Facilitation, administration and promotion of the FRDC Rock Lobster Post - Harvest Subprogram

Dr. Bruce Phillips
Principal investigator

Australian Government

Fisheries Research and Development Corporation

Project No. 2000/250

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FRDC Project 2000/250 Rock Lobster Post-Harvest Project 1: facilitation, administration and promotion

Principal Investigator

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Fisheries Research and Development Report
FRDC Project No. 200/250

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FRDC Project 2000/250 Rock Lobster Post-Harvest Project 1: facilitation, administration and promotion (RLPHS)

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Objectives:

1. Co-ordinate the FRDC Rock Lobster Post Harvest Subprogram.
2. Conduct an annual research workshop to present outcomes from the Subprogram and to define research objectives for subsequent years.
3. Facilitate travel of the Subprogram project principal investigators, industry representatives and Subprogram Leader to biannual scientific meetings.
4. Facilitate travel of industry representatives, Subprogram Leader of the enhancement and aquaculture Subprogram, and Subprogram Leader to biannual steering committee meetings.
5. Co-ordinate the preparation of Subprogram media releases and Workshop publications.
6. Integrate with other FRDC funded rock lobster research programs including the FRDC Enhancement and Aquaculture Subprogram.
7. Co-ordinate the preparation and distribution of a biannual Subprogram newsletter.
8. Develop and maintain a strategic plan for post-harvest rock lobster research.
9. Develop a strategic plan for the Subprogram.

Non-technical summary:

Outcomes achieved to date

An independent Subprogram Leader, and a highly responsive Steering Committee that is composed of industry experts from across Australia have provided an effective and efficient system for directing relevant research activities to ensure continued and increased profitability for the Australian rock lobster fisheries. The Steering Committee, under the Subprogram Leader's direction, worked as an integrated group, rather than a collection of individuals, and carefully selected and recommended projects for funding, and then followed their progress and offered advice to principal investigators in the national interest. The industry representatives on the Steering Committee have also acted to provide research facilities within the industry as the best locations to conduct research, and ensuring the uptake of successful projects as soon as they occur.

The research conducted by the Subprogram has significantly improved Australia's understanding of the physiology and biochemistry of lobsters, from the time of capture through to processing in a variety of product forms. This is of considerable assistance in investigating methods of better handling, cooking, and/or processing lobsters for live export.

Studies to alleviate leg loss in western rock lobster have yielded impressive results. However, the introduction of cold-water stunning is not yet endorsed by the Subprogram, as we are awaiting confirmation that it will not cause increased mortality on undersized lobsters, not adversely effect the egg production or cause increased mortality of breeding females.

The dramatic effects of hypo-salinity in causing leg loss has been a major achievement, and its rapid take up by industry will cause a major reduction in leg loss and a consequent multi million dollar increase in value of the fishery.

A major method of communicating research results from the Subprogram has been the Code-of-Practice. The newly revised Code incorporates in a subtle way the best ideas from the results in a form endorsed by the industry. As a result this leads to improved post-harvest handling practices, and thereby to increased safety and profitability of the fishery.

Studies of the methods of cooking of western rock lobsters, with the intention of alleviating the blackening of the flesh after thawing, have already indicated the source of the problem and a likely solution. Further tests will be undertaken to confirm if the solution can be carried out under commercial conditions.

The mission of the RLPHS is to ensure that Australia obtains the maximum value for its rock lobster catch.

The markets for rock lobsters, and the forms in which they are sold, are constantly changing. There has been a dramatic change from frozen products to live marketing of a large portion of the catch. Almost all the southern rock lobsters are now all exported live. However, because of fluctuations in the global markets and very high catch levels, much of the western rock lobster catch is now sold in a variety of product forms. Due to these changes, industry constantly faces new challenges to retain, maintain, and expand markets and profitability.

The purpose of the Subprogram is to work with industry to identify the problems that the industry faces in meeting these challenges. It then seeks to support research studies to provide answers to the problems in a cost effective and timely manner. The outputs of the research are rapidly provided to industry in a form that allows industry to capture the benefits of the research for the Australian industry.

An independent Subprogram Leader, and a highly responsive Steering Committee that is composed of industry experts from across Australia have made this possible. The Steering Committee, under the Subprogram Leader's direction, worked as an integrated group, rather than a collection of individuals, and carefully selects and recommends projects for funding, and then follows their progress and offers advice and assistance to the principal investigators, in the national interest. The industry representatives on the Steering Committee have also acted to provide research facilities within the industry as the best locations to conduct research, and ensuring the uptake of improved post-harvest handling practices as soon as they are identified.

Subprogram Strategic Plan

RLPHS established a Strategic Research Plan for the needs of the Subprogram, and this has been updated annually. A copy of the 2002-2007 plan is included as **Appendix 6**.

Subprogram management and operating procedures

To ensure that research conducted within the Subprogram was relevant and met the above criteria, a Steering Committee was established to:

1. Provide industry feedback and views;
2. Review existing research based on FRDC contractual obligations;
3. Prioritise new proposals and provide a priority list for other agencies;
4. Ensure outcomes are commercially focussed;
5. Co-ordinate industry and research provider involvement - optimum use of resources;
6. Facilitate extension and technology transfer.

Scientific and Steering Committee meetings

Annual scientific committee meetings were convened and all meetings were minuted and actioned. Detailed copies of the minutes have not been included in this report for the sake of brevity and the confidential nature of some of the discussions. In addition, direct discussions between the Steering Committee and the principal investigators were encouraged. Written reports and brief presentations by principal investigators were followed by discussion at each Steering Committee Meeting.

Six Steering Committee meetings were held between July 2000 and June 2003. These meetings were held in Hobart, Geelong, Adelaide and Perth.

Subprogram workshops

An annual workshop was convened in New Zealand between April 2 and 7 2001, by RLEAS and in conjunction with the New Zealand Rock Lobster Industry Council. A full set of proceedings was produced from this workshop and papers relevant to this Subprogram were included. See **Appendix 3.**

An annual workshop was convened in Cairns on 29 May 2002 jointly with the RLEAS Subprogram. The workshop was well attended with over 77 participants. A copy of the proceedings of the workshop is included as **Appendix 3**.

Communications

Subprogram newsletters were used as a primary form of communication between the Subprogram and industry.

Annual Operating Plans

Three annual operating plans for the RLPHS were prepared over the course of this project. A copy of the annual operating plan for 2002 has been included in this report as **Appendix 5.**

Collaboration and additional funding opportunities

Additional funding opportunities were investigated for the Subprogram. Additional funding was provided from the Geraldton Fishermen's Cooperative, the Western Rock Lobster Development Association, Development and Better Interest Fund, an Industry Development Unit of WAFIC in Western Australia, in support of the leg loss and hypo-salinity studies.

KEYWORDS: rock lobster, post-harvest, profitability

ACKNOWLEDGEMENTS

A special thanks to the members of the Steering Committee, Stephen Hood, Nick Polgeest, Richard Stevens, Rodney Treloggen, Patrick Hone (FRDC), Kym Redman and Glenn O'Brien for their commitment over the course of the project.

The efforts of Dr Robert van Barneveld, Leader of the FRDC Rock Lobster Enhancement and Aquaculture Subprogram is also acknowledged.

We wish to thank Tim Bray of the Department of Fisheries, Roger Edwards and the South Australian Lobster Industry, Katy Saunders and Seafood Industry Victoria, and Rodney Treloggen and the Tasmanian Rock Lobster Fishermans Association for assisting with distribution of the Subprogram Newsletter.

Background

This Rock Lobster Post-Harvest Subprogram (RLPHS) was initially established in 1996. In 1999 an independent review of the Subprogram was commissioned by FRDC and conducted in September 1999. The review recommended the continuation of the Subprogram, with an increased requirement for industry to play a more dominant role in determining what, if any, research is carried out in the post-harvest sector. A new project 2000/250 was established and commenced on 1 July 2000.

A summary of the Subprogram projects and activities since 2000 is presented below:

	00-01	01-02	02-03	03-04	04-05	05-06
1994/132.02 – Code of Practice. PI Richard Stevens.						
1996/344 – Physiological studies of stress and morbidity during post-harvest handling and storage of western rock lobster: 11 Standard autopsy techniques and immune system competency. PI Professor Louis Evans						
1996/345 - Physiological studies of stress and morbidity during post-harvest handling and storage of western rock lobster: 1 Physiological Stress Indicators. PI Dr Brian Paterson						
1999/202 – Rock Lobster Autopsy Manual. PI Professor Louis Evans						
2000/250 – Facilitation, administration and promotion of the FRDC Rock Lobster Post-Harvest Subprogram. PI Professor Bruce Phillips						
2000/251 – Development of a method for alleviating leg loss during post-harvest handling of rock lobsters. PI Dr Glen Davidson						
2000/252 – Optimising water quality in rock lobster post-harvest process. PI Dr Brad Crear						
2001/235 – Striking a balance between melanosis and weight recovery in western rock lobster. PI Hannah Williams						
2002/237 – A code of Practice for Handling Rock Lobster. PI Richard Stevens						
2001/255 – Quantifying and controlling hyper-and hypo-saline- induced post-harvest leg autonomy in the western rock lobster. PI Dr Glen Davidson						
2002/ 238 – Quantification of shell hardness in southern rock lobster. PI Dr Caleb Gardiner						
2002/239 – The effect of on board cold water stunning on the survival and growth of caught and returned western rock						

lobsters. PI Dr Glen Davidson						
2003/241 Rock Lobster Post-Harvest subprogram: strategic planning, project management and adoption PI Professor Bruce Phillips						
2003/342 Value-adding the southern rock lobster fishery-optimising flesh quality of under-valued large lobsters for the sashimi market PI Dr John Carragher						

A new Steering Committee for the Subprogram, **comprising only industry members,** was appointed in 2000. These members were selected on the basis of their expertise in the post-harvest area; at the same time ensuring that expertise on both the southern and western rock lobster fisheries would be available. In addition the Subprogram attempts to assist the industry in Queensland and Torres Strait, which is concerned with tropical rock lobsters. However, there is no official member with expertise in this area.

Subprogram Projects

The following are the summary details projects within the Subprogram up to June 2003.

Projects at start of Subprogram

1994/134.02: Code of Practice

Principal Investigator: Mr Richard Stevens
Abercromby Management Services P/L
21 Eckford Way
Duncraig ,W.A. 6023

Project Objectives:

To produce a Code of Practice for the handling of rock lobster.

1996/344: Physiological studies of stress and morbidity during post-harvest handling and storage of western rock lobster: 11. Standard autopsy techniques and immune system competency

Principal Investigator: Dr Louis Evans
Curtin University of Technology
Aquatic Science Research Unit
GPO Box U1987
Perth W.A. 6845

Project Objectives:

1. To identify suitable immune system parameters which can be used to evaluate stress responses and health status in captive lobsters and to

apply those parameters in a study of stress induced by post-harvest handling procedures.

2. To investigate the causes of mortality in captive lobsters held in processing factories. This study will focus on bacteriological and histopathological examinations and will result in the development of a standard protocol for the autopsy of lobsters.

3. To evaluate the influence of temperature change on immunological and physiological stress responses.

4. To study the influence of hormonal secretions on immunological and physiological stress responses.

5. To investigate innovative techniques which will boost immunocompetence but not adversely affect marketability of live product.

1996/345: Physiological studies of stress and morbidity during post-harvest handling and storage of western rock lobster: 1 Physiological Stress Indicators

Principal Investigator: Dr Brian Patterson
Centre for Food Technology
Department of Primary Industries
19 Hercules St., Hamilton Qld 4007

Project Objectives:

1. Identify key physiological stress parameters that either describe stress levels and/or predict likely further mortality in lobsters after harvest and apply these parameters in studies aimed at improving post-harvest handling practices.

2. Obtaining baseline measurements of physiological parameters in resting undisturbed lobsters, with reference to interactions between season and locality and the effects of moult stage and other biological variables.

3. Identifying physiological parameters, through field studies aimed at studying the effect of harvest and post-harvest handling on lobsters, which can be used to evaluate deviations from baseline values in captive lobsters.

4. Identifying physiological parameters through controlled laboratory experiments using identified stressors which can be used to evaluate deviation from baseline values in captive lobsters.

5. Develop simple methods of measuring one of the stress parameters identified in objectives 3 and 4 for use in lobster processing factories in the evaluation of stress levels in selected lobster shipments.

6. Apply the results and understanding of harvest and post-harvest handling gained from the field work in objective 3, and the stress parameters identified in objectives 3 and 4, in a study or studies of lobster post-harvest handling practices aimed at developing improved post-harvest procedures.

7. Use the findings of earlier sub-objectives to make recommendations for improvements in handling practices described in the recently published Code of Practice.

8. Use the findings to develop detailed knowledge and understanding of the physiological processes involved in the stress responses in lobsters which can be used by processing companies and fishers to devise improved methods of post-harvest handling and transport.

1999/202: Rock Lobster Autopsy Manual

Principal Investigator: Dr Louis Evans
Curtin University of Technology
Aquatic Science Research Unit
GPO Box U1987
Perth W.A. 6845

Project Objectives:

The publication of an autopsy manual to be used in the lobster industry.

2000/250: Facilitation, administration and promotion of the post-harvest Subprogram

Principal Investigator: Dr Bruce Phillips
Curtin University of Technology
Muresk Institute of Agriculture
GPO Box U1987
Perth W.A. 6845

Project Objectives:

1. Co-ordinate the FRDC Rock Lobster Post-Harvest Subprogram.

2. Conduct an annual research workshop to present outcomes from the Subprogram and to define research objectives for subsequent years.

3. Facilitate travel of the Subprogram project principal investigators, industry representatives and Subprogram leader to biannual scientific committee meetings.

4. Facilitate travel of the industry representatives, Subprogram leader of the Enhancement and Aquaculture Subprogram, and Subprogram leader to biannual Steering Committee meetings.

5. Co-ordinate the preparation of Subprogram media releases and workshop publications.

6. Integrate with other FRDC funded rock lobster research programs including the Enhancement and Aquaculture Subprogram.

7. Co-ordinate the preparation and distribution of a biannual Subprogram newsletter.

8. Develop and maintain a strategic plan for post-harvest rock lobster research.

9. Develop a strategic plan for the Subprogram.

New Projects added to the Subprogram

2000/251: Development of a method for alleviating leg loss during post-harvest handling of rock lobsters

Principal Investigator: Dr Glen Davidson
Geraldton Fishermen's Cooperative (originally Department of Zoology, UWA)
PO Box 23
Geraldton WA 6531

Project Objectives:

1. To identify a cold-water immersion treatment that rapidly immobilises western rock lobsters, while allowing swift recovery from immobilisation upon return to ambient temperature seawater. To investigate the effect of season/acclimation temperature on effectiveness of cold stunning in western rock lobsters. To investigate the use of sea sprays vs. immersion for cold stunning in western rock lobsters.

2. To investigate, in captivity, the effectiveness of the preferred treatment (identified in objective 1) for reducing leg loss in western rock lobsters.

3. To test the accuracy of factory grading of cold stunned western rock lobsters vs. untreated controls.

4. To describe the occurrence of leg loss, morbidity and mortality of western rock lobsters subjected to cold stunning prior to episodes of handling during the post-harvest process (i.e. at the time of pot-pulling and sorting, prior to factory grading) and to compare these to the performance of animals handled using current methods.

5. To investigate the effects of multiple simulated pot capture and release events, either with or without cold stunning, on growth, leg loss and survival of undersized western rock lobsters.

6. To compare, in captivity, effects of handling, with and without cold stunning, on the reproductive success of setose, tar spot and ovigerous female western rock lobsters. To investigate the effects of limb loss on the reproductive success of female western rock lobsters.

7. To conduct a survey to determine the extent and nature of leg loss in the southern rock lobster fisheries of Tasmania and South Australia.

2000/252: Optimising water quality in rock lobster post harvest

Principal Investigator: Dr Stephen Battaglene (originally Dr Brad Crear)
University of Tasmania
Tasmanian Aquaculture and Fisheries Institute
Marine Research Laboratories
Nubeena Crescent, Taroona, Tasmania, 7053

Project Objectives:

1. Production of a manual on optimising the provision of oxygen during rock lobster post-harvest processes.

2. Determine the median lethal concentration (LC-50) of ammonia to adult southern and western rock lobsters (stressed and unstressed).

3. Determine the physiological consequences of exposing lobsters to sub-lethal ammonia concentrations, and the consequences of further exposing lobsters to acute post-harvest stressors.

4. Production of a manual on ammonia problems during rock lobster post-harvest processes.

Quantifying and controlling hyper- and hyposaline-induced post-harvest leg autotomy in the western rock lobster

2001/255 Principal Investigator: Dr Glen Davidson
Geraldton Fishermen's Cooperative
PO Box 23
Geraldton WA 6531

Project Objectives

1. Survey salinity concentrations of surface films on individual lobsters and on relevant contact surfaces on boats and within factories.

2. Describe the relationship between autotomy and exposure to seawater of various salinities for lobsters of various sizes and moult stages.

3. Quantify leg loss during industry standard freshwater "drowning" procedures.

4. Compare responses to ionic and non-ionic solutions to elucidate the potential role of other contaminants, and the possible nature of the receptors and stimuli.
5. Investigate the relationship between daily environmental conditions and levels of post-harvest leg loss.

6. Field-test practical solutions for hyper/hyposaline-induced autotomy and make recommendations to industry.

2001/235 Striking a balance between melanosis and weight recoveries in western rock lobster

Principal Investigator: Dr Hannah Williams
Curtin University of Technology
School of Public Health
GPO Box U1987
Perth W.A. 6845

Project Objectives:

1. To establish the impact of temperature and food additives on the activity of *Panulirus cygnus* haemolymph phenol oxidase (PO) in vitro.

2. To establish the impact of current commercial practices on weight recovery and melanosis formation.

3. To establish the impact of post-harvest transportation on PO activity, weight recovery and melanosis formation.

4. To determine the effects of anti-browning agents on weight recovery and melanosis formation.

5. To validate the use of experimentally determined cooking profiles for improvement of cooked weight recoveries and prevention of melanosis.

6. To formulate recommendations and guidelines that will enable industry to apply the findings of the study.

2002/237 Code of Practice

Principal Investigator: Mr Richard Stevens
Abercromby Management Services P/L
21 Eckford Way
Duncraig ,W.A. 6023

Project Objectives:

To produce a Code of Practice for the handling of rock lobster.

2002/238 Quantification of shell hardness in southern rock lobster

Principal Investigator: Dr Caleb Gardner
University of Tasmania
Tasmanian Aquaculture and Fisheries Institute
Marine Research Laboratories
Nubeena Crescent, Taroona, Tasmania, 7053

Project Objectives

1. To calibrate the rate of change in shell hardness before and after the moult of southern rock lobsters relative to lobster size, sex, region and temperature.

2. To identify the region of the exoskeleton that is most suited for measuring hardness.

2002/239 The effect of on board cold water stunning on the survival and growth of caught and returned western rock lobsters (*Panulirus cygnus)*

Principal Investigator: Dr Glen Davidson
Geraldton Fishermen's Cooperative
PO Box 23
Geraldton WA 6531

Project Objectives

1. To determine the effect of commercial capture with or without cold-stunning on the survival and growth of returned protected western rock lobsters.

2. To observe and film in the wild the behaviour of western rock lobsters caught and returned with or without cold-water stunning.

Need

The catches of Australia's rock lobster fisheries are at or near their maximum level. However, adding value to the rock lobster catch by way of enshrining maximum quality on delivery to the processing factories, 100% survival of live lobsters shipped to overseas destinations, perfect cooking regimes for the product processed for this market, a continuous maintenance and upgrading of handling conditions, health and safety conditions, and respect for community welfare concerns, will ensure continuing and improved profits for the industry.

A major objective of the Subprogram has been to ensure delivery to the processing factories of rock lobsters that are intact (no limbs missing) and healthy and strong, so that they are in a condition suitable for live export. Not all such lobsters are exported live. However, this provides the processor/marketer with the greatest choice, and lobsters cooked and frozen that are in this condition, provide greater percentages of flesh recovery. The studies conducted under the Subprogram have shown that rock lobsters at the time of capture are healthy and vigorous and that all of the reductions in condition are the result of less than perfect handling and transport conditions on the way to the factory. Additional studies to find ways of reducing these problems, and education of the participants, are still needed.

The markets for rock lobsters and the forms in which they are sold change constantly. The change from frozen products to live marketing of a large portion of the catch has been dramatic. Because of this the industry faces new challenges to retain maintain and expand markets and profitability.

The purpose of the Subprogram is to identify with industry the best possibilities to assist industry in meeting these challenges. It then seeks to support research studies to provide answers in a cost effective and timely manner. The outputs of the research are then disseminated to industry in a form suitable to allow industry to capture the benefits of the research for the Australian industry.

Objectives

1. Co-ordinate the FRDC Rock Lobster Post-Harvest Subprogram.

2. Conduct an annual research workshop to present outcomes from the Subprogram and to define research objectives for subsequent years.

3. Facilitate travel of the Subprogram project principal investigators, industry representatives and Subprogram Leader to biannual scientific meetings.

4. Facilitate travel of industry representatives, Subprogram Leader of the enhancement and aquaculture Subprogram, and Subprogram Leader to biannual steering committee meetings.

5. Co-ordinate the preparation of Subprogram media releases and Workshop publications.

6. Integrate with other FRDC funded rock lobster research programs including the FRDC Enhancement and Aquaculture Subprogram.

7. Co-ordinate the preparation and distribution of a biannual Subprogram newsletter.

8. Develop and maintain a strategic plan for post-harvest rock lobster research.

9. Develop a strategic plan for the Subprogram.

Methods

Industry consultation and communication

The Subprogram Leader promoted the activities of the RLPHS through industry newsletters and direct communication with industry organisations and representatives. Heavy reliance was placed upon ongoing maintenance of the Steering Committee with representatives from the rock lobster wild fishing sectors and processing sectors across Australia for the provision of strategic direction and advice.

The Subprogram leader presented the work of the Subprogram to the National Lobsters Congress in September 2001.

The Subprogram leader presented the work of the Subprogram to the working group devising a Strategic Plan for the southern rock lobster fishery in Melbourne.

Strategic planning

Strategic planning for the RLPHS was based on discussions of the Steering Committee and ongoing consultation between the Subprogram Leader and members of industry and researchers in Australia.

Communication with FRABs

Communication with FRAB's was via distribution of an annual operating plan for the RLPHS in December of each year combined with direct communications. The Subprogram Leader also attended the annual FRDC FRAB workshop to promote the activities and objectives of the RLPHS.

