Live Transport of Crustaceans in Air-

Prolonging the Survival of Crabs

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Summary

We studied the live transport of crustaceans in air, using the spanner crab *Ranina ranina* as an example, and developed guidelines for handling live spanner crabs which we presented to an industry workshop. Our findings were also of general relevance to the live shipment of other oceanic crab species.

The spanner crab fishery has burgeoned over the last couple of years through interest in the live market. The choice of spanner crabs as a topic of study was therefore timely. The handling practices used on boats in this fishery, (storing crabs out of water), were appropriate for handling "live" crabs destined for cooking but we found that more careful handling was required for the live export market.

While spanner crabs appeared to tolerate being stored in air, our studies showed that this tolerance was misleading. The crabs stressed quickly when they arrived on deck and became quiescent. Their blood pH fell rapidly, a symptom called acidosis. Quiescence was their only means of dealing with acidosis.

In practical terms, any time that a spanner crab was out of water was too long. Spanner crabs are stored in air twice after harvest, first while on the boat and again when actually exported. The conditions experienced by the crabs stored in air at ambient temperature on boats were much worse than those of crabs cooled for export. At the very least, the crabs should be cooled down or sprayed with cold seawater when stored in air on boats. The best way to store crabs on boats would be submerged in live wells- but there are problems with this because the unrestrained crabs can injure each other.

We also tested other methods of alleviating stress. Spanner crabs cannot buffer low pH in the blood, unlike many other commercially harvested crustaceans. We sought to correct this using a dip treatment. However, this did not improve their survival. For an animal that cannot correct acidosis, spanner crabs survive for an extraordinary period in air. The stressed crabs may linger on because they "shut down" and keep the acidosis from reaching fatal levels.

Using the results of our research, we presented guidelines for handling spanner crabs at an industry workshop on December 9-10th 1993. This workshop attracted favourable comment from the industry and copies of several papers were published in the May edition of *Queensland Fisherman*.

We concluded that the way the crabs are currently being handled on boats is too stressful. Physiological studies show that spanner crabs, like other oceanic crabs, are not well equipped to survive in air. Yet even if you store them cold on the boat they still die after a few days in tanks on shore. This mortality is an impediment to the maturity of the industry. The crabs may be succumbing to bacterial infections caused by injury and we recommend that their claws are immobilised by banding after capture.

Background

It is well recognised that there are considerable opportunities for the niche marketing of live crustaceans in Asian countries, particularly in Taiwan and Japan. Crucial to the profitability of these exports is the requirement that most of the animals reach the market alive; air-freight is an expensive way to ship "dead" weight. Although techniques for live transport of crustaceans have been developed along general principles and "rule of thumb", success is not guaranteed by this approach. Specific knowledge of the strengths and weaknesses of individual species is required to underpin these practises and provide a basis for confidence in their success.

The spanner crab *Ranina ranina* is an unusual marine crab that spends much of the time buried in the sea floor. It has a wide, if patchy distribution in the Indian Ocean, through the archipelagos of South East Asia into the western Pacific, and on to Japan and Hawaii. It supports minor fisheries in a number of countries.

In Australia, the fishery is a relatively recent phenomenon. There was a time when spanner crabs were rubbish, by-catch! Serious fishing for spanner crabs begin in the late 1970's as an adjunct to the sand and mud crab fisheries (*Portunus pelagicus* and *Scylla serrata*) using gear that was developed from that used by recreational fishermen. In the years that followed, refinements were made to the "dillies" or tangle nets used to harvest the crabs so that the crabs were easier to disentangle from the traps and were therefore less likely to be injured (Sumpton, W. 1993. How to minimise net damage to spanner crabs. *The Queensland Fisherman* April 1993 23-25).

The spanner crab fishery is based largely in Queensland and northern New South Wales. To begin with, the annual catch of spanner crabs remained at around a few hundred tonnes, all of which was destined for the cooker. At the time that this research was proposed, it was worth in the order of one million dollars, based on an annual catch of about 500 tonnes for which the fisherman gets $1-2 per kilogram.

Interest in exporting live spanner crabs to Taiwan reached a head in 1991 with exports by some commercial operators as well as pilot exports by the applicants (Queensland Department of Primary Industries). Prices for live spanner crabs in Taiwan and Japan were in the order of $10-25 per kilogram. The results obtained were promising provided that transit temperature was controlled within certain limits and damage from rough handling was prevented. Nevertheless, problems with poor survival still occurred.

The fishery has not looked back since then. During the course of this study it has burgeoned from relative obscurity, leap frogging over the other crabs to become the most valuable live crab fishery in Australia. The beach price for the crabs has doubled (to about $4.00 per kg, about half of the value of mud crabs) while there has been a five-fold increase in the annual catch, which now exceeds 2000 tonnes. The fishery is now estimated to be worth 11 million dollars.

This rapid growth has raised concerns both about the management of the fishery and its ability to consistently produce good quality live crabs. An extension was sought to specifically address these concerns about treatment of the crabs on boats, and to run an industry workshop. The rationale for this was that handling methods that arose when the fishery was producing a cooked product were not necessarily those best suited for storing and transporting crabs for live export.

The workshop also helped to galvanise moves within the spanner crab fishery for a reassessment of the management regime applied of the fishery because of concerns about the rate at which it was expanding. Live spanner crabs are now a very valuable resource and keeping that resource going is high on everyone's agenda. The Queensland Fish Management Authority (QFMA) and Queensland Commercial Fishermans Organisation (QCFO) have made major progress in this area during 1994.

Need

When export of live crabs was first established, survival of spanner crabs exported to Taiwan was not reliably close to 100%. The handling problems encountered during these early exports prompted a reappraisal of existing methods of live transport. Later, during the project itself, it became clear from our own results and discussions with fishermen that the post-harvest handling of the crabs was probably the major cause of the problems. The need for work in this area was so pressing that the project was extended and its aims expanded to cover this. As part of this change in emphasis, a workshop was held on harvesting and post-harvest handling of live spanner crabs.

At its conception, the project was primarily concerned with improving the survival of crabs by developing new ways of transporting them. Existing live transport methods relied to a certain extent upon the physiological characteristics of a given species and also by avoiding temperature extremes during transport. The development of a new export industry for spanner crabs hinged upon the fact that not all crustaceans show the same tolerance of handling stress. Furthermore, transit temperature cannot always be reliably controlled, particularly during airport stopovers. This project sought to improve the tolerance of live handling and transport shown by the spanner crab by intervening in the physiological problems that arise when crustaceans are handled and exposed to higher than optimal temperatures during transport out of water. It was expected that this work applied both to the immediate post-harvest handling of the crabs at sea as well as to their subsequent transport and distribution.

The expansion of this project was necessary in order to keep pace with the rapid development of the live spanner crab industry. The initial success of the first exporters, including exports by IFIQ to Japan and work by IFIQ staff for one of these companies, Poulos Bros, has paved the way for an increase in interest in the harvesting and export of live spanner crabs. The most tangible benefit of this was for the fishermen, raising the potential value of the catch. IFIQ continued to provide advice to new entrants to this industry. Discussions with processors and fishermen showed that the rapid increase in exports was putting pressure on the price, making it crucial to cut costs in Australia.

An important cost is crabs that die in tanks before they can be packed and exported. The survival of crabs arriving from crabbers differs. This is in part a consequence of the rapid expansion in the live trade and the entry of fishermen into the industry who have not previously harvested this species and are unfamiliar with its peculiarities. Yet, part of the mortality is due to injury. This can be controlled by emphasising that care must be taken when removing crabs from the nets, information which is already available following research on the harvesting of spanner crabs conducted by QDPI Fisheries Branch and NSW Fisheries (FIRTA 90/5). However, even if crabs are uninjured, reducing post-harvest mortality is complicated by the range of techniques currently used by fisherman to hold spanner crabs on their boats. A price premium itself is no incentive if fisherman have no information about what equipment and how much extra effort is required to reduce this mortality.

Objectives (a change from the original application is underlined)

- To increase knowledge of the techniques required for successful live transport of crabs destined for export or domestic markets.

- To put this knowledge to commercial practise in developing guidelines and protocols.

Sub-objectives:

(a) To investigate changes in ion concentrations and acid-balance of the blood of the spanner crab *Ranina ranina* in air.

(b) Study the physiological role that water in the branchial chamber plays during storage of *Ranina ranina* in air.

(c) To use the knowledge gained from these studies to prolong the survival of spanner crabs held on boats and during trial exports.

(d) To apply the chosen techniques to live transport of other commercially important species.

All objectives were achieved. The second objective, "To put this knowledge to commercial practise ..." began with the industry workshop and is ongoing (see Further Developments).
Sub-objectives (a) and (b) were met. Sub-objective (c) was only met in part. The technique that we developed to prolong the survival of crabs during trial exports, buffering their blood pH, was not successful so these trial exports were not conducted. Sub-objective (d) was also contingent on the success of the buffer treatment.

Methods

In the original proposal, this project sought a method that would reduce the acidosis experienced by aquatic crabs when they are first lifted from the water. However, during the course of the study, on-board handling practices assumed importance as an area of study, so an extension was applied for which considered this.

The plan of operation of the original application was divided into two sections. The first section involved testing the effects of a preliminary buffer formulation on survival of spanner crabs during controlled trials and during commercial shipments by cooperating agents. Work on the second part of the project, the physiological studies, was conducted in parallel to the first part and was used to assess the mechanism underlying the technique. It was anticipated that any modifications arising from the results of the physiological studies could be applied to the survival and export trials.

When the importance of handling crabs on boats was recognised a third section was added in the application for the extension to this project. This section related to workshops/seminars and research work on live crab boats and at processors.

Effect of buffer formulations on survival during transport.

Crabs were treated as if for ordinary commercial shipments but also dipped either into a buffered or into a "blank" solution (i.e. sea-water) prior to packing them. The treated crabs were then stored in controlled conditions. The ability of this buffer formulation to prolong survival was intended to be trialled on other selected species of live seafood, (eg. prawns, crayfish etc.) as they became available, but this work was conditional on the buffer actually prolonging the survival of crabs.

Changes in acid-base balance and ionic regulation in *Ranina ranina* during emersion.

The blood chemistry of crabs in air with and without buffer treatment was studied. Blood gas and acid-base status was monitored using recognised techniques based on the Radiometer Blood Microsystem. Measurements of the constituents of branchial fluid were not made when it became clear that spanner crabs could not buffer their blood pH naturally. Blood lactate concentration was measured using diagnostic kits and UV-visible spectrophotometry. The ionic composition of the blood was measured using ICP-Mass spectroscopy and a chloride titrator. Changes in the gas composition of the air around the crabs due to respiration were intended to be monitored using a gas chromatograph, however these observations were not conducted. Instead, a more detailed study of anaerobic metabolism (that causing lactate to accumulate) was undertaken because results indicated that spanner crabs were not as robust as the industry believed.

Studies on boats and preparation of guidelines for discussion at an industry workshop

1. Changes in blood pH, ionic regulation and lactate concentration in *Ranina ranina* during storage on boats and recovery in holding tanks.

There were two experiments. In the first, blood samples were taken from crabs handled in different ways, upon arrival at processors on shore. The samples were intended to be taken back to the lab and the pH of each measured. But as it turned out, this work was conducted at Bundaberg (Central Queensland), too far from the laboratory for any pH measurements of transported blood samples to be reliable. This itself was another reflection of changes within the fishery. It was originally expected that most of the work could be conducted at Mooloolaba, however Bundaberg proved to be a more practical base of operations for some experiments.

In addition to what we proposed to do, we also recorded the subsequent survival of crabs from the different treatments during storage in a tank at Bundaberg, and conducted preliminary observations of crabs experiencing road transport. This additional work arose as a result of recommendations from the workshop.

The blood sample was split and part assayed for lactate using diagnostic kits and part assayed for various ions (particularly calcium). Ammonia measurements were not conducted. The information from the lactate assay was sufficient to show that spanner crabs experience an extraordinary amount of physiological stress during handling.

The second experiment was conducted on a boat by periodically sampling blood of crabs from the point of harvest, during storage on the boat and then during recovery at the processors. The same range of assays (with the exception of blood pH, because of the delay involved between sampling and measurement) was conducted on these samples.

2. Advisory workshops

Preliminary meetings were held in January and February of 1993 to discuss the issue and convey existing knowledge to fishermen as well as to describe the experiments intended to compare different on-board holding methods.

Following this initial consultation, a more formal workshop was held in Brisbane to discuss the on-board handling guidelines, the results of the previous handling studies, as well as to reinforce other harvesting and management issues, where appropriate. A booklet of guidelines, developed over the preceding months, was given to participants and also formed the basis of a series of papers that appeared in *Queensland Fisherman*. The workshop proceedings is included in Part 4 of this report.

Recommendations arising from the Brisbane workshop were addressed over the closing months of the project.

Detailed Results

The detailed results of the project are presented, appropriate statistical tests applied, and discussed in the draft publications in the general circulation part of this report (Part 2).

These include:

- an extended summary of the project suitable for inclusion in *Australian Fisheries*
- a study of crabs in different storage systems on boats
- a study of what happens to crabs when they are stored out of water, and why they don't die as fast as they might
- a preliminary study of crabs stored in seawater sprays that showed some very interesting results
- an analysis of why it is not a good idea to store spanner crabs in pure oxygen atmospheres

A confidential report is also attached (Part 3) that discusses the results of the buffer experiments (see also Intellectual Property).

Benefits

The results of this research give the live spanner crab industry a sound basis with which to standardise its practises and minimise stress and damage and thereby reduce mortality rates.

The explosion of interest in live transport of spanner crabs means that the flow of benefits presented in both the original application and the extension were rendered obsolete by the flow of events. A revised flow of benefits is presented here.

QLD	55%
NSW	30%
SA	5%
Tas	5%
WA	5%

The spanner crab fishery occurs in Queensland and NSW, and the findings of this work have and will continue to be disseminated to all concerned with this industry. Liaison will continue between us and NSW Fisheries to ensure that fisherman and processors are informed. Enquiries will also be encouraged from NSW crab processors wishing to export live spanner crabs. A larger flow of benefits is given to Queensland, reflecting the distribution of the resource and because the live export industry has a head start in this

state.

Originally, it was anticipated that this work would apply to all live crab fisheries, but feedback from local processors suggested that live transport of live blue swimmer or blue manna crabs (*Portunus pelagicus*), was not commercially attractive at that time. Since the conclusion of the project, a study of live transport of *Portunus pelagicus* commenced in Western Australia with support from the National Seafood Centre and Dr Paterson was invited to visit the applicants to familiarise them with the Queensland work. This contact will continue.

South Australia is included because these results will be of use to the small fishery for the sand crab *Ovalipes australiensis*, and possibly in the future for transport of *Portunus pelagicus*.

Intellectual Property

The intellectual property arising from this research is a method that buffers blood pH in crabs while they are stored out of water. This method is described in the confidential section of this report (Part 3).

It did not succeed in making spanner crabs live any longer out of water, but that may be because of the way that spanner crabs respond to storage in air. This method may be of use in prolonging the survival of certain other species, if the need and opportunity arises. Also, buffering the blood pH may have unforeseen benefits in terms of texture. Subsequent work, during the development of a Code of Practice, may show this.

Further Development

The recommendations of this work are that...

- Spanner crabs should be stored on boats or transported by road in cool, moist conditions (Temperature 16-20°C, 100% Relative Humidity)

- A practical way should be found to restrain spanner crabs after capture to reduce physical injury when crabs are crowded together- in particular by banding the claws

- A study should be conducted on the physiological effects of seawater sprays when storing crustaceans for long periods because of the questions raised in this study.

It was not possible to cover all aspects recommended by the workshop so an application was put to the Queensland Fishing Industry Research Advisory Committee (QFIRAC) to examine spray storage systems, particularly the fact that some mud crab storage systems may not function to their full potential (see the preliminary study of crabs in sprays in part 2 of this report). Just because the crabs "survive" does not mean their condition

cannot be improved.

QFIRAC preferred that a proposal should be submitted to the National Seafood Centre to establish a code of practise for the spanner crabs fishery. However, DPI is already facilitating an application by the Queensland Commercial Fisherman's Organisation (QCFO), Australian Prawn Farmer's Association (APFA) and Seafood Marketers Association of Queensland (SMAQ) to the Commonwealth Department of Industry Science and Technology for a program entitled "Quality Assurance in the Queensland Seafood Industry." Spanner crabs are one of six industry sectors covered by this application. A major objective of this exercise will be to build on the experience of participants in this growing industry and the results reported here to develop a Code of Best Practice for the spanner crab fishery.

It is anticipated that industry liaison during the development of the Code will re-iterate the gaps in our present knowledge and provide support for further research of a more specific nature at some point in the future.

Staff

Brian Paterson, Physiologist, (Project Leader, 80%)
Bruce Goodrick, Senior Food Technologist (10%)
Stephen Grauf, Temporary Technician (100%) (Funded by the project)
Ross Smith, Technician (20%)
Paul Exley, Temporary Technician (100%, replacing Mr Grauf) (Funded by the project)

Final Cost

The FRDC Statement of Receipts and Expenditure has already been forwarded.

Distribution

Complete report

FRDC 10 copies (1 copy unbound, plus disc copy, Word Perfect 5.1)

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National Fishing Industry Training Council
CSIRO Division of Fisheries Library, Hobart
CSIRO Division of Fisheries Library, Cleveland

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Dr Ian Brown, DPIQ
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Professor Nigel Forteath, University of Tasmania, Launceston

South Australia

Library, Aquatic Sciences, South Australian Research and Development Institute.

Western Australia

Library, Bernard Bowen Laboratory, WA Fisheries, Waterman.
Dr Richard Stevens, Western Australian Fishing Industry Council
Mr Patrick Spanoghe, Curtin University of Technology

PART 2

COLLECTED PAPERS

COLLECTED PAPERS

Project review

Supporting papers

B. Paterson, P. Exley and R. Smith (Editors). *Live transport of crustaceans in air-prolonging the survival of crabs*. Report to the Fisheries Research and Development Corporation (Project 92/71), Department of Primary Industries, Queensland, December 1994. pp 1-5.

OUT OF THE FRYING PAN INTO THE FLYER: HARVESTING, STORAGE AND TRANSPORT OF LIVE SPANNER CRABS

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Introduction

The spanner, red frog or Kona crab *Ranina ranina* is an unusual marine crab that spends much of the time buried in the sea floor. It has a wide, if patchy distribution in the Indian Ocean, through the archipelagos of South East Asia into the western Pacific, and on to Japan and Hawaii. It supports minor fisheries in a number of countries.

In Australia, the fishery is a relatively recent phenomenon. There was a time when spanner crabs were regarded as rubbish, by-catch! Serious fishing for spanner crabs begin in the late 1970's as an adjunct to the sand and mud crab fisheries (*Portunus pelagicus* and *Scylla serrata*) using gear that was developed from that used by recreational fishermen. In the years that followed, refinements were made to the "dillies" or tangle nets used to harvest the crabs so that the crabs were easier to disentangle from the traps and were therefore less likely to be injured (Sumpton, 1993).

At first the annual catch of spanner crabs remained at around a few hundred tonnes, all of which was destined for the cooker. Interest in exporting live spanner crabs to Taiwan reached a head in 1991 with exports by some commercial operators as well as pilot exports by the Queensland Department of Primary Industries. The fishery has not looked back since then. In just a couple of years it has burgeoned from relative obscurity, leap frogging over the other crabs to become the most valuable live crab fishery in Australia. The beach price for the crabs has doubled to about half of the value of a mud crab, while there has been more than a five-fold increase in the annual catch, which has now exceeded 2000 tonnes and it has not stopped yet.

This rapid growth has raised concerns both about the management of the fishery and its ability to consistently produce good quality live crabs. Handling methods that arose when the fishery was producing a cooked product are not necessarily those best suited for storing and transporting crabs for live export.

Harvesting for live export

Most crustaceans destined for live transport, such as lobsters or crabs are usually trapped or potted rather than trawled indiscriminately. Pots and traps cause little or no damage to the product. However, the tangle nets used to catch spanner crabs are an exception to this general rule. These nets can snare the crabs so well that it is a laborious process to

remove them without injury. Membranes between segments of the legs are easily torn and the crabs are in danger of bleeding to death. This problem has been recognised for some time, since crabs below a certain carapace length (100 mm) must be returned to the sea (Kennelly *et al.* 1990) and some attention has been paid to re-designing the nets, reducing the "drop" (tightening the net on the frame) and finding an optimal mesh-size to avoid injuries (Sumpton 1993). These modifications have become even more imperative now that most of the catch is marketed alive.

When taken from the water, the crabs scramble about for a few moments and then become inactive. Spanner crabs are not aggressive, and fishermen don't tie them up or restrain the claws; a practise that is mandatory when handling mud crabs. The unrestrained crabs are usually stored on deck in plastic baskets. This practise arose when the crabs were returned to shore alive and has continued because there does not seem to be a problem.

The crabs survive on deck for long periods and do not appear to be under much stress. However, this is not the whole story. Spanner crabs are typically packed and exported within 12 hours of being landed, without the period of recovery or "purging" that is common when shipping other live products. The exporters avoid storing the crabs because the crabs weaken and die after 2 to 4 days, apparently because of bacterial infections. As disease resistance in crustaceans is possibly influenced by injury and stress, it is possible that the period that they spend out of water on the boat is causing this mortality.

Storage and transport at sea

Our experiments show that any time that a spanner crab spends out of water is really too long. The amazing thing is that they are not killed by the stress they experience on the boats. Amongst live crustaceans of commercial importance, spanner crabs are apparently equipped the least to tolerate storage out of water, and it is no wonder that they become very inactive in air. They are suffocating.

The minimal recommendations first given for storing spanner crabs in air (Paterson, 1994) were developed from knowledge of other crab fisheries. Undamaged crabs can be held for several hours in air without dying, as long as they are held in a moist, shaded or dark, cool place (eg. under a wet hessian bag) they should not be exposed to direct sunlight or allowed to warm up. The crabs should not be disturbed or handled unnecessarily and it is important to avoid shocks such as dropping or knocking the container holding the crabs.

The apparent robustness of this crab when stored out of the water is very deceptive. Spanner crabs accumulate large amounts of metabolic wastes very soon after leaving the water, and it appears to be these wastes that are responsible for the crabs becoming so inactive. By shutting down in this way, the crabs are able to linger on for a surprisingly long time. They reduce any further accumulation of these wastes, which if allowed to proceed unchecked would have killed the crab in a few hours.

The obvious way to avoid stressing the crabs is to store them underwater. The spanner crab boats in Hawaii use "live wells" to hold the catch (Ian Brown personal communication). However, this practice requires careful thought. We tried storing spanner crabs in an aerated live-well and got very poor results. The crabs were probably too active because of the warm temperature of the surface water, causing them to fight too much!

If a wet well is not used, there are still ways of reducing the rate at which harmful metabolic wastes accumulate in the crabs. Cooling the crabs down, even from the moment they arrive on the boat, will have a major impact on the stress of storing them out of the water. The deck temperature might be as much as ten degrees hotter than the ocean the crabs live in. Keeping the crabs comparatively cold, (16-20°C) reduces their need for oxygen and may even allow them, in their quiescent state, to subsist for a time on the limited amount of oxygen that they can get out of the atmosphere. Rather than just keeping the crabs in a cold room, spraying cold seawater over the crabs reduces even further the rate at which wastes accumulate.

Using low temperature and water sprays to reduce the stress experienced by crabs in air may only be possible on large vessels. Small speedboats that operate close to shore may continue to store crabs out of water with minimal intervention to alleviate stress. Perhaps by using just a deck spray or keeping the crabs relatively cool in insulated boxes. However, as the fishery has developed, landings are being made at ports north of Brisbane, where the boats tend to be larger in size and more suitable for conversion. Still, the northern expansion of the fishery brings with it another issue, that of road transport.

Road transport

The stress that the crabs experience on the boats is only the beginning. Many boats do not unload at the export premises so a period of road transport of varying duration and distance is necessary. This is particularly true for boats currently operating from Round Hill Head, whose crabs must be trucked to Bundaberg over a bumpy unsealed road (124 km). Furthermore, it is not unknown for crabs to be driven all the way from Bundaberg to Brisbane (368 km).

Currently, the crabs transported in this way were already stressed on the boat so it is not known what complications are introduced by road transport. We drove a couple of cases of crabs from Bundaberg to Round Hill Head and back again. This was a "worst case" attempt to see if handling crabs in this way was able to stress and kill them. The shock and vibration of the road journey does not seem to have a discernible effect on the further accumulation of metabolic wastes in the crab and they all survived when unloaded at Bundaberg. However, the crabs appeared to "drown" when submerged, becoming very weak and lethargic. This phenomenon also occurs in mud crabs. In that species, the crabs having difficulty breathing when returned to seawater after more than 3 days in air (Varley and Greenaway 1992). Until a more detailed study can be conducted, we recommend that spanner crabs are transported by road in enclosed vehicles at low temperature (16-20°C) and high humidity.

Live holding prior to export

In comparison with exporters of live rock lobsters, spanner crab exporters seem to be in an extraordinary hurry. They often export their crabs within 12 hours of receiving them. This is not so fast that it does not allow the crabs to fully recover from the physiological stress of storage on the boats (it may only take a couple of hours to do that), however it means that the crabs (which have fed on baited traps) are not left in a tank for several days to defecate and clear their guts of food and wastes prior to export. The exporters would certainly like to hold their crabs for longer. This would allow them to play the market more successfully, to level out the "boom, bust" pattern that factors such as weather impose on the fishery. However, keeping spanner crabs for long periods in storage tanks has proved to be a major hurdle. They tend to die.

In the early years, one of the explanations for this was inexperience, for example inadequate biological filtration and aeration of the water in the storage tank. The conditions within the storage tank must be such that the crabs are allowed to recover and are not stressed any more. As a general rule, the oxygen tension in the tank should exceed 70% of saturation at all times. This is relatively easy to achieve in practice, using blowers or vane pumps, so we do not anticipate that oxygen level will ever be critical in a correctly designed system.

As spanner crabs are marine animals, care must be taken that the salinity in the tanks does not deviate too far from that expected under oceanic conditions (about 35ppt). This is particularly important when the holding tank must be filled from an estuary or creek, where the salinity can fall very low after rain. To give you an idea of the crab's tolerance of salinity change, we were able to successfully keep spanner crabs for a day or so at a salinity of 26 ppt by slowly reducing the salinity from 32 ppt over the course of a day. When we tried to take some crabs down further, to 19 ppt, half of them died!

Crabs still die in storage tanks even though these problems with water quality have been solved. If spanner crabs are left overnight after capture, weak or dead crabs (eg. crabs whose claws hang limply when they are lifted from the water) must often be removed from the catch the following day. And the next day there are some more dead crabs to be removed and so on. The mortality can certainly be influenced by the way the crabs are handled on the boat, however, regardless of the method used to store them on the boat, a large percentage of the catch still succumbs within 5 days of capture. Keeping the crabs cold on the boat delayed the onset of mortality by a few days.

