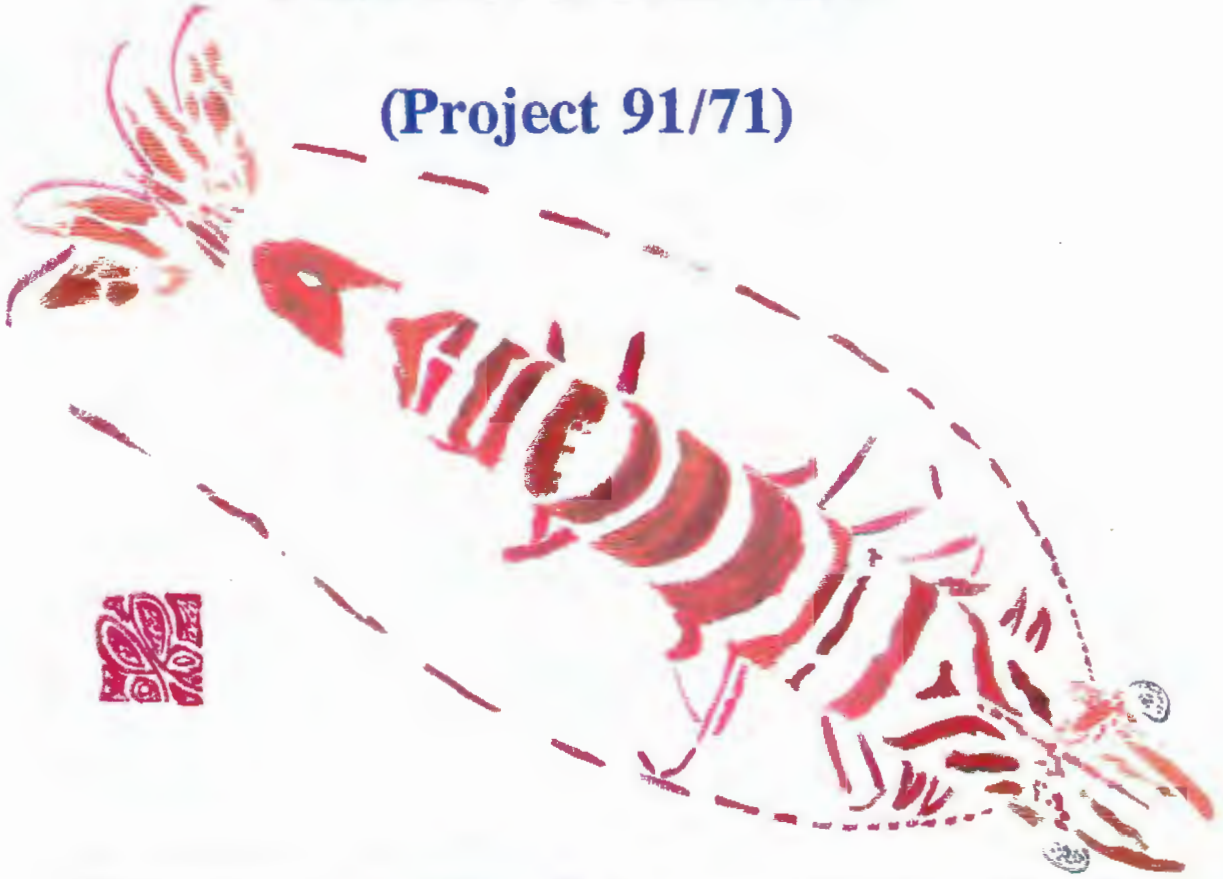
Report to the Fisheries Research and Development Corporation

# TRANSPORTATION AND POST-HARVEST HANDLING OF OCEAN CAUGHT PRAWNS DESTINED FOR LIVE EXPORT.

## (Project 91/71)



Bruce Goodrick, Brian Paterson and Stephen Grauf

DPI
QUEENSLAND
DEPARTMENT OF
PRIMARY INDUSTRIES

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# TRANSPORTATION AND POST-HARVEST HANDLING OF OCEAN CAUGHT PRAWNS DESTINED FOR LIVE EXPORT

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Bruce Goodrick, Brian Paterson and Stephen Grauf

August 1993

## PROJECT INFORMATION

Project title: **Transportation and post-harvest handling of ocean caught prawns destined for live export**

Research organisation:

International Food Institute of Queensland
Department of Primary Industries
19 Hercules St Hamilton, Brisbane 4007

Project leader: Mr Bruce Goodrick

Duration of project: 1991-1992

Staff involved: Dr Brian Paterson, Mr Stephen Grauf

Cooperating agent(s): Scarborough Trawler Seafoods Pty Ltd

### OBJECTIVES

To facilitate the live export marketing of brown tiger prawns from the Australian natural prawn fishery.

### SUB-OBJECTIVES

Devise a practical system for transporting live penaeid prawns at sea and on land prior to packing for export.

Determine appropriate methods for capture, on-board handling and storage of prawns intended for live export.

# FOREWORD

This report is a discussion of the findings of this project and gives recommendations for further work that is required. It is written in the form of a series of papers. Because of the need to pass on information in a timely fashion to industry, the first of the following papers is a summary report which is written in a form in which it can be submitted with minimal modification (inserting photographs) to an industry journal. Readers wishing to go further into the methodology used, the results obtained and their significance, or who wish to read more detail on an area summarised in the first paper are invited to read the supporting papers. These have also been written with a view to submitting them to appropriate journals and industry magazines, and consequently, each is written with a different audience in mind.

Bruce Goodrick, Senior food technologist
Brian Paterson, Physiologist
Stephen Grauf, Technician

(Editors)

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## SUMMARY

Following our successful development of a sawdust-based method for the live export of farmed kuruma prawns, *Penaeus japonicus*, from Australia, this project aimed to use this method to export brown tiger prawns, *P. esculentus*, from the wild fishery. However, it was not possible to export this species, using the method developed previously, because the brown tiger prawn did not survive well in sawdust. This method was however adequate for domestic transport of brown tigers and other prawns, for example, for use as aquaculture broodstock.

The inability of the brown tiger prawn to survive for long periods in sawdust could be due to harvest stress but it may also be a characteristic of the species.

To meet the first sub-objective, a system was developed for holding live prawns on trawlers which was also suitable for hauling prawns overland to the packing facility.

The second sub-objective related directly to the issue of capture stress. Prawns from the wild fishery are harvested by trawling. This is a very stressful method of capture and while the condition of the prawns may be improved by shortening the trawl time, this has consequences for the commercial viability of the process.

Prawns that are not killed outright in the trawl net show symptoms of extreme fatigue beyond that observed in other cases where live crustaceans are handled. A significant percentage of the prawns subsequently developed symptoms of tissue breakdown ("white-spot") and were unusable. However, the physiological symptoms of the remaining prawns suggested that they recovered fully. When these prawns were packed in sawdust they survived for only 12-18h, whereas survival for at least 24h is required for marketing overseas.

So, even if less stressful methods of harvesting are adopted, or careful grading of weak trawled prawns is practised, the survival of the brown tiger prawn is still marginal in the context of live export.

Faced with what appears to be an intrinsic weakness of the brown tiger, compared to the longevity of the kuruma prawn in sawdust, the options for developing an export market using existing packing methods are limited. Alternative methods of packaging and storage could be studied in future, since the brown tiger prawn may be intolerant of the low temperatures used to transport kuruma prawns.

## RECOMMENDATIONS

1. That the dry transport method should be tested as a means of transporting aquaculture broodstock since it does not require sea water (avoiding corrosion problems in air-cargo holds).

2. That alternative methods of packaging and storing live brown tiger prawns be studied with a view to maximise their survival in air.

3. That research continue on seeking alternative methods of capturing live prawns.

B. Goodrick, B. Paterson and S. Grauf (Editors). *Transportation and post-harvest handling of ocean caught prawns destined for live export.* Report to the Fisheries Research and Development Corporation (Project 91/71), Department of Primary Industries, Queensland, August 1993. pp 1-10.

# TRANSPORTATION AND POST-HARVEST HANDLING OF OCEAN CAUGHT PRAWNS DESTINED FOR LIVE EXPORT

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SUMMARY

Following our successful development of a sawdust-based method for the live export of farmed kuruma prawns, *Penaeus japonicus*, from Australia, this project aimed to use this method to export brown tiger prawns, *P. esculentus*, from the wild fishery. However, it was not possible to export this species, using the method developed previously, because the brown tiger prawn did not survive well in sawdust. This method was however adequate for domestic transport of brown tigers and other prawns, for example, for use as aquaculture broodstock.

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## INTRODUCTION

Seasonal catches of wild brown tiger prawns *Penaeus esculentus* are currently exported from Australia in frozen "green" form, largely to Japan. While in the past this has been a lucrative market for larger prawns, the supply pressure from cultured species (especially the black tiger prawn *Penaeus monodon*) and the increased costs of commercial fishing operations has resulted in a diminishing return from this fishery. The larger brown tiger prawn does however, still command a substantial premium price in Japan. This species, and the japanese king or kuruma prawn *Penaeus japonicus*, have red or brown stripes and are favoured for their attractive appearance. The kuruma prawn is usually marketed in the live form and as such commands a superior price, (Ovenden *et al.*, 1993).

**Table 1.** Prawn species discussed in this article.

| | |
|---|---|
| "TIGERS" | |
| Black tiger (or leader) | *Penaeus monodon* |
| Brown tiger | *P. esculentus* |
| Grooved tiger | *P. semisulcatus* |
| "KINGS" | |
| Eastern king | *P. plebejus* |
| Japanese king (or kuruma) | *P. japonicus* |

While it is possible to grow kuruma prawns in Australia, and the live transport technology has been successfully applied here (Goodrick *et al.*, 1993a), this species is uncommon in Australian waters. However, the similarity of the brown tiger and kuruma prawns suggests that a live market for the former could be developed using prawns from the wild fishery.

Though endemic to Australian waters, the brown tiger prawn is often caught alongside of a close relative, the grooved tiger prawn, *Penaeus semisulcatus*. This species closely resembles the brown tiger in appearance but it has a more cosmopolitan distribution throughout South East Asia, where it is known as the bear prawn or green tiger prawn. In Japan, there is a preference for the brown tiger prawn rather than the grooved tiger prawn because of the better colouration of the former. The brown tiger is more often maroon in colour than brown. Yet, in Australia, the two species are often lumped together as "tigers". Australian fishermen will have a strong incentive to recognise the difference if the brown tiger becomes more valuable through live marketing.

Prawns from the wild fishery in Australia are harvested by trawling. In Japan, a significant proportion of live kuruma prawns already come from the wild fishery (Ovenden *et al.*, 1993), where gentle harvesting techniques are employed. Even though these methods are not used in Australia, small numbers of Australian prawns are already trawled live for use as aquaculture broodstock or for "tag-and-release" studies so there appears to be no *a priori* reason why existing harvesting methods cannot be modified to minimise the stress of capture for larger quantities of prawns.

To know if prawns recover from harvest requires a better understanding of what actually happens to prawns inside trawl nets. Prawns and other crustaceans are most likely stressed in nets by exercising to exhaustion and of course by injury. A period of recovery on board the boat is probably necessary. This study has therefore considered the harvesting and on-board holding of brown tiger prawns so that the species can then be examined using live transport methods developed by the authors from extensive experiments with aquacultured kuruma and black tiger prawns.

## HARVEST STRESS AND RECOVERY

Trawling is a very stressful method of capture. This is particularly true when large amounts of by-catch are found in the net. The condition of the prawns may be improved by shortening the trawl time but this has consequences for the commercial viability of the process. The time required for setting and retrieving the net is more or less constant, reducing the fishing time of the net if short trawl shots are used.

Prawns caught in a trawl net are apparently very active, though an indication of the magnitude of the stress involved in capture can be seen in the "live" prawns that are apparently paralysed when they reach the sorting tray. Physiological studies show that prawns which survive trawling have been severely stressed.

There is a special group of compounds in organisms called adenylates, usually denoted by their abbreviations ATP, ADP and AMP. They function as the energy currency of metabolism, (Atkinson, 1977). That is, moving muscles during swimming or walking consumes ATP, (adenosine triphosphate) which must then be replaced. ATP is generated from food or "energy" reserves in the animal. Ordinarily, the prawn uses oxygen to completely break down its food (e.g. sugars), to simple wastes, such as ammonia and carbon dioxide. Large amounts of ATP are generated in the process. However, a prawn that is stressed or exercised to exhaustion is unable to get oxygen fast enough to meet the demand for ATP. Instead, the sugars are only incompletely broken down, and accumulate as lactic acid. Large amounts of this compound are typically present in trawled live prawns, whereas lower amounts are present in prawns harvested on farms, (Paterson *et al.*, 1993).

The prawns that were not killed outright by injury showed symptoms of extreme fatigue beyond that observed in other studies where live crustaceans are handled (Whiteley and Taylor, 1992), however, similar levels of fatigue occur when black tiger prawns are stored in sawdust for 24 h (Paterson, 1993a). Lactic acid production does not generate very much ATP, so the concentration of ATP falls, and that of the low "energy" molecule AMP (adenosine monophosphate) rises. Usually, the prawn then fatigues, (i.e. it is "exhausted") and begins to recover, restoring its ATP reserves. However, recovery did not occur in the trawl net. Under extreme physiological stress, when too much AMP is present, a change occurs in the prawn's metabolism and some of the AMP breaks down to a related compound, inosine monophosphate (IMP) (Figure 1), a flavour enhancer in seafood, (Komata, 1990). It is sobering to note that the physiological literature suggests that the metabolism of animal cells collapses irreversibly when stressed to this degree (Sylvestre and le Gal, 1987). Trawled prawns typically accumulate high levels of IMP in their muscles while they die, (Paterson *et al.*, 1993).

Prawns remain fatigued for long periods during trawling and profound changes occur in their metabolism. Some of these changes are possibly debilitatory. Lactic acid concentration and other measures normally applied to the study of stress in crustaceans could not distinguish prawns that were apparently healthy (jumping around) from prawns that were capable of only weak movements (i.e. paralysed). All of the trawled prawns were fatigued, having similar levels of lactate and IMP, and the paralysed prawns were apparently just more fatigued than the active ones, (Paterson, 1993b). The levels of various ions or "salts" in the blood were therefore measured in an attempt to explain the onset of paralysis in the prawns. The results were not encouraging.

A rise occurred in the potassium ion level in the blood of trawled prawns. This is of concern because potassium is the principal ion found **inside** muscle and other tissues, suggesting that the blood has been contaminated by cell contents, perhaps by outright rupture of cells. To put things in a more familiar context, blood potassium levels rise in people when the blood supply to tissues is interrupted, and cardiac arrest is an extreme case of this, (Kleber, 1990). The ratio of potassium concentration either side of cell membranes is an important factor in nerve function so it is probably not surprising that the prawns were paralysed after trawling.

