ROCK LOBSTER ENHANCEMENT AND AQUACULTURE SUBPROGRAM:

PILOT STUDY OF DISEASE CONDITIONS IN ALL POTENTIAL ROCK LOBSTER AQUACULTURE SPECIES AT DIFFERENT GROWTH STAGES

Professor Louis Evans

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

curtin University of Technology Western Australia

ROCK LOBSTER ENHANCEMENT & AQUACULTURE SUBPROGRAM

Project No. 1998/304

1998/304-Pilot Study Of Disease Conditions In All Potential Rock Lobster Aquaculture Species At Different Growth Stages

Principal Investigator:
Professor Louis H. Evans[1]

Co-Investigators:
Dr Brian Jones[2], Dr John Norton[3], Dr Judith Handlinger[4], Dr Japo Jussila[1], Dr ElenaTsvetnenko[1], Dr Annette Thomas[3]

**1 Aquatic Science Research Unit, Curtin University of Technology,
2 Fisheries WA, 3 Queensland DPI, 4 Tasmania DPIF**

2003
ISBN

TABLE OF CONTENTS

1998/304 PILOT STUDY OF DISEASE CONDITIONS IN ALL POTENTIAL ROCK LOBSTER AQUACULTURE SPECIES AT DIFFERENT GROWTH STAGES

Principal Investigator: Professor Louis Evans
Address: Aquatic Science Research Unit
Muresk Institute
Curtin University of Technology
Kent St
Bentley 6102
Telephone**:** 08 9266 4500 Fax: 08 9266 4422

OBJECTIVES:

1. To establish a national network of rock lobster health and disease personnel.
2. To conduct a symposium on health and disease management in lobster aquaculture and long-term holding facilities.
3. To collate and document the current state of knowledge on rock lobster diseases.
4. To provide a disease diagnosis service for existing FRDC rock lobster projects in Queensland, South Australia and Western Australia.

NON-TECHNICAL SUMMARY

OUTCOMES ACHIEVED TO DATE
A lobster health network was successfully established following a workshop of health personnel, as part of this project. A description of dissection and examination procedure for lobster autopsy was produced following the workshop. An International Symposium on Lobster Health Management was convened in conjunction with the Third international Lobster Congress in South Australia. Members of the research team provided a disease diagnostic service in their respective states.

Due to the infancy of rock lobster aquaculture, disease conditions associated with production are poorly understood. Similarly, the prevalence of disease conditions in wildstock and their likely impact in aquaculture systems or extended holding systems has yet to be determined. While other factors associated with the establishment of rock lobster aquaculture are perceived as a higher priority, it is recognised that health monitoring and the early identification of diseases that may affect production is critical. In the short term there is a need to establish a mechanism for the monitoring of disease conditions of juvenile and adult lobsters in land-based and sea-based holding systems. This will not only provide industry with a means of assessment of moribund lobsters, but will facilitate the identification of health research priorities.

The principal diseases of spiny lobsters comprise bacterial diseases (gaffkemia, shell disease, vibriosis), fungal infections (systemic and superficial infections) and parasitic infections (Sindermann 1990; Bower *et al.* 1994; Evans *et al.* 2000). Not all of these

diseases have been reported from Australian spiny lobsters. For example, two of the major disease conditions observed in holding facilities for clawed lobsters in North America, gaffkemia (Stewart 1980) and 'bumper car' disease (Cawthorn 1997), have yet to be observed in Australian lobsters. Shell disease, on the other hand, is ubiquitous and has been reported from numerous clawed and spiny lobster species from both the Northern and Southern hemispheres (Getchell 1989; Evans *et al.* 2000).

In order to minimise disease outbreaks in cultured lobsters and manage disease conditions that do occur, protocols need to be developed for cost-effective health management. In addition, personnel need to be trained in lobster disease diagnosis and health management and detailed information on likely disease conditions is required. This project aimed to address aspects of these varying needs by conducting a pilot study of disease conditions in all potential rock lobster aquaculture species at different growth stages. A grant of $19,999 was awarded to the project team by FRDC and additional funds were provided by organisations whose staff participated in the project.

PROJECT PARTICIPANTS

The project was coordinated by Professor Louis Evans, Aquatic Science Research Unit, Curtin University of Technology. Co-investigators of the project were Dr Brian Jones, Fisheries WA, Dr John Norton and Dr Annette Thomas, QLD DPI, Dr Judith Handlinger, TAS DPIF and Dr Japo Jussila and Dr Elena Tsvetnenko, Curtin University of Technology.

PROJECT OUTCOMES

The project outcomes for each of the four objectives were as follows:

1. Establish a national network of rock lobster health and disease personnel

A one-day planning workshop, held in Perth in June, 1998, facilitated the sharing of knowledge and information between fish health personnel from four Australian states and established a national network of rock lobster health and disease personnel. Approximately 25 industry representatives and project participants attended the workshop. Details on the workshop proceedings is attached (Appendix 1). A description of the dissection and examination procedure for a lobster autopsy was produced following the workshop (Appendix 2).

2. Conduct a symposium on health and disease management in lobster aquaculture and long-term holding facilities

The lobster health management symposium was held in Adelaide in September, 1999 and was attended by approximately 70 industry, government, FRDC and research personnel. The program included three keynote addresses from international experts in lobster/crustacean health management as well as presentations from Australian and New Zealand lobster aquaculture and enhancement researchers. An Abstract Proceedings (Appendix 3) was distributed at registration. A full Proceeding comprising ten written papers (Appendix 4) is being placed on the Muresk Institute of Agriculture, Curtin University www site and will be advertised in a special edition of Marine and Freshwater Research containing papers from the Florida lobster conference. Copies of the Proceedings are available upon request. Two panel discussions, one on 'Health

Management Issues in Lobster Aquaculture' and the other on 'Live Lobster Export' were a feature of the symposium and encouraged active information exchange between researchers and industry personnel. Synopses of these two panel discussions (Appendix 5) have been included in the Proceedings.

3. Collate and document the current state of knowledge on rock lobster diseases

Associate Professor Louis Evans and Dr Brian Jones collaborated with Dr James Brock, Department of Agriculture, Hawaii, in the preparation of a review of the current state of knowledge of rock lobster diseases. The review will be published in the second edition of the book 'Spiny Lobsters: Fisheries and Cultures' edited by B.F. Phillips and J. Kittaka. Information concerning the current state of knowledge of rock lobster diseases is also documented in the Proceedings of the International Symposium on Rock Lobster Health Management.

4. Provide a disease diagnosis service for existing FRDC rock lobster projects in Queensland, South Australia and Western Australia.

Fish health personnel from the four different states have conducted a series of investigations on disease conditions in rock lobsters. These investigations included a detailed study of immune parameters and histopathology in healthy and moribund tropical rock lobsters in Queensland, an investigation of shell disease and poor health in southern rock lobsters in South Australia, a study of disease conditions in puerulus and juvenile southern rock lobsters in Tasmania and a study of the effect of air exposure and holding conditions on rock lobster immune parameters in Western Australia. Detailed reports on some of these investigations are appended (Appendices 6-10).

Keywords: lobster**,** disease, growth stages, physiology, stress, *Panulirus cygnus, Jasus sp.*

ACKNOWLEDGEMENTS

The advice, assistance and funding support from the Fisheries research and Development Corporation (FRDC) and the critiques and review by colleagues from the FRDC Rock Lobster Enhancement and Aquaculture Subprogram is gratefully acknowledged. We also wish to thank the staff of lobster processing facilities in Western Australia, South Australia and Queensland for their support.

PILOT STUDY OF DISEASE CONDITIONS IN ALL POTENTIAL ROCK LOBSTER AQUACULTURE SPECIES AT DIFFERENT GROWTH STAGES

FINAL REPORT

1. BACKGROUND

Due to the infancy of rock lobster aquaculture, disease conditions associated with production are poorly understood. Similarly, the prevalence of disease conditions in wildstock and their likely impact in aquaculture systems or extended holding systems has yet to be determined. While other factors associated with the establishment of rock lobster aquaculture are perceived as a higher priority, it is recognised that health monitoring and the early identification of diseases that may affect production is critical. In the short term there is a need to establish a mechanism for the monitoring of disease conditions of juvenile and adult lobsters in land-based and sea-based holding systems. This will not only provide industry with a means of assessment of moribund lobsters, but will facilitate the identification of health research priorities.

The principal diseases of spiny lobsters comprise bacterial diseases (gaffkemia, shell disease, vibriosis), fungal infections (systemic and superficial infections) and parasitic infections (Sindermann 1990; Bower *et al.* 1994; Evans *et al.* 2000). Not all of these diseases have been reported from Australian spiny lobsters. For example, two of the major disease conditions observed in holding facilities for clawed lobsters in North America, gaffkemia (Stewart 1980) and 'bumper car' disease (Cawthorn 1997), have yet to be observed in Australian lobsters. Shell disease, on the other hand, is ubiquitous and has been reported from numerous clawed and spiny lobster species from both the Northern and Southern hemispheres (Getchell 1989; Evans *et al.* 2000).

2. NEED

Effective health management of rock lobsters held in captivity is of critical importance in long term holding of rock lobsters and in lobster aquaculture. However, in order to achieve effective health management, data and information is required on diseases that are known to occur in rock lobsters and protocols need to be developed for prevention and treatment of these diseases. Information should be provided to fishers on the factors contributing to the development of disease in post-harvest lobsters. In addition, information sharing and training opportunities are required for personnel likely to be involved lobster disease diagnosis.

This project aimed to address aspects of these varying needs by conducting a one year pilot study of disease conditions in all potential rock lobster aquaculture species at different growth stages. A grant of $19,999 was awarded to the project team by FRDC and additional funds were provided by organisations whose staff participated in the project.

3. OBJECTIVES

The aims of the project were to:

- establish a national network of rock lobster health and disease personnel
- conduct a symposium on health and disease management in lobster aquaculture and long-term holding facilities
- collate and document the current state of knowledge on rock lobster diseases
- provide a disease diagnosis service for existing FRDC rock lobster projects in Queensland, South Australia and Western Australia.

4. ACHIEVEMENT OF PROJECT OBJECTIVES

The approach followed to achieve the four project objectives and the outcomes of these activities are described below.

4.1 Establish a National Network of Rock Lobster Health and Disease Personnel

The establishment of the network of rock lobster health and disease personnel was commenced by conducting a planning workshop in Perth on 24th June, 1998. Approximately 25 industry representatives and project participants attended the workshop which comprised a one day workshop of presentations by project participants and other FRDC losbter researchers followed by a second day of meetings between the project participants.

A workshop proceedings was produced and circulated on the day of the workshop. Details of the revised version incorporating changes recommended by the Sub-Program Leader is attached (Appendix 1).

A standardised approach to conducting an autopsy on rock lobsters was discussed during the meetings held after the workshop. Comments and suggestions were incorporated into a draft version of the autopsy procedure and this version, which describes the dissection and examination procedure, was produced and circulated to project participants for comment (Appendix 2). Further expansion of the description of the autopsy procedure including details of hemolymph sampling and testing will be presented in the final report of the subsequent FRDC project performed by the project team – FRDC 99/202: Rock Lobster Autopsy Manual.

4.2 Conduct a Symposium on Health and Disease Management in Lobster Aquaculture and Long-Term Holding

The International Symposium on Lobster Health Management was held at the South Australian Research and Development Institute (SARDI), Adelaide, from Sunday, 19th September to Tuesday, 21st September, 1999. The Symposium preceded the Lobster Congress held at the Stamford Hotel in Adelaide from 21st – 25th September. Over 70 participants from industry, government organisations and research agencies attended the symposium. Presenters included research personnel from Australia, New Zealand, USA, Canada, Finland and Norway.

The aim of the symposium was to provide a forum for information exchange between scientists and industry personnel on health management of lobsters and other commercially important crustaceans. Key speakers at the Symposium included Professor Kenneth Söderhäll, University of Uppsala, Sweden, an international authority on crustacean immunity, Professor Bob Bayer, Lobster Institute, University of Maine, USA, an international expert on lobster health management and Professor Rick Cawthorn, Atlantic Veterinary College, University of Prince Edward Island, Canada, the Director of Lobster Health Research Centre the mandate of which is to apply the principles of veterinary medicine to the postharvest sector of crustacean fisheries and crustacean aquaculture, in particular clawed lobsters.

The program comprised eight sessions over two days, the first three sessions on each day comprising keynote addresses and submitted presentations and the last session a panel discussion. The session and panel discussion topics were as follows:

Day 1

- Health management in lobster aquaculture and long term holding
- Immunity and health assessment
- Lobster health management studies
- Health management issues in lobster aquaculture

Day 2

- Stress assessment techniques
- Stress and health management
- Lobster postharvest and enhancement
- Live lobster export
- Crustacean stress and immunity

An Abstract Proceedings of the presentations was distributed to participants at the conference (Appendix 3). Full written papers were submitted by some of the participants and a peer review of submitted papers was conducted.

A full Proceedings comprising eleven written papers (Appendix 4) is being placed on the Muresk Institute of Agriculture, Curtin University www site and will be advertised in a special edition of Marine and Freshwater Research containing papers from the Florida lobster conference. Copies of the Proceedings are available upon request.

Two panel discussions, one on 'Health Management Issues in Lobster Aquaculture' and the other on 'Live Lobster Export' were a feature of the symposium and encouraged active information exchange between researchers and industry personnel. Synopses of these two panel discussions (Appendix 5) have been included in the Proceedings.

4.3 Collate and Document the Current State of Knowledge on Rock Lobster Diseases

Associate Professor Louis Evans and Dr Brian Jones collaborated with Dr James Brock, Department of Agriculture Hawaii, in the preparation of a review of the current state of knowledge of rock lobster diseases. The review will be published in the second edition of the book 'Spiny Lobsters: Fisheries and Culture' edited by B.F. Phillips and J. Kittaka. Information concerning the current state of knowledge of rock lobster diseases is also documented in the Proceedings of the International Symposium on Rock Lobster Health Management.

4.4 Provide a Disease Diagnosis Service for Existing FRDC Rock Losbter Projects in Queensland, South Australia and Western Australia

Fish health personnel from the four different states have conducted a series of investigations on disease conditions in rock lobsters. These investigations included a detailed study of immune parameters and histopathology in healthy and moribund tropical rock lobsters in Queensland, an investigation of shell disease and poor health in southern rock lobsters in South Australia, a study of disease conditions in puerulus and juvenile southern rock lobsters in Tasmania and a study of the effect of air exposure and holding conditions on rock lobster immune parameters in Western Australia. Detailed reports on some of these investigations are appended (Appendices 6-10).

5. BENEFITS

The Australian rock lobster industry will benefit from this research by having improved information on disease conditions likely to occur in aquaculture or long term holding conditions. The aims of the project in collating information on lobster diseases and in promoting collaboration and communication between lobster health personnel throughout Australia was successfully achieved.

6. FURTHER DEVELOPMENT

While this project achieved the stated aim of collating information on rock lobster diseases there is still a significant lack of understanding of factors contributing to lobster diseases and of disease conditions occurring in Australian lobsters. For example, no virus diseases have as yet been described in spiny lobsters. Viral diseases are of major health concern in most aquaculture species, the recent losses in production in shrimp aquaculture through viral diseases being a prime example. A survey of disease conditions in wild stock lobsters, as has been proposed in Tasmania, will address this concern and make a significant contribution to knowledge of disease conditions in Australian rock lobsters.

One of the major disease conditions likely to affect the quality of product in long term storage of lobsters is tail rot, a form of shell disease. Investigations on the etiology of this condition, and on approaches to minimise its occurrence in cultured or stored lobsters, should be given a high priority. Simplifying and improving methods of diagnosing and monitoring lobster health is another priority area for research.

Support and funding for disease studies in aquatic animals is currently being provided by the Australian Federal Government through the recently established AQUAPLAN initiative. Given the importance of disease prevention and health management in any aquaculture operation, consideration should be given to providing support through AQUAPLAN to maintain and expand the network of lobster health personnel established through this FRDC project. This could be achieved through sponsorship of an annual workshop of lobster health management as well as post-graduate student scholarships in this area.

7. CONCLUSION

This project was successful in:

- Establishing a network of rock lobster health personnel
- Conducting an international symposium on lobster health management
- Collating information on the current state of knowledge on spiny lobster disease conditions, and
- Providing a disease diagnostic service for existing FRDC rock lobster projects in Queensland, South Australia and Western Australia.

The way forward for lobster health management is the development of effective procedures for preventing diseases in lobster aquaculture and live holding facilities and for treating disease outbreaks when and if they occur.

8. REFERENCES

Bower, S. M., McGladdery, S. E. & Price, I. M. (1994) Synopsis of infectious diseases and parasites of commercially exploited shellfish. *Annual Review of Fish Diseases*, **4**, 1-199.

Cawthorn, R. J. (1997) Overview of 'bumper car' disease – impact on the North American lobster fishery. *International Journal for Parasitology*, **27**, 167-72.

Evans, L. H., Jones, J. B. & Brock, J. A. (2000) Diseases of spiny lobsters. In: *Spiny Lobster Management* (Ed. by B. F. Phillips, J. Kittaka & J. S. Booth), pp. 586-600. Gray Publishing, Kent, UK.

Getchell, R. G. (1989) Bacterial shell disease in crustaceans: a review. *Journal of Shellfish Research*, **8**, 1-6.

Sindermann, C. J. (1990) *Principal Diseases of Marine Fish and Shellfish,* 2nd edn., Vol. 2*, Diseases of Marine Shellfish.* Academic Press, San Diego.

Stewart, J. E. (1980) Diseases. In: *The Biology and Management of Lobsters*, Vol. 1 (Ed. by J. S. Cobb & B. F. Phillips), pp. 301-42. Academic Press, New York.

APPENDIX 1

PROCEEDINGS, ROCK LOBSTER DISEASE WORKSHOP

June 1998

Table of Contents
Aim and Scope of Workshop
Outcomes of Workshop

(Copies available from Associate Professor Louis Evans, Aquatic Science Research Unit, Curtin University of Technology, Kent Street, Bentley WA 6102)

curtin
University of Technology
Perth Western Australia

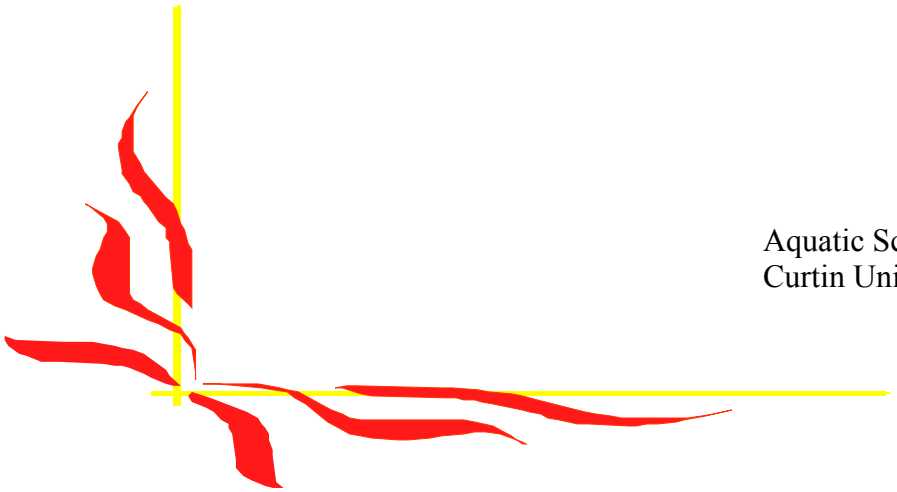
FRDC ROCK LOBSTER

AQUACULTURE SUB-PROGRAM

ROCK LOBSTER DISEASE WORKSHOP

Technology Park, Bentley
24th June, 1998

PROCEEDINGS



Aquatic Science Research Unit
Curtin University of Technology

TABLE OF CONTENTS

AIM AND SCOPE OF WORKSHOP

The main aim of the workshop was to provide a forum for researchers and industry personnel to discuss current and likely future problems arising from disease outbreaks in postharvest and cultured lobsters and to identify future research priorities.

The workshop comprised one day of presentations on topics relating to lobster stress physiology, immunity and disease processes, and case studies, followed by a general forum.

APPENDIX 2

LOBSTER AUTOPSY PROCEDURE

FRDC REPORT

LOBSTER AUTOPSY PROCEDURE

Aquatic Science Research Unit
Curtin University of Technology

TABLE OF CONTENTS

CURTIN UNIVERSITY OF TECHNOLOGY
AQUATIC SCIENCE RESEARCH UNIT, MURESK INSTITUTE OF AGRICULTURE

LOBSTER AUTOPSY PROCEDURE

1. Lobster Collection

It is preferable that lobsters on which an autopsy is to be conducted are held in an aquarium or a holding tank with good aeration and water quality conditions prior to the autopsy. Delivery to the laboratory in an esky or foam container with an ice bottle included to reduce temperature is an alternative collection procedure but it should be noted that prolonged air exposure will alter blood chemistry and vigour index results. Lobsters should be cooled down prior to dissection by placing in the freezer section of a refrigerator for at least 5 minutes.

2. Vigour Index

Prior to collection of the hemolymph sample and dissection a vigour index should be performed. The method for conducted a vigor index is described in Appendix 1.

3. Gross Observations and Morphometric Measurements

The following measurements and observations should be recorded on the results sheet: Sex, orbital carapace length, wet weight, moult stage. If the lobster is from an aquaculture facility, condition factors may also be of diagnostic significance. These are obtained by weighing the whole hepatopancreas or tail muscle, removing a 1-5g sample, recording the wet weight of the sample, drying the sample and calculating the desired condition factor (Wet or dry hepatosomatic ratio or wet or dry tail muscle ratio) using the equation : Wet or dry weight of organ/Total body wet weight x 100.

4. Hemolymph Collection

Hemolymph should be collected from the base of the fifth walking leg. Different samples are required depending on the test to be performed (e.g. total or differential hemocyte counts, clotting time, hemolymph protein or other biochemistry tests or bacteriology). Whether or not the hemolymph is collected into a preservative solution and if preservation is required, the nature of the solution, will vary depending on the diagnostic test. Information on these tests will be provided in the expanded version of the dissection procedure to be published in the final report of the FRDC project: FRDC 99/202.

In order to collect the hemolymph the surface of the membrane between the dorsal area of the carapace and the tail is swabbed with 70% ethanol. The hemolymph is then collected with a sterile syringe with a 24 gauge needle, both of which should be stored on ice or kept in the refrigerator prior to use.

5. Dissection Procedure

The following dissection procedure is recommended:

Remove the lobster from the aquarium, place in a refrigerator for approximately 2 hours to induce finaesthesia and place it on a dissection board (Fig. 1). Heavy duty rubber gloves should be worn to avoid cuts or wounds. If skin damage is accidentally sustained it is recommended that the area be immediately washed and swabbed with a suitable disinfectant (e.g. betadene).

Using a sharp knife remove the proximal area of the carapace just behind the antennae (Fig. 2) and then dissect away all walking legs (Figs. 3 & 4). Cut along the junction of the carapace and the abdomen to remove the tail (Fig. 5). The specimen is now ready for removal of body tissues (Fig. 6). Cut through the thoracic region just internal to the two branchiostegal lines (i.e. two longitudinal cuts from the distal edge of the carapace to the proximal cut surface, about 1cm on either side of the centre of the carapace) (Fig. 7). Carefully cut away the exoskeleton from underlying tissues to reveal the heart, hepatopancreas and gonads (Figs. 8 & 9). If the lobster is close to moult there may be a thin sheet of unhardened chitin underlying the exoskeleton - this should be stripped off with forceps. Remove the left brachiostegite to reveal the gill chambers by cutting along the line. Follow the same procedure to remove the right brachiostegite.

6. Tissue Sample Collection

The removal of tissues should be performed as rapidly as possible as to avoid autolysis. The most sensitive tissues with respect to autolysis are the hepatopancreas and the antennal glands. These should be the first tissues to be removed. Tissue samples should be removed from the following organs and placed in the fixative: hepatopancreas (two samples, one from the proximal lobe on one side of the body and the other from the distal lobe on the other side of the body; see Fig. 9), antennal gland (two samples, comprising one half of each gland; see Fig. 10), gills (one podobranch from each branchial cavity; see Fig. 11), heart (two samples, obtained by cutting the heart in half and then taking one half of each section; see Fig. 8), midgut (one sample taken close to the junction of the midgut and the hindgut), hindgut (one sample taken approximately half way down the length of the organ;), ventral nerve (one sample taken from the proximal region of the tail) and abdominal muscle (two samples taken from the abdominal muscle immediately adjacent to the carapace; see Fig. 12). Each tissue piece should be approximately 0.5-1 cm^3. The volume of fixative to tissue should be at least 20:1.

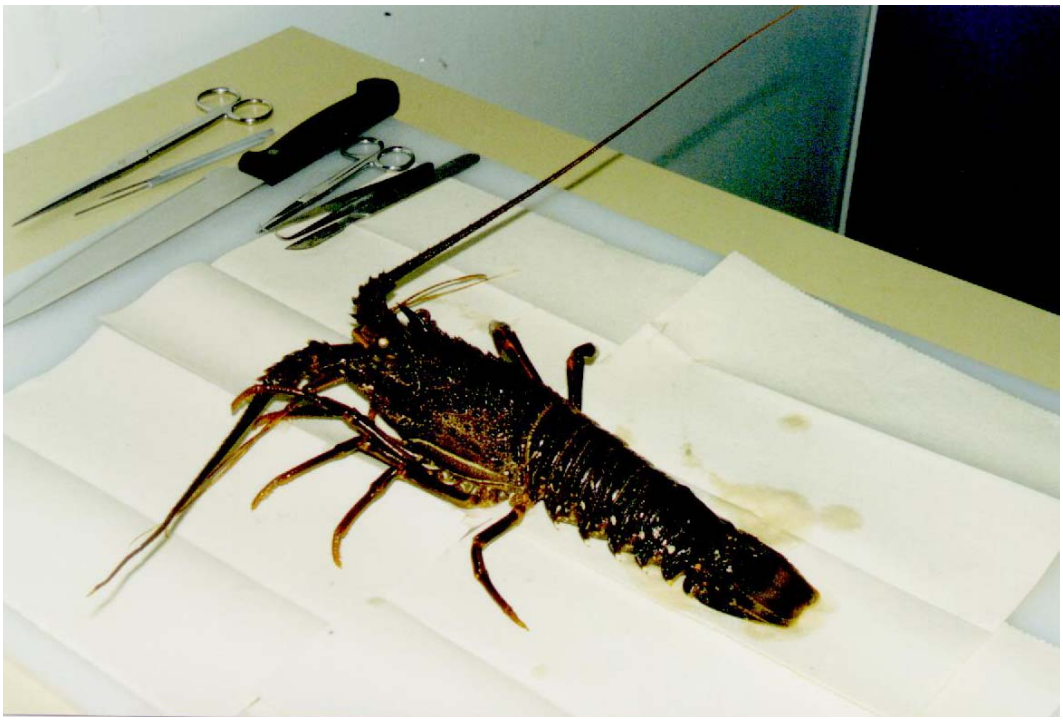


Figure 1 Preparation of lobster for dissection

Figure 2 Removal of proximal section of carapace

Figure 3 Removal of walking legs

Figure 4 All walking legs removed

Figure 5 Separation of tail and abdomen

Figure 6 Lobster ready for removal of tissue specimens

Figure 7 Location of branchiostegal line

Figure 8 Location of heart

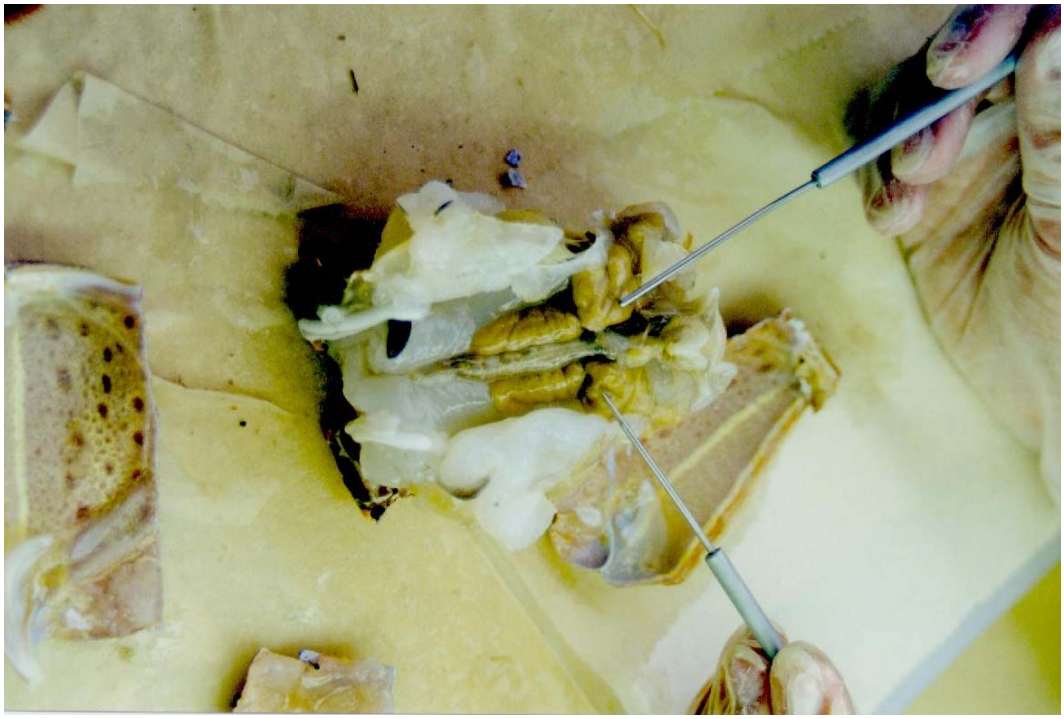


Figure 9 Location of hepatopancreas

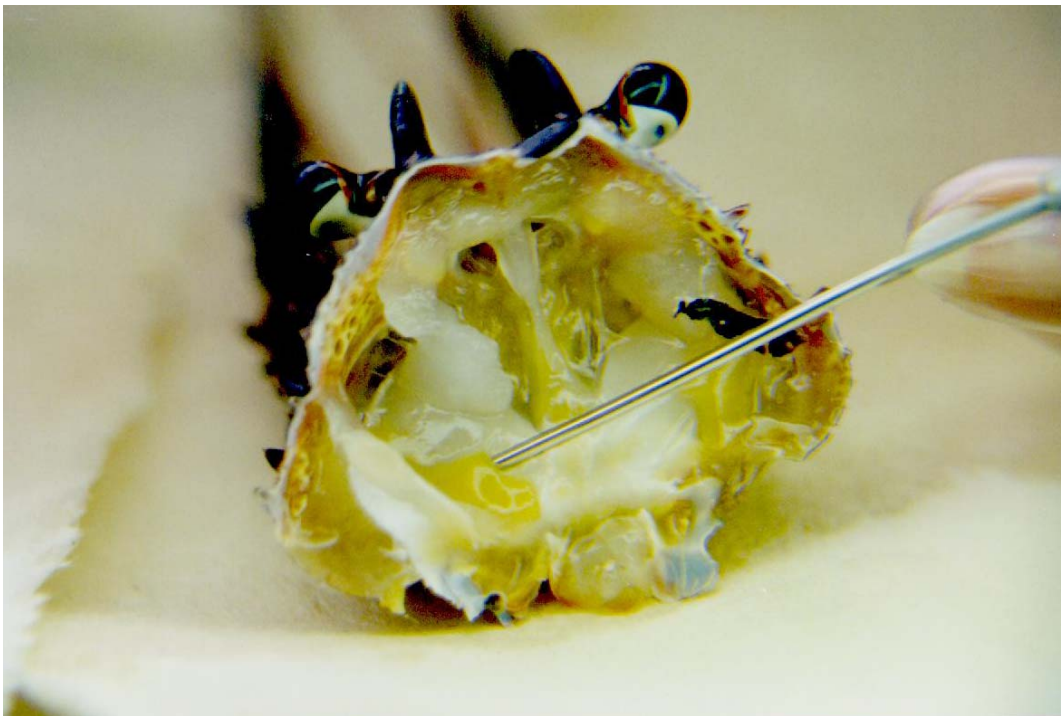


Figure 10 Location of antennal gland

Figure 11 Location of gill filament

Figure 12 Location of muscle sampling site

7. Fixation and Processing

Various fixatives are suitable for histopathological examination of lobster tissues. Buffered formalin is not an ideal fixative but can be used if a more suitable fixative is not available. Fixatives of choice include Bouins fixative and buffered formalin prepared in seawater. The latter fixative has been found to highlight the granular cells which are of diagnostic significance in disease diagnosis based on histopathological examination. It is suggested that tissue sections are mounted in two or more paraffin blocks to minimise processing costs.