This delayed mortality is characteristic of syndromes referred to as "vibriosis" (a bacterial infection) in other live crustacean industries. Simply, the crabs get sick. For example, Morrissy et al (1993) mentioned that yabbies (*Cherax destructor*) die within two to three days of arrival from a "systematic bacterial infection" if they are transported with "dirty" water (i.e. containing a high bacterial load) still trapped around the gills. The bacteria need not come from outside of the animal. Bacteria such as *Vibrio* can apparently always be found in crustacean blood, and these chronic infections typically flare up during handling and transport into outright septicemia (Sizemore 1985, Ellender et al 1992).

Physical injury during handling is one way that these infections can occur. When you try to pick a crab out of a basket, you very often get that crab and several others attached! This cannot be good. When the crabs are crowded, they will injure each other regardless of whether or not they are underwater. Cooling the crabs down may have the benefit of making them less active in storage and less likely to injure each other, and thus promote fewer opportunities for bacterial infections to take hold. Further studies of crab's dying in tanks could be undertaken to see if binding the claws has a significant effect on survival.

Conclusions

The way that spanner crabs are currently handled on boats is too stressful. Physiological studies show that spanner crabs are not well equipped to deal with being stored out of water. The crabs should be stored on boats either in live wells (taking care to avoid fighting and injury), at low temperature in humid air or under cold seawater mists or sprays. Yet even if you store them cold on the boat they can still die after a few days in tanks on shore. The inability to store the crabs for long periods is an impediment to the maturity of the industry. The crabs may be succumbing to bacterial infections caused by injury and we recommend that serious thought be given to restraining the crab's claws to stop injury. Further work on this aspect is probably required.

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STORING SPANNER CRABS (*RANINA RANINA*) IN AIR AT LOW TEMPERATURES ON BOATS REDUCES PHYSIOLOGICAL STRESS AND IMPROVES SURVIVAL IN STORAGE TANKS ON SHORE

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SUMMARY

Commercial operators have difficulty keeping spanner crabs (Ranina ranina) *alive in storage tanks on shore for more than a few days. The way that the crabs are handled after harvest may contribute to this. We first studied changes in blood ion concentrations in spanner crabs stored in air on a boat to see if dehydration occurred. The results of this study pointed instead to "acidosis" being a potential problem. We then stored crabs on boats using three different methods, in the air (the current industry practice), in cold air (20°C) and in an aerated flow-through seawater tank ("live well"). For the survival trial, we kept the crabs in a recirculating seawater storage tank, recording crab mortality for several days. On receiving them at a factory we also took blood samples and measured blood lactic acid concentration (an important indicator of acidosis). We also studied whether road transport could further raise blood lactic acid concentration. The results show that the crabs were severely stressed by the time they reached the factory, with high levels of lactic acid in their blood. Unexpectedly, road transport added no more lactic acid than you would expect if the crabs stayed undisturbed in air for the same period. Apparently, scope for further lactic acid accumulation is limited when crabs have already been out of water for several hours. Crabs previously stored on the boat in cold air show the best survival in the tank on shore. The live-well was no better than storing crabs in air. We suspect that the crabs were fighting in the live well, since fishermen don't bind the claws. Cooling the crabs down on the boat reduces physiological stress and improves the survival of the crabs during the first few days of storage, but the crabs still begin to die after this. The crabs may be succumbing to bacterial infections and we recommend binding the claws to reduce injuries.*

INTRODUCTION

The spanner or Kona crab *Ranina ranina* has a wide, if patchy distribution in the Indian Ocean, through the archipelagoes of South East Asia, into the western Pacific, and on to Japan and Hawaii. It supports minor fisheries in a number of countries. The Australian fishery from spanner crabs is one of the largest in the world, with a landed catch in 1993 of about 2500 tonnes, of which most was caught in Queensland and the rest in New South Wales. The crabs are harvested using baited tangle nets, and are prone to injury when being removed from the nets. Studies show that damage to or loss of legs reduces the survival of undersized crabs (<100 mm carapace length) returned to the fishery (Kennelly et al 1990), prompting the redesign of the capture apparatus to minimise injury (Sumpton 1993).

When that work was done, crabs of legal size were kept "alive" for cooking and freezing on shore, but now most of the landed catch is exported live. The live market has reinforced the need to harvest crabs without injuring them, but the crabs are still held in air on the boat, in a similar method to that used when they were destined for the cooker.

Aquatic crabs and lobsters generally asphyxiate and desiccate when stored out of water. Carbon dioxide excretion and oxygen uptake is impaired and if the animal cannot satisfy its oxygen demand then lactic acid will accumulate in the blood (Vermeer 1987, deFur et al 1988, Uglow et al 1986, Whiteley and Taylor 1992). However, some crustaceans, for example the mud crab *Scylla serrata*, can respire reasonably well in air and accumulate no lactic acid, (Varley and Greenaway 1992). Other species can be cooled down. This reduces their metabolic rate and slows the accumulation of wastes such as carbon dioxide and lactic acid, (deFur et al 1988, Whiteley and Taylor 1990). In some circumstances the metabolic rate of a lobster at low temperature (10°C) can fall to the point where it is satisfied by the limited rate of oxygen uptake that is possible in air (Whiteley et al 1990).

Dehydration is another potential problem (Tyler-Jones and Taylor 1986) but the literature is ambivalent about whether survival is improved by keeping the product damp or spraying water on it (Hunt et al 1986, Simonson and Hochberg 1986, Vermeer 1987). Presumably some species are more susceptible than others and different circumstances may influence the rate of desiccation.

The spanner crab fishery in Hawaii avoids these problems completely by using boats equipped with "live wells" (Ian Brown, personal communication), a practice similar to that applied to other live crustaceans (such as rock lobsters). The "dry" practice has apparently arisen and continued in the Australian fishery because there does not seem to be a problem. The crabs are usually alive when they reach the shore. But spanner crabs handled this way, sometimes with an intervening period of transport by road, often die after a couple of days storage in seawater tanks. This means that they must be exported before the typical "purging" process used on other live products and also means that the crabs cannot be held over in tanks if the price is poor.

Most of the boats currently used for harvesting spanner crabs are small and fast- this rules out many of the obvious suggestions for improving the condition of the crabs during storage at sea (eg. water sprays and cool rooms). Before a decision is made about upgrading to a larger boat or changing the facilities on a small boat information is needed of how wet wells and chilling the crabs compares to existing practices in terms of the survival of the crabs on shore.

In this study we kept spanner crabs on a boat under a variety of conditions, then returned them to shore and stored them for several days in a recirculating seawater aquarium to see how treatment on the boat effected the survival of the crabs. At the same time we also examined the effects of an intervening period of road transport between landing the catch and actually placing it in a storage tank. Measurements were obtained of lactic acid and inorganic ion concentrations in the blood of the crabs to shed some light on the stresses they were experiencing.

METHODS AND MATERIALS

Effect of harvesting, storage in air and submersion on the concentration of major cations in the blood of spanner crabs

This experiment was conducted on a commercial crab boat offshore from Mooloolaba in southern Queensland. For this experiment SCUBA divers took blood samples from crabs in tangle nets on the sea bed (depth about 20 metres) prior to winching the nets on board. A hole was punched into the carapace, above the pericardium, using a metal spike fitted with a plastic sleeve to restrict the depth of penetration. These samples (about 0.4 ml) were taken using disposable plastic syringes (1 ml) that were modified to be self-filling by fitting a plastic spring to the plunger. A rubber gasket was mounted around the needle to prevent the needle penetrating too far into the crab and to stop seawater entering the wound.

Further blood samples (about 0.4 ml) were taken from crabs removed from the tangle net on the boat. Other crabs were sampled after they had been in air for 3 hours. The remainder of the catch was returned to shore and driven to the laboratory at IFIQ where blood samples were taken from crabs that had been in air for 7 hours. The remaining crabs were then submerged. Further groups of these crabs were sampled after 1 and 3 hours submersion. The blood samples were frozen after sampling and analysed for major inorganic cations (below).

Effect of storage method on blood lactic acid and ion concentration

On the boat

Crabs were harvested off the coast of Bundaberg in southern Queensland in another commercial crab boat. Crabs taken from the net were distributed randomly amongst three experimental treatments. Sixty crabs were stored in each treatment. Each treatment could be accommodated in two of the plastic baskets normally used in the fishery for keeping crabs.

One treatment involved storing crabs in water and the other two treatments involved crabs stored out of water. The wet treatment (WET) used a 180 litre tank which seawater entered from the vessel's deck hose. The water in the tank was aerated using a battery powered air pump and an array of air stones tied to a grid on the tank bottom. The tank had baffles to prevent surging. The two dry treatments were crabs stored in plastic baskets and covered with a damp towel (DRY) which is similar to the methods currently using in this fishery and a second treatment (DRY/COLD) where crabs were placed in insulated boxes and a polystyrene sheet placed over them along with 1 kg of frozen gel-ice.

The required number of crabs for each treatment were gathered over 19 shots (a shot involves harvesting a long line with 10 tangle nets (each 1 m^2) attached). Within each

treatment the crabs were held in air for varying periods depending on the time of day that they were caught. The crabs from each net were distributed between the treatments in such a way that time spent out of water was independent of treatment.

After all crabs required for these three treatments were captured, the remaining crabs harvested were kept as surplus in baskets, covered with a wet piece of polyurethane foam and 1 kg of frozen gel ice, (the method used by this particular fisher) and returned to shore for the road transport experiment.

Sorting on shore
The crabs from the storage experiment were unloaded from the boat and crabs that were not in baskets at this stage (WET and DRY/COLD) were each transferred to a pair of baskets. The crabs caught after those used in the storage experiment were then randomly distributed amongst 6 baskets (ie. 30 crabs per basket). These crabs were destined for the road transport experiment.

At this stage, 1 ml samples of blood were taken from four crabs chosen at random from each basket of crabs from the storage experiment and from two baskets of surplus crabs. Each sample was injected into a 1 ml microcentrifuge tube and a 0.4ml sub-sample was immediately mixed into an equal volume of 0.6 mol/l perchloric acid in a second 1 ml microcentrifuge tube. Both vials (whole blood and perchlorate extraction) were then frozen in dry ice and returned to the laboratory in Brisbane for further extraction and analysis.

After completing sorting and blood sampling, the crabs from the storage experiment (WET, DRY and DRY/COLD) were placed in a recirculating seawater aquarium (18.9°C). At this stage these crabs had been in air for between 7 and 13 hours. This aquarium was cooled to 17.7°C overnight.

Effect of road transport on blood lactic acid and ion concentration

At the same time, two baskets of the randomly sorted, surplus crabs (the baskets from which crabs had blood samples taken) were also placed in the aquarium. These crabs were the controls for the road experiment (CONTROL) and had been in air for between 4 and 7 hours. A further two baskets of the surplus crabs were placed on the floor beside the aquarium and surrounded with cardboard. These were the blanks for the truck experiment (AIR). The remaining two baskets of crabs (ROAD/AIR) were tied onto the back tray of a four-wheel drive vehicle, covered with a tarpaulin and driven to and from The Town of 1770. This is the same road normally used when trucking crabs from the Town of 1770 to Bundaberg (124 km) and thence to Brisbane (a further 368 km south). On arriving back at the recirculating seawater aquarium, about 6 hours later, blood samples were taken from 8 crabs in each of the AIR and ROAD/AIR treatments, (these samples were partitioned into whole blood and perchlorate extracted blood as before), and the remaining crabs from both of these treatments (which at this stage had been out of water for between 10.5 to 13.5 hours) were then placed in the aquarium beside the CONTROLS.

Recording survival of crabs during subsequent storage

The number of dead crabs in each treatment in both experiments was counted twice on the following day (morning and afternoon) and then again twice daily for the following four days.

Measuring blood lactate and ion concentration

The frozen partially-extracted blood samples were thawed and the supernatant was removed by centrifuge and then neutralised with 3 mol/l KOH in order to remove the resulting precipitate. The extracts were frozen prior to determination of L-Lactic acid using a commercially available kit (Boehringer-Mannheim cat no: 139 084) and a UV-visible spectrophotometer at a wavelength of 340 nm. Appropriate dilutions of the raw extract, or modifications to the total reagent volume, were made to bring the sample values within the standard curve.

The concentrations of sodium (Na^+), magnesium (Mg^{2+}), potassium (K^+) and calcium (Ca^{2+}) were measured in whole blood by inductively-coupled plasma mass spectrometry (ICP-MS). The coagulated sample (200 to 400 mg) was digested in 2.5 ml of nitric acid in low pressure teflon bombs, placed in a microwave oven for 40 min at 280 watts. Each digested sample was then diluted to 50 ml with distilled water and analysed by ICP-MS, with standards.

RESULTS

Effect of harvesting, storage in air and submersion on the concentration of major cations in the blood of spanner crabs

The blood sodium concentration of crabs on the seabed was significantly higher than that of crabs arriving on deck (Figure 1). None of the other ions changed in concentration during harvest though similar drop was seen in the mean calcium concentration though this was not significant at 5%. During storage on the boat, the concentrations of potassium and calcium increased between 3 and 7 hours in air, while there was a trend for sodium concentration to decrease though this was not significant at 5%. When the crabs where placed in a tank that was hyperionic in these ions with respect to the seawater that the crabs came from the only ion to change significantly after 3 hours was potassium, which fell abruptly on submersion.

In spanner crabs, the blood magnesium concentration was close to that of sea water and no changes in this parameter were seen during harvesting and post-harvest handling.

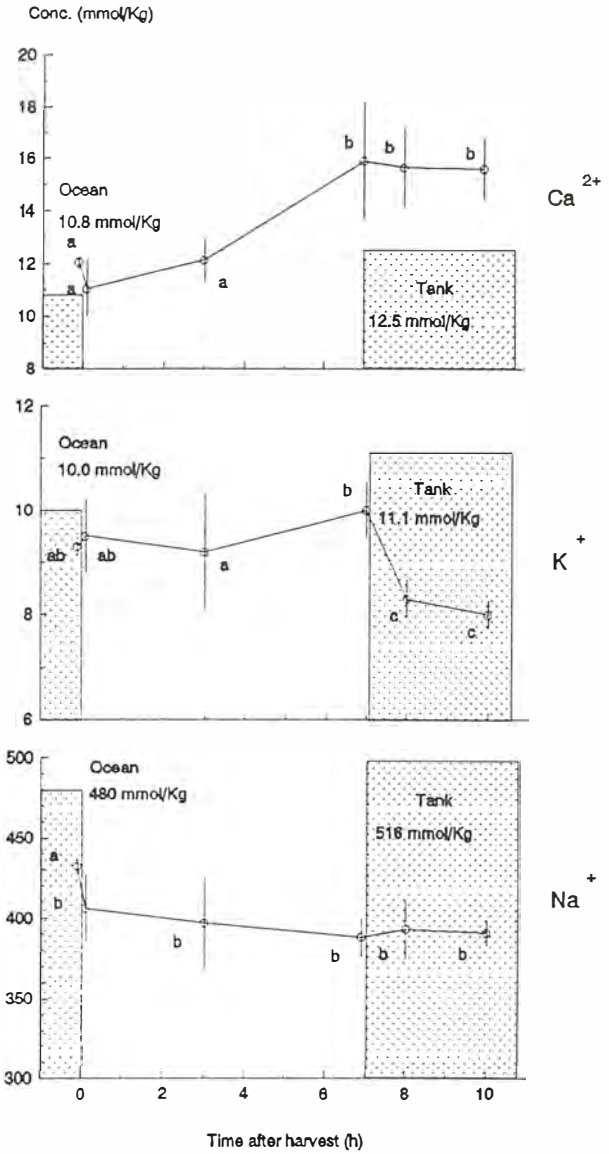

Figure 1. Changes in levels of major cations in the blood of spanner crabs after harvest. Shaded area refers to ocean or tank water. Points with the same letter are not significantly different.

Blood lactic acid and ion concentration at the factory

Storage method

Table 1 shows the effect of different treatments on the lactic acid concentration in the blood of spanner crabs. The lowest concentrations were seen in the live well (WET) and when crabs were stored at low temperature (DRY/COLD). The highest concentration was seen in crabs stored in air on deck (DRY). The highest Na^+ and lowest Mg^{2+} concentrations were also seen in this treatment. Treatment on the boat had no significant effect on blood Ca^{2+} and K^+ concentration.

Road transport

Unexpectedly, driving crabs to the Town of 1770 and back to the factory had no significant effect on the blood lactic acid concentration of spanner crabs (Table 2). However, significant differences where seen in blood Na^+ and Mg^{2+} concentration. Blood Na^+ concentration of crabs after the road trip (ROAD/AIR) was significantly lower than that of crabs that remain undisturbed beside the storage tank (AIR). Blood Mg^{2+} concentration increased significantly during the period in crabs that were not transported (AIR), although there was no significant difference between the concentrations of the air and ROAD/AIR crabs in Table 2. This reversed the trend seen above when Mg^{2+} concentration fell in the DRY treatment on the boat.

Survival at the factory

Storage method

Crabs were stored on the boat using different treatments (60 crabs per treatment) and delivered to a recirculating seawater storage tank on shore, where their mortality was followed for several days (Figure 2). Crabs from the DRY/COLD treatment on the boat showed the best results. Mortality was quite low in this treatment for the first 3 days but afterwards, mortality rate increased. About half of the crabs in the other treatments had died after 5 days of storage.

Road transport

These crabs had only been in air (primarily on the boat) from 4 to 7 hours when the first group was placed without delay in the storage tank (CONTROL on Figure 3). Mortality in this group was negligible during the first day but thereafter increased dramatically. Initially, mortality rate was highest in crabs that had been transported by truck for 6 hours between being landed and being placed in the holding tank (ROAD/AIR). Crabs that spent this period undisturbed in air (AIR) showed a cumulative mortality profile that was not significantly different from that of the crabs that were placed in the tank without the 6 hour delay.

Table 1. Effect of different treatments on the boat on mean concentrations (±SD) of lactic acid (mmol/l) and ion concentrations (mmol/kg) in the blood of spanner crabs *Ranina ranina* arriving at a factory. Eight crabs sampled per treatment. *ns* indicates no significant difference between treatments. In each column, means assigned different letters are significantly different.

	Temp (°C)	Time in air (h)	Lactic acid	Na	Mg	Ca^{ns}	K^{ns}
WET	27-28	4-5	22.5±5.38*a*	441±8.1*a*	45±0.0*a*	16.2±1.20	12.5±1.45
DRY	25-28	7-13	39.6±14.70*b*	479±21.2*b*	43±2.0*b*	16.1±2.77	13.9±2.11
DRY/COLD	19-21	7-13	23.4±11.84*a*	461±24.4*a*	45±2.1*a*	14.6±2.27	12.1±0.90

Table 2. Effect of road transport on mean concentrations (±SD) of lactic acid (mmol/l) and ion concentrations (mmol/kg) in the blood of spanner crabs *Ranina ranina* arriving at a factory. Eight crabs sampled per treatment. *ns* indicates no significant difference between treatments. In each column, means assigned different letters are significantly different.

	Time in air (h)	Lactic acid^{ns}	Na	Mg	Ca^{ns}	K^{ns}
CONTROL	4-7	27.1±8.75	483±20.6*ab*	42±1.8*a*	14.2±2.65	13.4±0.85
AIR	10-13	35.1±11.41	498±46.4*a*	47±2.9*b*	17.2±2.61	12.5±0.76
ROAD/AIR	10-13	38.5±11.07	451±25.9*b*	44±2.7*ab*	15.3±2.87	12.3±1.85

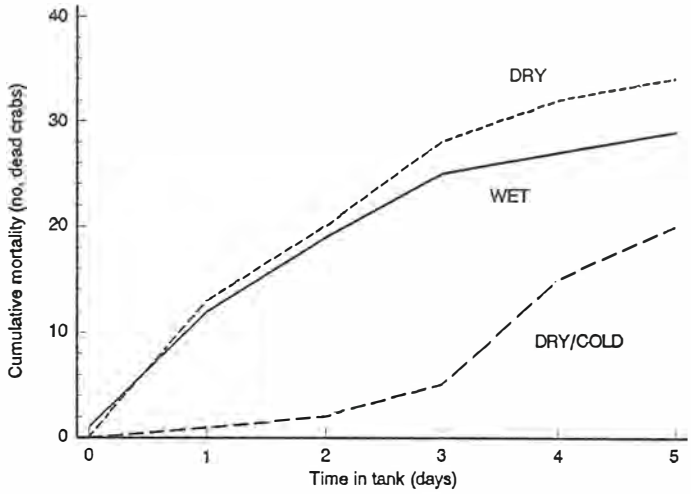

Figure 2. Effect of on-board handling method on the subsequent survival of spanner crabs in a seawater storage tank.

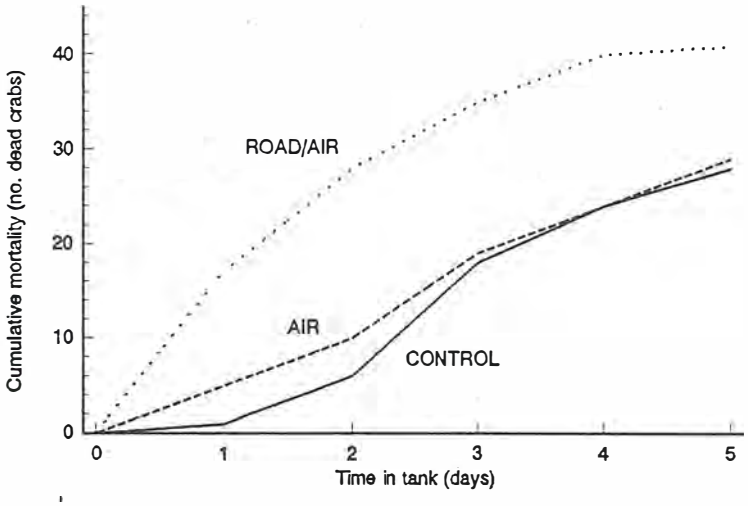

Figure 3. Effect of road transport on the subsequent survival of spanner crabs in a seawater storage tank.

DISCUSSION

You would expect that storing the crabs in water would be considerably better than any kind of storage out of water. This proved not to be the case. The best survival in this experiment was found when crabs were stored in cold air rather than stored in a tank of continually refreshed sea-water. However, before we go looking for spanner crabs on beaches it is worthwhile acknowledging that crowding unrestrained crabs in a "live well" is probably not a good idea.

Storage on boats

Crustaceans intended for live transport and storage are typically harvested using traps or pots. Spanner crabs are no exception to this. The advantage of these kinds of capture devices is that the crabs are not stressed during capture. Underwater video recordings and observations made by SCUBA divers show that the crabs do not struggle in the tangle nets (or "dillies") while the nets are being winched to the surface. The only area od concern is that when the dilly is at the surface and if the boat is rolling in a swell, the snared crabs are jerked about by the rise and fall of the net frame.

We have no explanation for the apparent fall in blood Na^+ concentration between the sea floor and the deck of the boat. A similar response occurred in the blood calcium concentration, and though this was not significant at 5%, it tends to rule out contamination of the sample with seawater as an explanation. The blood magnesium concentration did not change, but this is already close to that of seawater so no inferences can be drawn from this fact.

After they have been freed from the net, any undersized crabs are returned to the fishery and the crabs of legal size are typically placed in plastic baskets, which can hold up to 30 kg of crabs. The crabs struggle urgently when lifted from the water and continue to scramble about for a short period when placed in the baskets. After this, the crabs become relatively quiescent, though occasionally a basket of crabs might "wake up" and all start moving again. The practice of keeping the crabs in baskets, out of water, began before the live market for this product arose and it has continued because there does not seem to be a problem. The crabs are still alive when the catch is landed. However, this is not the whole story. Spanner crabs are typically packed and exported within 12 hours of being landed, without the period of "purging" that is common practice when shipping other live products. The exporters are in so much haste because, after a delay of 2 to 4 days, a large proportion of the crabs in the tank weaken and die, apparently because of bacterial infections. As disease resistance in crustaceans may be influenced by injury and stress, it is possible that the period that they spend out of water on the boat is preventing the crabs from being stored for long periods in tanks on shore.

When blood samples were taken from the crabs on arrival at the factory and analysed they had very high concentrations of lactic acid in the blood. Anything done to the crabs

after that had a relatively small impact on the amount of lactic acid in the blood. The lowest values on "arrival" (about 20 mmol/l), were seen in crabs sampled from the WET and DRY/COLD treatments. Much of this lactate may have accumulated in the "cold" crabs while they were being weighed and sorted into baskets, loaded onto a truck and driven next door at ambient temperature. Similarly, the WET tank was drained to reduce weight prior to the vessel returning to shore.

The lactate concentration was higher in the crabs that stayed on deck all of the time in the baskets. These are extraordinarily high values, and evidence that there is something seriously wrong with the way that this crab is routinely handled. European and Norway lobsters *Homarus gammarus* and *Nephrops norvegicus* accumulate about 8 to 10 mmol/l of lactic acid in their blood during post-harvest handling (Spicer et al 1990, Whiteley and Taylor 1992). Lowery and Tate (1986) associated levels of lactate at around 40 mmol/l with "morbidity" when blue crabs *Callinectes sapidus* were deprived of oxygen underwater.
To some extent, the extraordinarily high lactic acid concentrations may reflect a complete failure in respiratory gas exchange during long periods out of water. Crabs that sustain moderate levels oxygen uptake in air, such as *C. sapidus* and *S. serrata* show only a small rise in lactate concentration or no change at all (deFur et al 1988, Varley and Greenaway 1992).

Surprisingly, the blood showed no changes in ionic concentration that were consistent with dehydration. Taylor et al (1987) found that dehydration increased the concentration of potassium and chloride in the blood of the freshwater crayfish *Austropotamobius pallipes*. Perhaps, rather than concentrating in the blood, the ions were redistributing within body compartments. The concentrations of some ions changed as a function of the time they spent out of water and significant differences were demonstrated between handling treatments. A non-significant trend for blood sodium concentration to decrease is seen in Figure 1. This pattern was not seen when crabs were stored on deck in a later study, where sodium concentration increased (Table 1). In contrast, transporting the crabs by truck has reduced the sodium concentration relative to crabs left undisturbed in air for the same period (Table 2). There was also an non-significant decrease in the blood sodium concentration of dehydrated crayfish *A. pallipes*, apparently as this ion entered the muscles (Taylor et al 1987).

The rise in calcium concentration demonstrated in the Mooloolaba study (Figure 1) was not seen in the later work where crabs had been out of water for various periods (Table 1 and 2). When spanner crabs are out of water the calcium concentration of the blood rises more slowly than in some other crustacean species, (deFur and McMahon 1984, Whiteley and Taylor 1990). Rising calcium concentration often, but not always, accompanies acidosis in crustacean blood (Taylor and Innes 1988, Varley and Greenaway 1992).

Road transport

In this study, the factory was located near the dock and it was only necessary to load the crabs onto a truck and drive next door. However, spanner crabs are sometimes driven long distances after landing, a practise which lengthens the delay before they are submerged. However there is more to road transport than simply keeping the crabs in air for longer.