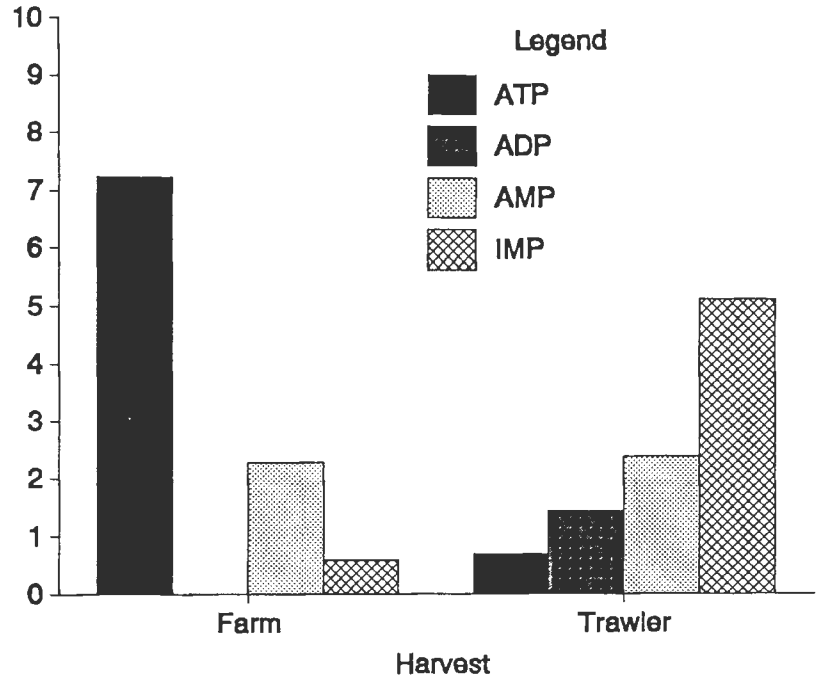


**Figure 1.** Adenylate and IMP concentrations in brown tiger prawns caught by net at a farm or by trawling at sea.

The period of prolonged fatigue and rise in blood potassium level is also undoubtedly related to the incidence of tissue necrosis or "white spot" in the trawled prawns. This phenomenon has attracted some interest in the past, (Rigdon and Baxter, 1970; Ross, 1976). Opaque regions of the muscle appear to signify permanent damage to the integrity of the muscle, so a significant proportion of the catch must be down graded and does not meet the standard for packed live prawns. Losses of live prawns through injury and stress will have a great influence on the economic viability of this practise. About half of the catch is dead to begin with, and up to half or more of the survivors are discarded during storage because they are injured or damaged internally (eg. unable to stand upright).

## ALTERNATIVE WAYS OF HARVESTING LIVE PRAWNS

The obvious solution to this problem would be to harvest the prawns using a less stressful technique. In Japan, wild kuruma prawns are harvested in a number of ways, using tangle nets, or slow beam-trawling. One method of capture involves the use of what amounts to a monofilament drift net. While prawns could be caught using this method with a minimum of stress, in Australia this kind of net is illegal. Other alternative techniques, such as trapping, have been advocated as a means of capturing high quality prawns, but these techniques have yet to be applied commercially, (Rick Buckworth personal communication).

So, at present, trawling is the only method available to collect wild prawns for live transport. Prawns apparently take more than an hour to recover from trawling. Despite the profound, indeed, pathological nature of trawl stress, the physiological symptoms of the surviving prawns suggested that they eventually recovered, at least in terms of the parameters measured in this study, (Paterson, 1993b). The usual balance between the concentrations of the adenylates ATP and AMP was restored, indicating that prawns are tolerate severe metabolic stress.

This finding was anticipated, since trawled prawns are already used successfully as aquaculture broodstock.

However, the apparent recovery shown by most of the surviving trawled prawns does not necessarily mean that they are in the best possible condition to withstand further stress during storage out of water.

## HOLDING AND TRANSPORTING PRAWNS

After capture, prawns must be placed in a well-oxygenated tank of sea water to allow them to recover. A system for holding live prawns on trawlers was developed by us which was also suitable for hauling prawns overland to the packing facility, (Goodrick *et al.*, 1993c). This tank was tested by successfully hauling prawns from Cooktown to Cairns. The live prawns are stored in trays to aid handling, an extension of the techniques applied to farm-grown kuruma prawns in the previous study (Goodrick and Paterson, 1992).

The holding system shown in Figure 2 is an extension of the concept of a "swim-tank" to include an option for aeration, pre-chilling sea-water from the deck hose and for storing the prawns in mesh-based, stackable wooden trays. Living prawns are taken from the swim tank and placed in the wooden trays inside the bin. As each tray is filled with prawns (about 2 kg per tray) an empty tray is placed over it and the process is repeated until all trays are filled. The lid of the bin is then fitted over the last tray but this is not clamped down tightly while the bin is at sea (unless the sea is rough), allowing water to overflow. Water circulation and aeration is achieved using air-stones placed in a frame on the floor of the bin and connected through the bung to an air pump.

Large capacity aquarium aerators are available and if the vessel does not have 240V AC power, an inverter can be purchased to run the air pump on DC. Water supplied from the deck hose can be cooled by running through an inexpensive plastic heat-exchanger (a length of 13 mm irrigation polypipe) placed in an ice slurry, such as in an RSW tank. When operating in Moreton Bay, where the sea temperature is in the mid- to high twenties during Summer, cool the water to 15 to 20°C. In North Queensland waters, cooling to about 20-25°C should be adequate. Reducing the temperature stops the prawns from swimming around and reduces their appetite. The temperature of water leaving the heat exchanger can be regulated using a tap to adjust the flow rate. The holding tank used was an industrial style fish-bin that could easily be transferred to and from a fishing vessel in dock and was also suitable for use with a fork-lift. A valve was included in the lid to allow aeration to continue when the lid was sealed, for example during road haulage.

## SURVIVAL OF BROWN TIGER PRAWNS IN SAWDUST

Live transport experiments were conducted on brown tiger prawns brought back to the laboratory. Unfortunately, the brown tiger prawn showed the poorest survival of the three tiger species available (Goodrick *et al.*, 1993b). Given the low price of black tiger and grooved tiger prawns in the fresh frozen market, it is not likely that live export of these species to Japan would be viable, especially as strong competition could be expected from other countries. When wild brown tiger prawns that had apparently recovered from harvest were cooled at a rate of 3°C/h to 12°C and packed in sawdust, they survived for only 12 to 18h at this temperature. Survival for at least 24h is required for marketing overseas. Interestingly, wild grooved tiger prawns caught alongside brown tiger prawns survived longer in sawdust than the latter (Figure 3). Only small numbers of living prawns were available for these studies. Where larger numbers of prawns are caught, on a commercial scale, it would be possible to exercise more discrimination in selecting the prawns for packing.

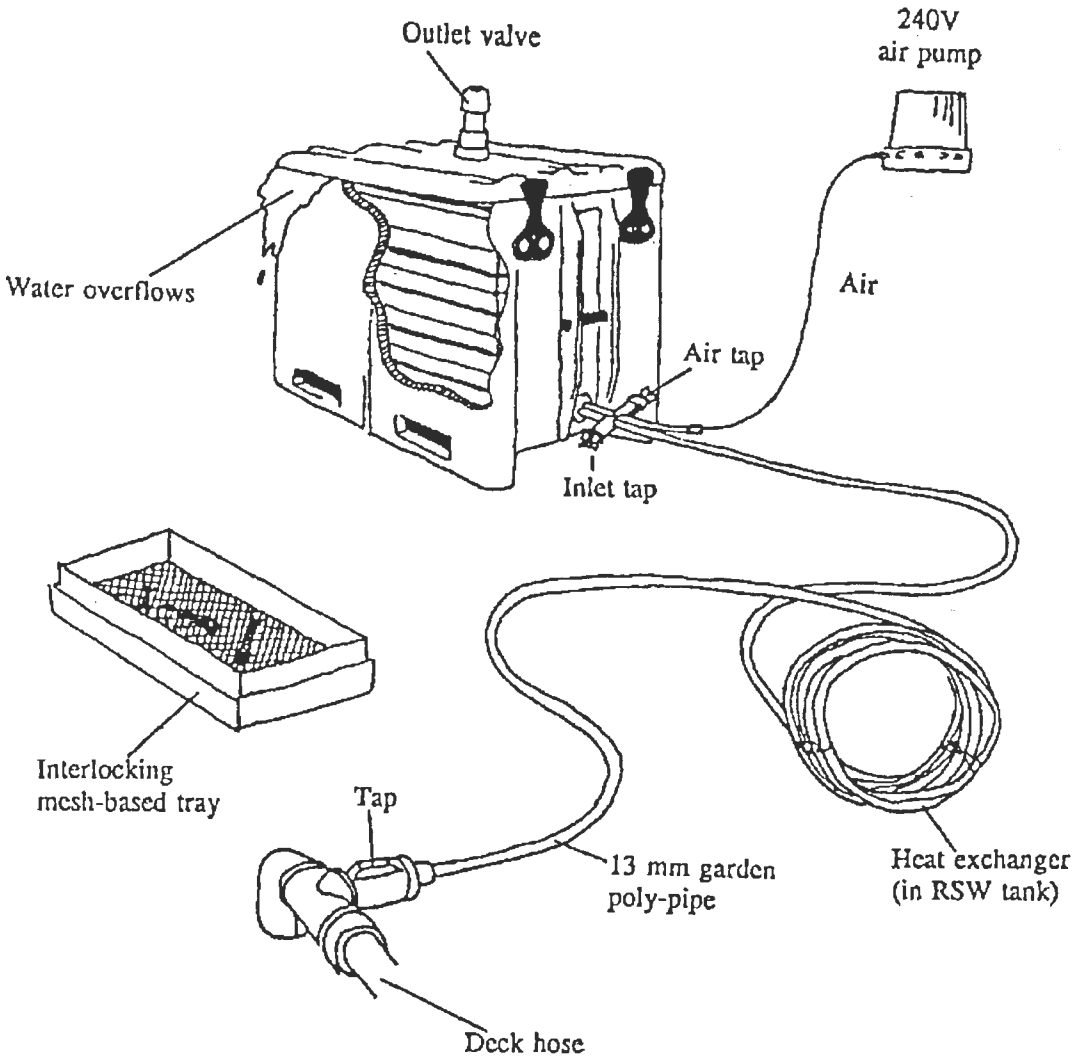


**Figure 2.** Portable tank for storing live prawns caught at sea. The tank shown is an Xactics 309F or similar, which has been modified to allow water flow and aeration. The water is diverted from the deck hose through a length of 13 mm garden poly-pipe, part of which is rolled up as a heat exchanger (immersed in the RSW tank), and then enters the bung of the bin. The temperature of water leaving the heat exchanger can be regulated using a tap on the deck hose to adjust the flow rate. Living prawns are taken from the swim tank and placed in floating, mesh floored wooden trays that stack inside the bin. As each tray is filled with prawns (about 2 kg per tray) an empty tray is placed over it and the process is repeated until all trays are filled. The lid of the bin is then fitted over the last tray but this is not clamped down tightly while the bin is at sea (unless the sea is rough), allowing water to overflow. Water circulation and aeration is achieved using air-stones placed in a frame on the floor of the bin and connected through the bung to an air pump.

Of course, the poor survival of these prawns may be partly caused by the harvest technique. An obvious way to find out whether this is so is to study brown tiger prawns harvested from a prawn farm, where harvesting was less stressful, (Paterson *et al.*, 1993). Brown tiger prawns are not commonly grown in farms but a crop was available for experiments at Cooktown. Yet, even when harvested using a gentler method, cultured brown tiger prawns did not survive in sawdust for as long as black tigers caught at the same farm did, though this result was confounded by evidence that the brown tiger prawns at the farm were in poor condition. Further studies of cultured brown tiger prawns are required to establish a "base-line" of survival in sawdust against which to compare the performance of trawled prawns. These studies should be done when more farmed brown tigers are available.

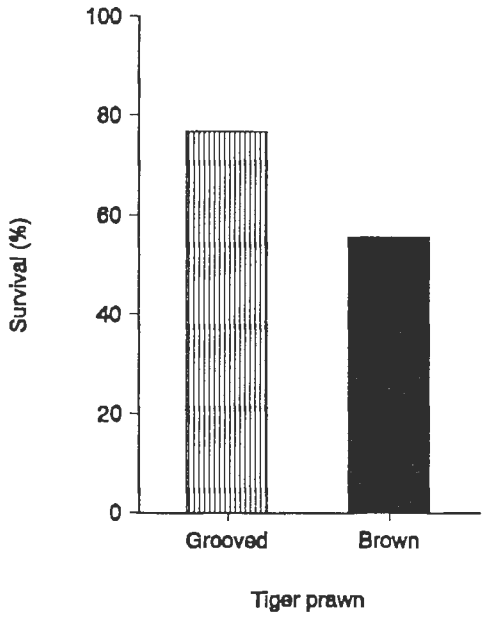


**Figure 3.** Survival of grooved (n=65) and brown tiger prawns (n=25) after 24h in sawdust at 12°C.

The metabolic responses of the brown tiger prawns in air were broadly similar to those seen previously in the black tiger, that is, the animal was not able to sustain its metabolic energy reserves for long periods in sawdust at 12°C, (Paterson, 1993b). Using the index of stress developed in the previous study of black tiger and kuruma prawns, (Paterson, 1993a) the metabolism of the brown tiger prawn is clearly not suited to live transport.

After about 12h in sawdust, the fall in energy reserves stimulated a rise in the concentration of lactate in the muscle tissue (Figure 4), and as noted above, this is a symptom of extreme physiological stress. However, unexpectedly, the IMP concentration of the brown tigers increased at a faster rate than it did in the black tigers. IMP is formed in crustaceans by an enzyme that is normally present at very low levels of activity. However, as alluded to above, the activity of the enzyme rises during stress, causing large amounts of IMP to accumulate. The results of this study suggest that the enzyme remains at a higher than expected level of activity long after the prawns have apparently recovered from harvesting. If this phenomenon is a lingering symptom of trawl stress then many other aspects of the animal's metabolism may also be out of balance, a situation which could compromise the survival of the prawns during commercial handling.