APPENDIX 1

Table 1. Lobster Vigour Index

Somatic response	Classes					
	0(d)	1(m)	2(w)	3(h)	4(vh)	5(a)
Defensive born response	-	-	-	-	-	+
Vigourous tail flip	-	-	-	-	+	+
Appendages movements	-	-	-	+	+	+
Firm tail	-	-	+	+	+	+
Eyestalk response	-	+	na	na	na	na

Legend:
a = defensive
vh = very healthy
h = healthy
w = weak
m = moribund
d = dead
na = not applicable

Spanoghe, P. 1996. An investigation of the physiological and biochemical responses elicited by *Panulirus cygnus* to harvesting, holding and live transport. Doctoral Thesis. School of Biomedical Sciences, Curtin University, Perth, Western Australia. 378 pp.

APPENDIX 3

ABSTRACT PROCEEDINGS, INTERNATIONAL SYMPOSIUM ON LOBSTER HEALTH MANAGEMENT

September, 1999

Program
Symposium Aim
Organising Committee

(Copies available from Associate Professor Louis Evans, Aquatic Science Research Unit, Curtin University of Technology, Kent Street, Bentley WA 6102)

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Curtin
UNIVERSITY OF TECHNOLOGY

ROCK LOBSTER ENHANCEMENT & AQUACULTURE SUBPROGRAM

International Symposium

on

Lobster Health Management

Abstract Proceedings

19-21 September, 1999
Adelaide

Convened by

AQUATIC SCIENCE RESEARCH UNIT,
CURTIN UNIVERSITY OF TECHNOLOGY

Sponsors

Fisheries Research and Development Corporation
South Australian Research and Development Institute
Muresk Institute of Agriculture,
Curtin University of Technology

TABLE OF CONTENTS

SYMPOSIUM AIM

The overall aim of the symposium is to provide a forum for information exchange between scientists and industry personnel on health management of lobsters. This information exchange will be achieved through the presentation of papers on topics of direct relevance to lobster health management and through panel discussions on issues raised by participants.

ORGANISING COMMITTEE

Associate Professor Louis Evans	-	Symposium Convenor; Abstract Proceedings Editor
Dr Brian Jones	-	Abstract Proceedings Editor, Committee Member
Dr Judith Handlinger	-	Committee Member
Dr Ruth Reuter	-	Committee Member
Dr John Norton	-	Committee Member

APPENDIX 4

TABLE OF CONTENTS, PROCEEDINGS, INTERNATIONAL SYMPOSIUM ON LOBSTER HEALTH MANAGEMENT

September, 1999

(Copies available from Associate Professor Louis Evans, Aquatic Science Research Unit, Curtin University of Technology, Kent Street, Bentley WA 6102)

International Symposium

on

Lobster Health Management

Proceedings

19-21 September, 1999
Adelaide

Convened by

AQUATIC SCIENCE RESEARCH UNIT,
CURTIN UNIVERSITY OF TECHNOLOGY

Sponsors

Fisheries Research and Development Corporation
South Australian Research and Development Institute
Muresk Institute of Agriculture
Curtin University of Technology

TABLE OF CONTENTS

APPENDIX 5

SYNOPSES OF PANEL DISCUSSIONS, INTERNATIONAL SYMPOSIUM ON LOBSTER HEALTH MANAGEMENT

1. Health Management Issues in Lobster Aquaculture
2. Lobster Live Export

INTERNATIONAL SYMPOSIUM ON LOBSTER HEALTH MANAGEMENT ADELAIDE, 19-21 SEPTEMBER, 1999

PANEL DISCUSSION SUMMARY

Health Management Issues in Lobster Aquaculture

1. INTRODUCTION

A panel discussion on 'Health Management Issues in Lobster Aquaculture' was held at the conclusion of the first day of the lobster health management symposium. The discussion was moderated by Dr Robert van Barneveld, Sub-program Leader, FRDC Rock Lobster Enhancement and Aquaculture Sub-program and was attended by approximately 70 symposium participants. Participants included industry personnel, rock lobster researchers, government and FRDC representatives and international visitors.

At the commencement of the panel discussion Dr van Barneveld reviewed the FRDC Rock Lobster Enhancement and Aquaculture Sub-program and suggested that discussion could be directed at four main topics:

- Research and industry priorities with respect to lobster aquaculture and enhancement
- Research directions
- Industry feedback on research program
- Other issues

Discussion was held on these and other topics raised by symposium participants. A summary of these discussions along with the names of principal contributors to the discussion is given below.

2. DISCUSSION TOPICS

2.1 Research Directions and Priorities

2.1.1 Health management practices in post-harvest storage and aquaculture

An audience member commented that health management is of prime importance to successful long term storage and aquaculture. In response, another participant stressed that it was while it was important to develop and use health management techniques, the major priority was in the education of fishers, handlers and airlines as well as in developing understanding of environmental conditions of holding/rearing systems which optimise health and survival.

A West Australian processing industry member described the approach taken in his company to optimise survival during live marketing. He indicated that extensive studies had been undertaken through working with individual fishers right through to markets in Japan. This work has yielded a good data base on lobsters from different sources and their fate with respect to survival during transport. He commented that the most healthy lobsters were those delivered direct to the factory from local fishing boats – these had a low rejection rate at grading and an almost zero mortality on storage. Providing fishers follow best practice procedures mortalities can be reduced to a small proportion of residual health compromised animals. However, he stressed the need for education of fishers on best practice post-harvest handling procedures.

An audience member from New Zealand supported the above views and commented that the major factor affecting lobster health in post-harvest was how the animals were treated before they came into the factory.
(Mike Leach, Colin McDonald, Wayne Hoskins, Ben Diggles)

2.1.2 Disease investigations

The suggestion was made that spiny lobsters are very robust and that disease issues were not a high priority. Instead, it was suggested that identifying optimal conditions for aquaculture should be the main priority. This suggestion was hotly disputed with one audience member stating that this was a very short sighted view. He went on to express the view that diseases were inevitable in aquaculture and generating data and information on occurrence of disease conditions though investigation and monitoring of mortalities was essential.

This opinion was supported by another participant who commented that there was a dearth of information on lobster diseases and the task of identifying pathogens and managing disease outbreaks would be simplified if more information was available. The issue of the need for identification of health indicators for use in health management and health monitoring was raised by another participant. The opportunity to improve health through the use of probiotics was also mentioned.

One audience member commented that there were three main areas where research was required:

- Knowledge of disease threats
- What effect diseases have on the host and the compounding effect of stress in disease outbreaks
- Treatment options

However, the same individual also commented that there was an urgent need to identify optimal conditions for rearing lobsters and went on to say that the emphasis between these two research areas would probably change if disease outbreaks occurred.

The need for investigations on aquaculture system design was supported by another audience member who suggested that a high priority should be given to documenting current rearing techniques for puerulus and juveniles and conduct research on improving these techniques.

(Andrew Jeffs, Judith Handlinger, Brian Jones, Kenneth Söderhäll, Ben Diggles, Louis Evans; two audience members)

2.1.3 Genetic and disease risks from lobster aquaculture and enhancement

The question was raised as to whether disease risks to wild stock lobsters exist from aquaculture or long term storage in coastal locations. This question stimulated a wide ranging discussion on issues relating to disease risks, genetic issues and risks to biodiversity through escape of cultured lobsters to the wild. A summary of comments and questions is given below:

Disease issues

- There is a real risk of disease from aquaculture or long term storage
- If the pilchard viral disease is used as an example it can be confidently concluded that a similar scenario could occur in the rock lobster industry. Any unexplained mortalities should be investigated as quickly as possible.

Genetics issues

- Genetics is likely to be an important area for research - observations in the United States have suggested that the source of lobsters from the wild influences survival during long term storage

- Is there any genetic work being done on rock lobsters?
- Little work is currently being conducted on spiny lobster genetics
- Genetic analysis is being conducted in lobster stock enhancement trials in Europe but it is still too early for definitive results

- Will escape of genetic stocks into the wild affect wildstock?
- Whether or not escapees affect wildstock biodiversity isn't really known but ecologists tend to support the view that they do
- It is unlikely that cultured animals reared on artificial diets would outcompete wildstock animals
- There is likely to be little risk to the genetic integrity of wild stock lobsters from cultured escapees since lobster aquaculture is still in its infancy

The comment was made that effort should be placed in trying to enhance the lobster papulation in the wild rather than conducting on-shore aquaculture – issues such as maximising shelter, food supply, predator protection were of prime importance. Another audience member commented that research on artificial habitat for settlement and early juvenile predator protection was being conducted in New Zealand.
(Ross Gould, Gil Waller, Kenneth Söderhäll, Andrew Jeffs, Judith Handlinger, Mike Leach, Knut Jörstad, Wayne Hoskins)

2.2 Shell Disease

A discussion was held on the likely etiological factors in shell disease, tank rot and tail erosion. It was questioned whether shell disease is always preceded by a physical injury to the lobster. An outbreak of shell disease in which trauma due to poor trap

design in a southern rock lobster fishery was described while another participant commented that shell disease results from high stress, poor nutrition and high temperatures. The likelihood of the condition having varied and interacting causes was stressed.
(Judith Handlinger, Mike Geddes, Louis Evans, audience member)

2.3 The Use of Antibiotics in Lobster Aquaculture and Enhancement

Following on from one of the presentations during the paper sessions a member of the audience commented that antibiotics should not be used in lobster aquaculture or enhancement. The question was raised as to responsibility for enforcement of antibiotic usage. It was indicated that enforcement was ensured through HACCP and through regulation by government agencies.
(Bob Bayer, Steve Hood, audience member)

2.4 Technical Topics

Two questions were asked relating to the storage of lobsters in tanks.

2.4.1 Tank design

Q - How should a tank be set up so as not to get dead areas?

A – Refer to the Lobster Institute web page for information on holding systems for lobsters

2.4.2 Lobster holding systems

Q – What is the optimal holding system for lobsters?

A1 – The Clearwater system uses low temperatures for extended holding of Maine lobsters

A2 – Comment on experience in holding system at Port Lincoln – using natural and artificial feeds

2.4.3 Variation in lobster size

Q – Is there any information to explain why the size of lobsters from different catch zones differ?

A1 – Size differences are also seen in wildstock lobsters in New Zealand. It is probably due to differences in habitat. Have also looked at size differences in cultured phyllosoma and found that egg quality can influence lobster size.

A2 – There has been some work on Panulirus argus which showed that it was possible to identify which broodstock contributed to different groups of settled lobsters.
(Gil Waller, Mike Geddes, Colin McDonald, Glen Davidson, Andrew Jeffs, Bob Bayer, Jean Lavelle, audience member)

3. SUMMARY

Dr Robert van Barneveld summarised the discussions by concluding:

- Health management is an issue in rock lobster aquaculture and enhancement
- Holding conditions is also an issue, particularly as they influence health
- Resources should be directed towards monitoring mortalities

Meeting notes compiled by:

Associate Professor Louis Evans
Convenor
International Symposium on Lobster Health Management

INTERNATIONAL SYMPOSIUM ON LOBSTER HEALTH MANAGEMENT ADELAIDE, 19-21 SEPTEMBER, 1999

PANEL DISCUSSION SUMMARY

Lobster Live Export

1. INTRODUCTION

A panel discussion on 'Lobster Live Export' was held at the conclusion of the second day of the lobster health management symposium. The discussion was moderated by Professor Bruce Phillips, Sub-program Leader, FRDC Rock Lobster Post-Harvest Sub-Program, and was attended by approximately 50 symposium participants. Participants included industry personnel, rock lobster researchers, government and FRDC representatives and international visitors.

Professor Phillips introduced the topic by describing the current activities of the FRDC Rock Lobster Post-Harvest Sub-program. He indicated that the first major project in the program – Physiological Studies on Stress and Morbidity during Post-Harvest Handling and Storage of Western Rock Lobster *Panulirus cygnus* – was nearing completion. The project comprised two sub-projects, one managed by Dr Brian Patterson, QLD DPI, focussed on physiological parameters and the other by Assoc. Prof. Louis Evans, Aquatic Science Research Unit, Curtin University of Technology, focussed on immune parameters. In addition to this major project, two other projects within the Sub-program had been completed, one on the development of a Code of Practice for post-harvest handling of rock lobsters, conducted by Richard Stevens, WAFIC, and the other a study of the influence of environmental conditions in holding systems on lobster physiology, a PhD study conducted by Dr Brad Creer, University of Tasmania under the supervision of Professor Nigel Forteath.

An FRDC funded project aimed at publication of a rock lobster autopsy manual had also been recently approved. This project is being lead by Assoc. Prof. Louis Evans, Curtin University of Technology and is being jointly conducted with Dr Brian Jones, Fisheries WA and other fish health personnel from South Australia, Tasmania and Queensland.

Professor Phillips informed the group of a recent decision by the Sub-program Steering Committee to endorse three new projects for 2000 and beyond – investigations of leg loss in post-harvest lobsters, truck transport conditions and ammonia tolerance. The use of hides as rock lobster bait was also considered to be an important issue and had been investigated recently in another FRDC project conducted by Richard Stevens, WAFIC. He then opened the discussion by inviting participants to raise issues of relevance to live lobster transport.

Discussion was held on topics raised by symposium participants. A summary of these discussions along with the names of principal contributors to the discussion is given below.

2. DISCUSSION TOPICS

2.1 Use of Hides for Lobster Bait

The topic of the use of cow hides as bait for lobster traps was raised. Two issues were discussed in relation to this topic – a marketing concern, in particular the presence of hairs and hide fragments in the gut of lobsters when they are served at a restaurant and secondly, whether lobsters are adversely affected by eating hides. From comments by audience members it appeared that there had been no research on this topic.

Another issue which was raised related to possible adverse impacts of regurgitated hide material on water quality in holding systems. This query lead to a discussion on purging of lobsters. A comment was made that the presence of excess wastes in effluents would be a concern for regulatory authorities but this had not been in issue in the lobster processing industry to date. A lobster processor said that purging of lobsters took about three days.
(Glen O'Brien, Mick Olsen, Ross Gould, Bruce Phillips, Ross McGregor, Louis Evans)

2.2 Quality of Post-Harvest Lobsters

The question of how to improve the quality of post-harvest lobsters was raised by a lobster processor. One avenue was to pay a price differential for different quality of product. This suggestion stimulated considerable discussion. It was pointed out that small boats may not be able to install elaborate equipment required to maintain optimal condition of lobsters following capture. As a result it was not appropriate to pay a different price for different quality lobsters. Another audience member stated that it was clear in the South Australian industry that different boats delivered lobsters of different quality. A price differential would appear to be the only way to change this situation. Another processor disagree strongly with this view, saying that the best avenue for improving quality was through education of the fishers. He went on to describe the approach used in his company – assessing catch, providing published information, encouraging peer pressure – and stated that this approach had resulted in a significant improvement in product quality. Another participant agreed with this view and commented that in Western Australia there had been a marked improvement in post-harvest handling procedures. However, another audience member made the observation that while the information was available to fishers, installation of correct equipment and use of best practice handling procedures were not always the first priority.
(Stephen Hood, Glen O'Brien, Bruce Phillips, Wayne Hoskins, two audience members)

2.3 Live transport conditions

Some questions and comments related to issues of environmental conditions used to transport lobsters. A processor asked what avenues could be taken to enhance to survival of lobsters over long air flights - what is presently known with respect to optimal purging time, environmental temperature, treatment chemicals etc? Another

participant enquired as to whether the optimum temperature and oxygen levels had been determined for live lobster transport. In reply, it was pointed out that these conditions were very species specific. The need for a better understanding of lobster biology was stressed. A comment was made that improvements in survival had been achieved through improvements in engineering but that what was now needed was a better understanding of lobster biology. Difficulties with attitudes of airline companies were also discussed – it was suggested that the airlines were more concerned with passengers than with freight.
(Bruce Phillips, Glen O'Brien, Mick Olsen, Louis Evans, Steven Hood, two audience members)

2.4 Education of Fishers

The need for improved approaches to education of fishers was a common thread through much of the discussions. The question was raised as to how fishers could be encouraged to use the Code of Practice to improve their handling procedures. It was commented that while they may read to information provided it did not necessarily follow that they would improve their practices. Another participant made the comment that coastal tours were not very effective and that research was required on the best approaches to ensuring fishers have access to and use information. It was commented that fishers listen most to other fishers and to staff from processing companies or Cooperatives.

Some suggestions for improving information transfer included:

- Training Fisheries WA staff in patrol boats and in depots in best practice procedures
- Conducting regular seminars for fishers as well as for researchers
- Developing information briefs similar to those used in the agriculture industry
- Setting up websites
- Encouraging more interaction between fishers and researchers
- Producing more video material
- Disseminating information in fisheries journals

(Ron Gould, Brian Jones, Bruce Phillips, Glen O'Brien, Mick Olsen, Louis Evans, Steven Hood, two audience members)

Meeting notes compiled by:

Associate Professor Louis Evans
Convenor
International Symposium on Lobster Health Management

APPENDIX 6

CASE STUDIES 1 & 2
PILOT STUDY OF DISEASE CONDITIONS IN ALL POTENTIAL ROCK LOBSTER AQUACULTURE SPECIES AT DIFFERENT GROWTH STAGES: QUEENSLAND PROJECT ON *PANULIRUS ORNATUS*

Norton, J.H.[1] and Linton, L.[2]

[1] Queensland Department of Primary Industries, Oonoonba Veterinary Laboratory, PO Box 1085, Townsville, Qld 4810

[2] Queensland Department of Primary Industries, Northern Fisheries Centre, PO Box 5396, Cairns Mail Centre, Qld 4870

FRDC ROCK LOBSTER ENHANCEMENT AND AQUACULTURE SUBPROGRAM

Project 4: Pilot study of disease conditions in all potential rock lobster aquaculture species at different growth stages: Queensland Project on *Panulirus ornatus*

John H. Norton[1] and Larnie Linton[2]

[1] Queensland Department of Primary Industries,
Oonoonba Veterinary Laboratory,
PO Box 1085, Townsville, Qld 4810

[2] Queensland Department of Primary Industries,
Northern Fisheries Centre,
PO Box 5396
Cairns Mail Centre, Qld 4870

Abstract

Thirty juvenile lobsters, caught as pueruli from the wild, were divided into 3 groups each of 10 lobsters and fed 3 different diets: a natural diet of bait prawns, a commercial *Penaeus monodon* pellet and a kuruma *Penaeus japonicus* commercial pellet. A second group of 6 moribund adult lobsters was collected from processing factories. Haemolymph samples were aseptically collected for aerobic bacterial culture, total blood cell count, magnesium, lactate, total protein, potassium and phenoloxidase activity. Histopathology was done on the tissues.

Of the juvenile lobsters, there was a significant difference between the wet weights of lobsters to the natural and monodon diets. Magnesium, lactate, total protein and potassium were not significantly different for the three groups of juvenile lobsters. This data was not available for the adult lobsters. No significant differences were found for phenoloxidase for the three groups of juvenile lobsters. However, all three phenoloxidase tests were significantly different in a comparison between the 30 juvenile lobsters and the 6 adult lobsters. For the juvenile lobsters, the total haemocyte counts recorded for lobsters fed the natural diet were significantly higher than those of lobsters fed the monodon diet. The total haemocyte count of lobsters fed the kuruma diet was also significantly higher from that of lobsters fed the monodon diet. These counts were not available for the adult lobsters. Aerobic bacterial cultures for the 30 juvenile lobsters yielded no isolates in 17 lobsters, *Pseudomonas* sp. in 9 lobsters, *Vibrio* sp. in 1 lobster, *Aeromonas* sp. in 2 lobsters and *micrococcus* sp. in 1 lobster. Of the 6 adult lobsters, no bacteria were cultured from 3 lobsters while multiple species were cultured from the other 3 lobsters. Histopathology revealed minimal lesions in either group of lobsters.

Total haemocyte counts might be a useful indicator of nutritional status, while the phenoloxidase test might be used to indicate poor health.

Introduction

With increasing interest in the aquaculture of rock lobsters, there was a need to commence health testing of Australia's indigenous species of rock lobsters and in particular of *Panulirus ornatus*, Queensland's premier rock lobster. The aim was to acquire knowledge of the normal anatomy and histology of these crustaceans, as well as to develop practical biochemical and haematological tests. These latter tests would be used to assess the health status of wild-collected and aquaculture-reared rock lobsters.

Materials and Methods

Lobsters were obtained from 2 sources: (1) thirty wild-caught pueruli used in a grow out experiment over 12 months at the QDPI's Northern Fisheries Centre (NFC), Cairns and (2) six debilitated wild-caught adult lobsters that were unsuitable for live export from packing houses (PH) in Cairns.

NFC Lobsters: 30 young adult lobsters *Panulirus ornatus*, 10 from each of 3 separate diet treatments (natural, monodon and kuruma). The natural diet consisted of bait prawns while the monodon diet was a *Penaeus monodon* commercial pellet and the

kuruma diet was a high protein *Penaeus japonicus* commercial pellet. These lobsters had been reared in a flow-through system and fed daily. The tanks were regularly cleaned of waste, food, cast exoskeletons, etc.

PH Lobsters: Six lobsters which were lethargic and judged to be unsuitable for the live export trade were made available for necropsy. These lobsters had been caught in the Torres Strait, transported by aircraft to Cairns (840 km) and placed into chilled, recirculation tanks prior to overshipping within a few days. Debilitated lobsters came either from rejects following air transport to Cairns (PH lobsters Nos. 1,2,3) or from their inability to survive in the holding tanks in Cairns (PH lobsters Nos. 4,5,6).

Gross Examination

Each lobster was weighed, the carapace length measured (from between the horns to the rear of the cephalothorax) and sex recorded. The following observations were also recorded for the NFC lobsters: the number of appendages lost, the vigor index (scale of 0 to 5) (Spanoghe and Bourne 1997), the presence and severity of tail blisters (0, 1, 2) and the presence and severity of tail erosion (0, 1, 2).

Haemolymph (collection and processsing)

Each lobster was bled from the pericardial sinus. The membrane at the rear of the carapace was disinfected with 70% alcohol or a 1:50 dilution of 10% povidone iodine (Betadine). The haemolymph sample was collected with a 21 gauge needle and a 5ml disposable syringe. Haemolymph samples were left to clot at 4°C for three days when the ability of each sample to clot was recorded. Each clot was then broken up, and the haemolymph lysate and serum spun off at 1000g for 5 minutes.

Bacteriology

A drop of aseptically collected haemolymph was applied to each of 2 bacteriological plates (MAV and TCBS) which were incubated aerobically at 25°C for 48 hrs. Any colonies were identified by routine diagnostic methods.

Haematology

A 0.5ml sample of haemolymph from each of the NFC lobsters was added to 4.5ml of 10% seawater formalin. A total haemocytic count was performed using the mammalian white cell method on a standard haemocytometer (Benjamin 1969). The total haemocyte count (THC) per ml of haemolymph was calculated as follows:

$$\text{THC} = \frac{\text{cells counted (4 x lmm squares) x 10 (dilution) x 10 (depth)}}{\text{4 (no. of squares counted)}}$$

$$= \text{cells counted in the 4 x 1mm squares x 25}$$

Biochemistry

Chilled haemolymph samples from NFC lobsters were analysed for magnesium, lactate, total protein and potassium by routine biochemical methods. This choice of tests was partly based on the work of Paterson et al. (1997). Chilled haemolymph samples from both NFC and PH lobsters were analysed for phenoloxidase activity (Hernandez-Lopez *et al* 1996).

Post Mortem Examination

Each lobster was killed by rapidly cutting with a hacksaw through the cephalothorax, directly behind the eyes and the horns to remove the brain or cephalic ganglion. An eye was collected. The tail was removed from the body by twisting it out of the cephalothorax. A cut was made around the anus with scissors, and the hindgut was pulled free with a pair of forceps.

Two parallel cuts were made along the dorsal aspect of the cephalothorax and the dorsal section of the carapace was removed to reveal the internal organs. The following organs were excised: heart, gills, stomach, haematopoietic tissue, hepatopancreas, abdominal muscle, antennal gland and epidermis. The nerve cord was removed by making two parallel cuts with a hacksaw along the ventral cephalothorax. The collected tissues were placed in 10% seawater formalin. A pleopod sample was collected and the moult stage estimated (Turnbull 1989).

Histopathology

Tissues were cut and stained with haematoylin and eosin, according to routine methods. Tissue sections were examined by light microscopy and lesions classified according to Appendix A where possible.

Statistics

One way analysis of variance was used to compare diets for the NFC juvenile lobsters and to compare phenoloxidase activity between PH adults and NFC juvenile lobsters.

Results

The weight, carapace length, sex and moult stage of each of the juvenile NFC lobsters are listed in Table l(a). There was a significant difference ($p<0.05$) between the weights of the NFC lobsters on the natural and on the monodon diets only. No significant difference was found for carapace length and moult stage for the NFC lobsters. Similar details for the adult PH lobsters are listed in Table l(b). Results of a gross examination for appendages lost, vigor index, tail blisters and tail erosion for the juvenile NFC lobsters are listed in Table 2. These data are unavailable for the adult PH lobsters.

Table l(a) Diet, weight, carapace length, sex and moult stage of juvenile NFC lobsters *P. ornatus.*

Lobster No.	Diet	Weight (gm)	Carapace Length (mm)	Sex	Moult Stage
1	Natural	215.3	52.4	M	C1
2	"	158.3	53.5	F	D1
3	"	332.2	71.1	M	C
4	"	295.2	67.5	F	C
5	"	244.1	65.0	F	C
16	"	346.5	73.5	M	C
17	"	176.4	58.6	M	C
18	"	318.9	70.7	M	C
19	"	186.7	56.8	F	B
20	"	234.6	63.7	M	C
6	Kuruma	224.1	63.8	F	C
7	"	180.3	59.4	M	D2
8	"	258.8	66.5	M	C
9	"	159.0	56.7	M	C
10	"	146.1	53.4	F	D1
21	"	109.9	50.5	M	C
22	"	189.3	58.3	F	C
23	"	212.5	62.3	M	C
24	"	45.3	35.8	F	D2
25	"	341.2	75.0	M	C
11	Monodon	91.0	45.9	F	C
12	"	116.1	51.1	M	C
13	"	316.9	70.2	F	C
14	"	198.4	63.6	M	C
15	"	161.6	56.1	F	C
26	"	126.1	49.9	F	D2
27	"	233.1	64.3	M	D2
28	"	133.6	53.2	M	C1
29	"	158.6	55.5	M	D1
30	"	100.7	47.7	F	C

Table 1(b) Weight, carapace length, sex and moult stage of adult PH lobsters *P. ornatus*

Lobster No	Export Operator	Weight (gm)	Carapace Length (mm)	Sex	Moult stage
1	A	730	94	M	D0
2	A	680	89	F	D2
3	A	450	78	M	B
4	B	860	98	M	C
5	B	700	93	F	C
6	B	980	104	F	C

Table 2 Results of a gross examination including number of appendages lost, vigor index, tail blisters and tail erosion in juvenile NFC lobsters fed different diets

Lobster No	Diet	No. Appendages Lost	Vigor Index (0-5)	Tail Blisters (0,1,2)	Tail Erosion (0,1,2)
1	Natural	1	5	0	1
2	"	0	5	0	1
3	"	2	5	0	1
4	"	1	5	0	1
5	"	0	5	0	1
16	"	2	5	0	1
17	"	0	5	0	1
18	"	0	5	0	1
19	"	0	5	0	1
20	"	0	5	0	1
6	Kuruma	0	5	0	1
7	"	0	5	0	1
8	"	0	5	0	1
9	"	1	5	0	1
10	"	0	5	0	1
21	"	0	5	0	1
22	"	0	5	0	2
23	"	0	5	0	1
24	"	0	5	0	1
25	"	2	5	0	1
11	Monodon	0	5	0	1
12	"	0	5	0	1
13	"	0	5	0	1
14	"	0	5	0	1
15	"	0	5	0	1
26	"	0	5	0	2
27	"	0	5	0	1
28	"	1	5	0	1
29	"	1	5	0	1
30	"	0	5	0	1

Results of the biochemical tests for the juvenile NFC lobsters are presented in Table 3 for haemolymph magnesium, lactate, total protein and potassium. No significant difference ($p>0.05$) was recorded for magnesium, lactate, total protein and potassium. Similar data are not available for the adult PH lobsters. Haemolymph phenoloxidase results are given in Table 4(a) for the juvenile NFC lobsters and in Table 4(b) for a comparison of the adult PH lobsters and the juvenile NFC lobsters. No significant difference was found for the phenoloxidase results (including laminarin, trypsin and spontaneous tests) for the NFC lobsters. However, when the 30 NFC lobsters were compared with the 6 PH lobsters, the difference was highly significant ($p<0.01$) for all 3 tests for phenoloxidase. Table 5 includes the total haemocyte counts for the juvenile NFC lobsters. The total haemocyte count was significantly different ($p<0.01$) between the NFC lobsters on the natural diet and those on the monodon diet. There was also a significant difference ($p<0.05$) in total haemocyte counts between the NFC lobsters on the kuruma and monodon diets. Similar data are not available for the adult PH lobsters. The results of aerobic bacterial cultures on aseptically collected haemolymph

samples are presented in Table 6(a) for the juvenile NFC lobsters and in Table 6(b) for the adult PH lobsters. For the juvenile NFC lobsters, the number of organs examined is given in Table 7, while the type and classification of histological lesions are given in Tables 8, 9 and 10. Information on the type and classification of lesions from the adult PH lobsters is given in Table 11.