The Town of 1770, near Round Hill Head in Central Queensland, is one of the most northerly of the major spanner crab ports in Queensland. Crabs landed there are transported by truck to factories, not only in Bundaberg (124 km away on an unsealed road) and even as far as Brisbane (368 km further south of Bundaberg). In this study we have not attempted to simulate an actual shipment of crabs, but rather we wished to demonstrate that transporting crabs by road was capable of killing them. We admit that the stress experienced by these crabs went beyond that you would expect from crabs carried over the same road in a heavily laden truck. The stress experienced by commercial shipments of crabs would presumably lie somewhere within the range of mortality described here. The objective of improving the survival of the crabs would be to bring the mortality closer to that expected if you just held the crabs in air for the same period.

The crabs were still alive when we placed them in the holding tank in Bundaberg. This emphasises the extraordinary ability of this crabs to take punishment of this magnitude without giving any external signs of injury- giving a false sense of security to people handling them. The crabs appeared to "drown" soon after being submerged. They became very weak and lethargic and the mortality rate the next day speaks for itself. We suspect that the crabs were unable to obtain oxygen when returned to the water. A similar phenomenon has been described by Varley and Greenaway (1992) when mud crabs are submerged after they have been in air for several days.

Contrary to our expectations, even a "worst case" attempt at road transport like that conducted here did not cause a significant increase in the blood lactic acid concentration in spanner crabs above that you would expect from leaving them in air for the same time. Disturbance increases the blood lactic acid concentration of *H. gammarus*, apparently by increasing locomotor activity and metabolic rate (Taylor and Whiteley 1989, Whiteley et al 1990). Perhaps spanner crabs are already too exhausted to respond in this way: the lactic acid concentration is already too high. The crabs may actually have fatigued during the initial struggling on deck. Disturbance may not increase the activity and the blood lactic acid concentration of a crab that has already fatigued (Burke 1979).

Recovery in storage tanks on shore

The stress of storing crabs and lobsters out of water is such that the crabs need to be submerged and allowed to recover in seawater before they are further stressed by being taken out of the water again (Whiteley and Taylor 1992). This recovery process may take several hours to complete. Ordinarily this would not be a problem. Rock lobsters are kept

in tanks for several days before being packed for export. However, spanner crabs are usually exported about 12 hours after arriving at the factory. Once lobsters are returned to the water, the blood pH and carbon dioxide levels return to normal within about an hour (Whiteley and Taylor 1992). However, we have seen that spanner crabs are able to build up high levels of lactic acid in their blood and it will probably take them several hours to remove this from their blood (Bridges and Brand 1980, Paterson et al 1994) and this time may not be much less than the recovery period that the crabs currently receive before they are exported.

Potassium concentration in the blood fell abruptly when spanner crabs were submerged following 7 hours in air on a boat. The speed of this change suggests either that the K^+ has left the blood and entered the tissues, (perhaps as crab recovers from acidosis) or that the blood itself has been diluted. The latter option seems unlikely in an osmotic conformer placed in a hyper-osmotic medium. Perhaps some of the crab's tissues lose water to the environment and the K^+ moves into tissues to help restore cell volume (Kregenow, 1981).

Mortality during purging

Another reason to put the crabs in storage tanks is to allow them to rid their alimentary tracks of undigested bait and faeces and thereby to reduce their metabolic rate prior to export. Rock-lobsters are given 4-5 days to "purge." Yet, regardless of the method used to store spanner crabs on the boat, a large percentage of the catch still succumbs within 5 days of capture. All that keeping the crabs cold on the boat did was to delay the onset of mortality by 3 days. This is in keeping with industry experience. At best they can not keep the crabs past this point without loosing a significant part of the catch. This delayed mortality is characteristic of syndromes referred to in the literature as "vibriosis" (a bacterial infection) in other live crustacean industries. Simply, the crabs get sick. For example, Morrissy et al (1993) mentioned that yabbies (*Cherax destructor*) die within two to three days of arrival from a "systematic bacterial infection" if they are transported with "dirty" water (i.e. containing a high bacterial load) still trapped around the gills. The bacteria need not come from outside of the animal. Bacteria such as *Vibrio* can apparently always be found in crustacean blood, and these chronic infections typically flare up during handling and transport into outright septicemia (Sizemore 1985, Ellender et al 1992).

The high mortality in the live well treatment was unexpected. It suggests that low temperature per se rather than submersion in water has a beneficial effect on the crabs. Certainly, the lactic acid content of the blood was not a good indicator of subsequent survival results. Aquatic crustaceans survive better out of water anyway if they are cooled down (deFur et al 1988) but there may be a further benefit here from reducing physical injury. Spanner crabs are not "banded" or "tied" after harvest. The crabs are not as dangerous to people as mud crabs but when you try to pick a crab out of a basket, you very often get that crab and several others attached! This cannot be good. When the crabs are crowded, they will injure each other regardless of whether or not they are underwater. Perhaps with spanner crabs, the cooled crabs were less active in storage and less likely to injure each other, and thus promote fewer opportunities for bacterial

infections to take hold.

To conclude, spanner crabs can build up extraordinarily high concentrations of lactic acid during routine handling and storage in air after harvest. Cooling the spanner crabs down on the boat reduces this physiological stress and improves the survival of the crabs during the first few days of storage, but the crabs still begin to die after this. The inability to keep spanner crabs for long periods in tanks on shore remains a serious impediment to the maturity of the industry. The crabs may be succumbing to bacterial infections and we recommend binding the claws to reduce injuries.

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A LACTIC ACID "CEILING" ARRESTS PROFOUND ACIDOSIS IN THE BLOOD OF AN ION-CONFORMING CRAB, *RANINA RANINA*, STORED OUT OF WATER AT 25°C

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SUMMARY

Respiratory gas levels, acid-base balance and the dynamics of lactic acid accumulation were studied in the blood of the marine crab Ranina ranina *while stored out of water at 25°C. This crab spends much of the time buried in the sea floor. It is marketed live and is typically stored out of water after harvest for up to 8 hours or more. This practise has persisted because the crabs are still alive when they reach shore. Spanner crabs could not regulate the chloride ion concentration of their blood during changing salinity and could not regulate their blood pH while in air, showing an uncompensated respiratory and metabolic acidosis during the first couple of hours out of water. In this respect they resemble other crabs that are ionic conformers. Storing the crabs in a high oxygen atmosphere exaggerated this acidosis, by generating more carbon dioxide than usual. After 3 hours in air, the rate of acidosis slowed. Blood calcium concentration did not change and there was no evidence of large rise in total carbon dioxide concentration in the blood that normally accompanies compensation responses. Instead, the fall in blood pH mirrors the rise in lactic acid concentration in the blood. Both symptoms remain more or less constant after 3 h out of water. Apparently the spanner crabs only means of surviving for long periods in air is to stall the entry of lactic acid into the blood, perhaps by reducing its demand for oxygen.*

INTRODUCTION

The spanner or Kona crab *Ranina ranina* spends much of the time buried in sandy substrates on the sea floor (Skinner and Hill 1986) and shows morphological and behavioural adaptations to sustaining gill ventilation while buried that are broadly similar to those of other burying crabs (Taylor and Atkinson 1991). One practical consequence of this is that the crab's retain relatively large amounts of water in their gill chambers when removed from the water.

Australia has the largest spanner crab fishery in the world, with a landed catch in 1993 of almost 2500 tonnes. The crabs are harvested using baited tangle nets (Sumpton 1992) from areas of clean sand in relatively deep water (20 to 60 m) but they are sometimes found stranded by exceptionally low spring tides- buried in exposed sand bars. In the Australian fishery they are stored dry after harvest for up to 8 hours or more until the boat returns to shore. In Hawaii, the crabs are stored in seawater, in boats containing "live wells." In Australia, the "dry" practise arose when the crabs were brought alive to

shore for cooking and then sold as a frozen cooked product, but it has persisted even though a considerable fraction of the catch is now exported live to Taiwan. Other seafood industries based around live marketing, such as the Western Rock Lobster *Panulirus cygnus*, rely upon live wells to deliver product to storage tanks on shore. But with spanner crabs, there has never seemed to be a problem. The crabs apparently survive quite well on the boat, as long as they are kept shaded and are not allowed to dry out.

The spanner crab, like other primarily aquatic crustaceans, is expected to lack the adaptations found amongst intertidal and semi-terrestrial crustaceans (eg. mud crabs *Scylla serrata*) that allow high levels of oxygen uptake to be sustained in air (Taylor and Innes 1988). The gills of aquatic crustaceans are widely thought to "collapse" and become matted together when no longer buoyed up by water (deFur et al 1988). Under these circumstances, exchange of gaseous oxygen and carbon dioxide (and bicarbonate ions) is curtailed and the animal rapidly asphyxiates. Oxygen deprivation leads to an increased reliance upon anaerobic metabolism, which in crustaceans (as in fish) leads to lactic acid accumulating in the tissues and blood. The metabolic acidosis, coupled with the respiratory acidosis caused by elevated carbon dioxide levels in the blood can, through the Bohr Effect, reduce the oxygen affinity of haemocyanin and thereby erode the crabs capacity to sustain limited amounts of oxygen transport in air (Taylor and Innes 1988).

The fact that spanner crabs survive as well as they do in air suggests that they have some way of containing the consequences of asphyxiation. We can use this species to test the hypothesis recently advanced by Burnett and McMahon (1987), that residual water in the gill chamber of crabs that are strong ionic regulators is an important avenue for acid-base regulation. On the basis of its oceanic habits, the spanner crab is presumably a stenohaline ionic conformer which nevertheless traps large amounts of water in its branchial chamber when taken from the water. It is of interest to see whether this species is capable of metabolic "compensation" for acidosis while out of the water by raising the total carbon dioxide and calcium concentration of the blood. These symptoms may indicate that dissolution of carapace carbonates plays a role in correcting changes in blood pH when the crab is removed from the water.

This sub-tropical/tropical crab experiences a seasonal range of seawater temperature of 19 to 26°C off the southern Queensland coast (Skinner and Hill 1986), however, this temperature range can probably be widened to between 15 and 29°C if populations south and north of this point are considered. Crabs stored out of water on boats are probably exposed to a similar range of temperature. In this study, we examined changes in blood gas levels, acid-base balance and the accumulation of lactic acid in the muscle and blood of *R. ranina* stored out of water at 25°C to see if this species showed a classical compensation response to acidosis. We also studied the concentration of chloride ions in the blood of this species at different salinities to confirm that it is indeed an ionic conformer. The results show that the apparent "robustness" of this crab, which has encouraged such a blasé approach to its handling, does not stand up to physiological scrutiny.

METHODS AND MATERIALS

Spanner crabs were purchased from a commercial supplier (Mooloolaba Fisheries) and stored in a recirculating sea-water holding tank at IFIQ. Experiments at IFIQ began at least 2 days after the crabs arrived, to allow any crabs weakened by handling to die. When crabs were required for acid-base measurements in Sydney, they were packed after one to 2 days storage in Brisbane and air-freighted in 15kg batches to a smaller re-circulating sea-water aquarium at the School of Biological Sciences, University of New South Wales. Experiments using crabs in Sydney were started on the day after they were placed in the aquarium.

Ionic regulation in the spanner crab.

Blood samples were taken from spanner crabs arriving at the dock and from crabs that had been sitting in a commercial storage tank overnight. The main objective was to see how well this species could regulate the magnesium concentration its blood with respect to seawater and also to see if the level of calcium in the blood could give a clue to possible acidosis occurring when the crabs are stored out of water on the boats.

To specifically look for evidence of blood chloride regulation in this species, crabs were put in recirculating seawater aquariums (34 ppt) and allowed to recover overnight. The next day the salinity of two aquariums (12 crabs each) was reduced gradually (in 1-2 ppt increments during the course of the day) to 26ppt and left at that level overnight. On the next day, the salinity of one of these low salinity aquariums was gradually reduced further still, to 19ppt and left at that salinity overnight. Blood was sampled (about 0.3 ml) from 10 crabs exposed to normal salinity (34 ppt) and from those crabs surviving at the lower salinities (26 and 20 ppt). A Corning Chloride Analyser (Model 925) was used to measure the concentration of chloride in the water in each tank and in suitably diluted crab blood.

Acid-base balance while stored in air

The crabs were lifted from the water in the recirculating seawater aquarium (temperature, 18°C) and packed into 20 l plastic Nally bins. No attempt was made to drain fluid from the gill chamber when transferring them to tubs. The tubs of crabs, containing a small amount of standing seawater to maintain humidity, were stored in a 25°C controlled temperature room. Some crabs were carried from the aquarium in a tub of seawater and placed in aerated tubs of seawater in the CT room and allowed to warm up to 25°C. Samples of blood were taken from some crabs after 3 hours in air and from others after 7 hours. Blood samples were also taken from immersed crabs at that temperature.

Effect of hyper-oxia on acid-base balance while stored in air

The experiment was carried out in a controlled temperature cabinet containing a water bath (25°C). The treated crabs were stored out of water for 3h because the previous experiment showed that the most dramatic change in blood parameters occurred within this time. Two treatments were used; either ordinary atmosphere (which has an oxygen

partial pressure of about 22 kPa) or an atmosphere augmented using a cylinder of oxygen (>40 kPa). Other crabs were placed into the cabinet as controls, stored for the same period in a tub containing aerated seawater. Six crabs were used in each treatment.

Blood pH and dynamics of lactic acid while stored out of water

Crabs were taken at random from the aquarium (N=8) using a scoop net and blood samples were taken for determination of pH, calcium and lactate concentration. Immediately after removing the blood sample, each crab was weighed and then chopped in half down the mid-line, both to rapidly destroy the CNS and provide quick access to the muscles at the base of the legs which were removed into bioassay bags and frozen in a dry-ice/acetone bath.

Other crabs were then removed from the tank and stored in plastic baskets with mesh-floors, in humid conditions at 25°C in a air-conditioned wet-processing laboratory. Blood and muscle samples were taken from three more groups of 8 crabs that had been in air for 3, 7 and 10 hours respectively. At each sampling time, blood and muscle samples were also taken from two to three crabs that remained in the tank, until 8 control samples were taken.

Recovery of blood lactic acid concentration

During this study it became clear that spanner crabs could accumulate very high levels of lactic acid in their blood. In general, crustaceans cannot rapidly metabolise an "oxygen debt" of this magnitude. Under commercial conditions, the crabs might only be left in the tank for 12 h before they are taken from the water again, and exported. This practise raises the question of how effective spanner crabs were at reducing the circulating levels of lactic acid in this time frame.

For this experiment, crabs that had been in air for 4 to 7 hours were purchased at the dock and transported, at ambient temperature, to a recirculating seawater aquarium (temperature 22°C). Blood samples were taken from 5 crabs, selected at random, on arrival at the laboratory and then the rest of the catch was submerged in the aquarium. Blood was sampled from groups of five crabs after they had been allowed to recover in the tanks for 1, 3, 7, 14 and 21 hours.

Sampling blood

The carapace was rapidly drilled or punctured above the pericardium, using surface grooves as a guide. Samples of blood were taken from crabs subjected to various treatments using either an ice-cold glass Hamilton syringe or glass tuberculin syringes and 22 gauge needles. Where gas-tight sampling was not required, disposable plastic insulin syringes were used. The samples were handled on ice to retard clotting, though sample coagulation was never really a problem.

Acid-base and blood gas determination

The pH of each blood sample was determined using a Radiometer G299A microcapillary electrode, and a further volume of sample was injected into the measuring chamber for the PO_2 and PCO_2 electrodes, connected to a Radiometer BMS3 Mk2 and PHM 73 Blood gas monitor. The blood gas analyser was thermostated to the experimental temperature (25°C) and calibrated using precision buffers and humidified gas mixtures (10% O_2 and 1% and 2% CO_2 respectively), prepared using gas mixing pumps.

After measuring blood gas parameters, a 25μl subsample was analysed for total CO_2 (i.e. $[CO_2+HCO_3^-+CO_3^{2-}]$) using a PCO_2 electrode and Radiometer PHM 71 Acid-base analyser by the method of Cameron (1971). The analyser was calibrated using 25μl injections of 10 mmol/l $NaHCO_3$ in 500 mmol/l NaCl.

The remainder of the blood sample was added to a labelled vial and frozen for later calcium and chloride determination using an autoanalyser and ion-specific electrodes. A 1/6 dilution was used to bring the concentration of both ions within the dynamic range in the instrument.

Muscle and blood lactic acid determination

Crab blood samples were diluted 1:1 with 0.6 mol/L ice-cold perchloric acid. The supernatant was removed after centrifuging and then neutralised with 3 mol/L KOH and the precipitate removed by centrifugation. The extracts were frozen prior to determination of L-Lactic acid using a commercially available analysis kit (Boehringer-Mannheim cat no: 139 084) and a UV-visible spectrophotometer at a wavelength of 340 nm. Appropriate dilutions of the raw extract, or modifications to the total reagent volume, were made to bring the sample values within range of the standard curve.

Crab muscle (about 20 g) 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 30 min, the potassium perchlorate precipitate was removed by filtration. The filtrates were then frozen for subsequent HPLC analysis. The concentration of L-lactic acid in the extract was measured using an HPLC method (Bio-Rad).

RESULTS

Blood ion levels

The blood of *R. ranina* had a magnesium concentration very close to that of seawater (Table 1). Ion levels were lower in the crabs in the storage tank, perhaps because of the lower salinity in the water or because of stress and dehydration of crabs stored on the boat. There was wide variation in blood calcium concentration in crabs arriving at the dock, with values ranging from 14.0 to 18.9 mmol/kg. *R. ranina* could not regulate the chloride concentration its blood when stored at different salinities (32, 26 and 19 ppt) (Figure 1). High mortality amongst the crabs stored at 19 ppt meant that only 6 crabs survived to be sampled (50% mortality).

Table 1. Mean concentrations of minor cations in the blood of *R. ranina* on arrival on shore (n=9) and after acclimation overnight in a storage tank (n=10).

	Concentration (mmol/Kg)		
	Mg	K	Ca
on arrival	46.6±1.2	16.0±0.8	16.5±2.0
acclimated	38.4±1.7	11.9±0.4	14.5±0.2
ocean water	53.5	9.7	14.0

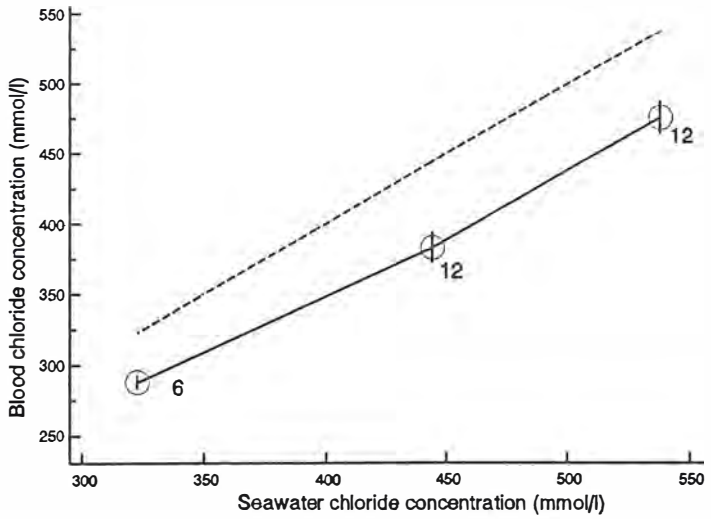

Figure 1. Effect of salinity on the mean (±SD) chloride level in haemolymph of *Ranina ranina*. Dashed line shows isoionic line. Sample size shown for each point.

Acid-base balance while stored out of water at 25°C

When *R. ranina* was stored out of water for 3 h there was a significant fall in blood oxygen tension (PO_2), and a significant rise in carbon dioxide tension (PCO_2) (Table 2). Blood pH fell significantly but there was no significant rise in total carbon dioxide concentration (CCO_2). The concentrations of calcium (Ca^{2+}) and chloride (Cl^-) ions increased in the blood after 3 h in air. Further storage out of water (to 7 h) did not cause any further changes in blood parameters.

Table 2. Effect of storage in air on blood parameters in *R. ranina* at 25°C

	Period in air (h)		
	0	3	7
n	8	6	6
PO_2, kPa	7.82[a] (3.52)	2.23[b] (0.52)	4.00[b] (1.21)
PCO_2, kPa	0.39[a] (0.18)	0.78[b] (0.18)	0.80[b] (0.10)
CCO_2, mmol/l	2.25[a] (0.45)	2.67[a] (0.37)	2.92[a] (0.31)
pH	7.306[a] (0.056)	7.005[b] (0.145)	6.910[b] (0.059)
Ca^{2+}	14.9[a] (2.22)	18.5[b] (1.81)	18.0[b] (0.69)
Cl^-	525[a] (84.2)	629[b] (44.1)	617[b] (28.2)

Effect of aerial hyper-oxia (>40 kPa) on acid-base balance while in air

The mean oxygen tension in the blood of spanner crabs stored in oxygenated air for 3 hours was not significantly different from that of crabs submerged in aerated seawater for that time (Table 3). When crabs were stored in ordinary atmosphere for this period, the mean oxygen tension in the blood was significantly lower than that of crabs submerged in aerated seawater.

Removing crabs from the water caused a significant increase in both carbon dioxide content and partial pressure and a significant fall in blood pH (Table 3). These symptoms were exaggerated in crabs stored in an oxygen-rich atmosphere (>40 kPa). Blood calcium concentration did not change significantly in the emersed crabs.

Table 3. Effect of aerial hyperoxia on the blood acid-base balance of *R. ranina* stored in air at 25°C. For explanation of table, see Table 2.

	Treatment PO_2, kPa		
	submerged	Out of water (3h)	
	22	22	>40
n	6	6	7
PO_2, kPa	8.13[a] (4.19)	3.20[b] (0.76)	6.13[ab] (1.87)
PCO_2, kPa	0.31[a] (0.06)	0.68[b] (0.11)	1.28[c] (0.26)
CCO_2, mmol/l	2.8[a] (0.70)	4.9[b] (0.81)	6.2[b] (1.23)
pH	7.409[a] (0.106)	7.230[b] (0.065)	7.093[c] (0.044)
Ca^{2+}	11.2[a] (0.6)	11.4[a] (0.8)	11.9[a] (0.4)

Blood pH and dynamics of lactic acid while stored out of water

When crabs were stored in air at 25°C there was an initial rapid acidosis, with blood pH falling from 7.66±0.19 to 6.96±0.10 in 3 hours (Figure 2). Thereafter the rate of acidosis slowed dramatically, falling to 6.87±0.05 after 10 hours in air. But pH is a logarithmic function of the hydrogen ion (H^+) concentration (i.e. $pH = -\log[H^+]$). For example, a decrease in the rate at which pH falls with time may actually show a linear rise in H^+ concentration. The change in the rate of acidosis shown in Figure 2 is an exaggeration of the real situation. However, expressing the pH data as H^+ concentration (in mmol/l) does not change the conclusion that the rate of acidosis falls with time spent in air. The H^+ concentration rises from $2.35\times10^{-5}\pm0.75\times10^{-5}$ mmol/l to $11.3\times10^{-5}\pm2.37\times10^{-5}$ mmol/l in the first 3 hours, and thereafter rises only gradually to $12.9\times10^{-5}\pm1.5\times10^{-5}$ mmol/l after 10 hours in air.

The calcium level in the blood did not change significantly during the experiment though there was considerable variation in the blood calcium level in emersed crabs versus submerged ones. The calcium concentration was 16.7±0.15 mmol/kg in resting submerged crabs and was 17.4±2.13 mmol/kg after 10 h in air.

The change in pH was mirrored by changes in the lactic acid concentration in the blood (Figure 2). Resting submerged *R. ranina* had a blood lactic acid concentration of 0.14±0.07 mmol/l. Circulating lactic acid concentration increased one hundred fold, reaching 14.38±4.05 mmol/l after 3 hours in air at 25°C. Thereafter, the lactic acid concentration did not change significantly for a further 7 hours.

The concentration of lactic acid in the muscles at the base of the legs of submerged, resting crabs was 2.96±0.58 mmol/kg. The changes in lactic acid concentration of the muscle was similar to that seen in the blood, though of a smaller magnitude, reaching 6.88±0.63 mmol/kg after 10 hours in air (Figure 2). Apparently, the lactic acid concentration in the blood of emersed crabs was higher than that in the muscle.

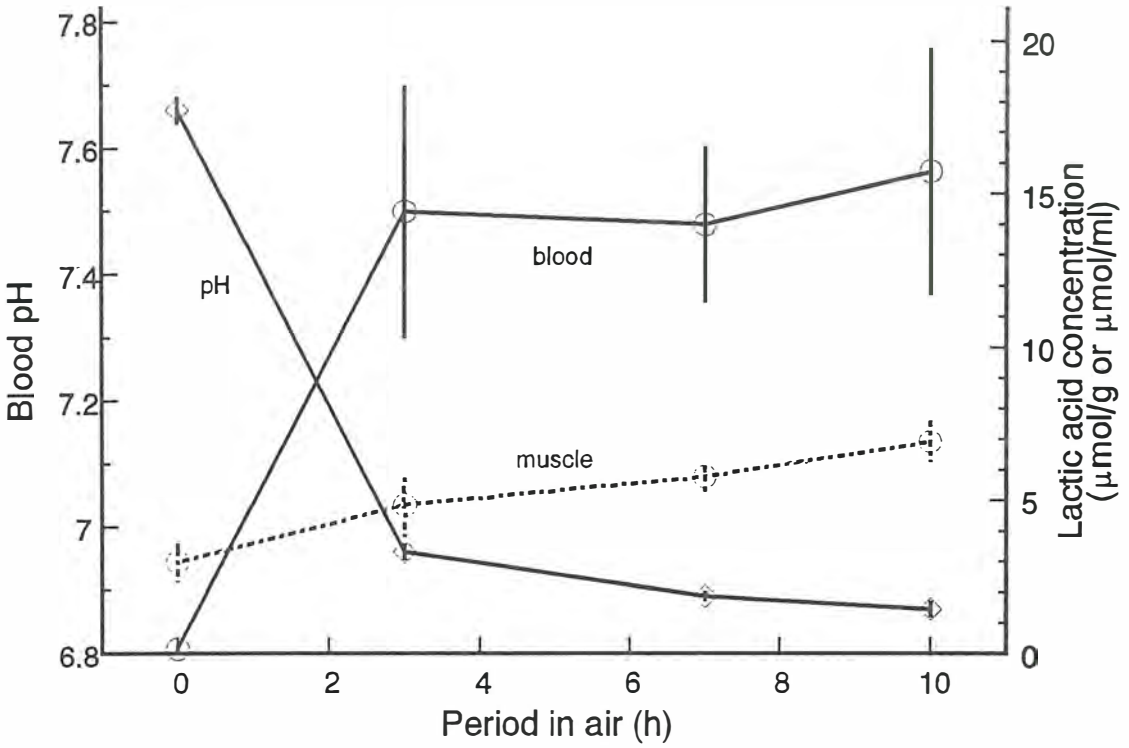

Figure 2. Dynamics of lactic acid accumulation in *R. ranina* stored in air at 25°C. Each sample shows mean (±SD) of eight crabs.