Development of new research proposals

New research proposals were developed through the use of facilitated strategic planning meetings. The Subprogram Leader convened meetings with relevant researchers and research institutions to:

1. Define the planned outcomes of the new proposal;
2. Manage an indicative budget for the research as defined by the Steering Committee;
3. Identify which researchers/institutions are best placed to undertake the research;
4. Promote collaboration between researchers and institutions where appropriate;
5. Seek external expertise and inputs as required.
6. Ensure the new proposal meets the objectives of the Subprogram and that the research remains relevant and focussed.

The Subprogram Leader ensured new research proposals were distributed to FRABS and the RLPHS Steering Committee for comment and ratification before the proposals were submitted to FRDC, and facilitating adjustments to the proposals prior to submission.

Co-ordination of research reports

The Subprogram Leader collated progress and final reports from projects within the each year for delivery in a common format to FRDC. These reports were distributed to members of the Steering Committee for comment and review.

Review of research progress and direction

The RLPHS Steering Committee interviewed Principal Investigators of each project within the Subprogram twice annually as part of the Steering Committee meetings. Principal Investigators were expected to report progress against contracted milestones, justify any changes in research direction, and demonstrate the research program was making a valuable contribution towards the achievement of the subprogram objectives. The Steering Committee made recommendations to the FRDC Board in relation to potential changes to the objectives of the research program, or instances where project progress is unsatisfactory.

Co-ordination of research extension

A major function of the Subprogram Leader was the organisation and delivery of an annual research workshop to highlight the activities and outputs of the RLPHS. Workshops were convened with presentations from invited speakers and researchers aimed at delivering key messages to end-users for use in practical rock lobster aquaculture and enhancement systems.

The Subprogram Leader compiled a subprogram newsletter at least annually or as required highlighting research outcomes, developments in rock lobster post-harvest processes and events relevant to the RLPHS. The Subprogram Leader was also be responsible for the approval of all media releases and scientific publications arising from research projects within the Subprogram using the RLPHS Steering Committee communication policy as a guide.

Liaison with FRDC

The Subprogram Leader was the conduit for communications between FRDC and Subprogram participants in relation to project contracts, project reports, new submissions and general correspondence. The Subprogram Leader also represented the RLPHS at the annual FRDC FRAB and Subprogram meetings in Canberra.

Results/Discussion

Subprogram mission and content

At the time of establishment, the RLPHS consisted of the following projects:

1994/134.02: Code of Practice

Principal Investigator: Mr Richard Stevens
Abercromby Management Services P/L
21 Eckford Way
Duncraig ,W.A. 6023

Project Objectives:

To produce a Code of Practice for the handling of rock lobster.

1996/344: Physiological studies of stress and morbidity during post-harvest handling and storage of western rock lobster: 11. Standard autopsy techniques and immune system competency

Principal Investigator: Dr Louis Evans
Curtin University of Technology
Aquatic Science Research Unit
GPO Box U1987
Perth W.A. 6845

Project Objectives:

1 To identify suitable immune system parameters which can be used to evaluate stress responses and health status in captive lobsters and to apply those parameters in a study of stress induced by post-harvest handling procedures.

2 To investigate the causes of mortality in captive lobsters held in processing factories. This study will focus on bacteriological and histopathological examinations and will result in the development of a standard protocol for the autopsy of lobsters.

3 To evaluate the influence of temperature change on immunological and physiological stress responses.

4 To study the influence of hormonal secretions on immunological and physiological stress responses.

5 To investigate innovative techniques which will boost immunocompetence but not adversely affect marketability of live product.

1996/345: Physiological studies of stress and morbidity during post-harvest handling and storage of western rock lobster: 1 Physiological Stress Indicators

Principal Investigator: Dr Brian Patterson
Centre for Food Technology
Department of Primary Industries
19 Hercules St., Hamilton Qld 4007

Project Objectives:

1. Identify key physiological stress parameters that either describe stress levels and/or predict likely further mortality in lobsters after harvest and apply these parameters in studies aimed at improving post-harvest handling practices.

2. Obtaining baseline measurements of physiological parameters in resting undisturbed lobsters, with reference to interactions between season and locality and the effects of moult stage and other biological variables.

3. Identifying physiological parameters, through field studies aimed at studying the effect of harvest and post-harvest handling on lobsters, which can be used to evaluate deviations from baseline values in captive lobsters.

4. Identifying physiological parameters through controlled laboratory experiments using identified stressors which can be used to evaluate deviation from baseline values in captive lobsters.

5. Develop simple methods of measuring one of the stress parameters identified in objectives 3 and 4 for use in lobster processing factories in the evaluation of stress levels in selected lobster shipments.

6. Apply the results and understanding of harvest and post-harvest handling gained from the field work in objective 3, and the stress parameters identified in objectives 3 and 4, in a study or studies of lobster post-harvest handling practices aimed at developing improved post-harvest procedures.

7. Use the findings of earlier sub-objectives to make recommendations for improvements in handling practices described in the recently published Code of Practice.

8. Use the findings to develop detailed knowledge and understanding of the physiological processes involved in the stress responses in lobsters which can be used by processing companies and fishers to devise improved methods of post-harvest handling and transport.

1999/202: Rock lobster autopsy manual

Principal Investigator: Dr Louis Evans
Curtin University of Technology
Aquatic Science Research Unit
GPO Box U1987
Perth W.A. 6845

Project Objectives:

The publication of an autopsy manual to be used in the lobster industry.

2000/250: Facilitation, administration and promotion of the post-harvest Subprogram

Principal Investigator: Dr Bruce Phillips
Curtin University of Technology
Muresk Institute of Agriculture
GPO Box U1987
Perth W.A. 6845

Project Objectives:

1 Co-ordinate the FRDC rock lobster post-harvest Subprogram.

2 Conduct an annual research workshop to present outcomes from the Subprogram and to define research objectives for subsequent years.

3 Facilitate travel of the Subprogram project principal investigators, industry representatives and Subprogram leader to biannual scientific committee meetings.

4 Facilitate travel of the industry representatives, Subprogram leader of the Enhancement and Aquaculture Subprogram, and Subprogram leader to biannual Steering Committee meetings.

5 Co-ordinate the preparation of Subprogram media releases and workshop publications.

6 Integrate with other FRDC funded rock lobster research programs including the Enhancement and Aquaculture Subprogram.

7 Coordinate the preparation and distribution of a biannual Subprogram newsletter.

8 Develop and maintain a strategic plan for post-harvest rock lobster research.

9 Develop a strategic plan for the Subprogram.

New Projects added to the Subprogram

2000/251: Development of a method for alleviating leg loss during post-harvest handling of rock lobsters

Principal Investigator: Dr Glen Davidson
Geraldton Fishermens Cooperative (originally Department of Zoology,UWA)
PO Box 23
Geraldton WA 6531

Project Objectives:

1. To identify a cold-water immersion treatment that rapidly immobilises western rock lobsters, while allowing swift recovery from immobilisation upon return to ambient temperature seawater. To investigate the effect of season/acclimation temperature on effectiveness of cold stunning in western rock lobsters. To investigate the use of sea sprays vs. immersion for cold stunning in western rock lobsters.

2. To investigate, in captivity, the effectiveness of the preferred treatment (identified in objective 1) for reducing leg loss in western rock lobsters.

3. To test the accuracy of factory grading of cold stunned western rock lobsters vs. untreated controls.

4. To describe the occurrence of leg loss, morbidity and mortality of western rock lobsters subjected to cold stunning prior to episodes of handling during the post-harvest process (i.e. at the time of pot-pulling and sorting, prior to factory grading) and to compare these to the performance of animals handled using current methods.

5. To investigate the effects of multiple simulated pot capture and release events, either with or without cold stunning, on growth, leg loss and survival of undersized western rock lobsters.

6. To compare, in captivity, effects of handling, with and without cold stunning, on the reproductive success of setose, tar spot and ovigerous female western rock lobsters. To investigate the effects of limb loss on the reproductive success of female western rock lobsters.

7. To conduct a survey to determine the extent and nature of leg loss in the southern rock lobster fisheries of Tasmania and South Australia.

2000/252: Optimising water quality in rock lobster post harvest

Principal Investigator: Dr Stephen Battaglene (originally Dr Brad Crear)
University of Tasmania
Tasmanian Aquaculture and Fisheries Institute
Marine Research Laboratories
Nubeena Crescent, Taroona, Tasmania, 7053

Project Objectives:

1. Production of a manual on optimising the provision of oxygen during rock lobster post-harvest processes.

2. Determine the median lethal concentration (LC-50) of ammonia to adult southern and western rock lobsters (stressed and unstressed).

3. Determine the physiological consequences of exposing lobsters to sub-lethal ammonia concentrations, and the consequences of further exposing lobsters to acute post-harvest stressors.

4. Production of a manual on ammonia problems during rock lobster post-harvest processes.

Quantifying and controlling hyper- and hyposaline-induced post-harvest leg autotomy in the western rock lobster

2001/255 Principal Investigator: Dr Glen Davidson
Geraldton Fishermen's Cooperative
PO Box 23
Geraldton WA 6531

Project Objectives

1. Survey salinity concentrations of surface films on individual lobsters and on relevant contact surfaces on boats and within factories.

2. Describe the relationship between autotomy and exposure to seawater of various salinities for lobsters of various sizes and moult stages.

3. Quantify leg loss during industry standard freshwater "drowning" procedures.

4. Compare responses to ionic and non-ionic solutions to elucidate the potential role of other contaminants, and the possible nature of the receptors and stimuli.

5. Investigate the relationship between daily environmental conditions and levels of post-harvest leg loss.

6. Field-test practical solutions for hyper/hyposaline-induced autotomy and make recommendations to industry.

2001/235 Striking a balance between melanosis and weight recoveries in western rock lobster

Principal Investigator: Dr Hannah Williams
Curtin University of Technology
School of Public Health
GPO Box U1987
Perth W.A. 6845

Project Objectives:

1. To establish the impact of temperature and food additives on the activity of *Panulirus cygnus* haemolymph phenol oxidase (PO) in vitro.

2. To establish the impact of current commercial practices on weight recovery and melanosis formation.

3. To establish the impact of post-harvest transportation on PO activity, weight recovery and melanosis formation.

4. To determine the effects of anti-browning agents on weight recovery and melanosis formation.

5. To validate the use of experimentally determined cooking profiles for improvement of cooked weight recoveries and prevention of melanosis.

6. To formulate recommendations and guidelines that will enable industry to apply the findings of the study.

2002/237 Code of Practice

Principal Investigator: Mr Richard Stevens
Abercromby Management Services P/L
21 Eckford Way
Duncraig ,W.A. 6023

Project Objectives:

To produce a Code of Practice for the handling of rock lobster.

2002/238 Quantification of shell hardness in southern rock lobster

Principal Investigator: Dr Caleb Gardner
University of Tasmania
Tasmanian Aquaculture and Fisheries Institute
Marine Research Laboratories
Nubeena Crescent, Taroona, Tasmania, 7053

Project Objectives

1. To calibrate the rate of change in shell hardness before and after the moult of southern rock lobsters relative to lobster size, sex, region and temperature.

2. To identify the region of the exoskeleton that is most suited for measuring hardness.

2002/239 The effect of on board cold water stunning on the survival and growth of caught and returned western rock lobsters (*Panulirus cygnus)*

Principal Investigator: Dr Glen Davidson
Geraldton Fishermen's Cooperative
PO Box 23
Geraldton WA 6531

Project Objectives

1. To determine the effect of commercial capture with or without cold-stunning on the survival and growth of returned protected western rock lobsters.

2. To observe and film in the wild the behaviour of western roc lobsters caught and returned with or without cold-water stunning.

Subprogram identity

The RLPHS logo was developed as a clear identifier for all Subprogram documents.

Subprogram management and operating procedures

To ensure that research conducted within the Subprogram was relevant and met the above criteria, a Steering Committee was established to:

1. Provide industry feedback and views;
2. Review existing research based on FRDC contractual obligations;
3. Prioritise new proposals and provide a priority list for other agencies;
4. Ensure outcomes are commercially focussed;
5. Co-ordinate industry and research provider involvement - optimum use of resources;
6. Facilitate extension and technology transfer.

After a selection process including FRDC endorsement, membership of the Steering Committee included Bruce Phillips (Chair), Patrick Hone (FRDC), Glenn O'Brien (WA), Stephen Hood (WA), Nick Polgeest (VIC), Richard Stevens (WA), Kym Redman (SA), Rodney Treloggen (TAS) and Robert van Barneveld (FRDC Rock Lobster Enhancement and Aquaculture Subprogram).

The Steering Committee met in approximately March and September each year to review project progress and establish research priorities. Advice from the September Steering Committee meetings was sent to all Fisheries Research Advisory Bodies so that they were aware of the subprogram research priorities. All new projects relating to rock lobster enhancement and aquaculture were assessed by the Steering Committee and were submitted to the FRDC Board via the subprogram. An annual subprogram workshop was held each March to extend research results to industry and researchers.

In addition to the RLPHS Steering Committee, a Scientific Committee was established to:

- To conduct scientific reviews of all projects; ensuring that research to be undertaken is achievable;
- To ensure scientific objectives are met;
- To foster and develop collaboration;
- To coordinate new funding applications.

The Scientific Committee reported to the Steering Committee through the Subprogram Leader.

The participants of the Scientific Committee consisted of the Subprogram Leader, Principal Investigators of the subprogram component projects and direct industry collaborators (as required). Tenure was dependent on the funding term for a particular project. The Scientific Committee met biannually, and if possible, the meetings were held to coincide with other events to minimise travel costs. An annual research workshop ensured that research results and project progress was disseminated to a wider audience and that all members of the rock lobster aquaculture industry and other interested parties could benefit from the research.

Principal investigators were also brought to communicate directly with the Steering Committee via interviews at the Steering Committee meetings and to only hold informal meetings on scientific content of the projects.

Scientific and Steering Committee meetings

Six Steering Committee, and three Scientific Committee meetings were held between July 2000 and June 2003. All meetings were minuted and actioned. Detailed copies of the minutes have not been included in this report for the sake of brevity and the confidential nature of some of the discussions.

Subprogram workshops

A workshop was been convened in New Zealand in conjunction RLEAS and with the New Zealand Rock Lobster Industry Council between April 2 and 7, 2001. A full set of proceedings was produced from this workshop.

A full list of publications arising from workshops is contained under References.

Communications

The RLPHS Steering Committee facilitated the orderly release of information produced by, and meeting the needs of Subprogram participants.

All media releases, publications and presentations produced as a result of Subprogram activities were vetted by the Subprogram Leader

Subprogram Newsletter:

- The Subprogram Leader before distribution edited the Newsletter.

Workshop Proceedings:

- Researchers supplied a disk copy of presentations from the workshop as well as more comprehensive supplementary documentation to include in the proceedings within 2 weeks of the workshop.

Scientific Publications:

- Were developed with the input of all appropriate co-authors
- Were submitted through the normal publications review channels of the institution of the primary author
- Were then provided to the Subprogram Leader who examined it and provided comments back to authors.

Subprogram Media Releases:

- All media releases should have been sent to the Subprogram Leader via e-mail for review and distribution to the Steering Committee
- Once reviewed by the Steering Committee any suggested editing were forwarded to the Subprogram Leader for collation
- All editing was then forwarded to the author for preparation of a final draft
- A proof of print media was sought by the Subprogram Leader prior to release. If the media outlet was not prepared to release the proof of the text, then approval will not be granted for publication. However, this was not always possible.
- A transcript of radio and television interviews was requested or heard by the Subprogram Leader prior to release. If the media outlet is not prepared to release a transcript, then approval will not be granted for release. However, this was not always possible.

Unsolicited Media Enquiries/Interviews:

- Where possible arrange an appropriate future time to discuss the topic so that a brief note can be circulated via e-mail to the Steering Committee detailing;
- Who the media contact is and what organisation they represent
- The topic to be covered
- Details of what issues will be discussed
- Discussion should be restricted to research you have or are conducting (refer to appropriate scientist if required) not issues of a policy or political nature
- Upon completion of the interview a brief summary of what transpired should be sent to the Subprogram Leader
- It should be noted that planned media releases are the preferred option whenever possible.

Subprogram Conference Presentations:

- The conference presentation abstract should be sent to the Subprogram Leader via e-mail for review and distribution to the Steering Committee
- The final conference presentation should have been sent to the Subprogram Leader via e-mail for review and distribution to the Steering Committee
- The final conference paper should have been sent to the Subprogram Leader via e-mail for review and distribution to the Steering Committee
- All conference presentations should have used the standardised Subprogram presentation format.

Copies of the newsletter prepared during the course of this project have been included in **Appendix 4.**

Subprogram publications

A special effort is made by the Subprogram to produce publications, which can be readily used and implemented by industry. Each publication is carefully vetted by the Subprogram Leader and then the Steering Committee to ensure the text and form of presentation are in a suitable form.

The Subprogram has now released the following publications:

> Optimising Water Quality, Bradley Crear and Grant Allen
>
> Recirculating Systems NH_3, Bradley Crear, Jennifer Cobcroft and Stephen Battaglene
>
> Rock Lobster Health and Diseases: A Guide for the lobster industry, Frances Stephens, Seema Fotedar and Louis Evans
>
> Best Practice in the Western Australian Lobster Industry, WAFIC, Richard Stevens

Each of these has been well received and is in constant demand. Each of these is included in **Appendix 7**.

The publication, Best Practice in the Western Australian Lobster Industry, produced by WAFIC, is also available as a Video or DVD.

Subprogram Web site

A web site was established for the Subprogram. Information on the Rock Lobster Post-Harvest Subprogram including all of the newsletters can be accessed by visiting the web-site www.frdc.com.au/research/programs/rlph/index.htm

Annual Operating Plans

Three annual operating plans for the RLPHS were prepared over the course of this project. A copy of the 2002 annual operating plan has been included in this report (see **Appendix 6**).

Priority setting and new research projects

Project selection and development was conducted by the Subprogram Leader working with the Steering Committee.

All proposed projects were examined by the Subprogram Leader before presentation before the Steering Committee. Comments from the Steering Committee were provided direct to the proposed Principle Investigator on the day, and then in writing after the meeting. Draft copies of the revised proposals were subsequently circulated to the members of the Steering Committee for comment, prior to submission to FRDC.

Collaboration and additional funding opportunities

Additional funding opportunities were investigated for the Subprogram.

Additional funding was provided from the Geraldton Fishermen's Cooperative, the Western Rock Lobster Development Association, Development and Better Interest Fund, an Industry Development Unit of WAFIC in Western Australia, in support of the leg loss and hypo-salinity studies.

Benefits

An independent Subprogram Leader, and a highly responsive Steering Committee that is composed of industry experts from across Australia have provided an effective and efficient system for directing relevant research activities to ensure continued and increased profitability for the Australian rock lobster fisheries. The Steering Committee, under the Subprogram Leaders direction, worked as an integrated group, rather than a collection of individuals, and carefully selects and recommends projects for funding, and then follows their progress and offers advice to principal investigators in the national interest. The industry representatives on the Steering Committee have also acted to provide research facilities within the industry as the best locations to conduct research, and ensuring the uptake of successful projects as soon as they occur.

The research conducted by the Subprogram has significantly improved Australia's understanding of the physiology and biochemistry of lobsters, from the time of capture through to processing in a variety of product forms. This is of considerable assistance in investigating methods of better handling, cooking, and/or processing lobsters for live export.

Studies to alleviate leg loss in western rock lobster have yielded impressive results. However, the introduction of cold-water stunning is not yet endorsed by the Subprogram, as we are awaiting confirmation that it will not cause increased mortality on undersized lobsters, not adversely effect the egg production or cause increased mortality of breeding females.

The dramatic effects of hypo-salinity in causing leg loss has been a major achievement, and its rapid take up by industry will cause a major reduction in leg loss and a consequent multi million dollar increase in value of the fishery.

A major method of communicating research results from the Subprogram has been the Code-of-Practice. The newly revised Code incorporates in subtle way the best ideas from the results in a form endorsed by the industry to increase their safety and profitability.

Studies of the methods of cooking of western rock lobsters, with the intention of alleviating the blackening of the flesh after thawing, have already indicated the source of the problem and a likely solution. Further tests will be undertaken to confirm if the solution can be carried out under commercial conditions.

Further Development

As a result of this project, a further submission was made to FRDC for continuation of the Rock Lobster Post-Harvest Subprogram. This project was funded and will be on-going until June 2006, conditional on the Board support following a review to be conducted by the FRDC after the first year.

Planned Outcomes

The first priority of the Subprogram was to increase the value of the rock lobster production in Australia. The dramatic success of the alleviation of appendage loss project has clearly demonstrated that the research undertaken by the Subprogram will achieve this outcome. These studies are not fully completed but have already affected boat design, and improved handling practices on the boats and operations in the processing factories.

Because of the reduced appendage loss, improved prices area possible for the lobster catch, and the industry can better select its markets for its products.

The Subprogram structure has increased the level of cooperative research on areas of industry interest, mainly because of their meeting together at the time of the annual workshops and when presenting progress reports of research to the Subprogram Steering Committee. These direct presentations by the researchers to industry have significantly improved cooperation and mutual respect between the two groups, and led to more rapid uptake by industry of research outcomes. All Subprogram publications are carefully edited by the Steering Committee before release to ensure that the material is in a suitably form to ensure this uptake.

Research results have been adopted into the newly released revised Code of Practice for the Western Rock Lobster Fishery, and will similarly be adopted into the planned Code of Practice for the southern rock lobster fisheries.

The beneficiaries of the research conduced by the Subprogram have been the rock lobster fishing industry including both fishers and processors, and the State and Federal agencies responsibly for the rock lobster fisheries.

Conclusion

An independent Subprogram Leader, and a highly responsive Steering Committee that is composed of industry experts from across Australia have made this possible. The Steering Committee, under the Subprogram Leaders direction, worked as an integrated group, rather than a collection of individuals, and carefully selects and recommends projects for funding, and then follows their progress and offers advice and assistance to the principal investigators, in the national interest. The industry representatives on the Steering Committee have also acted to provide research facilities within the industry as the best locations to conduct research, and ensuring the uptake of improved post-harvest handling practices as soon as they are identified.