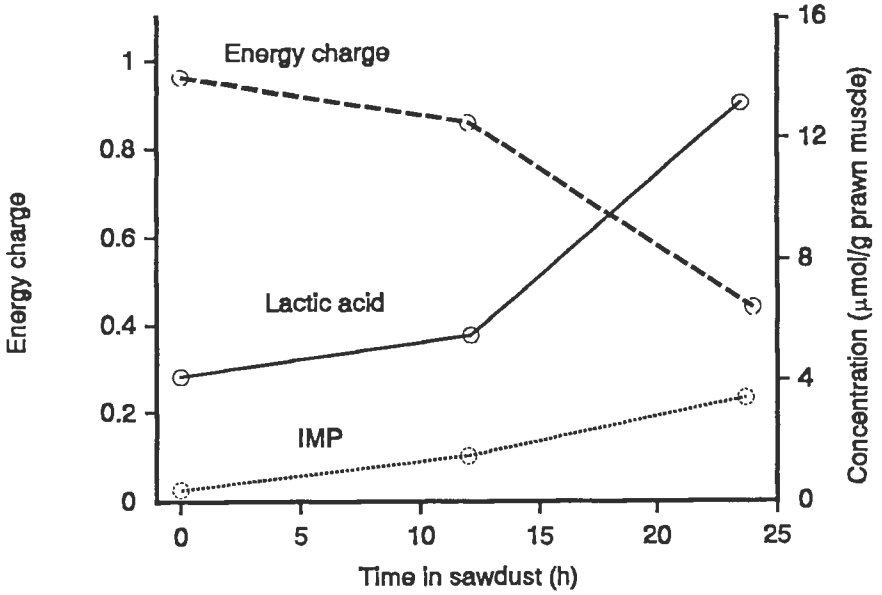


**Figure 4.** Metabolism of brown tiger prawns stored for 24h in sawdust at 12°C.

Furthermore, observations when handling brown tiger prawns at this temperature suggest that a temperature of 12°C may be too cold. The survival of the prawns may possibly be extended slightly by cooling to higher temperatures (about 15°C) rather than the 12°C routinely used to ship kuruma prawns, though there are doubts that this temperature would immobilise the brown tigers. This suggestion does however parallel data obtained in the previous study that indicated that survival of the black tiger prawn was slightly better at 16°C than at 12°C (Goodrick and Paterson, 1992). It is possible that tropical brown tiger prawns are less cold-tolerant than the sub-tropical/temperate kuruma prawn, though this does not explain why brown tigers should die sooner than grooved tigers or black tiger prawns in sawdust. Further work is required on the ideal storage and handling conditions for brown tigers.

Even if less stressful methods of harvesting are adopted, or careful grading of trawled prawns is practised, the survival of the brown tiger prawn is still marginal in the context of live export. Faced with what appears to be an intrinsic weakness of the brown tiger, compared to the longevity of the kuruma prawn in air, the options for developing an export market using existing packing methods are limited. Since brown tigers, at best, are expected to survive for 18 to 24 hours this means that sending them through auction at Tokyo (Tsukiji) or Osaka is probably not the best way to sell them in Japan. Rather, the prawns could be sold directly to wholesalers with live-holding tanks so that the product is placed in tanks as soon as possible after arrival in Japan. This way the survival of the prawns during subsequent wet storage can be maximised.

You may ask why we are studying all of these "tiger" prawns when the kuruma prawn is blatantly a "king" prawn in everything but its stripes. This is a question of marketability and not of biology. Our preliminary trials with eastern king prawns (*P. plebejus*) suggest that they probably have what it takes to be shipped around just like kuruma prawns are. But another market must be found for them because they are not a ready substitute for kurumas. The problem of trawl stress also remains, since the only eastern king prawns we've studied so far were caught as "by-catch" from a prawn farm during our previous study of kuruma prawns.

## CONCLUSIONS

The brown tiger prawn is apparently less tolerant of live transport than the grooved tiger and black tiger prawns and none of these species survives as well as the kuruma prawn does in sawdust. Unless the catch is large enough to permit the rigorous selection of strong prawns, the brown tiger will not survive long enough out of water to allow routine export using existing methods. However, the survival of all of these species is adequate to allow shipment of aquaculture broodstock over short distances using this method. Even if further refinements of the transport technique give a survival of 95% at sale in Japan, the earlier losses of prawns during harvesting, and grading, threaten the viability of the enterprise. In the longer term, less stressful harvesting techniques must be adopted, so that the prawns are in the best possible condition to withstand the stress of live transport.

## ACKNOWLEDGMENTS

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# LIVE STORAGE AND TRANSPORT OF PRAWNS CAUGHT AT SEA

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SUMMARY

A transportable prawn holding tank is described which can be used for storing prawns on board trawlers and for hauling them overland to a packing facility. This tank supports an environment that allows wild prawns to recover from harvesting while at sea and while being taken to a packing facility on shore. Holding live prawns on a trawler at sea is relatively simple. However, the likely causes of stress when carrying prawns overland in a closed body of water must be considered if the processing facility is not located right at the dock. Even if these factors are controlled, the loss of prawns due to harvest stress and injury compromise the commercial viability of the exercise.

## INTRODUCTION

The live export of farmed kuruma prawns, *Penaeus japonicus*, from Australia to Japan has recently been established, (Goodrick and Paterson, 1993), raising interest in the live export of prawns from the ocean. Kuruma prawns are commercially insignificant in the wild fishery of Australia, but the brown tiger prawn *P. esculentus*, resembles the kuruma closely enough to suggest that it may find a niche in the Japanese live seafood market.

The export of wild prawns presents new problems. On a prawn farm, the product is handy to the processing facility, and can be harvested using methods that cause very little stress, (Paterson *et al.*, 1993). However, wild prawns are trawled offshore, and stored for a day or more on board before reaching shore. Even after they are landed, the prawns may have to be transported overland for several hours to a packing facility, where they must then be allowed to recover before being packed.

Trawlers are not built with wet-wells, a necessity in many live seafood boats, but the deck hose is a reasonable substitute. Vessels that already collect live product for research or commercial purposes (research trawlers and broodstock harvesting) already make use of this facility. However, once the prawns reach shore, hauling them overland is potentially a more serious problem because of the consumption of oxygen and build-up of metabolic wastes (carbon dioxide and ammonia) in the water (Paterson and Goodrick, 1991). Similar problems occur when transporting live fish in water (Berka, 1986) and the methods developed here are simply an extension of the handling techniques applied to farm-grown kuruma prawns by Goodrick and Paterson (1993).

This paper describes a transportable storage tank for holding live prawns on a trawler which is also suitable for hauling prawns overland. While holding prawns at sea is a relatively straight-forward process, the possibility that the processing facility is not close to the dock requires that some attention is paid to the likely causes of stress when hauling prawns in a closed body of water.

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## HARVESTING LIVE PRAWNS

Prawns from the wild fishery in Australia are harvested by trawling. In order to maximise the survival of trawled prawns for live marketing, the trawling operation must be geared to reduce stress and damage to the prawns during capture. Essentially, this means modifications to the nets (eg. using a lighter tickler chain) reduction in shot duration and/or reduction in trawl speed. Either way the costs of capture are significantly increased due to the reduced bottom time and/or the total swept area.

Since the bottom time is reduced, the percentage of time spent fishing is also reduced and hence the effective catch rate. Where the actual time from the start of "winching up" to re-establishing bottom contact may be approximately 20 to 30 minutes, a short shot of say 15 minutes, designed to maximise survival, may result in an effective fishing time of only 30% compared to 75% for a "normal" shot duration of say 90 minutes.

While all efforts are made to minimise damage to the product, trawling causes losses ranging from 0 to 30% depending on the quantity and type of bycatch and the bottom conditions. One solution to this problem is to harvest the prawns using a less stressful technique. In Japan, wild kuruma prawns are harvested in a number of ways, using tangle nets, or slow beam-trawling. One method of capture involves the use of what amounts to a monofilament drift net. While prawns can be caught using this method with a minimum of stress, in Australia this kind of net is illegal. Other techniques, such as trapping, have been advocated as a means of capturing high quality prawns, but these techniques have yet to be applied commercially, (Rick Buckworth, personal communication).

## HOLDING PRAWNS AT SEA

Some aquatic crustaceans such as crabs and lobsters can be stored dry on the deck after capture, however, this method is unsuitable for prawns. Many species of prawn are quickly stressed when removed from the water, so live prawns or shrimp should be stored in water (Whyte and Carswell, 1982). Live prawns taken from the catch can be placed temporarily in a swim tank before being transfered to the holding bin. Prawns that have been injured by trawling can therefore be sorted from the catch at an early stage.

Regardless of the design of the boat being used, prawns must be stored in a tank in which the environmental conditions (temperature, oxygen, nitrogenous wastes, pH) enable them to recover from harvesting without any further stress. Fortunately, holding prawns at sea is quite simple, since seawater can be diverted from the deck hose into a tub or swim tank. Consequently, low oxygen and rising ammonia levels are not a problem because the seawater is continually being refreshed, flushing away wastes and providing oxygen. Nevertheless, when storing large quantities of prawns, an air pump should be installed to mix the tank contents and to provide oxygen during periods when the engines are turned off or when the vessel is entering harbour (when there is reason to suspect pollution or low salinity).

To be practical, a holding system for live prawns must be compact, since it must not get in the way of the crew during trawling. For small vessels (about 10 to 12 m) one simple practice involves placing plastic tubs on deck, each fed by a hose branching off the deck hose. Prawns can be sorted by first putting them in a "swim tank" for a few minutes recovery, and then prawns that are able to sit upright can be transferred in buckets to the plastic tubs. A piece of onion bag, shade cloth or net can be added to the tub, as the prawns will readily grasp this material. An inverter connected to the power supply can allow a 240V AC aquarium-style air pump to be used to assist aeration and mixing of the water.

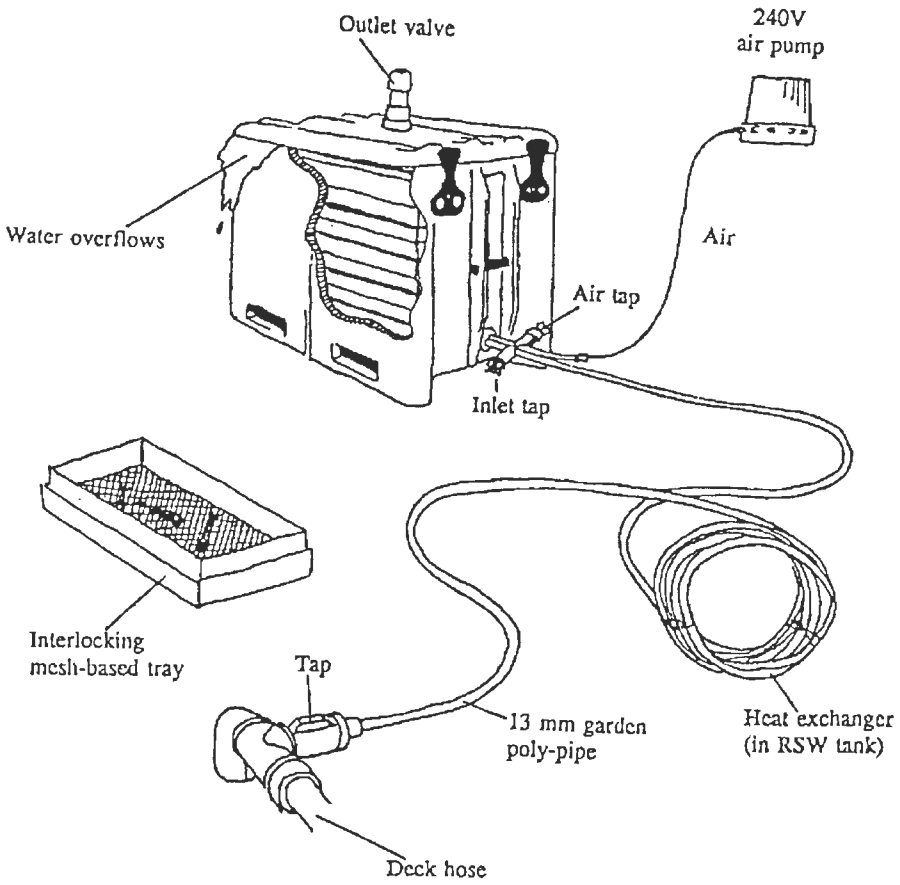


**Figure 1.** Portable tank for storing and transporting live prawns caught at sea.

The problems arising from crowding prawns together can be reduced by cooling them to a temperature between 17 and 20°C, thereby reducing their activity, feeding, oxygen consumption and excretion of wastes, (Paterson and Goodrick, 1991; Paterson, 1993). Water supplied from the deck hose can be pre-cooled by running through an inexpensive plastic heat-exchanger placed in an ice slurry, such as in an RSW tank. For example, 25 metres of 13 mm "poly" irrigation pipe can be wound into a coil and placed in-line between the deck hose and the tanks. To control the degree of cooling, a tap can be added to the pipe to control the flow rate (at a faster flow rate, the water is cooled less) or alternatively, the coil can be lifted partly out of the ice slurry (reducing the area exposed). It is not necessary to use a metal cooling coil (eg. stainless steel) and copper should not be used because it is toxic to crustaceans.

For larger vessels, with 240 V AC power supply, the holding system shown in Figure 1 is just an extension of the concept of a "swim-tank" to include an option for aeration, pre-chilling the sea-water and for storing the prawns in mesh-based, floating wooden trays. These trays must be stackable and interlocking so that after each tray is filled with prawns (up to about 10kg per tray) an empty tray is placed on top and this tray is then filled, and so on until all trays have been used. A mesh lid is placed on the top tray to stop prawns from leaping out when the bin lid is removed.

The catch in the trays should be checked regularly (every hour or when adding prawns) to remove sick, injured and dead prawns and return these to the "green" catch. When sorting the live product, the trays can be removed one by one and placed in an adjacent swim tank- checking for prawns that are lying on their side, discoloured or obviously damaged. After sorting, all trays are then returned to the holding bin. By keeping the prawns in trays, it is not necessary to handle them.

The holding tank illustrated (Figure 1) is an Xactics 309F with external dimensions of 109x56x71 cm and internal dimension of 91x49x56 cm. Other bins can be used, as long as they have a well-fitting lid. The wooden trays must be tailor made to fit neatly inside. The operator may wish to install several smaller units rather than one large tank as small tanks are easier to handle and transfer to shore. The lid can be strapped on during heavy seas to reduce water loss and surging within the tank. A valve is included in the lid (Figure 2) to allow aeration to continue when the lid is sealed (eg. during hauling overland below).

The idea of making this large bin transportable is so that the entire tank can be removed from the trawler upon reaching port. Prawns jump around and damage themselves when removed from the water, so the trays of prawns should never leave the water until they arrive at the packing facility and are transferred to the final holding tank. If dock facilities do not allow the bin to be lifted, then individual trays can be taken from the bin, placed into a carry bin of shallow sea-water, and then carried by hand to the nearby processing plant or to a second transport bin waiting on a truck for shipment to a distant plant ( eg. up to a couple of hours away).

## TRANSPORTING PRAWNS OVERLAND

A number of methods are available for carrying prawns overland, depending upon the period, distance and number of prawns involved. In the past, prawn broodstock have been air-freighted by putting two or three prawns in plastic bags of seawater, with oxygen gas in the head space, and then packing these bags in a foam carton (Robertson *et al.*, 1987). This method is similar to that used for the transport of fish and prawn fry/larvae and is covered in reviews such as Berka (1986). Similar methods are sometimes used to send live prawns to market. For example, most kuruma prawns arrive at the Tsukiji Markets in boxes of sawdust, but some wild prawns arrive in foam tubs of seawater aerated with portable air-pumps.