Table 3 Results of biochemical tests including haemolymph clotting, magnesium, lactate, total protein and potassium of juvenile NFC lobsters on different diets

Treatment (No. of Lobster/Group)	Clotted	Not Clotted	Magnesium (m mol)	Lactate (m mol)	Total Serum Protein (g/L)	Potassium (m mol)
			Mean (range)			
Natural (10)	1	9	9.35[a]* (8.00-11.44)	11.78[a] (5.00-20.00)	59.56[a] (17.6-96.00)	10.71[a] (9.55-12.42)
Kuruma (10)	4	6	9.32[a] (8.08-11.28)	13.88[a] (9.00-19.00)	57.70[a] (32.00-93.60)	10.48[a] (9.28-12.35)
Monodon (10)	5	5	9.57[a] (8.08-11.12)	11.40[a] (5.00-20.00)	48.10[a] (17.00-90.40)	10.03[a] (8.81-11.22)

* In each column, values followed by the same letter do not differ significantly (p>0.05)

Table 4(a) Haemolymph phenoloxidase activity (change in absorbance at 492nm per ml per minute) from juvenile NFC lobsters on three different diets

Treatment (No. of Lobster/Group)	Phenoloxidase activity – mean (range) Laminarin	Trypsin	Spontaneous
Natural (10)	541[a]* (247-855)	834[a] (617-1104)	448[a] (234-609)
Kuruma (10)	521[a] (96-1191)	898[a] (352-1780)	457[a] (39-1046)
Monodon (10)	566[a] (273-901)	943[a] (717-1443)	511[a] (227-654)

* In each column, values followed by the same letter do not differ significantly (p>0.05)

Table 4(b) Haemolymph phenoloxidase activity (change in absorbance at 492 nm per ml per minute) for 6 adult PH lobsters and 30 juvenile NFC lobsters

Treatment (No. of Lobster/Group)	Phenoloxidase activity – mean (range) Laminarin	Trypsin	Spontaneous
Adult PH(6)	165[a]* (100-340)	446[a] (340-580)	143[a] (80-290)
Juvenile NFC (30)	542[b] (96-1191)	891[b] (352-1780)	472[b] (39-1046)

* In each column, values followed by a different letter differ significantly (p<0.01)

Table 5 Total haemolyte counts from juvenile NFC lobsters fed three different diets.

Diet Treatment (Lobster No.)	Total Haemocyte Count Means (range)
Natural (10)	2.02[a]* (0.76-3.16)
Kuruma (10)	1.70[b] (1.15-2.65)
Monodon (10)	1.10[ab] (0.54-2.11)

* Values followed by (a) differ significantly ($p<0.01$);
* Values followed by (b) differ significantly ($p<0.05$)

Table 6(a) Results of aerobic bacterial cultures of aseptically collected haemolymph samples from juvenile NFC lobsters on three different diets

Lobster No.	Diet	Bacterial Isolate
1	Natural	NAG*
2	"	*Pseudomonas* sp.
3	"	*Vibrio* sp.
4	"	*Pseudomonas* sp.
5	"	NAG
16	"	"
17	"	"
18	"	"
19	"	*Aeromonoas* sp.
20	"	"
6	Kuruma	NAG
7	"	*Pseudomonas* sp.
8	"	NAG
9	"	"
10	"	"
21	"	"
22	"	*Pseudomonas* sp.
23	"	NAG
24	"	*Micrococcus* sp.
25	"	*Pseudomonas* sp.
11	Monodon	*Pseudomonas* sp.
12	"	*Pseudomonas* sp.
13	"	NAG
14	"	NAG
15	"	*Pseudomonas* sp.
26	"	NAG
27	"	*Pseudomonas* sp.
28	"	NAG
29	"	"
30	"	"

*NAG = No aerobic growth

Comment:

1. *Aeromonas* and *Micrococcus* suggests contamination during sampling or processing of the samples.
2. Of the Pseudomonads a number of these appear to be of marine origin including *Alteromonas*, a subspecies of the Pseudomonads.

Table 6(b) Results of aerobic bacterial cultures on aseptically-collected haemolymph samples from adult PH lobsters

Lobster No.	Export Operator	Bacterial Isolates
1	A	No aerobic growth
2	A	No aerobic growth
3	A	No aerobic growth
4	B	*Aeromonas* sp., *Flavobacterium* sp., *Pseudomonas* sp., *Vibrio* sp.
5	B	*Aeromonas* sp. *V. harveyi*, *V. splendidus II, Vibrio* sp.
6	B	*Pseudomonas* sp. *V. harveyi, Vibrio* sp.

Table 7 The numbers of each organ examined histologically from each group of juvenile NFC lobsters on three different diets

Tissue/Organ	Diet Group		
	Natural	Kuruma	Monodon
Heart	8	9	9
Antennal Gland	3	3	5
Hepatopancreas	10	10	10
Abdominal Muscle	10	10	10
Gills	10	10	10
Ventral Nerve	10	10	9
Midgut	0	0	0
Hindgut	10	10	10
Stomach	10	10	10
Eyes	10	10	10
Epidermis	10	10	10
Gonad	N/A*	N/A	N/A
Haematopoietic Tissue	9	10	8

* N/A = Not available (immaturity)

Table 8 Histological lesions found in each organ from each of the groups of juvenile NFC lobsters on a different diet

Diet Group	Organ	Lesions
Natural	Gill	Multiple focal inflammation (1)*
	Hepatopancreas	Inflammation of the interlobular vessels (1)
Kuruma	Heart	Small granulomas on epicardium (1)
	Hepatopancreas	Small granuloma (1)
Monodon	Heart	Focal inflammation (1)
	Gills	Focal inflammation (1)

Comment:
Some bacterial fouling of gills in all lobsters in all groups (no pattern)

* Numbers of lobsters affected.

Table 9 Coded classification* of histological lesions in organs of juvenile NFC lobsters on three different diets.

Lobster No.	Heart	Antennal Gland	Hepatopancreas	Abdominal Muscle	Gill	Ventral Nerve	Midgut
1	NA**	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
2	NA	1.2.2	1.3.2	1.4.2	1.5.2	1.6.2	NA
3	1.1.2	NA	1.3.2	1.4.2	1.5.3	1.6.2	NA
4	1.1.2	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
5	1.1.3	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
16	1.1.2	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
17	1.1.2	1.2.2	1.3.2	1.4.2	1.5.2	1.6.2	NA
18	1.1.2	NA	1.3.2	1.4.2	1.5.4	1.6.2	NA
19	1.1.2	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
20	1.1.2	1.2.2	1.3.2	1.4.2	1.5.2	1.6.2	NA
6	1.1.2	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
7	NA	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
8	1.1.3	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
9	1.1.2	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
10	1.1.2	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
21	1.1.2	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
22	1.1.2	1.2.2	1.3.2	1.4.2	1.5.2	1.6.2	NA
23	1.1.2	1.2.2	1.3.2	1.4.2	1.5.2	1.6.2	NA
24	1.1.2	NA	1.3.4	1.4.2	1.5.2	1.6.2	NA
25	1.1.2	1.2.2	1.3.2	1.4.2	1.5.2	1.6.2	NA
11	1.1.2	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
12	1.1.2	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
13	1.1.2	NA	1.3.2	1.4.2	1.5.4	1.6.2	NA
14	1.1.2	1.2.2	1.3.2	1.4.2	1.5.2	NA	NA
15	1.1.2	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
26	1.1.2	NA	1.3.2	1.4.2	1.5.2	1.6.2	NA
27	NA	1.2.2	1.3.2	1.4.2	1.5.2	1.6.2	NA
28	1.1.2	1.2.2	1.3.2	1.4.2	1.5.2	1.6.2	NA
29	1.1.2	1.2.2	1.3.2	1.4.2	1.5.2	1.6.2	NA
30	1.1.2	1.2.2	1.3.2	1.4.2	1.5.2	1.6.2	NA

* 1.1.2 = No abnormality detected
1.2.2 = No abnormality detected
1.3.2 = No abnormality detected
1.3.4 = Small nodule encapsulation
1.4.2 = No abnormality detected
1.5.2 = No abnormality detected
1.5.3 = Focal hemocytic accumulation
1.5.4 = Small nodule encapsulation
1.6.2 = No abnormality detected

** NA = Not available

Table 10 Classification of histological lesions in organs of juvenile NFC lobsters on three different diets

Lobster No.	Diet	Hind Gut	Stomach	Eyes	Epidermis	Gonod	Haematopoietic
1	Natural	NAD*	NAD	NAD	NAD	NA**	NAD
2	"	NAD	NAD	NAD	NAD	NAD	NAD
3	"	NAD	NAD	NAD	NAD	NA	NA
4	"	NAD	NAD	NAD	NAD	NA	NAD
5	"	NAD	NAD	NAD	NAD	NAD	NAD
16	"	NAD	NAD	NAD	NAD	NA	NAD
17	"	NAD	NAD	NAD	NAD	NA	NAD
18	"	NAD	NAD	NAD	NAD	NA	NAD
19	"	NAD	NAD	NAD	NAD	NA	NAD
20	"	NAD	NAD	NAD	NAD	NA	NAD
6	Kuruma	NAD	NAD	NAD	NAD	NA	NAD
7	"	NAD	NAD	NAD	NAD	NA	NAD
8	"	NAD	NAD	NAD	NAD	NAD	NAD
9	"	NAD	NAD	NAD	NAD	NA	NAD
10	"	NAD	NAD	NAD	NAD	NA	NAD
21	"	NAD	NAD	NAD	NAD	NA	NAD
22	"	NAD	NAD	NAD	NAD	NA	NAD
23	"	NAD	NAD	NAD	NAD	NA	NAD
24	"	NAD	NAD	NAD	NAD	NA	NAD
25	"	NAD	NAD	NAD	NAD	NA	NAD
11	Monodon	NAD	NAD	NAD	NAD	NA	NAD
12	"	NAD	NAD	NAD	NAD	NA	NAD
13	"	NAD	NAD	NAD	NAD	NA	NAD
14	"	NAD	NAD	NAD	NAD	NA	NA
15	"	NAD	NAD	NAD	NAD	NA	NA
26	"	NAD	NAD	NAD	NAD	NA	NAD
27	"	NAD	NAD	NAD	NAD	NAD	NAD
28	"	NAD	NAD	NAD	NAD	NA	NAD
29	"	NAD	NAD	NAD	NAD	NA	NAD
30	"	NAD	NAD	NAD	NAD	NA	NAD

* NAD = No abnormality detected
** NA = Not available

Table 11 Histological classification of lesions from adult PH lobsters

Tissue	Histopathological Classification Exporter ( ) Lobster No ( ) (A) 1	(A) 2	(A) 3	(B) 4	(B) 5	(B) 6
Heart	1.1.2	1.1.2	1.1.2	NA[a]	1.1.3(1)[b] 1.1.8(1)	1.1.2
Antennal Gland	NA	NA	NA	NA	NA	1.2.9(2)[c]
Hepatopancreas	1.3.5(2) 1.3.8(2)	1.3.5.(2) 1.3.8(2)	1.3.4.(1) 1.3.8(0)	1.3.3.(2) 1.3.8(0)	NAD 1.3.8(1)	NAD 1.3.8(1)
Abdomin M	1.4.6	NAD	NAD	1.4.3(1)	1.4.3(1)	NAD
Gills Filaments	NAD	NAD	NAD	1.5.3(1)	NAD	NAD
Vent Nerve	NA	NA	NAD	NAD	NAD	NAD
Midgut	NA	NA	NA	NA	NA	NA
Hindgut	NA	NAD[d]	NAD	NAD	NAD	NAD
Stomach	NAD	NAD	NAD	NAD	NAD	NAD
Eyes	NAD	NAD	NAD	NAD	NAD	NAD
Cuticular Epidermis	NAD	(e)	NAD	NAD	NAD	NAD
Gonad	NAD	NAD	NAD	NAD	NAD	NAD
Haematopoietic	NAD	NAD	NAD	NA	NAD	NAD

(a) NA = Not available.
(b) 0 = Not present; 1 = Minor presence; 2 =Marked presence.
(c) Large zone of inflammation
(d) NAD = No abnormality detected.
(e) Shell disease; epidermal hyperplasia.
1.3.3; 1.4.3; 1.5.3 = Focal hemocytic accumulation
1.3.4 = Small nodule
1.3.5 = Medium sized encapsulation
1.3.8 = Apparent increase in phagocyte rosettes

Discussion

Within the NFC lobsters, the monodon diet gave poorer growth suggesting that this diet is not ideal for juvenile lobsters. The differences in the total haemocyte counts between the NFC lobsters on the 3 diets suggest that this test might be a more sensitive indicator of nutritional differences than the biochemical tests which failed to detect any differences between lobsters on the 3 diets.

However, although differential haemocyte counts were attempted, no confidence was held in attempts to differentiate semigranulocytes from hyalinocytes. Hence, these counts were omitted as unreliable.

In the necropsy examinations, difficulty was experienced in finding the antennal gland. It did not have the distinctive greenish colour which was seen in West Australian rock lobsters. The midgut also was not collected because of its small size (a few millimeters only in length) (Patterson 1968). In the necropsy of live lobsters, there may also be need to consider chilling as a form of anaesthesia as part of an animal ethics protocol prior to euthanasia. The moult staging was relatively easy when the method of Turnbull (1989) was used. The haemolymph clotting pattern for

lobsters on the 3 diets appeared to be random and consequently is of no use as an indicator of nutritional status.

The high prevalence of bacterial contaminants in the haemolymph samples suggests a need for an improved antiseptic collection technique. The use of a sterile swab to apply the 70% alcohol would ensure a more thorough disinfection of the frequently concave membrane behind the rear edge of the carapace where the needle is inserted. Alternatively, the use of a povidone iodine solution on non-edible lobsters would improve the efficiency of disinfection. The difference in bacteriology results between the lobsters from Exporter A and Exporter B may be explained as follows. The moribund lobsters from Exporter A were bled soon after arriving by air from Torres Strait and had not been exposed to a contaminated environment. In contrast, the moribund lobsters from Exporter B were bled after they had spent several days or more in chilled, heavily stocked (and heavily contaminated) holding tanks in Cairns.

The differences in the phenoloxidase activity (all 3 tests) between the NFC and the PH lobsters suggest that this test has potential as a health indicator. The lower concentrations attained in the phenoloxidase test in the PH lobsters, compared to the NFC lobsters, also suggest that some of this chemical was expended in overcoming bacterial infections and tissue damage. For the other biochemical tests ie. magnesium, lactate, total protein and potassium, the lack of any significant difference between the 3 diet groups of NFC lobsters suggests that a diet change may be insufficient to affect the haemolymph concentration of any of these chemicals. Furthermore, the range of values for magnesium, lactate and potassium for the NFC lobsters are close to the normal ranges given for lobsters when first caught from the wild (Paterson *et al.,* 1997). Further tests on moribund lobsters are needed to evaluate these biochemical tests further.

Acknowledgments

The authors thank M/s Kelly Field for the phenoloxidase tests and the staff of the Oonoonba Veterinary Laboratory for haematology, bacteriology and histopathology. Mr R. Meyer is thanked for statistical analyses.

References

Benjamin, M.M., 1969. Haematology in "Outline of Veterinary Clinical Pathology", 6th edition, Iowa State University press, Iowa.

Hermandez-Lopez, J., Gollas- Galvan, T. and Vargas-Albores, F., 1996. Activation of the prophenoloxidase system of the brown shrimp (*Penaeus californiensis* Holmes). *Comparative Biochemistry and Physiology* **113C**: 61-66.

Paterson, B.D., Grauf, S.G. and Smith, R.A., 1997. Haemolymph chemistry of tropical rock lobsters (*Panulirus ornatus*) brought onto a mother ship from a catching dinghy in Torres Strait. *Marine and Freshwater Research* **48**, 835-838.

Paterson, N.F., 1968. The anatomy of the cape rock lobster, *Jasus lalandii* (H. Milne Edwards). *Annals of the South African Museum* **51**, 1-232.

Spanoghe, P.T. and Bourne, P.K, 1997. Relative influence of environmental factors and processing techniques on *Panulirus cygnus* mobidity and mortality during simulated live shipments *Marine and Freshwater Research* **48**, 839-844.

Turnbull, C.T., 1989. Pleopod cuticular morphology as an index of moult stage in the ornate rock lobster, *Panulirus ornatus*. *Australian Journal of Marine and Freshwater Research* **40**, 285-293.

APPENDIX A

HISTOPATHOLOGY FIGURES

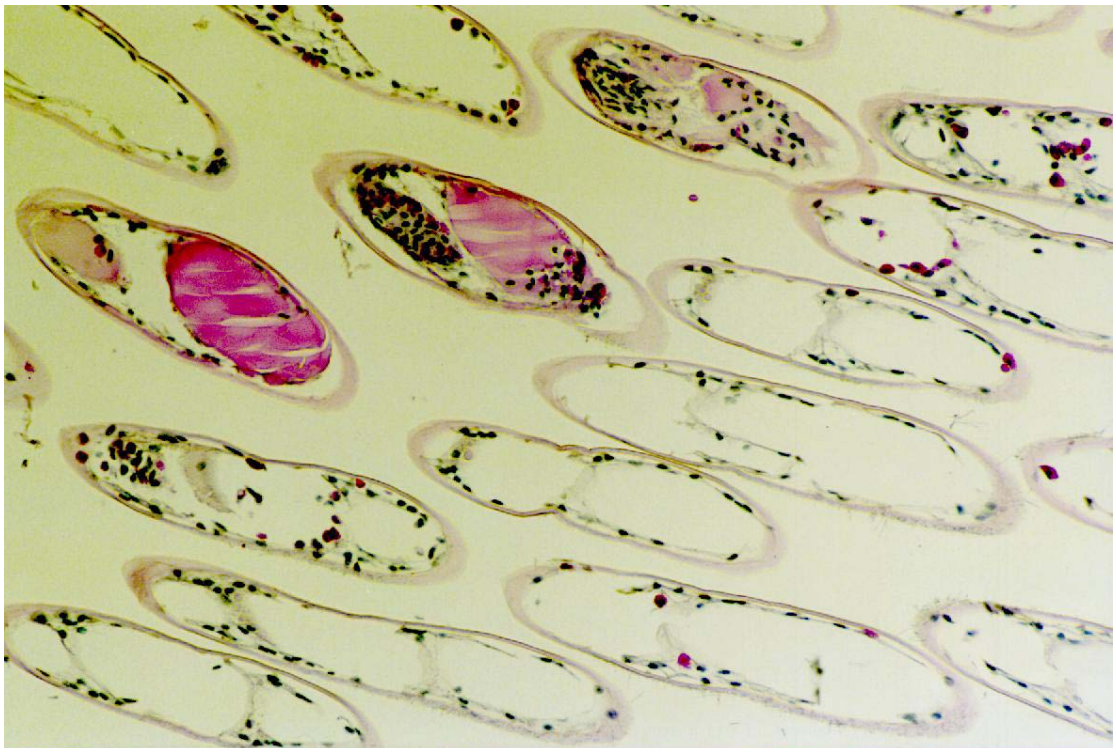


Figure 1 Gill granuloma (low power)

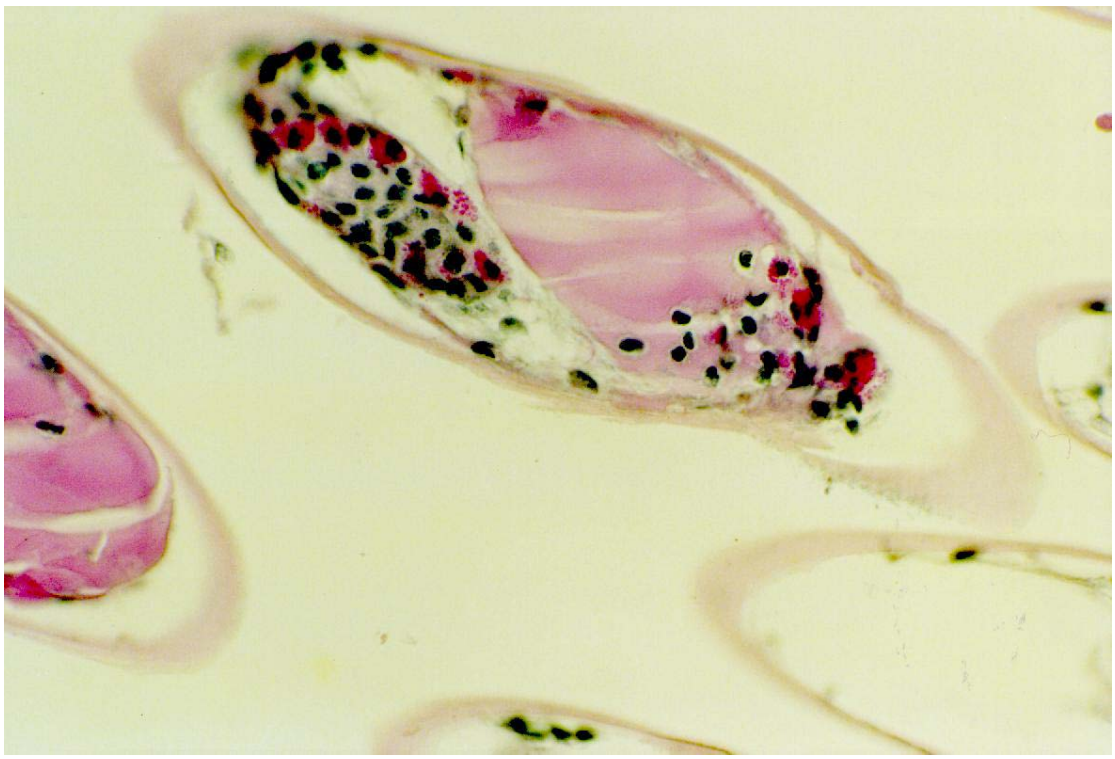


Figure 2 Gill granuloma (high power)

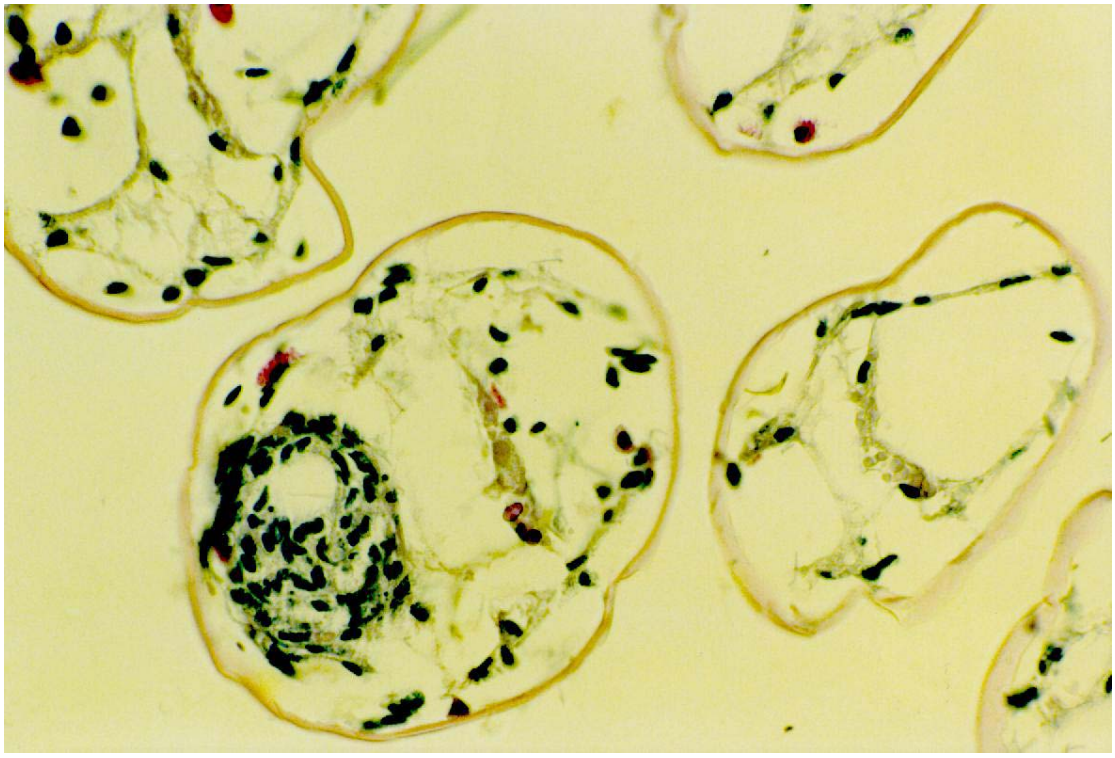


Figure 3 Old gill granuloma (high power)

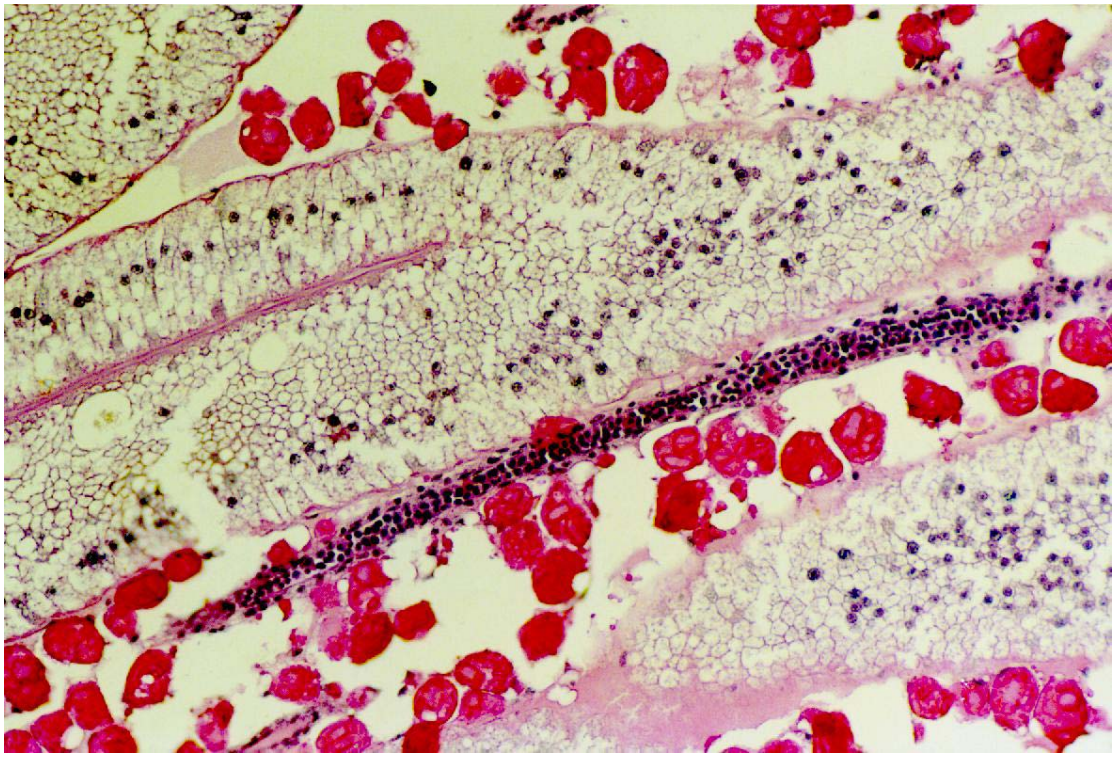


Figure 4 Hepatopancreas – terminal arteriole cuffing

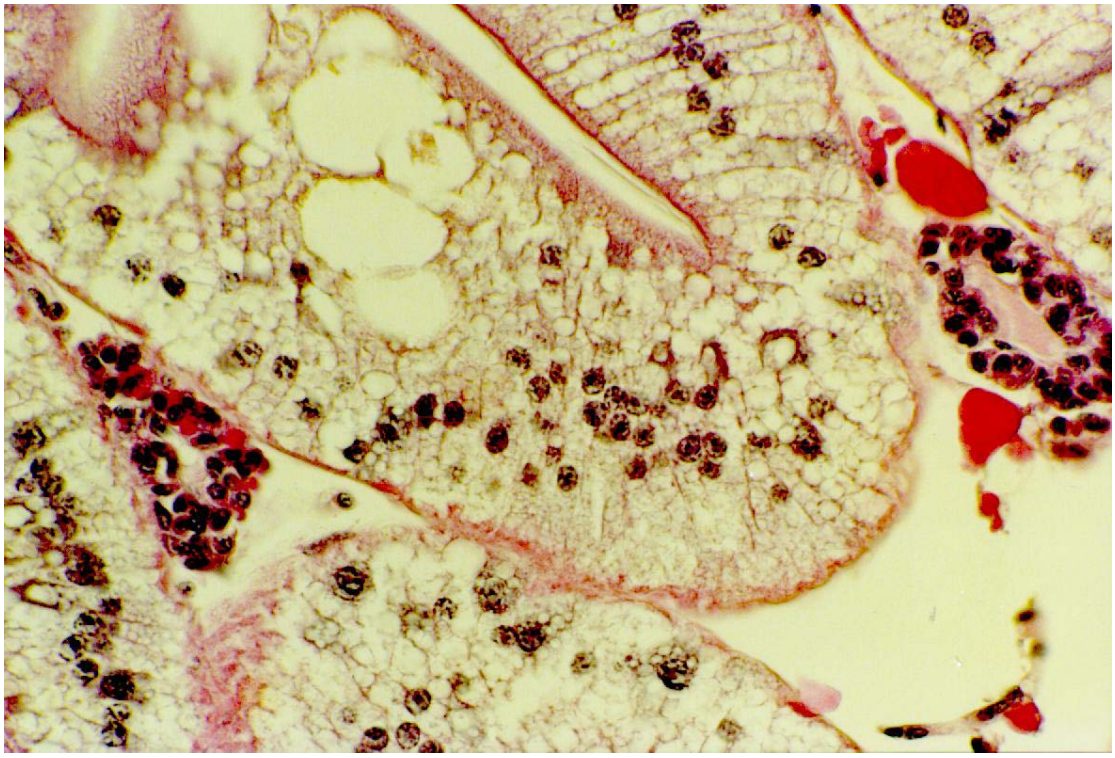


Figure 5 Hepatopancreas – terminal arteriole cuffing

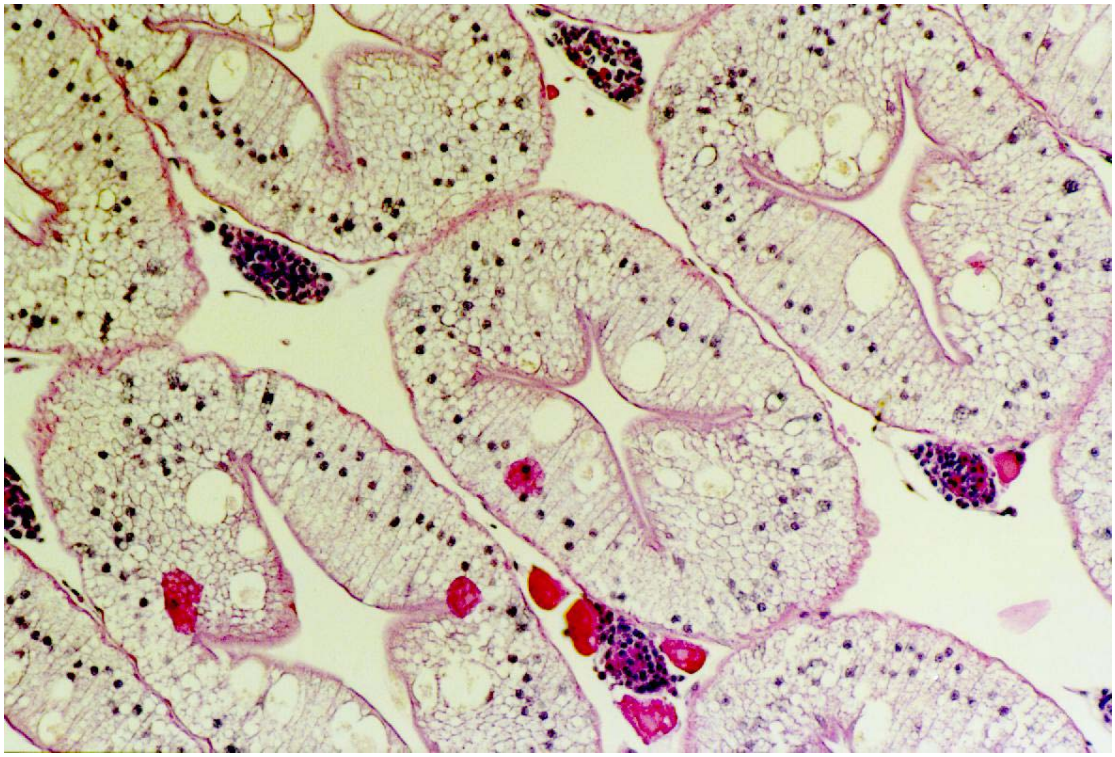


Figure 6 Hepatopancreas – terminal arteriole cuffing

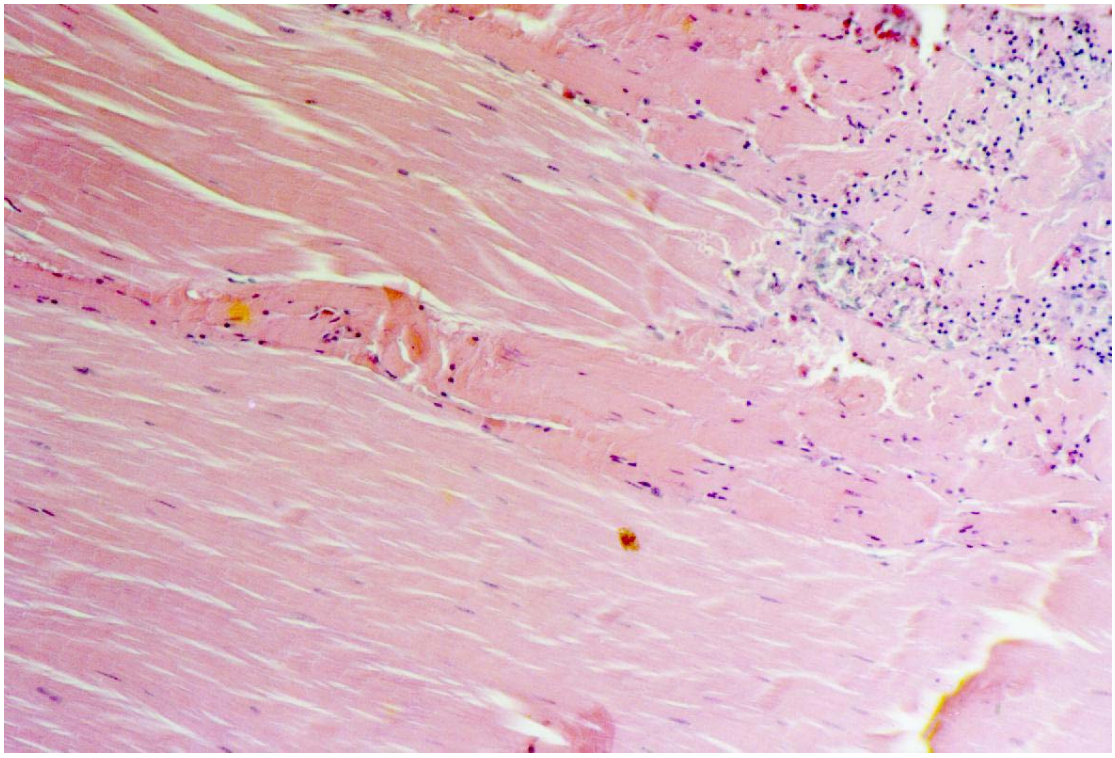


Figure 7 Skeletal muscle necrosis (low power)