Recovery of blood lactic acid concentration

Crabs purchased from a fisher had a lactic acid concentration prior to being submerged in the recirculating sea-water aquarium of 8.65±1.68 mmol/l. The lactic acid concentration had all but returned to "normal" (0.50±0.35 mmol/l) after 7 hours in the tank at 22-23°C (Figure 3) and the subsequent decline in lactic acid concentration was prolonged.

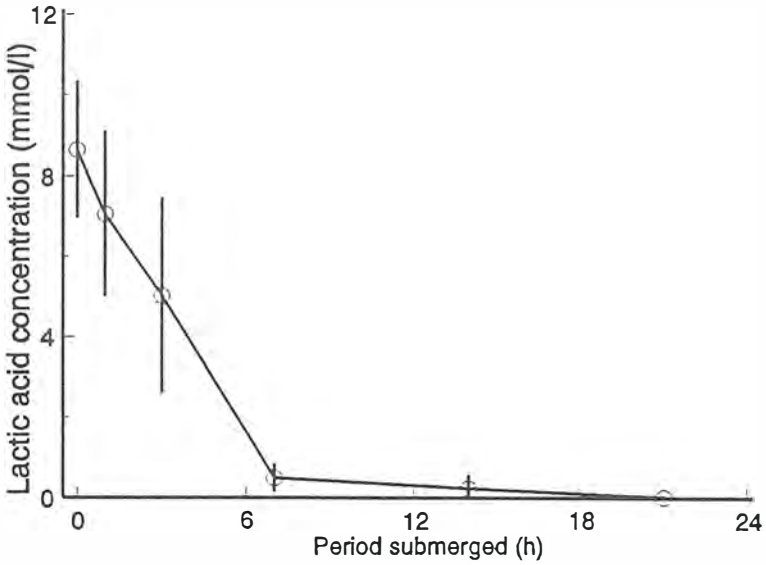

Figure 3. Fall in mean (±SD) concentration of lactic acid in blood of *R. ranina* placed in storage tank. Each point shows data from 5 crabs.

DISCUSSION

Spanner crabs survive for as long as they do out of water because of a dramatic fall in the rate of blood acidosis during long periods out of water. To explain why this happens we should consider how the spanner crab responds to being taken from the water. The crabs scramble around for a few minutes and then become quiescent. Crabs that have been in air for 3 hours are severely hypoxaemic (blood oxygen tension is very low) and acidotic. The blood pH can fall by as much as 0.6 units, which is amongst the largest fall recorded in a crustacean during functional or environmental anaerobiosis (Adamczewska and Morris 1994). A fall in blood pH of similar magnitude occurs when the lobster *Panulirus argus* is stored out of water (Vermeer 1987) but the acidosis is less pronounced in other species of aquatic crustaceans that are stored in air during commercial handling (Whiteley and Taylor 1993, Varley and Greenaway 1992, deFur et al 1988).

The level of lactic acid in the muscle and blood of resting, submerged spanner crabs at 25°C is similar to those reported from crabs and other decapod crustaceans (respectively, 1.25 to 2.94 mmol/kg Albert and Ellington 1985, Gade 1984; 0.04 to 0.4 mmol/l Forgue et al 1992, Zainal et al 1992, Albert and Ellington 1985). From this resting level, the concentration of lactic acid in the blood of spanner crabs rises very rapidly when they are removed from the water, in three hours exceeding the highest values obtained in comparable studies.

When aquatic crustaceans such a crabs or lobsters are emersed, lactic acid either does not accumulate in the blood at all (Varley and Greenaway 1992) or rises to levels of up to 10

mmol/l over periods up to 9 hours in a variety of species (deFur and McMahon 1984b, deFur et al 1988, Spicer et al 1990, Johnson and Uglow 1985).

An acidosis of respiratory and metabolic origin like that seen in spanner crabs also occurs in other marine crabs such as *Cancer productus*, *Maia squinado* and *Liocarcinus puber* stored out of water (deFur and McMahon 1984, Taylor and Innes 1988, Johnson and Uglow 1986) and in the European lobster *Homarus gammarus* when stored in air at 20°C (Whiteley and Taylor 1990). A fall in blood pH of this magnitude should have profound consequences for the survival of the crabs. For example, the spider crab *Maia squinado* rapidly succumbs to an acidosis induced failure of oxygen transport when taken from the water (Taylor and Innes 1988). The prognosis for spanner crabs is not so bad, after the rapid acidosis, the fall in blood pH slows abruptly.

This marine ion-conforming crab fits into the hypothesis put forward by Burnett and McMahon (1987, discussed further in Burnett 1988): that crabs that are osmotic regulators are better equipped than other crabs are to use residues of water in the gill chamber to regulate their blood pH while in air. Acidosis is not arrested by buffering, that is, by a "compensation" response. The spanner crab is unable to buffer this acidosis by a large rise in the total carbon dioxide concentration of the blood. Total carbon dioxide concentration did rise to different degrees in Table 2 and 3, perhaps reflecting differences in the unmeasured "metabolic" contribution to the acidosis (lactic acid) and seems to reflect nothing but carbon dioxide accumulating in the blood because the gills are no longer working. This is demonstrated most clearly by storing the crabs in an oxygen-enriched atmosphere. While this treatment augments the flow of oxygen into the crab, even more carbon dioxide accumulates than would normally be the case, which in the spanner crab forces the blood pH down even more than usual. This finding also helps to explain why McLeese (1965) found that storing American lobsters *Homarus americanus* in oxygen-enriched air actually killed them faster than holding them in normal atmosphere. While blood oxygen tension in spanner crabs falls dramatically while they are in air, the "common sense" reaction of treating this by giving them more oxygen is probably the last thing they need. Aquatic crustaceans in general have difficulty excreting carbon dioxide while in air (Taylor and Innes 1988) and spanner crabs in particular cannot deal effectively with the acidosis arising from high carbon dioxide levels in their blood.

The rise in total carbon dioxide concentration as crustaceans compensate for acidosis while out of water is generally accompanied by a rise in the calcium concentration in the blood (Burnett 1992). The spanner crab shows a rise in blood calcium concentration even though it does not buffer its blood pH. In both *C. productus* and *H. gammarus* the rise in calcium concentration is already evident after only 3 hours in air (deFur and McMahon 1984b, Whiteley and Taylor 1990) but in spanner crabs if any rise does occur, it sometimes takes several hours and is not primarily related to the initial acidosis. Crabs sampled directly from the boat showed a wide range in calcium concentrations (Table 1). These crabs have been in air for anything between three and eight hours at temperatures possibly in excess of 25°C. The experiment in the controlled temperature room showed that both chloride and calcium concentrations changed in the same manner (Table 2),

suggesting that a more serious disturbance in ionic regulation (eg. dehydration) has occurred. This was not seen when a later experiment was conducted in a controlled temperature cabinet containing a water bath (Table 3) nor in the later study of lactate accumulation. Contrast this reaction to that of the lobster *H. gammarus*. When emersed at high temperature (20°C) the concentration of bicarbonate and calcium ions in the blood of the European lobster rises as expected even though this does not stop the acidosis.

If the crab is unable to buffer its blood pH, then the only mechanism it can use to slow down the rate of acidosis is to reduce the rate at which acidic wastes accumulate in the blood. It can do this by becoming inactive, as *E. albidigitum* does, and thereby reduce the demand for oxygen and hence the metabolic shortfall that appears as lactic acid (Burnett and McMahon 1987). Or it might simply retain this waste in the tissues where it appeared or sequester it somewhere else in the body (Taylor and Wheatly 1981, Tyler-Jones and Taylor 1988). Measurements of lactate concentration in the basal muscles of the walking legs suggest that this tissue is not a sink for these ions. A similar pattern of accumulation occurs in both compartments, though this is attenuated in the muscle. The concentration gradient between the muscle and blood appears inverted, as if lactate was being actively removed from the tissue. Further observations are required to clarify this.

One important factor slowing lactic acid production could be that anaerobiosis during the initial period of struggling in air forces the crab to become quiescent; it fatigues. The changes in lactic acid concentration when spanner crabs are taken from the water are similar to those of a crab exercised to exhaustion. In an exercised crab, the concentration of lactic acid in the blood rises rapidly and then begins to fall slowly again once the animal fatigues and begins to oxidise or convert the lactate back into glycogen (McDonald et al 1979, Burke 1979, Henry et al 1994). Therefore, an aquatic crab that exercises in air, when gas exchange is impaired, might accumulate a considerable amount of lactic acid before fatigue sets in. However, a similar pattern of a rise and plateau in blood lactate concentration is sometimes seen in crustaceans exposed to environmental anoxia, (Pritchard and Eddy 1979, Hill et al 1991, Taylor and Spicer 1987, Zebe 1982).

The fact that so much lactate is produced in *R. ranina* before it fatigues may relate to the crab's burying lifestyle. Like other burying crabs it may rely on anaerobic metabolism when buried deep in the sediment (Bridges and Brand 1980), as suggested by the speed with which this species can reduce the concentration of lactic acid in its blood upon re-submersion. The spanner crab may also possibly go anaerobic when burying rapidly into the sand to escape from predators. Incidentally, the speed with which these crabs can process an oxygen debt of this magnitude is quite fortuitous because they are typically spelled in on-shore holding tanks for as little as 12 hours before being exported.

Apart from fatigue, a range of other factors may determine the rate of anaerobic metabolism in the tissues of a crab in air. Central to these is the efficiency of gas exchange at the gills and the success of oxygen transport to the tissues given the acidosis that occurs when crabs are taken from the water (Burnett 1992). After the initial low immediately that the crabs are taken from the water, the blood PO_2 of spanner crabs rises. One factor contributing to this change could be that water is draining out from amongst

the gill lamellae, increasing the surface area available for oxygen diffusion (Taylor and Wheatly 1981), though a fall in oxygen demand at the tissues may also explain it. The gills of spanner crabs are apparently more robust than those of some marine portunids since the gills of *R. ranina* do not collapse like those of the blue crab *Callinectes sapidus* (deFur et al 1988).

Even though the gills of *C. sapidus* collapse when the crab is stored out of water, it is still able to meet much of its metabolic demand aerobically and only a small amount of lactic acid accumulates (deFur et al 1988). There are a number of reasons why this happens. To begin with, clumping of the gill platelets may assist gas exchange, by fleeing platelets from the main water-logged mass of the gill (deFur et al 1988). Secondly, the metabolic rate of a crab or lobster in air is lower than that when submerged, so it does not need as much oxygen. Lastly, a reduced demand for oxygen can be partially or completely met by improvements in oxygen transport to the tissues arising from a reversal of the acidosis (reversing the Bohr effect) and direct effects of lactate and calcium ions on the oxygen-binding properties of haemocyanin (Taylor and Whitely 1989, Whiteley and Taylor 1990, Burnett 1992). Whether the ceiling of lactate concentration in the blood of emersed spanner crabs can be explained by a return to aerobic metabolism depends on how successfully the crab is able to counter the deterioration in oxygen carrying capacity (Bohr Shift) that must inevitably accompany a fall in blood pH of 0.6 units.

A rapid rise in blood lactic acid concentration during the first few hours that they are out of water, followed by a plateau, is almost without precedent in the literature, and the general impression gained is that the rate of lactic acid accumulation is continuous while species are stored in air. There could be more than one reason for this. Studies of commercially important species, such as *Cancer productus* and *Callinectes sapidus* stored in air are typically only a couple of hours in duration, so we can not say that the lactic acid concentration of the blood will not approach a ceiling after that point. However, the primary explanation for the rapid rise in lactate concentration followed by a ceiling could be the manner in which the crabs or lobsters are emersed.

The other cases in the literature where the concentration of lactic acid in the blood rises rapidly, subsequently reaching a plateau or even decreasing, occur when the animals are taken from the water by hand (Taylor and Wheatly 1981, Spicer et al 1990). Disturbance during commercial handling increases the activity and metabolic rate of emersed *H. gammarus* (Taylor and Whiteley 1989, Whiteley and Taylor 1990). Under these circumstances they show a rapid uncompensated acidosis similar to that seen here in spanner crabs (Taylor and Whiteley 1989, Whiteley and Taylor 1990). However, if left undisturbed this species accumulates lactate at a steady rate (Whiteley and Taylor 1990).

Perhaps if you remove any crustacean from the water by hand the lactate concentration in its blood will rise most rapidly in the first couple of hours, because it is disturbed and active at the very time when its oxygen uptake fails. This may in part explain why Spicer et al (1990) found that lactate accumulated at the same rate in the norway lobster *Nephrops norvegicus* regardless of whether they were placed on ice or stored at 10°C. The lobsters placed on ice will not cool down immediately to 0°C (Spicer et al 1990), so

if the initial disturbance dominates their metabolic response then the iced lobsters may accumulate just as much lactate as lobsters that are not iced. Experiments dealing with the responses of aquatic crustaceans that leave the water voluntarily must be designed in such a way that this "artefact" is eliminated, often by draining the water away from around the animal. However, from a commercial perspective this response is quite real: Crabs and lobsters cannot be harvested without handling them.

The apparent robustness of the spanner crab when stored on the deck of a fishing boat, is very misleading. Any period in air stresses spanner crabs. They show a rapid and uncompensated acidosis when lifted from the water and only survive long periods out of water during commercial handling by becoming quiescent in response to severe acidosis. Once this happens no more lactic acid enters the blood, and the acidosis slows down. The crabs cannot buffer their blood pH so rather than leaving them to their own devices, they should be cooled down as quickly as possible after they leave the water, for example by briefly dipping them in cold seawater or by placing them immediately under a spray of cold seawater. This would reduce their activity and metabolic rate from the very start and curtail the magnitude of the initial acidosis.

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SPRAYING SEAWATER OVER SPANNER CRABS (*RANINA RANINA*) STORED IN AIR REDUCES THE RATE OF ACIDOSIS AND LACTATE ACCUMULATION

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SUMMARY

The spanner crab Ranina ranina *is marketed live and is typically stored out of water after harvest for up to 8 hours or more. Seawater sprays have been suggested as a means to improve the survival of the crabs during live handling. In this study, spanner crabs were stored out of water for up to 12 hours, either in humid air at 19°C or under a sea-water spray at the same temperature. The pH and accumulation of lactic acid in the blood of these crabs was measured to see whether the spray played a physiological role other than to keep the crabs wet. In crabs stored out of water with or without the spray the blood pH decreased significantly in the first 3 hours but reached a "plateau" after that and did not change significantly for the next 9 hours. The blood pH reached a significantly lower plateau in crabs that were stored in air alone and the fastest rise in blood lactic acid concentration also occurred in this treatment. The sea-water spray not only improved the crab's ability to regulate its blood pH when stored out of the water (probably by increasing the rate of CO_2 excretion) but it also reduced the animal's reliance upon anaerobiosis to meet its energy needs during storage in air. When storing spanner crabs out of water, it is recommended that sea-water sprays be used, to reduce the physiological consequences of asphyxiation. Water sprays used for storing other live crustaceans may alleviate the stress of storing out of water to a greater extent than has hitherto been realised.*

INTRODUCTION

The spanner or Kona crab *Ranina ranina* is marketed live in Taiwan. In Hawaii, the boats used to harvest this species are equipped with "live wells," (Ian Brown personal communication) allowing the catch to be stored submerged until it is returned to shore. However, the Australian fishery often uses small speed boats and the crabs typically remain out of water after harvest for up to 8 hours or more until the boat returns to shore, where the catch can be transferred to seawater storage tanks.

Aquatic crustaceans are prone to desiccation when stored out of the water if not kept in cool humid conditions (Taylor et al 1987). Seawater sprays are sometimes used to keep the catch wet, with the tacit assumption that the spray is the next best thing to keeping the product submerged. Spraying seawater over crabs that are stored in air may sound like common sense. However, this assumes that the water sprayed over the outside of the crab comes in contact with the gills, the organs which are the primary site of gas exchange and ionic and acid-base regulation (Henry and Wheatly 1992).

When aquatic crustaceans are lifted from the water, exchange of oxygen and carbon dioxide (and bicarbonate ions) is curtailed and the animal rapidly asphyxiates unless it can reduce its demand for oxygen or in other ways compensate for the fall in blood pH that occurs as carbon dioxide accumulates in the blood, (Taylor and Innes 1988). If oxygen demand is not reduced then lactic acid may accumulate in the blood, since the animals rely increasingly upon anaerobic metabolism to meet their energy demands, a change that contributes to the fall in blood pH (Taylor and Innes 1988).

Seawater sprays may have a physiological benefit if they allow water to accumulate in the gill chamber. Burnett and McMahon (1987) have proposed that the water that remains in the gill chamber while crabs are in air continues to play a major role in excretion of carbon dioxide and regulation of blood pH, particularly in species that can regulate the chloride concentration of their blood in the face of changing salinity. Apart from its role in ionic regulation, the Cl^-/HCO_3^- ion exchanger on the crab gill is also an important mechanism of pH regulation, since movements of bicarbonate ions (an ionised form of carbon dioxide) influence blood pH. deFur et al (1983) found that small (ie. < 100g) red rock crabs *Cancer productus* were better able to regulate their blood pH if they were exposed to air while buried in sand, than when exposed without a substrate. Apparently, the buried crabs draw enough water into their gill chambers from the sand around them to continue to excrete carbon dioxide even though their oxygen uptake remains depressed (deFur et al 1983).

As an oceanic crab, spanner crabs cannot regulate the chloride concentration of their blood, so it is unlikely that they use ionic regulation to control the pH of the blood when stored in air. Nevertheless, a spanner crab in a seawater spray may draw enough water into its gill chamber to continue carbon dioxide excretion. Some species of aquatic crustaceans show air-gulping or a partial emersion response during aquatic hypoxia-simultaneously taking advantage of the oxygen available in the air while continuing to use the water as a medium for CO_2 excretion (Taylor and Spicer 1988). However, the case for a physiological role for seawater sprays is not clear cut. Varley and Greenaway (1992) recently found that a spray did not favour carbon dioxide excretion in mud crabs, *Scylla serrata* stored at a fish market.

In this paper we examined the pH and lactic acid concentration in the blood of spanner crabs stored at 19°C either in air or in a seawater spray to see whether the spray played a measureable physiological role beyond that of keeping the crabs wet. We wanted to see if spray systems used for the storage of this and other crustaceans contribute more to the physiological well being of the product than has hitherto been realised.

MATERIALS AND METHODS

Live spanner crabs were purchased from a commercial supplier (Mooloolaba Fisheries) and stored in a recirculating sea-water holding tank (temperature 19°C) at IFIQ. The experiment began 4 days after the crabs arrived.

Experimental protocol

A group of 40 crabs (430.4±134.2 g, range 332-1088g) were taken from the tank using a scoop net and placed in plastic tubs, 10 crabs per tub. Two tubs of crabs were placed under the spray (Spray) and two tubs (No spray) were placed floating on the top of the water, covered and held in position by the lid of the tank (temperature 19°C, and >90% humidity). The remaining crabs in the tank were used as controls. For the spray treatment, water from the holding tank was sprayed over the crabs using a submersible pump connected to a manifold fitted with garden micro-irrigation sprays. The excess flow from the pump was released back into the tank using a tap and bypass.

After 3 hours, blood samples were taken from 8 crabs in each treatment (No spray and Spray) and from 4 submerged crabs. This process was repeated again 12 hours after the start of the experiment.

Blood sampling and analysis

Samples of blood (about 0.8 ml) were taken from the crabs using an ice-cold glass Hamilton syringe and 22 gauge hypodermic needle. A hole was carefully punched through the carapace above the pericardium to allow easy penetration of the sampling needle.

The pH of each blood sample was determined anaerobically using a Radiometer G299A microcapillary electrode connected to a Radiometer BMS3 Mk2 thermostated to the experimental temperature (19°C) and calibrated using precision buffers. After measuring the pH, a 400 μl sample of the blood was mixed with an equal volume of ice-cold perchloric acid (0.6 mmol/l) in a labelled vial and the precipitated blood was stored in a freezer until ready to continue extraction and determination of lactic acid concentration (Boehringer-Mannheim cat. 139084).

RESULTS

Blood pH

The crabs in the No spray treatment were more acidotic (pH fell lower) than the crabs in the spray (Figure 1a). Blood pH of crabs resting in the aquarium at 19°C was 7.77±0.05 (mean±SD, n=8). When crabs were stored in humid air or a seawater spray at that temperature the blood pH fell significantly in both treatments during the first 3 hours but thereafter did not change significantly (Figure 1). The blood pH of 8 crabs stored for 3 hours in the spray treatment was 7.62±0.03, significantly higher than 8 crabs in the air treatment, 7.44±0.10.

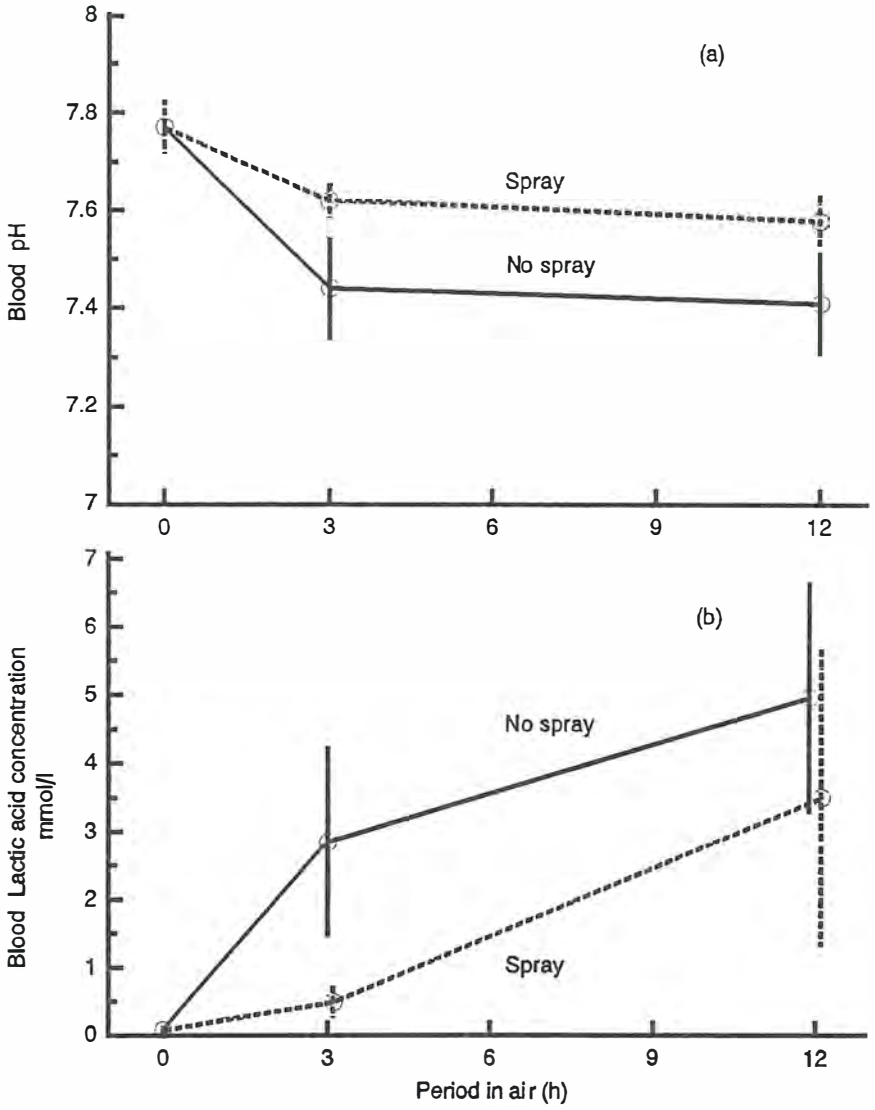

Figure 1. Effects of spraying seawater on the mean pH and lactic acid concentration (± SD) in the blood of spanner crabs stored out of water. Each point represents 8 crabs.

Blood lactic acid concentration

Lactic acid accumulated more rapidly in the No spray treatment, particularly in the first 3 hours of the experiment (Figure 1b). The concentration of lactic acid in the blood of submerged crabs was 0.08±0.03 mmol/l, rising rapidly to 2.84±1.36 mmol/l after three hours. Lactic acid accumulated more slowly in the sprayed crabs (0.48±0.20 mmol/l after 3 hours) but because the data shows heterogeneity of variances it is necessary to transform the raw data using log transformation and the log means were then compared using an LSD (t) pairwise comparison (Table 1). This showed that the rise in lactate concentration in the blood was significantly slower in the sprayed crabs, though it was apparently only delayed since lactate concentration rose later in the experiment.

Table 1. Comparison of mean lactate concentration (mmol/l) in the blood of crabs in the spray and no spray treatments after log transformation. Log means sharing the same letter are not significantly different at 1%.

		Treatment			
	Submerged	Spray		No spray	
		3h	12h	3h	12h
Log means	-1.1164*a*	-0.3649*b*	0.4594*c*	0.3907*c*	0.6585*c*
back transformed means	0.076	0.432	2.880	2.459	4.555

DISCUSSION

Spraying cold seawater over spanner crabs stored in air allows them to ease to a certain extent the physiological symptoms of asphyxiation, particularly during the first few hours in air when the greatest change in blood pH occurs. The spray allows them to regulate the pH of their blood at a higher level than would otherwise be possible, even to the extent of reducing the rate of lactic acid accumulation in the blood.

The gill is the principle site of gas exchange, ionic regulation and acid-base regulation in aquatic crustaceans (Henry and Wheatly 1992). When aquatic crustaceans are removed from the water they experience a rapid fall in blood pH (acidosis) caused by both the failure to excrete carbon dioxide (CO_2) ("respiratory acidosis") as well as the accumulation of lactic acid in tissues starved of oxygen ("metabolic acidosis") (Taylor and Innes 1988).

In some species, this acidosis can in the short term be slowed and later on even reversed ("alkalosis") by increasing the bicarbonate ion concentration in the blood (Taylor and Whiteley 1989). In the freshwater crayfish *Austropotamobius pallipes* and the mud crab *Scylla serrata*, the blood pH returns to a point close to that of the submerged animal (Tyler-Jones and Taylor 1988, Varley and Greenaway 1992). *Ranina* shares with some other commercial species such as *Homarus gammarus* the ability to arrest acidosis during

commercial handling (Taylor and Whiteley 1989). Some species are not so capable. The spider crab *Maia squinado* and the velvet swimming crab *Liocarcinus puber* rapidly succumb to worsening acidosis and the lactic acid concentration in the blood continues to increase in crabs stored in air (Taylor and Innes 1988, Johnson and Uglow 1987).