When hauling many kilograms of prawns from the dock to a holding facility, some kind of tank truck or trailer is preferred, particularly if the trawler lands the catch several hours away from the processing facility and a journey by road is required.

Aeration and cooling are still crucial factors to be considered, particularly as the tank is now a closed body of water. A safe temperature to haul prawns is to cool them by about 5 to 10°C below the sea-water temperature at the time they were caught. If a different tank is used to haul the prawns, it to best to pre-cool

the water and have the salinity of the transport tank identical to that of the holding tank (though the prawns can probably withstand a 5ppt drop in salinity without complications).

The air outlet valve (Figure 2) mounted in the centre of the lid is necessary during land transport because of the high amount of aeration used. Bubbling with oxygen gas is less practical because it will not mix the water adequately. A 12 V DC air pump, powered from the vehicle's electrical system, is suitable. The valve is designed so that the water surface is reduced, eliminating problems with surging (The people who design traffic roundabouts never drive around with a tank of prawns on a truck!) while allowing air but not water to escape from the tank.

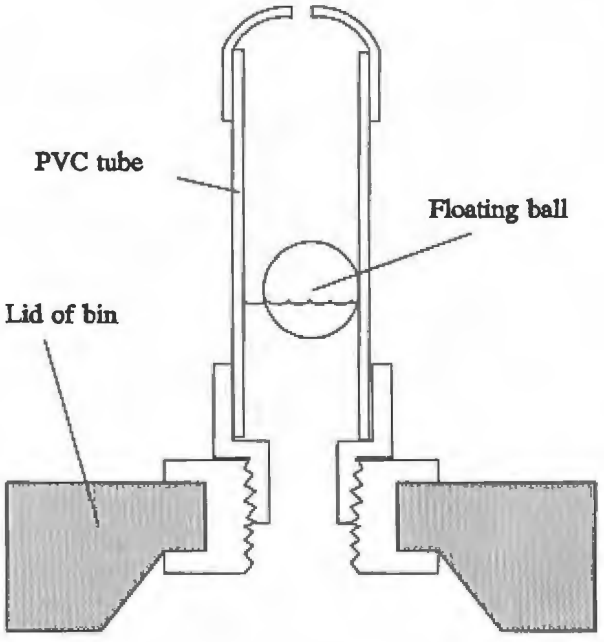


**Figure 2.** Detail of air outlet used in the prawn storage tank.

Prawns not only reduce the amount of oxygen in the water, they also excrete carbon dioxide and ammonia, waste products that are potentially toxic. The rise in carbon dioxide level (produce by the prawn's respiration) reduces the pH of the water, a change that is countered to some extent by vigorous aeration (blowing excess carbon dioxide out of the water). Reducing the pH however has the bonus effect of reducing the amount of the ammonia present in the toxic gaseous form (Paterson and Goodrick, 1991).

Ammonia concentrations will inevitably rise when large quantities of prawns are transported or stored in a fixed volume of sea-water, but this is not a problem in the short term- as long as other conditions (temperature and oxygenation) are optimal. Prawns can tolerate quite high levels of ammonia for short periods (Chen *et al.*, 1990; Chen and Lei, 1990), and it is not concievable that prawns would be transported long enough while wet to require biological filtration of the water.

## HOLDING PRAWNS ON LAND

When prawns reach the holding facility, they should be allowed to settle overnight (temperature 17 to 20°C) to recover fully from transport. Any prawns which subsequently become weak must then be discarded. A mortality of approximately 10% is common during overnight storage in the holding system as a subsequent result of capture stress and injury. A symptom of stress to pay particular attention to, is tail flesh that is

whiter than normal- evidence of internal damage ("white-spot" or "bruising"). This may take several hours to become obvious but it can involve 10 to 30% of the remaining prawns. These animals should not be packed.

Most live seafood exporters already have holding tanks installed which are suitable for storage of prawns. For long periods of storage, a biological filter can be used to remove ammonia from a holding tank by converting ammonia to nitrite (which is also toxic) and finally to nitrate. Mevel and Chamroux (1981) found that *P. japonicus* are particularly vulnerable to rising nitrite concentrations in closed aquarium systems, and in general it seems that nitrite build up is the more serious problem when holding live animals in closed systems, (Manthe *et al.*, 1985). A number of publications are available dealing with design and maintenance of biological filters and live holding systems (eg. Forteath, 1990) and it is not necessary to cover this issue here.

When enough prawns are available to send a shipment to domestic or overseas markets, then the cooling process can begin in a manner appropriate for the species concerned and to meet the logistic requirements of airline schedules. The further cooling and packing of wild caught prawns is considered in another paper of this series (Goodrick *et al.*, 1993).

## ECONOMIC VIABILITY OF CATCHING LIVE PRAWNS AT SEA

Losses of live prawns through injury and stress will obviously have a great influence on the economic viability of this practise. About half of the catch is dead to begin with, and up to half or more of the survivors are discarded during storage because they are injured or damaged internally (eg. unable to stand upright). The figures given here are estimates only, since the figures are subject to variation. Changes in capture technique and holding systems may well change the whole picture.

Therefore the costs of trawling live prawns are substantially higher than trawling prawns for chilled or frozen storage. The cost structure comparison is summarised in Table 1.

**Table 1.** The estimated cost factor introduced by changing from dead to live harvesting.

| | chilled /frozen | live | cost factor |
|---|---|---|---|
| effective bottom time | 75% | 30% | x 2.5 |
| trawl loss | - | 15%(ave) | x 1.17 |
| on-board storage loss | - | 10% | x 1.11 |
| on-land storage loss | - | 15% | x 1.17 |
| cumulative capture and storage cost increase (target species only)= | | | x 3.8 |

This is a broad estimate only and does not take account of the effect of lost fishing time on the reduced catch of non-target species, or of the residual value of the catch which is not suitable for live transport but otherwise useable as chilled or frozen product. A better idea of the viability of this process will be to

examine individual boats and consider differences in catch rate and by-catch between fisheries.

## CONCLUSIONS

Throughout the world, where species are destined for live marketing they are seldom caught by trawling. While the on-board holding and transport of live prawns is technically feasible, the losses of prawns due to the harvesting technique, presents a major obstacle to the exercise. There is therefore a large incentive to devise harvesting techniques which do not stress the prawns as much as those methods used currently.

## ACKNOWLEDGMENTS

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# THE CASE OF THE VANISHING IMP: ACCUMULATION OF INOSINE MONOPHOSPHATE (IMP) IN PRAWNS DURING HARVEST

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SUMMARY

The concentration of inosine monophosphate (IMP) and other ATP-related compounds, as well as the concentration of lactic acid, was measured in the abdominal muscle of several species of penaeid prawn. These prawns were harvested using different methods, from a prawn farm and also by trawling at sea. This study was conducted to examine the effect of harvesting on the accumulation of IMP in prawns and the possible role of this compound as a general measure of "freshness" or biochemical deterioration in prawns during subsequent post-harvest handling. A large proportion of adenosine monophosphate (AMP) was deaminated to IMP in trawled prawns, though in one trial, the "missing" AMP was already present as hypoxanthine in living brown tiger prawns, *Penaeus esculentus*, taken from the catch. The level of IMP in dead trawled *P. esculentus* and endeavour prawn, *Metapenaeus endeavouri*, was about 4.4 µmol/g, even though more than twice this amount of adenylates was estimated to be present in the live animal before trawling. *P. esculentus* harvested from a prawn farm had an IMP concentration of only 0.6 µmol/g in their tissues. Therefore, a high IMP concentration, whether in absolute or proportional terms, cannot be a general index of "freshness" in prawns since it is already an index of harvest stress.

## INTRODUCTION

The concentration of inosine monophosphate (IMP) is very high in trawled prawns (Fatima *et al.*, 1981). These authors suggested that the IMP concentration alone could be used as an index of "freshness" or biochemical deterioration in seafood, however the validity of this suggestion rests on assumptions about the level of adenylate nucleotides in prawn muscle and the changes in the rate of IMP accumulation during harvesting and death.

Ordinarily, ratios of adenylate breakdown products rather than concentrations of these compounds are used to chart the progress of biochemical deterioration in seafood. To calculate a K-value, for example, the concentration of the degradation products of IMP, namely inosine (HxR) and hypoxanthine (Hx) are divided by the total concentration of ATP-related nucleotides in the flesh (Ehira and Uchiyama, 1986). This gives a proportional change from the initial "fresh" state. To use IMP level itself, or indeed any concentration of a breakdown product of IMP as a measure of "freshness" implies that the total concentration of ATP-related compounds is a fairly conservative characteristic of animals. This may not be so.

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However, even if the proportion rather than the absolute concentration of IMP is taken as an index of early biochemical breakdown in prawn flesh, this assumes that high IMP levels are a characteristic of fresh prawns. One situation where this assumption may fail is when comparing prawns that are harvested in different ways, (for example wild versus farmed prawns). Another complication arises if prawns survive capture and excrete or metabolise the IMP that accumulated during harvesting.

Since IMP is a flavour enhancer in seafood, (Komata, 1990), the question of whether or not fresh prawns always have high IMP levels remains a significant issue even if this compound itself has no predictive value in terms of biochemical spoilage. IMP only acculumates in live crustaceans in response to severe physiological stress (Sylvestre and Le Gal, 1987; Paterson, 1993a). While the harvesting of cultured prawns is expected to involve less stress than the capture of wild prawns, and less IMP is expected to accumulate, Chen *et al.*, (1990) reported an IMP concentration of 6.9 µmol/g in cultured black tiger prawn, *Penaeus monodon*, immediately after harvest. This level is similar to that reported by Fatima *et al.*, (1981) from wild banana prawn *P. merguiensis*. This finding suggests that a high IMP concentration after harvesting may be a characteristic of farmed as well as wild prawns.

In this paper, the concentration of IMP and other ATP-related compounds, as well as the concentration of lactic acid, is considered in the abdominal muscle of several species of prawn harvested using different methods and from different sources. This study was conducted to examine the effect of harvesting on IMP accumulation and the implications of this effect on the "freshness" of prawns.

## METHODS

### Prawn species

Farmed prawns: black tiger prawn *Penaeus monodon*, brown tiger prawn *P. esculentus*
Wild prawns: brown tiger prawn, endeavour prawn *Metapenaeus endeavouri.*

### Different harvest methods and live prawns

#### At the farm

Live prawns were harvested from a farm near Cooktown in North Queensland. Brown tiger prawns, *P. esculentus* were harvested in the pond using a tunnel net. Prawns swimming in the pond followed the current created by a paddle wheel aerator and were guided between the V shaped wings of the net into a tunnel with a series of cones or funnels so that the prawns cannot swim back out. The prawns passed through this tunnel and were concentrated in a net basin or enclosure. Prawns were sampled directly from the enclosure. Black tiger prawns, *P. monodon*, were harvested from an adjacent pond using a cast net.

#### At sea

Live *P. esculentus* were captured by otter trawl near Cairns, North Queensland, on board a prawn trawler using 1-2h shots. Prawns were sampled immediately from the sorting tray.

### Freezing the prawns

Whole prawns were placed into a bioassay bag, which was then sealed and frozen rapidly in a dry ice-acetone bath. Samples from the farm and trawler were stored in an insulated box containing dry ice and taken back to the Department of Primary Industries (DPI) Northern Fisheries Laboratory in Cairns where they were stored for 1 to 2 days (-20°C) prior to being flown back to the International Food Institute of Queensland (IFIQ) laboratory in Brisbane for storage (-20°C) prior to assay.

Comparison of two trawled species

Commercially important prawns, brown tiger prawns, *P. esculentus* and endeavour prawns, *Metapenaeus endeavouri* were caught during sea trials of a benign prawn trawl, over a period of three nights (4th to 7th May 1992) in Moreton Bay, south east Queensland. The port-side net was a standard otter trawl used in this fishery while the starboard net was modified by adding a turtle excluder (TED) and a fish separator panel, to reduce by-catch. For each species the biochemical composition of the prawns was the same regardless of which net they were caught in, so data is only presented here for the standard trawl.

Five "commercially"-dead samples of each species were taken after the catch from each net was sorted, weighed and measured. The prawns were placed into labelled bags and frozen in a "snap" freezer (-30°C). The samples were then transported back to the IFIQ laboratory in an insulated box containing dry ice and stored at -20°C until analysis.

Equal weights of prawns were taken from each sample and the prawn abdominal muscle was prepared as below.

Extraction

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

Nucleotide and lactate assay

Nucleotide analysis was performed by an HPLC method similar to that reported by Ryder (1985), with a mobile phase of 0.06M $K_2HPO_4$ and 0.04 M $KH_2PO_4$ at pH 7 containing 50mL methanol/L and a flow rate of 2mL $min^{-1}$. ATP, ADP, AMP, IMP, inosine and hypoxanthine were assayed using a UV/visible detector at 254 nm. The balance between ATP and AMP, an equilibrium that is an important factor in the regulation of cellular metabolism, can be described by the "adenylate energy charge" (AEC) (([ATP]+½[ADP])/([ATP]+[ADP]+[AMP]), Atkinson, 1977). Since the prawns were either alive or only recently dead, the AEC of each prawn was calculated from the nucleotide concentrations.

L-lactic acid concentration in the same muscle extract was measured using the HPLC method of Morawski (1984). A Waters µ-Bondapak $C_{18}$ column was used for this assay with a mobile phase of 0.5% $(NH_4)_2PO_4$ solution at pH 2.8, and detection was accomplished by a UV/visible detector set at 214nm.

RESULTS

Different harvest methods and live prawns

The highest ATP concentrations and adenylate energy charge (AEC) were seen in the farmed prawns (Table 1). However, contrary to expectations the IMP concentration of *P. esculentus* was similar for prawns harvested at the farm or captured at sea. This was despite the ATP level and AEC in the trawled prawns being very low. The total nucleotide level (Total) was the same for all categories, but the hypoxanthine (Hx) concentration was remarkably high in the trawled prawns (and to a lesser extent in the cast-netted *P. monodon*). Upon capture, the K-value of these living trawled prawns was 37%, compared to 13 and 3% for the farmed *P. monodon* and *P. esculentus* respectively. After the sample of trawled prawns was taken, those prawns that remained were allowed to recover, apparently without ill-effect, in tanks of sea-water and taken back to shore for further live handling experiments (Goodrick *et al.*, 1993). The AMP and lactic acid concentration of the farmed *P. esculentus* were higher than the farmed *P. monodon*, and the AEC of the former prawns was relatively low.