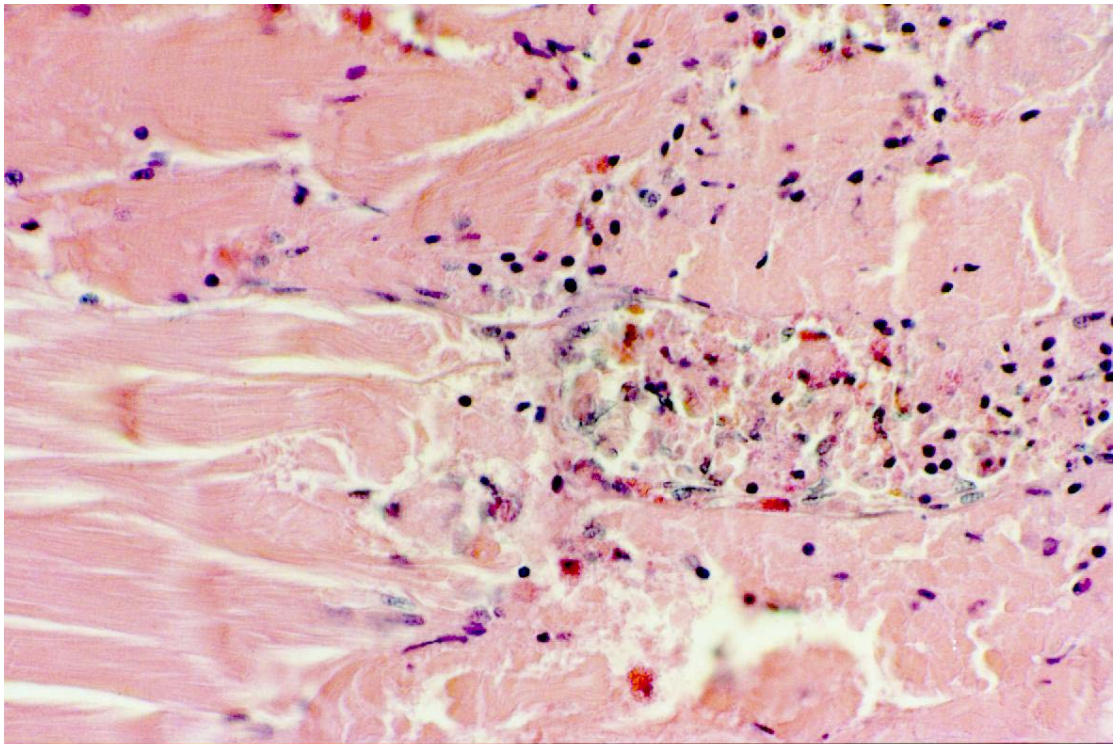


Figure 8 Skeletal muscle necrosis (high power)

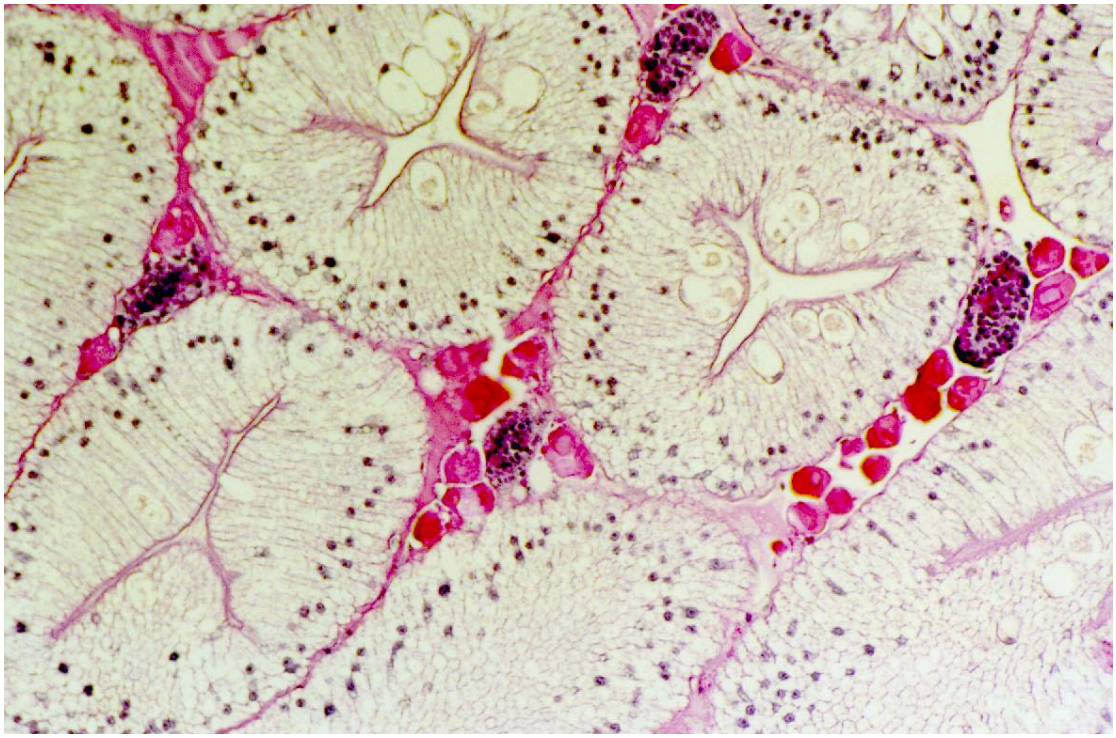


Figure 9 Hepatopancreas – terminal arteriole cuffing

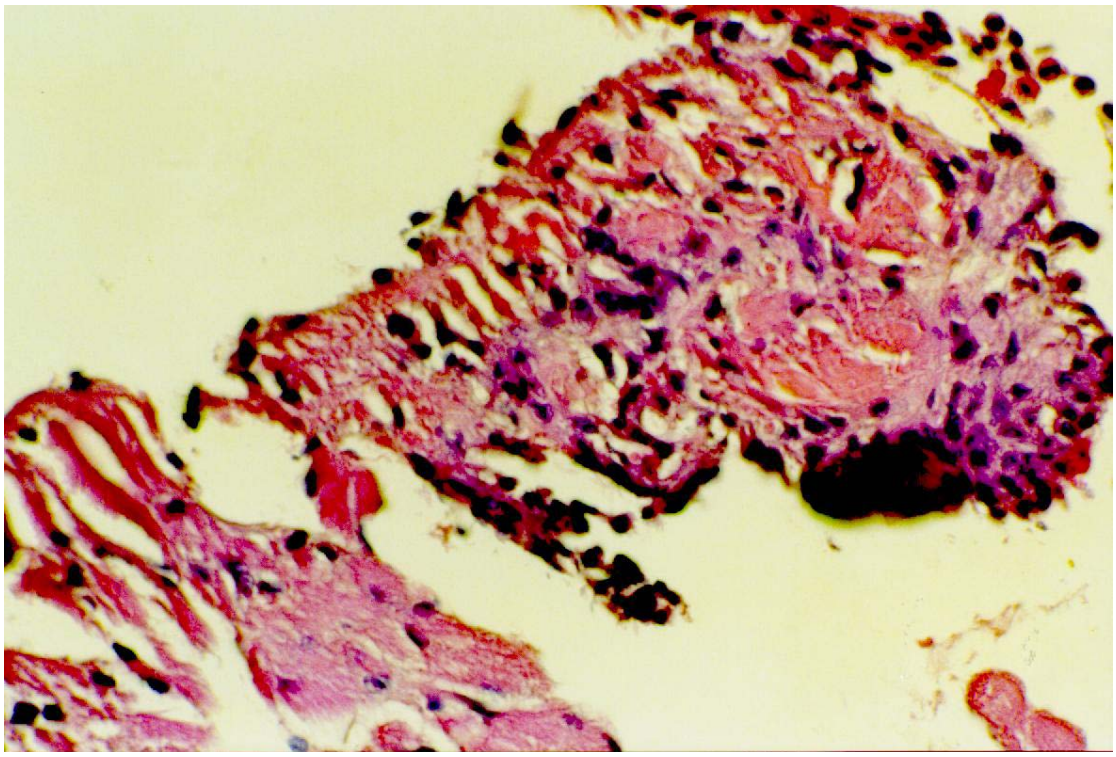


Figure 10 Heart – local necrosis and inflammation

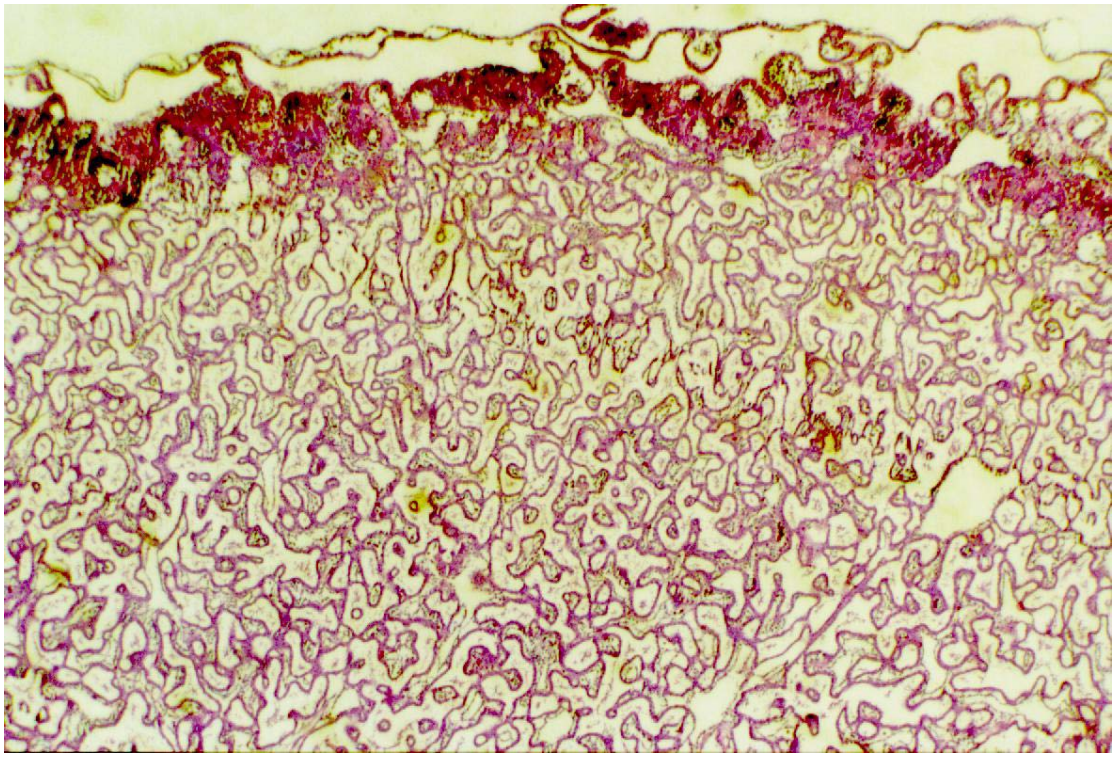


Figure 11 Antennal gland – zone of inflammation (low power)

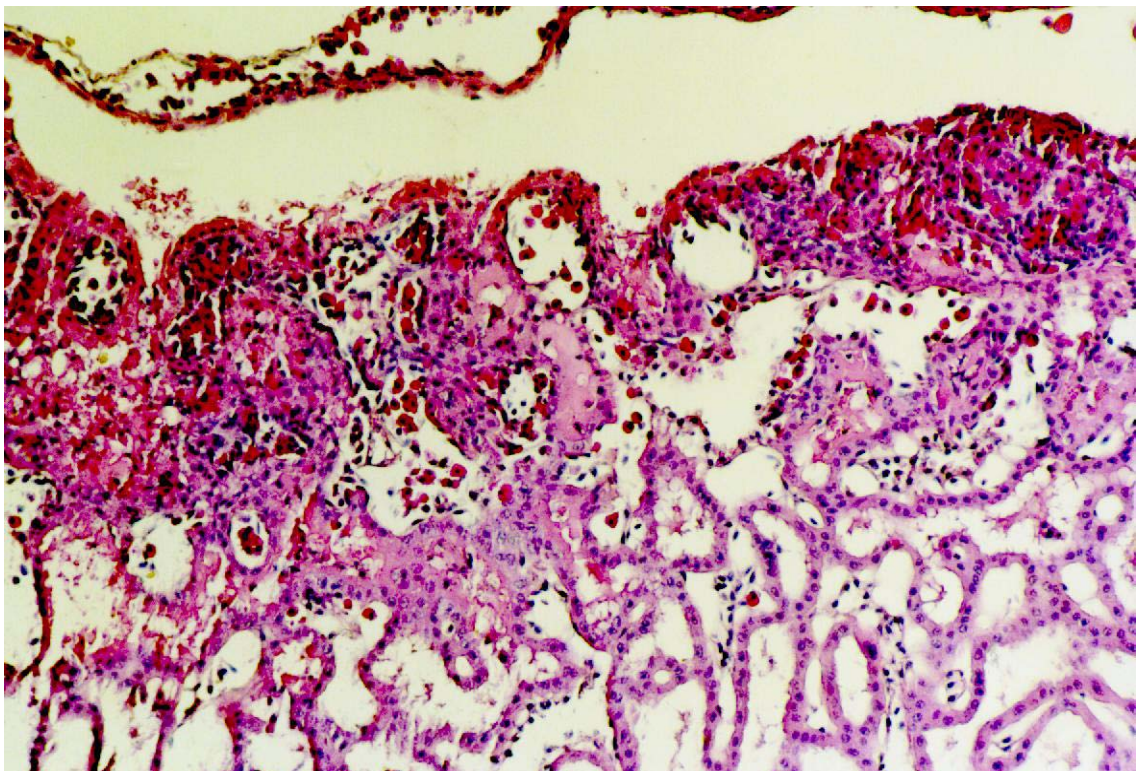


Figure 12 Antennal gland – zone of inflammation (high power)

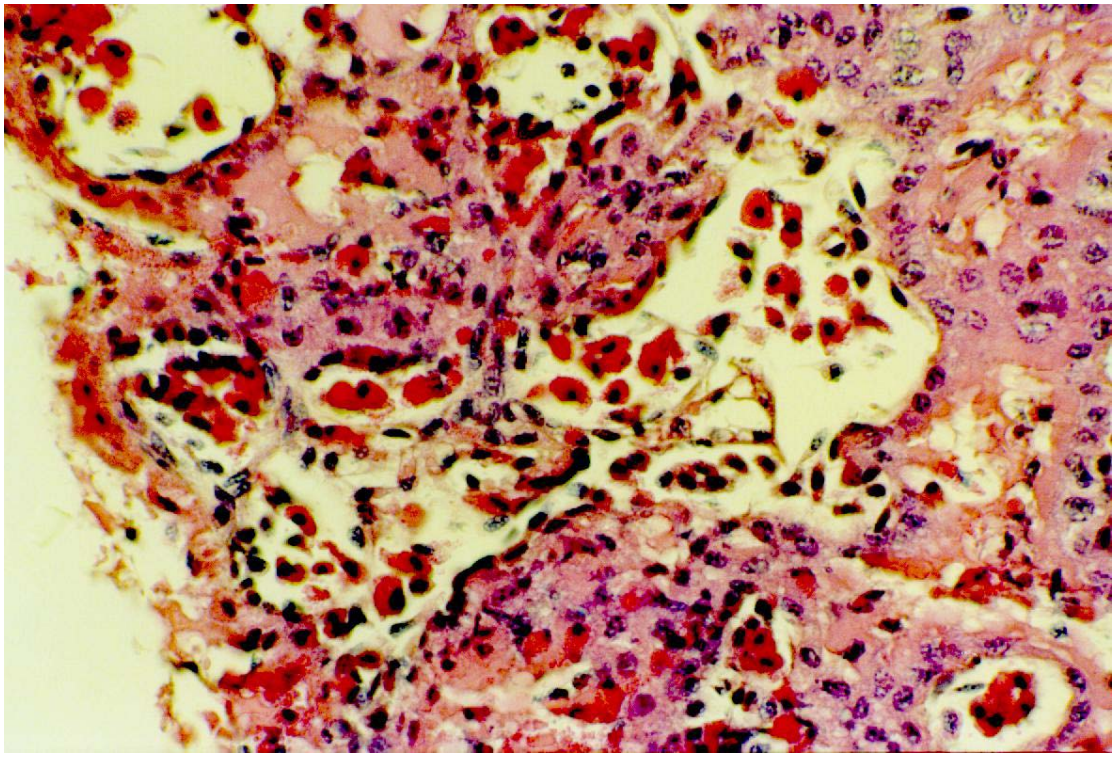


Figure 13 Antennal gland – zone of inflammation (high power)

APPENDIX 7

CASE STUDY 3
TAIL DISEASE IN SOUTHERN ROCK LOBSTERS (*JASUS EDWARDSII*)

R.E. Reuter[1], M.C. Geddes[2], L.H. Evans[3], and S.R. Bryars[2]

[1]Veterinary Pathology Services, 33 Flemington St, Glenside, South Australia 5065
[2]University of Adelaide, Adelaide, South Australia 5000
[3]Aquatic Science Research Unit, Curtin University of Technology, Kent Street, Bentley, Western Australia 6102

(Reprinted with permission from Proceedings, International Symposium on Lobster Health Management, Adelaide 1999; (L.H. Evans & J.B. Jones Eds))

TAIL DISEASE IN SOUTHERN ROCK LOBSTERS (*JASUS EDWARDSII*)

Ruth E. Reuter[1], Michael C. Geddes[2], Louis H. Evans[3], and Simon R. Bryars[2]

[1]Veterinary Pathology Services, 33 Flemington St., Glenside, South Australia, 5065;
[2]University of Adelaide, Adelaide, South Australia 5000;
[3]Aquatic Science Research Unit, Curtin University of Technology, Kent Street, Bentley, Western Australia 6102

ABSTRACT

Wild caught lobsters are commonly kept captive in holding facilities to maximise harvest returns. Tail disease due to chitin-destroying bacteria is considered a major problem overseas, in lobsters held in groups over winter. In South Australia the condition has been seen during the summer period. Tissue samples were collected in 1999 from five groups of lobsters being fed different diets, in sea-based and land-based holding systems at Port Lincoln, South Australia. Lesions were identified in the chitin on the tail and/or claw in 11 animals. Histopathologic examination of lesions showed inflammation often associated with cracks and fissures in the overlying chitin. Of the four samples cultured, *Vibrio alginolyticus* was isolated from all samples, with *Plesiomonas shigelloides* also obtained from one of the samples. *V. alginolyticus* and *Aeromonas hydrophila* had been cultured from similar lesions in lobsters from the same sea-based holding cages one year earlier. These organisms are commonly present in marine and estuarine environments. However it was considered that the handling and holding of the lobsters, in association with elevated water temperatures, could have predisposed them to invasion of damaged tissue by these organisms. *V. alginolyticus* has also been associated with skin damage, ulcers, anaemia, tail and fin disease in finfish, and mortality in eels after handling.

I. INTRODUCTION

Incidents of shell and tail disease in lobsters in holding facilities have been reported since the early 1930s (Hess, 1937). More recently, a survey of lobsters in lobster traps close to sewage disposal sites and relatively unpolluted areas identified a 7.9% incidence of shell disease. However, there was no significant statistical difference in prevalence between lobsters from the two sites (Ziskowski *et al*, 1994). A questionnaire circulated to producers by the Department of Marine Resources, University of Maine, USA revealed that shell disease was prevalent in lobsters in holding facilities, appearing between 3 weeks and 4 months of holding in up to 50% of the facilities surveyed (White, 1999). Chitinolytic bacterial disease of American lobsters (*Homarus americanus*) and shrimps (*Cancer* spp.) has been described as ubiquitous on the east coast of North America and common in lobsters stored in pounds (Bower *et al*, 1994).

The rock lobster fisheries in Southern Australia are a valuable commercial industry. Due to the variation in financial returns depending on season, market forces and other factors, there has been increasing interest in holding live lobsters for extended periods. However there have been problems experienced with mortalities, shell and

tail disease, and feeding in these situations. These factors have resulted in reduced condition and quality of the product. In December of 1997 a small pilot study was instituted to hold lobsters in cages over the Australian summer period, to identify some of the factors of importance in the production of shell and tail disease under these circumstances. Based on this study, an expanded trial was conducted in the 1998 summer period.

II. MATERIAL & METHODS

In November 1998 a feeding trial was initiated at Port Lincoln, South Australia, as part of the FRDC project 98/305 on live-holding of adult *Jasus edwardsii*. One hundred experimental lobsters were separated into 5 treatment groups of 20 animals each. Four of the groups were held in separate compartments in a sea-based cage system. One of these groups was not fed, while the other 3 groups were each fed on one of 3 diets: a hard, dry artificial pellet (SRLD1-98); a soft, moist artificial pellet (SRLD1-98); and live mussels (*Mytilus edulis*). The fifth treatment group was held in a land-based raceway system and fed on the hard, dry artificial pellet diet. The details of the feeding trial will be reported separately.

At the completion of the trial, 10 animals were selected for detailed examination from each of the experimental groups and from a lobster processing facility. The 10 lobsters from the processing facility were used as a control group as they were wild-caught and had not been exposed to long-term holding. The external surface of each lobster was examined for evidence of blisters, pitting, erosions or other damage to the carapace, appendages and tail. Sections from 4 representative lesions from the tails were cultured on 7% horse blood agar, MacConkey's agar, TCBS cholera medium and Saboraud's agar*. The plates were incubated at 24°C. Slices of tissue were placed in 10% seawater formalin, fixed at least 24 hours, processed routinely and stained with haematoxylin and eosin for microscopic examination.

* Oxoid Australia Pty. Ltd., Melbourne, Australia.

III. RESULTS

Gross examination

Examination of the lobsters revealed changes in the tail from blistering of the ventral surface to ragged edges and complete loss of some sections. The exoskeleton exhibited variably sized areas of pitting and erosion on the shell of the claw. Damage of this type was found in 11 of the 60 animals sampled. Affected animals were found in all of the groups.

Bacteriology

Culture of the tail samples yielded a heavy growth of *V. alginolyticus* from all 4 samples and a moderate growth of *Plesiomonas shigelloides* from 1 sample. Both organisms were sensitive to all antibiotics tested. Fungal agents were not identified in the specimens.

Histopathology

Sections taken from 7 samples of tail or shell revealed microscopic cracks and fissures of varying sizes in the superficial chitin, extending into the underlying soft tissue. There was haemorrhage, oedema, collections of haemocytes and, in two cases, small pockets of Gram negative bacteria in the areas of erosion on the surface and inflammation underneath the remaining fragments of cuticle. Thrombosis of blood vessels was evident in two cases.

IV. DISCUSSION

In the Port Lincoln area the water temperatures are highest in late January and February, when they may reach 21-24ºC around the lobster facilities. The water temperatures in the locations from which the lobsters originate usually range from 16-18ºC. In one overseas study, an association between increased water temperature and development of lesions was described (Taylor, 1948). In Tasmania, a seasonal count of *Vibrio* sp. identified in sediment from sites along the east coast peaked in the summer (Cameron *et al*., 1988). *V. alginolyticus* is commonly found in marine and estuarine environments and has been isolated from the water in marine fish tanks (Gilmour, 1977). *Plesiomonas shigelloides*, another member of the Vibrionaceae, has been associated with disease in rainbow trout overseas (Cruz *et al.*, 1986) Numerous species of Gram negative bacteria possess the enzyme chitinase, which destroys the chitin of the shell, resulting in pitting, erosions and severe shell damage. Many of the *Vibrio* sp. are considered secondary opportunists, causing disease only when fish or shellfish are under stress, or when there is already damage to the shell, allowing the bacteria to invade the underlying tissue. High mortality, ulcers or "red spot" in farmed sea bream (Colorni *et al.*, 1981) and eels (Austin & Austin, 1993) have been associated with extensive handling, suggesting the *Vibrio* organisms isolated were opportunists. In the study reported here, many of the lobsters in the holding facilities developed a range of exoskeleton and tail lesions. The splitting and cracking of the chitin seen microscopically could have allowed entrance of bacteria such as *V. alginolyticus*, commonly present in the water. Predisposing factors could have included handling of the animals, the stress of holding resulting in decreased immunocompetence, injury from fighting or abrasions from the cage wire, and elevated water temperatures during the period of holding.

ACKNOWLEDGEMENTS

We would like to acknowledge the assistance of Dr. R. Musgrove, Mrs. A Barnes and Ms. S. Fotedar in sample collection and preparation; and Mrs. K. McLennan for the microbiological examinations.

REFERENCES

Austin, B & Austin, D.A. (1993) Vibrionaceae representatives. In *Bacterial Fish Pathogens; disease in farmed and wild fish*. 2nd ed. pp 265-307.

Bower, S., McGladdery, S.E. & Price, I.M. (1994) Chitinolytic bacterial disease. In Synopsis of infectious diseases and parasites of commercially exploited shellfish. *Annual Review Fish Disease* **4**,129-130.

Cameron, D.E., Garland, C.D., Lewis, T.E., & Machin, P.J. (1988) A survey of Vibrionaceae in Tasmanian coastal waters, with special reference to bacterial species pathogenic to fish or shellfish. *Ausralian Journal Marine Freshwater Research* **39**, 145-152.

Colorni, A., Paperna, I. & Gordin, H. (1981) Bacterial infections in gilthead sea bream *Sparus aurata* cultured in Elat. *Aquaculture* **23**, 257-267.

Cruz, J.M., Saraiva, A., Eiras, J.C., Branco, R., & Sousa, J.C. (1986) An outbreak of *Plesiomonas shigelloides* in farmed rainbow trout, *Salmo gairdneri* Richardson, in Portugal. *Bulletin of European Association of Fish Pathology* **6**, 20-22.

Gilmour, A. (1977) Characteristics of marine vibrios isolated from fish farm tanks. *Aquaculture* **11**, 51-62.

Hess, E. (1937) A shell disease in lobsters (*Homarus americanus*) caused by chitinovorous bacteria. *J. Biol. Board. Can.* **3**, 358-362.

Taylor, C.C. (1948) Shell disease as a mortality factor in the lobster (*Homarus americanus*). *Maine Department Sea Shore Fisheries, Fisheries Circular* 4,1-8.

White, S. (1999) Shell disease survey produces preliminary results. *Lobster Bulletin*, Lobster Institute, University of Maine, Orono, USA **13**, 4.

Ziskowski, J., Spallone, R., Kapareiko, D., Robohm, R., Calabrese, A., & Pereira, J. (1994). Shell disease in American lobster (*Homarus americanus*) in the offshore, Northwest-Atlantic region around the 106-mile sewage-sludge disposal site. Abstr. Marine Environmental *Engineering*. The Gordon and Breach Publishing Group. OPA (Overseas Publishers Association) Amsterdam B.V.

