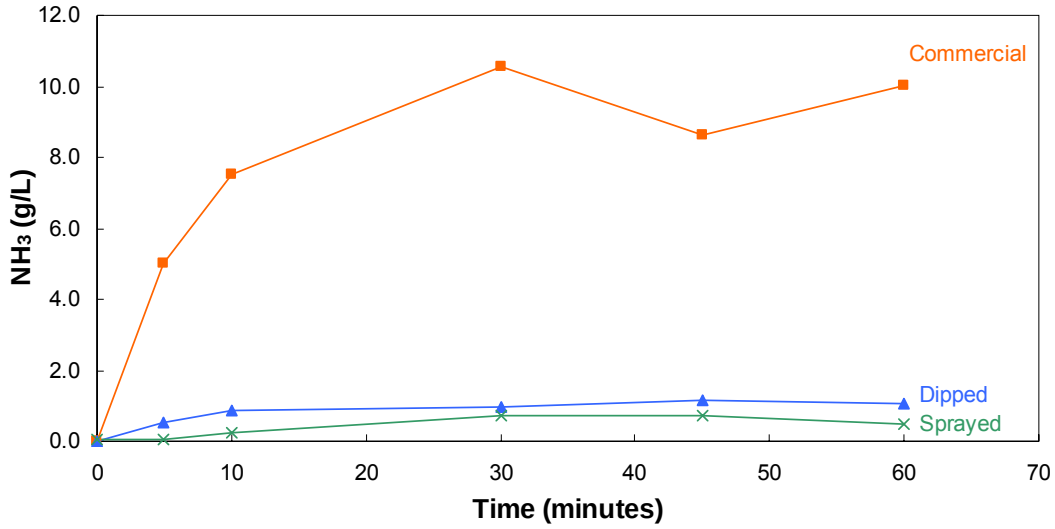

Figure 5.74. Excretion of ammonia immediately after re-immersion following 14 days held under different conditions.

5.5.2.2 Effect of dip duration on stress levels in mud crabs

During discussions with mud crab harvesters, it became evident that there was a great deal of confusion as to the benefits gained from dipping crabs (commonly referred to as 'dunking') during storage at base camp. Several harvesters strongly advocated the practice, others were more doubtful of potential benefits partially from the basis of the additional handling that 'dunking' would dictate. Having gained a better understanding of the biochemical changes that occur with different handling practices, the effect of different dipping times on the crabs was examined.

Experimental design
Five groups of 8 crabs were held emersed under commercial practice conditions for 7 days, then subjected to a dip in aerated seawater for times of 1, 5, 15, 30 or 60 minutes. For each dip time, a different group of crabs was used. Haemolymph samples were taken immediately post-dip for stress index analysis. Seawater samples (Plate 5.11) were also taken for determination of ammonia excretion rates. Crabs were then returned to a seawater holding tank and observed over the next 7 days for liveliness. Crabs were bled again at 14 days for further analysis.

Plate 5.11. Water sample being taken for ammonia efflux analysis.

Results and discussion:
The pH of haemolymph in crabs dipped for 1, 5 or 15 minutes did not change significantly from the rested (basal) level. Those crabs dipped for 30 minutes were highly variable with respect to the haemolymph glucose response between individual animals, with some showing an increase in pH and others showing a decrease. After a 60 minute dip, all crabs had a slightly higher blood pH than the resting level, indicating that 60 minutes is a period long enough for shifts in metabolism to occur.

The effect of dip-time on glucose levels in crab haemolymph is presented in Figure 5.75. All dipping times resulted in elevated glucose levels, with a 15 minute dip causing the greatest increase.

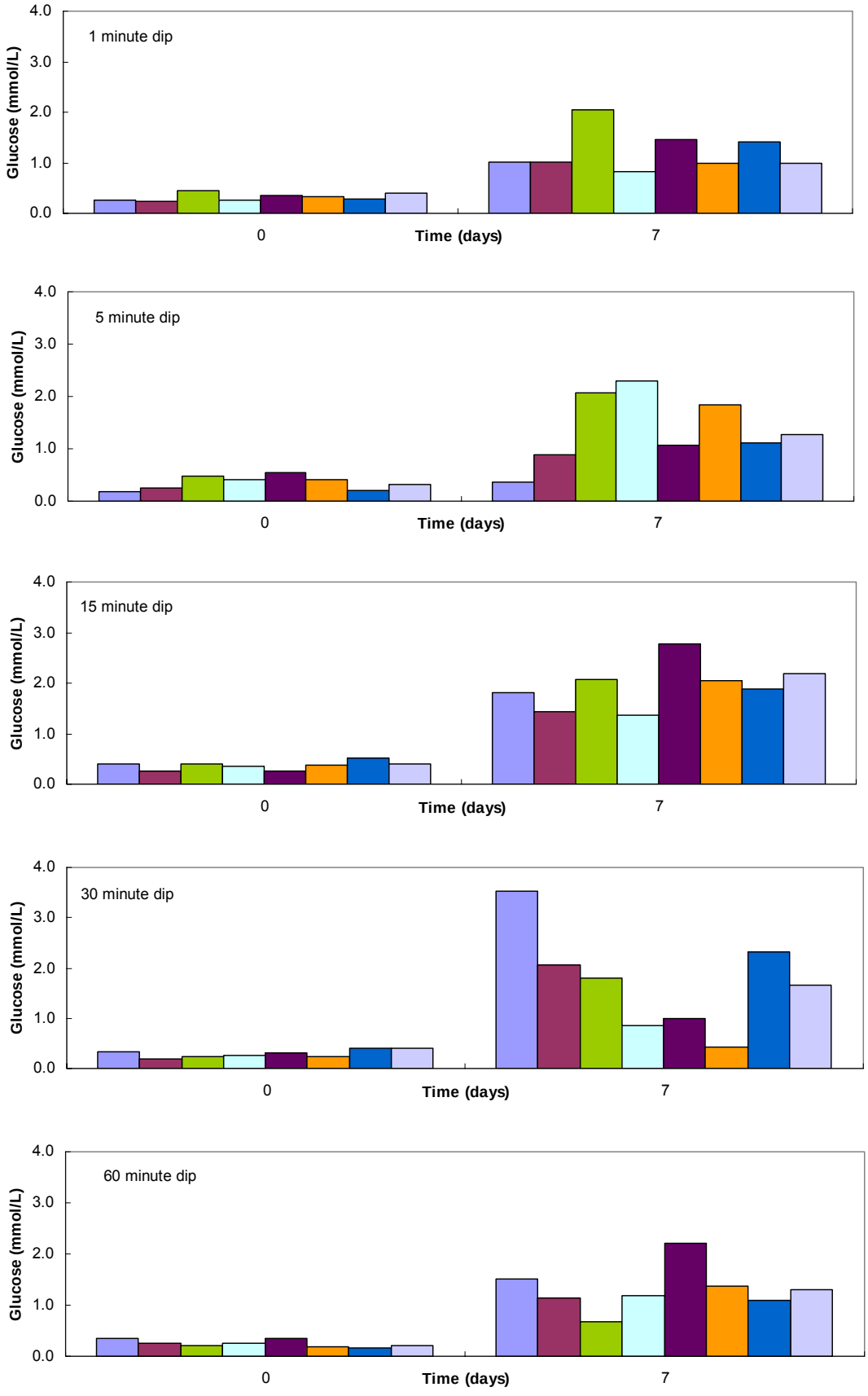

Figure 5.75. Haemolymph glucose levels in crabs after different dipping times.

A 5 minute or 30 minute dip resulted in the greatest variation in circulating glucose increase between individual crabs. This may suggest variable individual metabolic responses or perhaps it is an anomaly. Similar individual variation was subsequently observed in crab batches dipped for 5 or 30 minutes after a further 7 days storage post-dip, demonstrating the suggested metabolic effect is real. The differences in metabolic response could be due to aggression and movement inside the sacking.

Ammonia excretion immediately post-dip was slightly higher after a 1, 5, 15 and 60 minute dip as compared to control treatment crab. The ammonia excretion after a 30 minute dip was very high compared to the others. This may indicate the switching on of some hormonal trigger that causes a major shift in metabolism. This again points to a need to investigate exactly how mud crabs remove toxic ammonia from their systems while emersed.

An additional observation during this trial was the level and rate of faecal excretion. Records show that the amount excreted was greatest after the 1 minute dip and decreased to be least after a 60 minute dip indicating that the trigger for release is almost instant in mud crabs when they are re-immersed. This observation has high importance for handling practice at sectors further along the supply chain and emphasizes the critical need of re-immersing crabs in a recovery tank for a short period after the long dry transport phase, prior to crabs being transferred to retail holding tanks.

Observation and liveliness grading of the crabs during the 7 days after dipping indicated that only the 1 minute and 5 minute dips caused any effect. For crabs dipped for 1 minute, 2 of 8 were graded as ‘slow’ 7 days later, while for crabs receiving the 5 minute dip, 4 of 8 were ‘slow’ and one very ‘slow’ (almost dead). Although not investigated further in this study, such results imply that very short duration dips is very detrimental to mud crabs. A possible reason is that hormonal triggers that initiate detoxification processes are instigated immediately the crabs are re-immersed and when removed from the water, the processes are not readily reversible. This warrants further investigation to understand the mechanisms that are occurring upon re-immersion of mud crabs.

5.5.2.3 Inclusion of a recovery step within supply chains

Research investigations throughout this study have all shown that mud crabs are subjected to extreme stress factors along the supply chain to market and these are accumulative. One of the most obvious ways to reduce stress levels is to include a recovery step within the chain as occurs for all other seafood species presented for live marketing (prawns, rock lobsters, coral trout). However, inclusion of such a step would impose increased handling and infrastructure costs within the value chain. Consequently before such a practice could be recommended, a thorough investigation of the benefit to be gained would need to be undertaken. So, the question is: Would a recovery step at some phase of the transport chain reduce stress levels in the crabs and help them tolerate further on-transport to distant markets?

From recovery data obtained during other experimentation in which crabs were used for several different trials, it appears that mud crabs are capable of full recovery from the stresses imposed. This conclusion is based on measured biomarkers (stress indices) in the haemolymph illustrating return to basal levels within 5 days. Figure 5.76 shows the levels for glucose present in the blood of individual crabs after 5 days in the recovery tank.

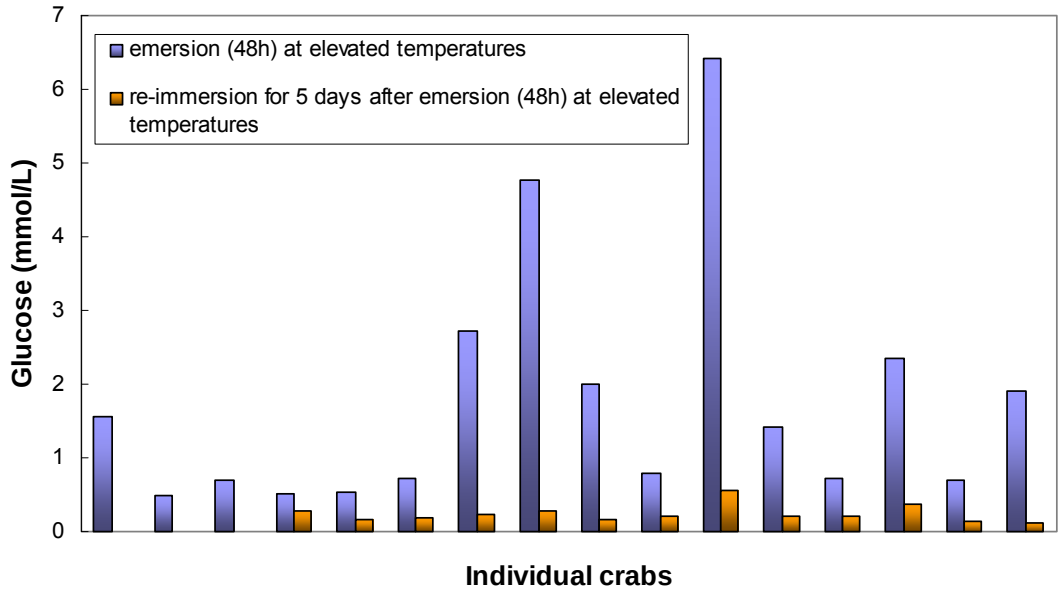

Figure 5.76. Haemolymph glucose levels in individual stressed crabs and after recovery in holding tank (*n* = 16).