**Table 1.** ATP-related compounds and lactic acid concentration (µmol/g) in live prawns caught using different harvest techniques.

| | Harvest | | |
|---|---|---|---|
| | cast net | tunnel net | trawling |
| species | *P. monodon* | *P. esculentus* | *P. esculentus* |
| ATP | 7.2±1.6 | 7.2±0.4 | 2.6±1.2 |
| ADP | 0.1±0.1 | nil | nil |
| AMP | 1.0±0.5 | 2.3±0.6 | 2.9±1.3 |
| *AEC* | 0.87 | 0.76 | 0.47 |
| IMP | 0.5±0.3 | 0.6±0.3 | 0.7±0.2 |
| HxR | 0.4±0.3 | 0.2±0.3 | 1.0±0.9 |
| Hx | 0.9±0.9 | 0.1±0.2 | 2.8±1.8 |
| Total | 10.0 | 10.0 | 10.1 |
| Lactate | 6.6±2.1 | 10.0±2.0 | 13.0±3.4 |

Comparison of trawled *P. esculentus* and *M. endeavouri*

When samples of dead prawns were taken after trawling, roughly equal amounts of AMP and IMP were present in both species, and ATP and Hx concentration was very low (Table 2). The AEC was very low in both cases, as befits a dead animal.

## DISCUSSION

Inosine monophosphate (IMP) concentration is an index of stress in living prawns (Paterson, 1993a), so the use of IMP concentration as a biochemical index of freshness in prawn flesh is questionable. However, the role of IMP as a flavour enhancer in seafood suggests that IMP concentration is still an important measure of seafood quality. After prawns are "harvested", that is, as soon as they can be touched by human hands (i.e. "handled"), trawled prawns have experienced more physiological stress than prawns harvested at a farm. This is not to say that farmed prawns will necessarily always have lower IMP levels than wild prawns, but only that the methods of harvesting used at sea favour the production of IMP before the prawns die.

So, before discussing the IMP concentration of farmed and wild prawns it will be useful to consider the events that lead to the formation of this compound. IMP forms in the tissues of trawled prawns as a result of stressful exercise within the cod-end. Typically, both IMP and lactate accumulate when fish exercise, for example when they struggle on a fishing line (Wells, 1987; Hochachka, 1985). However, IMP does not normally appear when crustaceans exercise (Raffin and Thebault, 1987).

A prawn exercised to exhaustion will fatigue in a few minutes, while a typical trawl shot lasts for an hour or more. During this time, the prawn's metabolic rate exceeds its rate of rate of oxygen uptake and as a result lactic acid accumulates quickly. Live *P. esculentus* caught in 15 minute trawls, on the same field trip that the data for Table 2 was collected, already had a lactic acid concentration of 10.5 µmol/g (Paterson, 1993b), similar to that of the dead *P. esculentus* studied here. Production of lactic acid may be reduced in response

to fatigue and death. In this context it is interesting to note that the lactic acid level in the dead *M. endeavouri* was significantly lower than in the dead *P. esculentus*. Apparently, the tail muscle of the former species has a lower capacity for anaerobic glycolysis, the pathway that produces lactic acid.

**Table 2.** ATP-related compounds and lactic acid concentration (µmol/g) in dead prawns caught using an otter trawl.

| | *P. esculentus* | *M. endeavouri* |
|---|---|---|
| ATP | 1.0±0.5 | 1.5±0.2 |
| ADP | 0.6±0.1 | 0.5±0.1 |
| AMP | 5.8±1.1 | 4.7±0.4 |
| *AEC* | 0.18 | 0.26 |
| IMP | 4.4±0.9 | 4.4±0.2 |
| HxR | 0.3±0.1 | 0.2±0.1 |
| Hx | 0.1±0.1 | 0.3±0.1 |
| Total | 12.2 | 11.6 |
| Lactate | 12.1±2.3 | 8.4±3.1 |

Shrimps and crayfish normally fatigue at an adenylate energy charge (AEC) of between 0.5 and 0.7 (Onnen and Zebe, 1983; Gade, 1984; England and Baldwyn, 1983). IMP is apparently formed when fatigued prawns continue to consume ATP at a rate faster than they can restore their energy charge. ATP production may be inhibited by accumulation of metabolic wastes associated with exercise and disturbances in blood circulation and gill ventilation. As a result, ATP and ADP are consumed, and the AEC falls below 0.5. The AEC seen here in trawled *P. esculentus* (Table 1) is similar to that reported by Paterson (1993b). AMP is not the only nucleotide that accumulates, some of this compound is converted into IMP. In a physiological context, this process prevents high levels of AMP from building up in the cells. The enzyme responsible, AMP deaminase, is not present in high enough amounts in the tissues of invertebrates for it to convert all AMP to IMP (Suwetja *et al.*, 1989). Consequently, relatively large amounts of AMP persist even after death.

From the discussion above, it is clear that IMP concentration rises because of pathological changes in the prawns metabolism. Living brown tiger prawns caught in 15 min trawls, mentioned above, already had 5.1 µmol/g of IMP in their tissues (Paterson, 1993b), similar to that shown for dead prawns caught with the same gear that night (Table 2). Similar levels of IMP were reported by Fatima *et al.*, (1981) in recently trawled *P. merguiensis*, 5.8µmol/g (ten hours after capture) and these authors proposed that the IMP concentration could be used as an index of freshness. However, an objection to this idea is that the concentration of IMP in a given sample of prawns may be determined by the amount of adenylate nucleotides (ATP, ADP and AMP) originally present in the animal.

The total nucleotide pool in different species of crustaceans ranges between 6 and 12µmol/g (Suwetja *et al.*, 1989; Matsumoto *et al.*, 1991; Paterson, 1993a) and it probably changes in response to environmental conditions (Giesy *et al.*, 1981). Lack of knowledge about the size of the nucleotide pool is one of the reasons why measuring hypoxanthine concentration alone cannot give a reliable indication of biochemical spoilage (Ehira and Uchiyama, 1986). However, the "total pool of purine nucleotides" is too nebulous a concept to describe the processes of feedback inhibition at work to regulate the size of the adenylate pool in the living

prawn. Of most interest to this discussion is the kinetics of AMP deaminase as it converts the adenylate pool to IMP. Amongst vertebrates, where the activity of this enzyme is more pronounced, mechanisms exist to protect the adenylate pool from shrinking too far during exercise, (Atkinson, 1977).

An interesting fact to emerge here is that despite the concentration of purine nucleotides being between 10 and 12 µmol/g before harvest, the level of IMP seen in prawn flesh reaches a peak of only 5 to 6 µmol/g (Flick and Lovell, 1972; Fatima *et al.*, 1981; Chen *et al.*, 1990; this study). The rapid conversion of AMP to IMP (for an invertebrate) that occurs in response to the stress of trawling apparently slows down once the animal dies. This prevents all of the adenylates from converting to IMP before the IMP itself begins to degrade to inosine and hypoxanthine during chilled storage. An alternative explanation could be that a change in enzyme activity, such as end-product inhibition of AMP deaminase, occurs if the IMP level rises, either above a certain concentration, or above a particular fraction of the adenylate pool. If this threshold value of IMP concentration is independent of the size of the adenylate pool then IMP concentration may indeed be quite valid as a measure of "freshness" in trawled prawns.

At the risk of proposing yet another "quality" index, the ratio of IMP to the total nucleotide pool (usually ATP+ADP+AMP+IMP) ought to give an adequate picture of this process. Of course, any mechanism that degrades or recycles large amounts of IMP in the prawns while they are being captured will render meaningless the idea of using IMP to chart the biochemical deterioration of the dead prawn. Table 1 shows an extraordinarily high hypoxanthine level in living brown tiger prawns- a k-value of 37%! This finding is difficult to explain, since it does not fit with the results from the later study (Table 2), (see also Paterson, 1993b) nor with data in the literature (Fatima *et al.*, 1981). However, before discounting this data it is worth noting that fluctuations occur in the total nucleotide pool when prawns recover from trawling (Paterson, 1993b). The enzyme substrates measured when calculating the k-value are presumably a major pathway for a living prawn to rid itself of purine wastes such as IMP.

Trace amounts of the adenylate pool appear in the blood as urate, an end product of purine catabolism, when crabs are deprived of oxygen (Lallier *et al.*, 1987), though in this case the waste appears to come from the hepatopancreas (mid-gut gland) (Dykens and Shick, 1988; Lallier and Walsh, 1991; Dykens, 1991) an organ which may be less able to defend its nucleotide pool during stress than muscle tissue. Perhaps, under some circumstances, IMP breakdown begins before the living prawns arrive on deck. Xanthine dehydrogenase and/or xanthine oxidase (the enzymes that convert hypoxanthine via xanthine to urate) show negligible activity in crab muscle (Dykens and Shick, 1988). Under this circumstance, it is not surprising that purine breakdown in prawn muscle would stop at hypoxanthine.

The whole idea of high IMP levels as a sign of freshness fails when prawns are harvested without IMP accumulating. The kuruma prawn *P. japonicus* is marketed live and consequently is harvested with a minimum of stress from farms and from the sea. IMP is not apparently a significant component of the nucleotide profile of fresh *P. japonicus* (Nakamura and Ishikawa, 1986; Matsumoto and Yamanaka, 1991; Suwetja *et al.*, 1989) though IMP accumulates if the prawns are stressed during transport in sawdust (Furusho *et al.*, 1988, Paterson, 1993a).

In this study, farmed *P. esculentus* showed an IMP concentration of only 0.6 µmol/g when harvested. This contrasts with the results of Chen *et al.*, (1990), who found an IMP concentration of about 6.9 µmol/g in fresh *P. monodon* at a prawn farm. The high IMP level may have been caused by crowding the prawns in the net or by a delay in handling after harvest, since it isn't clear how soon after harvest the prawns were frozen. If post-harvest handling is not responsible, then disease or pollution may be involved, since AMP deamination does occur in response to a number of environmental stressors (Reddy and Rao, 1990; Sylvestre and Le Gal, 1987).

Another unusual feature of the data of Chen *et al.*, (1990) is that the total nucleotide pool falls from 18 to 12µmol/g during the first 10 h of post-harvest transport and thereafter remains constant for the remainder of the experiment. The fall in the nucleotide pool occurs when the sum of the adenylates shrinks from 12 µmol/g down to 6 µmol/g without producing a corresponding rise in IMP concentration. There is no evidence of any purine degradation that might account for this change. The IMP concentration remains relatively

constant and its degradation products (HxR and Hx) do not begin to accumulate until much later in the experiment. So, what happened to the IMP?

If we assume that the fresh prawns have been handled identically to the prawns subsequently used during the experiment, that is they represent the true "zero" state of the prawns, this "mass balance" problem could be solved if you assume that the 6 µmol/g of IMP that was initially in the prawns really has disappeared (eg. excreted from the animal), which is the last thing you want to happen to a flavour enhancer. Similar inconsistencies in mass balance of nucleotides occur in other experiments with living prawns, especially when they recover from trawl stress (Paterson, 1993a & b) and one of the treatments used by Chen *et al.*, (1990) did use live prawns. However, the fall in the nucleotide pool also occured in dead prawns (held on ice), and it is unlikely that these prawns would excrete the missing nucleotides. Perhaps an alternative degradation pathway, such as adenosine formation (Cheuk *et al.*, 1979), a compound currently out of favour as an intermediary in purine degradation (Suwetja *et al.*, 1989), would account for the difference. Evidently, the metabolism of purines in harvested prawns is a more complicated picture than the word "degradation" implies.

The authors note that the high ATP level in their prawns was evidence of the "non-stressed" state of the prawns. The AEC of these prawns, at 0.67, was consistent with exhausting exercise (c.f. the shrimp *Crangon crangon*, Onnen and Zebe, 1983) and though it was less than that of the farmed prawns reported here, it was not as low as the AEC of trawled prawns (Table 1). The IMP level still seems extraordinarily high for this AEC value, (Sylvestre and Le Gal, 1987) and, as outlined above, any significant level of IMP in a crustacean is evidence of severe metabolic stress.

The data presented here show that farmed prawns need not accumulate high levels of IMP during harvest. The words "need not" are important because we have only sampled prawns at the point that they can first be handled. Obviously, there may be as much scope for bad handling at a farm as there is for good handling on a boat. If aquacultured prawns can have lower levels of this compound after harvest than trawled prawns do, then studies are required to follow this initial difference through the post-harvest handling of the product. Handling may have a more equilizing effect on nucleotide composition than our initial study of harvested prawns indicates.

To conclude, IMP is formed under some circumstances in prawn flesh during harvest and as such can be used as an index of the physiological stress of harvesting. Prawns are harvested from farms and at sea using different methods and under a variety of environmental conditions. Consequently, even if empirical data gathered in the future shows that trawled prawns tend to enter storage with a characteristic concentration of, (or proportion of nucleotides present as) IMP in their flesh, the contribution of the harvest method to IMP production in prawns means that in general the proportion of purines present as IMP in a product cannot tell how far the process of nucleotide degradation has progressed. Perhaps it has not even started.

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# MINIMAL HARM AT HARVEST TIME: ALL BROWN TIGER PRAWNS (*PENAEUS ESCULENTUS*) ARE FATIGUED BY TRAWLING BUT SOME ARE MORE FATIGUED THAN OTHERS

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SUMMARY

Biochemical composition of abdominal muscle and ion content of the haemolymph was measured in brown tiger prawns *Penaeus esculentus* caught by otter trawl. This work was done to see if prawns that were still jumping when they arrived on the trawler had not been in the net for long enough to fatigue. The adenylate energy charge (AEC) was very low in trawled prawns (less than 0.5) and there was no difference in the tissue lactate and inosine monophosphate concentration or the tissue AEC of the jumping prawns and the prawns that were only capable of feeble movements. Haemolymph calcium ($Ca^{2+}$) levels were also similar regardless of the level of activity. However, the haemolymph of moribund prawns showed higher potassium ($K^{+}$) level than that of jumping prawns, as if they have been fatigued for so long that they are unable to control potassium leakage from their cells. Prawns that were allowed to recover in holding tanks restored levels of energy metabolites and blood ion composition that were apparently normal. All of the *P. esculentus* were fatigued by trawling and differences in appearance and behaviour of the prawns were probably a consequence of being fatigued for different periods. Despite the apparent recovery of these prawns, the period of low AEC during trawling may cause longer term damage.

## INTRODUCTION

Understanding the stress that crustacea experience during harvest is important when management practises require their return to the sea, for example when size or sex restrictions apply (Smith and Howell, 1987, Sumpton *et al.*, 1989), or when only the claws of the animal are "cropped" (Simonson and Hochberg, 1986). Post-harvest mortality in animals returned to the fishery can never be known exactly, (Nielsen *et al.*, 1989). In contrast, when the catch itself is marketed live, there is no error in recording mortality, and an obvious commercial incentive to reduce it.