The amount of lactic acid that accumulates when spanner crabs are stored in air at 19°C is similar to that seen in other commercial species (deFur and McMahon 1984, deFur et al 1988, Spicer et al 1990, Johnson and Uglow 1985) though emersion at higher temperatures leads to a more dramatic accumulation of lactate (Paterson et al 1994 a&b). Low temperature is commonly used to reduce the metabolic rate and hence the rate of acidosis when aquatic crustaceans are stored in air (Whiteley and Taylor 1990).

Some aquatic crustaceans accumulate very little lactic acid during exposure to air but in other species it rises progressively (Burnett and McMahon 1987, deFur et al 1988, Whiteley et al 1990, Whiteley and Taylor 1990). *Ranina* stored in air shows a different pattern. After an initial rapid increase, the rate of lactic acid accumulation slows, a response also shown by the norway lobster *Nephrops norvegicus* during emersion (Spicer et al 1992). The profile of acidosis and the apparent ceiling of lactate concentration in the blood of aquatic crustaceans may not be wholly a response to emersion. For example a similar response, a rapid rise followed by a plateau is found when crabs exercise underwater (McDonald et al 1979, Booth and McMahon 1985) and exercise in air is expected to exaggerate this response (Burke 1979). Once the crabs stop exercising and become quiescent in air they may cease producing lactic acid but be unable to re-oxidise the lactic acid already produced. This topic is the subject of a separate study (Paterson et al 1994b).

How does the spray work?

The rate of acidosis of crabs in a cold spray is less than you expect from crabs kept in humid air at the same temperature. The principle effect of the spray, in this species at least, seems to be to control the rate of acidosis during the first few hours of emersion.

Contrary to expectations, crabs stored in the spray accumulated less lactic acid during the initial few hours of the experiment. A reduced level of anaerobic glycolysis is not something that would be predicted on the basis of our hypothesis. Small amounts of water entering the gill chamber may act as an important sink for carbon dioxide excretion during the acidosis at the beginning of emersion but it would not be expected to favour oxygen uptake, (deFur et al 1983). Carbon dioxide is very much more soluble in water than oxygen is.

The link with anaerobic metabolism may be indirect. There are several possibilities. Perhaps the crabs in the spray treatment were more quiescent than those in the air treatment. Casual observations during sampling suggest the opposite, that the crabs from the spray treatment were more vigorous than the crabs from the air treatment, and this may be a direct consequence of the degree of acidosis and lactic acid accumulation. If the two groups of crabs have a similar demand for oxygen, then perhaps the wetting effect of

the spray may enhance gas exchange at the gill. But this could just as reasonably retard oxygen uptake, by keeping the gill water-logged and reducing the surface area available to exploit atmospheric oxygen (deFur et al 1988).

If oxygen demand at the tissues is equal and the efficiency of the gill remains the same, then perhaps the blood of the crabs in the spray treatment is better able to deliver oxygen to the tissues. The degree of acidosis, and consequently the magnitude of the predicted Bohr Effect, is less in crabs in the spray treatment. With the low blood oxygen tensions typical of emersed crabs, the Bohr effect will reduce the ability to load oxygen at the gills (deFur and McMahon 1984a, deFur et al 1988).

Some species of commercial crab or lobster can negate the Bohr effect and continue or resume aerobiosis when stored out of water because of mediating effects of lactate and calcium ions on the binding properties of the animal's oxygen transport pigment, haemocyanin (Taylor and Whiteley 1989, Burnett 1992). This may indeed be one of the factors contributing to the reduction in lactic acid accumulation rate seen in quiescent crabs stored for long periods in the no-spray treatment, but further work is required to demonstrate this.

Interestingly, after the initial delay in the rate of lactic acid accumulation in the spray treatment, the level of this metabolite in the blood rose in crabs stored for longer periods in the spray. But Figure 1b may show not so much a rise in the rate of anaerobiosis, caused for example by a later failure in oxygen transport to the tissues, but a delayed release of the lactate accumulated in the tissues during the initial struggling on exposure to air. Further work is required to show this, particularly to see if crabs stored in sprays on boats also show this later rise in lactate concentration.

Conclusions and further work

Spray systems are already used for keeping freshwater crayfish (*Cherax* spp.) and mud crabs *Scylla serrata*. It would be hard to find someone who said it was not a good idea. Yet despite the practise having a status close to being "common sense" the literature is curiously ambivalent about its efficacy. Some studies report that keeping crabs or lobsters wet or damp using sprays, "periodic wetting" or wet sacks improves their survival (Witham 1971, Simonson and Hochberg 1986) while others report no effect at all (McLeese 1965, Vermeer 1987). The reason for the discrepancy may relate to the amount of water actually passing over the crabs as well as to causes of mortality unrelated to the spray system.

In some cases, water sprays may do nothing more than wet the crabs. Given the clear benefit of storing spanner crabs in a spray system, it is interesting that Varley and Greenaway (1992) found elevated total CO_2 concentration and CO_2 tension in the blood of mud crabs *Scylla serrata* stored in a spray system at a fish market. The crabs were apparently using their normal mechanism for buffering acidosis in the absence of contact with water. The industry thinks that just because the crabs "survive" in these storage units that there is no problem. However, we would argue that they might as well turn off the

spray, because the only reason why the crabs survive is because they are remarkably tolerant of stress. The best practise for a crab processor to follow would be to improve the condition of the crabs by making sure that the storage method they use is as stress free as possible.

The sea-water spray not only improves the spanner crabs ability to regulate its blood pH when stored out of the water (probably by improving the rate of CO_2 excretion) but it apparently also reduces the animals reliance upon anaerobiosis to meet its energy needs during storage in air. When storing any aquatic crabs out of water, it is recommended that refrigerated sea-water sprays be used, to reduce the physiological consequences of asphyxiation.

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ADDING OXYGEN TO ASSIST CRUSTACEANS DURING LIVE TRANSPORT- AND WHY IT HARMS SPANNER CRABS

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SUMMARY

Aquatic crustaceans generally have difficulty getting oxygen and excreting carbon dioxide when they are lifted from the water. Carbon dioxide typically accumulates in the blood and, amongst other things, forces the pH of the blood to fall unless internal mechanisms are able to arrest this change. Adding oxygen, in solid or gaseous form, to the container is sometimes suggested as a way to assist the respiration of these animals during live transport but no studies have been conducted of the physiological consequences of this. The spanner crab Ranina ranina *is a good example of a crab that is poorly suited to transport out of water, so oxygen might conceivably be of practical use for shipping this species. We found that storing spanner crabs in an oxygen-enriched atmosphere enhanced the flow of oxygen into the crab. However, this did not do the crab any good at all- because it produced more carbon dioxide than the crab normally would when held in air. This species is unable to cope with excessive amounts of carbon dioxide and its blood pH falls to a lower level if oxygen is added than if the crabs were left in ordinary air. This result calls into question other instances where oxygen is used to help live seafood to "last the distance" while transported out of water. "Aquatic" species which have high rates of oxygen consumption in air may require a shot of oxygen if confined in a closed box. Fortunately, crustaceans like mud crabs and freshwater crayfish which are more tolerant of being out of water are just those forms that are already adapted to deal with excessive levels of carbon dioxide in their blood. Perhaps you can only use oxygen to help the tough species last longer, but not to carry the weak species over the finishing line.*

INTRODUCTION

Aquatic crustaceans generally have difficulty getting oxygen (O_2) when they are stored out of water because their gills collapse. When crustaceans cannot get enough oxygen to satisfy their needs they go "anaerobic" and produce lactic acid rather than carbon dioxide (CO_2) as a waste product of metabolism (de Fur et al 1988) and the acidity (pH) of the blood and tissues falls- a symptom called acidosis. Adding oxygen to the container, either directly as a gas, or using a chemical generator, is sometimes suggested as a way to assist the respiration of crustaceans and other seafood stored out of water. Yet, there appears to have been no attempt to investigate the physiological consequences of this intervention on the animals themselves.

Adding oxygen is not the "common sense" solution that it first seems. McLeese (1965) reported that oxygen-rich air actually killed American lobsters (*Homarus americanus*)

Adding oxygen is not the "common sense" solution that it first seems. McLeese (1965) reported that oxygen-rich air actually killed American lobsters (*Homarus americanus*) faster than ordinary atmosphere. Excretion of carbon dioxide is also a limiting factor during live transport, probably more so because carbon dioxide dissolves readily in blood as the bicarbonate ion (HCO_3^-). This ionised form of carbon dioxide is readily excreted when the animal is submerged but remains in the blood when the animal is in air.

Rising concentrations of carbon dioxide dissolved in the blood also cause the pH of the blood to fall (Taylor and Whiteley 1989, Whiteley and Taylor 1990). So, regardless of whether or not a crustacean is producing lactic acid, its blood becomes more acidic in air. This symptom is thought to be a major cause of death when aquatic crustaceans are shipped out of water, (Vermeer 1987, Whiteley and Taylor 1992) since metabolic processes require the blood and tissues to remain within the "normal" range of pH. Some species of crustaceans that voluntarily leave the water can actually slow down the acidosis and reverse it. In the commercial sphere, acidosis is usually slowed by keeping the product cool during shipment. This lowers its metabolic rate, reducing the build up of wastes, and in some circumstances can reduce the metabolic rate to the point that it is easily satisfied by the limited amount of oxygen uptake that is possible in air (Whiteley et al 1990).

POSSIBLE EFFECTS OF TOO MUCH OXYGEN

Acidosis may be the reason why oxygen killed the lobsters mentioned above. Storing product in a pure oxygen atmosphere is expected to increase the rate of oxygen consumption. This may avoid the production of lactic acid by anaerobiosis but it would still cause a higher than usual amount of carbon dioxide to accumulate in the blood. This accumulation of carbon dioxide has the potential to exaggerate rather than circumvent the acidosis experienced by some species when held in air.

But acidosis even occurs if submerged crustaceans are exposed to high amounts of oxygen. Physiological studies show that raising the oxygen level of water above normal saturation causes acidosis in submerged crabs, apparently because heart activity and blood circulation is retarded by the abnormal amounts of oxygen present in the water (Wheatly, 1987). Put simply, the crabs are so overwhelmed by all the oxygen that they don't breathe properly and the CO_2 level in their blood rises (Sinha and Dejours 1980). Corresponding studies have not been conducted on aquatic crustaceans stored in high oxygen atmospheres to see if abnormally high levels of oxygen also occur in the blood under these circumstances.

Despite these physiological misgivings about the use of oxygen to assist crustaceans and other seafood during live transport, the practice is well entrenched in a number of aquaculture industries, for example transport of fish and fish fry, prawn broodstock and post larvae and even when transporting freshwater crayfish in air. There must be other factors coming into play to determine whether or not oxygen treatment is suitable for the transport of live seafood.

The spanner or Kona crab (*Ranina ranina*) is marketed live in Taiwan, where it has a reputation of not enduring live transport very well. We have been studying the performance of this species during simulated live transport and storage to try to find ways of improving its survival in air. In this simple experiment, we looked at gas levels and pH in the blood of spanner crabs stored in air under high oxygen conditions to see whether it would be any use to export them in oxygen. The results come some way to explaining why oxygen may work for some species but not others.

THE EXPERIMENT

The crabs were purchased from a commercial supplier in Mooloolaba (Qld) and rested for 2 days in a recirculating seawater aquarium at the International Food Institute of Queensland (IFIQ) and then packed with 1 kg of gel-ice and wood wool into polystyrene boxes and air-freighted to Sydney. The crabs were left overnight to recover in a recirculating seawater aquarium at the School of Biological Sciences at the University of New South Wales, Kensington Campus (UNSW) before conducting the experiment.

The experiment was carried out in a controlled temperature cabinet containing a water bath (25°C). The treated crabs were stored out of water for 3 hours because previous experiments showed that the most dramatic change in blood pH occurred within this time. Two treatments were used; either ordinary atmosphere (which has an oxygen partial pressure of about 22 kPa) or an atmosphere augmented using a cylinder of oxygen to at least twice the normal level (>40 kPa). Other crabs were placed into the cabinet as controls, stored for the same period in a tub containing aerated seawater. Six crabs were used in each treatment.

Samples of blood were taken from crabs in the different treatments and blood gas levels (in kilopascals, kPa) and pH was measured using a Radiometer Blood Micro-System and Blood Gas Monitor thermostated to the experimental temperature (25°C) and calibrated using precision buffers and known mixtures of gases. Total carbon dioxide concentration in the blood (carbon dioxide CO_2 plus its associated ions, bicarbonate HCO_3^- and carbonate CO_3^{2-}) was measured according to the method of Cameron (1971) and expressed in mmol/l (millimoles per litre).

RESULTS AND DISCUSSION

The mean oxygen tension in the blood of spanner crabs stored in oxygenated air for 3 hours was not significantly different from that of crabs submerged in aerated seawater for that time (Figure 1). When crabs were stored in ordinary atmosphere for this period, the mean oxygen tension in the blood was significantly lower than that of crabs submerged in aerated seawater.

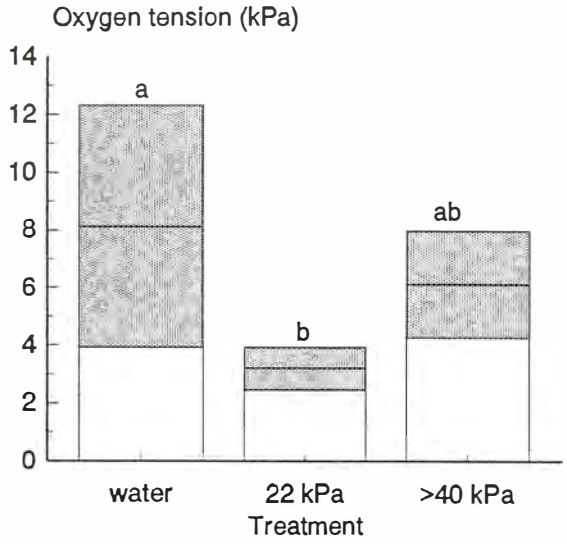

Figure 1. Mean oxygen (O_2) partial pressure (kPa) in the blood of spanner crabs stored at 25°C for 3 hours in aerated seawater ('water'), normal atmosphere ('22 kPa') and in an oxygen-enriched atmosphere ('>40 kPa'). Shaded region of each histogram represents one standard deviation above and below the mean. Six crabs per treatment. Bars with the same letter over them are not significantly different at 5%.

Removing crabs from the water caused a significant increase in both carbon dioxide content and partial pressure and a significant fall in blood pH (Figure 2). These symptoms were exaggerated in crabs stored in an oxygen-rich atmosphere (>40 kPa).

Storing the crabs in an atmosphere that was high in oxygen compensated for the inefficiency of the gills in air by increasing the diffusion gradient between the air and the blood. Nevertheless, abnormal amounts of oxygen did not appear in the blood. When the crabs were stored in "hyperoxia" (>40 kPa) the level of oxygen in the blood was similar to that you expect to see in a submerged crab. This could arise because the gills were wet with stagnant water, with little surface area in contact with the air (de Fur et al 1988).

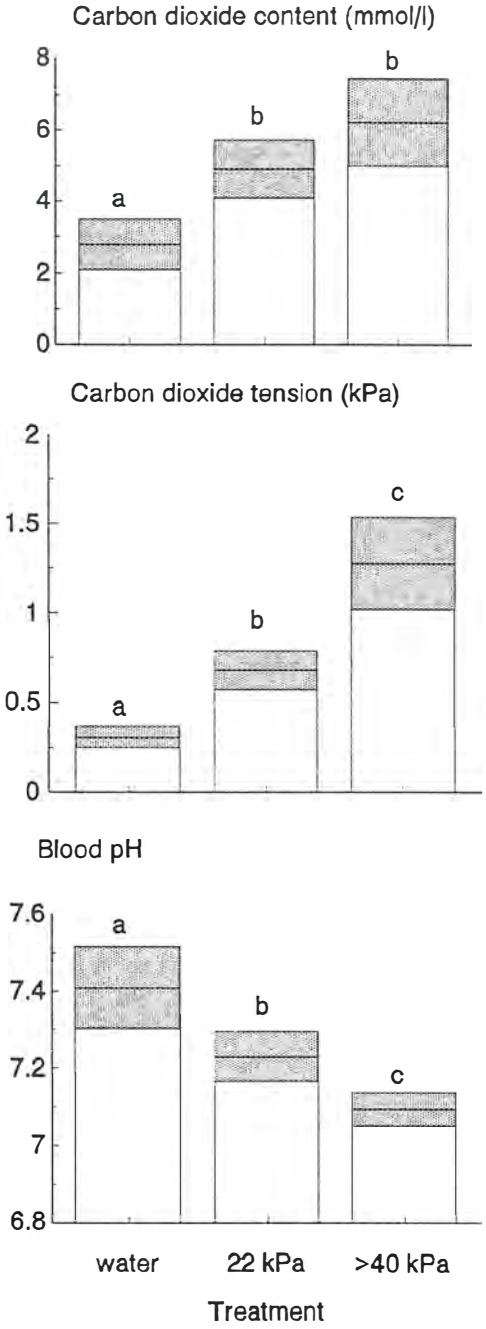

Figure 2. Mean carbon dioxide (CO_2) content, CO_2 partial pressure (kPa) and pH in the blood of spanner crabs stored at 25°C for 3 hours in aerated seawater ('water'), normal atmosphere ('22 kPa') and in an oxygen-enriched atmosphere ('>40 kPa'). Shaded region of each histogram represents one standard deviation above and below the mean. Six crabs per treatment. For individual graphs, bars with the same letter over them are not significantly different at 5%.

However the high oxygen partial pressure in the blood of crabs from the high oxygen treatment could be misleading. We did not measure the oxygen *content* of the blood in these crabs, which includes the oxygen that is present in solution as a gas, as well as the far larger proportion that is usually bound to a special transport pigment called haemocyanin. This pigment gives the blood a blue-grey colour when oxygen is bound to it. The pH of the blood was so low when crabs were stored in air (Figure 2) that the contribution of the haemocyanin to oxygen delivery to the tissues is likely to be curtailed by a property of this pigment known as the "Bohr effect." As the pH of the blood falls, oxygen no longer associates as readily with the pigment molecule so the blood cannot "carry" as much oxygen to the tissues as it used to. When this happens the oxygen content falls and the blood also becomes colourless. Taylor and Innes (1988) noted that acidosis developed so rapidly in the blood of the spider crab *Maia squinado* that the crabs probably asphyxiated because the blood pH falls too low for the haemocyanin to carry oxygen to the tissues. A rapid fall in blood pH of similar magnitude occurred when the spanner crab was stored in air.

The crabs did not breathe any easier in the oxygenated atmosphere, that is, their blood physiology did not become more like that of submerged crabs. On the contrary, the oxygen treatment exaggerated the symptoms seen when crabs were stored out of water. The spanner crab's big problem is that it cannot handle acidosis. Adding oxygen makes the situation worse, not better. Even if the high oxygen partial pressure prevents the crabs from becoming anaerobic, avoiding the production of lactic acid (not measured here), it still causes a higher than usual amount of carbon dioxide to accumulate in the blood (both in terms of partial pressure kPa, and total content mmol/l). This build up of carbon dioxide exaggerates the acidosis experienced by spanner crabs stored in air.

You might object that spanner crabs are normally transported out of water at temperatures much lower than 25°C, under circumstances when their metabolic rate is reduced, but the conclusion still holds. Increasing their oxygen uptake rate at 15°C would still produce more carbon dioxide than usual, a situation which spanner crabs are not equipped to deal with at any temperature. In this experiment, a temperature of 25°C was chosen to allow these results to be compared with our other experiments on spanner crabs (which are sometimes stored out of water on boats at temperatures higher than 25°C) and to published literature on other species.

What about other species?

This result calls into question other instances where oxygen is used to help the live seafood to "last the distance" while transported out of water. This practice arises because the shippers fear that the product will consume all of the oxygen from the air in the container during transport. While you might think this unlikely when dealing with an aquatic animal in air, "back of the envelope" calculations using published data (eg: Taylor and Wheatly 1981) suggest that this outcome is quite possible when shipping species that voluntarily leave the water under some circumstances, such as the mud crab *Scylla serrata* and various species of freshwater crayfish (eg *Cherax quadricarinatus*). Cutting holes in the side of the box is a simpler solution, though this makes it difficult to stop the

contents from warming rapidly during transport. Oxygen is an alternative when the box must remain cool and air-tight. Fortunately, species with a relatively high rate of oxygen consumption in air are able to handle the correspondingly high rates of carbon dioxide accumulation and begin to reverse the acidosis (Varley and Greenaway 1992). An oxygen-rich atmosphere may not be as stressful to these species as it is to the spanner crab. Perhaps you can only use a shot of oxygen to help a tough species to live longer- not to assist a weak species. It would be interesting to see whether the level of dissolved oxygen in the blood of these "air-breathing" species rises to extraordinary levels when they are stored in an oxygen-rich atmosphere.

The issue of storing fish and crustaceans in water with extraordinary amounts of oxygen in it also pays further attention. Disturbances in gill ventilation and blood circulation are likely, though while the gills are underwater these organs can play an important role in regulating the internal physiology of the animal (Wheatly 1987). Any stress occurring under these circumstances must obviously be reconciled against the certainty that the animals concerned, such as prawn broodstock or live fish destined for export, would soon die without oxygen. For many species of fish and crustaceans, it would be better to start with too much oxygen in the water rather than to end up with too little.

CONCLUSIONS

Storing spanner crabs in an oxygen-rich atmosphere is not likely to help them to survive any longer. This treatment may increase the flow of oxygen into the crab, overcoming the impaired functioning of the gills in air, but the spanner crab is unable to cope with the consequences: augmented production of carbon dioxide, promoting acidosis in the blood. Storage in atmospheres rich in oxygen may only be appropriate for species that are better able to respire in air and which can regulate the pH of their blood under these circumstances.

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PART 3

CONFIDENTIAL MATERIAL

B. Paterson, Paul Exley and Ross Smith (Editors). *Live transport of crustaceans in air- prolonging the survival of crabs*. Report to the Fisheries Research and Development Corporation (Project 92/71), Department of Primary Industries, Queensland, December 1994. pp 59-69.

DOES ACIDOSIS REALLY KILL SPANNER CRABS IN AIR? BUFFERING BLOOD ACIDOSIS DURING STORAGE OUT OF WATER DOES NOT DELAY DEATH

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SUMMARY

Spanner crabs experience a rapid fall in blood pH (acidosis) during the first couple of hours in air. Acidosis is thought to be a major causes of mortality when live crustaceans are transported in air. This symptom is usually treated using low temperature, this reduces the animal's metabolic rate and consequently the rate at which acidic wastes build up in its blood. But controlling temperature is not always possible in practise. In this study, we attempted to prolong the survival of spanner crabs in air at constant temperature by dipping them beforehand in a solution of sodium bicarbonate (Baking Soda) in seawater. The dip (5g $NaHCO_3$ per litre of seawater) slowed down the rate of acidosis in the crabs by raising the blood bicarbonate concentration. However, several trials showed that the dipped crabs did not live any longer than controls that were sham dipped in ordinary seawater. The degree of acidosis, though extreme, stabilises in the sham-dipped crabs. They stopped short of dying and lingered on for quite a while, allowing the blood pH of the dipped crabs to catch up to them. Crabs dipped in this solution after capture showed no improvement of survival during long term storage in holding tanks. Buffering blood acidosis using sodium bicarbonate does not prolong the survival of spanner crabs. Perhaps the dip treatment stresses the crab and this counteracts the anticipated benefit of controlling acidosis. However, the survival of the treated crabs was no worse, so the effect of the dip may be neutral. Acidosis may not be what kills spanner crabs in air. Rather, the control crabs may also stop acidosis reaching fatal levels and all crabs survive long enough in air for something else to kill them.

INTRODUCTION

Acidosis is thought to be a major cause of death when crustaceans are transported since metabolic processes require the blood and tissues to remain within a certain "physiological" range of pH (Vermeer 1987, Whiteley and Taylor 1990). The acidosis occurs when aquatic crabs are lifted from the water because they have difficulty taking in oxygen and excreting carbon dioxide, resulting in a decline in blood pH as carbon dioxide and lactic acid (produced when oxygen supply is limited) accumulate in their tissues and blood. After this initial acidosis, crustaceans usually compensate or buffer the change in pH by adjusting the ionic composition of the blood, particularly by a large increase in the concentration of bicarbonate ions. However, the spanner crab is unusual amongst crustaceans that are transported live in that it is unable to compensate for falling pH in its blood by raising the total CO_2 concentration (largely present as bicarbonate ion) (Paterson et al., 1994a). The crabs inability to compensate for the accumulation of carbon dioxide

and lactic acid make it vulnerable to acidosis during export.

From a practical perspective, acidosis is usually addressed by cooling the animals down during shipment. This lowers their metabolic rate, reducing the production of CO_2 and lactic acid, and in some circumstances can reduce the metabolic rate to the point that it is easily satisfied by the limited amount of oxygen uptake that is possible in air (Whiteley et al., 1990). However, keeping the product cold requires frozen coolants that add to the cost of the packaging and air-freight. Any saving in weight and cost is obviously a benefit where the profitability of the market is marginal. Further, a physiological method of prolonging survival may help to reduce the variation in survival between shipments-seeing the product arriving at the market in a more vigorous condition.

One way to do this might be to simulate the normal buffering process that occurs in crustaceans. A major mechanism of pH regulation in aquatic animals involves exchange of bicarbonate ions at the gills, linked to movements of chloride ions in the other direction (Dejours et al., 1982). When contact with the water is lost, the animal may depend upon dissolution of $CaCO_3$ reserves in the exoskeleton and/or digestive gland to release bicarbonate ions into circulation (Cameron 1985, Spaargaren 1990, Truchot 1984). Manipulating the bicarbonate level in the surrounding water does effect the blood pH of crustaceans (Truchot 1984, Spaargaren 1990), and preliminary tests showed that dipping spanner crabs beforehand in a solution of sodium bicarbonate slowed the rate of acidosis when they were stored in air.

This is apparently the first time anyone has suggested that Baking Soda be used to treat acidosis when transporting live crustaceans in air. This compound is proposed as an anaesthetic for transporting fish in water, however this purpose requires much lower concentrations than that used in this study and the active constituent is CO_2 rather than the bicarbonate ion per se (Booke et al., 1978, Takeda and Itazawa, 1983). Oral doses of sodium bicarbonate are already used to alleviate acidosis in terrestrial vertebrates by raising the total carbon dioxide concentration of the blood. For example this compound is given to treat acidosis in diarrhetic calves (Grove-White and White 1993) and it is a component of the "milkshakes" that are believed to improve the performance of racehorses (Lloyd et al 1993). Ingested bicarbonate changes the carbon dioxide and pH level of blood and disturbs the breathing patterns of terrestrial vertebrates such as horses and humans. However, these effects on respiration will not apply in the same way to aquatic organisms because these forms use the oxygen level in their blood rather than carbon dioxide to control gas exchange. The anaesthetic and toxic effects of carbon dioxide on fish are associated with a failure of the red blood cells to transport oxygen. However, crustaceans don't have red cells and nothing is known about what CO_2 levels will kill crustaceans.