Of critical importance with respect to the application of a recovery practice within the industry is exactly how quickly mud crabs recover. Investigations have already demonstrated the benefits attained from a recovery step. Thorough analysis of the full benefits of a recovery step, including how quickly and for how long the recovery is sustained, was undertaken. Most other seafood species (prawns, rock lobsters, and coral trout) presented for live marketing have a recovery step in the supply chain and this is usually undertaken soon after harvest to minimise the effects of capture stress.

Mud crabs are a hardier animal than most other seafood species. They are one of very few species that will voluntarily leave aquatic environments of toxic or poor-quality and they are able to survive up to 3 weeks emersed. However, as mortalities still occur at high rates when stress levels are severe, the practise of a recovery step was considered likely to be beneficial to reduce stress and minimise mortalities. Specific details on procedures for a recovery step need to be identified.

Experimental design
Two different recovery experiments were performed to observe stress biomarker indicators:

1. 7 crabs (mean weight 1.7kg) were emersed for 3 days, wrapped in double-folded damp hessian inside a 40kg plastic crate. Crab haemolymph was the collected for stress biomarker analysis, followed by a 2 hour purge in aerated seawater. After purging and a 10 minute drain, crabs were placed into a recirculating bio-filtered seawater tank. Daily bleeding for analysis of stress indicators was performed over the following 3 days.

2. 12 crabs (mean weight 1.2kg) were packed into a double-layered damp hessian lined 40kg plastic crate and emersed for 8 days. Crabs were then bled for stress indicator analysis followed by a 2 hour purge in aerated seawater with a 10 minute drain. Crabs were then placed into a recirculating

bio-filtered seawater tank for 24 hours. Haemolymph samples were extracted for analysis at 2, 6 and 24 hours after the purge.

Results and Discussion

It was evident from the results of the first experiment that crabs recover within 24 hours and maintained that condition for the following 3 days. The second trial was performed to determine how quickly a stressed crab's biomarkers return to normal levels.

Regardless of whether a crab has been emersed for 3 or 8 days, their weight returned to the original wet weight within 2 hours, an increase of 4% from immediate post-emersion. This gives an immediate benefit to the industry as crabs are sold by weight. Therefore, crabs exposed to a recovery step are more profitable to the industry by way of total live weight, as well as improved condition of the crab leading to less mortality further along the supply chain.

Crabs held emersed under ideal conditions for an extended period will typically display a large range of pH values (pH 7.3 – 7.8 or 0.5 pH units). For this trial, crabs were emersed for 8 days, but similar results have been observed after 14 days emersion. However, 21 days emersion causes pH to rise to levels pH >7.9 which is indicative of highly stressed crabs.

On immersion in a recovery tank (1crab to 10L of aerated seawater) for 2 hours, the mud crabs pH values increase slightly (0.05 pH unit) and display a typically wide range of values between animals. The increase continues for the next 4 hours with a total average increase of 0.15 units. The increase is likely to be due to immersion in the higher pH seawater (pH 8.0) since it will be readily absorbed into the animal's system to counteract previous dehydration effects. Additionally, the increase in pH could be related to metabolic processes like ammonia release, or to stress added from the crabs changing from a calm inactive state during emersion to an active aerated immersed environment (Plate 5.12).

Plate 5.12. Active crabs in aerated purge tanks.

Within 24 hours, the haemolymph pH for all animals will resolve to a basal level around pH 7.45 ± 0.1 (Figure 5.77). The pH of the crabs' haemolymph then remains at this 'rested' state whilst crabs continue to be immersed.

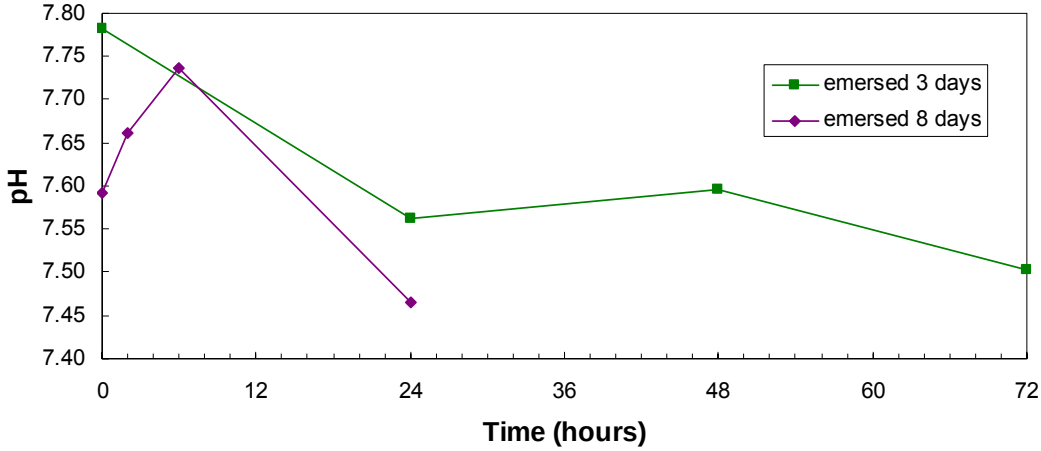

Figure 5.77. Mean haemolymph pH in crabs in the post recovery period.

As previously identified, glucose levels <1.0 mmol/L indicate a 'rested' crab. Values after the 3 day emersion trial are still within this range and after 8 days slightly above. However, both trials show a complete resolution to basal levels over 24 hours, with a drop of 0.8 mmol/L haemolymph glucose within 6 hours (Figure 5.78). As with pH, the spread of values for glucose circulating in the haemolymph immediately following extended emersion is large (0.3 – 4.0 mmol/L). This spread of values decreases during the recovery period, indicating that all crabs fully recover with 24 hours. It is likely that this recovery is achieved within 12 hours.

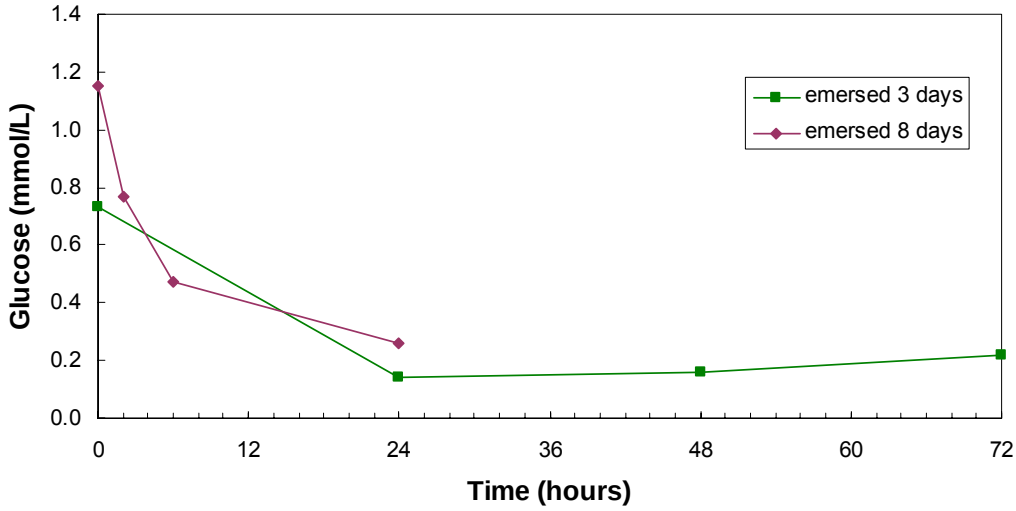

Figure 5.78. Average haemolymph glucose in crabs in the post recovery period.

Lactic acid is not a major stress indicator in crabs held emersed under ideal conditions, even when the period is extended up to 3 weeks. In fact, when held in damp hessian crates, lactic acid levels are lower than those found in crabs in the

wild or held in recirculating tanks. Hence, results for these trials remained in the same range as basal levels (<1.0 mmol/L).

Ammonia is excreted rapidly upon re-immersion of the crabs. Initially, a 2 hour purge seemed effective. Mud crabs emersed for extended periods, however, were still excreting ammonia at a steady rate at this time. After 3 hours, the ammonia efflux rate had diminished (Figure 5.79, and refer section 5.3.3.5). It is recommended that a minimum 3 hour purge be used crabs that have been held emersed >7 days.

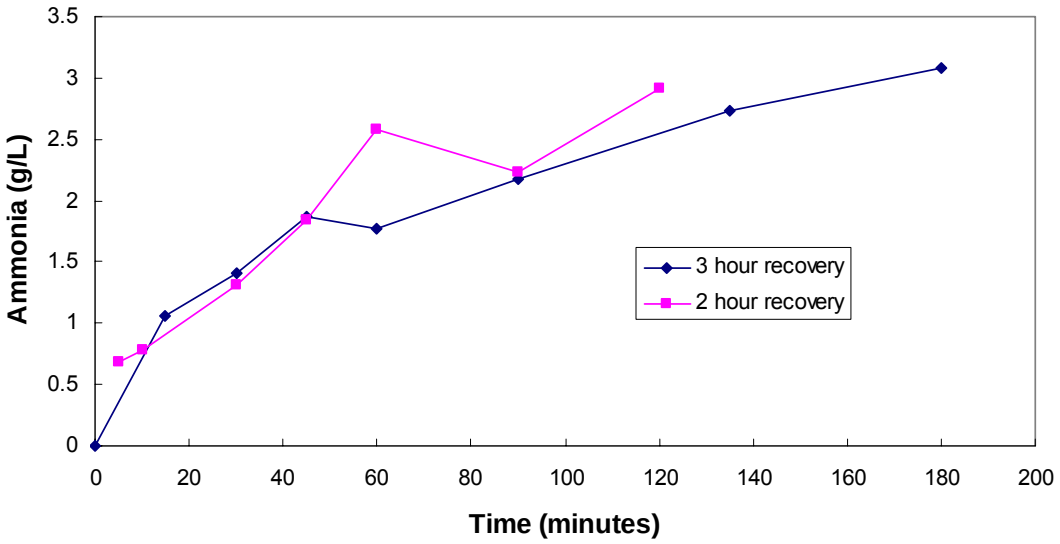

Figure 5.79. Ammonia excretion upon re-immersion.

The results from these trials support previous findings that a recovery step at strategic stages in the supply chain will result in healthier, livelier crabs in the market place. If industry facilities allow, this purge step can be followed by holding crabs in a bio-filtered seawater tank for 12 hours to allow animals to achieve maximum vigour. Following a recovery stage, on-going holding of crabs has been identified as being practical and beneficial for the crabs in either a spray system or, ideally, in tanks. Alternatively, refreshed crabs can be stored dry in crates with a daily dipping for at least 2 hours in aerated seawater. For ongoing holding of crabs in a bio-filtered seawater tank, it is vital that emersed crabs be purged for 3 hours in a separate tank prior to transferring to holding tank so that the water quality does not deteriorate. The water from the purge step should not be re-used.