This is a very successful subprogram and strongly supported by industry, particularly in Western Australia. The Subprogram publications:

Optimising Water Quality, Dr Bradley Crear and Dr Grant Allen

Recirculating Systems $NH_{3,\ Dr}$ Bradley Crear, Dr Jennifer Cobcroft and Dr Stephen Battaglene

Rock Lobster Health and Diseases: A Guide for the lobster industry, Dr Frances Stephens, Seema Fotedar and Professor Louis Evans

Best Practice in the Western Australian Lobster Industry, WAFIC, Richard Stevens

Are well received by industry in both Australia and New Zealand, and we receive requests for these publications from many other countries.

The newsletter has also been successful. The format is straight forward, and includes a range of material in each issue. We printed about 4500 of the first two issues, but the demand has increase to approximately 8000 per issue.

The industry has strongly supported the application to FRDC for renewal of the Subprogram, clearly indicating there appreciation of its value to the rock lobster industry.

References

1 In the August September 2003 Edition of *Fishing Today* the "FRDC Rock Lobster Post-Harvest Subprogram" was described.

2 In the February/March 2003 Edition of *Fishing Today* the project by Brad Crear and Mark Powell on "Optimising water quality in rock lobster post-harvest processes", is described.

3 A final report of a project "Application of health indices of dietary regimes and live transport stressors in the southern rock lobster, *Jasus edwardsii.*" is available. This has been submitted to FRDC by Dr Louis Evans and colleagues.

4 Several papers presented at the Lobster Health Management Symposium in Adelaide are to be published in 2001 by the Aquatic Science Research Unit, Muresk Institute of Agriculture, Curtin University of Technology. Enquires should be sent to this Unit.

5 Jussila, J., McBride S., Jago, J. and Evans, L. H. (2001) Hemolymph clotting time as an indicator of stress in western rock lobster (*Panulirus cygnus* George). Aquaculture 199, 185-193.

6 Paterson, D. B., Davidson, G.W. and Spanogue, P. T. (2001) Identifying stress when western rock lobsters are stored out of water: The average and individual blood lactate concentrations. Contact Aquatic Science Research Unit, Curtin University of Technology, Perth.

7 Paterson, D. B., Davidson, G.W. and Spanogue, P. T. (2001) Measuring total protein concentration in blood of the western rock lobster (*Panulirus cygnus* George). Contact Aquatic Science Research Unit, Curtin University of Technology, Perth.

8 Powell, M., Crear, B. and Allen, G. (2001) Lobsters in the toilet: Acid-base effects of ammonia exposure in the spiny lobster *Jasus edwardsii* . Presented at the Australian and New Zealand Society of Comparative Physiology and Biochemistry , Adelaide, December.

The following are the papers presented by members of the Subprogram at the Workshop in Cairns in May 2002:

Physiological studies on stress and morbidity during post-harvest handling of western rock lobsters: Prof Louis Evans (Curtin University of Technology, WA)

Physiological stress indicators: Dr Brian Paterson (Queensland Department of Primary Industries)

Optimising water quality for live holding of rock lobsters: Dr Bradley Crear (TAFI, Tasmania)

Alleviating leg loss in western rock lobsters: Dr Glen Davidson (Geraldton Fishermen's Cooperative)

Effects of hyper and hyposaline seawater on leg loss: Mr Wayne Hosking (Geraldton Fishermen's Cooperative)

Striking a balance between melanosis and weight recoveries in western rock lobsters: Ms Hannah Williams (Curtin University of Technology)

A recent scientific paper of interest:

Williams, H. G., Davidson, G. W. and Mamo, J. C. (2003). Heat –induced activation of Polyphenoloxidase and western rock lobster (*Panulirus cygnus*) hemolymph: Implications for heat processing. Journal of Food Science 68: 1928-1932.

Appendices

Appendix 1-Intellectual Property

As a result of this project, a further submission was made to FRDC for continuation of the RLPHS. This project was funded and will be on-going until June 2004.

Appendix 2 - Staff

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Curtin University of Technology, GPO Box U1987, Perth, WA 6845, Australia
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Appendix 3-Workshop Publications

Proceeding of the Workshop held in New Zealand

Proceedings of the Workshop held in Cairns

RLEAS Workshop 2001

Developments in Rock Lobster Enhancement and Aquaculture III

RLEAS Publication No. 6

Edited by Dr Robert van Barneveld

April, 2001

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ROCK LOBSTER ENHANCEMENT & AQUACULTURE SUBPROGRAM

Proceedings of the Third Annual Rock Lobster Enhancement and Aquaculture Subprogram Workshop – Wellington, New Zealand, 2001

Venue

"Te Papa"

Wellington, New Zealand

April 4, 2001

The Rock Lobster Enhancement and Aquaculture Subprogram is supported by the Fisheries Research and Development Corporation.

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Enquiries or questions relating to this document should be directed to Dr Robert van Barneveld, RLEAS Leader, c/- Barneveld Nutrition Pty Ltd, 19-27 Coonan Rd, South Maclean, QLD, 4280.

Ph: 07 5574 8611 Fax: 07 5547 8624 Email: robvanb@dove.net.au

Strategic directions for Australasian rock lobster enhancement and aquaculture research.

Robert van Barneveld

Leader, RLEAS

Why is there interest in rock lobster aquaculture in Australia ?

The Australian rock lobster fishery is an important marine resource making up 25% of Australia's total fishery landings and presently worth around $450 million per annum. However, while many are currently well managed, most Australian rock lobster fisheries are at their maximum sustainable capacity. Small increases in the value of production may be made in the future by increasing sales of live lobsters and/or targeting periods of high demand, but total gains are likely to be minimal. The real potential for significant growth appears to be through some form of aquaculture.

What form could rock lobster aquaculture take in Australia ?

Rock lobster aquaculture could proceed in a number of ways:

1. On-growing of adults through a moult to increase weight whilst allowing sale at periods of peak demand/ value,
2. On-growing of wild-caught puerulus (newly-settled juveniles) to a small (and potentially very valuable) market size of around 200-300 g, and
3. Culture of phyllosoma from eggs through the 11 larval stages to puerulus and subsequent ongrowing to market size as above.

4. In addition, the potential exists through improved survival rates, for aquaculture to provide stock for reseeding and enhancement of the wild fishery.

Ongrowing of adults

Investigations into the ongrowing of adult southern rock lobsters (*Jasus edwardsii*) have been ongoing since 1994, mainly in South Australia. The lobsters are held in cages at sea and are presently being fed with natural diets (trash fish/mussels). There is the potential to achieve weight gains of around 20% by growing the animals through the annual moult, representing a 60% return on investment. Some difficulties have been encountered with the renewal of leases for sea cages in South Australia, and hence interest in this form of aquaculture is changing focus to land-based raceway systems.

Ongrowing of wild-caught juveniles

In Tasmania (southern rock lobster, *J. edwardsii*), Western Australia (Western rock lobster, *Panulirus cygnus*) and Queensland (tropical spiny lobster, *Panulirus ornatus*) there is a growing interest in the potential for capturing wild puerulus and ongrowing them to a small market size. The basis for this is that there is thought to be high mortality of wild puerulus in their first year post settlement (anywhere from 75-97%) . However, recent results are showing that, if these animals are brought ashore and ongrown in tanks, the mortality is minimal (2% in Tasmania). Therefore the theory is that aquaculturists can ongrow the 'excess' that would have died in the wild. The animals are caught in collectors deployed at sea and quite large numbers have been caught in Tasmania.

A major issue associated with the on-growing of wild caught juveniles is how to compensate the wild fishery for their removal. This has been addressed in Tasmania after considerable discussion, by an agreement that 25% of all captured puerulus will be returned to the wild after one year of growth in captivity. This will ensure that there is no negative impact on the wild fishery and in fact, will probably lead to enhancement of the fishery. The success of this method obviously depends on the survival rate of released juveniles.

Culture of puerulus from eggs

Spiny rock lobsters have a complicated life cycle. The eggs hatch as tiny spider-like transparent larvae or phyllosoma. The phyllosoma drift in ocean currents for up to two years until they are ready to settle on a substrate and metamorphose into puerulus. The phyllosoma phase involves 11 distinct morphological stages and up to 17 moults (*J. edwardsii*). Culture of phyllosoma to puerulus has been successfully achieved in Japan and New Zealand in very small numbers. The phyllosoma can be fed on Artemia or chopped mussel flesh, but nutrition seems to be the major problem. The time to settlement can be greatly

reduced in culture compared to that of wild larvae. In the long term, the culture of lobsters from eggs may prove to be the answer to the future sustainability of rock lobster aquaculture.

In all cases there are many issues that require further research before rock lobster aquaculture can become a commercial reality. Although there are a number of commercial groups on-growing adult rock lobsters, there is presently no commercial aquaculture of puerulus or larvae in Australia.

Why do we need a pro-active research program ?

The Australian rock lobster fishery is a valuable resource because it represents a unique source of a number of high quality, high value rock lobster species grown in a pristine environment and hence sought after in many markets. It is clear that a number of overseas countries are investigating the potential for rock lobster aquaculture and if successful in developing aquaculture systems through closure of the life cycle, the current Australian market for spiny lobsters could be threatened. Success in culturing lobsters overseas would also result in a "reactive" research program in Australia that may not result in beneficial outcomes for all potential stakeholders in the rock lobster industry. Further to this, given the diversity of rock lobster species in Australia it is unlikely that development of aquaculture systems will be the same in each state, hence a coordinated research approach is desirable to ensure limited research resources are optimised and that the Australian industry as a whole pursues common goals.

- Development of a proactive Australian research program for rock lobster enhancement and aquaculture allows:

- Representatives of the wild capture fishery and aquaculture sector to work together to ensure the highest research priorities are pursued and that all consequences associated with the development of a rock lobster aquaculture sector are considered;

- An national approach to the research using rock lobster species from across Australia;

- Development of strategic international research alliances with countries such as New Zealand who have an advanced research program, and who share similar industries issues to Australia;

- An identifiable centre for rock lobster aquaculture research and a focal point for representatives of the wild fishery, aquaculture sectors and government agencies.

What is the role of the Rock Lobster Enhancement and Aquaculture Subprogram ?

The Fisheries Research and Development Corporation established the Rock Lobster Enhancement and Aquaculture Subprogram (RLEAS) in July, 1998 following consultation with industry and scientists. The Subprogram was established with the following objective or "mission":

"To provide technology for use in Australian rock lobster enhancement and aquaculture systems so they can be internationally competitive and can operate in harmony with the wild fisheries".

What would we like to achieve through the Rock Lobster Enhancement and Aquaculture Subprogram

Delivery of technologies that facilitate the development of a viable rock lobster aquaculture industry in Australia, with adequate consideration and contingency for:

- Protection of the wild fishery in terms of economic and social viability;
- Neutral or positive impact on the wild fishery in terms of stock numbers;
- Commercial viability of closing the life cycle of rock lobsters;
- Increasing profitability and wealth for Australasia;
- Economic and marketing assessments to ensure aquaculture and wild fishery products do not compete for common markets.

What are the specific requirements and subsequent research needs to develop successful rock lobster aquaculture and enhancement systems ?

Species selection for aquaculture

- Viable rock lobster species with a short larval phase, fast growth potential at high densities, disease resistance and high market value that does not compete with existing wild capture sectors for common markets.
- Species well suited to aquaculture that is native to the wild fishing sector in the region of the aquaculture enterprise.

At present, research is focussing on dominant wild capture species, while perhaps more resources could be directed towards comparative morphology and aquaculture potential of other less common species, particularly some of the tropical species.

Puerulus collection

- Collect large numbers of puerulus from the wild for on-growing in aquaculture systems.
- Identify when surplus numbers of puerulus exist so that they can be captured and on-grown to increase survival rates.
- Minimise labour inputs for collection.
- Alternative sites for puerulus collection in conjunction with existing aquaculture enterprises.

Research in Western Australia and Tasmania has examined the development of collection methods for puerulus from the wild. Large 'fluffy' collectors set at different depths and in different areas off the Western Australian coast have been trialed with varying levels of success. This research has recently been extended to Tasmania where a number of different collector types on long lines are being examined. The Western Australian research has been hampered by low puerulus settlement, but has found that inshore collectors are more successful than those situated at a distance off shore. In Tasmania a survey of commercial aquaculture facilities has revealed some interesting spatial patterns of settlement on submerged structures while the research component has led to the development of suitable cost-effective collector types.

Biological neutrality

- Collect puerulus from the wild without an influence on the number of mature lobsters reproducing or the viability of the wild fishery.

Historical data on the settlement of puerulus in specific areas in Western Australia has been used to assess the potential impact of puerulus removal on subsequent wild populations of adult lobsters. Extensive statistical analysis has been employed to assess a range of scenarios. The large numbers of puerulus involved and the high mortality rates in the regions examined suggest that removal of puerulus would have a minimal impact on settlement rates in these regions.

Larval rearing/Propagation

Closure of the rock lobster life cycle to permit commercially viable culture of lobsters from eggs to adults through:

Pre-Breeding

- Ensure broodstock are collected/maintained at adequate size/condition.
- Optimise nutrition, environmental conditions for maintaining condition of broodstock.
- Know the history of individual broodstock
- Optimise health.

Breeding

- Ensure correct ratio of males:females
- Provide optimum conditions for incubation, mating
- Mate at correct time for successful fertilisation
- Maximise gamete production
- Disease control, maximise hygiene

Hatch-out stage

- Provide a system to catch/select larvae
- Provide adequate conditions for hatching out

Larval phase

- Maximise survival
- Optimise growth
- Synchronise development (eg moults)
- Minimise length of larval phase & selection to achieve high quality puerulus
- Most efficient culture system

Puerulus Phase

- Maximise moults
- Appropriate settlement conditions

Nursery/Juvenile Phase

- Optimise growth
- Maximise survival
- Grading to minimise size differences
- Transport from nursery and deliver to market or grow-out or wild

- Optimise conditions & preparation for grow-out
- Wean onto a suitable diet

Others

- Health (optimise)
- Efficiency of mass production systems (particularly larval phase)
- Optimum nutrition
- Minimise cost/unit production
- Minimise environmental impact
- Select appropriate site

Broodstock selection

- Selection of broodstock
- Maintenance for broodstock
- Initiate breeding program
- Optimise genetic strategy
- Optimise genetic integrity

Research on propagation of the southern rock lobster in Tasmania has resulted in the successful culture through 10 of it's 11 larval stages in around 9 months. The outcomes of an International Workshop organised by the Tasmanian Aquaculture and Fisheries Institute through the Rock Lobster Enhancement and Aquaculture Subprogram suggest that the problems involved in larval rearing make it a risky proposition. However, economic and biologically feasibility on a commercial scale do appear to be achievable. Subsequent research on the rearing of rock lobster phyllosoma suggests that the nutrition of these larval stages is limiting and new techniques need to be developed for the delivery and improved utilisation of feeds. It is hypothesised that nutritional status of the phyllosoma towards the end of their larval phases has a significant influence on settlement.

Nutrition

- Multiple sources of nutrients for all growth phases.
- Robust diets that can be altered depending on the availability of raw materials while still supplying the same nutrients.
- Commercially produce a manufactured diet that is water stable, attractive, easy to handle, store and transport, shelf stable and cost-effective.
- Diets suitable to support optimum growth of all phases of the production cycle.

- Minimal impact on the surrounding water quality through nutrient loads.
- Diets that support optimum survival of juveniles during their first year of development.
- Nutritional manipulation of moult cycles.
- Nutritional enhancement of lobster product quality in live-held adults.

Nutrition research is being conducted on tropical, western and southern rock lobsters. All stages from early juveniles through to adults are being examined and cost effective manufactured diets are being evaluated. Recent work has shown that the protein content of diets for southern rock lobsters should be approximately 450 g/kg with lipid levels around 100 g/kg. The most noteworthy outcomes of nutrition experiments in all states to date are the apparent superiority of mussels over other diets and the distinct colour differences observed in lobsters fed different diets. It has been demonstrated that inclusion of approximately 100 mg/kg of carotenoids in southern rock lobster diets produces lobsters which are close to the natural colour of wild caught juveniles.

On-growing of juveniles and system requirements

- Cost effective tanks or sea-based holding cages that promote optimal growth and feed conversion performance.
- Husbandry procedures to ensure optimal growth (stocking density, water temperature, water quality, light, feeding regimes etc).

On-growing of juvenile rock lobsters takes place in tanks and the animals grow best on a diet of fresh mussels. Artificial diets are readily accepted, but the growth and survival rates are not as good as with mussels. Despite this, artificial diets do support exceptional growth of southern rock lobsters if supplemented with mussels three times per week. Hides are placed in the tanks to reduce cannibalism that can occur at the moult. Under these conditions a marketable size can be reached in 2 years (Tasmania) or a weight of 500 g in 1 year (Queensland) with very low mortality levels. System design research is defining environmental requirements of juvenile and adult tropical and southern rock lobsters as well as identifying system design criteria for on-growing of adults. A recent experiment in Tasmania has shown that a temperature of 18-22° C is optimal for growth and survival of southern rock lobsters. Further research is required to identify optimal growing conditions in South Australia. Both dry and moist manufactured feeds have been examined as cheap alternatives to fresh mussels, yet cannibalism at the moult and a disease causing blackening and necrosis of the tail sections requires further attention.

Health

- Disease-free aquaculture environment.
- Disease-monitoring to ensure disease transfer to the wild fishery is not possible.

In the absence of identifiable diseases, it is difficult to priortise health research. Linkages with existing health programs such as "AQUAVETPLAN" may provide the best opportunities to ensure the above requirements are met.

Economics and marketing

- Aquaculture reared lobsters of appropriate size and colour for premium markets that do not compete with existing lobster markets.
- Capacity to support premium markets for both aquaculture reared and wild caught lobsters.

Enhancement

- Reseeding of juvenile aquaculture reared juveniles that survive to increase wild fishery stocks and egg production levels.

As an adjunct to the above research in rock lobster aquaculture and enhancement, research underway in Tasmania is concentrating on the survival of wild caught on-grown juveniles after release back into the wild. Using electronic tagging methods released juveniles have been tracked for up to two weeks with no mortalities recorded.

Propagation of rock lobsters – nutrition, health and environment

Bradley Crear

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The FRDC project 99/315 (The development of rock lobster propagation techniques for aquaculture in Australia) was developed following a FRDC workshop (FRDC 98/300 – Hart and van Barneveld, 2000) in Hobart in 1999. The workshop was convened to discuss the potential for the development of rock lobster propagation in aquaculture systems. 99/315 was a one-year preliminary project to allow methodologies and expertise to be developed, to enhance the structure and design of a longer-term project. It was a multi-institutional project, with 6 research providers (from 3 states) being involved. Research focussed on 3 different species of rock lobster. Due to the preliminary nature of the research the objectives of 99/315 were varied:

Objective 1 - Develop an artificial diet acceptable to phyllosoma of three species of rock lobster, that is water stable and easily manipulated.
Objective 2 - Examine mass culture systems and determine environmental requirements for phyllosoma of three species of rock lobster.
Objective 3 - Develop hormonal control of moulting in rock lobsters.
Objective 4 - Determine the health status of phyllosoma of southern rock lobster under culture conditions.

The major outcomes of 99/315 were:

OBJECTIVE 1:
Characterisation of the morphology and function of the mouthparts and foregut indicate that J. edwardsii phyllosoma ingest soft fleshy foods such as gelatinous bodied zooplankton. Changes in the structural characteristics were observed with age. The results suggest that early stage phyllosoma would benefit from a diet comprising soft gelatinous items, while late stage phyllosoma are better prepared to deal with larger, fleshy prey. The observed structural characteristics should serve as a guide in the development of formulated diets.

Stage 5-11 wild-caught J. edwardsii phyllosoma that were collected from two sites in New Zealand. Changes in the fatty acid composition of J. edwardsii phyllosoma during ontogeny may reflect changes in their diet and physiology, and may also provide information on the nutritional importance of some

fatty acids. Therefore, the fatty acid data for natural phyllosoma provides a useful framework to compare with cultured phyllosoma, and live diets used in their culture.

As a first step in the production of a suitable formulated diet, exploratory work was initiated to see if a microbound diet could be developed with appropriate characteristics, such as water stability, buoyancy and attractiveness. The form of the diet was not suitable for the Stage 3-4 *Jasus edwardsii* phyllosoma it was trialed on. However, the feeding of Artemia in alginate pellets resulted in better survival and possibly growth of mid-stage larvae, suggesting that inert pellets of some form are suitable. The formulated diet will need to be redesigned to improve its physical characteristics to a form suitable to both the culture system and the developmental stage of the phyllosoma.

The size and composition of Artemia were shown to have a significant effect on the growth, survival and composition of *J. edwardsii* phyllosoma. Phyllosoma could be cultured to Stage VIII when fed only Artemia of sufficient size (□ 1.5 mm) but survival and growth were poor when fed 0.8 mm Artemia. The combination of 1.5 mm Artemia plus mussel gonad resulted in lower survival of larvae than feeding of Artemia alone (□ 1.5 mm). The lipid composition of dietary Artemia was affected the lipid composition of early stage *J. edwardsii* phyllosoma. With respect to growth and survival of the phyllosoma, low protein and HUFA, but high carbohydrate and 18:2(n-6) diets were not adequate enrichment diets, whereas high HUFA, high protein diets were.

OBJECTIVE 2:

The research investigated the propagation of southern, tropical and western rock lobsters and all 3 species were successfully hatched from eggs. All species have produced larvae successfully in captivity and larval rearing facilities have been developed in Tasmania, Western Australia and Queensland. The New Zealand upwelling system was trialed in Queensland and Western Australia. This research identified that culture systems (both mass culture and research) and methods need to be developed to suit the specific biological, environmental and behavioural requirements of each species. This research was especially important for the tropical species where there have been limited attempts at larval rearing.

OBJECTIVE 3:

This study has shown that sufficient material can be collected from the first few phyllosoma developmental stages, ie Stages I to IV, to allow a temporal analysis of hormonal content of phyllosoma before, during and after moult and metamorphosis. The extraction of hormones (ecdysteroids and methyl farnesoate) from phyllosoma and their separation into the various classes has been optimised. Day 1 phyllosoma larvae were shown to contain ecdsyone as one of the main ecdysteroids present. Methyl

farnesoate was also demonstrated to be present in phyllosomas. Further work is required to further simplify quantification of ecdysteroids and methyl farnesoate, including the development of suitable enzyme linked immunoassays.

OBJECTIVE 4:

Microbial infections appeared to have a major impact on the health status of phyllosoma in all studies. The major source of the microbial load appears to be the Artemia, therefore methods of minimising the transfer of the load to the phyllosoma need to be developed. Additionally, Artemia may be an inadequate diet therefore some of the health problems may be exacerbated because the phyllosoma are in poor nutritional condition.