This paper examines the mechanism by which the bicarbonate dip affects the pH of crab blood and then considers whether crabs live any longer if they are treated in this manner prior to being stored out of water. The results suggest that despite the fact that the dip does alleviate acidosis in spanner crabs, this symptom may not be the factor that ultimately kills spanner crabs during live storage and transport.

HOW DOES THE BUFFER WORK?

This experiment was conducted alongside that of a study described elsewhere in the public release section of this report (see the "Acid-base balance during emersion" experiment described in Paterson et al 1994a). Briefly, the crabs were stored out of water at 25°C for up to 7 hours. In order to test the dip, additional crabs were treated in the same way as the untreated crabs described in the other paper except that 30 minutes prior to taking the crabs from the water a weighed quantity of sodium bicarbonate was added to the water bathing them, in order to raise the bicarbonate concentration by 60 mmol/l. The gas levels and acid-base balance of this treated group of crabs was then followed for 7 hours, corresponding to the times that samples were taken from the untreated crabs as described previously.

Exposing crabs to a medium of excess bicarbonate prior to emersion has a dramatic effect on their CO_2 equilibrium (Figure 1). The extra bicarbonate added to the medium raises both the total CO_2 concentration and the carbon dioxide tension (PCO_2). While the carbon dioxide tension in the blood (PCO_2) is similar to the CO_2 tension in the medium, the total CO_2 in the blood (about 15 mmol/l) is lower than that outside of the animal (about 62 mmol/l). Blood chloride and calcium levels were significantly lower in the crabs exposed to the extra bicarbonate, but this difference disappeared when the animals were stored in air for 7 h. The high partial pressure and total CO_2 content persisted in air though there was a gradual fall in both parameters. Changes in oxygen tension of the blood of the treated crabs were not significantly different from that of the untreated crabs and this data is not presented.

These results confirm that exposing spanner crabs to a solution of sodium bicarbonate (Baking Soda) prior to storing them in air slows down the rate of acidosis. It does this by raising the "total carbon dioxide" concentration of the crab's blood. This means that bicarbonate ions are moving into the animal from outside, and augmenting the natural carbonate buffer system present in the blood of these crabs. While the crabs are submerged in the dip solution, the ionic content of their blood is disturbed, but this is corrected once the animal is removed from contact with the bulk of the solution. The volume of the crab's blood will then exceed the volume of any solution remaining in the gill chamber, bringing the ambient medium rapidly into equilibrium with the blood. The disturbance in chloride ions was expected because the mechanism by which crustaceans regulate their chloride ion (Cl^-) concentration is linked to movements of bicarbonate ions (Dejours *et al* 1982) though it is interesting that this effect was noted even in a crab that is unable to regulate the chloride ion content of its blood in the face of changes in the salinity of its environment (Paterson et al 1994a).

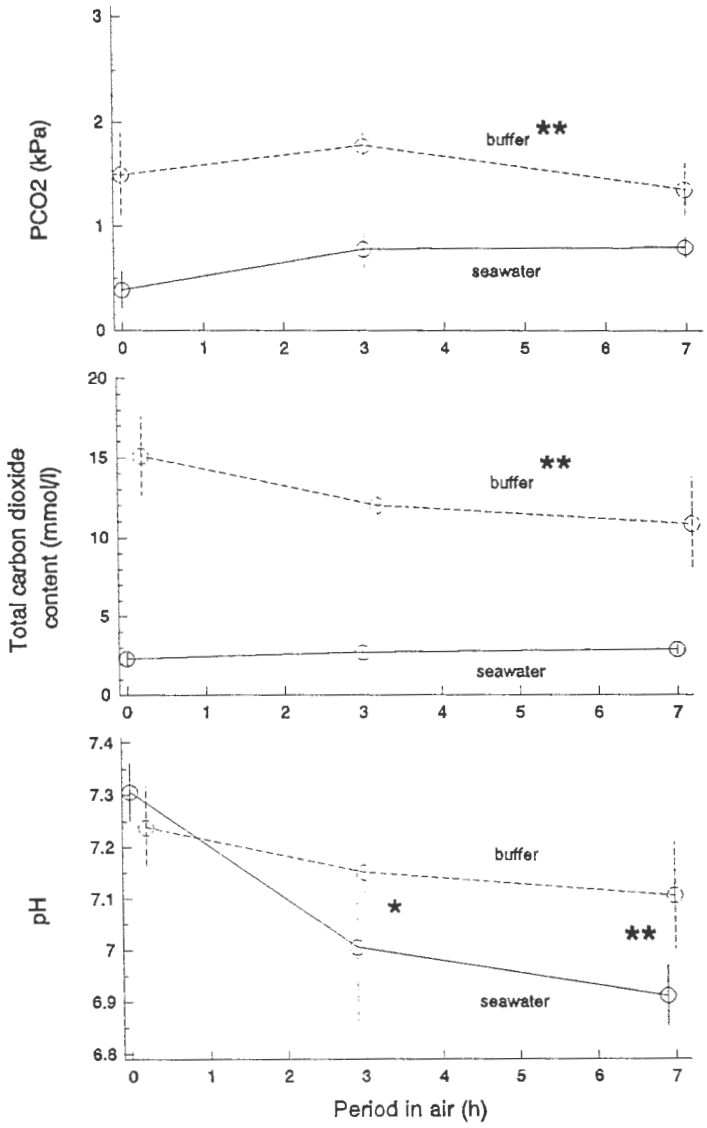

Figure 1. Effect of the buffer treatment on the carbon dioxide levels (PCO_2), total carbon dioxide concentration (mmol/l) and pH of blood from spanner crabs stored in air at 25°C. The solid line shows crabs stored beforehand in ordinary seawater. Asterisks on the label ("buffer") show that the treated crabs remained significantly different throughout the experiment. Otherwise, one or two asterisks indicate that at that time the treatment mean was significantly different from "seawater" crabs at 5 and 1% respectively.

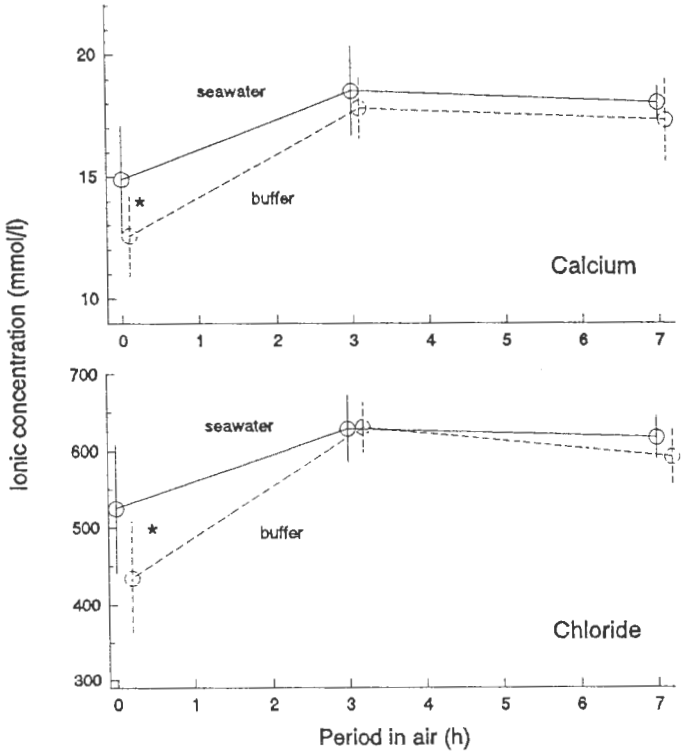

Figure 2. Effect of the buffer treatment on the mean calcium and chloride ion concentration (mmol/l, ±SD) in the blood of spanner crabs stored in air at 25°C. The solid line shows crabs stored beforehand in ordinary seawater. One asterisks indicates that at that time the treatment mean was significantly different

The effect of the bicarbonate treatment is transient at 25°C. CO_2 and HCO_3^- levels decrease and the crabs eventually return to a more natural carbonate equilibrium. In the long term, the blood pH of the treatments begins to equalise, largely because the acidosis in the untreated crabs slows down, eventually allowing the treated crabs to catch up.

Another experiment confirmed that the blood pH of the two groups eventually merges. This trial simulated export conditions. Crabs were held beforehand in the holding tank at 16°C and the experiment was conducted at a lower temperature, under conditions similar to those of the preliminary experiment described in the original application. Two storage temperatures were used (10 and 15°C) and the time of storage was extended to 48 hours to approximate the time taken for exported crabs to reach the market.

When crabs were packed from this tank and stored in humid air at 10°C for 48h the blood pH was significantly higher in the dipped crabs (Table 1), confirming the preliminary results reported in the original proposal. However, when crabs were stored at a temperature of 15°C, there was no difference between the blood pH of dipped crabs and crabs which not been dipped (Table 1). The higher metabolic rate of the crabs at

15°C accelerates the changes in blood pH so that the two treatments merged together within 48 hours storage out of water.

Table 1. Bicarbonate buffer treatment and the survival and blood pH of *Ranina ranina* stored in air for 48h at different temperatures.

		Treated crabs stored in air for 48h			
	control (16°C) (0h)	10°C		15°C	
		seawater	buffer[2]	seawater	buffer
survival[1]		8	7	4	5
pH					
mean	7.64	7.38	7.51**	7.28	7.22[NS]
SD	0.05	0.05	0.06	0.08	0.03
N	8	7	6	4	5

SD is standard deviation, N is number of crabs from which blood samples were taken
[1] number of crabs alive out of 9
[2] seawater + 5g $NaHCO_3$/litre
** buffer treatment significantly different ($P < 0.01$) from seawater treatment
[NS] not significant

Having demonstrated how the treatment works, it remained to show whether this treatment actually improves the survival of the crabs in air.

SURVIVAL EXPERIMENTS

Unfortunately, the buffer treatment did not repeatedly prolong the survival of the crabs. Neither did it kill them faster. Several trials showed that there was no significant difference between the survival of crabs dipped in the buffer solution and crabs sham-dipped in seawater.

The experiments involved taking a group of crabs, allocating them at random into two groups and dipping them in a bath of the buffer (baking soda in seawater) or in plain seawater. After a series of preliminary experiments the following protocol was adopted. The experiment was conducted within two days of capture, to keep the work within the commercial time frame. If we waited any longer the sample of crabs may be biased by

weeding out the "weak" crabs before the experiment was conducted. They die in the tank rather than when you want them to die, in the experiment. It seemed reasonable to assume on the basis of preliminary tests that it is the weak crabs that respond best to the buffer treatment.

So, up to 50 crabs per treatment were stored at high humidity in a controlled temperature room at 20°C and left in there for about 16 hours, since experience showed that little mortality occurred during this time. After this, the crabs were examined for signs of life every 2 hours until about half the crabs had died in one treatment. The dead crabs were not replaced after each census. For our purposes, death was defined as any crab whose eyestalks protruded and would not retract when touched, or whose walking legs hung limply when handled and which did not resist manipulation (some crabs had already retracted their eyestalks when they died). The data were then subjected to a 'survival' analysis which compared the time taken for a dipped crab to fail, that is, die, against that of the blank or "sham-dipped" crabs. Essentially the analysis is based on the Proportional Hazards Model (PHM) as described by Cox (1972).

The results of the last experiment are shown in Figure 3. There was not any point doing any statistics on a result as unequivocal as this. The early trials gave results tantalising enough for us to continue to the experiment that produced the results shown in Figure 2. However, no significant difference could be demonstrated in these early trials, even the one shown in Figure 3. Samples sizes in these early trials were small for various reasons (such as delays because the crabs had the habit of never getting caught when we were actually in a position to conduct the experiment, and died in the tanks in the meantime).

If there was something to these early indications the phenomenon disappeared as the fishery developed. If the buffer dip has any effect on the survival of the crabs it seems to be quite minor, and requiring much larger sample sizes to demonstrate, and this sits starkly against the clear benefit of this treatment in slowing acidosis. One possibility that could be addressed is that the dip only helps weak crabs live longer and has no effect on the strong ones. It is tempting to say that better experience of the crabbers and improved handling methods on the boats led to a progressive improvement in quality of the product and removed the weak crabs from contention but this is just speculation.

Work in this area was therefore terminated. No significant effect on the buffer in prolonging the survival of crabs could be demonstrated. It was felt that we would be better occupied examining the on-board handling of the crabs since this was a major issue in the fishery and because we could see that handling on the boat was influencing the survival of the crabs that we were experimenting upon. This work was covered in Paterson et al (1994b).

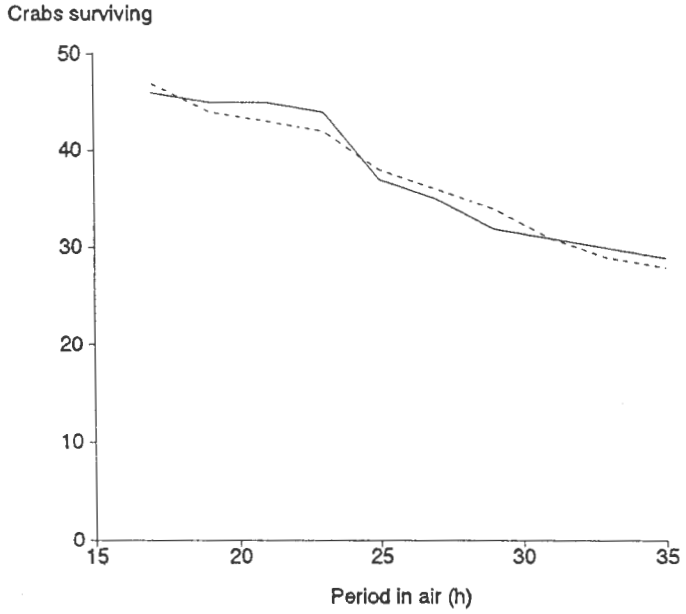

Figure 3. Crabs surviving during storage in air at 20°C (n=50 for each treatment). Dipped crabs shown by dashed line. Blank or "sham-dipped" crabs indicated by solid line.

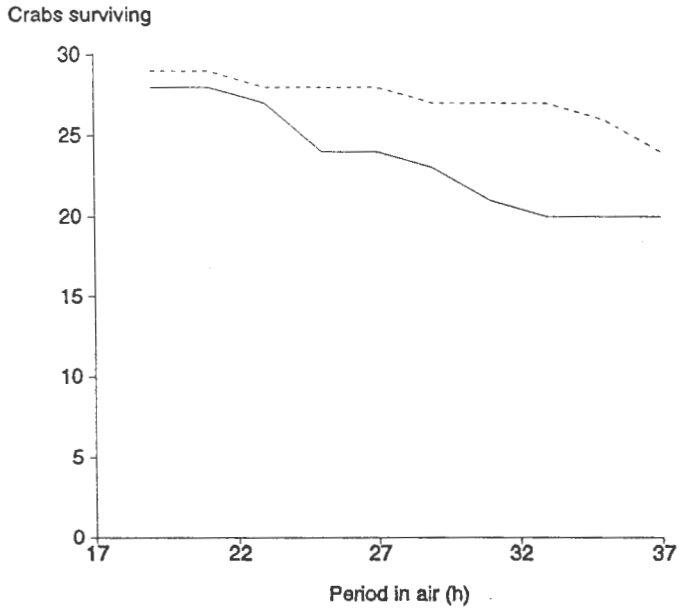

Figure 4. Crabs surviving during storage in air at 20°C (n=30 for each treatment). Dipped crabs shown by dashed line. Blank or "sham-dipped" crabs indicated by solid line.

TREATING CRABS ON BOATS

The data above shows that the effect of the buffer wears off with time, presumably as a new internal equilibrium is reached. When spanner crabs are exported, they may be out of the water for almost two days so perhaps we are expecting too much for the buffer to keep the crabs alive. However, the buffer apparently remains functioning in the crabs blood at least for the time that they are held on boats after harvest so perhaps the dip is more suitable as a post harvest treatment.

We tried that in conjunction with the on-board handling experiment described in the first paper of the main report (Paterson et al 1994b). Briefly, the crabs were harvested and stored on deck in a variety of ways. Some crabs were stored on deck in lug baskets (DRY), similar to a common practise then in use in the fishery. The DIP treatment was identical to the DRY treatment except that the crabs were dipped beforehand in a solution of the buffer.

Crabs dipped in this solution after capture showed high lactic acid concentrations (46.6 ± 11.35 mmol/l) but this was not significantly different from the DRY treatment (39.6 ± 14.7mmol/l). The crabs seemed to struggle more when treated with the dip, but this does not seem to have made a major impact on the blood lactic acid concentration when samples were taken on arrival at the factory, after the crabs have been in air for several hours.

When these dipped crabs were returned to the shore, they died at the same rate in the tanks as the DRY treatment did. So easing the acidosis experienced by the crabs on the boat did not circumvent this problem.

WHY DIDN'T THE BUFFER KEEP THE CRABS ALIVE FOR LONGER?

The rationale of this study was that, if acidosis was a major cause of death when crustaceans are transported out of water, then treating that acidosis using a dip should have a beneficial effect on survival. The fact that it didn't leaves us with two possible explanations. Firstly, that the beneficial effects of the dip are outweighed by its side-effects and secondly that it is not acidosis that kills spanner crabs when they are stored out of water.

The side-effects of any treatment that raises the carbon dioxide level of the blood relate to the fact that it is a metabolic waste product- it is potentially toxic or at least will suppress metabolism. Carbon dioxide is toxic to fish in high concentrations, but at sub-lethal levels, handicaps the fish because haemoglobin in the blood cells can no longer carry as much oxygen to the tissues as it used to, and the fish is forced into in-activity. Whether this happens when crustaceans are exposed to high bicarbonate concentrations is uncertain; they don't use blood cells to transport oxygen, and oxygen transport is going to be interrupted anyway when the crab is taken out of the water. The very fact that this relatively large dose of bicarbonate (which as it turns out is still in the physiological range found in other crustaceans, eg. Whiteley and Taylor 1990, 1992) did not increase or

decrease the rate of mortality suggests that the symptoms are neutral.

Acidosis, while itself stressful, may not be what is killing the crabs. The spanner crabs ability to endure what seems to be a catastrophic fall in blood pH may actually help to explain why the dip did not work. For a study of the causes of mortality when spanner crabs are stored out of water, we have been having an extraordinary amount of difficulty killing them. We may have tried using this treatment on the wrong species of crab. The rate of acidosis eventually slows down in the blank crabs as well (see Paterson et al 1994a for a discussion of this phenomenon). They stop short of dying and linger on for quite a while, allowing the blood pH of the buffered crabs to catch up to them. Despite their different rate of acidosis, the two groups of crabs die at the same rate suggesting that it is another factor correlated to time in air that is killing the crabs. Dehydration can be ruled out, since the experiments were conducted at high humidity. However, the "missing" factor might be the accumulation of other metabolic wastes, such as ammonia, which are not as readily subject to practical intervention as carbon dioxide is.

Apparently, the buffer did not work in this species because the blank crabs did not allow the acidosis in their blood to reach fatal levels. Some other species of marine crabs die much faster when removed from the water, such as *Ovalipes australiensis* (Messner 1985), so this dip may prove useful when applied to them.

Another issue to consider is that even if the dip has no effect on survival of the crabs, then perhaps it still has some benefit in terms of the quality of the product. If the crabs spend long periods of time with their tissue and blood pH at a low level, then perhaps this has an effect on the texture of the flesh. However, we are not aware that texture per se has been identified as a problem by the spanner crab industry.

CONCLUSIONS

Buffering blood acidosis using sodium bicarbonate does not prolong the survival of crabs. Perhaps the dip treatment stresses the crab and this counteracts the anticipated benefit of controlling acidosis. However, the survival of the treated crabs was no worse, so the effect of the dip may be neutral. Acidosis may not be what kills spanner crabs in air. Rather, the control crabs may also stop acidosis from reaching fatal levels and all crabs survive long enough in air for something else to kill them. What that something else is remains unknown.

ACKNOWLEDGEMENTS

Stephen Nottingham provided valuable statistical assistance when devising the survival experiments. Peter Greenaway (University of New South Wales) provided access to facilities for the physiological studies. This research was supported by the Fisheries Research and Development Corporation (Project 92/71).

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Workshop on Harvesting and Post-harvest Handling of Live Spanner Crabs

9th and 10th December, 1993

AIRPORT INTERNATIONAL MOTEL
528 KINGSFORDSMITHDRIVE,HAMILTON, BRISBANE.

DPI DEPARTMENT OF PRIMARY INDUSTRIES
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Workshop on Harvesting and Post-harvest Handling of Live Spanner Crabs

Brian Paterson
Seminar Co-ordinator
IFIQ

DPI QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES AGRIBUSINESS

Brisbane, 1994

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SUMMARY AND RECOMMENDATIONS

HARVESTING AND POST HARVEST HANDLING OF LIVE SPANNER CRABS: WORKSHOP SUMMARY AND RECOMMENDATIONS

Brian Paterson
Physiologist
IFIQ

Introduction

The export of live spanner crabs (*Ranina ranina*) from Queensland has recently become a burgeoning industry. This expansion has coincided with research into the harvesting and post harvest handling of spanner crabs supported by the Fisheries Research and Development Corporation (FRDC) and its predecessors. This workshop was proposed as a means to gather the industry and research sectors together to discuss the results of this research work.

Right from the beginning it became obvious that there was also considerable interest in issues beyond the narrow "technical" arena, specifically relating to aspects of marketing and management of the fishery. The shift to "live" marketing has seen unprecedented interest in the fishery. This workshop was therefore an unique opportunity to gather people together to provide an "industry-wide" perspective of these developments.

The workshop program covered two days. The first day examined the management of the fishery and the marketing of the crabs and provided an opportunity to discuss current issues relating to the status and management of the spanner crab fishery. The second day considered technical aspects of live spanner crab marketing; the methods of harvesting, storage and export of live crabs. Results from current and previous research by the DPI were presented for discussion and the importance of appropriate handling at all stages of the process was emphasised.

The purpose of this workshop was for everyone to gain an appreciation of current knowledge and to identify the most pressing issues that remained to be addressed. This report summarises the information covered and includes a list of those issues for consideration (or research) raised during discussion with the industry. Some of these issues could be left to individuals or companies to pursue for their own gain, but other issues are more appropriately handled on an industry-wide basis by organisations such as QCFO, QFMA and DPI. Some have been addressed in research conducted since the workshop, and are discussed in the final report of project 92/71 "Live transport of crustaceans in air-prolonging the survival of crabs".

Marketing and management

Virtually all of the live crabs are currently exported to Taiwan. Under these circumstances, the rise in price from about $2 to over $4 per kilo in the last couple of

years has seen increasing numbers of fishermen and boats entering the fishery. This change may not yet be reflected in fisheries statistics, which have a recognised lag-time, but the fishermen were in general agreement that some kind of spanner crab bonanza was under way, which may ultimately threaten the sustainability of the resource if it is not curbed in time.

Laurie Gwynne of the Queensland Fish Management Authority (QFMA) summarised the management regime currently in place and invited comment. Up till now, the spanner crab fishery has been regulated using a month-long seasonal closure in November-December (loosely based on the breeding season), a minimum size limit (>100 mm carapace length) that strongly favours the retention of large males and lastly by restrictions on the number and configuration of the traps that a boat can carry. The fishermen present had an opportunity to provide feedback to the QFMA on these strategies and to comment on whether a "live crab" endorsement was an option for the fishery. In the ensuing discussion, some of the fishermen told of having seen female crabs carrying eggs outside of the official closure period, so a review of its timing was suggested. Voluntary reporting of these "berried" females through the existing logbook mechanism was proposed so that the information could be provided quickly to the scientists and managers responsible.

Apart from the existing management regime, calls have come from spanner crab fishermen for some kind of restriction to be placed on entry into the fishery or for a quota to be placed on catches. This brings understandable cries of dismay from those outside who also want a piece of the action. Fisheries managers end up in a difficult situation. There are about a thousand licences that are able to harvest spanner crabs, though only a small proportion do so at the moment. Obviously, if too many fishermen enter the fishery this may reduce the available catch and the livelihoods for individual fishermen. It also threatens to cause oversupply in the market and may ultimately even endanger the sustainability of the fishery. But at present the Managers have no actual evidence that the fishery is under threat. The statistics coming in do not make it easy to interpret what is happening. As Ian Brown (Fisheries Branch) explained in his overview, the fishery has been expanding onto virgin grounds at the same time that the extra effort has appeared. DPI Fisheries Branch (in cooperation with fishermen) will investigate the potential size of the fishery we are now dealing with so that more realistic decisions can be made about its management.

Regulating the profitability of a fishery (rather than only managing the sustainability of the resource) through restricted entry and/or quotas is always going to be a controversial subject. However, the workshop was told that something like this has been achieved in certain other fisheries so there is no reason why it can not come about for spanner crabs as well. It was left to the fishermen to make it clear to the QFMA that their own experience "on the grounds" raised concerns in their mind about the effect of increasing fishing activity, in advance of any warning signals coming via logbook statistics and other sources.

The increasing activity in the catching sector has ramifications all of the way through to the market. Oversupply remains a potential problem. Add to this the effect of weather on effort and the supply to the market becomes quite volatile. Sometimes there are no crabs at all and sometimes there are just too many. While the landed price given to the fishermen has increased over the last few years, the market price has fallen, squeezing the processors and exporters somewhere in the middle.

Everyone knows that it is dangerous to rely on a single market but the hard bit is to find other markets for live spanner crabs. The crabs are unrecognised in other "Chinese" countries such as Singapore and Hong Kong, and while small quantities have been marketed in Japan, this market is not going to absorb a significant portion of the catch. Ray Teh (SEA Foods International) noted that a major factor restricting the market for live spanners in Asia is that Chinese cuisine is not all the same. Cantonese chefs don't use spanner crabs.

One way to smooth out the supply of crabs is to hold them in tanks for longer periods, either in Australia (a topic discussed below) or in the receiver's tanks in Taiwan. However, the receivers have as much trouble holding the crabs as their counterparts in Australia and solving this problem will probably require cooperation at both ends of the export chain.

Of course, sometimes the buyers want lots of crabs. Ray explained the importance of understanding the ups and downs of the market in Taiwan, taking into account festivals like Chinese New Year. In particular, he warned processors and exporters not to try exporting too many crabs during Ghost Month.

Harvesting and post-harvest handling

The second day of the workshop was intended to put aside the important management issues and to concentrate solely upon the technical aspects of producing top quality live crabs. The crabs are harvested using dillies or tangle nets and stored in air on the deck of the boat until it returns to shore in the afternoon. The crabs are transferred to a live storage tank where they are cooled and stored overnight prior to packing for export.

One recognised problem is that processors sometimes have a lot of trouble holding crabs for long periods in their tanks. The crabs are packed and exported without the three or more days of "purging" (allowing the product to clear faeces from the gut) that is mandatory in allied fisheries, such as rock lobsters. It is possible that one of the reasons why survival of crabs at the market is so unpredictable, with episodes of poor survival, is because the crabs are exported prematurely.