5.5.2.4 Comparison of a salt water or fresh water recovery dip

Through measurement of identified stress biomarkers in mud crab haemolymph in this study, we have highlighted the benefit of a recovery step in the supply chain, as occurs for other live seafood products. To this point, recovery was in full salinity seawater. However, access to plentiful and continuous quantities of fresh seawater could be a challenge for some sectors of the supply chain. *Scylla serrata* is an euryhaline crab and it is reported that they have survived for over 6 hours in pure fresh water (Davenport and Wong 1987). These trials investigated whether fresh water is as effective as salt water in the purge stage as measured by haemolymph stress biomarkers. Also of significant importance to the industry and marketplace, is

the question in this scenario of whether a fresh water dip has any effect on the ultimate flavour of the cooked crab. Therefore, this was also investigated by trained sensory assessors.

Experimental design
Crabs were held emersed for 9 days in 40kg plastic crates lined and covered with double-folded damp hessian. Crabs were then exposed to one of three recovery procedures:

- 7 crabs were purged for 2 hours in aerated seawater then held emersed for a further 24 hours in hessian dampened with saltwater

- 8 crabs were purged for 2 hours in aerated town water that had been pre-aerated for 24 hours (to remove chlorine). Crabs were then held emersed for a further 24 hours in hessian dampened with town water

- 8 crabs were purged for 2 hours in aerated town water that had been pre-aerated for 24 hours (to remove chlorine). Crabs were then placed into a recirculating bio-filtered seawater tank for 24 hours.

Crabs were bled for haemolymph analysis immediately prior to immersion in the recovery tank, after the 2 hour purge and 24 hours post-recovery. Ammonia levels within the seawater and town water recovery phase were monitored at 0, 5, 10, 30, 45, 60, 90 and 120 minutes.

Sensory Triangle Test
A further 12 crabs were held emersed for 6 days in a 40kg plastic crate lined and covered with double-folded damp hessian. Crabs were then immersed in either aerated fresh town water or seawater for 3 hours. Crabs were euthanased by immersion for 2 hours in free draining ice, cooked to an internal temperature of 80°C in an industrial steamer (Plate 5.13), then cooled in free draining ice overnight. Ice slurry or boiling water was not used to ensure minimisation of any effect on the flavour of the meat from fresh or salt water uptake during the cooking or cooling process. Picked claw and body meat from the two treatments was kept separate for sensory analysis (Plate 5.13).

Plate 5.13. Crabs in commercial steamer and crabmeat pickers in action.

Two triangle tests (claw meat, body meat) were preformed by 38 consumers who have experience in seafood sensory analysis. The triangle test is widely used for discriminative applications in sensory analysis. The aim is to determine whether or not detectable differences exist between two samples. For each test, assessors received a total of three coded samples of crabmeat. They were told that two samples were the same and one sample was different and asked to select the sample they considered different.

Results and discussion

No discernable difference between treatments was evident in the mean haemolymph pH (Figure 5.80).

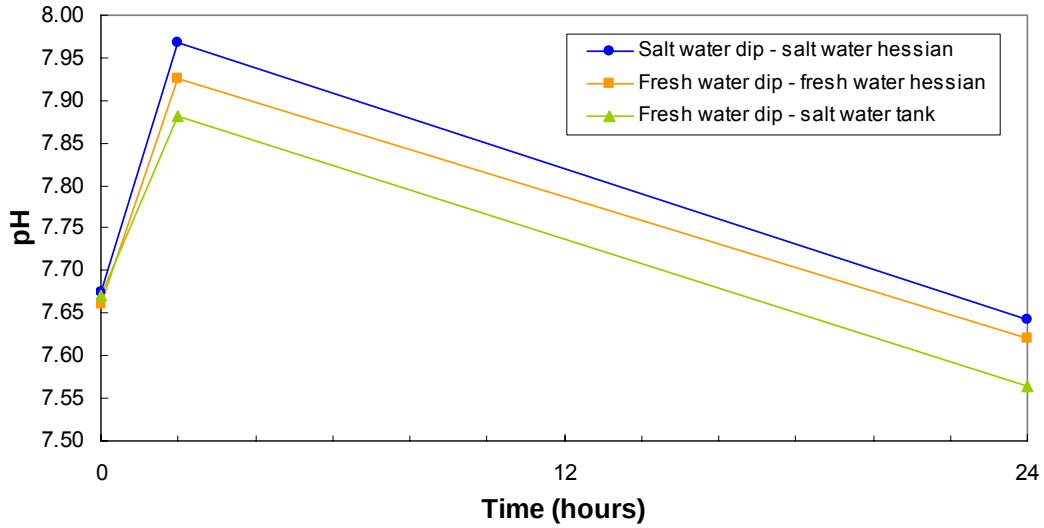

Figure 5.80. Haemolymph pH in crabs held in salt or fresh water.

All crabs returned to basal pH values with 24 hours irrespective of treatment and remained at these 'rested' levels for a subsequent 6 days (Figure 5.81).

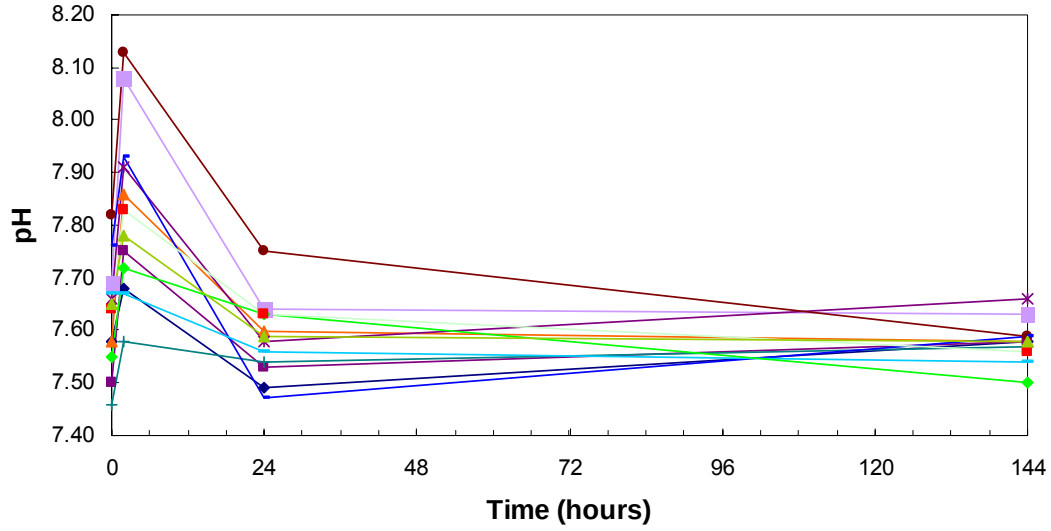

Figure 5.81. Haemolymph pH in crabs during recovery.

After 9 days emersion, initial haemolymph glucose levels were not elevated to the level that is associated with a stressed animal. However, all glucose levels dropped after the 2 hour recovery step, irrespective of the water type used (Figure 5.82).

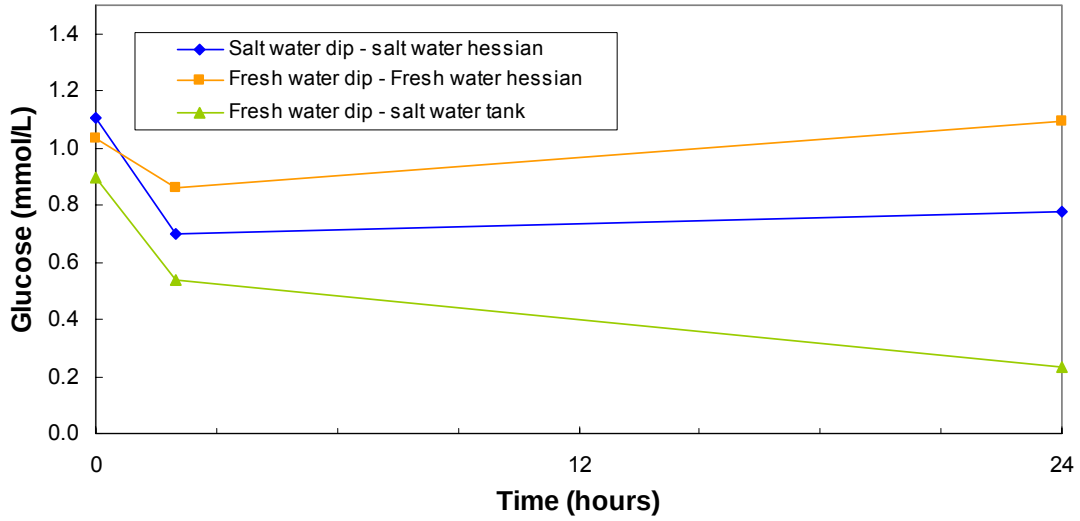

Figure 5.82. Haemolymph glucose in crabs held in salt or fresh water.

As previously demonstrated, crabs that were subsequently returned to a seawater tank continued to show a drop in haemolymph glucose values to completely 'rested' levels. The glucose levels in crabs that were subsequently held emersed in damp hessian remained at fairly constant values, while crabs recovered in town water followed by dampened hessian storage had slightly increased glucose levels. From the glucose values alone, it appears that town water is as effective as seawater for use in a recovery step, followed by the best scenario of returning the crabs to a recirculated bio-filtered seawater tank or a spray system. If this is not available, crabs can be stored emersed and kept in hessian dampened with seawater in preference to town water.

Further trials showed that crabs tolerate a 2 hour recovery step using town water straight from the tap equally as well as the previously aerated water used in the above trials, negating the need to bubble off any chlorine. It is essential that when when crabs are immersed aeration is applied to the water.

Importantly, ammonia that accumulated in the crabs during emersion was excreted at comparable rates in both town and seawater (Figure 5.83). It is established that mud crabs are able to excrete in excess of 340mg of ammonia per kilogram per day during re-immersion after 7 days held emersed (Ruscoe *et al.* 2002). Our results indicate that the toxic metabolites can be released equally effectively in town water or seawater. The benefit of this to the industry is enormous, negating the need to obtain fresh seawater at any point in the supply chain for a recovery step.

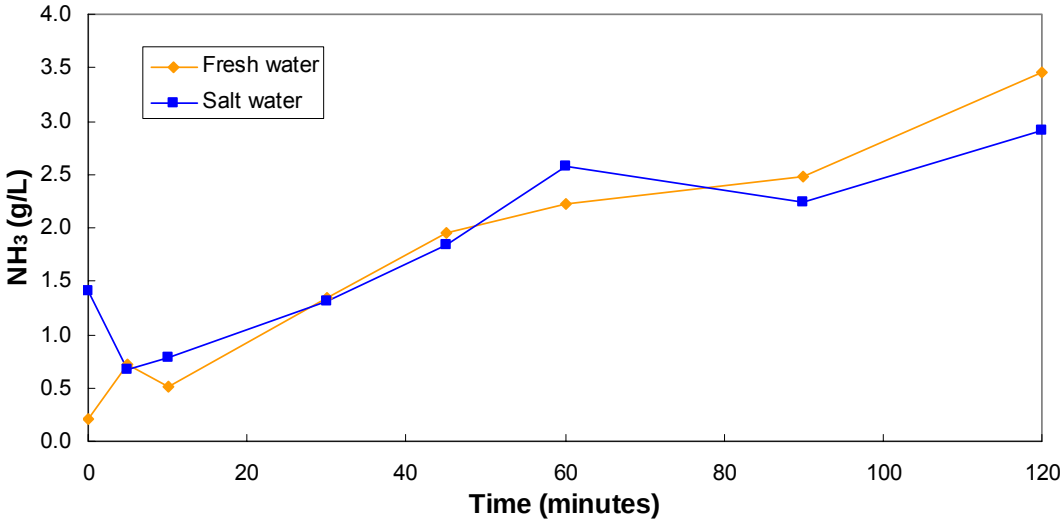

Figure 5.83. Excretion of ammonia in recovery waters.

Evaluation of the cooked crabmeat by a panel of consumers indicated that there is no effect on flavour, aroma, texture or appearance when crabs are recovered in town water as opposed to seawater (Table 5.15). The significance of this result is that a town water recovery step can be recommended with the confidence that it will have no detrimental effect on crabmeat at the consumer level.