The information obtained in 99/315 was used to develop further submissions to the FRDC for funding of projects focusing on nutrition and larval hormones. These projects were funded (2000/214 and 2000/263, respectively).

Project 2000/214 focuses on nutrition, as the results of 99/315 and the experience of other researchers worldwide suggest that inadequate nutrition is the most limiting factor in the production of spiny lobster larvae. It has the following research objectives:

1. To demonstrate that nutrient supply is a limiting factor in the growth and survival of rock lobster phyllosoma by the identification of:
   - 1.1 - which nutrients are critical?
   - 1.2 - which nutrients are rock lobster phyllosoma adapted to digest?
   - 1.3 - whether we can manipulate growth through manipulation of nutrient supply

2. To reduce the reliance on live feed for rearing of RL phyllosoma by the identification of:
   - 2.1 - what attractants are required to make formulated diets attractive to phyllosoma?
   - 2.2 - what factors influence consumption of formulated diets?

Like 99/315, this project is also multi-institutional, involving researchers from Curtin University, Fisheries WA, Tasmanian Aquaculture and Fisheries Institute, CSIRO (Commonwealth Scientific and Industrial Research Organisation), QDPI (Queensland Department of Primary Industries) and NIWA (National Institute of Water and Atmospheric Research – New Zealand). The collaboration with NIWA is seen as a very important development, as it will allow research synergies between Australia and New Zealand to be taken advantage of. The total number of species being studied is four (Jasus edwardsii –

southern or red rock lobster; J. verreauxi – eastern, packhorse or green rock lobster, or pawharu; Panulirus cygnus – western rock lobster; P. ornatus – tropical rock lobster).

The presentation will present major results from Project 99/315 as well as some recent results from project 2000/214.

References

Hart, P. and van Barneveld, R., 2000. Technical potential for rock lobster propagation in aquaculture system. RLEAS Publication No. 3. FRDC Project 98/300 Final Report, 98 pp.

Determination of the optimum environmental and system requirements for the growout of juvenile southern rock lobsters (Jasus edwardsii)

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The southern rock lobster, *Jasus edwardsii* is native to southern Australia and New Zealand and in both countries there is a considerable interest in its aquaculture potential. Culture methods for ongrowing need to be developed and optimised. Research into optimising culture systems and environmental requirements is being undertaken at the Tasmanian Aquaculture and Fisheries Institute (TAFI), Marine Research Laboratories. The research is funded by the Tasmanian Government and the Fisheries Research and Development Co-operation (through the Rock Lobster Enhancement and Aquaculture Subprogram).

Determination of the optimal culture conditions will to a large extent determine the methods used for growing out lobsters. It is important that the systems are developed in a cost effective manner, therefore although it would be necessary to optimise survival, it may mean that it is not economically essential to maximise growth (eg. Temperature may be kept lower than that for optimum growth). A good understanding of the effects of culture conditions will lead to the development of the most cost-effective techniques.

Juvenile southern rock lobster will approach 350g after 3 years in culture at a constant 18^0C. Survival of lobsters over 2.5 years has been greater that 85% under optimal conditions. The majority of mortalities occur during the small juvenile stages with lobsters greater than 2-g suffering little mortality. The majority of the mortalities are due to cannibalism of the newly moulted lobsters.

We have investigated the affect of the provision of hides, the affect of temperature and the affect of photoperiod on growth and survival pf small (<30g) juvenile lobsters. The provision of hides improved survival pf lobsters (98% compared with 86% over a 4 month period.) However, it did not result in increased growth. Hide design is a continuously developing process, which is influenced by factors such as lobster assize and system design. It needs to provide for lobsters needs as well as ensuring that the management techniques are practical.

Temperature is a major environmental factor affecting growth of lobsters. The effect of temperature on growth is going to define the culture system used and/or where it is sited. To date various results have been achieved, however studies in NZ and Australia had suggested an optimal temperature for growth of 18-20^{0}C. In previous studies we had determined that the growth during spring (water temperature of 13-18^{0}C) was approximately 80% that at a constant temperature of 18^{0}C. Growth during winter (10-12^{0}C) was approximately 40% of that at 18^{0}C. As we wished to optimise growth we investigated temperatures of 18,20,22 and 24^{0}C. Growth and survival was reduced at 24^{0}C. The results suggest the lobsters were suffering thermal stress and that 24^{0}C is close to the upper thermal limit. The optimum temperature range was calculated to be 19-210C. Culturists would need to consider the economic advantages of lobsters reaching market size in the shortest possible time against increased costs associated with heating water.

Photoperiod was shown to affect growth but have no affect on survival. Lobsters in 24 hours dark (0 light), 12 hours dark (12 light), and 6 hours dark (18 light) had 10-15% greater growth compared to lobsters grown in 0 hours day (24 light) or 18 hours dark (6 light). This information will be especially necessary in the development of designs and management protocols for indoor systems. The use of multiple daily photoperiods to increase growth will be investigated in future trials.

Future research plans to concentrate on defining the optimal environmental and system requirements of larger juveniles (>100g). However, there is the need to further investigate methods of optimising survival of small juvenile, as that appears to be the stage when the majority of mortalities occur.

Feed development for the growout of juvenile southern rock lobsters (Jasus edwardsii)

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The southern rock lobster, *Jasus edwardsii* is native to southern Australia and New Zealand and in both countries there is considerable interest in its aquaculture potential. Culture methods for ongrowing need to be developed and optimised. The availability of suitable formulated diets is recognised as being critical to successful commercial production.

Research into development of the diets is being undertaken at the Tasmanian Aquaculture and Fisheries Institute (TAFI), Marine and Research Development Co-operation (through Rock Lobster Enhancement and Aquaculture Subprogram). It involves collaboration between scientists at the TAFI Marine Research Laboratories (Hobart), TAFI School of Aquaculture (Launceston) and the CSIRO Marine Research Laboratory at Cleveland in Qld.

Currently little knowledge about the nutritional requirements of rock lobsters is available. However, rapid growth of the prawn farming industry has resulted in the development of many formulate diets for prawns. Prawns and lobsters have similar feeding strategies and rely on chemical cues to locate food. Thus, lobster can be slow to locate and consume feeds and those characteristics of prawn diets, such as high attractiveness, palatability and water stability, should suit the feeding behaviour of lobsters. An initial trial showed that lobsters detected the presence of food (antennae waving noted) and began a searching pattern immediately after the introduction of prawn pellets to the tank.

As a first step we investigated the growth response of lobsters to a formulated prawn diet. The lobsters ate the diet well but growth and survival was reduced compared to that with mussels. The ready acceptance and consumption of the formulated diet was a very positive result, which indicated there was potential for the development of diets for *J. edwardsii.*

The next step investigated the suitability of a wide range of formulated diets. We also incorporated some mixed diets to determine how much of the mussel diet could be replaced with a formulated diet without affecting growth or survival. For that study we used the diet that had produced reasonably poor growth and survival in the original study. We had two mixed treatments (A= mussels 4 days/week + formulated

diets 3 days/week; B= mussels 1day/week + formulated 6day/week). In this study we found that some of the formulated diets produced promising growth and survival although still less than that of mussels. In general, the *Penaeus japonicus* diets produced better results than the *P.monodon* diets. However, we achieved extremely promising results with the mixed diets. We found that we could replace mussels with the formulated diet 6day/week without affecting the growth or survival. When the formulated diet was fed 3 days/week growth was actually greater than when fed solely on mussels.

With most of the diets tested the Food Conversion Ratio (dry weight food consumed : wet weight growth) was between 1.5:1 and 2:1, which indicated that the lobsters were using the diets well. The colour of the lobsters at the end of trial covered a broad range, going from light pink to dark red. The final colour was correlated to the carotenoid concentration in the diet. This showed that the final colour could be controlled, which may be important for marketing purposes.

Most of the research with diet development had been conducted in lobsters less than 30g in weight. In some of the more recent studies we have investigated lobsters in the size range of 70-250g. The results with the larger lobsters has been very encouraging, with growth rates equivalent to that with mussels. However, the survival is still lower, with the majority of the mortalities due to cannibalism at the moult. Further research is being conducted on this size range.

The final step we are going through is the development of diets specifically for the growout of southern rock lobster. These diets need to be cost-effective. Unfortunately, the 'off the shelf' formulated diets providing good growth are very expensive. To achieve that goal we need to define the nutritional requirements of *J.edwardsii*. Our current knowledge of the nutritional requirements include:

- Optimal digestible protein level and optimal g\digestible protein: digestible energy ratio for small (<20g) juveniles.
- Apparent digestible protein and energy values for fish meal based diets
- Carotenoid inclusion level (at least for optimising colours)

We have also completed a study to determine the optimal digestible protein level for large juveniles (>70g). It is likely that the nutritional requirements will vary with lobster size.

Planned research will see the determination of the optimal lipid level and optimal cholesterol level. As carotenoids can be an expensive component of the diet there is a need to further examine its role to determine if its inclusion level can be lowered without adversely affecting growth and survival. Maybe it will only need to be included in the diet at high levels toward the end of the growout cycle when there is

a need to change the colour for market purposes. We will also be examining the role that attractants play in the attractiveness and palatability of diets.

The implications of this research for the industry are the development of a cost-effective growout diet coupled with appropriate feed management techniques. These should lead to lower production costs, less feed wastage and lower labour costs. The decreased reliance on mussels will decrease or prevent the variation in quality/quantity, the need to handle and store large quantities of mussels and the potential for the introduction of disease.

Reducing rock lobster larval rearing time through hormonal manipulation

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Lobsters (*Macrura Reptantia*), whether fresh, frozen, or preserved, are one of the most valuable seafood commodities. World landings are dominated (>62%) by the Astacidea, or clawed lobsters, from the north Atlantic. The Palinuridea, or rock lobsters (also referred to a spiny lobsters) made up approximately 33% and the Scyllaridae, or slipper lobsters, 2% of world landings in 1998. Of the Palinuridea, 28% of total landings are from temperate species of the genera *Jasus* and *Palinurus* with 72% being of subtropical and tropical species. In Australia, the western rock lobster (*Panulirus cygnus*) is by far the most valuable wild fisheries rock lobster species and accounts for 20% of the total value of all fisheries production in Australia. The red (*Jasus edwardsii*) and green (*J. verreauxi*) rock lobsters from the southern coast of Australia are also of significant importance in wild fisheries value. In contrast, the ornate rock lobster (*P. ornatus*) from the northern tropics, although part of a wild fisheries is of minor importance.

The Rock Lobster Enhancement and Aquaculture Subprogram (FRDC) has, as one of its two main goals, the development of a rock lobster aquaculture sector. If a rock lobster aquaculture sector develops successfully the vast majority of product would be targeted for export markets. The subtropical and tropical species of the Palinuridea already form the largest proportion of the world's wild rock lobster fisheries and hence already have a significant global market share, especially *P. argus* from the Caribbean which serves a major proportion of seafood commodities of the multi-billion dollar Caribbean tourist market. The global import trade (129,957 mt, US$1.7 billion) is approximately equivalent in tonnage and value for fresh (chilled) (68,428 mt, US$12,052/mt) and frozen (61,529 mt, US$13,634/mt) product (1998). The main lobster importing countries (including Astacidea and Palinuridea) of live and frozen product in order of size are USA, France, Italy, China, Spain, Japan, and Canada.

The selection of suitable aquaculture candidates does not necessarily depend on the importance of specific species that presently form important wild fisheries sectors. Suitable candidates with aquaculture potential would include attributes such as commanding a high price in an established market, rapid growth to a marketable size on inexpensive, readily available diets, disease resistant, having high tolerance to variable environmental conditions, possesing life history traits favorable to farming conditions, and, if they are to form a 'ranching' aquaculture sector e.g. puerulus grow-out, to be readily

available from the wild. Although not a commercially important wild fisheries species in Australia the subtropical and tropical Palinuridea meet many of these aquaculture selection criteria.

One of the most critical and challenging s of the closed-life-cycle production of a marine species is success in the larval rearing phase. The most successful aquaculture crustacean group is the penaeid prawns. They meet most of the attributes required by aquaculture candidates and most importantly have a short larval phase. Indeed, the only large scale commercial aquaculture successes have been with crustacean species with a short larval phase (Figure 1). Of the Palinuridea the tropical rock lobsters have some of the shortest planktonic phases. We have select *Panulirus ornatus* as one of our model species due to its relatively short larval phase. From a stock of 11 females some breeding statistics include an average female body mass of 1698±157 g, a maternal phase of 23 days, mean hatching dates for three cycles occurring on 23 Dec, 20 Jan and 13 Feb, an average of 32 days between hatchings, an average of 161±27 gms of phyllosomas per hatching, 100% of females have successful hatchings and a mean hatch of 1,098,600 phyllosomas per hatching.

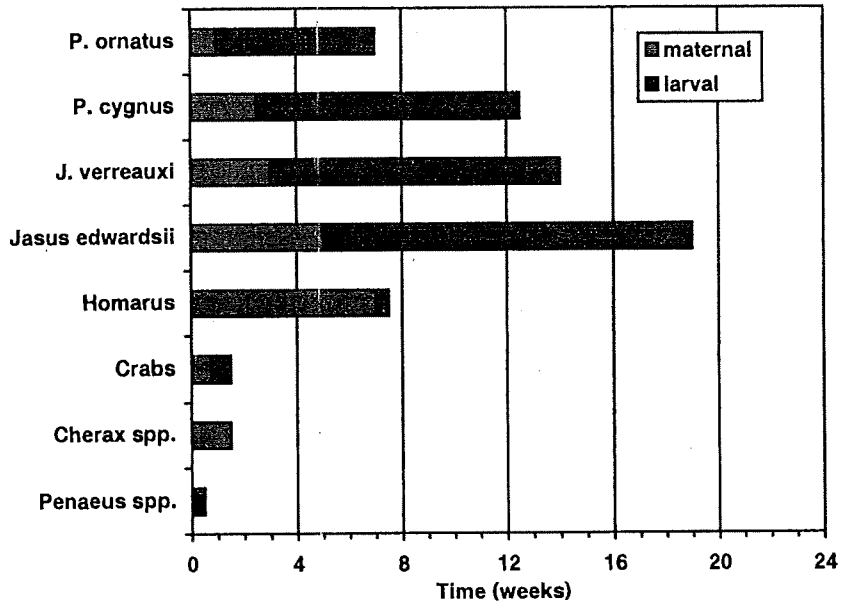

Table 1. *Length of the maternal and larval phase of various crustacea.*

Larval development in rock lobsters is anamorphosis through a series of gradual changes with a final metamorphosis characterized by a radical change in morphology and function from phyllosoma (larvae) to puerulus (postlarvae). One technological challenge for larval rearing includes the significant changes in physical size between phyllosoma Stages I to XI (Figure 2). Although there may be similarities in total

body length across species associated with each of the phyllosoma stages, the number of moults, and more especially the length of the intermoult period, can vary significantly between species (Figure 3). For a closed-cycle breeding technology to be developed for rock lobster aquaculture there is a requirement to examine ways in which the lengthy larval period can be significantly shortened.

Larval crustacean moulting and metamorphosis appears to the under the same endocrine controls as those found in adult Crustacea. Several major endocrines (hormones) influence and modulate moulting and metamorphosis. Although relatively little is known of the details of these endocrine processes there is a significant understanding of these processes in the near relatives of crustacea - the insects -which can be used as a model to understand the processes in Crustacea.

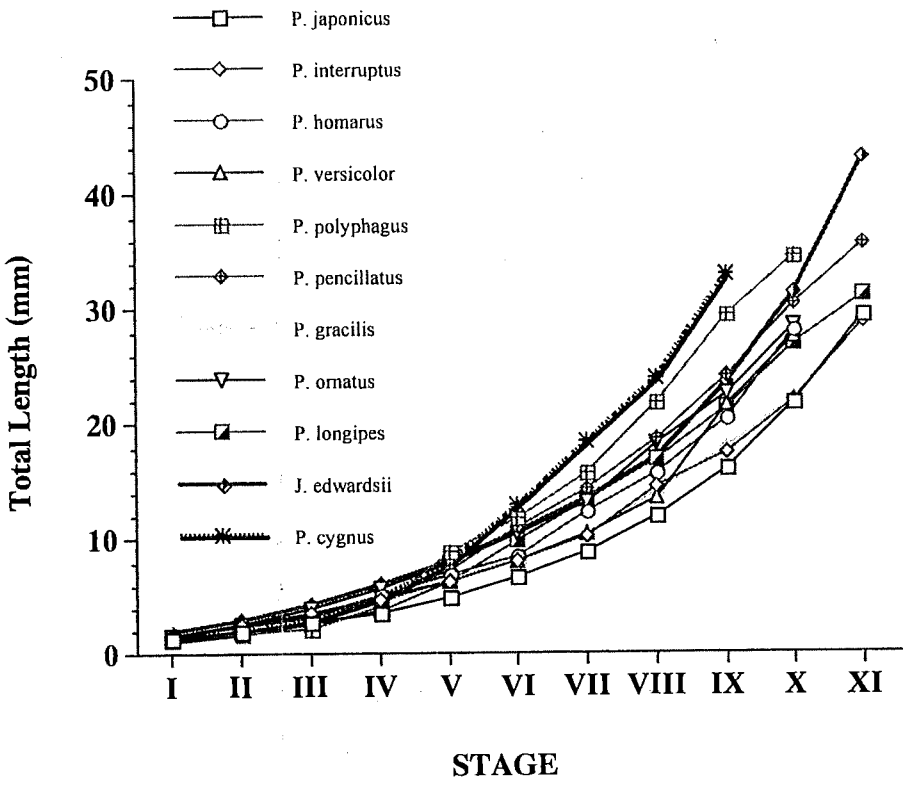

Figure 2. *Total body length for the various stages of phyllosoma development in various species of the Palinuridea.*

The various transformations through the larval stages and moults are under endocrinological and molecular controls. All major endocrine organs, including the X-organ-sinus gland complex in the eyestalk, the Y-organ (the source of ecdysteroids) and the mandibular organ (the source of the juvenile-like hormone methyl farnesoate) are present in larvae. In Astacidea lobsters episodic pulses of ecdysteroids are correlated with larval transformations. We have demonstrated the presence of

ecdysteroids and methyl farnesoate in day 1 larvae of *P. ornatus*. Much of the temporal and tissue specificity of ecdysteroid responsiveness is modulated by the presence or absence of specific nuclear receptors. The family of hormone nuclear receptors are highly conserved. In order to identify nuclear receptor genes we used PCR amplification from genomic DNA using a degenerate primer to a highly conserved region of nuclear receptor DNA binding domain. We sequenced 34 clones, 30 from *P. ornatus* and 4 from *P. versicolor* (Fig. 3). Several clones exhibited good protein alignments with known receptor-like proteins. Four sequences were used to design specific primers for amplification of each receptor gene from *P. ornatus* RNA samples. These new primers will be used, in conjunction with Oligo-(dT) primers to amplify the full length receptor genes from *P. ornatus* larval RNA. Once the full length genes have been amplified and cloned, their sequences can be determined and used for designing paired primers for use in Real-Time PCR assays to screen for differential expression of each receptor gene at various stages of larval development.

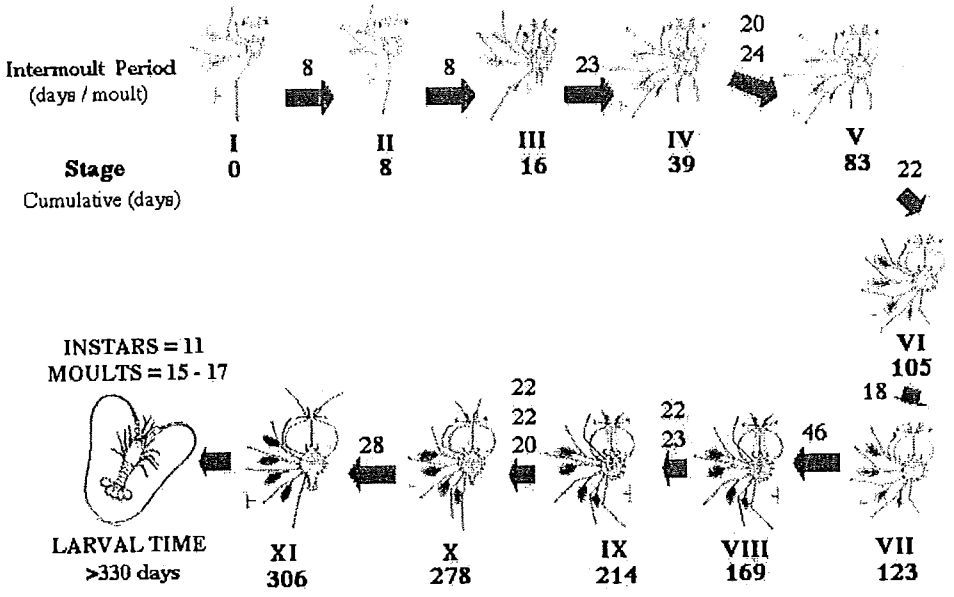

Figure 2. *The larval cycle of Jasus showing the changes in phyllosoma morphology for the XI stages (not to scale). Individual intermoult period, in days, for which there may be several per instar stage, shown above arrows. Cumulative days as larvae indicated below Roman numerals.*