Wayne Sumpton (Fisheries Branch) summarised the work done to reduce injury to the crabs during harvest. The gear first used to harvest spanner crabs was never really suited to catching live crabs. But even before the live market took off it was necessary to find ways of harvesting the crabs without injuring them. Undersized crabs had to be returned

to the sea and laboratory experiments showed that many of the injured crabs probably died from loss of blood. However, work conducted recently in both Qld and NSW found that injury can be reduced by relatively straightforward adjustments to the mesh size and the tension or "drop" of the net as well as by careful handling of the crabs during removal. The higher prices paid for the crabs give an added incentive to reduce injury because now the processor gets to count the cost of the dead crabs in the tank.

It is common practise for the crabs to be stored on deck in lug baskets. Brian Paterson (IFIQ) told the fishermen that the crabs have difficulty breathing in air and are stressed severely by being held under these circumstances. Basic guidelines were presented for storing crabs in air, with emphasis on the need to keep the crabs moist, cool, in the dark and undisturbed. The difficulties being encountered in holding the crabs for long periods in tanks will probably require a major rethink about the way the crabs are handled on boats in the future. They are probably being stressed too much on the boats. It was also suggested that the fishermen think about banding the claws of the crabs to reduce injuries.

There was a lot of discussion about the relative merits of different holding systems on the boats, (e.g. water sprays, refrigeration and wet wells) and it was agreed that this area should be given priority during the remaining period of the current project (FRDC 92/71), both to find out whether wet wells are the answer they appear to be (in comparison with sprays or refrigerated holds) and to find out how long the crabs can be held in air without problems developing later in the tanks.

Another major issue is road transport. Crabs are being drawn from a much wider area than they have been in the past, in some cases the crabs must be trucked for a couple of hours from the dock to the tank where they will be held prior to export. The consequences of this practise for survival are unknown.

Bruce Goodrick (IFIQ) told the workshop that efficient and reliable storage tanks were an important component of the export operation. Prior to export the crabs need to be given the opportunity to recover from harvest and on-board handling.

When storing large quantities it is important to stop the environment within the tank from deteriorating. The operation of recirculating seawater tanks is straightforward but all the same this should not justify a blasé attitude. Equipment for cooling and aerating the seawater is essential, as is a reliable source of that seawater. Also pivotal to the functioning of these systems is the "biological" filtration of water, encouraging the growth of bacteria that will safely consume the ammonia excreted by the crabs. There is a wealth of information available about this kind of technology and Bruce discussed some of the practical aspects of their operation. In particular he noted that the filters are apt to "starve" if the tank is left without crabs for a couple of weeks or more (e.g. during bad weather and the closure) and that operators should think about feeding the filter at this time with artificial sources of ammonia and nitrite.

Despite the best efforts of the processors, spanner crabs have proved to be a difficult species to keep in tanks. Some of the crabs die within three to four days of arrival,

perhaps because of compounding effects of injuries and infections triggered by entering a new environment. The processors get around this by exporting the crabs as soon as possible after arrival. Preliminary experiments show that changed conditions on the boats influence the mortality rate in the tanks. This tank mortality is a major hurdle to overcome before the industry can reach maturity, when holding tanks can be used to manage the timing and quantity of exports.

Brian Paterson (IFIQ) noted that the method used to ship the crabs is not very different from that applied to other live crustaceans, such as rock lobsters and there seems little scope for improving the method without redesigning the carton around the crab. Live transport experiments have been undertaken at IFIQ and attention has been paid to identify the physiological changes that the crabs experience during live shipment. Spanner crabs suffocate readily in air. The level of oxygen in their blood falls sharply and they accumulate large amounts of carbon dioxide. Controlling the temperature that the crabs experience during shipment is an important aspect of the method, but attempts to prolong the survival of the crabs by other means have not been as useful. Storing the crabs in air with a higher level of oxygen in it does nothing to curb the accumulation of carbon dioxide in the crab - in fact it exaggerates it! An attempt to treat the crabs with a dip that reduces the consequences of high carbon dioxide levels in the blood does not let them live any longer. The effect does not persist for long enough. However, the method works over the time frame required to hold crabs on the boats so it may be more useful under these circumstances.

Issues for consideration and further research

The following issues were identified during the workshop as worth attention when reviewing the management regime of the fishery and when establishing research priorities.

Marketing and management

- Recommend to QFMA that there should be a specific spanner crab endorsement and a "freeze" on licenses based, for example, on demonstrated activity in the fishery before a certain date (using log book returns etc). Operators were encouraged to write to QFMA voicing their concerns.

- Pay attention to product quality, training and improved handling practises.

- Identify the size and extent on the fishery as well as variations in the time of spawning.

- Introduce total protection of females (as in some areas legal-sized females are being landed).

- Introduce ITQ's (though this was only desirable from live market perspective).

Harvesting and post-harvest handling

- Compare the performance of crabs under different holding regimes on boats (wet wells, water sprays, cooling, dip).

- Determining the maximum time that crabs can be held in air without having problems when put into holding tanks.

- Investigate ways of improving survival of crabs during road transport from remote ports.

- Identify whether the crabs run out of oxygen in the cartons during export.

Further research on the post-harvest handling of spanner crabs has been completed since the workshop. This work has largely addressed the first two points under "Harvesting and post-harvest handling" but preliminary observations have been made on road transport of crabs. The Final Report of project (FRDC 92/71), which will be available soon, discusses this more recent work in full.

Conclusions

The workshop was well attended, (76 people attended) with representatives from major live crab exporters, a majority of fishermen and key government and fisheries management personnel. It actually exceeded our conservative expectations in terms of numbers of registrations. This was no doubt due to the enthusiasm and concern of the fishermen and other operators as well as the efforts of representatives of the Queensland Commercial Fisherman's Organisation (QCFO). In future a much bigger venue will be required.

The workshop attracted favourable comment by virtually all who attended and achieved its objectives of encouraging transfer of research results to the industry and fostering discussion amongst various parts of the research and industry community. Major steps were taken toward a reassessment of the management regime applied to the fishery, a process which will be ongoing. Work on the on-board handling of spanner crabs has also received an added boost, allowing priorities to be set for the remaining period of the project. The workshop ended by reiterating the note of caution expressed on the first day, that there is danger that the fishery will be over exploited. Fishermen were encouraged to voice any concerns they had about the future of the fishery to the QFMA.

This live crab workshop may become a regular event, though in a different format (probably only 1 day) along the lines of suggestions made by those present and to respond to the changing needs of the industry.

Acknowledgements

This workshop could not have come about without the help of many people. The workshop organising committee for want of a better title included Paul Exley, Bruce Goodrick, Brian Paterson, and Ross Smith. Thanks also to Ray Teh (SEA Foods International) Ian Brown and Wayne Sumpton (DPI Fisheries Branch) and Laurie Gwynne and Annette Magee (QFMA) for contributing to this event. Of course a lot of the credit must go to the fishermen and processors for their enthusiasm and in particular to Roger Honey and Allan Jones for their advice and assistance. This workshop and the research reported therein was supported by the Fisheries Research and Development Corporation and its predecessors (Projects 81/71, 90/5 and 92/71).

NOTES

PROGRAM

Thursday 9 December 1993

10.00 am	Registration (Tea/Coffee)	
11.00 am	Welcome	
11.10 am	Introduction	Brian Paterson
Session I:	***Marketing and Management***	Bruce Goodrick, Chair
11.30 am	The spanner crab fishery	Ian Brown
12.00	Lunch	
1.30 pm	Marketing	Ray Teh
1.55 pm	Forum discussion: Marketing live spanner crabs	
2.35 pm	Management regime - spanner crabs	Laurie Gwynne
3.30 pm	Afternoon tea	
4.00 pm	Forum discussion on managing the fishery	Bruce Goodrick
4.40 pm	Summing up (Day 1)	

Friday 10 December 1993

Session II	***Live holding on boats***	
9.00 am	Tightening up on spanners - harvesting live spanner crabs (*Ranina ranina*) without injuring them	Wayne Sumpton
9.25 am	A stacked deck - handling live spanner crabs (*Ranina ranina*) after harvest	Brian Paterson
9.50 am	Forum discussion on live harvesting and on-board holding	
10.45 am	Morning tea	

Session III	***Holding on land***	
11.00 am	Tanks for the crabs - live holding of spanner crabs (*Ranina ranina*) prior to export	Bruce Goodrick
11.25 am	Discussion on wet holding live crabs	
12.30 pm	Lunch	
Session IV	***Live transport***	
2.00 pm	Acid nous - live export of spanner crabs (*Ranina ranina*)	Brian Paterson
2.25 pm	Discussion on live transport of spanner crabs	
3.30 pm	Afternoon tea	
3.45 pm	Summing up (Day 2)	Brian Paterson

WORKSHOP ON HARVESTING AND POST-HARVEST HANDLING OF LIVE SPANNER CRABS:

ABSTRACTS AND PAPERS

THE SPANNER CRAB FISHERY - HISTORY AND STATUS

Dr I W Brown
Senior Fisheries Biologist
QDPI

Spanner crabs (*Ranina ranina*) are found throughout the tropical Indo-Pacific region from South Africa to Japan and Hawaii, and around northern Australia from the Abrolhos Is (WA) to NSW. Spanner crabs have been fished commercially around the Hawaiian Islands since before the Second World War, and to a minor extent around the Philippines southern Japan and the Seychelles (Indian Ocean).

The fishery has a recent history in Australia, going back little more than a decade. Commercially viable operation began around 1978-79 in the Mooloolaba-Caloundra area when it was found that crab "dillies", previously used by recreational fishers to catch mud crabs and sand crabs in estuaries, were effective at catching spanner crabs in offshore waters. During 1983-84 the annual catch of spanner crabs in Queensland was estimated to be around 300 tonnes, which is an order of magnitude greater than the highest annual catch reported from the Hawaiian fishery during its 35 years post-war history.

In the mid 1980s the fishery expanded south into NSW (significant landings began to be reported by Northern Rivers cooperatives) and north to Double Island Point. Between 1982-84 and 1987 individual fishing efforts increased from 90 to 198 net lifts per boat per day to and average catch rates declined from 1.6 to 0.6 kg/net as the stock was fished down.

Since 1990 there has been an overall increase in both catch and effort. Catches have more than doubled (from 450 to 1100 t) and effort has risen 80% from 0.5 to 0.9 million net-lifts. There has been a corresponding increase in catch-per-unit-effort (CPUE), from 0.6 to 1.2 kg net lift, attributable mainly to an expansion of effort into productive virgin grounds north of Bundaberg. CPUEs in this area have declined since fishing started in 1988, indicating a fishing-down of the stock, but they are still substantially higher than in the southern grounds. Recent assessment of available information suggests that catch rates in the traditionally-fished areas are steady, and that the resource is being adequately maintained. However a close watch must be kept on the rate of increase in fishing effort in the northern grounds, and effective means developed for controlling effort (or total catch) in this fishery. Questions relating to the size of the fished stock (particularly its northern limit) also need to be addressed, as this has a direct influence on the amount of effort the fishery can sustain.

NOTES

MANAGEMENT REGIME - SPANNER CRABS

Laurie Gwynne
QFMA

The spanner crab fishery commenced in earnest in the 1980s although some fishing had occurred prior to then. At the time it was seen as a small addition to the crab fishery which centred around mud and sand crabs. The legal ability of spanner crab fishermen to access this resource was accommodated under Queensland legislation in the crab fishery endorsement. Since the mid 1980s Queensland has seen an increasing interest and corresponding effort in the spanner crab fishery. This has necessitated closer attention to the management measures in effect in the fishery.

The cornerstone of management has been licensing of fisherman and registration of vessels. The introduction of offshore constitutional arrangements and the philosophy that no fisherman shall be disadvantaged has required the Authority to accommodate holders of Commonwealth and NSW licences under special provision. Some recent refinements to the crab fishery endorsement legislation have been directed at the methods of taking spanner crabs, in particular the construction and configuration of the harvesting apparatus ("dilly"). The recent intensive fishing pressure on the resource has required the Authority to closely examine its present management regime and to assess its adequateness.

The management regime

The major management interventions in the spanner crab fishery are:

- licensing of fishers;
- registration of commercial fishing vessels;
- closed seasons;
- apparatus;
- minimum legal sizes;
- recreational bay limits and pot limitations;
- processors licence;
- commercial buyers licence; and
- export registration (AQIS).

Under the FIOM Act, there are four endorsements which permit the taking of spanner crabs (Table 1). The most common licence is the F licence is the Queensland wide normal crab endorsement. The closed season is annually from midday 20 November to midday 20 December and is intended to correspond with the peak spawning time for spanner crabs. The present restrictions in the apparatus are shown in Table 2.

Table 1 Number of crab endorsements and number of vessels currently operating in the fishery, categorised by licence type.

Fishery	No. Crab Pot Endorsements	No. Vessels (by principal fishery)
Queensland	1 021	163
South Queensland Spanner Crab Fishery (ex Commonwealth)	11	10
South Queensland Spanner Crab + Line (ex Commonwealth)	7	7
Restricted Spanner Crab + Line (NSW Concessional)	25	25
TOTAL	**1 064**	**205 (+)**

Table 2 Restrictions on apparatus for use in harvesting spanner crabs.

Rigid Frame	1 m^2
Minimum Mesh Size	25 mm (single)
	51 mm (double)
Net Drop	10 cm
Max. No. of Dillies in possession or use	30
Max. No. of Dillies on a trot line	10
Float at each end of trot line	
Float on each single dilly	

Issues related to management

Some of the issues in relation to management which have been identified include:

- Changes in effort in the northern range of the fishery. Evidence of vessels working 7 days a week to satisfy marketeers demands.
- Trawlers increasing their effort by potting for spanner crabs during the day.
- Timing of the closed season.
- A increase in the number of pot lifts and days worked.
- Catch and effort have apparently not yet plateaued. CPUE appears stable however the rate of increase in effort is not sustainable.
- Introduction of purpose built vessels which are largely unsuitable for other fisheries.

Options

Some of the management options which may be implemented to manage the fishery on a sustainable basis include:

- TAC - causes a gold rush mentality;
- ITQ - spreads effort throughout season;
- separate endorsement;
- extended or varied closed season - midnight closures;
- changes in apparatus;
- gear restrictions on trawlers e.g., no trawl gear if using crab dillies;
- area closures;
- licence limitations.

Some of these issues will be discussed during the next two days and I'm sure all will be aired in future meetings of the Crab Management Advisory Committee (CRABMAC).

NOTES

TIGHTENING UP ON SPANNERS - HARVESTING LIVE SPANNER CRABS (*RANINA RANINA*) WITHOUT INJURING THEM

Wayne Sumpton
Fisheries Biologist
DPI

Spanner crabs comprise the largest crab fishery (by weight) in Queensland, with the 1991 catch exceeding 700 tonnes. Methods of catching spanner crabs differ from those of the other crab fisheries; entangling netting on frames is used rather than the traditional potting or trapping methods for mud or sand crabs. Methods of operation vary between fishers but essentially strings of tangle frames are set on the sea floor and lifted after 30 to 90 minutes.

The frames are generally square and the netting used is typically 25 to 50 mm mesh size and baited with whole mullet or other fish. Spanner crabs are entangled in the mesh when they try to reach the bait. When nets are retrieved, the crabs are removed. Any undersized crabs are returned to the water. Typically about half the crabs are below minimum legal size. Entangled crabs are removed from the nets with varying degrees of care. Those removed quickly by simply tearing them off the net may sustain severe damage resulting from breakage or loss of limbs. Many fishers believe that crabs are able to survive this type of damage since they often capture crabs that show signs of healing where limbs or parts of limbs have been damaged.

Some recent work by New South Wales Fisheries researchers has shown that significant mortality may be caused by less than careful disentangling of crabs from nets. They found that at times almost all crabs that were quickly pulled off nets and suffered major limb damage subsequently die. Even "tipping" - the removal of two or three "spades" (the last segment on the legs, called "dactyls" by scientists) - caused a mortality rate of up to 60%. These figures suggest that many thousands of crabs which are returned to the water may not survive to be able to grow and be re-caught at some later date as marketable-sized crabs.

For this reason the Department of Primary Industries Queensland (QDPI) in consultation with NSW Fisheries undertook and recently completed a project on spanner crabs. The aim was to assess alternative trapping designs and existing apparatus. The results showed that no non-damaging apparatus was efficient enough to replace the current designs. Sixteen trap types, ranging from collapsible sand-crab side-entrance traps to top-entrance and trapdoor-style traps, were tested on spanner crab grounds and in tanks at the Deception Bay research station. However none was a viable alternative.

The best catch rates obtained were about four marketable crabs per lift - but the trap had to be in the water for more than three hours. During the same time one of the normal frames could be set, lifted and cleared about three times, with an average catch of four

crabs per lift or a total of 12 crabs per frame in three hours. In other words, the best of the non-entangling traps produced only one-third as many spanner crabs as the standard entangling net and frame being used now. In comparison to the other crab fisheries the spanner crab fishery is a high-volume, low-individual-value fishery that could not remain viable if non-entangling gear had to be used.

In addition to non-entangling apparatus we also tested traditional tangle nets of differing mesh sizes and shapes. The idea was to see which type of net would minimise damage (while maintaining acceptable catch rates). We tested singly and doubly-hung netting, loose or tight net tension, various net ply, monofilament and multifilament. Mesh sizes tested included 25, 35, 50, 65 and 85 mm. Methods used to remove spanner crabs from nets differ widely from one fisherman to another but for our trials we used a fairly conservative way of removing crabs; that is, by only breaking the spades off limbs which could not be untangled in two seconds. Obviously more extreme methods would cause far greater damage.

Generally the middle-size mesh (35 to 65 mm) caused less damage to crabs than did the 25 mm and 85 mm nets while maintaining similar catches rates. Greater time was also required to clear crabs from the smallest and largest mesh sizes. Likewise doubly-hung nets took longer to clear and caused more damage to crabs than did singly-hung nets. However the most significant result related to the method of net hanging.

Nets that were tightly hung (with little or no drop) caused only half the damage resulting from loose-hanging and also took 30% less time to clear of crabs. The number of undersized crabs was also much greater in loosely hung nets but the number of marketable crabs in loosely and tightly hung nets was virtually the same. Present regulations in Queensland allow for a maximum drop of net in spanner crabs nets of 10 cm compared to 60 cm in New South Wales. A modest 10 cm drop would appear much more effective in minimising damage to crabs removed from nets and subsequently returned to the water.

Video recording of crabs walking over tangle nets revealed that once they had become entangled their mobility was much more restricted on tightly-hung nets. Once crabs became entangled they moved about attempting to free themselves but on a tightly hung net they gave up much sooner and remained fairly still after a short struggle. In contrast, crabs entangled in loosely hung nets struggled for longer periods, often twisting around and becoming increasingly enmeshed. Crabs in loosely hung nets also often buried back into the sand, thereby causing even greater entanglement.

We concluded that singly hung nets of a mesh size between 30 and 50 mm hung with a drop of less than 10 cm were best for minimising damage and clearance time, while also maintaining good catch rates. These nets are commonly used by commercial operators, although many still also use doubly hung nets and mesh of 25 mm. If you are a spanner crab fisherman concerned about the health of the resource then you should consider switching to single mesh layer nets. Where possible limit the number of spades you remove per crab to two or fewer. Crabs that have a whole limb removed or which have several spades removed are not likely to survive!

When you consider that about 700 tonnes of undersized spanner crabs are discarded each year, it is clear that every effort should be made to ensure that the maximum number of these crabs survive to be caught at some later date.

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NOTES

A STACKED DECK - HANDLING LIVE SPANNER CRABS (*RANINA RANINA*) AFTER HARVEST

Brian Paterson
Physiologist
IFIQ

Introduction

Interest in handling live spanner crabs (*Ranina ranina*) has a long history. The size restriction imposed on the fishery requires that under-sized crabs (<100 mm) are returned to the sea. Consequently, research has already been conducted on ways of harvesting spanner crabs without injuring them. Now that attention has swung to exporting live crabs, the need for correct handling on board is now more important than ever.

After harvest, some aquatic crustaceans such as crabs and lobsters are stored and transported out of water. Currently, spanner crabs are held on deck in lug-baskets or similar tubs. This is the simplest technique available for holding the crabs without using a purpose-built boat with a wet well. However, holding crabs in air after harvest is not optimal for any aquatic animal. This practise involves the same kind of stress involved when the crabs are later shipped overseas - but under some of the worst possible conditions. It is the fisherman's job to make sure that crabs are as "comfortable" as possible during this period on the boat.

Spanner crabs do not move very much when held in air. However, they will scramble around in a frenzy for a minute or so if disturbed. Our studies show that spanner crabs don't tolerate being removed from the water as much as other commercially important crabs. This leaves them very prone to injury when held in air for long periods. Various suggestions have been put forward for easing stress when keeping these crabs in air, such as wet wells or refrigerated water sprays. Yet, some fishermen still seem to be able to catch spanner crabs, hold them on deck in lug-baskets and then return them safely to shore. So it is not clear under what circumstances the extra effort and equipment is required.

This paper describes the minimum standard of handling that the crabs require on the boat and proposes a way to quantify the condition of crabs handled in different ways.

Holding crabs on deck

There is a good reason why spanner crabs go perfectly still when removed from the water. They are suffocating. The phrase "..it hurts to move..." comes to mind. Nevertheless, undamaged crabs can be held for several hours in air without dying, as long as certain requirements are met. The minimum requirements for holding live spanner crabs are shown in Table 1. These are *minimum* guidelines because you can obviously do more for

the crabs. The guidelines here are more or less how the crabs are treated at the moment. When holding live crabs, the fisherman can adopt the passive approach, of preventing things happening, or actively assist the survival of the crabs. Therefore, the guidelines are presented in two forms: things that MUST be done and things that MUST NOT be done. It is worth noting the difference between these lists - they are not simply the opposite of the other.

Table 3 Minimum requirements for holding live spanner crabs.

Guidelines for handling live spanner crabs in air

The crabs must remain

* moist
* cool
* in the dark
* undisturbed

They **MUST NOT**

* dry out
* warm up
* be exposed to bright light unnecessarily
* be handled or disturbed unnecessarily

At the very least the crabs should be kept covered and out of the wind so that they do not dry out. However, a spray system of some kind would actively stop water loss. While it is important that the crabs remain in the shade so that they do not warm up, actually cooling them (by about 5°C below air temperature) would further reduce the stress of being held out of water. The crabs can be cooled in a variety of ways, depending on the facilities available.

The crabs should not be disturbed or handled unnecessarily. It is important to avoid shocks such as dropping or knocking the container holding the crabs. Pouring a bucket of seawater over a basket of crabs to "freshen" them up, is probably the kind of disturbance they don't need.

Crabs and clawed lobsters are usually tied or banded during live handling, but the spanner crab is not dangerous enough to require this - at least not to the people handling the crabs. However the crabs can injure each other. Try picking one crab out of a basket of crabs. You get that crab and a few of his mates attached.

The claws of spanner crabs are used for crushing and they easily punch holes in the legs of other crabs in the catch. These injuries will eventually become infected when the crabs are held in live holding tanks on shore. This may not be a problem at the moment, but the longer that crabs are held in captivity the more likely it is that these infections will develop.

Now, if you are catching two to three hundred kilograms of crabs that is a lot of claws to band. It is tempting to say that this is all too much effort to go to. And it probably is a lot of effort at the moment while the crabs only stay overnight in the tank before export. Of course, some people probably said that it was too much effort to remove the crabs carefully from the nets.

It is not yet clear to what extent spanner crabs are likely to dry out in air. Various crustacean species seem to show different resistance to loss of water. It is also not clear whether even this minimal treatment still damages the crabs - there must be a reason why it is sometimes difficult to keep the crabs alive in the holding tanks on shore without them dying.

One way of finding out whether storing the crabs in air damages them is to discriminate between the first couple of baskets and the rest of the catch caught on the trip. Perhaps it is these crabs, the ones who spend the longest time in air, which are the ones discarded from the tanks the next day as too weak for export. Processors could easily cooperate with their fishermen to conduct this experiment. If the first catches of the day are the crabs that weaken in the tanks, then they probably need special attention on the boats.

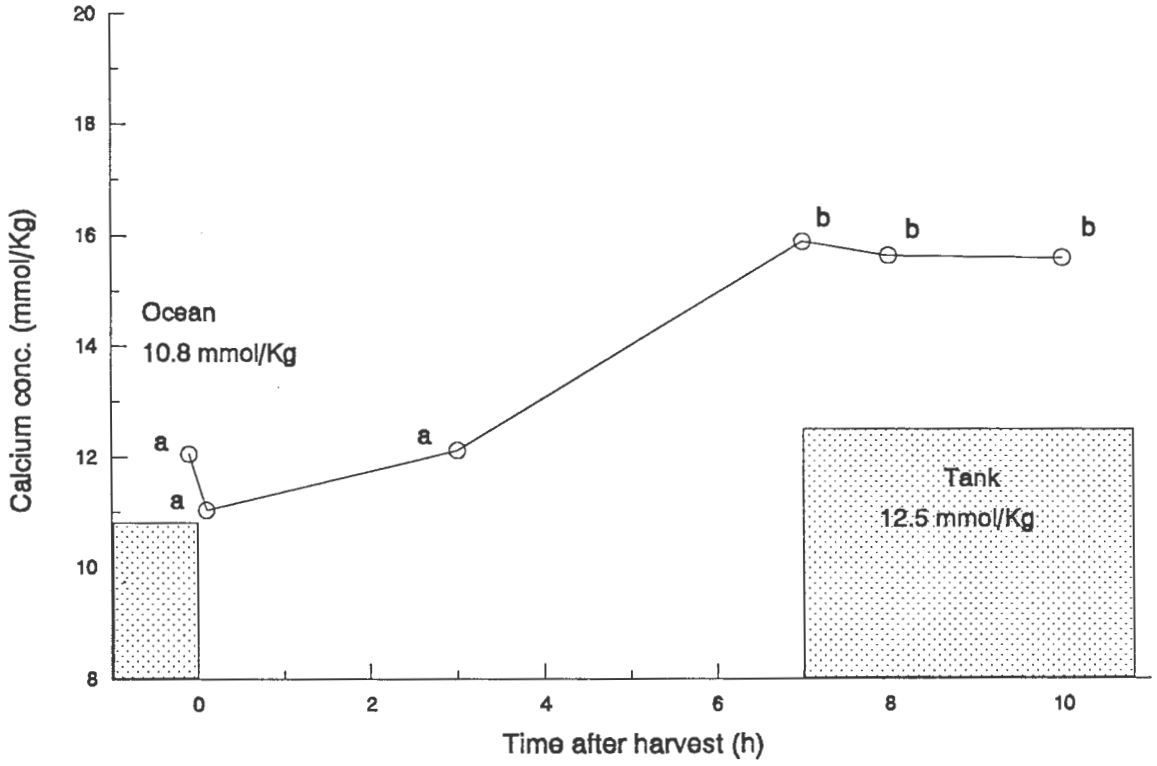

Figure 1 Changes in calcium concentration in the blood of spanner crabs during and after harvest. The height of the shaded blocks show the calcium concentration in the ocean and the storage tank. Points that have the same letter are not significantly different.