Table 5.15. Triangle test on meat from crabs recovered in different waters.

Meat	Total responses	Correct selection of different sample	Conclusion
claw	38	18	no significant difference, α>5%
body	38	14	no significant difference, α>5%

A study of live mud crabs at the Sydney Fish Markets showed that putting the crabs back into water (re-immersion) after an extended period of emersion is actually quite traumatic to the animal. Varley and Greenaway (1992) concluded that three days was about the maximum for keeping mud crabs in air without injuring them upon re-immersion. Crabs held in air for more than 3 days appear to suffocate, or as industry describe it ‘drown’, when put back into water. It is suggested this phenomenon may occur due to dehydration of the gills rendering them unable to operate effectively when first put back in water. For this reason, crabs should be individually re-immersed into a water environment and careful observation made of bubbles extruded from mouthparts (Plate 5.14). The general stress state of the animal should be monitored and at the first sign of difficulty, the crab should be raised out of the water for a few moments, then gently lowered back in and observed again. These actions need to be repeated until the crab is no longer blowing bubbles and is vigorously active in the hand holding it. Crabs can then be released fully into the water tank.

Plate 5.14. Bubbles from mud crab mouth that occurs upon re-immersion.

The researchers have witnessed an incident of high mortalities of NT crabs upon addition to a southeast Queensland retailer's holding tank. The retailer does not usually source NT crabs and failed to check the tank for dead crabs regularly. On this occasion, dead crabs left in the holding tank overnight induced the mortality of several dozen crabs. This occurred on a weekend and may have resulted in the mortality of the entire stock. The water quality was obviously compromised by the quantity of dead animals. NT crabs freighted to southern markets have been emersed for a week or more and subjected to severe stress. They need extra care over those harvested closer to market. In this case, a thorough 3 hour purge stage would not have compromised the holding tank water with ammonia efflux, allowed staff to identify individual slow animals that could be cooked immediately and improved the health of the crabs before they were added to the holding tank.

Project Objective

Modified handling methods trialed in industry

Through work achieved in this study, we have gained an understanding of which stress factors along the mud crab distribution chain impose the most stress on the crabs and have learned that such stresses are cumulative. We have also developed modified handling practices that reduce stress levels experienced by the crabs. However, this is only of value if the industry participants know about the findings. To this end, all through the investigations, we have been in continual communication with major mud crab stakeholders and, through their participation and keenness to improve handling methods for mud crabs, we were able to assess the application of modified methods *in situ* within the distribution chain.

Certain sections of the supply chain have introduced a recovery step to 'liven up' their crabs. These recovery methods varied to differing extents between harvesters, distributors and retailers. A certain amount of handling variation is inevitable due to viability of infrastructure and related to the point in the handling chain. However, it was considered valuable to work alongside industry stakeholders to demonstrate the benefits gained from adopting the methods developed and to establish their applicability within an industry environment.

5.5.2.5 Alternative holding system proposed by harvester

This mud crab harvester at Adelaide River, NT, is passionate about mud crab quality and has an active dislike of the hessian sacking used universally within the harvesting sector to store and transport mud crabs. The reason for hessian is to keep the crabs damp and dark and to prevent fly larvae contamination. However, the downside is that hessian is difficult to fully clean and remove crab discharges (blood, body fluids and faeces) between uses. Crabs get entangled in the hessian with limb damage or loss frequent. He and other crabbers also believe the hessian gives an unpleasant flavour to the crabmeat, although this was not investigated in this project. This harvester experimented with an alternative system for holding the crabs at camp. Discussions with us revealed:

Previous practice:

- standard hessian lined plastic 40kg lug baskets

Modified practice post-consultation:

- solid walled 200L tub kept uncovered in the shade. "*Also stops crabs heating up in the middle of the traditional lug baskets*" (Harvester, pers. comm.)
- holds about 2 baskets worth of crabs in the tub which has two 3/8" drain holes in the bottom
- tub about ½ filled with crabs and has no cover
- no aeration to the water is applied
- no hessian used at all
- tub is completely filled with seawater on the high tide, to capture cleanest water, once a day
- crabs start moving water through gills in less than 1minute
- crabs move around a little when immersed (top crabs get less immersion time)
- takes about 10 minutes to drain
- faeces are washed away leaving clean crabs
- has kept large old crab for up to 3 weeks ("*usually the hardest to keep alive for extended periods*", Harvester, pers. comm)
- crabs remain as lively as when caught

5.5.2.6 Confirmation of benefits from dipping daily

A daily 10 minute dipping trial simulating the procedures developed by the mud crab harvester was conducted to assess benefit gained in vigour of crabs. Haemolymph stress biomarker responses were measured.

Experimental design:
Mud crabs (n = 20) were placed in a 40kg plastic fish bin with four 5/32" holes drilled into the base. The number of holes and diameter was pre-determined to be

equivalent to the harvester's practice. Open steel mesh was positioned on top of the bin to stop crabs escaping and the bin was placed in a controlled air-conditioned room of 12 hours light/dark periodic cycle and temperature of 25°C. Mud crabs were immersed once per day by filling the bin with 40 litres fresh seawater, which was poured in slowly to minimise disturbance to the crabs. The seawater was allowed to freely drain through the drilled holes. Drainage time was approximately 15 minutes. Crabs were bled at days 0, 7, 14 and 21 for analysis of pH, glucose, lactate, protein and amino acids. Bleeding occurred prior to daily re-immersion. On days 7 and 14, mud crabs were gently sorted during bleeding process and re-packed in reverse order, with crabs previously positioned on top, now positioned on the bottom of the bin. After immersion on day 21, mud crabs were purged for 2 hours in aerated seawater (1:10 live weight (kg) : litre water). Ammonia of the purge water was monitored during this step.

Results and discussion:
Blood parameters measured in daily dipped crab showed only slightly lower biomarker levels compared to those obtained from crab held as per 'commercial' practice (damp hessian wrapped) (Table 5.16).

Table 5.16. Haemolymph parameters in crab treated with a 15 min dip and crab held as per commercial practice.

Treatment	Glucose (mmol/L)	pH	Lactate (mmol/L)	NH_3 in purge water after 90 mins (g/L)
15 min dip	0.88	7.72	0.25	8.67
'hessian – wrapped'	1.30	7.60	0.74	10.0

One of the most significant findings of this trial is evident from comparison of the stress biomarker levels with previously conducted dipping trials. The current trial is essentially a 15 minute dip every 24 hours, where as previous trials consisted of a daily 2 hour dip with aeration. Haemolymph glucose and pH values are relatively similar for each dip time (Table 5.17). Haemolymph lactate levels are slightly elevated during a 2 hour dip possibly because immersed crabs are more active than when held emersed. These elevated lactate levels could also be due to crabs having more time to swim around and interact with aggressive behaviour.

Table 5.17. Haemolymph values for 15 minute and 2 hours dipped crabs.

Treatment	Glucose (mmol/L)	pH	Lactate (mmol/L)	Ammonia in purge water after 90 mins (g/L)
15 min dip	0.88	7.72	0.25	**8.67**
2 h dip	0.92	7.52	0.89	1.10

As well, ammonia excretion in the purge water was nearly 8 times higher in crabs dipped for 15 minutes than those receiving a 2 hour period. This is likely due to the

shorter soak time not allowing the crabs to fully expel accumulated ammonia from their system from one day to the next. The results indicate that a daily 2 hour re-immersion step is more beneficial in allowing the crab enough time to excrete stored ammonia. However, for this extended period in purge water, it is critical that aeration of the water occurs. Without aeration, crabs will quickly deplete available oxygen (Figure 5.84) due to hyperventilation and there is also a rapid build up in ammonia within the purge water to a level that will be toxic to the crabs.

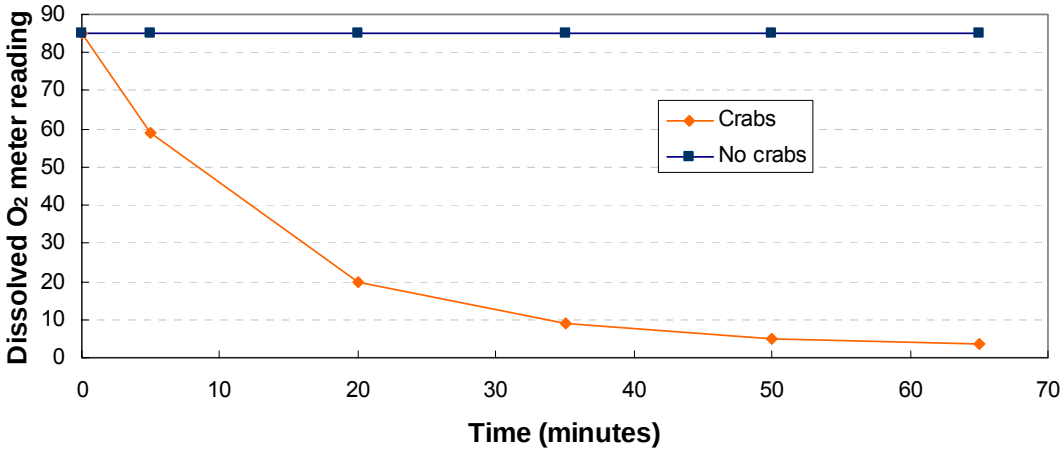

Figure 5.84. Depletion of oxygen by crabs in purge water (*n* = 3).

5.5.2.7 Recovery procedures developed

Analysis of stress indicator data obtained throughout this project concludes that returning mud crab to water allows physiological and metabolic recovery within the animal. We have demonstrated that even high levels of stress can be reduced under ideal conditions and the crab returns to full vigour. Occasionally, where stress levels are extreme, the crab has reached the point of no return and death is inevitable but this is rarely the case.

Successful reduction of stress and return to full vigour requires specific parameters within the recovery step. From experimental trials, it was found that the following factors are critical:

Prior to re-immersion:

- temperature equilibration before re-immersion – crabs and water should be the same temperature
- grading of crab – very slow, frothing should be cooked as these crab are unable to survive prolonged emersion or additional stress imposition from further transportation; crabs with a missing flipper are less likely to survive
- isolate damaged bleeding crabs, as blood in the recirculation system will increase inter-animal aggression (Dr. Brian Paterson, 2008, pers. comm.)

Recovery stage (re-immersion) parameters:

- 1:10 live weight (kg) : litre water - seawater or fresh water (aerated)
- crabs immersed tail-end first individually with mouth just under water
- hold until water starts flowing over the gills and out of the mouth (Plate 5.15)

- if the crab is lively and starts clawing it's face to pieces, fully release into water immediately
- if crab is not able to pump water across gills, then it is usually not able to recover successfully - cook
- 3 hours immersion
- dispose of water after crabs have been recovered

Plate 5.15. Water flowing from crab mouth during recovery step.

Holding/storage tank:

- monitor closely for next 2-4 hours as some, even seemingly healthy crab, may not fully recover
- depth of crabs in holding or spray tanks or bins max 3-4 crabs high
- even just-dead crabs must be destroyed - meat becomes soft, develops off-flavours and is a human health risk
- water quality must be monitored - pH, nitrates, ammonia, salinity
- regular water exchanges
- no feeding of crabs is required or advisable as feeding crabs in recirculation systems causes water quality deterioration

5.5.2.8 Recovery and short term holding trials with Brisbane retailer

The developed recovery step parameters (listed above) were confirmed in trials (total of 9 trials) conducted in conjunction with a major mud crab retail supplier between March and July 2008. Crabs that were slow or weak from sitting dry in the shop for 5-6 days were allowed to recover (3 hours) in ambient temperature aerated seawater or town water (section 6.1.2). Supply of excess crabs was also included in

recovery trials. All crabs were then held in a seawater recirculation tank at 24°C for a further 1-4 days or until required by the retailer.