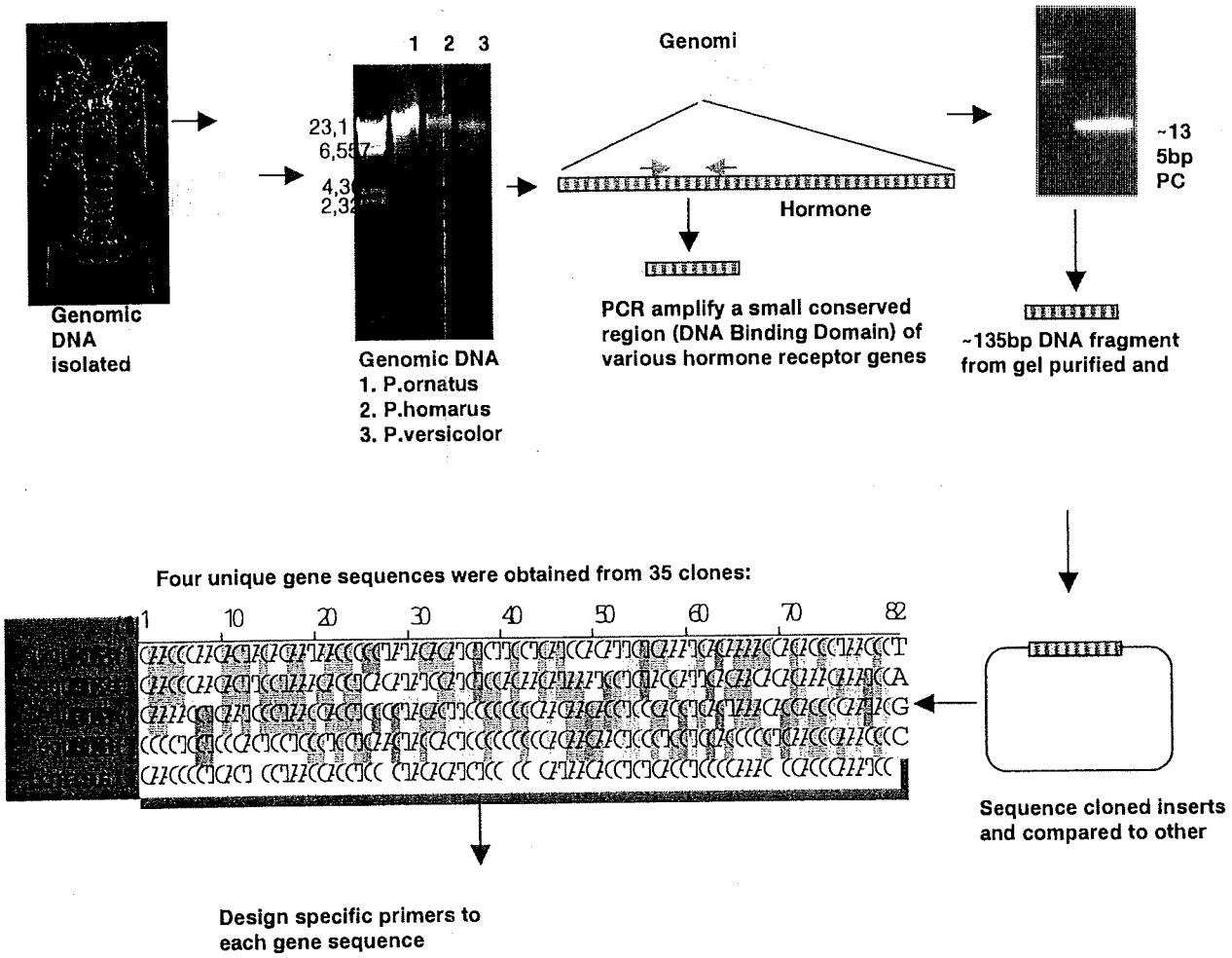

Figure 3. *Sequence of events to isolate nuclear hormone receptor genes.*

Manufactured feeds for juvenile and adult rock lobsters

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Development of a formulated diet for rock lobsters

Exploratory preference feeding studies were carried out with *P. ornatus* to assess the lobster's preference for alternative dietary formulations and presentation forms. Formulations based on fishmeal and containing a high inclusion (12 to 20%) of a dry crustacean meal were consumed more readily than those containing a high inclusion of dry squid meal. Diets provided as a steamed dry pellet were equally attractive to lobsters as those given as an alginate-bound moist pellet. Following this work, more comprehensive growth assay experimentation was carried out at Curtin to further evaluate the developed pelleted diets when fed juvenile *P. cygnus.*

At Curtin, a 9-week growth assay experiment utilising small *P. cygnus* juveniles (~1.4 g initial weight) was carried out to evaluate 5 dietary treatments – 2 formulations which were presented either as dry or moist pellets, and a diet of fresh mussel. Survival was excellent with only 4 animals not surviving out of the initial placement of 90. Lobsters fed the fresh mussel grew about two and a half times faster than those fed the pelleted diets (SGR of 2.63 cf 1.19 to 1.29 %/day, respectively) with no differences being seen between either formulation or pellet presentation form. Conclusions from this experiment were:

- The pelleted diets elicited a marked immediate feeding response in the lobsters but this behaviour rapidly diminished with time after feeding and was virtually absent after about 1 to 2 hours. By comparison, lobsters fed fresh mussel showed only a moderate immediate feeding response but this continued for at least 12 hours after feeding.
- The poor growth rate of the lobsters fed the pelleted diets was attributed to a poor consumption of the diet, which was thought to be caused by a rapid loss feeding attraction.

Further work to improve the attractiveness of the pelleted diet was carried out at CSIRO using *P. ornatus*. A series of pair wise preference comparison studies examined alternative formulations and

pellet presentation forms in an effort to improve the palatability and receptivity of the diet to the lobsters. The work showed that:

- Feeding fresh mussel and pellets together stimulated an enhanced consumption of both the pellet and the mussel. Moreover, the pellets were often preferentially and occasionally completely consumed before that of the mussel.
- Incorporation of a small amount of fresh mussel (5% wet weight and equivalent to ~1% dry weight) into the dry pellet formulation also stimulated a greater rate of pellet consumption in the absence of fresh mussel.

Recent research at TAFI compared the feeding response of *J. edwardsii* to fresh mussel, a commercial shrimp feed formulated for *Penaeus japonicus* that was 'as supplied' and after it had been immersed in water for 8 h. This work demonstrated that the shrimp feed 'as supplied' elicited a stronger feeding response than fresh mussel but that after 8 h immersion, though it still elicited a feeding response from the rock lobster, the response was less than that of fresh mussel.

In summary, the developed diets were well accepted by all three species of spiny rock lobsters but when fed to juveniles they were still markedly inferior to that of fresh mussel and the commercial shrimp feed made for *Penaeus japonicus*. Including a small amount of fresh mussel in the formulation prior to the production of dry pellets enhanced the lobster's receptivity to the diet but prolonging its attractiveness beyond about 2 to 3 hours was still a problem. The strong initial feeding stimulus elicited by the pelleted food was most likely due to the rapid leaching from the pellet of soluble amino compounds, and most likely free amino acids. The less immediate but more prolonged attractiveness of fresh mussel to the lobsters is thought to be due to a slower and more protracted leaching of amino compounds that act as feeding cues for the lobsters. Further research is underway to characterise the material that elicits the feeding response from mussel and potential ingredients of pelleted diets, and to design pellets with more defined leaching characteristics so as to prolong the feeding attractiveness of the feed pellet to the lobsters.

Protein and protein to energy requirements of rock lobsters

Although further research was needed to improve the lobster's attractiveness to the developed diet, there was an equally high priority to establish optimal dietary nutrient specifications if more rapid progress was to be made on developing practical diets for lobster grow-out culture. The highest research priority was to determine the optimal dietary specifications for protein and protein:energy for each of the three lobster species.

Accordingly, 12 diets constituting a 6 x 2 factorial examination of protein (6 concentrations that varied serially at 5% increments from 30 to 55%, as-fed basis) and energy (2 lipid concentrations of 6 and 10%, as-fed basis) were formulated and produced at CSIRO for distribution to partner laboratories for evaluation with small (initial weight of ~0.3 to 6g) juvenile *P. ornatus* (CSIRO), *J. edwardsii* (TAFI) and *P. cygnus* (Fisheries WA). The diets contained 5% fresh mussel as an attractant; protein and lipid contents were manipulated by serially varying de-fatted fishmeal (for protein) and fish oil (for lipid) at the expense of starch and diatomaceous earth. In experiments with *J. edwardsii* (TAFI) and *P. cygnus* (Fisheries WA), an additional diet of fresh mussel was included for comparative purposes. Apart from fresh mussel, all pelleted diets were fed restrictively (at 90 to 95% of satiety during acclimatisation) to ensure that any differences in growth could be attributed to nutritional, rather than to intake, effects. In parallel with the growth assay at TAFI, the efficiency of nutrient conversion/retention was calculated using comparative slaughter procedures. The apparent digestibility of the pelleted diets fed to *J. edwardsii* was also determined at TAFI. A subsidiary follow-on experiment with *P. ornatus* was carried out to compare an extruded *P. japonicus* shrimp diet with the best of the laboratory-pelleted diets at CSIRO. A second protein study of eight laboratory-pelleted diets of serially incremented CP (at 3% from 34 to 55%, air dry) but constant energy (8% lipid) was carried out at TAFI with larger (initial weight ~70 g) juvenile *J. edwardsii*; control diets of extruded *P. japonicus* shrimp feed and fresh mussel were also included for comparative purposes.

There was a close similarity in the response of all three species of juvenile lobsters to the laboratory-pelleted diets across the various experiments. Survival rate was generally high (>75%) in all experiments other than for the first experiment with *P. ornatus* where it was low (~60%) and markedly worse for the lobsters commencing the experiment at low weights of ~1.2 g. Further, survival rate was not significantly ($P>0.05$) affected by the diet fed – whether dry pellets or fresh mussel – in all experiments other than in the subsidiary second experiment with *P. ornatus* where it was better ($P<0.05$) for lobsters fed the extruded *P. japonicus* shrimp diet compared to those fed the laboratory-pelleted diet (100 vs 80%, respectively). Growth rate improved curvilinearly with increasing dietary protein concentration and was optimised at DM CP contents of about 49, 45 and 57% for *P. ornatus*, *J. edwardsii* and *P. cygnus*, respectively. Varying the lipid concentration of the pelleted diet had only a small effect on growth rate and this was species dependent. With *P. ornatus*, there was a tendency for the 10% lipid diets to produce higher growth rates, particularly at the higher CP concentrations whereas with *P. cygnus*, productivity was generally better at 6% than at 10% lipid. For *J. edwardsii*, dietary lipid content had no apparent effect on SGR. In the second TAFI experiment with larger *J. edwardsii* juveniles fed diets varying in CP at a constant DM lipid content of 8%, growth rate also improved curvilinearly with increasing dietary

CP; the asymptotic response occurred at about 59% DM. This was much higher than that observed for the small juveniles in the first TAFI experiment (~45% DM) and similar to the asymptote value of 57% (DM) found for *P. cygnus*. Based on these results, a dietary protein to energy ratio of not lower than 30 g CP per MJ GE (and similar on a digestible protein to digestible energy basis) is presently recommended.

Growth rates of all three species fed the laboratory-pelleted diets were comparatively poor: SGRs on the best diets were only 0.8, 1.1 and 2.8%/d for *P. ornatus*, *J. edwardsii and P.cygnus*, respectively. By comparison, *P. ornatus* lobsters fed the extruded *P. japonicus* shrimp diet under similar restricted feeding conditions grew at 2.0%/d while *J. edwardsii* and *P. cygnus* juveniles fed fresh mussel grew at rates of 1.3 and 6.1%/d, respectively. In the second TAFI experiment, lobsters fed an extruded *P. japonicus* shrimp diet or a diet of fresh mussel grew respectively, 16 or 30% faster than those fed the laboratory-pelleted diets. FCR responses for the pelleted diets in all experiments followed a similar pattern to those of growth rate as expected because of the imposed restricted feeding.

The apparent DM, CP and energy digestibility of the diets measured with *J. edwardsii* was low (viz. 59, 80 and 78%, respectively) but not markedly different from what has been reported with similar diets for other crustaceans. The far better growth of lobsters fed fresh mussel or the extruded *P. japonicus* shrimp diet compared to those fed the laboratory-pelleted diets points directly to a sub-optimal supply of a specific nutrient other than protein.

Conclusions drawn from the present work can be summarized as follows:

- Juvenile spiny rock lobsters grew best on pelleted diets that contained a reasonably high DM CP content of at least 50% (40% DCP) and a moderate DM lipid content of 6 to 8%. However, because of the comparatively poor growth rates observed in the present work, some caution must be exercised as to the validity of the optimum dietary protein concentrations stated above. For the rates of growth observed in the present work, much of the digested protein would have been catabolised for energy rather than being used for somatic growth of the animal. Thus, a more nutritionally optimal diet where food constituents other than protein are used as a major source of metabolic energy, will almost certainly have a lower protein content.

- A dietary protein to energy ratio of about 30 g CP per MJ GE appears to be optimal for juvenile *P. ornatus* and *J. edwardsii*; *P. cygnus* may require a higher dietary protein to energy ratio. Since the CP and energy apparent digestibilities of the diets were very similar (~80%) when measured in the present work with *J. edwardsii*, the optimal protein to energy ratio remains the same whether

expressed in units of digestible or gross protein and energy. Again for the reasons above, caution is advised against accepting this finding on protein to energy requirement until diets are developed that support much better lobster growth rates.

- Although there appears to be a general similarity in the way the three lobster species responded to dietary nutrient compositional changes, there were equally sufficient inter- species differences to warrant continued nutritional research on each species.

- The poor growth of lobsters fed the laboratory-pelleted diets and the markedly better growth on diets of fresh mussel or extruded *P. japonicus* shrimp feed, indicates that further diet development research is needed to improve the present compounded diet. The findings also suggest that the present formulation is limiting in one or more essential nutrients or alternatively, that critically important nutrients are being rapidly leached from the pellet and before the food is eaten by the lobster.

- Further research is needed both to improve the physical quality of the compounded pellet, and particularly to better control the rate at which soluble nutrients are leached from it into the surrounding water, and to determine the requirement for other essential nutrients that may be limiting productivity. A better selection of ingredients to enhance the palatability of the diet to the lobsters could improve consumption rates and also lead to better growth rates.

Nice Legs, shame about the waste! : Ways of controlling handling – induced appendage loss

Glen Davidson, Wayne Hosking

Geraldton Fisherman's Co-operative, Geraldton, WA

University of WA, Department of Zoology

Each year the western rock lobster industry loses an estimated 40-80 tonnes of product in the form of legs lost during post harvest. Aside from the sheer loss of weight, at $25-30/kg, leg loss is a significant problem because intact lobsters command premium prices. Industry estimates put the combined value of these losses at between $1-3 million/season.

In addition to these direct financial losses, the resource suffers as a result of leg loss due to 1) increased mortality of damaged returned lobsters (reproductive females and undersized) 2) reduced growth of damaged returned lobsters and 3) reduced reproductive success of damaged returned females.

Rock lobster aquaculture and enhancement operations may face similar problems, especially since cultured lobsters will be unavoidably exposed to increased handling and manipulation. All the sources of loss identified for a wild fishery will also apply when assessing the potential impacts of leg loss in a culture/growout scenario i.e.) loss of weight and value, reduced growth and increased mortality of stock and reduced reproductive success of broodstock.

As early as 1997, cold water stunning was suggested by sources in the western rock lobster industry as a potential method for preventing leg loss. Our aim has been to investigate the efficacy of this and other techniques for reducing leg loss, as well as to determine the potential impacts of useful techniques on the survival of retained lobsters, the survival and growth of returned lobsters and on the reproductive success of returned mature females. Successful strategies for alleviating leg loss in the wild fishery will also have application in culture/growout operations.

In this talk we will review the findings of the project to date. Results from experiments conducted in captivity, and from field trials of cold water stunning applied aboard a working commercial lobster boat and throughout the delivery chain to the processor will be presented, along with a brief economic assessment of the benefits conferred by the treatments.

Recent findings regarding previously undescribed causes of leg loss, a potential relationship between post-harvest handling and tail rot, and future directions for research will be discussed.

Investigation of tail fan necrosis in live-held adult rock lobsters

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Department Environmental Biology, University of Adelaide

[2]South Australian Research and Development Institute (Aquatic Sciences)

[3]Department of Microbiology and Immunology, University of Adelaide

This project originated as a recommendation from work on liveholding of the southern rock lobster, *Jasus edwardsii*, part of FRDC RLEAS Project 98/305, "Determination of optimum environmental and system requirements for juvenile and adult rock lobster holding and grow-out". One of the issues specified as a problem requiring further work was that of tail fan necrosis, then called "tail fan damage". This occurred in lobsters held in cages within floating pontoons and in onshore raceways in both summer and winter. As lobsters with necrotic tails may be rejected by processors, the problem was, and still is, seen as a significant obstacle in the development of the industry. Incidence of blistering and erosion reached 60% in some treatments during experiments run in summer.

The outcome of the recommendation was current project, the aim of which is to investigate potential causes of tail fan necrosis in live-held adult southern rock lobster. To date the two experiments have been carried out, both finishing in the week of the 26th of March, 2001

Field

450 lobsters were bought from local fishermen with 420 used for the experiment. The remainder were used to replace mortalities in the fist two weeks of the experiment

The experiment commenced on 27.11.00 in conjunction with FRDC 200/212 and was completed at the end of March. It was set up in seven outside tanks at Southern Australian Seafoods at Port Lincoln. The tanks were rectangular, about 4 metres long and were continuously aerated and supplied with water (flow-through). They were situated within a shade cloth enclosure. Temperature was monitored using data loggers. Each tank contained 4 PVC/oyster mesh cages – 28 in total. There were six treatments, four of feeding frequency (daily feed, bagged/daily fed, weekly fed and starved) and two of density (10 per cage (6.3/m^2) and 20/cage (12.7/m^2) (Fig 1). The bagged treatment contained lobsters that were put straight from the pot into individual fine mesh (0.5 mm) nylon bags, protecting the animals until they

could be released into the tanks. At that point the bags were removed. This was intended to control for damage that might have occurred on the boat and during the transport and potentially lead to tail fan necrosis. As it happens the frequency of damage (tail fan damage and limb loss) was much higher on the un-bagged lobsters than on those in bags when the experiment was started.

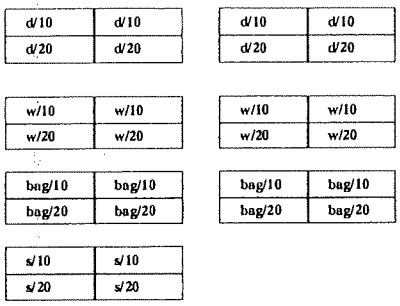

Figure 1. *Experimental design for feeding frequency density trial. d=daily feed, w=weekly feed, bag=bagged daily feed, s=starved.*

Tail fan state was assessed every two months as follows. Each of the five appendages of the tail are assessed individually for the presence of tears, scratches, blisters, holes and erosion. Each category was subdivided as appropriate, ie. small, medium, large in the case of the tears, blisters, holes and scratches and by percentage in the case of erosion . All subdivisions were made based on previous observations of damage.

Swabs were also from the tail fans of randomly selected lobsters from each treatment and plated out onto TCBS and Nutrient agar for assessment of microbial flora. The identity of the flora was determined by Dr Connor Thomas. Three lobsters were also randomly selected from each of the daily, weekly and bagged treatments and tissue samples taken from their tail fans for microbial fauna quantification and for examination by SEM. These lobsters were then placed on ice and bought back to Adelaide for condition assessment. At the end of the experiment, a further 60 lobsters were bought back to Adelaide for condition assessment.

Laboratory

90 Lobsters were caught over a three day period during normal fishing operations with the assistance of a commercial lobster fisherman. The pots were set in 30-50 feet of water off the south coast of Kangaroo Island. When pots were hauled up, males just over the legal limit (102mm Carapace Length) were separated from the catch. 65 of these lobsters were put into mesh bags and the remainder (25) left unbagged. The same technique was used in setting up the field experiment and had shown that bagged

lobsters sustained much less tail fan damage, and damage in general, than those left without bags, the standard commercial practice.

Lobsters were then transferred to a processor's tanks on the mainland and from there to the South Australian Aquatic Sciences Centre. During the journey they were treated in the same way as commercial lobsters, that is, housed in crates covered with damp sacking in a chiller truck (10-12°C). Once at SAASC they were in then kept in communal tanks ((15oC) for one week prior to the beginning of the experiment. At that point 60 lobsters were transferred to in individual 38l tanks and 20 to two further communal 500l tanks. Lobsters were removed from bags just prior to tank assignment. The remaining animals were used to replace any mortalities during the first week of the experiment. During the acclimation period and the experiment the water supply to all tanks was flow-through and all lobsters were fed 3 times per week at 2% body weight/day.

Individual Tanks

In the individual tanks the tanks were divided into two groups of thirty, kept at either 15°C or 23°C. Within each group lobsters were randomly assigned to one of three bagging treatments: bagged, un-bagged or bagged damaged, 10 lobsters per treatment. Each tank had its own individual water and air supply.

Bagged-damaged animals were purposely damaged before they were put into tanks. Four of the 5 tail fan appendages (the telson and three uropods (right to left)) out of each animal had small round holes (2mm) punched through them and cuts (7mm) made at opposite ends of the distal margins. All holes and cuts were made aseptically.

The tail fan states of all lobsters were assessed each fortnight as described above and photographs taken of all fans. Swabs for microbial analysis were also taken from 18 lobsters each fortnight (3 lobsters for each of the three bagging treatments from each temperature) and plated out onto TCBS and Nutrient agar for assessment of microbial flora.

Communal tanks

The lobsters in the communal tanks were used specifically for tissue samples, to follow the succession and quantity of microbial flora with the development of any tail fan necrosis. The lobsters were damaged using the same method as described previously. This time two uropods on the right side were damaged, the two on the other side kept as controls. The tanks were kept at 23°C.

Each fortnight, four lobsters were randomly selected and tissue samples taken. Standard tissue samples were aseptically removed from the tail fan of each animal using a 12mm hole punch. On the damaged side, each sample incorporated either a previously-made hole or a cut on a chosen uropod. A control tissue sample was also taken from a uropod in the undamaged side. These samples were placed in sterile vials and put on ice for transport to the microbiology lab. Samples from three of the lobsters were individually ground in PBS buffer and the solution plated out for microbial identification and quantification, the fourth was mounted for observation with SEM.

Preliminary results indicate

Laboratory

- The presence of marine species of *Vibrio*
- Blisters found in association with TFN are sterile, suggesting the fan infection is externally derived, not originating from a more general systemic infection.
- The use of bags significantly reduces initial tail fan damage and necrosis. The results show that if there is initial damage, necrosis is increased greatly.

Field

- The presence of marine species of *Vibrio*
- The use of bags significantly reduces initial fan damage, the final result remains to be analysed.

Future work:

1. To further develop and trial "bags" designed to protect lobsters from physical damage and ensuing Tail Fan Necrosis;
2. To trial alternative methods to treat TFN viz immunostimulant
3. treatment, bacteriocide "dipping" and bacterial treatment

The bag and Bagger prototype development would be carried out in collaboration with Greg Ward of Kingscote KI and Lenny Toumazos of the Fish Factory, Adelaide. The TFN treatment section would include experiments at SARDI and a trial of bagged animals at Port Lincoln, Southern Australian Seafoods (SAS) over the winter.

Development of growout systems for tropical rock lobster

Clive Jones

Northern Fisheries Centre, Department of Primary Industry, Qld

Several trials and experiments have been completed whose results enable us to define the basic requirements and likely performance of tropical rock lobster (*Panulirus ornatus*) in growout systems. Research was conducted at the Northern Fisheries Centre, a research facility of the Queensland Department of Primary Industries (QDPI) located in Cairns, Australia.