APPENDIX 8

CASE STUDY 4
APPLICATION OF HEALTH INDICES IN STUDIES OF DIETARY REGIMES AND LIVE TRANSPORT STRESSORS IN THE SOUTHERN ROCK LOBSTER, *JASUS EDWARDSII*

Louis H. Evans[1], Michael C. Geddes[2], Ruth E. Reuter[3], Richard Musgrove[4], Anne Barnes[1], Simon R. Bryers[2] and Seema Fotedar[1]

[1]Aquatic Science Research Unit, Muresk Institute of Agriculture, Curtin University of Technology, GPO Box U1987, Perth, Western Australia 6845
[2]Department of Environmental Biology, University of Adelaide, GPO Box 498 Adelaide, South Australia, 5000
[3]Veterinary Pathology Services, 33 Flemington St. Glenside, South Australia 5065
[4]South Australian Aquaculture Research and Development Institute, South Australia.

APPLICATION OF HEALTH INDICES IN STUDIES OF DIETARY REGIMES AND LIVE TRANSPORT STRESSORS IN THE SOUTHERN ROCK LOBSTER, *JASUS EDWARDSII*

Louis H. Evans[1], Michael C. Geddes[2], Ruth E. Reuter[3], Richard Musgrove[4], Anne Barnes[1], Simon R. Bryers[2] and Seema Fotedar[1]

[1]Aquatic Science Research Unit, Muresk Institute of Agriculture, Curtin University of Technology, GPO Box U1987, Perth, Western Australia 6845
[2]Department of Environmental Biology, University of Adelaide, GPO Box 498 Adelaide, South Australia, 5000
[3]Veterinary Pathology Services, 33 Flemington St. Glenside, South Australia 5065
[4]South Australian Aquaculture Research and Development Institute, South Australia.

EXECUTIVE SUMMARY

The influence of live transport stressors on immune parameters and on the induction of bacteremia in wild caught, adult lobsters *Jasus edwardsii* held in sea-based and land based holding systems with and without feeding was studied. Five different treatment groups of ten lobsters each were studied, one comprising lobsters held in factory tanks for two weeks at reduced temperature without feeding (control), three comprising lobsters held in an offshore cage system for four months either without feeding (unfed platform) or being fed mussels, *Mytilis edulis* (mussel diet) or a hard pellet diet (hard pellet platform) and one group which was held in a shore based raceway holding system for four months and fed the same hard pellet diet (hard pellet raceway). Control lobsters were subjected to a less stressful handling procedure prior to live transport (hemolymph sampling immediately after removal from factory tanks) than the other treatment groups which were harvested from cages or the raceway, transported to the factory and left in air for up to three hours prior to examination and hemolymph sampling. Three hemolymph samples were removed from lobsters in the latter four groups, including a pericardial puncture without prior sterilisation of the wound site. Immune parameters, total hemocyte counts (THC), proportion of circulating granular cells (%granular cells), clotting time, level of impaired clotting (failure to clot within 5 mins) and the prevalence of bacteremia were measured before and after live transport in all five groups.

The research questions addressed in the study and the answers to these questions, based on recorded observations, were as follows:

1. *Does Jasus edwardsii display similar levels of immune parameters and similar patterns of change in these parameters following prolonged air exposure to those of another spiny lobster species, Panulirus cygnus?*

The levels of circulating hemocytes and the proportion of granular cells in *J. edwardsii* tended to be higher than those to those previously reported for *P. cygnus* (Jussila *et al.* 1997; Fotedar *et al.* 2001) and bacteremia was lower in *J. edwardsii* examined in this study. Clotting time in *J. edwardsii* was considerably longer than in *P. cygnus* (mean clotting time of 2.6 mins in lobsters without impaired clotting in the

control group of *J. edwardsii* compared to 40-50 secs in *P. cygnus*) and the level of impaired clotting was also higher in *J. edwardsii*.

Comparison the levels of immune parameters in the two unfed groups of *J edwardsii* lobsters, one subjected to prolonged air exposure prior to sampling (unfed platform) and the other not exposed to air (control) showed some differences in the pattern of response to air exposure in this species compared to *P. cygnus*. The unfed platform lobsters had a higher level of circulating granular cells but similar clotting times and THC levels to those seen in the control lobsoters. The level of bacteremia, 10%, was the same in both groups. Air exposure of *P. cygnus*, on the other hand, led to a decrease in the proportion of circulating granular cells, an increase in clotting time, little change in THC and an increase in bacteremia (Fotedar *et al.* 2001). The results suggest that *J. edwardsii* may have different levels of immune parameters and different patterns of response of these parameters to air exposure but these findings should be confirmed with a larger sample size of lobsters and with treatment groups subjected to the same acclimatisation procedure prior to testing.

2. *Are immune parameters of J. edwardsii influenced by nutrition and, if so, does measurement of selected immune parameters have application as response measures in diet trials with this species*?

Starvation had a significant influence on the levels of THC of harvested lobsters sampled in Port Lincoln. The unfed lobsters (unfed platform and control) had significantly reduced THCs compared to lobsters that were fed mussels or the pelleted diet. There was no significant difference between mean values for any other immune parameter. The reduction in THC in unfed lobsters is in agreement with previous findings of lack of adequate nutrition on circulating hemocyte counts (Stewart *et al.* 1976; Norton *et al.* 2001) and suggests that this parameter may be usefully applied in assessments of dietary regimes for this lobster species. Further studies are required to determine whether other immune parameters also change with nutrient intake.

3. Are immune parameters altered following live transport and, if so, do these alterations provide evidence of decreased health status in the transported lobsters

The levels of immune parameters tended to increase (THC), decrease (%granular cells) or be unaffected (clotting time) following live transport. The prevalence of bacteremia in the 50 lobsters studied increased from 4% prior to transport to 40% after transport, with the mussel diet lobsters having the lowest level of bacteremia (20%prevalence) and the hard pellet raceway group having the highest (60%prevalence). Seventy two percent of surviving lobsters showed evidence of deterioration in health status following live transport, as evidenced by the presence of inflammatory lesions in body tissues. The occurrence of systemic inflammatory lesions in lobsters following live transport was accompanied by a significant decrease in %granular cells. However, the presence of inflammatory lesions showed no correlation with the occurrence of bacteremia. The results suggest that the proportion of circulating granular cells is affected by processes leading to inflammatory reactions in lobster tissues and, along with quantitative histopathology, may therefore be a useful measure of health status in lobsters. The presence of bacteremia, on the other hand, is more likely a reflection of stress status of lobsters and does not appear to have a strong correlation with tissue injury and inflammation. Application of other immune parameters in health assessment of lobsters should be further investigated.

I. INTRODUCTION

Live transport of seafood products is a well established marketing approach in clawed and spiny lobster fisheries (Riley *et al.* 1997; Spanogue 1996). Lobsters are harvested in pots, stored on board fishing vessels and then held for a further period of time in holding tanks or impoundments before being transported to market. Handling procedures aimed at minimising mortality during transport have been adopted for most lobster fisheries. For example, a code of practice for handling live Australian rock lobster, based on minimising air exposure time, heat stress and physical damage and on optimising environmental conditions in holding systems, ensures high survival of harvested product (Stevens *et al.* 1995).

While the mortality of lobsters during live transport can be minimised through the adoption of best practice handling procedures, excessive post-harvest stress caused by prolonged air exposure and poor handling practices is likely to result in reduced survival. Numerous workers have investigated survival of lobsters transported under different conditions (McLeese 1965; Whiteley & Taylor 1992; Spanogue & Bourne 1997; Riley *et al.* 1997). However, there have been few pathological studies aimed at determining the cause of morbidity and mortality in live lobster exports.

Mechanisms by which pathophysiological responses can cause cell injury and organ failure in vertebrates have been documented (Trump & Arstila 1975; Wood *et al.* 1983) and probably apply equally to invertebrate species. Physiological investigations of commercial shipments of *Homarus grammarus* (Whiteley & Taylor 1992), *Homarus americanus* (Riley *et al.* 1997) and *Panulirus cygnus* (Spanogue & Bourne 1997; Tod & Spanoghe 1997) suggest that death is likely to be due to failure of vital organ function through cell injury caused by acute hypoxemia, ammonia toxicity or intracellular acidosis. Opportunistic bacterial infections arising from either wounding or an impairment in the lobster's host defence responses has only been considered as a cause of death in one study conducted on commercial shipments of *Panulirus japonicus* (Sugita & Deguchi 1994)

The major stressors occurring during live transport and storage are air exposure, handling disturbances, minor wounding, exposure to temperature extremes or exposure to high levels of dissolved ammonia. Physiological responses to these stressors have been investigated in the laboratory in a number of different lobster species. Air exposure leads to internal hypoxia, respiratory and metabolic acidosis, elevated lactate levels and increased hemolymph ammonia concentrations (Taylor & Whiteley 1989; Spicer *et al.* 1990; Taylor & Waldron 1997; Schmitt & Uglow 1997). Similar physiological changes occur following physical disturbance (McMahon *et al*. 1978; Taylor & Whiteley 1989; Whiteley *et al.* 1990; Spanogue 1996) which is also accompanied by a rapid elevation in metabolic rate (McMahon *et al.* 1978; Winkler 1987; Hagerman *et al.* 1990). Elevated temperatures exacerbate the pathophysiological responses (Whiteley *et al.* 1990; Spanogue 1996) and lobsters have also been shown to be more vunerable to transport mortality when in the pre-moult stage of the moult cycle (Whiteley & Taylor 1992).

The pathophysiological responses to air transport stressors are likely to affect immunity in post-harvest lobsters. An immune parameter which has been studied in post-harvest lobsters and other crustaceans is the number of total circulating

hemocytes (THC) (Jussila *et al.* 1997, 1999). The number of circulating hemocytes found in crustacean blood (hemolymph) is affected by a range of physiological and pathological conditions. Decreases in total hemocyte counts have been reported following starvation in lobsters (Stewart *et al.* 1967) and freshwater crayfish (Evans *et al.* 1999a), in bacterial infections of lobsters (Stewart 1975) and crabs (Johnson 1976), in dinoflagellate infection of lobsters (Field & Appelton 1995), in moribund lobsters (Jussila *et al.* 1997) and in freshwater crayfish injected with foreign particles (Persson *et al.* 1987) or exposed to high levels of dissolved copper (Evans 2000) or other environmental toxins (Smith *et al.* 1995). Circulating hemocyte levels have also been shown in some crustacean species to be affected by sex and moult stage (Ravindranath 1974; Tsing *et al.* 1989; Evans *et al.* 1992) and fungal infections (Sequeira *et al.* 1996). Measurement of this and other immune parameters may be of value in assessing health and nutritional status of lobsters.

Immune responses to air exposure and live transport and nutrient deprivation have not been previously reported for the spiny lobster *Jasus edwardsii*. Information is also lacking on the influence of live transport stressors such as minor wounding, excessive handling, feeding prior to transport and prolonged air exposure on levels of immune parameters in this species and on the development of bacteremia in lobsters following live transport. The aim of this study was to evaluate the application of health indices (immune parameter assays, occurrence of bacteremia and quantitative histopathology) in the assessment of response to dietary regime (feeding mussels, an artificial hard pellet diet or not feeding) and live transport stressors (a combination of excessive physical handling, prolonged air exposure, feeding prior to shipment, minor puncture wounds and air shipment) in the southern rock lobster *Jasus edwardsii.*

II. MATERIALS AND METHODS

Experimental animals

Adult, wild caught lobsters, *Jasus edwardsii*, were obtained from a feeding trial conducted at Port Lincoln, South Australia. They were held from November 1998 to March 1999 in a either a sea-based holding system comprising steel cages lined with 3 mm oyster at a stocking density of 20 animals per cage or at a similar stocking density in a land-based raceway holding system. Lobsters in the sea-based trial were either not fed (unfed) or were fed a hard, dry artificial pellet (SRLD1-98) (pellet diet) or live mussels (*Mytilus edulis*) (mussel diet). The land-based trial comprised the pellet diet only. After four months holding, the animals were caught into baskets and transported to the processing factory where they were held for up to 5 hrs in a cold room before being selected at random for examination and hemolymph sampling. A further sample of 10 lobsters was also selected at random from the factory tanks and immediately sampled and examined, sampling being performed within one to two minutes after removal from the tank (control). These lobsters had been held at reduced temperature (approximately 14^{o}C) without feeding for two weeks prior to testing. All but two of the animals selected from the two groups were in intermoult or early pre-moult.

The feeding trial lobsters had three hemolymph samples taken, two from the ventral sinus following sterilisation with 70% alcohol and the third sample from the pericardial sinus. No sterilisation was performed prior to the pericardial sinus

sampling. Five lobsters from each group were sampled and then another five were sampled in the same treatment group sequence. The control lobsters were only sampled from the ventral sinus. All lobsters were then examined and returned to the cold room before being packed into foam boxes with sawdust and ice packs and transported by airfreight to the laboratory in Adelaide (approximately 1 hr flight time and 30 min road transport). Upon arrival at Adelaide the lobsters were transported to the laboratory and held overnight in a cold room before being examined, a hemolymph sample removed and dissected.

Experimental protocol

Each lobster was assessed for sex, carapace length, appendage loss, tail erosion, tail blisters and molt stage. Hemolymph samples were taken after swabbing the base of the fifth thoracic leg with 70% alcohol or from the pericardial sinus without sterilisation. A 0.2ml aliquot of the ventral sinus hemolymph sample was withdrawn into a sterile syringe containing 0.2ml of anticoagulant (1% glutaraldehyde in sodium cacodylate) and dispensed into an Eppendorf tube kept on ice. This sample was later used for total and differential hemocyte counts. Total hemocyte counts and differential hemocyte counts were performed as previously reported (Jussila *et al.* 1997). Due to the difficulties encountered in accurately differentiating between hyaline and semi-granular hemocytes, only %granular cells are reported. The granular cells were identified on the basis of large cell size, small nucleus and large number of red- pink granules. The pericardial sinus sample was used for moult stage, protein and metabolite estimation, the results of which will be reported elsewhere (Geddes *et al.* Final FRDC Report Project 98/305).

A second aliquot (about 0.3ml) was withdrawn from the ventral sinus into a sterile syringe. Two drops were placed on a marine saline agar plate, the plate carefully inverted and kept at room temperature for up to 2 days for bacteremia assessment. The rest of the hemolymph was transferred into an Eppendorf tube and 30µl was transferred into another Eppendorf then drawn into a capillary tube. The tube was repeatedly inverted until the hemolymph stopped moving. This process was timed and noted as clotting time. Observations were continued for 5 mins. Hemolymph samples which had not clotted after 5 minutes were recorded as impaired clotting. The clotting times were ranked 0 (0-1 min) - 5 (> 5 mins) to order to provide a numerical data set which would include the results from those lobsters in which no clot was observed within 5 minutes. The %prevalence of samples in each treatment group with impaired clotting was also determined.

Following hemolymph sampling in Adelaide, each lobster was dissected and approximately 0.5 cm^3 samples of heart, gills, hepatopancreas, abdominal muscle and antennal glands were placed in 10% seawater formalin, fixed for at least 24 hrs, processed and stained with haematoxylin and eosin for microscopic examination (Bancroft & Stevens 1977).

Mortality of lobsters following transport to Adelaide and overnight storage was observed in some treatment groups. Additional lobsters that had not had immune parameters measured in Port Lincoln were dissected to provide a sample size of 10 dissected lobsters per group.

Statistical analysis was performed using analysis of variance, chi-square test, Kruskal-Wallis test and the least significant difference test. The criteria for statistical significance was $P<0.05$ unless otherwise stated. Results are presented as mean ± s.e. unless otherwise stated.

It should be noted that the initial results analysis revealed that the mean value for THC for the first 5 lobsters sampled in the unfed platform group (15.25 $x10^6$ cells/ml) was significantly higher than that of the second group of 5 lobsters sampled (4.12 $x10^6$ cells/ml) while the mean THC for the first 5 lobsters sampled in the mussel diet group (6.44 $x10^6$ cells/ml) was lower than that of the second group of 5 lobsters (13.48 $x10^6$ cells/ml). The difference between the mean THC values for the two groups of mussel diet lobsters was not statistically significant. However, the clear lack of agreement between two sets of results from the first and second sample of lobsters from the two groups and the inverse pattern of THC values for the different treatment groups suggested that an error in initial labelling the lobster groups had occurred. Accordingly, all results for the first group of 5 lobsters in the unfed platform and mussel diet groups were transposed and the data analysis was performed on the rearranged data set. In order to validate this decision results for the protein levels in these lobsters have been requested from the other research group involved in this study but to date these results have not been provided.

III. RESULTS

Comparison of immune parameter levels in *Jasus edwardsii* with those of *Panulirus cygnus*

The levels of immune parameters in *Jasus edwardsii* from the three fed treatments measured in Port Lincoln were used in the comparison of immune parameters with *Panulirus cygnus*. Data for the latter species was obtained by averaging the control values reported in Fotedar *et al.* (2001) and from values for white and red lobsters reported in Jussila *et al.* (1997). The results are shown in Table 1.

Table 1 Comparison of immune parameters in *Jasus edwardsii** and *Panulirus cygnus***(mean±s.e.)

Species	THC ($x10^6$ cells/ml)	%granular cells	Clot time (mins)	Impaired clotting (%prevalence)	Bacteremia (%prevalence)
Jasus edwardsii	13.17±1.01	12.63±0.80	4.05±0.88	43(range 30-60)	0
*Panulirus cygnus***	6.1	9.6	0.8	4	37
*P.cygnus****					
White lobsters	5.6±0.7	8.0±1.9	ND	ND	ND
Red lobsters	5.3±0.7	10.7±1.4	ND	ND	ND

* mean values obtained by pooling data for fed lobsters sampled in Port Lincoln

** mean values obtained by pooling results obtained with control lobsters from Fotedar *et al.* (2001).

*** mean values obtained with healthy lobsters stored overnight in factory tanks following harvest (Jussila *et al.* 1997)

ND – Not determined

The levels of all immune parameters tended to be higher in *J. edwardsii* compared to *P. cygnus*. A marked difference was observed in the clotting time and in the %prevalence of impaired clotting. The prevalence of bacteremia was higher in *P. cygnus* than in *J edwardsii*.

Influence of prolonged air exposure on immune parameters in *Jasus edwardsii*.

The influence of prolonged air exposure on immune parameters was assessed by comparison of mean values obtained in the two unfed groups, control and unfed platform, the latter being exposed to air for up to 5 hr and the former being sampled immediately following removal from the factory tank. These results are shown in Table 2.

Table 2 Comparison of immune parameters in unfed lobsters subjected air exposure

Treatment Group	Immune Parameter			
	THC ($x10^6$ cells/ml)	%Granular cells	Clotting rank	Impaired clotting (%prevalence)
Control	7.48 ± 1.37	$7.58 \pm 0.68_a$	3.4 ± 0.4	30
Unfed platform	5.28 ± 0.79	$11.08 \pm 1.18_b$	4.2 ± 0.4	60

Mean values with different subscripts are significantly different ($P<0.05$).

There was no significant difference between the immune parameters THC and clotting time and between the prevalence of lobsters with impaired clotting. The proportion of granular cells in the lobsters exposed to minimum stress (control) was significantly lower than that of lobsters exposed to air for a prolonged period (unfed platform). The same prevalence of bacteremia was observed in both treatment groups (10%).

Influence of dietary regime on immune parameters

The influence of dietary regime on immune parameters was assessed by comparison of mean values obtained for immune parameters in lobsters from the diet trial sampled in Port Lincoln. The results are shown in Table 3.

Table 3 Immune parameters in different treatment groups in Port Lincoln samples (mean ± s.e.)

Treatment Group	Immune Parameter			
	THC (x10^6 cells/ml)	**%Granular cells**	**Clotting rank**	**Impaired clotting (%prevalence)**
Unfed platform	$5.28 \pm 0.79_a$	11.08 ± 1.18	4.2 ± 0.4	60
Hard-pellet platform	$12.13 \pm 1.14_b$	13.83 ± 1.66	3.8 ± 0.3	30
Mussel diet	$14.36 \pm 2.73_b$	12.95 ± 1.64	4.0 ± 0.3	40
Hard-pellet raceway	$13.01 \pm 0.98_b$	11.10 ± 0.54	4.1 ± 0.4	60

Mean values in the same column with different subscripts are significantly different ($P<0.05$).

Starvation had a significant influence on the levels of THC of harvested lobsters as determined by comparison of the mean values obtained for the four different treatment groups. The unfed lobsters had a significantly reduced THC compared to lobsters that were fed mussels or pelleted diet. The level of THC in this treatment group was similar to that of the other unfed group (control; Table 2). There was no significant difference between mean values for any other immune parameter. A large variation was seen in THC for lobsters in the mussel diet group (range: 3.27 to 28.53 x 10^6 cells/ml).

Influence of initial post-harvest stressors combined with air transport on stress and health parameters

Eighty two percent of lobsters survived the live transport and storage procedures but most of the lobsters subjected to prolonged air exposure prior to air shipment were in poor health, being lethargic, moribund or close to death. Six surviving lobsters from the treatment groups in which mortalities occurred (unfed platform, 3 lobsters); hard-pellet platform, 3 lobsters) and that had not been sampled for immune parameters in Port Lincoln were also sampled and dissected in Adelaide and their results included in the data analysis.

Changes in immune parameters following live transport in the individual treatment groups are shown in Table 4 and Figures 1-4. The levels of immune parameters tended to increase (THC), decrease (%granular cells) or be unaffected (clotting time) following live transport and these differences were significant for the control and unfed platform groups for THC and the unfed, hard pellet platform and hard pellet raceway groups for % granular cells. The %granular cells did not change following live transport in the control and the hard pellet raceway groups.

Table 4 Changes in immune parameters following live transport from Port Lincoln (PL) to Adelaide (AD)

Treatment Group		Immune Parameter				
		THC ($x10^6$ cells/ml)	%Granular cells	Clotting rank	Impaired clotting (%prevalence)	Bacteremia (%prevalence)
Control	PL	$7.48 \pm 1.37_a$	7.58 ± 0.68	3.2 ± 0.4	30	10
	AD	$14.64 \pm 3.83_b$	6.78 ± 0.80	3.1 ± 0.4	30	30
Unfed platform	PL	$5.28 \pm 0.79_a$	$11.08 \pm 1.18_a$	4.2 ± 0.4	60	10
	AD	$11.60 \pm 1.87_b$	$6.90 \pm 0.99_b$	4.1 ± 0.4	60	50
Hard-pellet platform	PL	12.13 ± 1.14	$13.83 \pm 1.66_a$	3.8 ± 0.3	30	0
	AD	14.30 ± 1.60	$9.13 \pm 1.09_b$	4.3 ± 0.3	80	40
Mussel diet	PL	14.36 ± 2.73	$12.95 \pm 1.64_a$	4.0 ± 0.3	40	0
	AD	13.25 ± 1.96	$7.85 \pm 1.56_b$	4.1 ± 0.4	60	20
Hard-pellet raceway	PL	13.01 ± 0.98	11.10 ± 0.54	4.1 ± 0.4	60	0
	AD	15.75 ± 2.33	11.48 ± 1.08	4.3 ± 0.3	60	60

Mean values for PL and AD from the same treatment group with different subscripts are significantly different ($P<0.05$)

The mean clotting ranks were similar in lobsters sampled in Adelaide compared to the corresponding Port Lincoln samples for all treatment groups. The clotting times for the control lobsters tended to be lower than those of other treatment groups in lobsters sampled both in Port Lincoln and in Adelaide but these differences were not significant. The mean clotting time for the Port Lincoln control lobsters (disregarding the three lobsters with impaired clotting) was 2.64 ± 0.19 mins. The level of impaired clotting in surviving lobsters tended to increase following live transport but the differences between mean levels before and after transport was not significant.

The prevalance of bacteremia in the 50 lobsters assayed before and after live transport increased from 4.2% prior to transport to 40% after transport. The %prevalence of bacteremia increased in all treatment groups following live shipment and there was a significant difference between the %prevalence of bacteremia in lobsters from the different treatment groups following live transport. The mussel diet lobsters had the lowest level of bacteremia and the hard pellet raceway group the highest level.

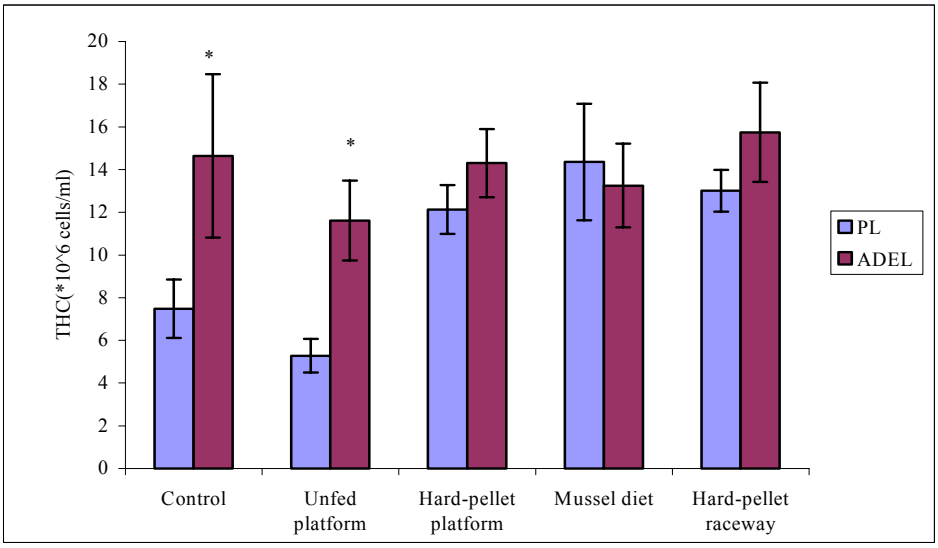


Figure 1 Effect of transport on total hemocyte counts
(*) Denotes a significant difference between mean values for Port Lincoln and Adelaide lobsters (P<0.05)

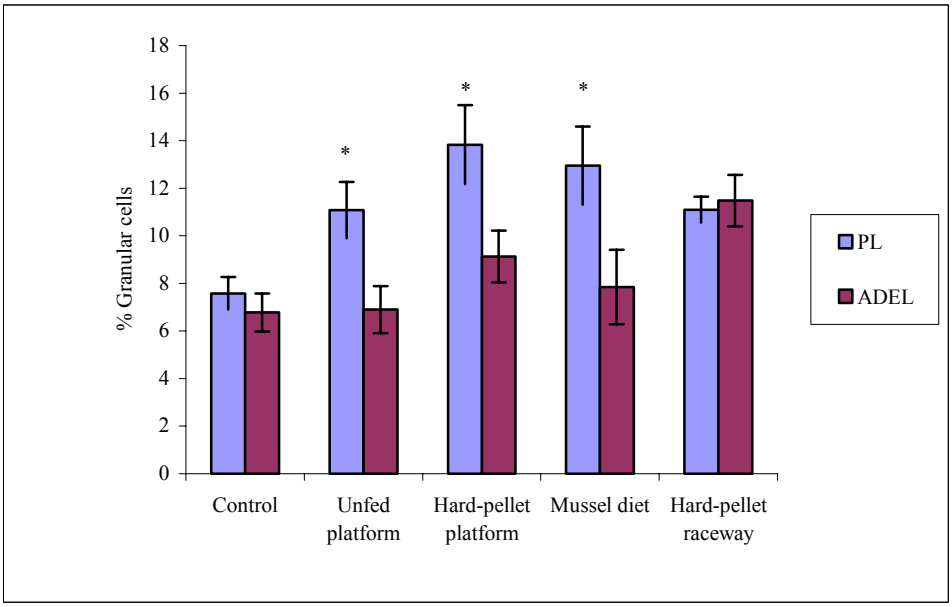


Figure 2 Effect of transport on proportion of granular cells
(*) Denotes a significant difference between mean values for Port Lincoln and Adelaide lobsters (P<0.05)

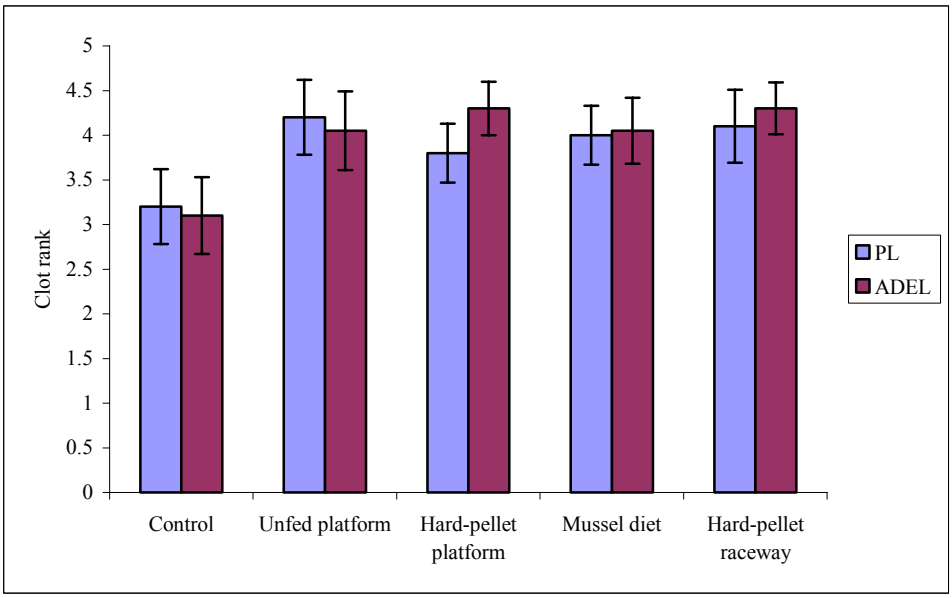


Figure 3 **Effect of transport on clotting ranks.**
(*) Denotes a significant difference between mean values for Port Lincoln and Adelaide lobsters (P<0.05)

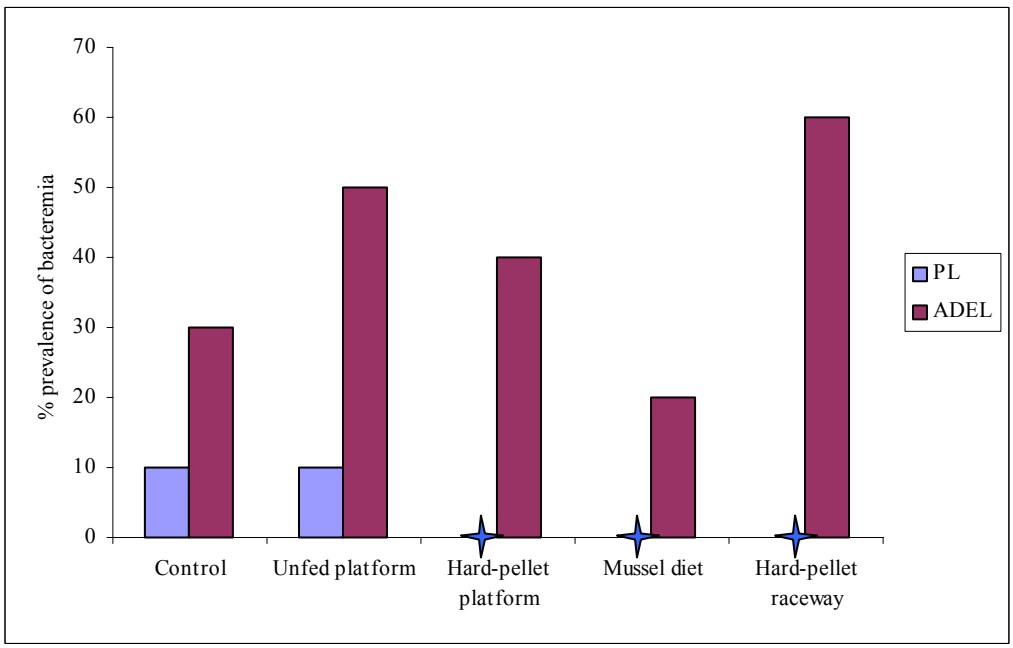


Figure 4 Effect of transport on prevalence of bacteremia

Histopathology was performed on all lobsters from the five treatment groups following live transport and a quantitative assessment of pathological lesions was performed on histological sections from thirty two lobsters from four treatments (control, unfed, mussel diet and hard-pellet raceway groups). Lobsters examined for

quantitative histopathology were assigned to three different groups depending on the level of histopathological response observed in tissue sections (nil response: no pathological lesions observed; focal response: isolated hemocytic inflammation or similar pathological lesions in one organ only; systemic response: hemocytic inflammation or similar pathological lesions in more than one organ). Immune parameters and bacteremia in these three groups are shown in Table 4.