Throughout the nine trials, a total of 132 crabs were recovered with only 3 mortalities. This equates to a 98% survival rate for 'slow' crab! After the 3 hour recovery step, all crab exhibited high vigour and liveliness and were able to be on-sold in excellent condition up to two weeks after initial purchase by the retailer. This is significant for the retailer economically as without this recovery step, the majority of these crabs would have been cooked instead of sold as live crab, hence not achieving highest revenue return. Based on the trial results, this retailer has now changed his handling methods to include a regular recovery step on consignment arrival, resulting in fewer mud crab mortalities and greater customer satisfaction.

5.5.3 By request from industry: Cooking and cooling mud crab

Throughout the life of this project, it has become apparent that different cooking methods and practices exist within the industry. Advice was regularly sought from us on appropriate cooking times and water salinity however, the post-cook cooling time or method was never queried. In order to provide requested information on the cooking procedure, we conducted two trials to determine if common-practice cooking and cooling times were adequate.

Common industry practice cooking method:

- euthanize crab in ice slurry (30-60 min) or fridge (3-4 h) or freezer (1 h)
- cook in seawater - 1kg for 18-20 minutes to obtain internal temperature of 80°C
- cool under running water, then in an agitated salted ice slurry (20-30 min)

Method Synopsis:

- euthanize crab (*n* = 9) (average weight 921g) in 15ppm seawater ice slurry for 60 minutes
- insert temperature probe through the synapse of the second walking leg into the centre of the crab (Plate 5.16)
- cook in seawater to obtain an internal temperature of 80°C
- rinse under running water, then immerse in 15ppm salted ice slurry until core temperature reaches 4°C (Plate 5.17)

Plate 5.16. Probe inserted to crab centre.

Plate 5.17. Logging temperature of crabs.

Results:
The results in Figure 5.85 show that a 1kg crab is successfully cooked to 80°C in 20 minutes, but requires up to **1.5 hours** cooling in agitated salted ice slurry.

When talking to industry about this finding, many retailers are shocked by the length of cooling time required. It is likely that an extended period of chilling is needed due to the crab shell being such a good insulator. This may be surprising to the industry because after a typical chill period (20 minutes), the temperature of the crab shell will feel cold to touch, and yet as demonstrated in our trial (Figure 5.85) the crab will still have a high internal temperature (>20°C). If crab is then placed in a display cabinet at ~4°C there may be potential loss of product quality. Time taken to achieve adequate temperature to avoid spoilage could be several hours. This critical information on a longer chilling requirement has been extended to key stakeholders within the industry.

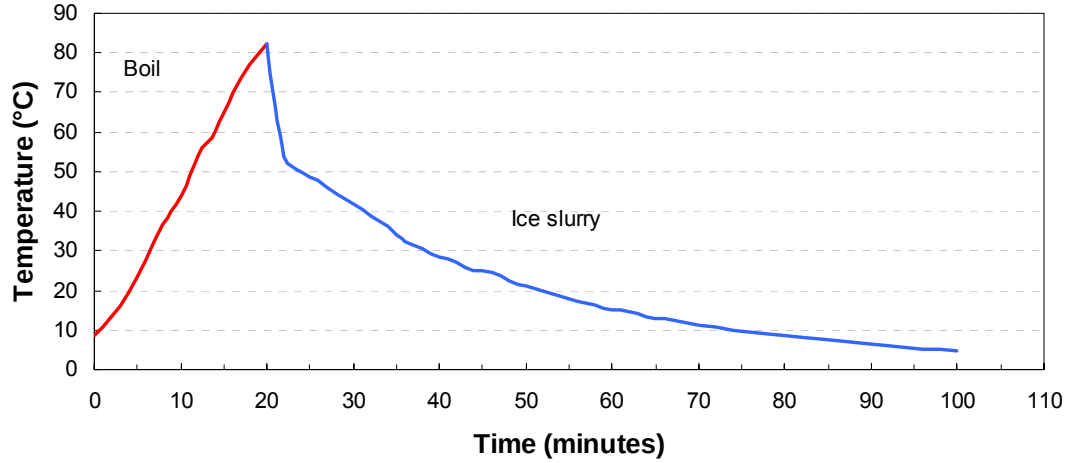

Figure 5.85. Core temperature of crabs during cooking and chilling.

Project Objective

Strategies for traceability of crabs through to market

Traceability is simply a record-keeping system designed to identify and track products from origin to consumption. Implementation of traceability is often initially driven by product safety issues as it can provide the ability to quickly trace back products at any point in their supply chain. It is important to note that frequently used term ‘product tracking’ and ‘product tracing’ have different meanings in the context of traceability. ‘Product tracking’ refers to the recording of information as the product moves through its supply and transport chain and the ability to identify in real time where the product is and what handling it has undergone. ‘Product tracing’ refers to the ability to follow a product back through the handling chain from the consumer to its source of origin.

Traceability is a relatively simple concept, however the actual process of creating an informational link between the origin of product and their handling/processing and distribution can be quite complicated. It requires strong communication relationships in both directions along the product supply chain and a level of vertical integration and co-operation surpassing that which usually exists.
Implementation of a robust traceability system has some major advantages:

- enhanced information management
- increased responsiveness to market demands
- creates a greater ability to respond to internal supply chain issues
- an ability to underpin market discrimination and advantage

Additionally, there is an increasing pressure from food regulators and authorities demanding the implementation of traceability systems for both domestic and international trade. For example, new traceability requirements are now mandatory in the EU and apply to all food, food-producing animals and all types of food chain operators from the farming sector to processing, transport, storage, distribution and retail to the consumer. Information on the name, address of producer, nature of products and date of transactions must be systematically registered within each operator's traceability system. This information is required to be kept for a minimum of 5 years and, on request, it must immediately be made available to relevant authorities.

Given the pressure in this direction, it is realistic to develop strategies for implementing traceability systems within any particular food supply chain including the live mud crab distribution chain. Currently, there is an increasing variety of product tracking and tracing systems being developed. These fall basically into three categories:

Paper-based

- simple
- inexpensive
- often already semi-existing
- easily understood
- time-consuming and labour intensive
- allows human error
- no ensured data integrity
- information retrieval and compilation is manual and complicated

Bar coded

- familiar style system, understood
- tried and tested, commonly used
- flexible, adaptable
- easily implemented into current practices
- readily available equipment and technical support
- minimal data collection
- no personalisation of labelling
- need additional equipment to read codes, requires capital investment
- can be relatively inexpensive

RFID (Radio Frequency Identification) and Smart tags

- sophisticated data collection
- enormous amount of data can be included
- provides full product history
- accurate
- tamper-proof
- environmentally resilient
- depending on tag design can also allow branding
- can be read-only and read-write
- expensive
- unfamiliar technology

- large capital investment
- requires specialised technical support

Industry stakeholders requested that the issues surrounding implementation of a traceability system for the mud crab supply chain be included within our research, with a view to developing strategies for through chain traceability. To this end, several detailed discussions were held with the commercial sectors of the industry and a workshop was conducted, July 2005 (in collaboration with Alan Snow, Seafood Services Australia) to initiate further joint discussion. A brief synopsis of all discussions follows:

Mud Crab supply chain

The relevant points identified for mud crab operations relating to traceability are:

- caught in geographically isolated locations
- infrastructure at harvest points are extremely fundamental
- a low technology industry
- long supply chain – catch to factory to market
- variable temperatures included in chain
- different transport methods used
- catch supply usually once per week
- crabs maintained as individual catcher lots
- usually sent to market by air freight – interstate and overseas
- some have investigated tagging of individual animals
- control is lost after pack-out step
- existing system identifies:-
    - catching areas
    - harvester - all crates are marked
    - weight and discards recorded
    - receival number
    - grade recorded
    - Info on delivery slip to wholesaler
    - can trace back if any complaints/issues emerge

Industry confirmed interest in a cost effective system that is simple and easy to use and implement.

Important issues identified

Two main challenges were repetitively raised – must be simple and cost-effective. Other points of note mentioned:

- a weeks supply consists of multi-day catches
- attachment of a tag to the crab – loss will mean traceability is compromised
- what unit should be identified – an individual crab?

The system will need to include the following aspects:

- store multiple information sets
- have a readable identification
- simple and efficient mechanism for attaching tag to crab
- tag must remain attached
- tag should be robust - environment is seawater to boiling pot
- be unique and tamper-proof
- capable of carrying brand

- easily removed if required but not able to be reused (at restaurant/consumer level)
- be cost effective!

Other factors to consider:

- information required or desired
  - data collection
  - scanning
  - data transfer and collation
  - information accessibility
- further tracing if crab processed (e.g. meat picked)
- tracing actually to consumer level
  - what is acceptable visually
  - great for a consumer or waiter to plug the id into a computer interface (e.g. palm pilot) and show crab history including pictures of where the crab was caught
- possibility of incorporating systems to meet the Clean Green Program requirements

6 Benefits and Adoption

Project Objective

Update industry of results through participation in trials

Consultation with major stakeholders and individuals throughout the supply chain readily illustrated a huge variation in handling practice within the wholesale / retail industry sector. Specific handling procedures were usually developed according to level of knowledge. Discussion and extension of the findings from this research project has led to improved handling practices resulting in improved live quality, fewer mud crab mortalities and greater customer satisfaction.

In this study we documented current live mud crab holding methods throughout the major industry wholesalers and retailers. Methods of holding included emersed, immersed, spray and daily dip/dunk. These are discussed separately below. The survey sheet used and comments received from various industry stakeholders are included in Appendices 6.1 and 6.2.

6.1 Crabs held emersed in various sectors of the industry

6.1.1 Darwin wholesaler and retailer

During the life of this project, we have had regular visits to and contact with a major mud crab retailer/wholesaler in Darwin. Through these consultations, the owner has made significant changes to his handling procedures and facilities. On one of our earlier visits, the entire stock of live mud crabs had been put in the cold room by a well-meaning staff member. Naturally they all died. Things have changed!

Existing practice:

- shed – large roller door entry, usually open for breeze
- graded on receival into unlined (no hessian) lug baskets and stacked against wall – exposed to breeze, machinery noise, people traffic and Darwin heat!
- stored overnight or for extended number of days in lug baskets
- 70-90% survival during storage

Modified practice post-consultation:

- Initial changes - quieter location and hessian cover draped over lug baskets
- Current modifications:
  - at ambient temperature
  - back of shed – less human traffic but still frequent disturbance from inquisitive visitors
  - purpose-built tank system (2) – fibre glassed thick styrofoam tanks
  - 40 cm deep water
  - bio-filter system used (commercial variety)
  - initially both tanks had excessive water flow that disturbed and moved crabs around like a washing machine; excessive frothing water. Water flow was subsequently reduced with bleed-offs in place

- holding tank 1 uncovered – arrival crabs, more frequent water exchanges
- holding tank 2 covered – stored crab, no water quality checks – if water surface foams, water replaced
- 95% survival during storage

6.1.2 Brisbane local retailer (1)

Due to proximity to our research laboratories we have had extensive dealing with this popular high-end retail outlet.

Existing Practice:

- Crabs were graded on arrival and slow crabs were cooked for sale
- crabs stored on shop floor in air-conditioned room, positioned in breeze/cool airflow
- dry fish bins, newspaper on bottom
- stores 5-6 days
- 80% survival during storage

Modified practice post-consultation:

- recovery on arrival in salted town water for 3 hours with intermittent addition of new running water to aerate water and remove dirt, mud, faeces, ammonia
- fish tubs position moved on shop floor - breeze free location and mesh lid
- normal supply is Wednesday - any crab left after weekend trade were given a purge step (2 hours salted town water)
- 98% survival during storage
- receiving good feedback from customers

6.1.3 Brisbane local retailer (2)

A large, well-established retail outlet.