I acknowledge the support of the Fisheries Research and Development Corporation through the Rock Lobster Enhancement and Aquaculture Subprogram (RLEAS) in enabling this research to be initiated, and the support of the MG Kailis Group over the past 12 months in continuing this research. I also acknowledge the technical assistance of Larnie Linton, Druce Horton and Will Bowman.

P. ornatus is a brightly coloured lobster endemic to the Indo-West Pacific region, and particularly abundant in northeastern Australia. In the Torres Strait in particular, the species supports a commercial fishery which lands approximately 500 tonnes (whole weight) of lobsters per year. The fishery has traditionally tailed and frozen its catch, but there is now an increasing proportion of that catch shipped live to Cairns, where it is graded, packed and exported as live product primarily to Asia. *P. ornatus* is a high value species fetching over $A45 per kilogram into markets in northern China. This market is seen as expanding with great opportunity for increased supply

Research activity was initiated by QDPI in 1997, with a preliminary assessment which indicated great potential. A formal research project was then established in 1998, supported by the RLEAS and over two years, various aspects were investigated including temperature, salinity and density for growout, and propagation. In 2000 a joint venture was established with MG Kailis P/L a diversified fishing and seafood company who fish and trade tropical rock lobster. This joint venture has a goal to commercialise tropical rock lobster aquaculture over the next 5 years through investigations of both growout and propagation.

Findings from a series of trials will be presented including, diet comparison, temperature and salinity tolerance, and assessments of density and shelter.

Diet Comparison

A diet comparison trial demonstrated that a commercial prawn diet designed for *Penaeus japonicus* was a suitable diet for *P. ornatus* (Figure1). It provided equivalent growth to a diet of fresh marine organisms including prawns, squid and scallops. A commercial prawn diet designed for *Penaeus monodon* proved to be inadequate for both growth and pigmentation of the shell.

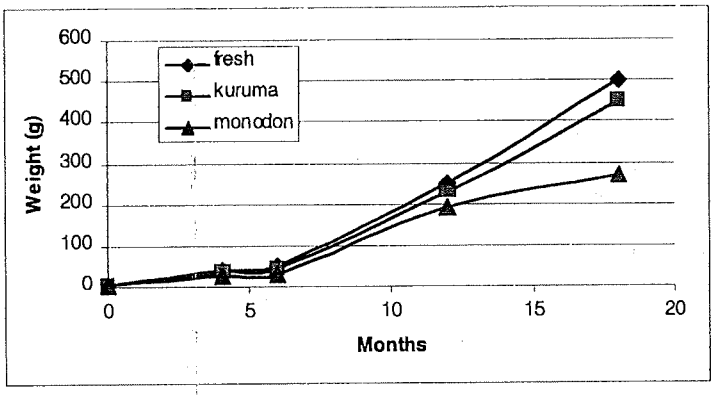

Figure 1. *Growth of P. ornatus in flow-through seawater systems, fed one of three diets; fresh, prawns, squid and scallops; kuruma, commercial diet for Penaeus japonicus; monodon, commercial diet for Penaeus monodon.*

Temperature Preference

Effect of temperature on growth and survival was investigated in a series of recirculating systems. The experiment demonstrated that optimal growth was achieved within the range of 25 to 28°C which provided the maximal moult increment and minimal intermoult period (Figure 2).

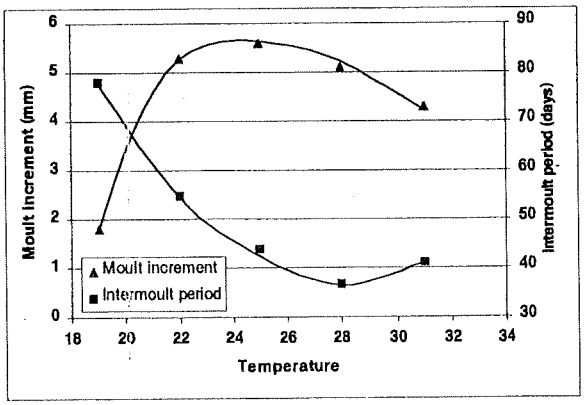

Figure 2. *Mean moult increment and intermoult period for P. ornatus grown at different temperatures.*

Salinity Tolerance

A salinity experiment was also conducted in a series of recirculating systems, maintained at set salinities. It examined the impact of 4 salinities from 20ppt through to full strength marine. While optimal growth was achieved at full marine conditions (Figure 3), *P. ornatus* demonstrated good tolerance and substantial osmo-regulatory capacity at salinities as low as 20ppt.

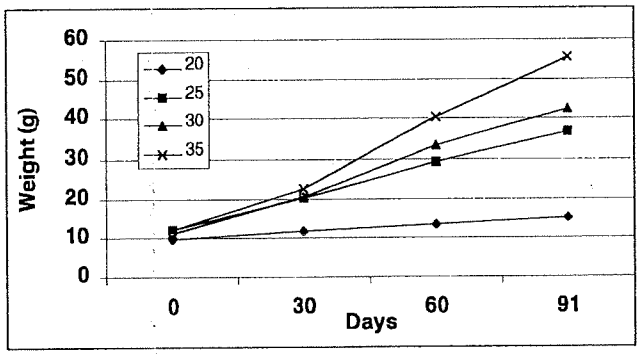

Figure 3. *Weight (g) of P. ornatus at 14 day intervals over 91 days, grown at four salinities.*

Density Assessment

A density experiment was conducted in large tanks with substantial numbers of lobsters to both examine density and a scaled up system with more relevance to commercial operation. The densities applied were equivalent to 14, 29 and 43 lobsters per square metre. The experiment was performed in a flow-through seawater system under ambient conditions. Four 3000l fibreglass tanks were used with plastic mesh divisions to create 3 compartments within each tank.

Each experimental unit was stocked with 60 wild caught juvenile lobsters of mean weight 3.2g. They were fed daily with a Penaeus japonicus prawn diet, which was supplemented regularly with fresh squid and prawns. At monthly intervals, lobsters were counted in each unit, and twenty lobsters were sub-sampled to measure weight and carapace length. The experiment ran for a period of 272 days.

Survival ranged from 45 to 58%, and although there appeared to be a negative correlation of density and survival, there was no significant difference between densities at harvest (Figure 4). Mortalities occurred consistently over the period, and were attributed to cannibalism of post-moult individuals.

Growth was exponential and equivalent for each density (Figure 5). There was no significant difference in final weight. Mean weight at harvest averaged over all three densities was 225 grams. A specific growth rate was calculated at 1.56% per day for the period.

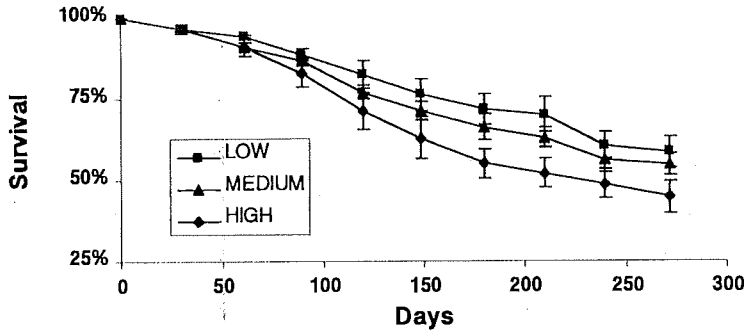

Figure 4. *Mean (for 3 replicates) (±s.e.) survival of ornate rock lobsters at three densities over 272 days.*

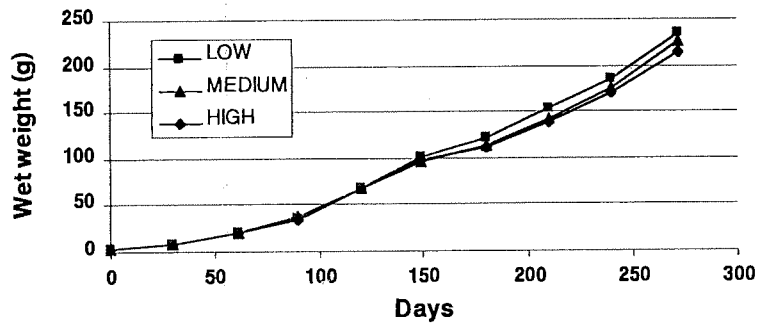

Figure 5. *Mean weight (g) of ornate rock lobsters at three densities cultured over 272 days.*

Despite the mortality experienced, numerically density was still relatively high at harvest, at 19 lobsters per square metre for the high density treatment, and biomass exceeded 4 kilograms per square metre.

Shelter Assessment

To address the issue of cannibalism, an experiment was designed to assess the impact of shelter and density on growth and survival. This experiment is underway at present, and will incorporate lobsters grown through to approximately 1kg.

Summary

Tropical rock lobsters perform well on a commercial prawn diet. Optimal temperature for growout is in the range of 25 to 28°C. The species is tolerant of reduced salinity, although performance is best in full marine conditions. The species is tolerant of relatively high densities, and even at the highest density applied, lobsters performed well. The growth rate was excellent and indicates that growth from 3g to 1kg at commercially viable densities may be achievable within an 18 month period. The growth for the post-puerulus to 3g however is less certain, and may take a couple of months. The flow-through seawater system supported at its maximum a biomass of over 4kg per square metre. Survival was lower than might be commercially acceptable, but was uninfluenced by density, suggesting other factors were responsible for the mortalities that occurred. It is likely that shelter and feeding strategy were the most influential factors, and that through manipulations of these reduced mortalities through grwout could be secured.

In conclusion, there is sufficient evidence now to confirm that growout of *P. ornatus* is likely to be commercially viable. The primary obstacle to commercial development is the definition of propagation technologies that provide consistent supply of high quality juveniles. There is no prospect of commercial exploitation of wild juvenile stocks. Questions of the relative suitability of flow-through seawater systems as compared with Recirculating Aquaculture Systems for gowout of this species are as yet unanswered, but will be an issue of economics rather than biological factors.

As a closing remark, it is worthy of note that sensory evaluations of the cultured lobsters from our work were made. This involved formal taste panel testing. Cultured *P. ornatus* were compared with wild caught tropical rock lobsters and wild caught western rock lobsters (*P. cygnus*). No significant differences between the species or between cultured and wild caught lobsters for either texture or taste parameters were found.

Rock lobster enhancement - Pilot scale project

Caleb Gardner, David Mills, S. Ibbott, S. Wilcox

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Overview

This pilot project evaluated enhancement of a coastal reef with rock lobster juveniles. Although we released juveniles that had been captured as puerulus and on-grown for one year before release, the concept of enhancement is much broader. Animals for enhancement could be obtained as juveniles from an area of high settlement and shifted to a suitable habitat at with low settlement, or it may one day be possible to release juveniles produced in hatcheries. The import point is that we need to be able to evaluate the success of the release operation and optimise survival.

This project was intended to evaluate our availability to measure survival of cultured animals relative to wild juveniles. Three objectives were defined to meet this main goal:

1. To develop methods to capture large numbers of juveniles (for controls monitoring survival)
2. To determine the extent of movement of reseeded animals after release (to isolate survival); and
3. To develop methods to assess relative survival of released juveniles.

The first two objectives to address methodological problems in estimating survival in the field, the third objective required a pilot scale run of estimating survival. Mathematical modelling of survival was a crucial aspect of the third objective.

In explaining what the project aimed to do, it is helpful to point out some common misconceptions about our goals. We only aimed to measure survival relative to wild animals not obtain absolute survival estimates. In fact, we were able to obtain survival estimates that were close to absolute estimates, but that precision is not vital if we're just comparing two groups. Also, we weren't attempting to optimise release – only test our ability to measure survival. The estimates of survival of reseeded animals that we obtained in this project should not be used for making management decisions – we chose a habitat that was useful for testing methodology, but it wasn't particularly typical of the Tasmanian coast.

We expect that the precision of our estimates is a minimum of what could be achieved because we released less than 500 juveniles which is a smaller sample that that which industry will release.

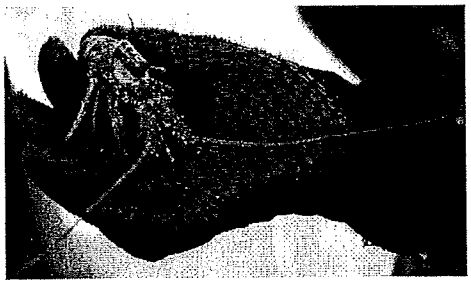
Objective 1: Developing methods to capture large numbers of juveniles

- A range of traps was tested. Only trammel nets were effective
- Catch rates may have been depressed by water temperature
- Catch rates by divers were sufficient

Objective 2: Determining the extent of movement after release

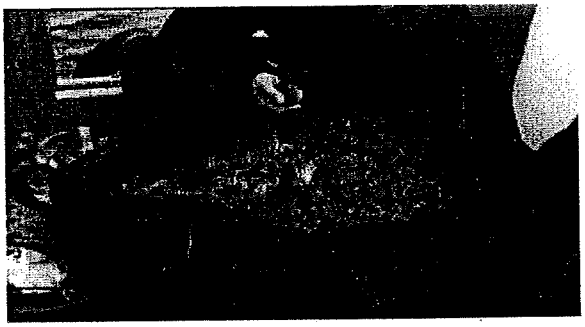
We needed to determine movement as we intended to measure survival by a technique called the 'Jackson Square'. Movement can bias survival estimates as released lobsters may have either dies or simply walked away from the search area. It's quite difficult to separate these two factors so survival estimates tend to be biased. The Jackson Square Technique overcomes this problem with a very simple concept: that the movement of lobsters out of a large square will be less than the movement out of a small square. The hitch is that the scale of movement of lobsters has to be in the same ballpark as the size of the squares – otherwise the mathematics of the Jackson Square Technique will fail.

We conducted trials on the movement of lobsters by attaching sonic tags that could be located by divers using a submersible detector.



Results from sonic tracking were that:

- Juvenile lobsters would occasionally cross sand – so this isn't a perfect barrier
- Reseeded lobsters didn't tend to head for home (as suggested in literature)
- Control (wild) animals from the same site didn't move less than control animals transplanted from nearby
- Reseeded juveniles moved a similar amount to wild animals;
- The ideal size for the Jackson Square was 32x32m.



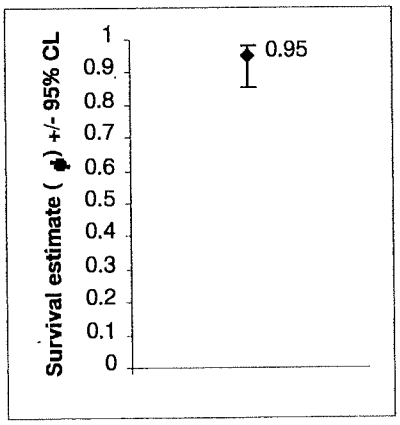
Unfortunately, many of these conclusions from the sonic tracking work turned out to be quite misleading, even wrong, in the context of a large scale release. Movement of juveniles in the large scale release was much greater than we were expecting. The reason? We suspect that lobsters behave quite differently when released in small numbers than when they were released as a large mass of 100s of individuals. Whatever the reason, the implication was that our Jackson Square was too small to provide useful survival estimates.

Fortunately, there was a solution to the problem which was to apply a different type of survival modelling. We tried to cover a larger area but this presents new problems. Most obviously, by spreading our effort over a larger area, we're more likely to miss some animals within our search. We overcame this by using a very new class of survival model that was developed for birds (multi-strata models). These models allowed us to estimate the probability of resighting a lobster in the different areas we surveyed, and also to estimate the movement of lobsters between different areas. Once we'd estimated movement and resighting probabilities, we were able to isolate survival parameters.

Objective 3: Estimating the survival of released juveniles

The main aim of this project was the 3rd objective which was to conduct a pilot scale run of a release operation, and test if we could obtain meaningful survival estimates. 427 cultures animals were released together with 153 control animals, which were wild animals from the same area. All of these juvenile lobsters were tagged on their antennae with a unique colour code. We then conducted diving surveys at 2-day intervals and recorded the codes of lobsters that were resighted. A summary of our observations and multi-strata survival modelling results are:

- Cultured juveniles moved much further than wild animals in large releases on the first day, but both groups were equivalent after that
- Our ability to resight cultured animals was the same as for control animals
- Our probability of resighting a mobster was related to which divers were searching and how much effort was put into searching a particular area (this may seem like we discovered the completely obvious, but it was important to be able to correct for both of these factors when estimating survival)
- Movement was greatest on the first day but tailed off towards the end of the 18 day survey
- Survival was no different between control and cultured animals
- Survival did not change during the course of the study (we were expecting mortality to be high initially then gradually reduce)
- And most importantly, our survival estimate was precise with remarkably narrow 95% confidence limits (in other words, the experiment was a success in demonstrating our ability to provide a meaningful data on the release of rock lobsters)



- We also identified several areas of the methodology that we think would improve estimates in future surveys.

Future Research

This project was intended to test our ability to conduct useful research on enhancement. It appears that we have a viable method for future research which we believe should focus oh:

- Formulating release protocols based on the anti-predator behaviour of juveniles
- Providing guidelines for optimal release (micro) habitat
- Evaluating the success of pilot scale releases for enhancement.

Evaluating the release and survival of juvenile rock lobsters released for enhancement purposes

Megan Oliver, Rob Stewart, Caleb Gardner[2], Alison McDiarmid

National Institute of Water and Atmospheric Research, New Zealand

[2]Tasmanian Aquaculture and Fisheries Institute, Hobart, Tasmania

Predation of hatchery reared individuals soon after their release into the wild environment can be a significant source of mortality in a range of fish and invertebrates (Olla *et al.*, 1998; Nodtvedt *et al*, 1999). Not only does the act of release disturb and agitate the individuals, which may make them more susceptible to predation, but also they may not recognise potential predators or take appropriate evasive action (Olla *et al.*, 1998). An additional problem in nocturnally active species, such as lobsters, is that hatchery raised individuals may emerge from shelter at inappropriate times thereby exposing themselves to a higher risk of predation. We undertook several experiments to investigate whether captive lobsters were more active during the day than their wild counterparts, and whether lobsters reared in captivity and then released into a wild environment recognised potential predators and took appropriate action compared to their wild conspecifics. Remote infra-red (IR) cameras permitted time-lapse video footage to be collected and emergence times and anti-predator responses to be analysed. Our results indicate that lobsters reared in captivity in the absence of predators and fed during the day (when it is most convenient for staff to feed them) are more active during the day than like-sized juvenile lobsters in the wild. However, when captive juveniles are released into a wild environment, they resume innate nocturnal emergence patterns. In addition, we found that captive lobsters responded in appropriate and similar ways to the presence of predators as their wild conspecifics. It was noted however from the field video footage, that predation pressure can vary significantly between sites. These preliminary results suggest that it is important to assess predator abundance when choosing appropriate sites for the release of juvenile lobsters. Overall it can be concluded that juveniles reared in captivity exhibit changes in their emergence patterns, but if they are displaced into a wild environment they display an innate response to the presence of predators and resume nocturnal activity cycles.

Potential impacts of puerulus collection on the biological neutrality of the West Australian rock lobster fishery and the relevance to other fisheries

Bruce Phillips[1,2], *Roy Melville-Smith*[2]

[1]*Curtin University of Technology, WA*

[2]*Fisheries WA, Perth, WA.*

The study was made to examine the impact of possible puerulus exploitation on the future catches in the wild fishery for the western rock lobster, *Panulirus cygnus,* in Western Australia, and to determine management measures which might be required to maintain 'biological neutrality'. A primary aim of management to maintain sustainability of the Western Rock Lobster Fishery is to maintain the reproductive capacity of the breeding stock at a level sufficient to replenish itself. Biological neutrality is in this context, the level of catch that would be needed to be forgone to compensate the reproductive capacity of the breeding stock if pueruli were removed for aquaculture.

This study was made using existing data on puerulus settlement, juvenile densities and mortalities, and recruitment rates to the fishery. It is not possible to follow the pueruli from the time of settlement right through to the breeding stock. Therefore it was assumed that adjusting the catch to compensate for removals of pueruli would provide sufficient protection of the breeding stock.

Because of constraints on time and resources, it was not possible to undertake a study of all areas of the fishery. However, data were available to permit examination of the 29oS - 30oS area to test the potential for puerulus harvesting. These latitudes encompass the area between Dongara and Geraldton, which are near the centre of the Western Rock Lobster Fishery.

Any exploitation of the early life history stages of an animal would be expected to result in some, though probably not proportional, change to the number of animals surviving to larger sizes. With this in mind, the approach taken in this study of modeling was the likely effect on future wild catches that might be predicted to result from the removal of various proportions of the levels of pueruli settlement.

The study was conducted in four parts.

(1) Catch-puerulus relationships;

Catch-puerulus relationships in the western rock lobster were used to assess relative mortality differences between different regions and different levels of puerulus settlement at the same region.

This section used fishers' compulsory catch and effort data, fishers' voluntary log book data and long term puerulus settlement data sets from research puerulus collectors moored at the Abrolhos Islands, Dongara, Jurien and Alkimos Western Australia.

The object of this part of the work was to estimate the likely contribution made by particular year classes of pueruli to commercial catches of red and white lobsters in subsequent seasons. In addition, the data were used to derive stock dependent mortality indices for the four areas of the fishery to examine changes in mortality indices in different areas of the fishery.

The model used differed from the power regression approach that has been used in the past for catch prediction, in that this new model was fitted assuming non-linear techniques. Conclusions from the analysis of the data confirmed what has been reported previously, namely that:

a) Recruitment into the commercial fishery is most dependent on settlement of pueruli in the coastal reefs taking place three and four years earlier. The contribution to the fishery made by pueruli settling five years earlier was estimated as being small and made little or no difference to the model fit.

b) That the contribution made by lobsters recruiting to the fishery three years after settlement, compared to those recruiting four years after settlement is different in the four regions that were examined. The percentage contribution made by three year old lobsters is highest at the Abrolhos Islands, followed by the north coastal areas.

c) Stock dependent mortality coefficients showed the highest stock-dependent natural mortality coefficient at the Abrolhos Islands followed by the northern coastal areas.

The important outcome is that it is clear that if pueruli are to be harvested in the future, that because of the different contributions to the fishery made by pueruli settling three/four years before recruitment in different regions, and because of differences in stock dependent mortalities in different regions, these factors would need to be taken into account in establishing harvesting procedures.