Table 4 Immune parameters and bacteremia in lobsters with nil, focal and systemic response histopathology

Parameter	**Nil response (9)**	**Focal response (12)**	**Systemic response (11)**
THC(x10^6 cells/ml)	18.31±3.46	12.14±2.16	11.75±2.29
%granular cells	$11.30±1.54_a$	$8.33±1.23_b$	$6.84±0.80_b$
Clotting rank	4.37±0.36	3.85±0.32	3.91±0.29
Impaired clotting (%prevalence)	66.7	25.6	36.4
Bacteremia (%prevalence)	33.3	50.0	54.5

Mean values in the same row with different subscripts are significantly different (P<0.05)
Numbers in brackets = number of lobsters with specified lesions

Nine lobsters (28.1%) showed no histopathological lesions, 12 lobsters (34.3%) showed isolated focal lesions (focal hemocytic aggregations or small nodules in either the heart, gill or antennal gland) while 11 lobsters (34.4%) had inflammatory lesions in more than one organ (mostly an extensive hemocytic aggregation in the heart resembling an inflammatory response to extensive cell injury along with lesions (focal hemocytic aggregations, small nodules and/or encapsulations) in at least one other organ). Three of the latter group had a focus of inflammation in the muscle tissues, and a further three lobsters displayed encapsulation and inflammatory reactions in the hepatopancreas, both features typically seen in bacterial infections. However, bacteria were only observed in one histological section, in the hepatopancreatic tubules of a lobster with systemic lesions.

The proportion of granular cells in the group showing no histopathological lesions was significantly higher than that of the group with systemic lesions (P<0.05) and THC tended to be lower in this group. There was no other significant differences between the immune parameters exhibited by the three groups. However, the immune parameters in the group with focal lesions tended to be similar to those of the group with systemic lesions.

Ten of the lobsters in the systemic response group had lesions in the heart suggestive of needle prick wounds or extensive cell injury from some other cause. Apart from tail lesions, the results of which are to be reported elsewhere, and the occasional

occurrence of coagulated hemolymph, mainly in antennal glands or gills, there were no other abnormal features seen in the tissue sections.

A comparison of the mean values for histopathology grades from the four groups subjected to quantitative histopathological examination is shown in Table 5.

Table 5 Prevalence of histopathological lesions in lobsters from different treatment groups

Treatment group	Number of lobsters	%Prevalence of histopathological lesions
Control	10	30.0_a
Unfed platform	6	83.3_b
Mussel diet	6	33.3_{ab}
Hard-pellet raceway	10	10.0_a

Mean values in the same column with different subscripts are significantly different ($P<0.05$)

The %prevalence of lobsters with systemic lesions was significantly lower in the control, mussel diet and hard-pellet raceway groups compared to the unfed platform group.

IV. DISCUSSION

The major findings of this study were that live transport of *Jasus edwardsii* led to a decrease in the proportion of circulating granular cells and an increase in the prevalence of bacteremia in lobsters which had been held for extended periods with and without feeding and exposed to different levels of handling stressors prior to live transport. In addition, seventy two percent of lobsters showed evidence a deterioration in health status following live transport, as evidenced by the presence of inflammatory lesions in body tissues. The occurrence of systemic inflammatory lesions in lobsters following live transport was accompanied by a significant decrease in %granular cells. Lobsters which had not been fed had a significantly lower mean THC than fed lobsters but there was no difference between other mean levels of immune parameters in lobsters fed different diets or held in different holding conditions.

Hemocytes play a central role in crustacean immunity and disease. Processes such as phagocytosis, clotting, nodule formation and encapsulation, foreign agent recognition, production of antimicrobial peptides and cytotoxicity are mediated by hemocytes or hemocyte factors and are vital mechanisms in the prevention of infectious disease (Söderhäll & Cerenius 1992; Bachere *et al.* 1995; Chisholm & Smith 1995). Granular cells, one of the three main types of hemocytes, are recognised as being the main repository of the prophenoloxidase system, through which foreign agent recognition is mediated (Söderhäll & Smith 1983), and are also active in other host defence processes (Hose & Martin 1989; Söderhäll & Cerenius 1992). Alterations in the levels of circulating granular cells are likely to affect host defence capacity and circulating levels of these hemocytes could therefore reflect health status.

The decrease in the proportion of circulating granular cells seen in the present study is similar to that observed following live transport and storage of *P. cygnus* (Jussila *et al.* 1997) and air exposure of *P. cygnus* (Fotedar *et al.* 2001). In the former investigation the proportion of granular cells was found to be lowest in moribund lobsters and other studies in our laboratory have confirmed this finding, some moribund lobsters having no granular cells present in circulation (unpublished observations). A high proportion of the Adelaide lobsters showed poor vigour, suggestive of poor health status and morbidity (Spanogue 1996). Overall, the results suggest that a decrease in the proportion of granular cells in spiny lobsters is indicative of decreased health status.

Reductions in hemocyte levels have been observed in a number of conditions including starvation (Stewart *et al.* 1967; Evans *et al.* 1999b) and infections (Stewart 1975; Johnson 1976; Field & Appelton 1995). Both of these responses were could explain differences in total hemocyte counts seen in this study, the low levels of THC in the unfed and the control lobsters reflecting a lack of adequate nutrition and the tendency for THC to decrease in lobsters with systemic inflammatory disease being suggestive of a response to bacterial infections or some other form of tissue injury resulting in the removal of hemocytes from the circulation. The reduction in THC in unfed lobsters is in agreement with previous findings of lack of adequate nutrition on circulating hemocyte counts (Stewart *et al.* 1967; Norton *et al.* 2001) and suggests that this parameter may be usefully applied in assessments of dietary regimes for this lobster species. No significant differences in other immune parameters were seen between the lobsters fed different diets, suggesting that these measures have limited application in assessment of response to diets in J. edwardsii. However, the post-harvest stressors to which the lobsters were exposed prior to hemolymph sampling may have masked differences in responses in lobsters fed different diets. Further studies should be performed on the efficacy of immune parameters in addition to THC in the assessment of lobster nutrition.

Some differences were observed in the levels of immune parameters in *J. edwardsii* compared to published values for another spiny lobster species, *Panulirus cygnus* (Jussila *et al.* 1997; Fotedar *et al.* 2001). The results suggest that *J. edwardsii* may have different levels of immune parameters and a different pattern of response of these parameters to air exposure but these findings should be confirmed with a larger sample size of lobsters and with treatment groups subjected to the same acclimatisation procedure prior to testing.

THC was shown to increase in all treatment groups following live transport, the final level being similar in all groups, regardless of the circulating numbers prior to transport. One explanation for these observations is that dehydration led to a decrease in total hemolymph volume and an apparent increase in hemocyte density. Dehydration may have had some influence on the THC levels after live transport but the rise in at least two of the treatment groups, control and unfed platform, was much higher than could be explained by tissue dehydration. Furthermore, prolonged air exposure in *P. cygnus* does not lead to a rise in THC (Fotedar *et al.* 2001). The increase in THC observed in this study may have been a response to the increased levels of bacteremia in lobsters following live transport.

An alternative explanation is that the increase in THC resulted from a delayed alarm stress reaction to handling. Previous studies with *P. cygnus* (Evans *et al.* 2000b) and freshwater crayfish (Hamann 1975; Evans *et al.* 1999a,b; Jussila *et al.* 1999) have shown that these crustacean species experience an alarm reaction to physical handling which is characterised by a rapid increase in the number of circulating hemocytes. The rise in THC following physical handling in *P. cygnus* and *Cherax tenuimanus* is a rapid response, occurring within two hours. If the rise in THC twenty four hours after stress exposure in *J. edwardsii* was an alarm reaction, it would appear that dynamics of immune parameter responses to post-harvest stressors in *J. edwardsii* differ from those of *P. cygnus*. Further studies are required to determine normal ranges for immune parameters in *J. edwardsii* and the pattern of response of immune parameters to post-harvest stressors.

While the mean clotting times showed no significant change following live transport, the prevalence of lobsters with impaired clotting tended to increase following live transport and was high at both sampling points in the raceway treatment group. An increase in prevalence of impaired clotting in lobsters exposed to extreme post-harvest stressors has also been observed with *P. cygnus* (unpublished data). These findings suggest that this parameter may have application in the assessment of transport stress in post-harvest lobsters. The presence of three animals with impaired clotting in the control group was surprising. Since the control animals were not fed during the two weeks storage in the processing tanks the impaired clotting reaction seen in these animals could be a reflection of poor nutrition. Starvation has been shown to reduce hemolymph protein levels in *Homarus americanus* (Stewart *et al.* 1967) and an inverse linear relationship was demonstrated between clotting time and hemolymph protein concentration in the same lobster species (Stewart *et al.* 1966). Further investigations on the application of the clotting time assay in the assessment of lobster health are warranted.

Lobsters sampled prior to air transport had a very low level of bacteremia (4.2%) but this level increased to 40% following transport and storage. Some of this increase probably resulted from the hemolymph sampling process providing a portal of entry for bacterial invasion. The appearance of the histopathological lesions in heart tissues of 10 of the animals examined was suggestive of an inflammatory reaction to a needle prick injury. However, the features also resembled those seen in moribund *P. cygnus* in which prior hemolymph sampling had not been performed (unpublished observations). Furthermore, similar increases in bacteremia have recently been reported for *P. cygnus* following air exposure under simulated live transport conditions (Tsvetnenko *et al.* 2001) and under laboratory conditions (Fotedar *et al*. 2001), both studies where prior sampling of lobsters was not performed. The results suggest that wounding is not the only cause of bacteremia in post-harvest lobsters and that other processes contribute to the invasion of the hemolymph by bacteria.

The isolation of bacteria from hemolymph cultures may be the result of stress reactions rather than a disease condition (Johnson 1976). Asymptomatic bacteremia, typified by the presence of gram-negative bacterial species in hemolymph cultures of apparently healthy animals, has been reported from various crustacean species including freshwater crayfish (Scott & Thune 1986; Wong *et al.* 1995) and crabs (Tubiash *et al.* 1975; Welsh & Sizemore 1985). However, bacteremia has also been widely reported from lobsters with bacterial disease including gaffkemia and vibriosis

(see review, Evans *et al*. 2000a) and was present in post-harvest blue crabs exhibiting increased mortality during moulting (Johnson 1976). A surprising finding was the absence of bacteremia in seven of the eleven lobsters showing histopathological evidence of a systemic inflammatory disease condition. The histopathological features seen in these lobsters were similar to, although less severe, than those reported for crabs and lobsters with systemic bacterial infections (Johnson 1976; Johnson *et al.* 1981). Either the lesions were not of bacterial origin or, as has been previously suggested, the mechanism for clearing bacteria from crustacean hemolymph is highly efficient (Smith & Ratcliffe 1980; White & Ratcliffe 1982).

The level of survival seen in this study was below industry average for *P. cygnus* (Spanoghe & Bourne 1997) and, presumably, *J. edwardsii*. The poor survival after live transport suggests that the degree of post-harvest stress to which lobsters were exposed was excessive compared to industry practices. The combination of systemic pathological lesions and low granular cell values suggests that all the lobsters in the systemic group would have been moribund at examination. While the cause of morbidity in these lobsters cannot be precisely identified, the presence of muscle and hepatopancreatic lesions suggestive of bacterial disease in six of lobsters in the systemic group suggests that opportunistic bacterial infections probably contributed to morbidity in these animals. Morbidity in other lobsters without these pathological reactions probably resulted from cell injury due to gross physiological disturbances such as acidosis or exposure to high ammonia levels, both characteristic physiological features of lobsters exposed to extreme adverse environmental conditions (Whiteley & Taylor 1992; Schmitt & Uglow 1997).

A major finding of this analysis was the identification of a different pattern of responses to exposure to live transport stressors by different treatment groups. The hard pellet-raceway group had a very low prevalence of lobsters with systemic lesions, a high mean proportion of granular cells and no mortalities. This pattern indicates that this group of lobsters were least affected by exposure to post harvest stressors. The unfed group on the other hand had the opposite pattern – the highest prevalence of lobsters with systemic lesions, a low level of %granular cells and 30% mortality. This group was likely to be the least healthy at the commencement of the trial, having been starved for several months, and therefore likely to have the lowest stress resistance.

Overall the results provide evidence of the validity of application of health indices in the investigation of live transport procedures for spiny lobsters. Measurements of THC, %granular cells, clotting time, %prevalence of impaired clotting, %prevalence of bacteremia and quantitative histopathology on a sample of 10 or more lobsters should enable differentiation of response to different treatment procedures as well as provide a measure of initial health status of lobsters used in trials of different procedures. If combined with measurement of protein and pH levels in lobster blood information on the likely cause of loss of health status following live transport could also be obtained.

While the indices used in this study were of value in differentiating the health status of different groups of lobsters, other measures of lobster health such as phenoloxidase levels and agglutination tests (Norton *et al.* 2001) should also be further investigated. Such indices would have application in improving lobster live transport technology, in

health monitoring during long term monitoring or aquaculture of lobsters and in resource management of lobster fisheries.

ACKNOWLEDGEMENTS

This investigation was kindly supported by the Fisheries Research and Development Corporation and Curtin University of Technology. We would also like to thank Ross McGregor, Lobster Australia Limited, for his assistance in the development of lobster health indices.

REFERENCES

Bachère, E., Mialhe, E., Noël, D., Boulo, V., Morvan, A. & Rodriguez, J. (1995) Knowledge and research prospects in marine mollusc and crustacean immunology. *Aquaculture* , **132**, 17-32.

Bancroft, J. D. & Stevens, A. (1977) Theory and practice of histological techniques. Churchill Livingstone, Edinburgh.

Chisholm, J. R. S. & Smith, V. J. (1995) Comparison of antibacterial activity in the hemocytes of different crustacean species. *Comparative Biochemistry and Physiology*, **110A**, 39-45.

Evans, L. H. (2000) Copper toxicity study in the freshwater crayfish, Cherax tenuimanus. In: *Abstract Proceedings 13th Biennial Symposium of the International Association of Astacology*, (Ed. by G. Whisson), p22. (abstract).

Evans, L.H., Fan, A., Finn, S.P., Dawson, S.A., Siva, C.J. and Lee, I.R. (1992). Nutritional status assessment studies in the freshwater crayfish, *Cherax tenuimanus*. In: *Proceedings of the Aquaculture Nutrition Workshop*, (Ed. by G. L. Allen and W. Dall), pp. 87-91. NSW Fisheries, Australia.

Evans L. H., Fotedar, S., Fan, A. & Jones, B. (1999a) Investigation of idiopathic muscle necrosis and circulating hemocytes in the freshwater crayfish *Cherax tenuimanus* exposed to acute and chronic stressors. *Freshwater Crayfish*, **12**, 356-70.

Evans, L., Paganini, M., Fan, A., Fotedar, S., Fotedar, R. & Jussila, J. (1999b) Acute and chronic stress studies in freshwater crayfish. *AQUA 1999, Proceedings Annual International Conference and Exposition of the World Aquaculture Society*, Sydney, Australia. p. 249.

Welsh, P. C. & Sizemore, R. K. (1985) Incidence of bacteremia in stressed and unstressed populations of the blue crab, *Callinectes sapidus*. *Applied and Environmental Microbiology*, **50**(2), 420-25.

Evans, L. H., Jones, J. B. & Brock, J. A. (2000a) Diseases of spiny lobsters. In: Spiny Lobsters: Fisheries and Culture, 2nd edn. (Ed. by B. F. Phillips & J. Kittaka), pp. 586 – 600. Fishing News Books. Blackwell Science Ltd, Oxford.

Evans, L., Chan, V., Fotedar, S., Jussila, J. & Tsvetnenko, E. (2000b) Influence of environmental stressors on immunocompetence in the spiny lobster *Panulirus cygnus*: Implications for lobster aquaculture. In: *AQUA 2000. Responsible Aquaculture in the New Millennium* (Ed. by R. Flos & L. Creswell), p. 206. European Aquaculture Society Special Publication, 28, Oostende, Belgium. (abstract)

Field, R. H. & Appelton, P. L. (1995) A hematodinium-like dinoflagellate infection of the Norway lobster *Nephrops norvegicus*: observations on pathology and progression of infection. *Diseases of Aquatic Organisms*, **22**, 115-28.

Fotedar, S., Tsvetnenko, E. & Evans, L. (2001) Effect of air exposure on immune parameters of the rock lobster *Panulirus cygnus*. *Marine and Freshwater Research* (in press).

Hagerman, L., Sondergaard, T., Weile, K., Hosie, D., & Uglow, R. F. (1990) Aspects of blood physiology and ammonia excretion in *Nephrops norvegicus* under hypoxia. *Comparative Biochemistry and Physiology,* **97A**, 51-5.

Hose, J. E. & Martin, G. G. (1989) Defense functions of granulocytes in the Ridgeback prawn *Sicyonia ingentis*, *Journal of Invertebrate Pathology*, **53**, 335-46.

Johnson, P. T. (1976) Bacterial infection in the blue crab, *Callinectes sapidus*: course of infection and histopathology. *Journal of Invertebrate Pathology*, **28**, 25-36.

Johnson, P. T., Stewart, J. E. & Arie, B. (1981) Histopathology *of Aerococcus viridans* var.*homari* infection (Gaffkemia) in the lobster, *Homarus americanus*, and a comparison with histological reactions to a Gram-negative species*, Pseudomonas perolens. Journal of Invertebrate Pathology*, **38**, 127-48.

Jussila, J., Jago, J., Tsvetnenko, E., Dunstan, R. & Evans, L. H. (1997) Total and differential haemocyte counts in western rock lobster (*Panulirus cygnus* George) under post-harvest stress. *Marine and Freshwater Research*, **48**, 863-7.

Jussila, J., Paganini, M., Mansfield, S. & Evans, L. H. (1999) On physiological responses, hemolymph glucose, total hemocyte count and dehydration of marron (*Cherax tenuimanus*) to handling and transportation under simulated conditions. *Freshwater Crayfish*, **12,** 154-67.

McLeese, D. W. (1965) Survival of lobsters, *Homarus americanus,* out of water. *Journal of the Fisheries Research Board of Canada* **22,** 385-394.

McMahon, B. R., Butler, P. J. & Taylor, E. W. (1978) Acid base changes during recovery from disturbance and during long term hypoxic exposure in the lobster, *Homarus vulgaris*. *Journal of Experimental Zoology,* **205**, 361-70.

Norton, J. H., Levy, N. and Field, K. (2001) A preliminary evaluation of three haemolymph tests to assess health status in tropical rock lobsters Panulirus ornatus. In: *Proceedings International Symposium on Lobster Health Management* (Ed. by L. H. Evans & J. B. Jones), Muresk Institute of Agriculture, Curtin University Publication (in press).

Persson, M., Cerenius, L. & Söderhäll, K. (1987) The influence of hemocyte number on the resistance of the freshwater crayfish, *Pacifastacus lenuisculus* Dana, to the parasitic fungus *Aphanomyces astaci. Journal of Fish Diseases*, **10**, 471-7.

Ravindranath, M. H. (1974) The hemocytes of an isopod *Ligia exotica* Roux. *Journal of Morphology*, **144**, 11-22.

Riley, J., Donahue, D., Ozbay, G., Bayer, R. (1997) Shipping and handling of live lobsters (*Homarus americanus*). In: *Marketing and Shipping Live Aquatic Products* , pp.73-76. Proceedings of Symposium on Marketing and Shipping Live Aquatic Products, Seattle.

Schmitt, A. S. C. & Uglow, R. F. (1997) Haemolymph constitutent levels and ammonia efflux rates of *Nephrops norvegicus* during emersion. *Marine Biology*, **127**, 403-10

Scott, J. R. & Thune, R. L. (1986) Bacterial flora of hemolymph from red swamp crawfish, *Procambarus clarkii* (Girard), from commercial ponds. *Aquaculture*, **58**, 161-5.

Sequeira, T., Tavares, D. & Arala-Chaves, K. (1996) Evidence for circulating hemocyte proliferation in the shrimp *Penaeus japonicus*. *Developmental and Comparative Immunology*, **20**, 97-104.

Smith, V.J. & Ratcliffe, N. A. (1980) Host defence reactions of the shore crab, *Carcinus maenas* (L.): clearance and distribution of injected test particles. *Journal of the Marine Biological Association of the U.K.,* **60**, 89-102.

Smith, V. J., Swindlehurst, R. J., Johnson, P. A. & Vethaak, D. A. (1995) Disturbance of host defense capability in the common shrimp, *Crangon crangon*, by exposure to harbour dredge spoils, *Aquatic Toxicology*, **32**, 43-58.

Söderhäll, K. & Cerenius, L. (1992) Crustacean Immunity. *Annual Reviews of Fish Diseases.* Pergamon Press Ltd, USA, pp3-23.

Söderhäll, K. & Smith, V. J. (1983) Separation of the hemocyte populations of *Carcinus maenas* and other marine decapods and prophenoloxidase distribution. *Developmental and Comparative Immunology*, **7**, 229-39.

Spanoghe, P. T. (1996) *An investigation of the physiological and biochemical responses elicited by Panulirus cygnus to harvesting, holding and live transport*. PhD thesis, Curtin University of Technology, Perth, Western Australia.

Spanoghe, P. T. & Bourne, P. K. (1997) Relative influence of environmental factors and processing techniques on *Panulirus cygnus* morbidity and mortality during simulated live shipments. *Marine and Freshwater Research,* **48,** 839-44.

Spicer, J. J., Hill, A. D., Taylor, A. C. & Strang, R. H. C. (1990) Effect of aerial exposure on concentrations of selected metabolites in blood of the Norwegian lobster *Nephrops norvegicus* (Crustacea: Nephropidae). *Marine Biology*, **105**, 129-35.

Stevens, R. Turner, D. & Whisson, G. (Eds) (1995) *A Code of Practice for Handling Live Rock Lobster*. Western Australian Fishing Industry Council, Perth, Western Australia.

Stewart, J. E. (1975) Gaffkemia, the fatal infections of lobsters (genus *Homarus*) caused by *Aerococcus viridans* (var.) *homari*: a review. *Marine Fisheries Reviews*, **37**, 243-54.

Stewart J. E., Dingle, J. R. & Odense, P. H. (1966) Constituents of the hemolymph of the lobster *Homarus americanus* Milne Edwards. *Canadian Journal of Biochemistry*, **44**, 1447-59.

Stewart, J. E, Cornick, J. W., Foley, D. M., Li, M. F. and Bishop, C. M. (1967) Muscle weight relationship to serum proteins, hemocytes, and hepatopancreas in the lobster, *Homarus americanus*. *Journal of the Fisheries Research Board of Canada*, **24**, 2339-54.

Sugita, H. and Deguchi, Y. (1994) Shipping. In: *Spiny Lobster management: current situation and Perspectives*. (Ed. by B. F. Phillips, J. S. Cobb and J. Kittaka), pp.503-9. Blackwell Scientific, Oxford.

Taylor, H. H. & Waldron, F. M. (1997) Respiratory responses to air-exposure in the southern rock lobster, *Jasus edwardsii* (Hutton) (Decapoda: Palinuridae). *Marine and Freshwater Research,* **48,** 889-97.

Taylor, E.W. & Whiteley, N. M. (1989) Oxygen transport and acid-base balance in the haemolymph of the lobster, *Homarus gammarus*, during aerial exposure and resubmersion. *Journal of Experimental Biology,* **144**, 417-36.

Tod, P. & Spanoghe, P. T. (1997) *Development of improved onshore storage and transportation protocols for the western rock lobster Panulirus cygnus*. Project Report 94/134.06. Fisheries Research and Development Corporation, Canberra, Australia.

Trump, B. F. & Arstila, A. V. (1975) Cellular reaction to injury. In: Principles of Pathobiology 2nd Ed. (Ed by M. F. LaVia & R. B. Hill), pp.9-96. Oxford University Press, New York.

Tsing, A., Arcier, J. & Brehelin, M. (1989) Hemocytes of penaeid and palaemonid shrimps: morphology, cytochemistry and hemograms. *J. Invertebrate Pathology*, **53**, 64-77.

Tsvetnenko, E., Fotedar, S. & Evans, L. H. (2001) Investigations of antibacterial activity in the spiny lobster, *Panulirus cygnus*. *Marine and Freshwater Research* (in press).

Tubiashi, H. S., Sizemore, R. K. and Colwell, R. R. (1975) Bacterial flora of the hemolymh of the blue crab, *Calinectes sapidus*: most probable numbers. *Applied Microbiology*, **29**, 388-392.

Welsh, P. C. & Sizemore, R. K. (1985) Incidence of bacteremia in stressed and unstressed populations of the blue crab, *Callinectes sapidus*. *Applied and Environmental Microbiology*, **50**, 420-25.

White, K. N. & Ratcliffe, N. A. (1982) The segregation and elimination of radiolabelled and fluorescent-labelled marine bacteria from the haemolymph of the shore crab, *Carcinus maenas* (L). *Journal of the Marine Biological Association of the U. K.*, **62**, 819-33.

Whiteley, N. M. & Taylor, E. W. (1992) Oxygen and acid disturbances in the hemoloymph of the lobster. *Hommarus gammarus during commercial transport and storage. Journal on Crustacea Biology*, **12**, 19-30.

Whiteley, N. M., Al-Wassia, A. H. & Taylor, E. W. (1990) The effect of temperature, aerial exposure and disturbance on oxygen consumption in the lobster, *Homarus gammarus,* (L). *Marine Behaviour and Physiology,* **17,** 213-22.

Winkler, P. (1987) Effects of handling on the in situ oxygen consumption of the American lobster. *Homarus americanus. Comparative Biochemistry and Physiology,* **87A,** 69-71.

Wong, F. Y. K., Fowler, K. & Desmarchelier, P. M. (1995) Vibriosis due to *Vibrio mimicus* in Australian freshwater crayfish. *Journal of Aquatic Animal Health*, **7**, 284-91.

Wood, C. M., Turner, J.D. & Graham, M. S. (1983) Why do fish die after severe exercise? *Journal of Fish Biology,* **22,** 189-201.

APPENDIX 9

CASE STUDY 5
EFFECT OF AIR EXPOSURE ON IMMUNE PARAMETERS OF THE ROCK LOBSTER *Panulirus cygnus*

S. Fotedar, E. Tsvetnenko and L.H. Evans

Aquatic Science Research Unit, Muresk Institute of Agriculture,
Curtin University of Technology, GPO Box U1987, Perth, Western Australia 6845

(Reprinted with permission from Marine and Freshwater Research (2000) Vol 51)

EFFECT OF AIR EXPOSURE ON IMMUNE PARAMETERS OF THE ROCK LOBSTER *Panulirus Cygnus*

Seema Fotedar, Elena Tsvetnenko and Louis Evans

Aquatic Science Research Unit, Muresk Institute of Agriculture, Curtin University of Technology, GPO Box U1987, Perth, Western Australia 6845;
fotedars@ muresk.curtin.edu.au

ABSTRACT

Live export of lobsters involves a series of post harvest operations which may have an adverse effect on lobster health. These effects can be assessed through measurement of selected immune parameters such as total hemocyte counts, hemolymph clotting time, bacteremia and the differential proportion of granular cells in lobster hemolymph. One of the main environmental stressors in the post harvest process is air exposure. This study aimed to determine the effect of air exposure on the health status of lobsters using immune parameters as indicators.

Lobsters were held in factory holding tanks for three days without feeding and then exposed to air for 2, 6, 12, and 18 h through placement in foam boxes. Controls were sampled at each time point by collecting lobsters from the same tank as the experimental animals. Experiments were conducted on adult, intermoult or early premoult, animals (10 animals /treatment group) with a different group of lobsters being sampled at each time point.

Air exposure caused a significant increase in clotting time at 12 and 18 h whereas the total hemocytes showed a decreasing trend, both indicative of a decrease in immuno-competence. The proportion of granular cells tended to be lower in air exposed animals compared to controls at three of the four time points and this difference was significant at 2 h exposure. In the air-exposed lobsters, the bacteremia levels tended to be higher than those in control animals at all time points and the difference was significant at 12 h exposure. As granular cells are a major component of the phagocytic system, low granular cell numbers and high bacteremia levels implied increased susceptibility to infection and lowered immunity. The results show that air exposure has a significant adverse effect on the immune system and hence on the health status of the lobsters.

Keywords: immune parameters, hemocytes, lobster, immunity, bacteremia

I. INTRODUCTION

Western rock lobster, *Panulirus cygnus* forms a valuable single species fishery in Western Australia. The product is sold in various forms, with live export being a major marketing approach. Live export requires rigorous selection of healthy lobsters that can survive aerial transport over long distances as well as retain good condition. Loss of condition may be accompanied by deterioration in flavor or texture (Paterson

& Spanoghe, 1997). The journey to the end of the marketing chain involves several post harvest operations, such as airfreight and handling, that are likely to act as stressors affecting health and condition. While a variety of crustaceans such as shore crab, *Carcinus maenas* and freshwater crayfish, *Austropotamobius pallipes* exhibit facultative air breathing (Taylor, 1982), palinurid lobsters are sublittoral organisms and stranding in air is extremely rare in natural populations. Thus, specific respiratory adaptations to such events are unlikely (Taylor & Waldron, 1997).