Stress and crabs

The symptoms that the crabs show when held out of water provide a means of comparing handling methods. Spanner crabs have trouble breathing in air, so various kinds of toxic wastes build up in their body which they cannot get rid of unless they are returned to the water. These wastes are acidic and as they build up inside the crab the increase in acidity begins to dissolve a small amount of the crab's shell. The amount of calcium in the blood rises, because the shell is largely made up of calcium carbonate (i.e. the same material as chalk). The rise in the concentration of calcium is not serious, but the wastes that cause it handicap the crab, making it slow and lethargic and ultimately stop it from making use of the small amount of oxygen it can breathe while in air. Without oxygen, the crab accumulates even more wastes and eventually dies.

The rate at which these wastes build up in the crabs depend on the temperature and whether or not the crabs scramble around in the basket. The crab's habit of going

motionless in air is its only means of reducing stress. Crabs will survive longer in air if they are cooled down, because this slows the rate at which the wastes build up. That is what exporters do with frozen coolants when shipping the crabs overseas. Cooling crabs down on a boat is less straight-forward but it will improve their well being.

Controlling the temperature of the crabs while handling and transporting them after harvest is difficult in some circumstances, for example on small boats. Therefore, we have been experimenting with a chemical dip that treats acidosis in live crabs, regardless of the temperature. Acidosis (the decline in the pH of the blood) is not as fast in the dipped crabs (Figure 2) and we are keen to test this dip under field conditions to see whether it improves the survival of the crabs during subsequent holding on shore. We originally hoped that the beneficial effects of the dip would allow it to be used during live export of the crabs (when acidosis is also a problem) but our laboratory trials show that the treated crabs do not live any longer than the untreated ones. The effects of the dip wear off after several hours, so it seems to be more suited to use when holding crabs at sea.

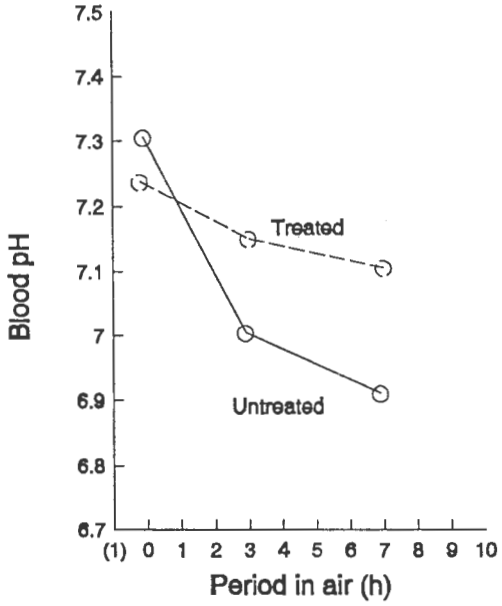

Figure 2 Slowing the onset of acidosis in spanner crabs held in air at 25°C.

Prospects

The poor performance of the spanner crab in air has prompted some people to advocate that the crabs be stored in water or that water sprays be used. It is not clear to what extent the extra effort and equipment is necessary or even what benefits follow. Storing crabs in air is very stressful, but the stress may be reversible, up to a point, and the crabs may survive well as long as they are submerged again in seawater upon reaching shore. Measuring the concentration of calcium in the blood is one way that different methods of

on-board holding can be compared, so that information is available to make meaningful decisions about changing over to new handling methods in the future.

TANKS FOR THE CRABS - LIVE HOLDING OF SPANNER CRABS PRIOR TO EXPORT

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Introduction

There are surely enough books, articles and papers in circulation about biological filters. We do not propose to write another one. But, a live holding facility is obviously necessary to accumulate stock prior to packing and export. During this time, the product is given the opportunity to recover from the stress imposed by harvesting and transport, and any weak or injured product can be sorted from the tank. That of course is the problem.

Spanner crab catches occur in bursts, largely because of bad weather keeping the fleet in. This causes fluctuations in supply to the market. There are either too many crabs or none at all. One way of levelling out the supply of crabs is to hold them over in tanks until the weather is bad, catches cease and supply to the market falls off. But there is a problem. The crabs do not always survive well in the tanks.

When compared with live seafood exporters in other parts of Australia, people who export live spanner crabs seem to be in an extraordinary hurry. The crabs leave the country about twelve hours after they reach the shore. In contrast, rock lobsters are first placed in a purging tank for a couple of days so that their guts can empty (the rationale for this is that starved animals have a lower metabolic rate and are better able to survive transport). Once purged, the lobsters are then transferred to the holding tank in preparation for export. Obviously, rock lobsters are more valuable, and may justify the extra labour costs involved in a more sedate operation but one suspects that much of the explanation for the problems with exports of spanner crabs is because they are shipped prematurely- before they've properly adjusted to captivity.

Experience shows that if you try to hold spanner crabs in tanks for more than a couple of days some of them die. These lost crabs may have been weakened and injured by stress after harvest. Yet, would some of the crabs that were apparently strong enough to be packed still have died even if you had left them in the tank? Does packing them only transfer the mortalities to the destination?

Holding crabs in tanks

This period of recovery in a tank is important when spanner crabs have been stored in air after harvest. If spanner crabs are like some other aquatic crustaceans then some aspects of their normal physiology may only require an hour to recover but in practice the crabs are usually rested overnight, and weak or dead crabs (e.g. whose claws hang limply when they are lifted from the water) are then removed from the catch the following day. When crabs are held for more than a day, it is advisable to remove the weak and dead ones at least once a day.

Sorting the catch means that only the stronger crabs will be packed, those which are in the best possible condition to be further stressed when exported, when they will be out of water again for 1 to 2 days.

Many live seafood exporters already have tanks installed for use with other species which are suitable for holding of spanner crabs. However, spanner crabs are one of the dirtiest live product on the market and the silt and body hairs that these crabs shed can do awful things to some biological filters. This is no doubt a problem that exporters have experienced at some time, and perhaps resolved in many and varied ways.

Obviously, when holding large quantities of product in a tank it is necessary to stop the environmental conditions from deteriorating. Many of the problems that arise when storing large densities of animals in holding tanks can be reduced by ensuring vigorous aeration and reducing the holding temperature, in the case of spanner crabs to between 16 and 18°C. Cooling reduces the activity, appetite and metabolic rate of the product and aeration provides oxygen as well as reducing the accumulation of waste gases such as carbon dioxide and ammonia in the tank.

As a general rule, the oxygen tension in the tank should exceed 70% of saturation at all times. This is relatively easy to achieve in practice, using blowers or vane pumps and aerators with a fine pore diameter. Large bubbles will help water movement but one large bubble has substantially less surface area for gas to move across than the same volume of air divided into smaller bubbles. We do not anticipate that oxygen level will ever be critical in a *correctly designed system.* Venturi aeration, if well designed into the system can also be advantageous, however with all aeration systems protection against loss of crabs through aeration failure should be incorporated into the design and if necessary a backup system should be considered.

As spanner crabs live in the ocean, care must be taken that the salinity in the tanks does not deviate too far from that expected under oceanic conditions (about 35 ppt). This is particularly important when the holding tank must be filled from an estuary or creek, where the salinity can fall very low after rain. At this stage we do not know the lowest salinity that these crabs can tolerate.

The crabs can be allowed to run loose in the holding tank or they can be corralled in some way. However, early attempts to use floating trays on a commercial scale were not

successful. Paradoxically, the best results in terms of survival in the tanks are obtained when the crabs are free inside the tank or corralled in cages or baskets on the tank floor. It is not clear why this should be so, but it may be a behavioural peculiarity of the animal. Obviously, there is a danger that crabs deep down in the pile will be asphyxiated in a poorly circulated tank.

The recent development of the spanner crab industry means that recommended levels of ammonia and nitrite in holding tanks must be obtained from practices applied to other crustaceans. Commercial operators of holding systems aim for a fairly rapid turnover of product, so the limits usually suggested for recirculating seawater systems (eg. 0.1 to 1.0 mg NH_3-N/l) are too conservative and under some circumstances may be difficult to maintain in practice. However an effective biofilter will ensure that increases in these ions are minimised during the storage period and reduced rapidly after crabs are removed from the tank.

A shipment of crabs spends so little time in the tank that it is arguable whether the concentrations of these wastes is a problem. However, using a biological filter is still recommended unless the fouled water is discarded after each batch of crabs goes through it. Otherwise, the water quality will deteriorate with successive batches of crabs. Once the practice of holding crabs for longer periods becomes entrenched, then biological filters will become essential. Crustaceans can tolerate surprisingly high concentrations of these wastes but only for short periods (less than 2 to 3 days).

The biofilter can be kept "alive" during periods when the tank is unstocked by routinely adding ammonia to the water. This practice of culture maintenance however, needs to be monitored and established on a trial and error basis.

Biofilters which have not been active for a period may similarly activated by addition of ammonia and possibly nitrate to encourage growth of the biofilter culture. New biofilters however need to have an active culture in addition to ammonia added to the tank, other wise the biofilter will take a long time to become established.

To ensure effective biofilter performance, tanks should not be overloaded and dead crabs need to be removed from the tank as soon as possible after death as spoilage bacteria and products of protein breakdown can have a detrimental effect on the biofilter.

Deaths in tanks

It is not clear why the crabs die during holding, but our preliminary data points to stress on the boat after capture as a contributing factor. Even the method of capture itself may be causing problems. Crabs are tangled by the joints of the legs and they are dragged, to the boat often hanging by a single leg. When the lines are retrieved at high speed the possibility of injury is intensified. Crabs that are injured or stressed on the boats will be weakened when submerged in the tanks and may succumb after a day or so to bacterial infections.

In one experiment, we cooled crabs on the boat by putting them into export style foam boxes with frozen coolants. Other crabs caught at the same time were treated in the usual manner in lug baskets. These crabs were then returned to our holding tank, which was partitioned so that we could see if there was any difference in survival of crabs handled in the different ways. We removed the dead crabs each day and kept a tally of deaths from each group for a week. The results are summarised in Figure 1.

Typically, there is a lag of a day of two before the number of deaths reaches a peak and then begins to taper off. The difference in survival between the two treatments was significant at 96 h, indicating that the handling of the product on the boats can influence the ability of the crabs to survive in holding tanks on shore. When the crabs had been held for six days, 80% of the crabs that were cooled on the boat remained alive, while only 63.6% of the crabs treated in the usual manner survived, again this difference is statistically significant.

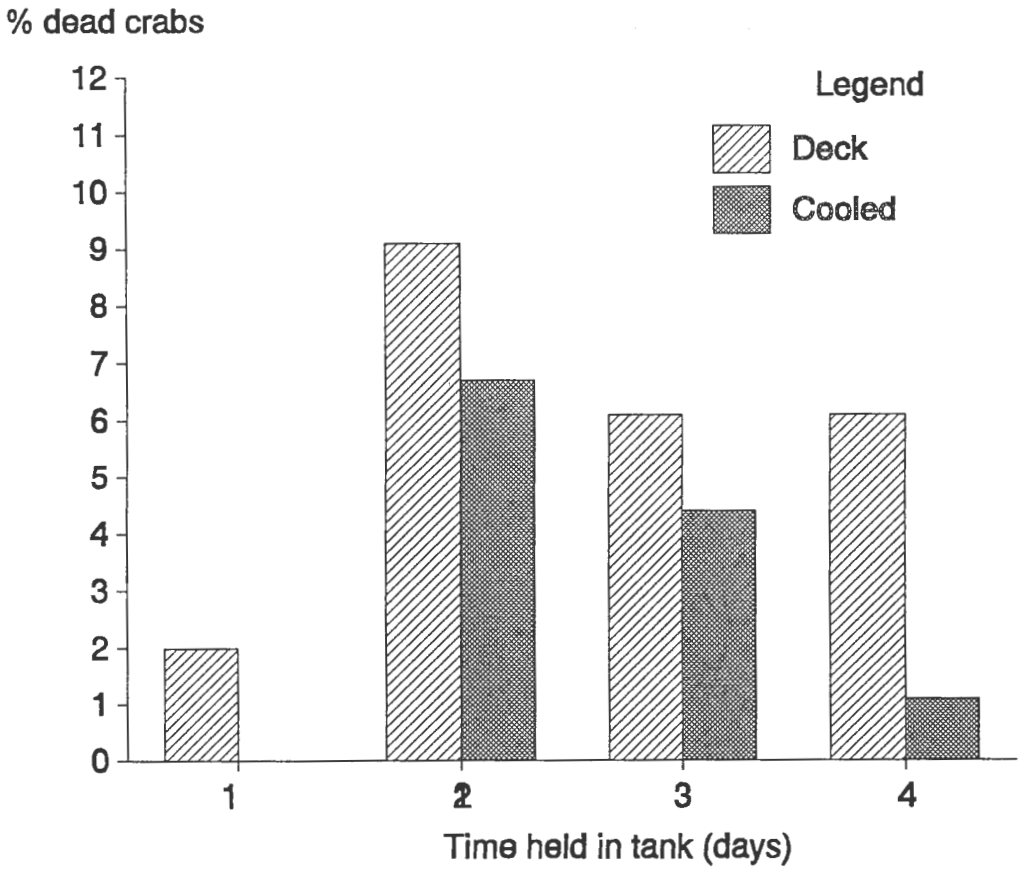

Figure 1 Crab deaths in holding tanks. Fewer crabs die if they are held beforehand at lower temperature on the boat.

We have not looked any further into the causes of these deaths. Autopsies will probably show bacterial infections but whether these have arisen through the crabs being injured and stressed in other ways would need to be studied. We have seen that when spanner crabs are resubmerged, certain properties of their blood do not return to normal suggesting that the period spent in air on the boat has injured them irreversibly.

Prospects

The marketing advantages to be gained from holding crabs for long periods in tanks mean that in the future, exporters will have to pay more attention to the reasons why crabs die. Part of the mortality is probably a delayed result of stress that the crabs experience on the boats and future changes in on-board handling will probably reduce mortality of crabs in the tanks and ease much of the urgency with which exporters currently handle the crabs.

NOTES

ACID NOUS - LIVE EXPORT OF SPANNER CRABS (*RANINA RANINA*)

Brian Paterson
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Introduction

Live exports of spanner crabs (*Ranina ranina*) have been under way for about 3 years. Spanner crabs are exported at a temperature of 12 to 16°C and under controlled conditions this species will survive for 2 to 3 days in air. However, survival of export shipments to Taiwan is not consistent, even when sent by experienced exporters. This variation in results (with mortality reaching 40% or more) may be caused by the handling that the crabs receive either on the boat or during the airflight. Much of the variation will probably be reduced by improvements in handling methods on the boats and greater care in the purging and grading the product for export.

Our research has sought to override this variation by using knowledge of the physiology of crustaceans to treat the symptoms of transport stress, rather than just trying to control the temperature of the crabs. Spanner crabs have difficulty breathing when they are lifted from the water. Adding pure oxygen is sometimes suggested as a way to assist the respiration of seafood under these circumstances. However, physiologist's have misgivings about this practise because it can allow unnaturally high levels of oxygen to enter the blood. The animal's body uses the level of oxygen in the blood to regulate the rate of blood flow, so it may misinterpret the seriousness of its predicament. However, apart from problems with oxygen, the build up of carbon dioxide in the crabs also limits their ability to survive in air.

High concentrations of carbon dioxide in the crabs cause the pH of their blood and tissues to fall (acidosis) and this symptom is thought to be a major cause of death when aquatic crustaceans are shipped in air. We have developed a method of slowing the progress of acidosis in the blood (independent of temperature) when spanner crabs are stored in air. Unfortunately, this method wears off too quickly within the time frame required for live exports and it may therefore be more suited to treating crabs stored on boats.

This paper discusses the method of cooling and packaging used to export live spanner crabs and examines the physiological principles underlying attempts to use other methods to prolong the survival of crabs.

Cooling and packing crabs

A variety of cooling methods have been applied to spanner crabs, and information on optimum rates of cooling are not available. These crabs can be cooled to a temperature of 12 to 16°C. The rate at which they are cooled depends upon the cooling capacity of the

live storage tank, but in general the tank is cooled down overnight so that the crabs are ready to be packed in the morning.

Once the product is cooled and lethargic, a final grading is carried out when packing them, both for size and also to remove any weak or injured animals (damaged or missing legs). Weak crabs can be identified by their lack of muscle strength, for example their claws hang limply when the crab is taken from the water.

The method used to ship spanner crabs is similar to that applied to many other live crabs and lobsters. Care must be taken with spanner crabs because of the amount of water that they can hold in the chambers in the shell where their gills are located. This water can drain out of the crabs during export, contributing to an excess "drip loss."

The crabs are packed tail-down into polystyrene boxes, the most practical way of fitting the crabs into the box. It is not known if any particular orientation of the crab is better than another when it comes to survival. However, to efficiently pack the crabs another way would probably require re-designing the box. A layer of sponge foam or similar on the bottom of the box is required to cushion the crabs during handling of the carton, otherwise the shock would be transmitted directly onto the narrow tail of the crab. Excess space in the box is usually filled with wood wool and a frozen coolant is added (though not in direct contact with the crabs) to keep the contents of the box cold.

The packing method is so straight forward that when a long string of good results is suddenly broken you immediately suspect that there was either something wrong with the crabs you packed or that they were handled badly by the airline. Handling mishaps on the plane are going to happen from time to time. Handling of the crabs on the boats and in tanks is already improving. Eventually, we will reach a point where the only variation in results is caused by the crabs themselves, perhaps in conjunction with the time of year.

The industry has been around for long enough now that patterns may be emerging regarding the performance of the crabs from year to year. Some other live seafood products show better survival during the cooler months of the year. This same pattern is just as likely to emerge with spanner crabs, more so because of the problems arising when storing them on deck in summer.

Stress and live transport of crabs

Controlling temperature in transit is the usual method for prolonging the survival of live seafood. Yet, this variable cannot always be reliably controlled. So, is it possible to use physiological knowledge to treat crabs when they are stored out of water?

Spanner crabs have difficulty breathing when they are lifted from the water. People have tried filling the boxes with oxygen gas to help aquatic crustaceans breathe in air, but sometimes this kills the product faster than it would otherwise die if left untreated.

For example, farmers who ship live freshwater crayfish (*Cherax* species: yabbies, marron, red-claw crayfish) routinely use pure oxygen or oxygen generators to help the crayfish survive long journeys yet they have no rationale for doing so, other than a fear that the crayfish will use up all of the oxygen in the box. Yet, I am not aware of any experimental proof that adding oxygen is necessary. In contrast, there is a considerable amount of physiological evidence to suggest that this practise is potentially harmful!!!

When crabs are in air they have trouble taking in oxygen and excreting carbon dioxide, resulting in a decline in blood pH (acidosis) as wastes build up in their tissues and blood. The blood pH falls rapidly in the first hour or so after the animal has been removed from the water (Figure 1). Afterwards the animals internal mechanisms normally begin to compensate. However, the spanner crab, unlike many other crabs and lobsters, has difficulty preventing acidosis in its blood. Apparently, the acidosis eventually slows down in spanner crabs through an effect of low pH on metabolism itself rather than any compensatory mechanism.

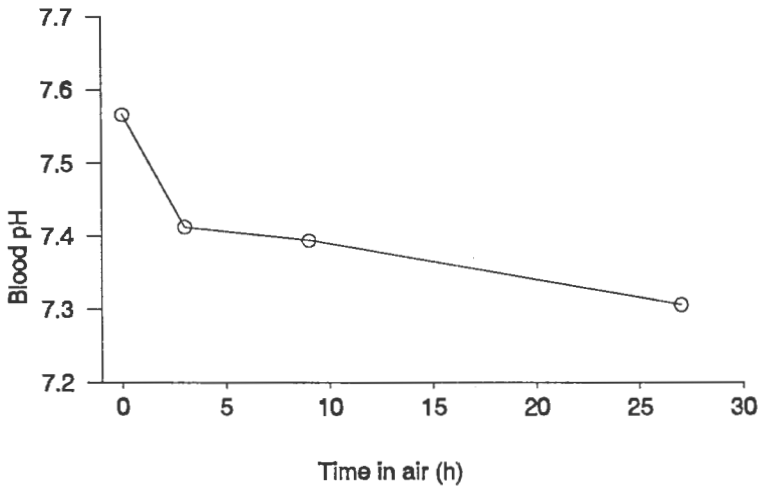

Figure 1 Decline in blood pH in spanner crabs held in air at 16°C.

Acidosis is thought to be a major cause of death when crustaceans are transported since metabolic processes require the blood and tissues to remain within the "normal" range of pH. Acidosis is usually slowed by keeping the product cool during shipment. This lowers its metabolic rate, reducing the build up of wastes, and in some circumstances can reduce the metabolic rate to the point that it is easily satisfied by the limited amount of oxygen uptake that is possible in air.

All that storing the crabs in an atmosphere of pure oxygen does is to increase the inward flow of oxygen (Figure 2). This may avoid the appearance of wastes such as lactic acid (produced when the crab is starved of oxygen) but it causes a higher than usual amount of

carbon dioxide to accumulate in the blood. This build up of carbon dioxide exaggerates and not reduces the acidosis experienced by spanner crabs held in air (Figure 3).

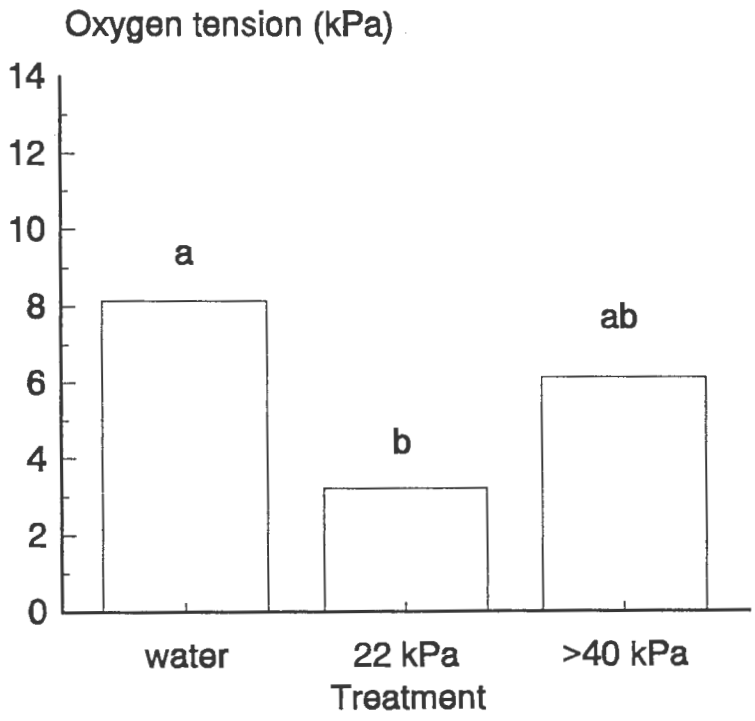

Figure 2 Mean oxygen (O_2) partial pressure (kPa) in the blood of spanner crabs stored at 25°C for 3 hours in aerated seawater ('water'), normal atmosphere ('22 kPa') and in an oxygen-enriched atmosphere ('>40 kPa'). Bars with the same letter on top are not significantly different.

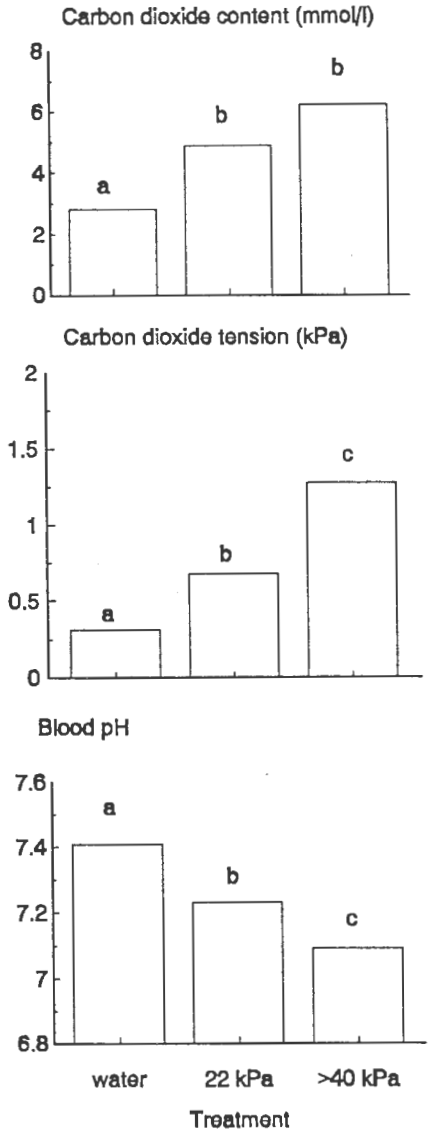

Figure 3 Mean carbon dioxide (CO_2) content, CO_2 partial pressure (kPa) and pH in the blood of spanner crabs stored at 25°C for 3 hours in aerated seawater ('water'), normal atmosphere ('22 kPa') and in an oxygen-enriched atmosphere ('>40 kPa'). Six crabs per treatment. For individual graphs, bars with the same letter over them are not significantly different.

We have developed a method of treating the crabs which slows the onset of acidosis when they are stored out of water. However, experiments show that the effects of the treatment do not persist long enough to prolong the survival of crabs held at 20°C for up to 36 hours. What may happen is that the pH in the blood of the untreated crabs falls so rapidly in the first few hours that it inhibits the crab's metabolism. Remember, the crabs also go very still in air. The acidosis slows down the rate of production of the very wastes that caused the acidosis in the first place. The untreated crabs remain in a sort of physiological "limbo" until the treated crabs catch up to them (after a point the blood pH

in the two treatments is the same) and by this stage the treatment has no effect on survival.

Prospects

Shipments of live spanner crabs with a poor outcome can arise from a number of causes. Occasional handling mishaps by the airlines are probably inevitable. Improvements in the methods of handling of the crabs after harvest and by taking time to purge the crabs on shore will probably make the export process itself more reliable, by ensuring the initial quality of the product.

The method used to export the crabs shows less scope for improvement. Refinements in the packaging materials used are feasible though always keeping in mind economic pressures. Physiological studies show that we can rule out using oxygen on these crabs. This result calls into question other instances where oxygen is used to help the product to "last the distance". Treating the product with oxygen during live transport may only work when the crustacean involved already has the ability to compensate for high levels of carbon dioxide and other acidic wastes in its blood.

Slowing the rate of acidosis in the crabs does not make them live any longer under experimental conditions within the time required to market them overseas. Nevertheless, the treatment we have developed for slowing acidosis will be most active during the flight when most handling problems and rises in temperature occur. Proving that this is beneficial during commercial shipments will not be straightforward. Mishaps are by their very nature unpredictable. As I've already pointed out, the time frame that this treatment persists for is similar to the length of time that crabs are held out of water after harvest, suggesting that it may prove most useful in lessening the stress imposed on the crabs at this time.

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