Existing Practice:

- stainless dry bin on shop counter
- high level of people disturbance
- slow crabs iced on death and sold green!
- not willing to proffer much information

Modified practice post-consultation:

- no change – not willing to accept advice

6.1.4 Brisbane seafood retailer (3)

The owner of this outlet received information about our research and extensive live mud crab holding knowledge from the previous retailer mentioned in this milestone. He adapted the town water daily dipping procedure to suit his own operating conditions and had instant benefits.

Existing Practice:

- 19°C air-conditioned shop
- held on shop counter

- dry fish bin, uncovered
- high level of people disturbance
- stored 5-6 days
- 90% survival during storage
- some mortalities, customer complaints at end of storage period

Modified practice post-consultation:

- daily immersion in running town water pouring from a height (to create aeration) for 2 hours
- stored 5-6 days – fewer mortalities
- lively crab after 6 days
- 95-97% survival during storage
- feedback from customers of good, cleaner flavour, improved vigour

6.1.5 Brisbane seafood retailer (4)

This long-standing Brisbane icon of quality seafood was renowned for their year round live mud crabs. The owner used to own trawlers on the Queensland east coast and has extensive experience in live crabs in both Australia and the UK.

Existing Practice:

- children's wading pool on shop floor
- sloping to allow dry end and semi submerged end to ½ way up carapace
- high level of people disturbance
- crabs slow after 4-5 days
- would not buy NT crab – "*too many die*"

Current Situation:

- no longer in business due to "*fishery buy-outs*" – a very grumpy pom!

Previous trials during our research relating to disturbance showed high lactate levels in crabs that were constantly exposed to human interference. Location of live crabs within a retail outlet is a trade-off between a less stressed animal and maximum exposure for increased sales. Perhaps, fewer crabs on display and a 2 hour recovery step each day would have achieved better results.

6.1.6 Melbourne seafood restaurant

This high-end specialist seafood restaurant is situated on Melbourne's South Bank. The owner has only recently added mud crabs to the menu and was experiencing more mortalities than his supplier said he should expect.

Existing Practice:

- quiet dry covered
- regular mortalities

Modified practice post-consultation:

- quiet, damp and covered
- reduced temperature fluctuations
- daily dip in town water

- improved vigour after 5-6 days
- fewer mortalities
- prefer NT crab – flavour

6.1.7 Bundaberg mud crab farmer

New to the mud crab farming industry, this aquaculturalist contacted us for advice on how to ship live mud crabs to Sydney.

Existing Practice:

- no experience with live supply chains

Modified practice post-consultation:

- gently hose and clean (scrub if necessary) crabs at harvest from pond
- hold in recirculated system, unfed for 24 hours prior to shipment to Sydney Fish Markets – allows crab to excrete faeces and de-stress after cleaning
- styrofoam eskies with air holes and damp newspaper
- soldier pack crabs face up
- 18°C optimum; not <16°C; not >25°C
- handle with care (including stickers on eskies)

6.1.8 Seafood Festival – mud crab races

Mud crab races are popular events at many seafood festivals in Queensland. The ordeal can be extremely severe on the animal and death is common.

Existing Practice:

- extreme stress situation
- crabs transferred from 18°C holding tanks to 30°C in full sun/shade
- extreme people interaction
- untied for races
- crabs immobile – most don't move unless nudged
- retied after race and returned to dry bins covered, doused a little with water bottle if they're lucky
- 10% mortalities during and immediately post-race
- 30-40% mortalities by end of day
- remainder slow, damaged and unrecoverable or saleable by end of day

Modified practice post-consultation:

- minimize crab stress = maximum crab liveliness = crowd enjoyment = crabs for sale post races = more funds raised for charity
- temperature equilibration with ambient
- aerated, covered tanks on-site at race venue
  - individual crabs immersed till water flowing over gills
  - slow or damaged crabs retired from further races
  - town water exchanges in holding tank

6.2 Crabs held immersed in various sectors of the industry

6.2.1 Darwin distributor

This outlet was a major distributor and wholesaler for mud crabs from the NT fishery.

Existing Practice:

- grade and pack (Plate 6.1) for supply to interstate and overseas markets
- tanks in sheltered outside shed
- crabs dumped into tanks
- held for 2-3 days depending on market demand / supply / price

Modified practice post-consultation:

- recovery tank (Plate 6.2)
  - individual crabs immersed till water flowing over gills
  - hold for minimum 2 hrs; maximum overnight
- holding tank, 2-3 days
- improved vigour

This business has since changed owners.

Plate 6.1. Crabs packed for interstate transport

Plate 6.2. Industry recovery tanks.

6.2.2 Cairns distributor and wholesaler

This is a Cairns based business turning over 200–300kg of crab per week. They perform a quality check on consignment arrival and grade out any weak or damaged crab (5% rejected at receival). They hold their crabs under the following conditions:

Existing Practice:

- 6000L seawater tank
- aerobic trawl mesh and coral bio-filter
- protein skimmer
- highly oxygenated via spray system
- lidded
- holding temperature 19-21°C
- obtain seawater at 21ppm then dilute to 16ppm

- fortnightly change half the quantity of water
- bi-weekly water quality tests – foam is an indicator of a problem
- stored on average for 2 days, (maximum 10 days)
- 96-97% survival during storage

Modified practice post-consultation:
No changes.

6.2.3 Mackay wholesaler and retailer

This supplier is a Mackay based business with a weekly turnover of 100-200kg. Quality checks are preformed on each consignment and graded as larger or smaller than 1kg to meet market demand. Consignment would contain on average: Dead 0-5%, Slow 5-10%, Empty 10-30%. Any slow or weak crabs are cooked. For retail sale they are stored in a holding tank.

Existing Retail Practice:
- air-conditioned room 20-22°
- no cover on tank, water 20°C
- water sourced from local river
- full water exchange 3-6 monthly
- recirculating commercial bio-filter system
- no water quality tests
- crabs stored 2-4 days
- 95% survival during storage

Modified practice post-consultation:
No changes.

6.2.4 Brisbane retail seafood market

This company has a strong relationship with their crabbers who only supply quality crabs sourced from either Gladstone or Mackay. Their suppliers know that every crab is graded for shell-flex on receival and any crab that is not 'A' grade is not paid for, consequently no empty crabs are supplied.

Existing Practice:
- spray each crab with hose to clean and grade for shell-flex. Grading: 'A' and one claws
- shed: large lidded recirculated tank
  - crabs held in submersed lug baskets. Crabs semi-active, some claw damage
  - 18°C seawater
  - external consultants maintain water – monthly checks
  - daily checks for dead or slow crabs
  - shell grit bio-filter
- shop – purpose built aquarium tanks
  - noisy, high level of people traffic – disturbance
- 90% survival during storage

Modified practice post-consultation:
No changes.

6.2.5 Brisbane Chinese restaurant specialising in live seafood

Existing Practice:

- aquarium tanks, aerated – water level ½ full
- no water quality checks
- irregular water exchanges

Modified practice post-consultation:

- aquarium tanks, aerated – water level ½ full
- regular water quality checks and exchanges
- livelier crabs

6.3 Crabs held in spray tanks

6.3.1 Brisbane wholesale and retail supplier

This company developed their own holding systems over many years of research and development. Their final system was a great success, easy to maintain and had very few mortalities. Previous trials we conducted based on their design showed the lowest stress biomarkers of any holding system for crabs stored up to 2 weeks (Plate 6.3).

Existing Practice:

- predominately source and supply Moreton Bay crab
- quiet back room, air-conditioned
- spray tank (garden micro-spray type), self-draining raised floor above a sump tank (one individual crab has lived in sump for months) submersible pump from sump
- bio-filter system
- 16ppm salinity
- crabs quiet, only stacked 3 crabs high
- 98% survival during storage

Current situation:
The company has recently gone out of business due to "*new fishing zones in Moreton Bay*".

Plate 6.3. Spray tank developed for our trials.

6.4 Crabs held emersed with daily dips

6.4.1 Mackay wholesaler and retailer

This supplier is a Mackay based business with a weekly turnover of 100-200kg.

Wholesale Practice:

- bulk dry fish bins on a slope to drain
- bins lid on, partly on, or sometimes off
- flushed with town water 2-3 times daily
- stored up to 24 hours, average 2-4 hours

Modified practice post-consultation:
No changes.

6.4.2 Bundaberg distributor

This distributor operates from knowledge gained through word of mouth from other industry stakeholders. They use 60L round rubbish bins half full of crab which is filled with town water from the bottom once a day, followed by an immediate drain. The rationale for the procedure is that the daily purge step eliminates waste products in a minimally disturbed environment that results in a better survival rate for the crabs. Good crab condition and survival are reported.

6.5 Summary of improved handling practices

Modified handling practices in use by industry have already reduced mortalities throughout the supply chain (Table 6.1). A 5% reduction in mud crab mortalities across the industry would equate to $0.5 million. Feedback has also indicated that overall mud crab quality has improved in relation to vigour, weight and flavour.

Table 6.1. Mortalities during storage in the retail sector.

	Existing practice %	Modified practice %
Emersed	10	4
Immersed	5	< 5

Appendix 6.1.

Survey sheet used with industry

All information will remain commercial-in-confidence.

Live Mud Crab Questionnaire

General Details		Comments / Issues
Business type	Distributor / Retailer / Wholesaler / Restaurant	
Business name		
Business owners name/s		
Postal address		
Location address		
Phone		
Address		
Contact name		
Mobile		
Contact email		
Years in business		
No. of kg purchased per annum	2007 / 2006 / 2005	

Receival & Holding		
Transport		
Transportation method used	Road / Air / Other	
Transportation cooling	Refrigerated / Unrefrigerated	
Time in transit for your receival (hrs / days)		
Source		
Harvest state (% each)	QLD / NT / NSW	
Harvest location - region(s)		
Receival		
Receival method	Foam / Cardboard / Lug / Other	
Internal packaging	Newspaper / Wood-wool / Seaweed / Other	
Receival weight avg (kg) - by consignment/week		
QA Assessment at receival	Yes / No	
Grading	Yes / No	
Grading method	Pre-graded / Visual / Shell flex / Other	

Grades	A / B / Diggers / Other	
Workers trained to grade	Yes / No	
Use of grading standards	Yes / No	
Rejected at grading (% for each)	Dead / Slow / Empty / Other	
Rejection reason		
Consignment feedback to supplier	Yes / No	
<u>Storing</u>		
Location (exposure to public e.g. fish tank, holding tanks)	Shop floor / Other	
Temperature control	Ambient / Air-conditioned	
Air Temperature °C		
Noise levels	Quiet / Some / Noisy	
Lighting conditions	Day / Artificial / 24hr / Timed	
Storage condition	Dry / Semi-submersed / Spray / Submersed	
Storage container cover type	None / Lid / Hessian / Other	
Storage period (avg, max)		
Survival rate during your storage (%)		

Storage Water		
Temperature °C		
Salinity		
Exchange (frequency / source)		
Bio-filter (type, capacity, flow)		
QA - tests	Yes / No	
Test types (pH, NH3 , N2)		
Test frequency		
Additional handling procedures		
Submersion (duration, salinity, oxygenated)		
Other		
Any other comments		

Appendix 6.2.