When caught by trawling, crustacea are expected to exercise enough to become anaerobic. Yet, the lobster *Nephrops norvegicus* shows no evidence of lactate accumulation in the haemolymph when trawled (Spicer *et al.*, 1990). This finding is unexpected because lactic acid is the principal anaerobic end product in crustacea (Gade, 1983; Gade and Grieshaber, 1986). If exercise provides a model for what happens to crustacea during trawling then they should fatigue as their reserves of arginine phosphate and adenosine tri-phosphate (ATP) diminish, and show metabolic acidosis as lactic acid accumulates in their tissues and haemolymph (England and Baldwyn, 1983; Onnen and Zebe, 1983; Waldron *et al.*, 1986). However, the abdominal muscle of trawled prawns differs from that of exercising crustaceans in having high levels of inosine monophosphate (IMP), a breakdown product of adenylate nucleotides (eg. Fatima *et al.*, 1981). The stress of trawling apparently increases the activity of the enzyme responsible, AMP deaminase. High levels of IMP in crustacean muscle is an indicator of pathological stress, and it also accumulates when live penaeid prawns

are stored in air (Paterson, 1993).

Brown tiger prawns, *Penaeus esculentus*, caught by otter trawl in north eastern Australia, usually die before reaching the trawler. This species is similar enough in appearance to the kuruma prawn *P. japonicus*, a species already marketed alive in Japan (Shigeno, 1979), that it seems to be a promising candidate for live export. However, this presupposes that the brown tiger prawn can be harvested with minimum stress from the wild. Some of the mortality in trawled prawns can be accounted for by injury but, even uninjured prawns that reach the boat alive show varying degrees of paralysis. Some of the prawns are still jumping when they arrive on the trawler, as if they have not been in the net for a long enough period for them to become fatigued. The remainder of the living prawns appear capable of feeble movements only.

In general, aquatic crustacea become lethargic at low environmental oxygen tensions (Taylor and Spicer, 1987) and both the lobster *Homarus gammarus* and the spiny crayfish *Panulirus argus* are sometimes unable to show tail-flips following storage in air (van der Meeren, 1991; Vermeer, 1987). Both kinds of respiratory distress (and their potential neurological side-effects) are accompanied by changes in energy metabolism, acid-base balance and haemolymph ion composition (Gade, 1984; Johnson and Uglow, 1985; Taylor and Whiteley, 1989). When crustaceans experience acidosis, one suggested means of buffering the haemolymph pH (especially while in air) is by the dissolution of calcium carbonate reserves in the exoskeleton (Truchot, 1983). Haemolymph calcium levels rise. Recently, Whiteley and Taylor (1992) found that both calcium and magnesium ion levels were high in emersed *H. gammarus* arriving weak at a seafood wholesaler. The unusual ionic composition of the haemolymph may account for the moribund appearance of these stressed lobsters (Whiteley and Taylor, 1992).

Since jumping prawns appear to be the healthiest of the catch, this study aimed to find out whether these prawns were really as unstressed as they looked. Reflexes such as the tail-flip response require proper functioning of the neuro-muscular system, so paralysis in trawled prawns could be associated with either fatigue (low ATP levels), failure of ion regulation, or physical damage to cells and tissues. These possibilities were considered in this study. As it turned out, the prawns showing tail-flips on the boat were already fatigued and prawns that did not had unexpectedly high potassium concentrations in their haemolymph.

## METHODS

Brown tiger prawns were caught by otter trawl on several day or night trips in Moreton Bay, south east Queensland. Set times of 15 or 60 minutes (respectively short and long shots) were used. Catches of this species were low, up to 40 prawns per shot, and most of these were dead, so whole live prawns and haemolymph samples were taken from repeated trawling. Some prawns were still able to show tail-flips on the sorting tray (jumping prawns) whereas others seemed to be paralysed and were capable of only feeble leg movements (non-jumping prawns). This difference was used to discriminate the health of prawns caught in long shots.

### Short shots

After 15 minute shots, living prawns were taken at random from the catch (regardless of how active). Ten of these prawns were frozen immediately (harvested prawns) and the remaining prawns were held on deck in an aerated tank of seawater (23°C) for 1 h before freezing a further 10 prawns. Sea-water was continually pumped through this tank. Prawns from different shots were separated in stacked, floating trays. After freezing the second sample, the remaining prawns were allowed to recover further in the tank and about 0.2 ml of haemolymph was taken (below) from the pericardial sinus of 10 prawns after 9 h. These samples were compared with the haemolymph samples taken from the prawns collected in the long shots, below, assuming that, under the circumstances, the prawns return to a state as close as possible to "normal".

Long shots

Jumping and non-jumping prawns (up to 3 per shot) were selected quickly from the catch. These prawns were either placed into a biossay bag and frozen intact for muscle analysis or had haemolymph samples taken for ion analysis.

Ten whole prawns of each group (jumping or non-jumping) were frozen in a slurry of dry-ice and acetone, and then removed to a ice-box containing surplus dry ice. During long trips this ice-box was stored in a "snap" freezer at -35°C and later stored in a -20°C freezer at the IFIQ laboratories prior to analysis of the samples.

In addition, 18 jumping and 17 not jumping prawns had about 0.2 ml of haemolymph withdrawn from the pericardial sinus (below the adrostral groove) using an ice-chilled hypodermic syringe (the syringes were kept cold to prevent clotting). These haemolymph samples were injected into 0.5 ml vials. The vials were sealed and stored, frozen, in the ice-box.

The remaining active prawns were transfered to a plastic tub on deck through which cooled sea-water (18 to 20°C) continually flowed. The water was cooled by passing through a plastic heat exchanger submerged in a refrigerated sea-water tank. Plastic mesh and/or old pieces of net were provided for the prawns to hold onto. The water was aerated. The prawns were allowed to recover for 4-5h and 10 prawns that were upright and showed no opaque areas in the abdominal muscle were frozen, in other words, those that appeared to be healthy, to see how "normal" their lactate and nucleotide concentrations were.

Long term recovery and storage experiment

Prawns from long shots were transported back to the laboratory where they were held in a recirculating sea-water aquarium (22°C). The metabolism of prawns stored in air at 12°C was investigated and compared with results of a previous study of the metabolism in air of *P. japonicus* and *P. monodon* (Paterson, 1993) to see if any long term symptoms of trawl stress were evident. The prawns were cooled in seawater from 22 to 12°C at a rate of 3 °C/h, and 8 prawns were immediately frozen for later analysis. Other prawns were packed 10 prawns per box into dry sawdust and stored in a cold room at 12°C. The onset of fatigue was monitored by removing boxes after 12 and 24h and immediately freezing the abdomen of 8 live prawns.

Biochemical analysis

Analysis of purine nucleotides and lactate by HPLC were performed using the same techniques used in a previous paper (Paterson, 1993), based on the methods of Ryder (1985) and Morawski (1984) respectively. ATP, ADP, AMP, IMP, Inosine and hypoxanthine were assayed. Negligible amounts of inosine and hypoxanthine were found. The "adenylate energy charge" (AEC), ([ATP]+½[ADP])/([ATP]+[ADP]+[AMP]: Atkinson ,1977), of each prawn was calculated from the nucleotide concentrations.

Electrolyte analysis

Magnesium, potassium and calcium concentration was measured in whole haemolymph by inductively-coupled plasma mass spectrometry (ICP-MS). The coagulated sample (100 to 200 mg) was digested in 2.5ml of nitric acid in low pressure teflon bombs, placed in a microwave oven for 40 minutes at 280 watts. Each digested sample was then diluted to 50 mL with distilled water and analysed by ICP-MS, with standards. The concentrations were determined at m/z, $^{25}Mg^{2+}$, $^{39}K^{+}$ and $^{43}Ca^{2+}$.

$Ca^{2+}$ and $Mg^{2+}$ were measured because concentrations of these ions have been reported to rise when crustaceans are stressed. $K^{+}$ was included because this ion is usually strongly regulated in crustacean haemolymph.

RESULTS

Short shots

The lactate and nucleotide composition of prawn caught in 15 minute shots is shown in Table 1. The prawns were extremely fatigued during capture and while the AEC did rise during recovery it remained at a "pathologically" low level. Lactate concentration was high after trawling and fell significantly within the recovery period. The ATP concentration increased significantly but the benefit of this to the AEC was countered by a rise in total adenylate pool (ΣAxP) of similar magnitude. Neither ADP nor AMP concentration changed significantly. The rise in ΣAxP cannot be ascribed to conversion of IMP back into adenylates, since the IMP concentration did not change dramatically (and it is only significant at P<0.10).

**Table 1.** Mean lactate and nucleotide concentrations (µmol/g, ±SD) and AEC of *P. esculentus* caught by otter trawl and distuguished by tail-flicks, and after 3-4 hour recover.

| | Sample | | |
|---|---|---|---|
| | Jumping | Not jumping | Recovered |
| n | 11 | 8 | 10 |
| Lactate | 16.4±4.0 | 14.7±5.0 | 3.1±1.2[!] |
| ATP | 2.0±0.7 | 3.0±1.2* | 5.7±0.6** |
| ADP | 1.5±0.7 | 2.2±0.9 | n.d. |
| AMP | 4.0±1.4 | 2.1±1.3 | 0.4±0.1** |
| ΣAxP | 7.4±1.1 | 8.9±1.3* | 6.1±0.6** |
| AEC | 0.37±0.15 | 0.45±0.14 | 0.94±0.01** |
| IMP | 1.6±0.7 | 1.7±0.8 | 0.6±0.2** |
| IMP+ΣAxP | 8.9±1.6 | 10.7±1.0 | 6.72±0.68* |

[!] data from 4 prawns, lactate not detectable (<2.00 µmol/g) in remaining prawns

In each row, asterisks denote significant differences (P<0.05 and 0.01) from first column.

Long shots

After one hour trawls, the "health" of the prawns was gauged according to whether or not they showed tail-flicks on the sorting tray.

In Table 2, there was no significant difference between ADP, AMP, lactate and IMP concentration of prawns in these two categories. However, surprisingly, the prawns that did not show tail-flicks had a significantly higher ATP concentration. The fact that AEC was not significantly different in the two groups is explained by the significantly higher total adenylate pool (ΣAxP) in the non-jumping prawns.

When jumping prawns were placed in the recovery tank for 3 to 4 hours there were significant changes in the levels of all nucleotides (ATP rising and AMP and IMP falling). The restoration of the AEC is partly due to the significant fall in the total adenylate pool (ΣAxP), indicating that most of the IMP has been lost from the system at this stage rather than being present as adenylates. Lactate concentration fell dramatically- and in some prawns was below the detection limit for the method.

Trawling had a significant effect on the calcium ($Ca^{2+}$) and potassium ($K^{+}$) concentration of whole haemolymph (Figure 1), whereas there was wide variation in the magnesium ($Mg^{2+}$) concentration and no significant differences between trawled prawns (8.37±4.42 and 10.38±4.20 mmol/l for jumping and non-jumping prawns) and prawns that had recovered from trawling for 9 hours (7.34±1.93 mmol/l). The variation was accounted for by only a few prawns and may have been caused by contamination with sea-water.

**Table 2.** Mean lactate and nucleotide concentrations (µmol/g, ±SD) and AEC of *P. esculentus* during recovery from trawling (15 minute shot).

| | Time after harvest | |
|---|---|---|
| | 0 h | 1 h |
| n | 9 | 11 |
| Lactate | 10.5±1.9 | 8.1±1.7** |
| ATP | 0.7±0.3 | 2.6±1.1** |
| ADP | 1.4±0.5 | 1.4±0.5 |
| AMP | 2.4±0.9 | 2.3±0.6 |
| ΣAxP | 4.5±1.3 | 6.2±2.0* |
| AEC | 0.32±0.06 | 0.52±0.06** |
| IMP | 5.1±2.1 | 3.5±2.2 |
| IMP+ΣAxP | 9.7±1.0 | 9.7±0.6 |

one or two asterisks denote significant differences from column one of $P<0.05$ and $P<0.01$ respectively.

The $Ca^{2+}$ level was high in the trawled prawns, but there was no significant difference in level between prawns that showed tail-flick and prawns that did not. However, prawns that did not jump had a higher $K^{+}$ concentration in their haemolymph than prawns that could, and both groups had significantly higher $K^{+}$ concentrations than the controls.

Long term recovery and storage experiment

The adenylate pool of 8 prawns after holding in the laboratory for a day, prior to storage in air was 9.6±1.4 µmol/g. This was higher than that seen in prawns up to 4 hours after capture (about 6 µmol/g, Table 1 and 2). The changes in the lactate and nucleotide concentrations when *P. esculentus* was stored at 12°C for 24h (Figure 1) were similar to those seen in *P. monodon* by Paterson (1993). Lactate concentration did not change significantly during the first 12h that the prawns were in air. However, IMP concentration increased prematurely (at 12h) before the AEC had fallen significantly (0.86±0.08, Figure 2). The increase in IMP concentration was small, and there was no significant change ($P>0.05$) in ΣAxP (8.1±1.7µmol/g) during this time. The continued rise in IMP concentration later at 24h storage is accompanied by a significant change in AEC and ΣAxP is now significantly different ($P<0.05$, 6.7±1.4µmol/g) from that seen at the start of the experiment. No significant change occured in total nucleotide concentration (IMP+ΣAxP), so the decrease in adenylate pool was matched by the rise in IMP concentration.

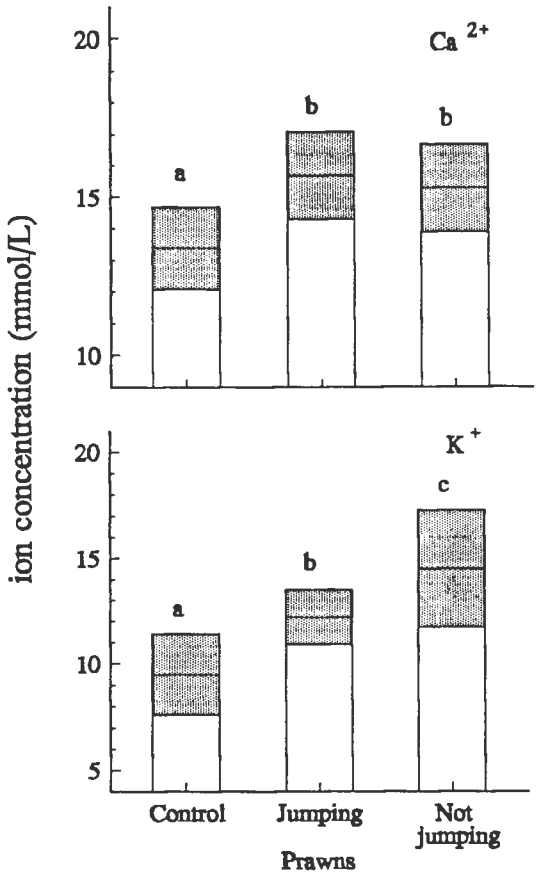


**Figure 1.** Ion concentration (mmol/L, mean±SD) in haemolymph samples. For each ion, samples with different letters are significantly different ($P<0.05$)

DISCUSSION

You might expect that a prawn which is still alive and kicking when it reaches the deck of trawler has not been in the net for long enough to be injured by trawling. However, prawns that survive trawling are already fatigued. The difference appears to be that the flicking prawns are not quite as fatigued as the prawns that cannot move but which aren't actually dead yet. The only benefit gained from shortening the length of the shot is therefore to reduce the time that the prawns remain fatigued for. Since trawl stress consists of various degrees of fatigue the symptoms observed go beyond those normally described in crustaceans under stress. "Exhaustion" is therefore the baseline data for what follows.