(2) Estimating mortality between pueruli settlement and recruitment to the fishery

An estimate was made of the rates of survival between settlement of the pueruli and recruitment into the fishery. To achieve this, it was necessary to have a measure of the absolute abundance of the pueruli, which could be linked with the subsequent catches of recruits.

Puerulus settlement data at Seven Mile Beach for 1987/88 and 1988/9 were used to estimate the number of pueruli/m2 that would have been likely to have settled over those years between 29oS and 30oS (an area encompassing the Seven Mile Beach study site, but approximately seven times larger). Aerial mapping techniques were used to identify all the hard surface areas within the 29°S to 30°S area to a depth of 20 m. The total surface area of this type of habitat between the shore and 20 m was estimated to be 382.3 million m2.

Data on recruitment to the western rock lobster fishery were available from fisher logbooks and compulsory catch and effort returns collected by Fisheries Western Australia.

The mortality rate between settlement and recruitment to the fishery was determined by dividing the number of recruits to the fishery in a particular season by the estimated puerulus settlement that gave rise to that recruitment. For the 1987/88 season, the estimated mortality was 98.11%, and for the 1988/89 season, 98.52%. In estimating these mortalities we assumed that there was constant mortality (M=0.226 year-1) from year two after settlement onwards. This is unlikely to be the case and these estimates are considered to be the upper limit of mortality in the first year.

To further investigate the mortality between the first year on the nursery reefs (puerulus settlement) and the second year, we assumed that the mortality rate was constant from the second year onwards through to recruitment. Under this scenario, it was found that the mortality for the first year after the settlement (age = 1 to 2 years) was 98.1% for the 1987/88 puerulus settlement, and 98.5% for the 1988/89 puerulus settlement. This means, that of the number settling as pueruli, only 2.3-3% survive their first year in the nursery reefs.

Clearly the young juveniles are subject to a very high mortality, particularly in the first year after pueruli settlement.

(3) Puerulus mortality model development

Mortality was modeled to decline with age rather than be constant after the first year, as in part 2 of this report. This change is considered to be biologically more defensible. As a result of this change, the estimate of mortality in year one after settlement decreased from 97-98% to about 80% (79.8% for the 1987/88 settlement and 83.9% for the 1988/89 settlement).

Using these data a model was developed to examine the likely impact that harvesting pueruli might have on the catches in the wild fishery. The model focused on the area between 29°S and 30°S, for which there was data available, and simulated the outcome of harvesting pueruli under various settlement levels (densities). Those densities were within the range that has been experienced over the 30 year period that pueruli settlement collectors have been in place at Seven Mile Beach.

Because of the high density dependent mortality that occurs between settlement of the pueruli and recruitment into the fishery, removal of a significant percentage (up to 30%) of pueruli for medium to high levels of puerulus settlement (300 to 1200 million puerulus settlement size) would result in a less than 10% reduction in catch.

The impact of puerulus removals on subsequent catch was estimated to be minimal except in the case of removal of very large numbers of pueruli. However, it would also be possible to counter these losses by effort reductions, and a set of tables allowing calculation of these reductions is provided.

If for example, it was decided to permit the harvesting of 20 million pueruli from the 29oS - 30oS latitude area, in a year when puerulus settlement was average (i.e. 600 million), and it was deemed desirable to reduce pot numbers to compensate for these puerulus removals, it would be necessary to remove approximately 1% of the effort in the 29oS - 30oS latitude area to achieve biological neutrality. This converts to a reduction in fishing effort of approximately 23,000 potlifts for the season.

The analysis of the impact of the 1993/94 management package for the western rock lobster fishery indicated that pot removals have less impact on the catch than has been calculated in this study. This is because at high levels of fishing effort a reduction of effort reduces peaks in the catch, thereby spreading it over a longer time without reducing the overall landings by anything like the proportional reduction in effort. On this basis the numbers of potlifts that need to be removed to achieve biological neutrality

could be double the figure that has been calculated. Further work will be necessary to clarify the actual effort reduction which will be necessary to compensate for pueruli removals.

(4) Integrating the detailed modelling work

The potential application of the models in estimating levels of puerulus exploitation and their likely effects on catches in the fishery were examined.

The results clearly show that there is a very high mortality between the time of puerulus settlement in the coastal reefs and the time the lobsters move offshore and recruit into the fishery. The mortality during the first year after the settlement (from ages 1-2 years) for *P. cygnus* is estimated to be either as low as 80% or as high as 97%. Assuming the two years of data that were available are typical and provide a reasonable estimate of abundance, only very small numbers of the settling pueruli survive to recruit into the fishery at about 4.5 years of age. The data on mortalities in other species of rock (spiny) lobsters are sparse, but consistent with the results we have obtained in this study.

It is inevitable that in a study such as this, dealing with major processes over periods of decades, it has been necessary to make a large number of assumptions in the calculations. It should also be noted that the data collected were not for the purpose for which they have been used in these analyses.

Much reliance has been placed on the reef density estimates of pueruli and juveniles made during the 1987/88 and 1988/89 seasons. These data are considered to be reliable. They are probably conservative as they are based on data on the number of pueruli observed by divers. The limestone reefs in which they were located may contain other caves and ledges in which divers cannot observe the pueruli and young juveniles.

The reef density estimates, which were estimated by bathometric contour lines from CALM satellite imagery data are likely to be much less accurate, and whether the juvenile lobster densities calculated in the Seven Mile Beach area can be extrapolated over the larger 29oS - 30oS region is also debatable. However, despite these potential errors in calculating absolute abundance of pueruli settling between 29oS - 30oS the most critical assumption is the mortality rate in the first year after settlement. The levels of settlement of *P. cygnus* pueruli observed (0.1778-1.3076 per m2) are similar or higher than Florida Spiny Lobster, *P. argus*, the only other species for which there are comparative data

The Florida studies found that the post-pueruli of *P. argus* are very flexible in their use of habitat. At times of high density they will make use of "less than perfect" shelter, allowing considerable numbers to survive. Clearly if numbers of pueruli are removed from an area, this allows the remaining post-pueruli to make use of the best shelter and other resources. It is not known if *P. cygnus* exhibits this flexibility. However, we do know that in years of high settlement, fishers report considerable numbers of recently settled lobsters in their pots which have been set in deeper water than where we normally record settlement. This means that the levels of puerulus settlement used in our analyses may be conservative, and that at times of high puerulus settlement even greater levels of settlement may occur than we have recorded. Some attempt has been made to take the uncertainty of variable settlement densities and reef area estimates into account by producing results from a number of different simulations.

By good fortune, since it has provided a good contrasting result, the number of pueruli estimated to have settled in 1988/89 was nearly 50% higher than those estimated to have settled in 1987/88 (483 million compared to 338 million), while the estimate of recruits available to the fishery surviving from those years was not nearly as marked (6.4 million from 1987/88 settlement and 7.1 million from 1988/89 settlement).

At this time the preliminary results of our attempts to design methods to catch large numbers of pueruli (Phillips *et.al.* submitted) suggest that the pueruli will be easiest to catch near the shore, and in selected locations with fringing reefs. This means that the removals would not take place evenly over the whole settlement area. The effect of removals in a small area is unknown, but could be examined.

A study carried out at Seven Mile Beach showed that pueruli and post-pueruli of *P. cygnus* are mainly eaten by small fish. None of these fish species live exclusively on rock lobster pueruli and they will therefore presumably adjust their diet based on the number of rock lobster pueruli available. We already know from the collector data that the annual levels of puerulus settlement vary considerably; hence the predators are already used to these kinds of fluctuations, and can respond appropriately.

Testing collector designs for commercial harvesting of western rock lobster *puerulus*

Roy Melville-Smith[1], *Bruce Phillips*[1,2]

[1]*Fisheries WA, Perth, WA.*

[2]*Curtin University of Technology, WA*

We have (i) tested modified western rock lobster sandwich collectors at different depths and distances offshore, (ii) tested different collector designs, (iii) examined the effect of collector size, and (iv) tested the effect of frequency of servicing the collectors. The only catches recorded in the onshore-offshore trials were on gear set at the inshore site (depths <5 m). Published data from the 1970s on the effect on catches of collector arrays and locations were re-examined with a general linear model. The analysis revealed marginally significant corner and layer effects, carry-over effects, and square-of-time effects. Five collector designs were therefore set in the shallows, two of which had replicates of three different sizes, and were checked over four lunar months during peak settlement. Sandwich collectors had significantly better catch rates than others ($P < 0.001$), and settlement rates were highly correlated with collector dimensions ($r = 0.72$). Daily servicing for seven days around the time of new moon, yielded catches 170% higher than those from a single monthly servicing ($P < 0.001$). Results indicate that tests for collectors must take into account corner, carry-over, neighbour, and layer effects and that to do so they must be set out in an array and repositioned after each sampling.

Appendix I:

Workshop Agenda

Rock Lobster Enhancement and Aquaculture Subprogram

Third Annual Workshop Agenda

Wednesday, April 4, 2001, "Te Papa", Wellington, New Zealand

9.00	Welcome (*Dr Patrick Hone*)
9.15	Strategic directions for Australasian rock lobster enhancement and aquaculture research (*Dr Robert van Barneveld*)

Lobster Propagation (Chair: Dr Andrew Jeffs)

9.45	Development of manufactured diets for rock lobster phyllosoma – attractants and feed intake (*Dr Michael Bruce*)
10.15	*Morning tea*
10.45	Propagation of rock lobster phyllosoma – nutrition, health and environment (*Dr Bradley Crear*)
11.30	Techniques for the manipulation of rock lobster phyllosoma larval phases (*Dr Mike Hall*)

Lobster Nutrition, Health and Housing (Chair: Pheroze Jungalwalla)

12.00	Manufactured feeds for juvenile and adult rock lobsters (*David Smith*)
12.30	*Lunch*
1.30	"Nice legs, shame about the waste !": Ways of controlling handling induced appendage loss. (*Wayne Hosking and Dr Glen Davidson*)
2.00	Causal factors of tail fan necrosis in live-held rock lobsters (*Dr Mike Geddes*)
2.30	Development of grow-out systems for tropical rock lobsters (*Dr Clive Jones*)
3.00	*Afternoon tea*

Capture and Release of Lobsters (Chair: Dr Patrick Hone)

3.30	Techniques for the assessment of survival of re-seeded aquaculture reared juveniles (*Dr Caleb Gardner and Dr Alison MacDiarmond*)
4.00	Progress on signature lipid profiling of wild and aquacultured *Jasus Edwardsii* (*Matthew Nelson*)
4.30	Potential impacts of puerulus collection on the biological neutrality of the West Australian rock lobster fishery and the relevance to other fisheries (*Prof Bruce Phillips*)
5.00	Testing collector designs for commercial harvesting of Western rock lobster puerulus (*Dr Roy Melville-Smith*)

5.30 Close

Workshop IV 2002

Rock Lobster Enhancement and Aquaculture Subprogram & Rock Lobster Post Harvest Subprogram

Developments in Rock Lobster Enhancement, Aquaculture and Post Harvest Practices

RLEAS Publication No. 7

Edited by Dr Robert van Barneveld & Dr Bruce Phillips

June 2002

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Proceedings of the Fourth Annual Rock Lobster Enhancement and Aquaculture Subprogram/Rock Lobster Post-Harvest Subprogram Workshop

Cairns, Australia, 29 May 2002

Venue

Cairns Cruising Yacht Squadron

Queensland, Australia

The Rock Lobster Enhancement and Aquaculture Subprogram is supported by the Fisheries Research and Development Corporation.

The Rock Lobster Post-Harvest Subprogram is supported by the Fisheries Research and Development Corporation.

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Introduction

Robert van Barneveld

RLEAS Leader

"Developments in Rock Lobster Enhancement, Aquaculture and Post-harvest Practices" records the proceedings of the fourth workshop of the Fisheries Research and Development Corporation (FRDC) Rock Lobster Enhancement and Aquaculture Subprogram (RLEAS), and the first combined workshop between the RLEAS and the FRDC Rock Lobster Post Harvest Subprogram.

The aim of these workshops is to present outcomes from core rock lobster research programs to industry and to promote interaction between scientists participating in the research programs. The workshops also represent an opportunity for both the wild capture sectors and the aquaculture sectors to provide feedback on the outcomes of the research and views on where the research programs might progress in the future.

This workshop provided participants with commercial views on the value of research into economics and marketing, developments in tropical rock lobster aquaculture and developments in temperate rock lobster aquaculture. This was complemented by presentations on proposed research into the enhancement of the western rock lobster fishery using techniques to improve puerulus settlement rates and survival, research underway in Tasmania to assure the health of aquaculture-reared juveniles destined for release into the wild fishery and progress on rock lobster reseeding research that is being undertaken in Australia and New Zealand.

From an aquaculture perspective, closure of the rock lobster life-cycle is seen as a key research outcome that will significantly influence how this industry develops in Australia. Accordingly, the RLEAS is investing heavily in rock lobster propagation research. This workshop was used to convey outcomes from this research program being undertaken in Australia and New Zealand. Recommendations arising from a recent review of this propagation research were also presented together with suggestions for future research directions.

Research has been underway for some time into techniques for the minimization and measurement of stress in live held lobsters. Research outcomes from projects underway within the rock lobster post-harvest subprogram dealing with stress and morbidity during post-harvest handling of lobsters were show cased as were ways to optimize water quality in rock lobster post-harvest processes.

The final component of the workshop dealt with ways to optimize post-harvest product quality for aquaculture and various markets. The highly successful research into leg loss in the western rock lobster and how this problem can be alleviated was conveyed to workshop participants through a series of entertaining presentations and videos. Researchers have also identified the causal factors influencing the development of tail fan necrosis in rock lobsters held in some aquaculture systems with details on the pathology, causes and ways to minimize the disease outlined during the workshop.

The concept of hosting these rock lobster subprogram workshops in different locations across Australia so that as many participants in the various rock lobster sectors can be exposed to the research directions and outcomes is maintaining favour with industry, research providers and research investors. The participation of the Queensland Rock Lobster Association in this event is gratefully acknowledged as is the involvement of the Minister for Primary Industries, Mr Henry Palaszczuk and the Queensland Department of Primary Industries.

Economics and Marketing : Establishing models for rock lobster aquaculture

Roger Edwards

SARLAC, SA, Australia

Background

- Development of lobster culture has been resisted if not impeded.
- Claims (untested) and cries such as:
  - Increased supply will decrease prices;
  - Why use industry's R&D $ to put them out of business;
  - It will never be economic;
  - Why do we want more supply – can't handle current;
  - Quality might ruin our image and impact price;
  - Aquaculture is a low priority compared to other investments.
- To counter this claim:
  - Markets are always changing and new opportunities are emerging;
  - Are lobster markets for all species fully developed?;
  - Have we fully explored value add products and new market potential for all species.
- Potential benefits claimed include:
  - Additional value added nationally through new volume and economic activity generated;
  - Adding value by enhancement of the current limited catch – feeding for weight gain, colour change, re-seeded fisheries & harvest strategies;
  - Economies of scale for existing export/transport/distribution infra-structure through higher volumes.

The questions

- Why undertake economic appraisal of the impact of aquaculture products?
- How you might approach this?
- What might we get from such analyses?

Why test the claims?

- Duty of care to demonstrate return on investment on R&D $;
- Choices - scarce R&D $ for propagation, re-seeding vs. grow out?;
- Choices - invest effort in tropical vs. southern lobsters etc;
- Who might benefit, who is worse off and by how much and/or relatively?;

- Wild fishery participants want to know – opportunity & risks;
- Investors want to know – opportunity & risks;
- Governments might want to know – regional development, employment flow-on effects, net benefits to Australia etc;
- To assume volume = lower price is simplistic and a nonsense – there are considerations of growing demand, market development, value adding and employment.

Supply & Demand

On the supply side

(1) improvements in aquaculture technology, management and production processes;

(2) a growing pool of investors with funds available;

(3) the fixed (declining) supply of 'substitute' product from the wild capture sector

On the demand side

(1) the income effect - as household income increases, demand for the product also increases;

(2) health issues – underlying strength in seafood demand; and

(3) food safety – recent fall in demand for red meat and a sharp increase in demand for white meat and seafood.

How do we go about it?

Stage 1 – Data Gathering

-Statistics can be gathered from (some) major markets.

-Look at trends in imports, consumption, elasticities and price.

-Look at future projections – consumption, product form, taste, drivers etc.

-Likely species, product form and aggregate volume scenarios can be explored with industry.

-Case studies of where it has happened before.

-Costs and operational details of production can be constructed .

Stage 2 – Market Mode

-Develop transparent economic models incorporate key production and market relationships.

-Build a framework for analysing how the market will respond to a significant change.

-Make assumptions where we don't know.

-Build the models to drop out, price, value added and employment impacts within aquaculture and wild sectors .

-Establish net benefits/costs .

Stage 3 – Analysis

- Test scenarios about volumes, costs, timing etc on price.
- E.g. - If x tonnes reach market y over time period z, what will happen to price and what other flow on impacts are there – value added & employment?
- Test the results for a range of assumptions e.g. future consumption in a particular market – test for nil, small and large increases.
- Might test for worst case and best case and likelihood.
- Report net impacts for a range of scenarios.

What do we get out of it?

- Feel for a range of impacts at different levels of supply of aquaculture product.
- Ranges of estimates for other positive and negative impacts.
- The 'winners' and 'losers' and potential impacts can be identified.
- Rank opportunities and negatives.
- Use rank to direct R&D investment and
- Use provide advice to investors and wild catch sectors.

What Value?

- It depends and we wont know until the work is done!!
- Models involve assumptions – too many.
- Require market and production data – poor data.
- Won't tell the whole story.
- Things will change.

Summary

- *Why analyse impacts?*
    - Host of questions about lobster culture that are unanswered
    - Duty of care to make wise R&D investments
    - Duty of care to at least attempt understand potential impacts of activities that R&D $ are being invested in.
- *How do we do it?*
    - Collect data
    - Build models
    - Analyse & present scenarios
- *What Value - depends?*
    - Get a feel for a range of scenarios
    - Direction/size of impacts
    - Winners & losers
    - Impact rank

Commercial development of tropical rock lobster aquaculture systems

James Fogarty

MG Kailis, Cairns, Qld, Australia

Background

Founded in 1962 by the late Michael George Kailis, a pioneer of Australia's rock lobster, prawn, pearl and tuna fisheries, the MG Kailis Group remains a family owned company, with a strong commitment to continuing research and development, and aquaculture.

The MG Kailis Group now has operations around Australia in many varying facets of the marine environment.

MG Kailis Operations

The major MG Kailis Group operations around Australia are as follows:

- Broome: The Group has been a leading Australian producer of cultured South Sea Pearls since 1975 and now farms at a number of locations along the North Western coast of Australia. The Group's activities cover all aspects of the pearling industry including catching, seeding, farming, harvesting and more recently propagation of pearl oysters.
- Exmouth: In 1995 a Hatchery operation commenced at Exmouth, principally involved in pearl oyster production, while also conducting Research and Development on other species (e.g. Tiger Prawns, Tropical Abalone)
- Exmouth Gulf: The Company has a fleet of prawn trawlers operating in the Gulf and a very modern processing facility on shore. Tiger, banana, king and endeavour prawn are the specie handled. It is currently involved in a reseeding project in this area for brown tiger prawn with FRDC, CSIRO and Fisheries WA. In addition there is a current project with tropical abalone involving the University of Queensland, the CSIRO and the ARC.
- Dongara: On the West Coast of Australia near Geraldton, this is the home of the Western Australian Lobster operation. The Company is a major player in this fishery with substantial processing facilities onshore including the development of a new live lobster holding centre on the Port Denison marina.
- Port Lincoln (SA): The company is involved in farming Southern Bluefin Tuna. This involves the capture of the tuna, usually by purse seining, and towing the catch live in nets back to holding farms for grow out.
- Cairns: This facility handles the purchase, handling and dispatch of prawn and lobster from the Gulf of Carpentaria, Torres Strait and the Qld East Coast.

Why Tropical Rock Lobster Aquaculture

- The Company has an ongoing involvement in the Western Australian, Tropical, NZ and US lobster fisheries and has identified that this high value product has a strong demand and an increasing consumption base.
- The Company is an active participant in the Tropical Rock Lobster in the two fisheries in Queensland waters.
- The Torres Strait fishery has been experiencing poor results for perhaps the last three years. This is basically a frozen tail Fishery with the availability of live product being quite small. Holding facilities on the outer Islands are virtually non-existent for lives. A number of islands do not have adequate freezing facilities.
- The Qld East Coast Fishery is concentrated in a very small area of the coast and is basically a Live Fishery.
- Currently the total intake from both fisheries in a whole weight measurement would be around the 500 tonnes per year with wild fluctuations.
- For the tropical lobster (*Panulirus ornatus*) there are basically 2 markets – a frozen tail market in the USA and a live market in China. The quantities available from Australia do not satisfy either of these 2 markets.
- The tropical lobster (in our view) is the fastest growing animal and is the most likely choice for commercial success.

Target Specie

- There is unsatisfied demand for tropical rock lobster in both markets.
- Research into the various specie of rock lobster tend to indicate that *P.ornatus* has the shortest grow out period to a commercial size.
- Other tropical lobster such as *P.pencillatus*, P.versicolor and P.longpipes may very well have easier and shorter grow out phases but they certainly do not have the market acceptance of *P.ornatus*.
- Current farming operations in Vietnam have proven the viability of growing from puerulus to an animal that is of marketable size.

Preferred Approach

- The Utopian solution would be to harvest juvenile lobster about 2cms in length from either the Torres Strait or the Qld East Coast and grow these out to a marketable size in sea cages as close to Cairns as possible.
- There does not appear to be sufficient juvenile lobster settlement along the coast to make this option viable. In addition we believe that there will be substantial resistance from commercial

fishermen in both fisheries to such a proposal. Further research work into this option could be addressed.

- Sea cages in this part of the world would also constitute a potential security problem.
- In addition the Qld East Coast in the main is contained within the Great Barrier Reef Marine Park. It is highly unlikely that the Marine Park Authority would allow this type of activity in the Park. Nevertheless it is important to point out that substantial cage culture of Tuna occurs in Port Lincoln without apparent environmental degradation problems occurring. We should keep an open mind on this one.
- This leaves us with closing the life cycle. A very difficult process and one, which at the moment from a commercial point of view has defied the best efforts of all concerned.
- Any project will probably have to be land based as approvals for at sea processes at least in Qld will be very difficult to obtain.
- Will it be an open system or a closed system? A closed system appears to be the most controllable and more easily managed but the costs will be higher.