Comments from industry stakeholders

every crab gets shell flex test. Crabs at fullest in March.
crabs cleaned with hose before storing
shed - large tank; shop - purpose built aquarium still tanks. opening window shallow water 6"
quiet shed; noisy shop
daily checks on every crab
NT too expensive
most deads in transport
counter - fish bin
daily dip in running town water for 2hrs
daily dip in running from a height town water for 2hrs 1st thing every morning
crabs normally 6day old would sometimes return complaints; now have improved strength, flavour (cleaner); has had return comments of good product from customers
lowered mortalities
was interested in why empty crabs were allowed and how long it takes them to become full in the wild
also that empty crabs were not as strong as full ones
Received from fishers by road. Despatched to markets\customers by air.
Received from fishers unrefrigerated.
Mixed modes of receival, from fishing crates, tubs & bins, to iceboxes. Generally without packing but some with damp hessian.
Despatched to markets\customers in styrofoam eskies with dry paper packing.
Yes. Assess for QA and Grading. Including: Weigh, sort\separate for LIVELINESS, SLOW, SOFT, DAMAGED, DEAD, WINGED, OTHER and grade into A & B and or <1 kg or >1 kg, depending upon market requirements.
Yes, workers trained. Informal on the job training and experience.
No grading standards\charts used. Graded by experience and on the job training.
Dead = 0 - 5%. Slow = 5 - 10%. Empty = 10 - 30%. Approximately 60 - 70% “A” grade crabs. Lower quality\slow\unlively crabs may be cooked on premises (not sold as live crabs). David can you please estimate a % that you would cook for me, Ta?
Dead, unlively\slow, empty, damaged (wingers\diggers).
Paperwork is provided back to the fishers detailing the quality of the crabs they have supplied. This feedback includes advice\instructions not to supply soft crabs.
Two modes of operation. Retail crabs sold from premises are kept in tanks. Crabs forwarded to market\customers kept in bulk bins.
Entire premises are either air-conditioned or cool rooms. All at ~20 - 22'C.
Described the noise levels as being "from quite to a bit noisy".

Artificial lighting used during business hours only (~7am - ~5pm).
Two modes of operation. Crabs in tanks. Crabs in bulk bins.
Crabs in tanks = no lid. Crabs in bulk bins = generally have the lid on by ~3/4, but sometimes off.
Trucked in.
Fish\crab crates\bins
~1800 - 3300 kg/week.
HACCP plan in place and used. HACCP plan outline supplied at interview.
Slow, foaming and more than 3 missing legs.
25% of crabs supplied from East Coast are empty. 100% of crabs from Gulf are full - Gulf fisherman use "self imposed code of practice\QA".
Phone calls are made to suppliers to discuss the quality of the crabs provided.
Warehouse type premises.
Artificially lit during business hours only.
Large (6000 L) tanks used.
Buy seawater at 21 ppm. This is then diluted with town water to 16 ppm, no treatment of town water as it is low in chlorine.
Fortnightly change half of the water.
Yes. Home made consisting of 6000L tank filled to ~4000L with ~500 kg of coral and trawl mesh above water line (awareness of aerobic and anaerobic requirements of filtration system). Active flow through protein skimmer system used. Highly oxygenated water maintained by high flow sprays into tanks.
Nitrate\nitrite, liquid based test kit used.
Every Wednesday and Saturday BUT visual inspection of bubbles on surface of tanks also provides an indication of water quality (no bubbles = OK, bubbles = something wrong).
Was extremely supportive and informative during this interview. He was very keen for any feedback and results\outcomes of this research. He also provided several other contact details of people with great knowledge in the industry.
Felt that "B" and "C" grade crabs should be banned. I feel that his intention here was that fishers\crabbers should not even keep or supply such crabs further along the supply chain. Return them to the water at capture.
Felt the number of crabbing licences should be cut back. For instance, in the Gulf, the 76 "N6" licensed barramundi fishermen should not be allowed to crab and only the 13 "C6" licensed crabbers should crab. While of the nearly ~900 (863?) East Coast "C1" licences only those who are "active" should be permitted.
Felt that the Queensland size and sex restrictions were appropriate and were maintaining a sustainable industry.
felt that the NT industry has suffered greatly due to their history of size and sex restrictions being different than Qld.
Felt that a code of practice for fishers\crabbers could be very useful. This should include respect for the crabs themselves, including knowledge and use of best handling practice and the environment as a whole.
Felt that training in best handling practice, care for crabs and responsibility to the industry and environment, for fishers\crabbers be mandatory (as part of the code of

practice).
When I asked who might "police\monitor\supervise" the fishers\crabbers obeying laws and or using code of practice and best practice - suggested that an organization like the RSPCA could be apt. Gave examples of fishers\crabbers who do not care so much leaving crabs uncovered in boats for extended periods during very hot days. Such practice would lead to crabbers discarding crabs that have died due to abuse etc.
Also gave examples of some crabbers who due to their knowledge\experience and care for their crabs are able to supply up to ~800 crabs without any dead. These crabbers also get very disappointed if during phone calls they hear that they have had some of their crabs die, they then attempt to remedy the situation.

7 Further Development

The researchers are in discussion with the NT Crab Fishermen's Association to determine appropriate modifications of the NT Mud Crab Code of Practice to incorporate the latest information. Additionally, brochures will be prepared specific to the different sectors of the supply chain. It is important to produce these in pictorial format due to the broad range of cultures of personnel within the fishery, often for whom English is not the primary language. The project findings will be presented at the NT mud crab management advisory committee meeting, May, 2009. Information guides are also being produced for the wholesale and retail sectors since the interest in improved handling and holding methods for mud crab was very strong within these sectors.

Further investigation will occur into the mechanisms of ammonia storage within mud crabs. During this project work, it was noted that ammonia does accumulate in crabs when held emersed and yet such accumulation was not toxic to the animals. The reason for this finding is not yet elucidated and requires further research. Haemolymph samples were taken from crabs and retained (at -80^{o}C) for future investigation.

Further work is also warranted on the possible correlation between colour of the haemolymph in mud crabs and moulting cycle and/ or stress level. This work would complement similar correlations carried out with Southern rock lobster (Musgrove and Babidge 2003).

8 Planned Outcomes

The major planned outcome of this project was increased survival of mud crabs throughout the handling supply chain. This was successfully achieved through identification of stress biomarkers within mud crabs which can be used as tools to understand which handling steps along the distribution chain impose the greatest stress to the crabs. With this information, we were able to develop alternative handling practices that minimised the stress experienced by the crabs and in so doing, improve survival rates.

Through working closely with industry stakeholder's right through the mud crab handling and distribution chain, we were able to demonstrate the benefits of alternative practices. The feedback from harvesters, wholesalers and the retail sector has indicated increased survival and improved vigor of mud crabs. Increased survival subsequently increases revenue throughout the commercial supply chain.

The information this research has gained provides a sound basis for commercial decisions with respect to operational options. Results have highlighted the importance of excluding commercially unsuitable crabs from harvest as they are less tolerant of stress imposed by distribution handling practices and will succumb to mortality much more rapidly than robust crabs. The information provided from this research has created an ability to supply distant markets, including export markets, with confidence. Sustained adoption of the results from this project will also result in improved market perception of mud crab quality leading to greater market demand.

Increased survival of the crabs within this fishery not only improves resource sustainability, but also improves public perception of commercial activities within the mud crab fishery. Greater resource sustainability has flow on effects for the recreational sector and the indigenous community.

9 Summary

9.1 Mud crab live supply chain

Mud crabs are harvested from estuarine areas in the northern half of Australia. As a seafood commodity to the retail industry sector, mud crabs are supplied only as live product. For the Northern Territory fishery, the physical demand on the animal is extreme as the crabs are harvested from remote locations, then transported to Darwin prior to shipment to market, often Sydney and further. The time length of the supply chain from capture to receipt within the retail sector can be up to 15 days, with the market requirement that the animal not only survive this period out of water but arrive at market lively and vigorous. Amazingly, most mud crab do. Within the Queensland fishery, transport chains can be equally as long for mud crab harvested from Cape York and the eastern side of the Gulf of Carpentaria, with similar high stress imposed on the animals.

Sometimes a significant portion of the catch has to be rejected prior to packing for transport to market, either domestic or export. Losses range from a frequent 5% and rising up to 35% during the wet season months. A small number of the rejected crabs are likely damaged by physical injury through capture or cannibalistic aggression due to close proximity with other crabs. During the wet season especially, a further small number of crabs may be rendered unacceptable through fly larvae contamination evident through the transport chain. However, the major portion of the rejects deemed too weak (graded as ‘slow’) to survive further transport are simply graded as ‘slow’ with no obvious reason.

For the crabs that don’t survive, mortality is caused by stress. Stress to a crab is induced by numerous factors, both environmental and physiological, some of which may not necessarily be lethal on their own but contribute accumulatively. How an individual crab responds to and/or deals with the stresses imposed during the supply chain is directly dependent on the physiological condition of the crab. ‘Condition’ is influenced by animal health, physical damage, moulting cycle phase and growth phase. Clearly, a crab not fully robust and in a weakened state will have less tolerance to deal with any level of stress imposed and surrender to mortality more rapidly. It is thought by industry that large crab (>1.5kg) do not handle lengthy transport chains as well as smaller crab.

Mortality, decreased vigour (slowness) and reduced quality of crabs during transport reduces the economic value and competitiveness of the industry and restricts access to distant markets. Our ability to reduce the wastage of crabs at this point in the chain relies on understanding the stress factors imposed on the crab and developing handling methods to minimise the stress. The NT Mud Crab Fisherman’s Code of Practice identifies broad best practice options for the harvesting of mud crab from remote locations, however does not currently include protocols for handling live crab at the end of the supply chain.

To maximise survival of mud crab through their long transport chain to market we need to reduce stress factors and levels. From the findings of our research, this is most effectively done by including a recovery step within the supply chain which has not been normal practice to date.

9.2 Stress biomarkers

Stress in a mud crab is induced by numerous factors, both environmental and physiological. Some factors may not necessarily be lethal on their own but contribute in combination to a high overall stress level. It is likely that stresses will be accumulative or synergistic. To determine the effect of different handling and holding conditions on the stress level in mud crabs, readily measurable parameters are required that correlate directly with animal stress.

9.2.1 Vigour index

The most obvious indicator of stress is a crab's physical liveliness, general robustness and response to external stimuli. To ensure the assessment of these attributes was objective, we developed a demerit point scale.

Score	Term	Description
0	dead	Dead
1	very slow, weak	Walking legs give very little resistance to pressure applied, pincers non-responsive, mouth parts may be drooping, foaming from mouth
2	slow	Walking legs have some strength but not actively moving, pincers have slow movement
3	lively	Walking legs strong and active, pincers active and aggressive

The index is based on limb movement and strength against gentle resistance. It is important to ensure crabs are at room temperature prior to assessing against the index.

9.2.2 Haemolymph glucose

Glucose is used as the major energy source in crabs and increases in haemolymph circulating glucose is indicative of activity or stress. Rested (unstressed) crabs have glucose levels in their blood of <1.0mmol/L although there is individual variation between crabs. Glucose levels rise quickly in immediate response to any form of stress imposition on the crab, but then tend to return to basal levels within a few hours as compensatory mechanisms come into play.

Glucose level can be correlated directly to stress level the crabs are subjected to:

Haemolymph Glucose (mmol/L)	**State of crab**
<1.0	'rested' crab
1.0 – 2.0	some stress has occurred
2.0 – 3.0	high stress but will recover under resting conditions
>3.0	extreme stress experienced

9.2.3 Haemolymph lactate

One of the by-products of anaerobic (no oxygen) respiration in a crab is lactic acid which accumulates in the blood as it cannot be converted further. The lactate levels obtained from all rested crabs illustrated that 91% had levels <1.3 mmol/L, suggesting that as an indicator a cut off level of <1.0 mmol/L could be used to indicate crabs that were not suffering from stress. Circulating lactate in crab haemolymph was shown to change rapidly in response to stress stimuli but varies in magnitude between individual crabs.

Lactate levels were extremely high in crabs at the point of death: >38.0 mmol/L and levels >10mmol/L were attained when crabs had been subjected to severely adverse treatment and holding conditions.

9.2.4 Haemolymph pH

The physiological basal pH of mud crab haemolymph is around pH 7.5. The pH is highly reactive to metabolic shifts within the crab and because of this the values obtained for rested crabs are extremely variable (pH 7.23 – 7.82). Crabs that had been subjected to severe stress of extreme temperature or extended emersion exhibited very high (>7.9) or very low (<7.2) haemolymph pH values. These crabs had a high likelihood of imminent death. However for some crabs subjected to similar conditions, pH remained within the 'rested' value limits. Hence, pH alone is not an effective indicator of stress levels in crab but is useful when combined with other indicator parameters.