A prawn exercised to exhaustion will fatigue in a few minutes, while a typical trawl shot, lasts for an hour or more. By-catch and other prawns in the cod-end will stimulate repeated tail-flips. Physiological literature shows that generally when crustaceans undergo heavy exercise, they go anaerobic and their tissue and haemolymph lactate concentration rises (Gade, 1984; Onnen and Zebe, 1983), causing acidosis which is accompanied by a rise in haemolymph calcium concentration (Waldron *et al.*, 1986). The changes in muscle lactate and haemolymph calcium levels in trawled prawns were consistent with this.

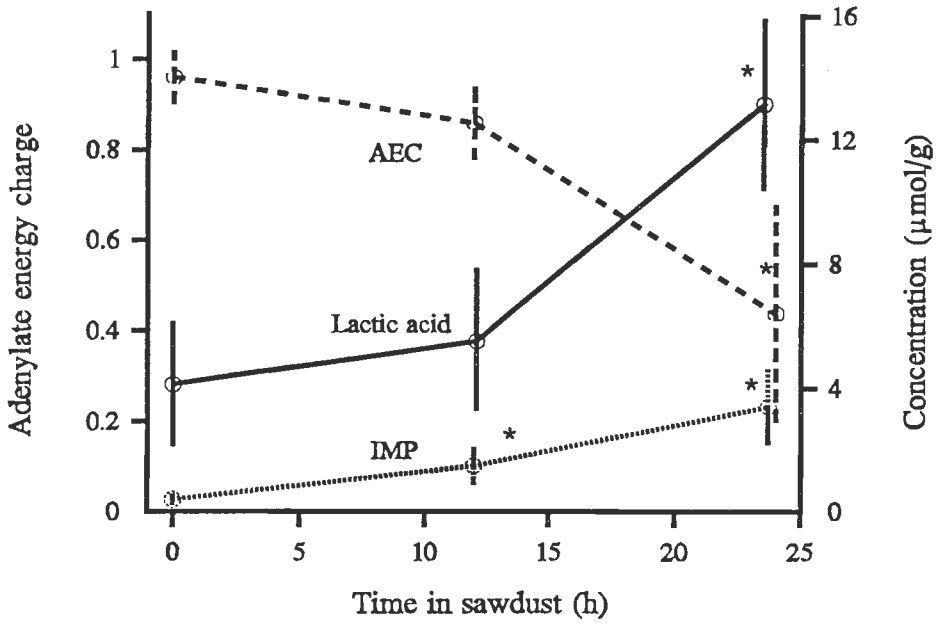


**Figure 2.** Effect of storing *P. esculentus* in sawdust at 12°C on mean energy charge (AEC), lactic acid and inosine monophosphate concentration (±SD, n=8) of abdominal muscle. Asterisk shows significant difference ($P<0.05$) from t=0.

"Escape swimming" or repeated tail-fliping occurs when *Nephrops norvegicus* is trawled (Newland *et al.*, 1992). However, Spicer *et al.*, (1990) found surprisingly low levels of lactate in the haemolymph of *Nephrops* immediately following trawling. In some crustaceans, such as *Crangon*, the primary anaerobic pathway used to fuel exercise is "phosphagen" (Onnen and Zebe, 1983). Fatigue occurs when the reserves of arginine phosphate reach a certain level and glycolysis is apparently rallied during recovery in order to restore the stores of ATP and arginine phosphate, (Gruschczyk and Kamp, 1990). However, another explanation for the absence of lactate from the haemolymph could be that it is retained in the tissue and only emerges into the haemolymph after the animal is brought on deck. For example, the concentration of lactate ions in the blood rises abruptly when the freshwater crayfish *Austropotamobius pallipes* is returned to water after storage in air, apparently because the ions are "washed" out of the tissues with the change in rate of blood circulation (Taylor and Wheatly, 1981). Interpreting the changes in haemolymph lactate concentration while trawled lobsters are stored on deck solely as an effect of emersion (eg. Spicer *et al.*, 1990) does not allow for these other possibilities.

Shrimps and crayfish normally fatigue at an adenylate energy charge (AEC) of between 0.5 and 0.7 (Onnen and Zebe, 1983; Gade, 1984; England and Baldwyn, 1983). As trawled prawns consume more and more ATP, the concentration of AMP rises. However, AMP is not the only purine nucleotide that accumulates in trawled prawns, some of this compound is converted into IMP. Normally, this does not happen when crustaceans exercise (Raffin and Thebault, 1987; Raffin *et al.*, 1988). IMP is a flavour enhancer in seafood (Komata, 1990) and the role of harvest stress in determining the IMP level of wild and cultured prawns is

considered in a companion study (Paterson *et al.*, 1993).

IMP is apparently formed when fatigued prawns continue to consume ATP at a rate faster than they can restore their energy charge. Energy metabolism may be inhibited by acidosis in the prawns, perhaps arising from a combination of exhausting exercise and disturbances in blood circulation and gill ventilation. The AEC continues to fall but the magnitude of that fall is lessened because deamination of AMP prevents high levels of AMP from building up in the cells.

According to the literature, the AEC values of trawled prawns are low enough to signify irreversible physiological collapse (Sylvestre and le Gal, 1987), but the prawns apparently do recover (below). The prawns appear to fatigue so quickly that regardless of the apparent health of the animal it is just as stressed or fatigued in terms of AEC, lactate and even IMP concentration whether it has been in the net for fifteen minutes or an hour. It is interesting that some of these fatigued prawns are still capable of showing tail-flips, but this could arise because the data presented here are averages for the whole abdomen- rather than measurements of discreet muscle blocks.

Prawns that didn't jump had a higher ATP concentration, (and a higher adenylate pool) than prawns that were jumping, no doubt because an inactive prawn has a lower metabolic rate. The adenylate pool was higher in the weaker prawns despite the same amount of IMP being present. This suggests that adenylates are no longer just being recycled. New adenylates may be arising from other pathological changes in the muscle (such as a breakdown in RNA synthesis).

Some of the prawns which are visibly paralysed upon capture did recover and begin swimming after a few minutes when placed in a tank of seawater, suggesting that this symptom was reversible. Similar behavioural and neurological problems apparently occur when live *Homarus gammarus* and *Panulirus argus* are stored and transported out of water (van der Meeren, 1991; Vermeer, 1987). Given that trawled live prawns are already used for a variety of purposes, such as tag and release studies, it is necessary to know how "real" this recovery is in terms of the normal physiological state of the animal.

With ATP concentration and the AEC as low as it is, it would be surprising if haemolymph electrolyte levels did not change. However, haemolymph calcium concentration was not higher in the prawns that did not jump on the sorting tray compared with the prawns that did jump. Whiteley and Taylor (1992) reported that unusually high levels of calcium and magnesium were seen in *Homarus gammarus* arriving at a wholesaler. In *P. esculentus*, magnesium levels may have risen during trawling but wide variance caused by a handful of extremely high values (that may result from injury and contamination of haemolymph with seawater) is obscuring the picture. Like many other crustaceans, prawns regulate the haemolymph magnesium level at a level considerably lower than that in seawater (Dall and Smith, 1981; McFarland and Lee, 1963).

Unexpectedly, it was haemolymph potassium that was significantly higher in the prawns that did not jump. Potassium is present in very large amounts in muscle tissue. The concentration in the haemolymph here is higher than that in sea-water, suggesting that either the potassium has left the cells or that the haemolymph volume has decreased. Potassium regulation in the haemolymph is necessary for sustaining nerve and muscle activity (Dall and Smith, 1981), and besides magnesium hyporegulation, potassium is one ion that is consistently regulated by many crustaceans living in a wide range of environments and habitats (Dall, 1984; Dall and Smith, 1981; McFarland and Lee, 1963; Moreira *et al.*, 1988).

High haemolymph potassium may arise in several ways. The AEC may be so low that the ion "pump" ($Na^+/K^+$ ATPase) is starved of ATP and cannot keep pace with the flow of $K^+$ out of the cells, or low pH in the cells has inactivated the enzyme (Kleber, 1990) or that some cells themselves have burst open, liberating their contents (such as ions and enzymes) into the haemolymph. The concentration of acid phosphatase, an enzyme normally found in cellular lysosomes, rises in the haemolymph when prawns are stressed (Dillon and Fisher, 1983)- and this syndrome may also explain the appearance of necrotic lesions inside the abdominal muscle of prawns after trawling (Rigdon and Baxter, 1970; Ross, 1976).

Recovery from trawling is not fast. Apparently it takes a couple of hours for the energy metabolism of the prawns to return to "normal", slower than recovery from "milder" stress such as exhausting exercise (Gade, 1984; Head and Baldwin, 1986). Recovery is associated with fluctuations in the total pool of adenylates ($\Sigma AxP$), something which normally does not happen when crustaceans exercise, (Gade, 1984; Onnen and Zebe, 1983). The mechanism behind the rise in the adenylate pool is unclear, but if the activity of AMP deaminase has increased in response to trawling, then perhaps the IMP itself is scavenged by the "purine nucleotide cycle" (i.e. Regnault, 1985), though further degradation and excretion would also be a potential fate for this compound.

Of course, recovery of the energy charge may not be a sensitive measure of internal damage. One way of testing the extent of this recovery from trawling is to test the physiology of the prawns in some way, to see if they show abnormal responses. Since this research was intended to examine the suitability of this species for live transportation in sawdust, an experiment was conducted to compare the metabolism of *P. esculentus* under these circumstances with that determined for *P. japonicus* and *P. monodon* in a previous study (Paterson, 1993).

*P. esculentus*, like *P. monodon* was unable to sustain the AEC when stored in sawdust at 12°C for 24h. Neither of these species are particularly suited for live transportation in comparison with the longevity of *P. japonicus* in sawdust. As reported previously for other prawns (Paterson, 1993), *P. esculentus* did not show significant anaerobic glycolysis in the abdominal muscle during the first 12h in air at this temperature, suggesting that other mechanisms such as arginine phosphate reserves are used to sustain the AEC. However, in contrast with the findings of the earlier study, the IMP concentration increased significantly while the AEC was still high. This is unusual for an invertebrate, (Raffin and Thebault, 1987). However, as alluded to above, the activity of the enzyme rises during trawling, causing large amounts of IMP to accumulate. The results of this study suggest that the enzyme remains at a higher than expected level of activity long after the prawns have apparently recovered from harvesting. If this phenomenon is a lingering symptom of trawl stress then many other aspects of the animal's metabolism may also be out of balance, a situation which could compromise the survival of the prawns during commercial handling.

To conclude, all *P. esculentus* are fatigued by trawling and any difference in the appearance and behaviour of prawns is probably a consequence of different degrees of fatigue, that is, the physiological consequences of, amongst other things, a very low AEC. Despite the pathological depths plumbed by these prawns during trawling, the survivors appear to recover surpisingly well, though the abnormal stress of trawling is reflected in fluctuations in the total adenylate pool during recovery, something not seen when crustaceans recover from exercise. However, the period of low AEC during trawling probably causes long term damage to prawns and this situation must be acknowledged if live prawns are harvested in this manner, for whatever reason.

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# LIVE TRANSPORT OF PRAWNS IN SAWDUST: A COMPARISON OF WILD AND CULTURED SPECIES

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SUMMARY

This paper considers the suitability of three species of tiger prawn for live transport using techniques already applied to the live export of the kuruma prawn, *Penaeus japonicus*. The survival of wild-caught brown tiger prawns, *Penaeus esculentus* was studied during simulated commercial shipment in sawdust at 12°C. The survival of trawled *P. esculentus* was compared with that of grooved tiger prawns *P. semisulcatus* caught at the same time. Since the stress of trawling may reduce survival, a study was also conducted using *P. esculentus* and black tiger prawns *P. monodon* from a prawn farm. This study was conducted to see if *P. esculentus* survived for a long enough period in air to allow live export to Japan.

Unfortunately, *P. esculentus* showed the poorest survival of the three species tested, with only 40 to 60% of prawns surviving 24h in sawdust at 12°C. *P. monodon* survived for the longest period in air, with about 90% survival at 24h. In part the poor survival of the *P. esculentus* may be due to trawl stress, but a study of farmed *P. esculentus* showed even less ability to survive in air, though these prawns may have been weakened by inadequate growout conditions. Measurements of haemolymph pH of both *P. esculentus* and *P. japonicus* during storage in sawdust at 12°C indicate that the former species can not regulate its haemolymph pH in air and also may not tolerate the low temperatures used to inactivate prawns prior to live transport.

A survival of 95% or more at the time of sale is necessary for live export. None of the tiger species studied here survives long enough to achieve premium prices. However, all three species survived well enough to be transported for short distances in a dry state. The ability of *P. monodon*, a popular aquaculture species, to survive in air for almost a day under these circumstances suggests that this technique may be a suitable way to transport prawn broodstock throughout Australia.

## INTRODUCTION

Recent live exports of farmed kuruma prawns (*Penaeus japonicus*) from Australia to Japan (Goodrick *et al.*, 1993a) have raised the question of whether these techniques are applicable to the ocean harvest. Kuruma prawns are commercially insignificant in the wild fishery of Australia, but the brown tiger prawn *P. esculentus* may find a niche in the Japanese live seafood market because of its striped colouration, similar to the red and white striped *P. japonicus*.

Though endemic to Australian waters, the brown tiger prawn is often caught alongside of a close relative, the grooved tiger prawn, *Penaeus semisulcatus*. This species closely resembles *P. esculentus* in appearance but it has a more cosmopolitan distribution throughout South East Asia, where it is known as the bear prawn or green tiger prawn. In Japan, there is a preference for *P. esculentus* rather than *P. semisulcatus* because of the

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better colouration of the former. The so-called "brown" tiger, *P. esculentus*, is more often maroon in colour than brown. Yet, in Australia, the two species are often lumped together as "tigers". Australian fishermen will have a strong incentive to recognise the difference if *P. esculentus* becomes more valuable through live marketing.

In Japan, a significant proportion of live *P. japonicus* already come from the wild fishery (Ovenden *et al.*, 1993), where gentle harvesting techniques are employed. However, prawns from the wild fishery in Australia are harvested by trawling. The live export of trawled prawns presents new problems. On a prawn farm, the product is handy to the processing facility, and can be harvested using methods that cause very little stress (Paterson and Goodrick, 1991). Trawling is a very stressful method of capture (Paterson, 1993c; Paterson *et al.*, 1993). An obvious way to find out whether this will cause problems for exporters is to study *P. esculentus* harvested from a prawn farm, where harvesting was potentially less stressful (Paterson *et al.*, 1993). *P. esculentus* are not commonly grown in farms but a crop of this species, as well as a crop of black tiger prawns *P. monodon*, was available for experiments at Cooktown, in North Queensland.