An understanding of the various changes occurring during exposure of lobsters to air is important to maintaining condition during commercial post harvest processing and live transport. Though several studies have examined the effect of aerial transport on physiology and survival of different crustaceans (McLeese, 1965; Winkler, 1987; Spicer *et al*., 1990; Whiteley & Taylor, 1992; Goodrick *et al*., 1993), few investigations have studied its impact on immuno-competence in rock lobsters. The immune system of crustaceans comprises cellular and humoral factors, implicated in functions such as phagocytosis, extracellular lysis, encapsulation and coagulation. The ability to carry out these functions at optimum capacity is influenced by environmental factors and stressors. Exposure to stressors leads to short and long term changes in immune parameters. Hamann (1975) observed an increase in circulating hemocytes in freshwater crayfish following handling stress. In a study on *Homarus americanus*, Stewart *et al*. (1967), showed decline in total hemocyte counts (THC) following starvation. Evans *et al*. (1999) reported significant alterations in a range of immune parameters in freshwater crayfish following exposure to acute and chronic stressors. In the present study the effect of air exposure on the health status was assessed using various immune parameters as indicators.

II. MATERIAL AND METHODS

Experimental animals

Adult western rock lobsters were obtained from a commercial supplier and held in a recirculating sea water system (temperature 15^{o}C) for three days without feeding prior to the commencement of the experiment. A total of 90 animals in the size range of 500-700g were sampled.

Experimental protocol

Lobsters were collected from holding tanks and exposed to air by placement in foam boxes for 2, 6, 12 and 18h duration. Controls were sampled at each time point by collecting lobsters from the same tank as experimental animals. Each group consisted of 10 animals with a different group of lobsters being sampled at each time point. Each lobster was assessed for moult stage *(*as per *Lyle & MacDonald, 1983)*, tail erosion and blisters.

Total and differential hemocyte counts

The base of 5th thoracic leg was cleaned with 70% alcohol. A 0.2ml aliquot of hemolymph was withdrawn into a 1ml sterile syringe containing 0.2ml of anticoagulant (1% glutaraldehyde in sodium cacodylate) and dispensed into an Eppendorf tube kept on ice. Total hemocyte count for individual lobsters were estimated with a haemocytometer under x100 magnification, using the anticoagulant

/hemolymph mixture. Cells were counted in both the grids and the mean used as hemocytometer count. The total hemocyte count was calculated as THC = (cells counted x dilution factor x 1000)/ volume of grid ($0.1mm^3$).

Differential hemocyte counts (DHC) were carried out as follows. One drop of hemolymph /anticoagulant mixture was placed on a slide and smeared. The smear was air dried and fixed in 70% methanol for 10min. Fixed smears were stained with May-Grunwald and Giemsa (10 min. in each) (Bancroft & Stevens, 1977) and mounted with coverslips. A total of 200 cells were counted on each slide. The criterion for identification of hemocytes was consistent with previously published information (Bauchau, 1981; Hose *et al.*, 1990). The granular cells were distinguished on the basis of large cell size, small pale nucleus and large number of eosinophillic granules in the cytoplasm and their proportion determined.

The mean values of THC and granular cells for treatment groups at each time interval were compared by one way ANOVA and individual pairs were compared using least significant difference. T-tests were used to determine differences between treatment groups at each time interval.

Bacteremia assessment

A 0.3ml aliquot of hemolymph was withdrawn into a sterile syringe. Five drops were quickly placed on marine saline agar plate, the plate carefully inverted and kept at room temperature (20- 22^0C) for up to 5 days. The number of colony forming units (CFU) were counted for each drop and CFU/ml calculated for each sample based on a total volume of 20 µl for each drop. The CFU/ml were ranked 1 (1-250 CFU/ml) to 12 (2751-3000 CFU/ml) with a final rank of 13 assigned to those samples in which the colonies were too numerous for an accurate count. Data were analysed using Kruskal –Wallis test for within treatment differences and Mann-Whitney test was used to compare treatment groups at each time interval.

Hemolymph clotting time

Hemolymph withdrawn into a sterile syringe was dispensed into an Eppendorf tube. A 30µl aliquot was quickly transferred into another tube and drawn into a capillary tube. The tube was repeatedly inverted until the hemolymph stopped moving and the time was noted. If the clot was not formed at the end of 90 seconds, it was recorded as ‘no clot’. The clotting times were ranked 0 (0-10 seconds) to 9(>90 seconds) to provide numerical data accounting for cases where no clot was observed in the 90 seconds. Data was analysed using Kruskal –Wallis test for within treatment differences and Mann-Whitney test was used to compare treatment groups at each time interval.

Data was processed with the aid of SPSS/PC+. The criterion for statistical significance was $P< 0.05$. Results are presented as mean ± s.e. unless otherwise stated.

III. RESULTS

All the lobsters belonged to intermolt (C) or early premolt (D0) stage and were suitable for the experiment. Slight tail erosion and a few tail blisters were observed in both the test and control groups of lobsters sampled. The THC at the start of the experiment was 6.65 ± 0.64 x 10^6 cells/ml (n=10). In the control group, the THC at 18 h was significantly lower than values at other time points (ANOVA, df=3, P = 0.001). The mean values for air exposed lobsters also showed a decreasing trend, but the change was not significant (ANOVA, df = 3, P = 0.59). There were no significant differences in hemocyte counts between control and air exposed lobsters at any time point (Fig. 1).

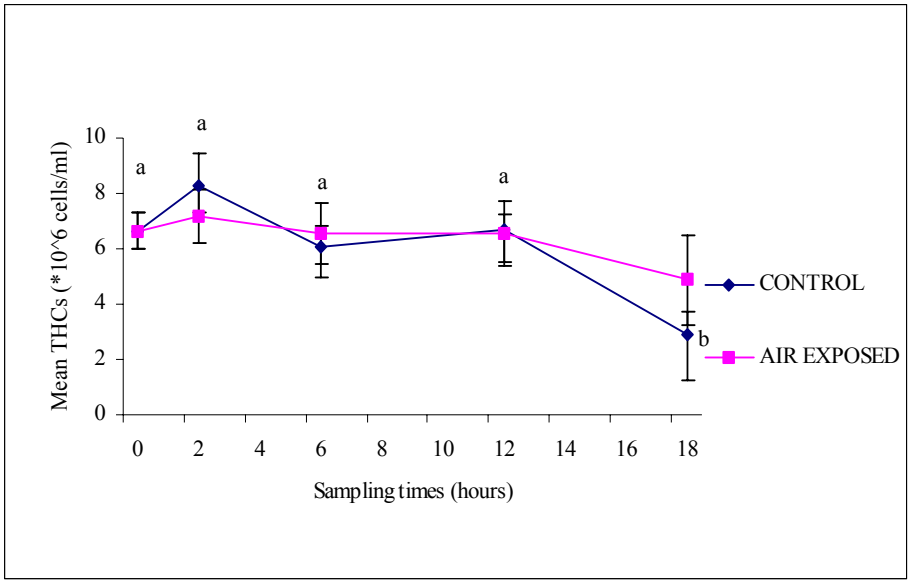


Figure 1 Mean total hemocyte counts for the air- exposed and control groups of western rock lobsters. *Different letters denote significant differences in mean values for the control groups (ANOVA, P<0.05).

The mean proportion of granular cells at the start of the experiment was 11.40 ± 2.06% (n=10). The mean values of granular cells in the control group varied at different time points and the18h mean value was significantly different to the 2h and 12h values (ANOVA, df =3, F =2.097, P = 0.11). The mean values for granular cells displayed an increasing trend in the air exposed lobsters but the differences were not significant (ANOVA, df = 3, F = 1.0, P =>0.40). The mean percent granular cells were significantly different, between control and air exposed animals, following 2h exposure (t = 2.760, df = 18, P <= 0.01) (Fig. 2).

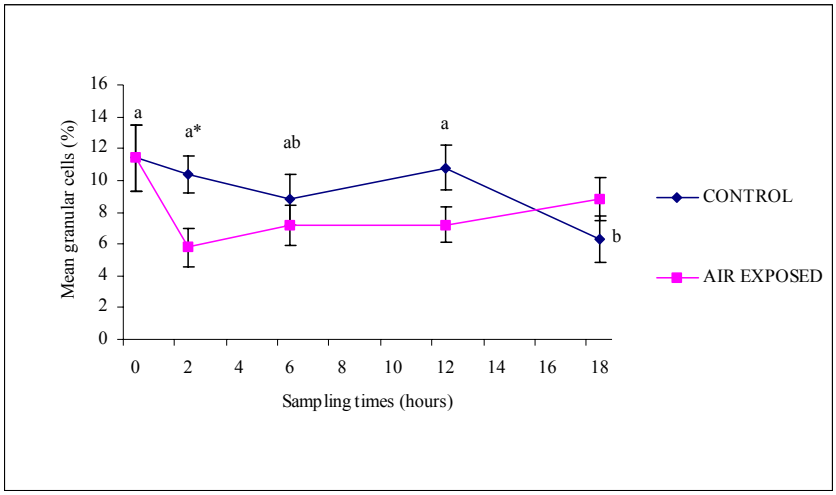


Figure 2 Mean percent granular cells for the air- exposed and control groups of western rock lobsters. *Different letters denote significant differences in mean values for the control groups (ANOVA, P<0.05). (*) denotes significant differences in mean values at the marked sampling point (t test, P<0.05).

The mean clotting rank at the start of the experiment was 4.0 ± 0.45 (n=10). The clotting ranks at different sampling times did not differ significantly in the control group (Kruskal-Wallis test, chi-square = 6.849, df = 3, P = 0.077), however, the differences were significant for the air exposed groups (Kruskal-Wallis test, chi-square = 19.800, df = 3, P = 0.000). Short-term exposure did not cause a significant change in clotting times. However, longer exposure of 12 and 18h caused a significant increase in clotting time in air-exposed animals ((z = -2.119, P = 0.03) & (z = -2.397, P = 0.01) respectively) compared to controls (Fig. 3).

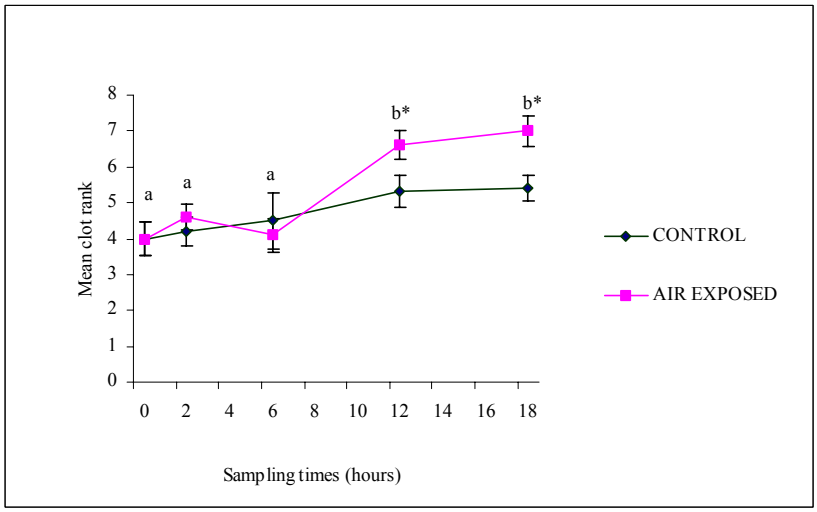


Figure 3 Mean clotting ranks for the air- exposed and control groups of western rock lobsters. *Different letters denote significant differences in mean values for air exposed lobsters (Kruskal – Wallis test, P< 0.05). (*) denotes significant differences in mean values at the marked sampling point (Mann-Whitney test, P<0.05).

The mean hemolymph bacteremia rank at the start of the experiment was 0.40 ± 0.16 and remained low in all control groups of lobsters (Kruskal Wallis test, chi-square = 3.702, df = 3, P = 0.295). The air-exposed animals had higher bacteremia levels but there were no significant differences within the treatment group (Kruskal Wallis test, chi-square = 6.422, df = 3, P => 0.09). The difference between air-exposed and control animals was significant after 12h exposure (z = -3.045, P<=0.002) and remained high after 18h exposure (z = -1.938, P =>0.05) (Fig. 4).

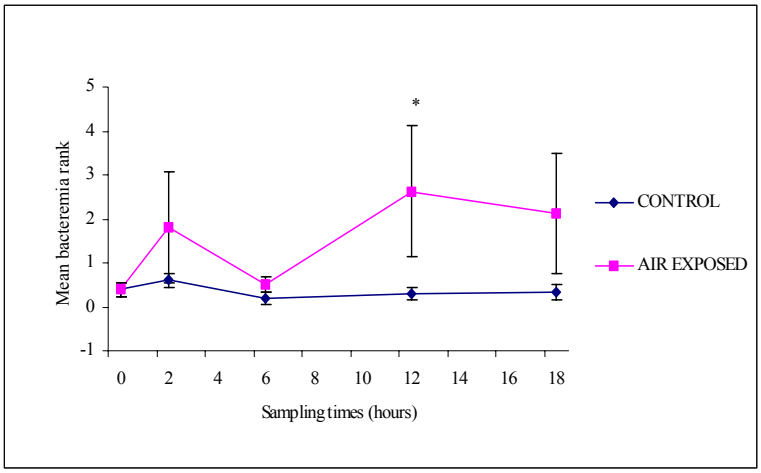


Figure 4 Mean bacteremia rank for the air-exposed and control groups of western rock lobsters. (*) denotes significant differences in mean values at the marked sampling point (Mann-Whitney test, P<0.05).

IV. DISCUSSION

The major findings of this study were that clotting time, the differential proportion of granular cells and the level of bacteremia in *Panulirus cygnus* showed some signs of being altered following air exposure. Clotting time and bacteremia levels were elevated and % granular cells tended to be lower in the air exposed lobsters compared to submerged controls. The findings are suggestive of a decrease in immuno-competence following prolonged air exposure and a consequent decline in health status.

The health status of an organism is a reflection of both its environment and the natural ability to overcome adverse conditions and potential enemies. This natural ability is a function of the immune system and can be assessed by measurement of selected immune parameters. Various environmental factors have been reported to affect hemocyte numbers or the proportion of different subtypes. These include sex, growth, moulting, starvation and parasites (Stewart *et al*., 1967; Ravindranath, 1974; Evans *et al*., 1992; Sequeira *et al*., 1995). Persson *et al.* (1987) found that the number of circulating hemocytes decreased dramatically following injection of foreign particles, leading to increased susceptibility to infection in freshwater crayfish (*Pacifastacus leniusculus*). Moribund lobsters were shown to have low THC in comparison to healthy lobsters in factory tanks (Jussila *et al*., 1997). Bacterial infections and starvation were shown to cause a decline in total hemocyte counts in *Homarus americanus* and in the blue crab, *Carcinus maenas* (Stewart *et al*., 1967 & Johnson,

1976 respectively). Sequeira *et al*. (1996) demonstrated that shrimp hemocytes had the capacity to proliferate following mitogenic stimulation. In this study the THC did not show any significant change following air exposure.

The proportion of granular cells was significantly lower in air exposed animals at 2h exposure and tended to remain lower than the control, but not significantly, except following 18h exposure. These changes could be associated with the change in hemocyte counts. Changes in differential counts could also be caused by changes in hematopoiesis (Sequeira *et al*. 1996). Bacteremic lobsters are known to show changes in the relative proportion of each type of hemocyte (Durliat & Vranckx, 1983). Jussila *et al*. (1997) observed the proportion of granular cells to be lower in moribund than in healthy group of lobsters. As granular cells are believed to be the main phagocytic cells in decapod crustaceans (Hose *et al*., 1990; Martin *et al*., 1996), the low values may be associated with increased phagocytosis and such cells becoming sequestered in tissues as observed by Cornick & Stewart (1968a).

Clotting is a primary mechanism of host defense against foreign organisms. Clotting involves cellular and plasma interaction. Many foreign particles that invade hemolymph can impair clotting ability (Durliat & Vranckx, 1983). Elevated clotting times can result from decline in total number of hemocytes or from the presence of increased number of damaged hemocytes associated with increased prevalence of bacteria (Durliat & Vranckx, 1983). High clotting times indicate a decreased ability to confine pathogens, pointing to lowered immune status.

Aquatic animals are in contact with a great number of bacteria in the water, sediment and/or food and hence harbor bacteria as temporary residents. Pathogenic bacteria colonize gills and gut of healthy crustaceans and may cause opportunistic infectious diseases when self defense mechanisms are suppressed during live transport (Sugita & Deguchi, 1994). The prevalence of bacteria in the hemolymph of stressed but apparently healthy crustaceans has been reported by other workers (Tubiash *et al*., 1975; Welsh & Sizemore 1985). Our results suggest that air exposure, even for short time periods, is a stressor that induces this condition. The high bacteremia levels are also suggestive of a decline in immune capacity and a likely increased susceptibility to infection.

Although the oxygen content of air is much higher than that of water, the gill structure of lobsters is not conducive to air breathing. Initially on emersion, the gills retain a film of water causing the filaments to clump by surface tension (Taylor & Waldron, 1997). This clumping results in impairment of gill function leading to internal hypoxia and hypercapnia (Taylor, 1982). As oxygen levels fall, lactic acid accumulates, pH falls and ammonia levels rise (Schmitt & Uglow,1997a). The animal responds by compensatory processes such as anaerobic respiration to maintain homeostasis (Taylor & Whiteley, 1989). Due to lowered energy production, other systems such as the immune system are likely to be compromised.
Aquatic animals such as lobsters are well adapted to life in water. Exposure to air creates a shock for the body systems and hence health. The combination of handling and prolonged air exposure is likely to exacerbate the stress reactions. The changes in immune parameters observed in this study *i.e*., high clotting times, low granular cell proportion and high bacteremia, imply that air exposure has an adverse effect on immuno-competence and consequently on the health status of lobsters.

ACKNOWLEDGEMENTS

We would like to thank Lobster Australia Pty Ltd., Fremantle for providing the lobsters and the facilities for this study. We would also like to thank Michael Burgess for technical assistance with the experimentation.

REFERENCES

Bauchau, A. G. (1981). Crustaceans. In '*Invertebrate Blood Cells*. Vol.2'. (Eds N. A. Ratcliffe and A.F. Rowley), pp 385-420. (Academic Press: Sydney.)

Bancroft, J. D. and Stevens, A. (1977). Theory and practice of histological techniques. Churchill Livingstone, Edinburgh.

Cornick, J. W. and Stewart, J. E. (1968a). Interaction of the pathogen, *Gaffkya homari*, with the natural defense mechanisms of *Homarus americanus. Journal of Fisheries Research Board of Canada* **25:** 695-700.

Durliat, M. and Vranckx, R. (1983). Analysis of clotting defects in diseased lobsters –I. Alterations in blood parameters. *Comparative Biochemistry and Physiology* **76A(1)**: 95-101.

Evans, L., Fan, A., Finn, S. P., Dawson, S. A., Siva, C. J. & Lee, I. R. (1992). Nutritional status assessment studies in the freshwater crayfish, *Cherax tenuimanus.* In *Proceedings Aquaculture Nutrition Workshop.* (Ed. by G. L. Allan & W.Dall), p87-91. Salamander Bay, Australia.

Evans, L., Fotedar, S., Fan, A. and Jones, B. (1999). Investigationof the idiopathic muscle necrosis and circulating hemocytes in the freshwater crayfish *Cherax tenuimanus* exposed to acute and chronic stressors. *Freshwater Crayfish* **12**: 356-370

Goodrick, G.B., Paterson, B.D. and Grauf, S. (1993). Air transport of live kuruma prawns (*Penaeus japonicus*) : Temperature control improves survival. *Food Australia* **45 (8)**: 400-403.

Hamann, A. (1975). Stress–induced changes in cell titer of crayfish hemolymph. *Zeitschrist suer Naturoforschung,* **30:** 850

Hose, J.E. and Martin, G.G. (1989). Defense functions of granulocytes in the ridgeback prawn, *Sicyonia ingentis. Journal of Invertebrate Pathology* **53:** 335-346.

Hose, J. E., Martin, G. G. and Gerard, A. S. (1990). A decapod hemocyte classification scheme integrating morphology, cytochemistry and function. *Biological Bulletin* **178:** 33-45.

Johnson, P. T. (1976). Bacterial infection in the blue crab, *Callinectes sapidus*: course of infection and histopathology. *Journal of Invertebrate Pathology* **28**: 25-36.

Jussila, J., Jago, J., Tsvetnenko, E., Dunstan, B. and Evans, L. H. (1997). Total and differential haemocyte counts in western rock lobsters (*Panulirus cygnus* George) under post –harvest stress. *Marine and Freshwater Research* **48**: 863-867.

Lyle, W. G. and MacDonald, C. D. (1983). Molt stage determination in the hawaiian spiny lobster *Panulirus marginatus*. *Journal of Crustacean Biology* **3**(2): 208-216.

Martin, G.G., Hose, J. E., Minka, G. and Rosenberg, S. (1996). Clearance of bacteria injected into the hemolymph of the ridgeback prawn, *Sicyonia ingentis* (Crustacea: Decapoda): role of hematopoietic tissue. *Journal of Morphology* **227**: 227-233.

McLeese, D. W. (1965). Survival of lobsters, *Homarus americanus*, out of water. *Journal of Fisheries Research Board of Canada* **22 (2)**: 385-394.

Paterson, B. D. and Spanoghe, P.T. (1997). Stress indicators in marine decapod crustaceans, with particular reference to grading of western rock lobsters (*Panulirus cygnus*) during commercial handling. *Marine and Freshwater Research* **48**: 829-834

Persson, M., Cerenius, L. and Soderhall, K. (1987). The influence of hemocyte number on the resistance of the freshwater crayfish, *Pacifastacus leniusculus* Dana, to the parasitic fungus *Aphanomyces astaci*. *Journal of Fish Diseases* **10**: 471-477.

Ravindranath, M. H. (1974). The hemocytes of an isopod *Ligia exotica* Roux. *Journal of Morphology* **144**: 11-22.

Schmitt, A.S.C. and Uglow, R.F. (1997a). Haemolymph constituent levels and ammonia efflux rates of *Nephrops norvegicus* during emersion. *Marine Biology (Berlin)* **127**: 403-410.

Sequeira, T., Vilanova, M., Lobo-Da-Cunha, A., Baldaia, L. and Arala-Chaves, M. (1995). Flow cytometric analysis of molt-related changes in hemocyte type in male and female *Penaeus japonicus*. *Biological Bulletin* **189**: 376-380.

Sequeira, T., Tavares, D. and Arala-Chaves, M. (1996). Evidence for circulating hemocyte proliferation in the shrimp *Penaeus japonicus*. *Developmental and Comparative Immunology* **20** (2): 97-104.

Spicer, J.I., Hill, A.D., Taylor, A.C. and Strang, R.H.C. (1990). Effect of aerial exposure on concentrations of selected metabolites in the blood of the Norway lobster *Nephrops norvegicus* (Crustacea: Nephropidae). *Marine Biology (Berlin)* **105**: 129-135.

Stewart, J.E., Cornick, J.W. and Dingle, J.R. (1967). An electronic method for counting lobster (*Homarus americanus* Milne Edwards) hemocytes and the

influence of diet on hemocyte numbers and hemolymph proteins. *Canadian Journal of Zoology* **45**: 291-304.

Sugita, H. and Deguchi, Y. (1994). Shipping. In 'Spiny Lobster Management: Current Situation and Perspectives'. (Eds B.F. Phillips, J. S. Cobb and J. Kittaka), pp. 503-509. (Blackwell Scientific: Oxford)

Taylor, E.W. (1982). Control and coordination of ventilation in crustaceans: responses to hypoxia and exercise. *Journal of Experimental Biology* **100**: 289-319.

Taylor, H.H. and Waldron, F. M. (1997). Respiratory responses to air-exposure in the southern rock lobster, *Jasus edwardsii* (Hutton) (Decapoda: Palinuridae). *Marine and Freshwater Research* **48**: 889-897.

Taylor, E.W. and Whiteley, N.M. (1989). Oxygen transport and acid-base balance in the haemolymph of the lobster, *Hommarus gammarus*, during aerial exposure and resubmersion. *Journal of Experimental Biology* **144**: 417-436.

Tsing, A., Arcier, J. M. and Brehelin, M. (1989). Hemocytes of penaeid and palaemonid shrimps: morphology, cytochemistry and hemograms. *Journal of Invertebrate Pathology* **53**: 64-77.

Tubiash, H. S., Sizemore, R. K. and Colwell, R. R. (1975). Bacterial flora of the hemolymph of the blue crab, *Callinectes sapidus*: most probable numbers. *Applied Microbiology* **29**: 388-392.

Welsh, P.C. and Sizemore, R.K. (1985). Incidence of bacteremia in stressed and unstressed populations of the blue crab, *Callinectes sapidus. Applied Environmental Microbiology* **50**: 420- 425.

Whiteley, N.M. and Taylor, E.W. (1992). Oxygen and acid-base disturbances in the hemolymph of the lobster *Homarus gammarus* during commercial transport and storage. *Journal of Crustacean Biology* **12**: 19-30.

Winkler, P. (1987). Effects of handling on the *in situ* oxygen consumption of the american lobster (*Homarus americanus*). *Comparative Biochemistry and Physiology* **87A (1)**: 69-71.

APPENDIX 10

CASE STUDY 6
A HEALTH MANAGEMENT STUDY OF LOBSTERS (*Panulirus cygnus*) IN A RACEWAY HOLDING SYSTEM

L.H. Evans, E. Tsvetnenko and S. Fotedar

Aquatic Science Research Unit, Muresk Institute of Agriculture,
Curtin University of Technology, GPO Box U1987, Perth, Western Australia 6845

FRDC CASE STUDY 6
A HEALTH MANAGEMENT STUDY OF LOBSTERS (*Panulirus cygnus*) IN RACEWAY HOLDING SYSTEM

Louis H. Evans, Elena Tsvetnenko and Seema Fotedar

Aquatic Science Research Unit, Muresk Institute of Agriculture, Curtin University of Technology, Kent St Bentley, Western Australia, 6102.

INTRODUCTION

Previous studies conducted on *Panulirus cygnus* held at a Western Australian seafood processing facility had identified an apparent lobster health problem, as measured by a suite of immune parameters. One possible explanation for a deterioration in health status could be uneven water flow patterns in the raceway tanks leading to differences in oxygen levels in different parts of the tank. Such differences could affect the health of lobsters held in the tank. The aim of this trial was to measure health parameters of lobsters held in the water inlet and water outlet of a raceway at a WA seafood processing facility and to measure oxygen levels at various parts of the tank.

After three days of the trial the preliminary results suggested that there was no difference in immune parameter results obtained in lobsters held in different locations in the tank. However, significant abnormalities in some immune parameters were observed. It was therefore decided to move some of the lobsters to tanks at Curtin University in order to determine whether these abnormalities would resolve when lobsters were moved to a different location.

EXPERIMENTAL APPROACH

Fifty lobsters were held in 3 baskets at each of the water inlet and outlet of a raceway ('water inlet' and 'water outlet' treatment groups). Another twenty-five lobsters were free-swimming at each of the water inlet and outlet ('free swimming' treatment group). This treatment was included to determine if holding in crates for extended periods had a detrimental effect on lobster health.

The lobsters were sampled from each treatment group at approximately 1000hrs on three consecutive days. All lobsters in the inlet and outlet groups were transferred to holding tanks at Curtin University immediately following the third sampling on the third day. The remaining lobsters in the free-swimming treatment group continued to be held at the processing facility. The lobsters at both locations were then sampled on day 8 and 10.

At each sampling period ten lobsters were removed and taken to the sampling station in a crate. Each lobster was assessed for vigour, sex, carapace length, appendage loss, tail blisters and tail erosion. Hemolymph was sampled for total hemocyte counts (THC) and differential hemocyte counts (DHC). A second hemolymph sample was extracted for bacteriology, assessment of clotting time, and hemolymph pH. A small piece of pleopod was also collected to determine moult stage. The group of lobsters

sampled first was rotated so as to avoid possible errors introduced due to stress reactions from the presence of the researchers.

RESULTS & DISCUSSION

Weather conditions

Throughout the period of the trial weather conditions outside the factory ranged from clear to overcast and raining heavily. The atmospheric pressure (a meteorological factor which might affect the pH and total circulating hemocyte counts in well acclimated lobsters) varied from 1012 – 1027 hPa (Table 1, Appendix 1). As has been observed in previous observations on partially acclimated lobsters (held to up to 10 days), there was no obvious correlation between the atmospheric pressure and THC and hemolymph pH results.

Water Quality

Water quality measurements (DO, pH and temperature) performed at the processing facility and Curtin University showed that water quality was adequate for western rock lobsters (Tables 2 & 3, Appendix 1). The oxygen levels in the raceway tank ranged from 83 – 90% saturation with no significant differences being observed in oxygen levels in different locations in the raceway tank (Table 3, Appendix 1).

The results obtained show no evidence of a variation in oxygen levels in different positions in the raceway tank.

Clotting Rank

The normal clotting time for post-harvest western rock lobsters tested within 5 days of capture is 25 – 49 secs which equates to 2 - 4 clotting rank. Clotting time has been found to increase in lobsters with a bacterial infection and decrease in lobsters experiencing an acute stress reaction (Evans et al., FRDC 96/344; Final Report). Some lobsters have been observed to develop a ‘no clot’ response in which no evidence of clotting is observed within 180 mins of observation. The proportion of losbters with ‘no clot’ reactions increases with decreasing health status.

All clotting ranks recorded during the period of the trial, with the exception of the outlet treatment group on Day 10, exceeded the clotting ranks expected for a healthy post-harvest lobster tested within 5 days of capture (Table 1, Appendix 2).

This observation suggests that the lobsters used in this trial had a decreased health status at the commencement of the trial. This was a surprising finding as the test animals were specifically selected to ensure a high health status.

Some significant differences ($p<0.05$) were observed between the mean values for clotting time for lobsters from different treatment groups over the first three days of the trial (Table 1, Appendix 2). The clotting time for lobsters in the free-swimming group on Day 1 was significantly lower than that of both the inlet and outlet treatment groups (Figure 1). Furthermore, in contrast to lobsters in the water inlet and outlet

groups, no lobsters in the free-swimming group exhibited a 'no clot' reaction on Day 1 of the trial (Table 1c, Appendix 2). No other significant differences were observed in lobsters held in different positions in the holding tank.

The lack of any significant difference between lobsters held at the inlet and the outlet of the tank suggests that water quality conditions which might affect lobster health were similar at both locations. The lower clotting time and absence of 'no clot' reactions in free swimming lobsters on Day 1 suggests that lobsters allowed to move freely around the holding tan had a higher health status compared to those restrained in baskets.

Clotting time for lobsters in the free-swimming treatment group increased significantly from Day 1 to Day 3, and then declined to levels similar to those recorded on Day 1. Clotting time also increased in the outlet group over the first three days of the trial. The mean % 'no clot' reactions progressively increased over the first three days of the trial and then decreased on Day 10 to the lowest mean value recorded for the whole trial. Clotting times and the %prevalence of 'no clot' reactions in the lobsters transferred to Curtin University were similar to those seen in the free swimming lobsters left at the processing facility on both sampling days.