9.2.5 Haemolymph urate

Uric acid is an end product of nitrogen metabolism and has been suggested as a marker of oxidative stress. It is considered that in some species of crab, urate positively modulates oxygen affinity of haemocyanin therefore its increased presence in the haemolymph would be a valuable compensating factor to hypoxic conditions. In mud crabs, urate levels measured in the haemolymph ranged from 4.34 – 238 µmol/L and rested crabs demonstrated basal levels anywhere within this range. Circulating urate level is subject to complex and not fully understood metabolic processes in crabs and hence not considered further as a useful stress indicator in mud crabs.

9.2.6 Haemolymph ammonia

Build up of ammonia in the haemolymph is prevented by rapid gaseous exchange across the gills when crabs are in water. However, during periods of emersion this cannot occur and so an increase in circulating ammonia in the crab blood would be expected. This can be measured and correlated to levels of stress experienced by the crab. Ammonia in crab haemolymph is present as either NH_3 or NH_4^+ and exchanges between the two forms depending on the pH of the blood. Both forms are toxic to the animal as levels increase.

Ammonia in rested mud crabs ranged from 0.66 – 21.85 mg/ml, with an average value of 7.17 ± 3.44 mg/ml. However, exactly the same range was observed in crabs whether in a resting state or after different stress factors had been imposed on them. This illustrates there was no apparent difference between haemolymph ammonia levels in stressed crab and rested crab and hence is not a useful indicator of stress.

9.2.7 Ammonia excretion rate

When crabs were re-immersed after emersion, the amount of ammonia exchanged into the water was rapid and considerable. The rate of ammonia excretion depended on the extent of stress the crabs were subjected to and therefore reflects the build up of ammonia within the crab. Mud crabs held quietly emersed for 7 days or more demonstrated a steady but slow ammonia efflux when re-immersed whereas crabs that had been subjected to severe physical disturbance had a faster and greater excretion rate. The data obtained for mud crab demonstrates that ammonia excretion rate seems to be a good indicator of stress level within the crab.

9.2.8 Total protein in haemolymph

The total amount of protein circulating in the blood of a crab is, in the first instance, correlated to feeding cycles in the animal and can be correlated with moult phase. However, total protein levels in crab haemolymph do increase under conditions of severe dehydration occurring due to prolonged emersion.

9.3 Stress factors

Stress to a crab is induced by numerous factors, both environmental and physiological, some of which may not necessarily be lethal on their own but contribute in combination. The foremost causes of stress identified in this study arise from changes in the crab's immediate environment and include emersion, handling and temperature changes. In this study we assessed the various stresses imposed on the crab along the handling/transport chain and determined the impact of each.

9.3.1 Emersion

Emersion refers to any period of time the crabs are out of water and held in air. This occurs frequently and for extended periods (up to 15 days) within the mud crab distribution chain. Usual commercial practice is to hold the crabs with claws tied in a crate lined with damp hessian. As mud crabs are aquatic animals, emersion causes respiratory metabolic stress due to the crab being unable to obtain sufficient oxygen.

Immediately post-emersion there is a rapid small increase in circulating glucose in the haemolymph in response to energy demands within the crab. With prolonged emersion, in the absence of other stress factors, glucose levels return to basal levels. Lactate levels also show an immediate small rise in response to emersion with a reflected decrease in pH of the haemolymph but again in the absence of other stimuli, levels return to basal values quickly.

The effect of emersion was evident from excretion rates of accumulated ammonia when crabs were re-immersed into seawater after emersion. There is a large and rapid efflux of ammonia immediately upon re-immersion and the amount and rate of excretion correlated with period of emersion.

Another effect of emersion is dehydration of the crabs with the consequent water loss from the crabs reducing the total weight of a crab and having implications for the harvester with respect to crab value. Also, it is likely to increase stress for the animal which may add synergistically to other stress factors. There is a rapid initial water loss during the first hour of emersion (~2-3% of body weight) and a slower but steady further water loss for 24 hours (to a total of ~4-8% body weight). Importantly,

it was found that crabs held with claws tied in a crate lined with damp hessian, as per commercial practice, also lost water rapidly during the first 6-8 hours. It is considered to result from behavioural aggression and greater animal activity when the crabs are in close proximity to each other under these conditions. However, while crabs crowded together in this way causes a rapid water loss initially, this stops after the first few hours and the humid atmosphere inside the damp hessian retains the moisture level sufficiently during further extended emersion.

9.3.2 Handling

Mud crabs are handled frequently at different points during distribution within the supply chain. Each handling event involves physical movement of the crabs and often a degree of shock, with all such disturbances adding stress impact to the crabs.

Mud crabs are checked regularly for liveliness and mortalities during storage but if carried out gently and with care, this practice does not impose severe stress on the crabs. Any slight immediate stress response that may occur during the process is readily recovered from by the crabs.

Handling of mud crabs through the supply chain involves physical displacement of the crabs at several points. Crates full of crab are moved for sorting and grading; loaded on transport vehicles, unloaded, often reloaded and transported long distances. In simulated disturbance trials, haemolymph lactate levels increased remarkably and half the crabs did not recover but died subsequently. This response indicates that sudden and severe jolting movement causes extreme stress to mud crabs.

Mud crabs are inherently aggressive animals and hence crabs in close proximity to each other causes high levels of physical activity, resulting in increased metabolic rates in the crabs and accumulation of end product biochemical compounds. In crowding situations, mud crabs show both aggressive and defensive behaviours often resulting in physical damage. To avoid crab damage and minimise the stress of aggression, commercial practice is to immobilise the crab claws by tying and it was found that this practice is not stressful for the crabs. However, external annoyance stimuli do impose stress on the crabs and the stress experienced correlates to the level of disturbance. Crabs that were frequently disturbed by human intervention all showed elevated stress biomarker levels directly corresponding to increased activity of the crabs. When disturbance ceased, the stress biomarkers returned to rested crab levels and the crabs showed full recovery within a few hours. It is concluded that mud crabs responded strongly and adversely to physical disturbance.

Through the supply chain mud crabs are frequently subjected to noisy environments, for example nearby generators. Trials demonstrated only slight elevation in stress biomarkers and so it is concluded that noise is not a major stress factor for the crabs.

The detrimental effect of breeze on exposed crabs has a common acceptance among experienced crab harvesters. Hence the industry practice of covering crabs with damp hessian and storing them in a draught-free position. Unfortunately, this practice is not always adopted further along the supply chain. Within wholesale and retail sectors, crabs are commonly exposed to strong air-disturbance, such as air-conditioned storage, regular opening/closing of doors and positions affected by natural breezes. Crabs subjected to constant breeze demonstrated extreme

changes in all haemolymph stress biomarkers. Lactate levels were excessively high in breeze exposed crabs (~20mmol/L) and they were graded as notably 'slow'. Breeze subjected crabs also exhibited significant weight loss, 12.5%, highlighting profit loss with these animals.

9.3.3 Temperature

Mud crabs are poikilothermic animals, taking on the temperature of the surrounding environment and they are without effective internal means of regulating their temperature. Hence, within limits, the higher the temperature the greater the metabolic rate and the faster the crab will suffer stress from lack of oxygen. Water and ambient temperatures can be excessively high during summer months in mud crab harvest locations. Additionally, the transport chain dictates periods of sudden temperature decrease. Holding crabs at constant temperature and minimising the temperature change is optimal for minimising stress and current commercial practice of holding crabs in damp hessian sacking is effective for this. However, it is noted that crabs are often subjected to sudden low temperatures during the supply chain and the damp hessian wrapping may not be effective in moderating sudden changes.

Mud crabs were held at temperatures between 10°C and 35°C for a two day period to establish the effect with respect to stress on the crabs. From the responses of the stress biomarkers in crab haemolymph, it was concluded that mud crabs best tolerate a temperature range between 25°C - 30°C. Temperatures outside this range impose an increasing extent of stress on the animals and temperatures in the low range (≤15°C) can result in extreme stress and death.

10 Conclusion

10.1 Mud crab live supply chain

Due to remote harvesting localities of mud crabs, the supply of live crab to interstate markets presents many challenges. Current holding and transportation practices, coupled with long lead times to market, impose considerable stress to the animal. This accumulation of stress was shown to reduce crab quality and dramatically increase mortalities. The implication to industry is substantial with reduced profits, competitiveness and marketable regions. This study identified industry feasible solutions to manage this build-up in supply-chain stresses of the crabs with a recovery stage highlighted as the most economically implementable.

10.2 Stress biomarkers

Metabolic changes within mud crabs are measurable through indicator parameters and these indices do correlate with stress level endured by the mud crabs. The biochemical indices of specific end-product compounds are inter-related however and hence, any one specific parameter measured alone does not necessarily tell the full story. Of all the haemolymph parameters we measured, circulating glucose was most directly responsive for indicating level of stress imposed on the crab. However, to gain an accurate picture it is better to assess several parameters together, the most useful appearing to be glucose, lactic acid and ammonia efflux.

10.3 Stress factors

Throughout the supply chain, mud crabs are predominately held emersed in damp hessian. As they are aquatic animals, emersion causes respiratory metabolic stress, accumulation of ammonia and dehydration of the animal. From the point of harvest, holding at camp, transport to and repackaging at distributors and eventual delivery to retail outlets, crabs are subjected to various handling conditions that can have accumulative or instant degradation effects on the live product. Some conditions have greater detrimental effects than others, such as breeze which dries the crab out and rough handling which can cause injury resulting in death. Exposure to extremes and sudden changes of temperature causes stress within crabs. The current commercial practice used by crab harvesters, using damp hessian to maintain constant temperature and create a humid environment, is a successful handling strategy.

Stress imposed on the crabs is accumulative along the supply chain with each handling phase and it was found important to develop protocols to reduce stress nearer market. The best strategy developed was the inclusion of a recovery step in the chain, where the crabs are purged for a short time then removed to fresh oxygenated seawater to rest prior to being placed into the marketplace. This step allowed for crabs to recover from stresses imposed throughout transport and handling and present to market in premium condition, showing maximum liveliness.

10.4 Handling recommendations

- Confirm legal size as it is illegal to hold undersized crab throughout the entire supply chain, not just on board

- Confirm that crab is not CUC (commercially unsuitable crab). Newly moulted crab are prone to stress and will not tolerate transport and temperature changes. CUC's returned to the water will become "A" grade crabs within weeks

- Tie crab's claws to restrict movement. This will minimise aggression and the possibility of damage to other crabs

- Wrap in clean damp hessian lined crate to limit disturbance, minimise moisture loss and stop direct breeze and sunlight affecting the crab

- Avoid placing crabs in direct wind/breeze onboard or in air-conditioning drafts in transport and holding. Direct breeze will cause mortality

- Keep quiet. Limit any loud noises as this will cause increased stress levels in the crab.

- Disturb as little as possible. Each time you disturb the crabs you are increasing stress levels

- Handle gently. Minimise the handling movements and apply care when legs are stuck in baskets or caught on another crab. Pulling on a leg that is stuck can cause bleeding increasing the risk of mortality

- Keep temperature constant. Avoid large variation in holding temperatures as the sudden change will cause stress. Allow crabs to slowly acclimatize to a new temperature and hold between 25°C – 30°C

- Remove weak or slow crab. These crab have been compromised at some stage and can be included in a purge/recovery step to help revive them but must be closely monitored

- All crab should have some form of purge and recovery step included in the handling chain. A three hour purge in aerated seawater (if seawater not available, town water) is recommended following extended emersion. This can be carried out on a daily basis if holding tanks are not available.

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12 Appendix 1: Intellectual Property

There is no intellectual property arising from this research project.

All results, findings and developed methods have already been extended into the mud crab industry and supply chains. All information belongs in the public domain.

13 Appendix 2: Project Staff

Principal Investigator:

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