This paper considers the suitability of *P. esculentus* (brown tiger), *P. semisulcatus* (grooved tiger) and *P. monodon* (black tiger) for live transport using techniques already applied to the live export of *P. japonicus* and includes a comparison of farmed and wild-caught *P. esculentus*.

## METHODS

### Survival of trawled *P. esculentus* in sawdust

Prawns were harvested by 1 to 1.5 h trawls during several trips on trawlers in Moreton Bay, South East Queensland. Living prawns were sorted from the catch and stored on board in aerated tubs of seawater. These prawns were taken back to the laboratory in plastic bags of seawater and a headspace of pure oxygen; or in floating wooden mesh-based trays inside of an aerated transport tank (Goodrick *et al.*, 1993b).

The prawns were allowed to recover overnight in a recirculating seawater aquarium (17 to 20°C) and then placed in a small tank and cooled at a rate of 2 to 3°C/h until they fell over (between 11 to 12°C), by pumping the water in this tank through a plastic heat exchanger emersed in an ice slurry. The prawns were then taken from the water and packed along with cold dry sawdust (stored at -30°C) into cardboard boxes. These boxes were then placed in a coldroom at a temperature of 12°C and boxes were opened after 12 to 24h of storage.

### Haemolymph pH of *P. esculentus* and *P. japonicus* in sawdust at 12°C

The haemolymph pH was studied in *P. esculentus* obtained as previously, during trawl trips in Moreton Bay, and *P. japonicus* was obtained from a commercial supplier (Moreton Bay Prawn Farm Australiasia Pty Ltd) and then allowed to recover overnight in the aquarium before the experiment. Prawns were cooled as before and packed into carboard cartons for 9 h at 12°C. Haemolymph samples were taken from the pericardial sinus of 6-8 prawns at 0, 3 and 9 h. Samples were taken using an ice-cold hamilton syringe and injected directly into the pH electrode. Haemolymph pH was determined using a Radiometer BMS-3 and microcapillary pH electrode thermostated to the experimental temperature. Rapid handling of the haemolymph samples allowed the pH to be measured and the electrode washed with saline before the sample clotted.

### Comparison of trawled *P. esculentus* and *P. semisulcatus* in sawdust

This trial was conducted at the Northern Fisheries Research Centre, at Cairns in North Queensland. Wild *P. esculentus* and *P. semisulcatus* were collected on board a trawler using commercial prawn gear and 1-2h shots and stored on deck in the same way as described above.

The prawns were off-loaded at a dock at the laboratory and taken by truck to an aquarium room where they

were placed in an aerated tank of seawater. This tank was cooled as described above until the prawns fell over. The prawns were then packed in a commercial live prawn carton, identical to that used for the live export of *P. japonicus* (Goodrick *et al.*, 1993a). About 30 prawns were packed into each inner carton and then these were placed along with frozen coolant sticks into an insulated outer carton. The complete carton was placed in an airconditioned room and the temperature inside and outside of the carton recorded using a data logger and thermocouples.

Comparison of farmed *P. esculentus* and *P. monodon* in sawdust

This study was undertaken in conjunction with the study of wild prawns at Cairns. Farmed prawns were harvested from a farm near Cooktown in North Queensland. Cultured *P. esculentus* were harvested using a tunnel net. Prawns swimming in the pond followed the current created by a paddle wheel aerator and were guided between the V shaped wings of the net into a tunnel with a series of cones or funnels so that the prawns cannot swim back out. The prawns pass through this tunnel and are concentrated in a net basin or enclosure. *P. monodon* were harvested from an adjacent pond using a cast net.

The prawns were placed in chilled seawater (about 18°C) in stackable trays inside a transport tank, modified industrial insulated box (Xactics 309F) (Goodrick *et al.*, 1993b). This tank was mounted on a trailer and towed back to the processing area. During this time the tank was aerated using a 12v DC air pump.

A large proportion of the *P. esculentus* harvested at the farm could not be packed. These prawns showed areas of opaque muscle when taken from the tunnel net. This symptom has been noticed previously by the authors in poorly harvested *P. japonicus* and in trawl caught *P. esculentus* which showed very poor survival in sawdust. However, in this case the symptoms of stress may have been caused by dietary problems and the "aging" of the pond itself (the crop was about 9 months old).

One carton of prawns (5kg of each species) was packed at the farm, stored overnight in an air-conditioned room in Cooktown before returning to Cairns the following morning by plane. When temperature logging was commenced at the laboratory in Cairns, 16h after it was packed, the carton had an internal temperature of about 16°C.

Several kilograms of each species were also transported from Cooktown to Cairns in the transport tank. The 12v air pump in this case was connected to the battery of the truck. A valve in the lid stopped water loss from surging, while allowing air to escape from the tank. On arrival at Cairns, these prawns were cooled and packed in a live prawn cartons in the same tank used previously to the trawl caught prawns (above).

RESULTS

Survival of trawled *P. esculentus* in sawdust

Survival of *P. esculentus* packed in sawdust at 12°C for periods ranging from 12 to 24h ranged between 45 and 70%, during three experiments (Table 1). The surviving prawns were often very weak and moved only slightly when unpacked.

Haemolymph pH of prawns in air at 12°C

*P. esculentus* had a higher haemolymph pH than *P. japonicus* when cooled to 12°C, (Figure 1). Acidosis occured when *P. esculentus* were stored for 9 hours in sawdust, whereas haemolymph pH did not change significantly when *P. japonicus* were stored in sawdust for the same period.

**Table 1.** Survival rate of *P. esculentus* caught on three different occassions and packed in sawdust at 12°C.

| Experiment | Number of prawns | Time in sawdust(h) | Percent alive (%) |
|---|---|---|---|
| I | 17 | 18 | 70 |
| II | 14 | 12 | 64 |
| | 20 | 24 | 45 |
| III | 21 | 24 | 62 |

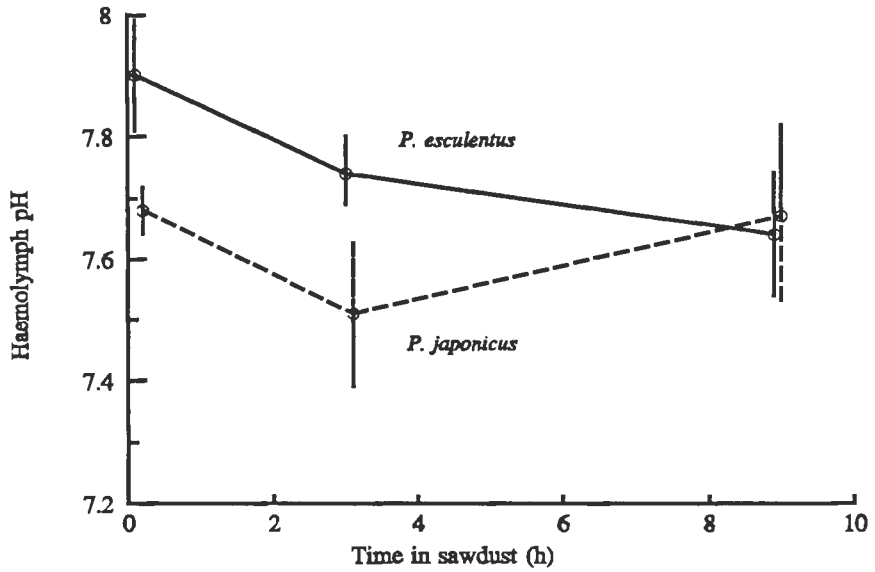


**Figure 1.** Effect of storage in sawdust at 12°C on the mean haemolymph pH (±SD, n=6 to 8) of *P. japonicus* and *P. esculentus*.

Comparison of trawled *P. esculentus* and *P. semisulcatus* in sawdust

A large proportion of the "tiger prawns" caught by the trawler were found to be grooved tiger prawns, *P. semisulcatus*, and only one *P. monodon* was caught (Table 2). Temperature measurements showed that the temperature within the carton was 12 to 13°C throughout the period of storage, while the room temperature fluctuated between 17 and 20°C. The *P. semisulcatus* showed only a slightly better survival (not significant at 5%) than the *P. esculentus* when the boxes were inspected at 24h.

**Table 2.** Survival rate of tiger prawns from the ocean and farm, stored in a commercial live prawn carton for 24h.

| Prawn | Number of prawns | Percent alive (%) |
|---|---|---|
| Trawled | | |
| *P. esculentus* | 25 | 56 |
| *P. semisulcatus* | 65 | 77 |
| Farmed | | |
| *P. esculentus* | 136 | 30 |
| *P. monodon* | 76 | 88 |

Comparison of farmed *P. esculentus* and *P. monodon* in sawdust

When the prawns were packed in sawdust at the prawn farm and flown to Cairns, at a storage temperature of 16°C, black tiger prawns proved to be the stronger of the two species, (Table 2).

When hauled from Cooktown to Cairns in a transport tank and then packed into sawdust, a marked difference was again found in the survival of the two species. The temperature in this carton remained at 10 to 11°C until the carton was opened and boxes removed to check survival after 12h storage. *P. monodon* showed 100% survival at 12h (n=30) and 76% survival after 29h (n=88). *P. esculentus* showed 75% survival after 12h (n=60) and only 8% survival after 29h (n=120). When the carton was closed again after 12h, the temperature had jumped up to about 14°C and increased steadily thereafter, to reach 18 to 19°C when the remaining boxes were opened. This perhaps explains the poor survival result at 29h.

## DISCUSSION

None of the tiger prawns studied here survived in air for as long as *P. japonicus* does. Indeed, of the three species studied, *P. esculentus* is apparently the least tolerant of storage in sawdust. This species shares with *P. japonicus* the habit of burying in the sea bed and reducing its metabolic rate by day, yet this life habit apparently does nothing to pre-adapt it to storage out of water. Other factors must be at work to govern the longevity of prawn species in air.

*P. japonicus* is blatantly a "king" prawn in everything but its stripes. It is the stripes that make it attractive but presumably it is its physiology that makes live transport possible. Our preliminary trials with eastern king prawns, *P. plebejus*, harvested as by-catch alongside *P. japonicus* at a farm suggest that this species also has what it takes to be shipped out of water, (Goodrick, Paterson and Grauf, unpublished data). Unfortunately this prawn is not as attractive as *P. japonicus* and is therefore no substitute for it.

Normally, when a prawn is taken out of the water it jumps about violently, injuring itself both physically and physiologically. *P. japonicus* is less likely to do this, even before you cool it down. So, right from the start it is clear that there is something unusual about the nervous system of *P. japonicus* and the prawn's metabolic response to being in air. If given the chance, this prawn will actually crawl around on a laboratory bench!

Prior to live transport, *P. japonicus* is processed by cooling the prawns in water to a point that they become paralysed with cold and fall over (Paterson, 1993b), usually at a temperature of between 10 and 14°C, (Shigeno, 1979). *P. japonicus* is remarkably tolerant of this treatment and can be cooled very quickly, (Goodrick *et al.*, in preparation). However, when *P. esculentus* are cooled to the point that they fall over and then packed in sawdust, they appear to be more paralysed than *P. japonicus* is under these cirumstances. The prawns become very lethargic and are unable to maintain muscle tension at the junction of the head and abdomen. These symptoms are not normally seen in *P. japonicus* unless stored at temperatures as low as 6°C, (Goodrick and Paterson, unpublished data). These observations suggest that *P. esculentus* does not tolerate the low temperatures required to process them before packing.

Physiological studies show that when penaeid prawns are held in air at 12°C, lactic acid does not begin to accumulate immediately in their abdominal muscle (Paterson 1993a & c). Yet, nevertheless, the haemolymph pH of *P. esculentus* continues to decrease during this time. This may arise from carbon dioxide accumulating in the blood (Taylor and Whiteley, 1989), or from lactic acid originating from other tissues. In contrast, the haemolymph pH of *P. japonicus* shows no significant change when stored in air under the same conditions. Apparently, the *P. japonicus* is better able than *P. esculentus* to regulate its blood pH when stored in air: a significant advantage in terms of live handling, (Vermeer, 1987; Whiteley and Taylor, 1990). The haemolymph pH data also corroborates with the idea that the *P. esculentus* studied here were too cold, since they started with a haemolymph pH at 12°C higher than that of *P. japonicus* at that temperature. Haemolymph pH normally rises as the temperature falls, (Truchot, 1983) and the pH of *P. japonicus* in this experiment (7.51±0.12 at t=0h) seems to be too low for inactive animals at this temperature. This suggests that the *P. japonicus* have a higher metabolic rate at 12°C than the *P. esculentus*- that is, they acclimate to the cold better than the latter species. These possibilities must be examined in future studies.

At present, trawling is the only method available to collect wild prawns for live transport, and this method of capture does involve an extraordinary amount of stress (Paterson, 1993c). Consequently, the survival of *P. esculentus* may be reduced by trawl stress. Many of the prawns show opaque patches or lesions in the tail muscle of varying size. We didn't catch enough prawns in this study to allow us to remove all prawns with minor lesions- we only discarded the prawns that were obviously injured and weak. Packing prawns that are already weakened by trawl stress is a problem, however, it is also worth pointing out that removing the weakest prawns is not likely to make the strongest ones live longer.

An obvious way to find out whether harvest stress is partly responsible for the poor survival is to study brown tiger prawns harvested from a prawn farm, where harvesting is less stressful, (Paterson *et al.*, 1993). Farmed *P. monodon* survived relatively well in air, confirming our previous observations (Goodrick and Paterson, 1992). Yet, even when harvested using a gentler method, cultured *P. esculentus* did not survive in sawdust for as long as the *P. monodon* caught at the same farm did, though this result was confounded by evidence that the *P. esculentus* at the farm were in poor condition. The better survival obtained from the trawled sample of *P. esculentus* as against the cultured one ($P<0.05$) cannot be generalised beyond the particular samples used here. This species is not a popular species for aquaculture in Australia, and its requirements for grow-out are not well known. Further studies of cultured *P. esculentus* are required to establish a "base-line" of survival in sawdust against which to compare the performance of trawled prawns.

These results emphasise the wide differences in stress tolerance of various penaeid prawns- differences that preadapt at least one species to live transport in sawdust but which appear to stop other species from lasting the distance. In order to transport these weaker species in the live state a way must be found to reduce the stress of capture for wild prawns as well as to find other ways of cooling and packaging them- to alleviate the fact that they have difficulty sustaining their metabolism when stored out of water, (eg. Paterson, 1993a and c).

For the purposes of live export, where prices are largely determined by quality and hence survival rate, none of the species studied here survives long enough in sawdust to reach Japan in good condition, though *P. monodon* comes close. Nevertheless, all three species survive well enough to be transported for short distances in a dry state. Prawn broodstock are normally transported in oxygenated containers of seawater, (Robertson *et al.*, 1987). Since domestic airlines insist on the use of "dry" packaging of seafood to prevent

corrosion damage to aircraft, this dry technique may prove suitable for shipping *P. monodon* broodstock to hatcheries.

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