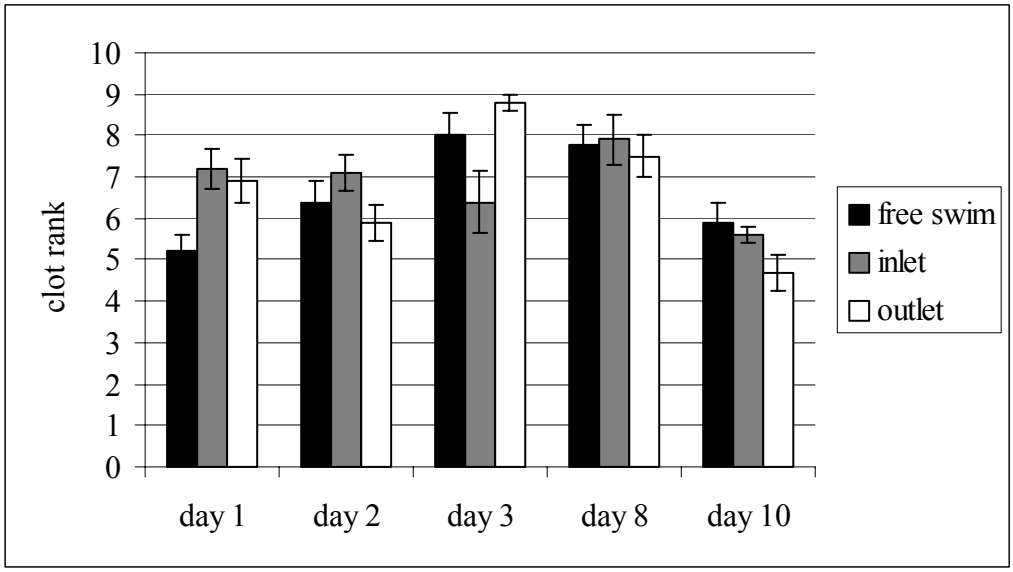


Figure 1 Clotting ranks for free-swimming lobsters and lobsters held at the water inlet and water outlet at the processing facility and at Curtin University.

The results suggest that the lobsters used in the trial were affected by an unknown agent/condition that caused an increase in clotting time. The long clotting times in Day 1 lobsters suggest that they were affected by this agent/condition before the commencement of the trial. The condition worsened over the first three days of the trial and then the lobsters started to recover. However, a complete recovery was not observed (see below; %granular cell section). The similar clotting times observed in lobsters in held at both processing facility and Curtin University suggests that this unknown agent/condition affected the lobsters at both locations and resolution occurred over a similar time frame at both sites.

Total Hemocyte Counts

The normal THC for post-harvest western rock lobsters tested within 5 days of capture is 3.97 – 8.79 x10^6 cells/ml (Evans et al., FRDC 96/344; Final Report). THC increases in lobsters experiencing an acute stress reaction and decreases in severely stressed lobsters and in lobsters with bacterial infections. THC also increases in late pre-moult (D1 stage and later). Late D stage lobsters are therefore not usually included in THC estimations. In this study all lobsters were found to be in C-D0 moult stages and were therefore suitable for study.

The THC for lobsters in the free swimming group and the water outlet group on Day 1 of the trial exceeded the normal range for post-harvest lobsters and the THC in the water inlet group was also at the high end of the normal range (Table 2, Appendix 2; Figure 2).

These observations suggest that the lobsters used for this trial were either experiencing an acute stress response on Day 1 of the trial or that they were affected by a condition/factor which caused an elevation in the number of circulating hemocytes.

There was no significant difference in the THC for each of the three treatment groups at any of the five sampling days (Table 2a, Appendix 2).

This result suggests that the two variables tested in the study (influence of position in raceway and comparison of health status of lobsters held at the processing facility and Curtin University) had no effect on circulating hemocyte counts.

There was a tendency for all THC values to decline over the first 3 - 8 days of the test and then to increase on Day10. The increase in THC from Day 8 to Day 10 was significant in the water outlet lobsters transferred to Curtin University (Table 2b, Appendix 2) but the difference in the Day 8 and Day 10 THC values for the other two treatment groups was not significant. Overall these changes were relatively minor and of doubtful health significance.

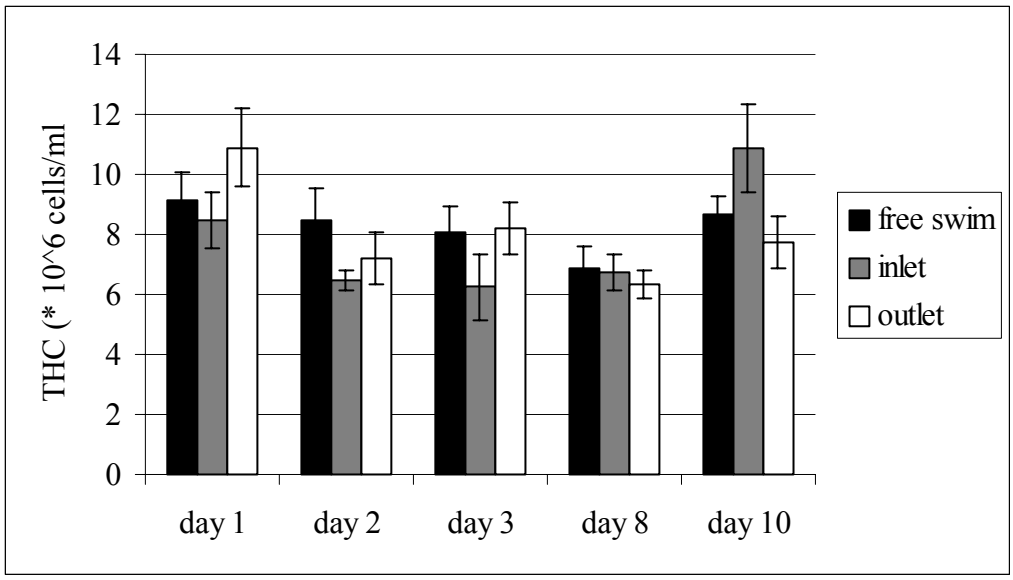


Figure 2 Total Hemocyte Counts for free-swimming lobsters and lobsters held at the water inlet and water outlet at the processing facility and at Curtin University

Hemolymph pH

No published normal range values are presently available for hemolymph pH in the western rock lobster. In the one data set presently available, the mean pH of 13 groups of 14 – 15 acclimated lobsters varied from pH 7.23 – 7.76 with low pH results showing a significant correlation with high THC values. These lobsters were post-harvest lobsters of normal health status held in a tank at the Muresk Marine Laboratory (MML) for a total period of 64 days during which time they were fed with mulies and mussels. Lobsters deprived of oxygen show a significant decrease in hemolymph pH.

The pH values obtained in this study ranged from pH 7.33 – 7.71 (Table 3, Appendix 2). This range is similar to that obtained in the above study. While there were statistically significant differences between the pH values for different treatment groups on two of the three first days of the trial, and between the values for the free swimming group over the five days of the trial, these differences are of doubtful biological significance. There was no evidence of abnormally low pH values, suggestive of lobsters deprived of oxygen.

The results provide no evidence to suggest that lobsters held in the raceway at the processing facility were exposed to low oxygen conditions.

The one major trend in the pH data was a significant decline in pH in all three groups on Day 10 (Table 3b, Appendix 2). The same pattern of change was seen in lobsters held at both the processing facility and Curtin University. This decline correlated with an increase in THC (Table 2b, Appendix 2). Similar patterns of decreasing hemolymph pH and increasing THC were also observed in the above described acclimation study at MML. The reasons for these variations are not known. In the previous study a correlation between these changes and alterations in atmospheric pressure was observed but there was no correlation apparent in this study.

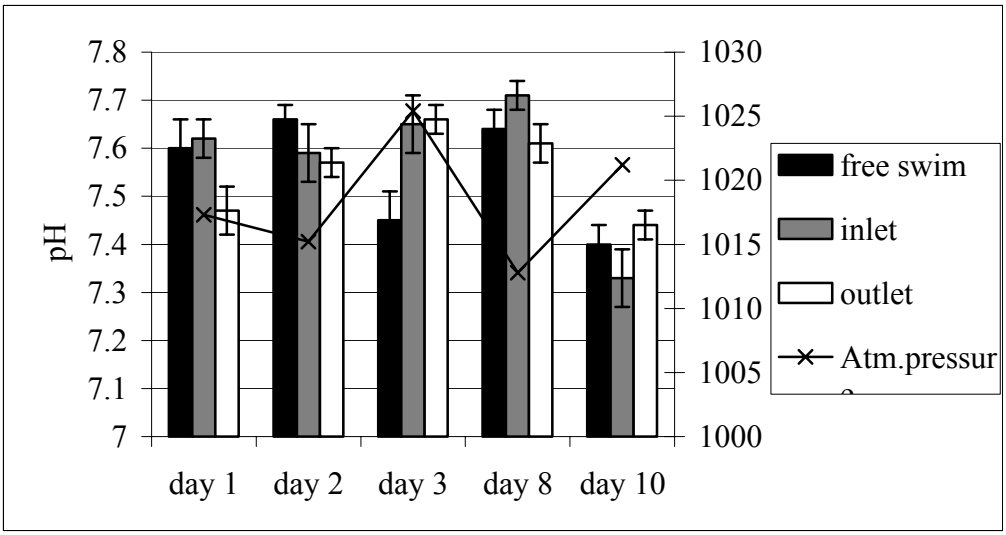


Figure 3 Hemolymph pH for free-swimming lobsters and lobsters held at the water inlet and water outlet at the processing facility and at Curtin University, compared to atmospheric pressure.

%Granular Cells in the Hemolymph

The normal range for %granular cells in post-harvest lobsters acclimated for 1 - 5 days following capture is 4.5 – 11.3% (Evans et al., FRDC 96/344; Final Report). %granular cells decrease in severely stressed lobsters and in lobsters with a bacterial infection.

No significant difference was observed between the %granular cells in the three treatment groups for the first three days of the trial (Table 4, Appendix 2; Figure 4).

This result suggests that holding lobsters in different positions in the raceway tank had no effect on the health status of the lobsters.

The %granular cells in lobsters on Day 1 of the trial were either above the normal range (free swimming and water inlet group) or at the high end of the normal range (water outlet group). The %granular cell values rose significantly in the free-swimming and water inlet groups on Day 2 and showed a similar trend in the water outlet group. The mean values obtained on Day 2 of this trial are the highest %granular cells values seen in western rock lobsters over the past 5 years. The mean values for the free-swimming group declined over Days 8 and 10 although this decrease was not statistically significant.

The results suggest that the lobsters used in the trial were affected by an unknown factor/condition that caused an increase in %granular cells over the first two days of the trial. The high levels of %granular cells in Day 1 lobsters suggest that they were affected by this factor/condition before the commencement of the trial.

Some differences were observed in the %granular cell results for the lobsters held at the processing facility and those held at Curtin University, the latter being higher, though not significantly different, to those held at the processing facility. These trends appear to be correlated to changes in bacterial content of the water, increasing trends in %granular cells being associated with high bacterial loads in the water ($\geq 3.25 \times 10^3$ colony forming units/ml) (see Figure 5).

Some indication of the nature of the unknown factor affecting lobsters used in this trial was obtained from examination of the stained blood smears obtained from the lobsters. Brown pigment deposits were observed in the granular cells. The deposits were seen in lobsters in all treatment groups on all days of the trial including Day 10 when the lobsters were presumably recovering. Subsequent blood tests conducted over the succeeding two weeks showed that the brown pigments persisted in the granular cells of some lobsters. These brown granules have not been previously observed in hemolymph smears of western rock lobsters by either the Curtin group or by Dr Brian Jones, FisheriesWA (Jones, pers. comm.).

While the nature of this pigment could not be determined it is known that granular cells phagocytose bacteria and other infectious agents and store the degraded organisms in vacuoles. It is possible that the brown pigment deposits resulted from phagocytosis of an infective organism.

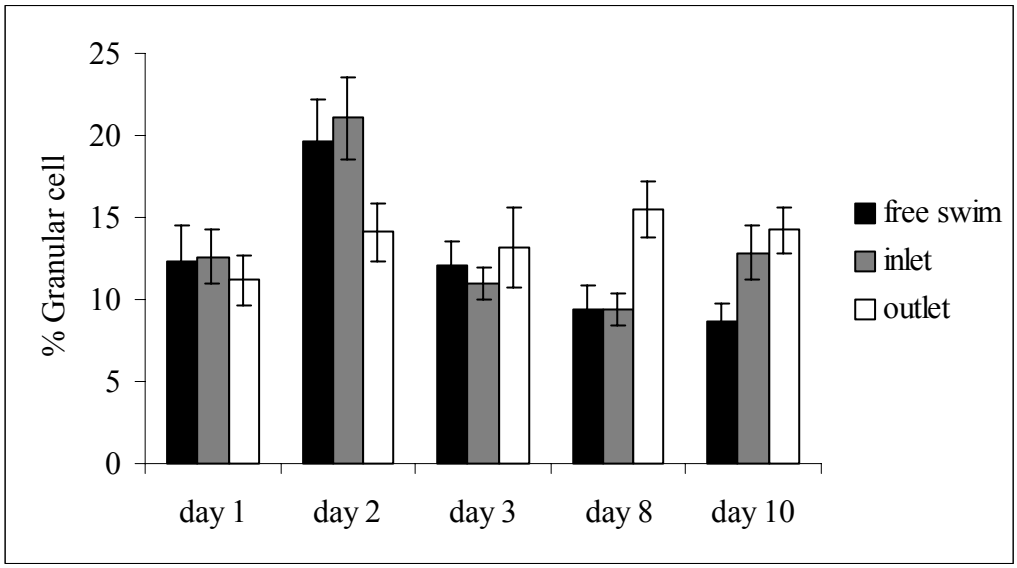


Figure 4 %Granular cells in hemolymph for free-swimming lobsters and lobsters held at the water inlet and water outlet at the processing facility and at Curtin University.

Bacteremia (Colony rank)

The normal range for bacterial colony rank is ≤ 0.5, equivalent to 12.5 colony forming units (CFU - an approximation to the number of bacterial cells present in the sample)/ml (Evans et al., FRDC 96/344; Final Report). Up to 50% of lobsters in a normal sample can exhibit this level of bacteremia. A non-stressed lobster is expected to have no bacteria in its hemolymph. However, post-harvest lobsters often display

mild bacteremia i.e colony ranks of up to 2 (50 CFU/ml). Values above this level are suggestive of a severely stressed lobster.

High levels of bacteremia were seen in seven of the 11 groups of lobsters tested at the processing facility (Table 5, Appendix 2). In addition, levels of bacteria in the water on Days 1,2 and 3 were higher than would be expected for good water quality ($\geq$3.25 x 10^3 CFU/ml; Table 2, Appendix 1).

These results suggest that the levels of bacteria in the raceway tank exceeded those normally seen in oceanic water but were less that levels reported to be associated with mortalities in hatchery tanks (Barton pers. comm.). Whether these levels of bacteria are a matter of concern is uncertain. However, the lobsters tested in this trial did exhibit higher levels of bacteremia than previously seen at this processing facility in the 1998/1999 season (Evans et al. 2000). This was particularly evident in water inlet lobsters tested on Day 2 when an exceptionally high level of bacteremia (4.7 colony rank) was observed.

The ambient water bacterial levels in the raceway tank fell on Days 8 and 10 and this fall was associated with a decrease in bacteremia in the free swimming lobsters tested on Day 10. The ambient water bacterial levels were initially low at Curtin University and corresponding bacteremia levels in the inlet and outlet treatment group lobsters were less than 0.5. However, following the introduction of lobsters to the system at Curtin University, the water bacterial levels increased to levels too numerous to count (bacterial colony rank 13) and this change was associated with an the increase in lobster bacteremia levels observed on Day 10 (Figure 5).

The results suggest that high levels of bacteremia in lobsters were associated with high levels of bacteria in the water. The health significance of excessive numbers of bacteria in lobsters is uncertain but is likely to be indicative of a low health status. Whether high bacterial loads contribute to the development of poor health caused by exposure to the unknown agent/condition present in lobsters in this trial is unknown.

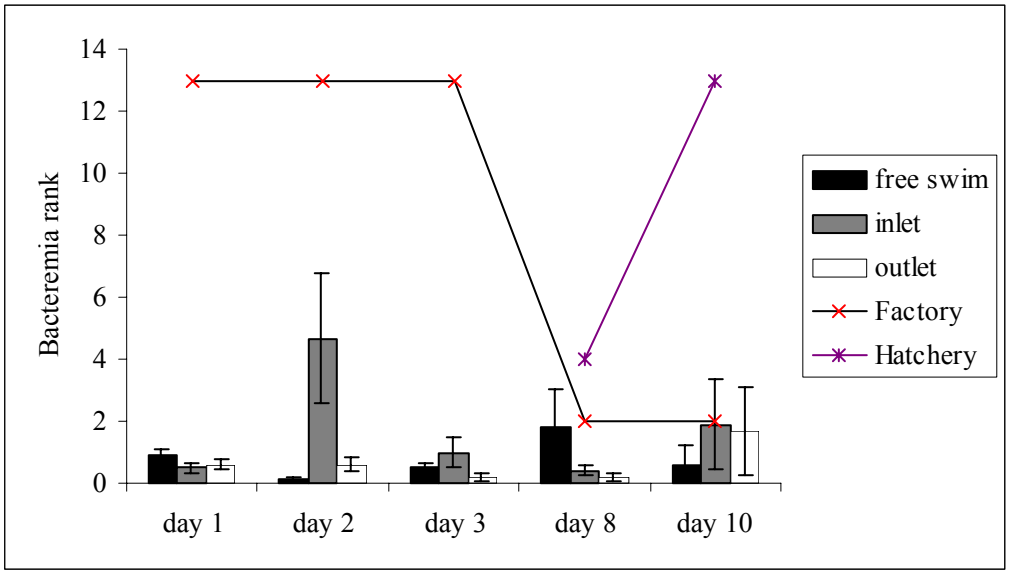


Figure 5 Bacteremia rank for free-swimming lobsters and lobsters held at the water inlet and water outlet at the processing facility and at Curtin University.

CONCLUSIONS

Overall these results provide no evidence to suggest that environmental conditions in different locations of the raceway tanks at the processing facility are associated with a deterioration in health status of lobsters stored in the tanks. In addition, immune parameters of lobsters held at the processing facility showed a similar pattern of variation to those held at Curtin University, suggesting that these variations were caused by factors common to both facilities.

The lobsters used in this trial had abnormally long clotting times which appeared to be associated with elevated levels of granular cells and increased bacteremia. The pattern of change of bacterial counts in ambient water samples shows that there were occasionally high levels of bacteria in the raceway tank and that a similar increase in bacterial load occurred at the Curtin Hatchery when lobsters were transferred from the processing facility to Curtin. The elevated bacterial load in the ambient water appeared to be associated with increased bacteremia in the lobsters.

Further studies should also be conducted on the agent causing the elevation of clotting time and %granular cells and the brown deposits in the blood cells of lobsters. It is possible that this agent was responsible for the occasional observation of excessive bleeding and poor survival in exported lobsters in the 1999/2000 lobster season.

APPENDIX 1

Meteorological and water quality observations

Fig. 1: Atmospheric pressure during period of trial

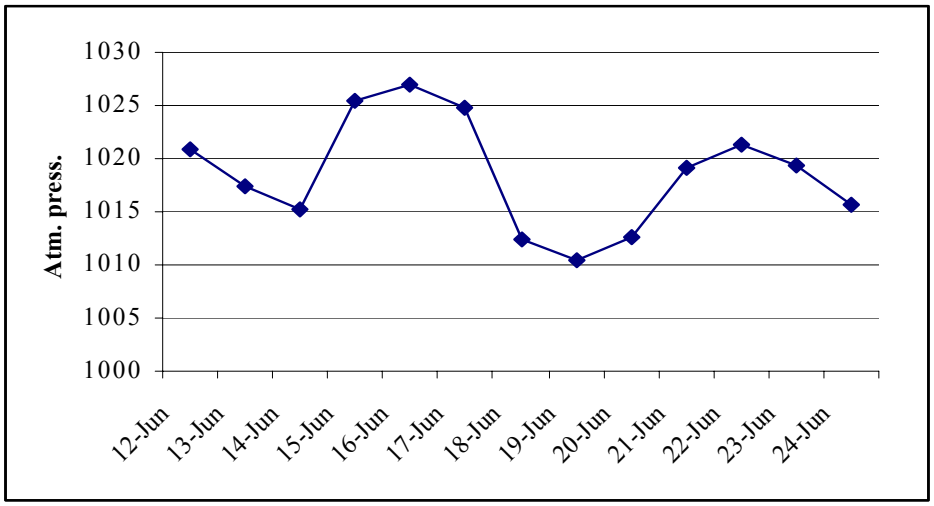


Table 2: Water Quality Parameters measured at Processing Factory and Curtin University

Date	Water pH		Temp (^{o}C)		Bacteremia (colony rank)	
	PF	CU	PF	CU	PF	CU
13.6.00	8.15	-		-	13*	-
14.6.00		-		-	13	-
15.6.00		-		-	13	-
20.6.00	8.35	8.25	15.5	15.8	2	4
22.6.00	8.26	8.20	16.1	15.4	2	13

* - colony rank 13 is assigned when there are too many bacterial colonies on the agar plate and an accurate count cannot be achieved.

PF: Processing Factory
CU: Curtin University

Table 3: Dissolved oxygen levels measured at Processing Factory and Curtin University

Site	Date sampled	Sample site	Dissolved Oxygen (% saturation)
Processing Factory	12.6.00	Water inlet	90
	12.6.00	Middle	89
	12.6.00	Outlet	84
Curtin University	20.6.00	Recirc. System	83
	22.6.00	Recirc. System	83

APPENDIX 2

STATISTICAL ANALYSIS OF LOBSTER IMMUNE PARAMETER VALUES

Table 1: Clotting rank

(a) Clotting ranks for free-swimming lobsters and lobsters held at the water inlet and water outlet at Processing Factory

Treatment	13.6.00	14.6.00	15.6.00	20.6.00	22.6.00
Free swimming	$5.2\pm0.4_{1}^{a}$	$6.4\pm0.5_{1}^{ac}$	$8.0\pm0.5_{1}^{b}$	7.8 ± 0.5^{bcd}	5.9 ± 0.5^{ad}
Water inlet	$7.2\pm0.5_{2}^{a}$	$7.1\pm0.4_{1}^{a}$	$6.4\pm0.8_{2}^{a}$	-	-
Water outlet	$6.9\pm0.5_{2}^{a}$	$5.9\pm0.4_{1}^{a}$	$8.8\pm0.2_{1}^{b}$	-	-

Mean values in the same row with different superscript letters are significantly different ($p<0.05$).
Mean values in the same column with different numbers are significantly different ($p<0.05$).

(b) Clotting ranks for free-swimming lobsters held at Processing Factory and lobsters transferred to Curtin University.

Treatment	20.6.00	22.6.00
Free swimming (Processing Factory)	$7.8\pm0.5_{1}^{a}$	$5.9\pm0.5_{1}^{b}$
Curtin Uni (previously water inlet)	$7.9\pm0.6_{1}^{a}$	$5.6\pm0.2_{1}^{b}$
Curtin Uni (previously water outlet)	$7.5\pm0.5_{1}^{a}$	$4.7\pm0.4_{1}^{b}$

Mean values in the same row with different superscript letters are significantly different ($p<0.05$).
Mean values in the same column with different numbers are significantly different ($p<0.05$).

(c) Percent prevalence of no clots

Treatment	13.6.00	14.6.00	15.6.00	20.6.00	22.6.00
Free swimming	0	20	60	50	10
Water inlet	30	10	30	60 (CU)	0(CU)
Water outlet	20	10	90	40(CU)	0(CU)
Mean %no clot	16.6	13.3	60	50	3.3

Table 2: Total Hemocyte Counts

(a) Total hemocyte counts for free-swimming lobsters and lobsters held at the water inlet and water outlet at Processing Factory.

Treatment	13.6.00	14.6.00	15.6.00	20.6.00	22.6.00
Free swimming	$9.12\pm0.93_1^{a}$	$8.48\pm1.07_1^{a}$	$8.10\pm0.85_1^{a}$	6.90 ± 0.72^{a}	8.65 ± 0.62^{a}
Water inlet	$8.46\pm0.92_1^{a}$	$6.47\pm0.34_1^{a}$	$6.25\pm1.11_1^{a}$	-	-
Water outlet	$10.89\pm1.31_1^{a}$	$7.21\pm0.89_1^{b}$	$8.19\pm0.85_1^{ab}$	-	-

Mean values in the same row with different superscript letters are significantly different ($p<0.05$).
Mean values in the same column with different numbers are significantly different ($p<0.05$).

(b) Total hemocyte counts for free-swimming lobsters held at Processing Factory and lobsters transferred to Curtin University.

Treatment	20.6.00	22.6.00
Free swimming (Processing Factory)	$6.90\pm0.72_1^{a}$	$8.65\pm0.62_1^{a}$
Curtin Uni (previously water inlet)	$6.72\pm0.61_1^{a}$	$10.87\pm1.46_1^{a}$
Curtin Uni (previously water outlet)	$6.33\pm0.45_1^{a}$	$7.75\pm0.88_1^{b}$

Mean values in the same row with different superscript letters are significantly different ($p<0.05$).
Mean values in the same column with different numbers are significantly different ($p<0.05$).

Table 3: Hemolymph pH

(a) Hemolymph pH for free-swimming lobsters and lobsters held at the water inlet and water outlet at Processing Factory

Treatment	13.6.00	14.6.00	15.6.00	20.6.00	22.6.00
Free swimming	$7.60\pm0.06_1^{a}$	$7.66\pm0.03_1^{a}$	$7.45\pm0.06_1^{b}$	7.64 ± 0.04^{a}	7.40 ± 0.04^{b}
Water inlet	$7.62\pm0.04_1^{a}$	$7.59\pm0.06_1^{a}$	$7.65\pm0.06_2^{a}$	-	-
Water outlet	$7.47\pm0.05_2^{a}$	$7.57\pm0.03_1^{b}$	$7.66\pm0.03_2^{c}$	-	-

Mean values in the same row with different superscript letters are significantly different ($p<0.05$).
Mean values in the same column with different numbers are significantly different ($p<0.05$).

(b) Hemolymph pH for free-swimming lobsters held at Processing Factory and lobsters transferred to Curtin University.

Treatment	20.6.00	22.6.00
Free swimming (Processing Factory)	$7.64\pm0.04_1^{a}$	$7.40\pm0.04_1^{b}$
CurtinUni (previously water inlet)	$7.71\pm0.03_1^{a}$	$7.33\pm0.06_1^{b}$
CurtinUni (previously water outlet)	$7.61\pm0.04_1^{a}$	$7.44\pm0.03_1^{b}$

Mean values in the same row with different superscript letters are significantly different ($p<0.05$).
Mean values in the same column with different numbers are significantly different ($p<0.05$).

Table 4: %Granular cells

(a) % Granular cells in hemolymph for free-swimming lobsters and lobsters held at the water inlet and water outlet at Processing Factory.

Treatment	13.6.00	14.6.00	15.6.00	20.6.00	22.6.00
Free swimming	$12.30\pm2.21_1^{ac}$	$19.65\pm2.52_1^{b}$	$12.05\pm1.46_1^{ac}$	9.40 ± 1.49^{c}	8.70 ± 1.06^{c}
Water inlet	$12.60\pm1.67_1^{a}$	$21.06\pm2.52_1^{b}$	$10.94\pm0.97_1^{a}$	-	-
Water outlet	$11.20\pm1.53_1^{a}$	$14.10\pm1.81_1^{a}$	$13.15\pm2.43_1^{a}$	-	-

Mean values in the same row with different superscript letters are significantly different ($p<0.05$).
Mean values in the same column with different numbers are significantly different ($p<0.05$).

(b) % Granular cells for free-swimming lobsters held at Processing Factory and lobsters transferred to Curtin University.

Treatment	20.6.00	22.6.00
Free swimming (Processing Factory)	$9.40\pm1.49_1^{a}$	$8.70\pm1.06_1^{a}$
CurtinUni (previously water inlet)	$9.35\pm0.99_1^{a}$	$12.83\pm1.64_{12}^{a}$
CurtinUni (previously water outlet)	$15.5\pm1.75_2^{a}$	$14.22\pm1.43_2^{a}$

Mean values in the same row with different superscript letters are significantly different ($p<0.05$).
Mean values in the same column with different numbers are significantly different ($p<0.05$).

Table 5: Bacteremia (colony rank)

(a) Bacteremia (colony rank) in hemolymph for free-swimming lobsters and lobsters held at the water inlet and water outlet at Processing Factory and at Curtin University.

Treatment	13.6.00	14.6.00	15.6.00	20.6.00	22.6.00
Free swimming	$0.9\pm0.2_1^{a}$	$0.1\pm0.2_1^{b}$	$0.5\pm0.2_1^{abc}$	1.8 ± 1.3^{ac}	0.6 ± 1.9^{bc}
Water inlet	$0.5\pm0.7_1^{a}$	$4.7\pm0.1_1^{a}$	$1.0\pm0.5_1^{a}$	-	-
Water outlet	$0.6\pm0.6_1^{a}$	$0.6\pm2.1_1^{a}$	$0.2\pm0.1_1^{a}$	-	-

Mean values in the same row with different superscript letters are significantly different ($p<0.05$).
Mean values in the same column with different numbers are significantly different ($p<0.05$).

(b) Bacteremia (colony rank) for free-swimming lobsters held at Processing Factory and lobsters transferred to Curtin University.

Treatment	20.6.00	22.6.00
Free swimming (Processing Factory)	$1.8\pm1.3_1^{a}$	$0.6\pm1.9_1^{a}$
Curtin Uni (previously water inlet)	$0.4\pm0.2_1^{a}$	$1.9\pm4.4_1^{a}$
Curtin Uni (previously water outlet)	$0.2\pm0.1_1^{a}$	$1.7\pm4.3_1^{a}$

Mean values in the same row with different superscript letters are significantly different ($p<0.05$).
Mean values in the same column with different numbers are significantly different ($p<0.05$).

(c) % Prevalence of bacteremia

Treatment	13.6.00	14.6.00	15.6.00	20.6.00	22.6.00
Free swimming	80	10	50	50	10
Water inlet	50	67	60	40	22
Water outlet	60	50	20	20	33

(d) % Prevalence of rank 13

Treatment	13.6.00	14.6.00	15.6.00	20.6.00	22.6.00
Free swimming	0	0	0	10	0
Water inlet	0	33	0	0	11
Water outlet	0	0	0	0	11

APPENDIX 11

INTELLECTUAL PROPERTY

There was no intellectual property generated in this project.

APPENDIX 12

PROJECT PARTICIPANTS

PROJECT PARTICIPANTS

Principal Investigator

Assoc Prof Louis Evans
Curtin University of Technology
Aquatic Science Research Unit
GPO Box U1987
Perth WA 6001

Co-Investigators

Dr Brian Jones
Fisheries WA

Dr John Norton
Queensland Department of Primary Industries

Dr Judith Handlinger
Tasmanian Department of Primary Industries and Fisheries

Dr Japo Jussila
Curtin University of Technology

Dr Elena Tsvetnenko
Curtin University of Technology

Dr Annette Thomas
Queensland Department of Primary Industries