Progress

- The Group has a joint venture arrangement with the QDPI to examine the possible propagation of Tropical Lobster from phyllosoma on a commercial basis.
- The project has been operating for around two years with some pleasant successes and also some failures.
- The process is enabling the Company to develop a very strong research team within the Group, which will enable it to take advantage of breakthroughs as they occur.
- Whilst propagation is the current focus we would expect resources to be moved to grow out as commercial propagation becomes an attainable result.
- A commercial operation is not attainable at this stage.
- The Company at this stage is coming to terms with the complexity of the process and will continue the project based on outcomes and hopefully steps forward in the learning process.

Developing southern rock lobster aquaculture in Tasmania

Pheroze Jungalwalla

Tassal, Tasmania, Australia

Introduction

Tassal Ltd is a producer, processor, marketer and exporter of high quality aquacultured product, mainly Atlantic salmon.

Why the interest in alternate species ?

A corporate decision to make a strategic investment in alternate species.

Drivers

- Amortise skills and resource base
- Increase product range
- Spread risk

Why Southern Rock Lobster ?

For any new species to to achieve Industry status, need high aggregate score on three parameters:-

1.Knowledge of biology of animal (medium score for Southern Rock Lobster (SRL))

2.Existence of profitable market (high score for SRL)

3.A "marginal edge factor"

In our case, this is perceived to be our aquaculture and marketing expertise, and the fact that SRL is a highly prized cool water species.

Source of stock

If the aim is to establish an Industry based on aquaculture of SRL, then puerulus collection is an essential but interim measure.

On-growing puerulus will allow us to learn much about health, nutrition, growth, and husbandry of the animal under aquaculture whilst we await closure of the life cycle.

Culture methods

Likely to vary with individual operator's perceptions of local advantage, and business confidence level.

Range

- Tidal pond culture
- Floating raceways
- On-shore tanks with flow through water usage
- Intensive recirculation facility (allows photo-therm control)

List roughly in order of increasing capex and increasing control over growing conditions

Progress to date

- Pueruli salvaged off salmon farm nets
- Some purpose built collectors deployed on salmon leases
- Pragmatic trials in collaboration with TAFI
- Water borne growth suppressants
- Flow rate vs growth
- Hides
- Survival, growth, and flesh quality on manufactured vs wet feeds

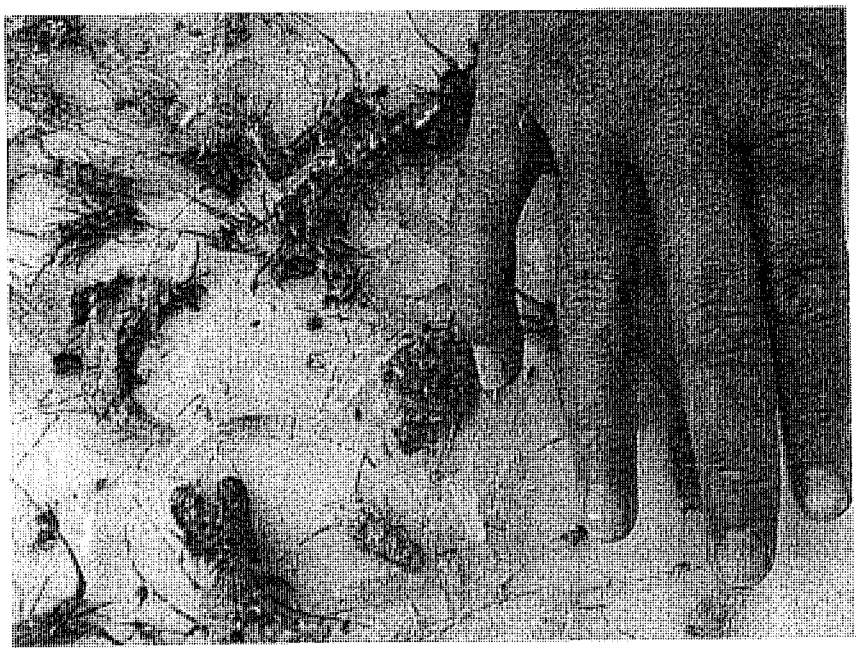

Figure 1. *Pueruli salvaged*

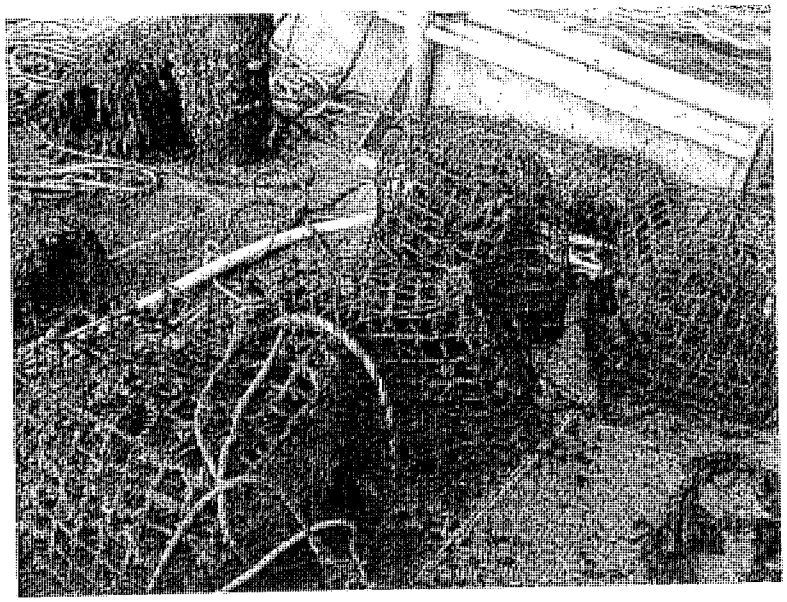

Figure 2. *Mills collectors on deck*

Trials with TAFI

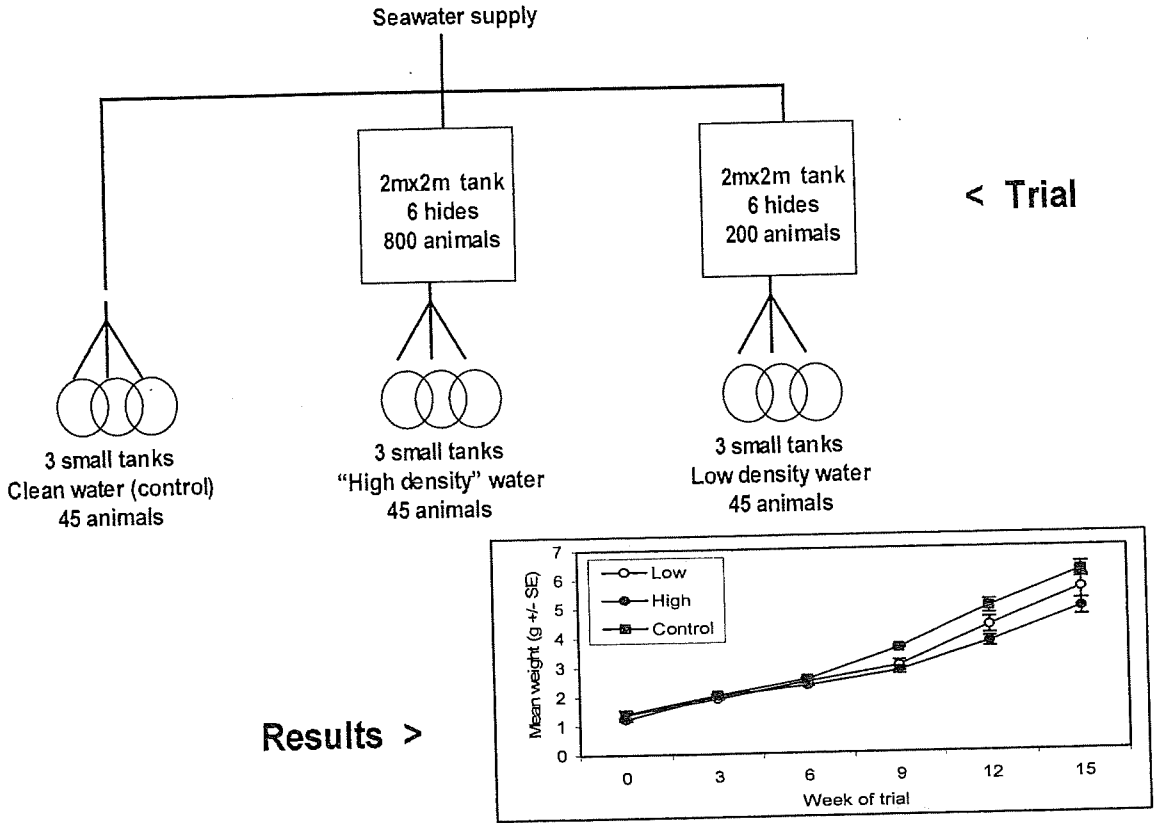

Figure 3. *Water borne growth suppressants*

Trials with TAFI

Trial:-	Medium flow	High flow
High stock density (n = 100/rep) (2 trmnts x 3 rep = 600 animals)	High density – Med flow S M L	High density – High flow S M L
Medium stock density (n = 100/rep) (2 trmnts x 3 rep = 600 animals)	Med density – Med flow S M L	Med density – High flow S M L

Results:-

- **Size had greatest effect on specific growth rate (SGR)**
- **Stocking density also affected SGR**
- **Flow did not affect SGR**

Figure 4. *Flow Rate vs Growth*

Results

- Size had greatest effect on specific growth rate (SGR)
- Stocking density also affected SGR
- Flow did not affect SGR

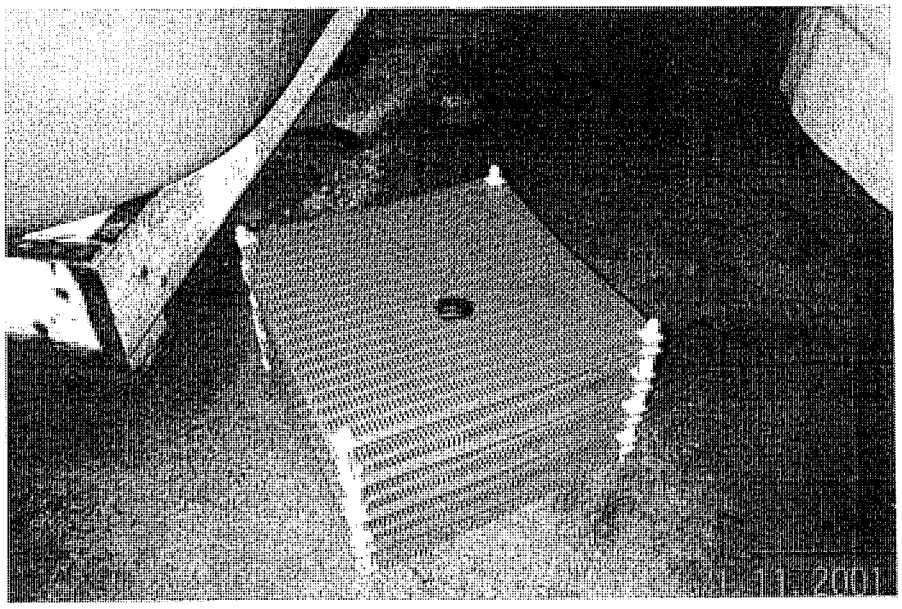

Figure 5. *Stacked plastic sheet hide, on floor*

Survival, growth, and flesh quality

Trial:

Small (200 – 300gr) animals on "wet" and on "manufactured" diets.

Results

- Survival better on "wet" diet
- Weight gain slightly better on "manufactured" diet
- Trial

Trial:

Comparison of lipid (fat) content of animals fed "wet" *cf* "manufactured" diets.

Results

- Overall lipid contents similar
- Fatty acid profiles marginally different

Research & Development Directions

Tasmanian aquaculture perspective

- Jasus is the appropriate species
- Major research effort must be aimed at propagation (esp. phyllosoma > puerulus)
- Small scale trials in collaboration with industry, targeting key components of juvenile culture, is a valuable R&D activity.

Enhancement of the Western Rock Lobster Fishery

Prof. Bruce Phillips[1] & Roy Melville Smith[2]

Curtin University of Technology, WA[1], Department of Fisheries, WA[2]

The FRDC has approved a new project 2002 which will commence on 1 July 2002.

This project, which was developed through the Enhancement and Aquaculture Subprogram, is an extension to FRDC project 98/302, "Aspects of puerulus settlement and the question of biological neutrality in the western rock lobster fishery."

The items of relevance for this new project arising from the biological neutrality project were:

- Pueruli were caught only in inshore areas;
- The settlement rate correlated with collector size;
- There is a very high mortality after pueruli settlement.

This high mortality is illustrated in Figure 1. Up to 97% of the pueruli and early juveniles die in the first year after settlement.

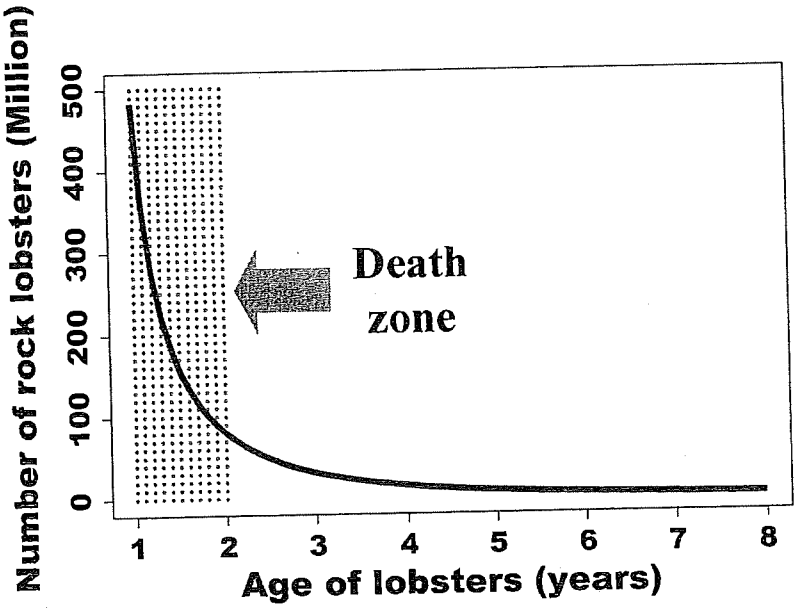

Figure 1. *Mortality Rate*

This new project is designed to see if we can design apparatus to increase survival of the pueruli and early juveniles in the shallow coastal areas If successful, this could lead to increased commercial catches.

The objectives of the new project are:

- To investigate in the laboratory, the number, size and positioning of holes suitable for post-pueruli shelters in an artificial reef environment ;
- To estimate the number of shelters that would be needed in order to make a scientifically measurable impact in a study area, and a preliminary estimate of what would be needed to provide an impact in a regional commercial catch and effort (CAES) reporting area;
- Design, in conjunction with coastal engineers, suitable puerulus/post puerulus enhancement structures that could be built in the future to test as a device to enhance local rock lobster populations ;
- To undertake a benefit cost analysis for the various options for enhancing western rock lobster.

The project will be a series of laboratory experiments lasting about a year using animals caught on collectors, followed by a series of desktop studies and planning sessions with engineers and economists.

We know from a previous study which I and others from CSIRO conducted (FIRDTF 1986/83) that, the early juveniles shelter in holes on hard surfaces, and that the most suitable hole size for the very early juveniles is 12 mm in diameter and about 50 mm deep. Larger juveniles require a larger hole size. The laboratory experiments will determine the most desirable position for these holes, i.e. on the upper, lower, or side surfaces of an object, and the most effective distribution by way of distance apart of the holes, on the most desirable side. Two of the block types to be used in the experiments are shown in Figures 2 and 3.

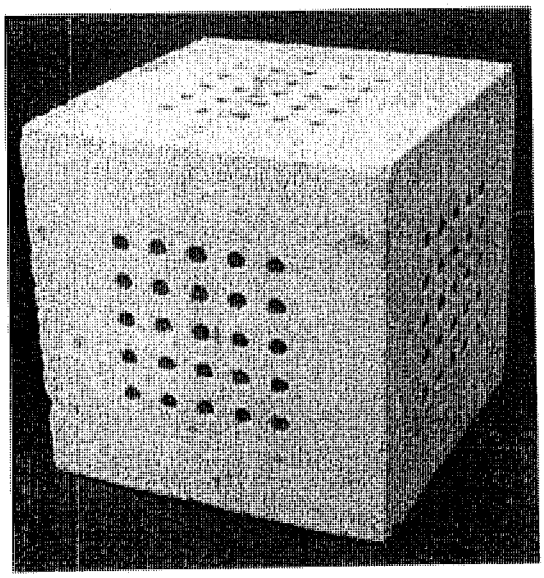

Figure 2 *Block Type*

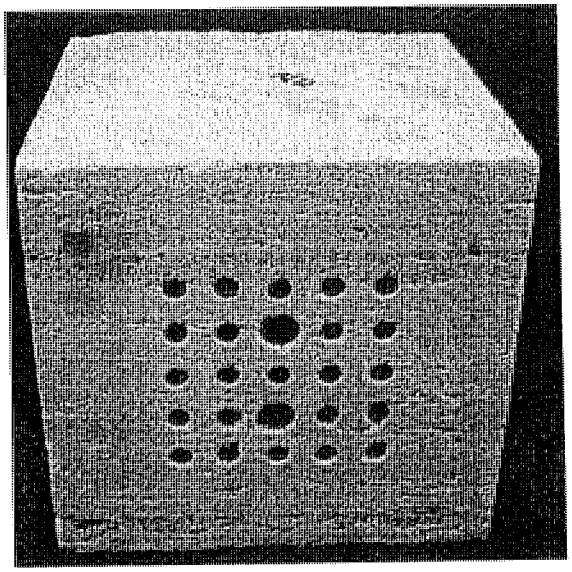

Figure 3 *Block Type*

this stage there is little to report, as the project has not yet started. However, to repeat for clarity the research plan for the two year of the project is:

- Laboratory studies to determine the hole sizes and distributions
- Design of settlement habitats
- Estimates of number and dimensions of settlement habitats needed for a scientific field test
- Benefit cost analysis

Field trials are not part of the current project. If this project is successful, a subsequent project to field test the apparatus, and to conduct a scientific test of the possibilities of enhancing early juvenile survival in a test area, will be developed.

This new project will need to include, not only the effects of the apparatus on the early juvenile lobsters, but also its effects on the animals in the ecosystem into which they are introduced.

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Phillips, B.F., Melville-Smith, R., Rossbach, M. and Cheng, Y.W (in press) Examining puerulus harvesting and the question of biological neutrality in the western rock lobster, and tecniques for large scale harvesting of lobster pueruli. Final Report on Project 98/302 to FRDC

Health assurance for southern rock lobsters *(Jasus edwardsii)*

Judith Handlinger, Barry Munday, Stephen Pyecroft and Caleb Gardner.

Tasmanian Aquaculture and Fisheries Institute, Fish Health Unit, DPIWE Animal Health Laboratory, Tas, Australia

Background

Basis of Aquaculture Initiative

Because of the long larval stage (up to 18 months), larval culture is still under development. Short term culture of juvenile *Jasus edwardsii* will rely on puerulus capture. As wild harvest is already limited for sustainability, there is concern that puerulus collection is stock neutral. It is aimed, therefore to return a proportion of on-grown farm stock to the wild to neutralise effects of collection (after approximately one year on-growing). The number to be reseeded is to exceed the number estimated to survived naturally .

Rock Lobster Health Issues include:

- Those common to a mature wild harvest industry with little history of disease investigation
- Those common to new aquaculture species
- Specific issues as a result of the proposed conditions imposed to for establish aquaculture sector.

Health issues associated with puerulus return

For neutrality to succeed, there is a need both to minimise the impact of returned animals, and to maximise survival of returned animals. To minimise the effect of returned animals, data on health of both wild and cultured animals is required, and to assess risk of disease transfer, a knowledge of disease distribution. To assess if there will be adequate survival of returned animals, we need to ensure only healthy stock are returned ensure we have knowledge of their normal behaviours (being addressed through other research), and have an estimate of the likely % natural survival: currently using maximum estimates, but there is a high likelihood that this may vary between settlement localities.

Health issues for the aquaculture initiative:

Few diseases of lobsters are known, confirming good aquaculture potential. However there have been few lobster disease studies (worldwide). It is typical of a wild harvest industry that only major diseases are detected. Culture unmasks disease because of crowding, possibly stress, and better observation.

Knowledge of the more common diseases is needed also to manage health of stock in culture, as well as to assess risks of returning stock to wild

The aim of project

To establish what diseases are present in Tasmanian bioregions (6 main areas), compare disease prevalence of wild and aquacultured animals, conduct a risk analysis of these findings, and to make recommendations whether, and under what conditions, to return juveniles to the wild.

The reasons for examining the health of released juveniles are that although there is regarded as only a small risk of spread of disease, the consequences of such spread are unknown. This is a standard, responsible action to avoid regional shift of diseases, address wild fishers concerns, and incidentally to assess the significance of common diseases for the better health management in culture.

Long term expectations or outcomes of the project are that there will be an on-going part-industry funded surveillance program for aquaculture, similar to other industries. Base-line data, of both wild and cultured stock, is needed for such a program. The study will have other Incidental advantages, such as supporting quarantine policy.

Methods

Statistically-relevant numbers of representative groups of juvenile wild southern rock lobsters (40/gp, 6 regions, collected by diving in conjunction with stock surveys, as far as possible), are being examined. This will provide 95% confidence that 1 infected animal will be seen if a disease is present at a prevalence of 10% or less, if the test is 75% efficient. The project will similarly examine cultured rock lobster stocks, using a similar sample number per farm, after a minimum culture period of at least 6 months, during which health will be monitored. All lobsters are examined grossly, then histologically. Lobsters with clinical signs of illness or lesions will be further examined including by bacterial culture. In samples with no animals showing clinical signs or gross lesions, bacterial culture of haemolymph of a random sample of lobsters is undertaken to provide a minimum of approximately 10% of lobsters cultured.

The prevalence of diseases in farmed and wild samples will be compared, and a risk analysis undertaken to determine the probability of adverse health consequences resulting from the release of cultured rock lobsters.

Progress

Constraints to progress included a delay in commencement of aquaculture based on *puerulus* collection (only 1 small batch collected so far, health of these being monitored). Farm sampling has been therefore delayed until next year. Wild sampling has proceeded to ensure the opportunity is not

missed. Samples have so far been received from 5 wild sites (2 samples incomplete). Examination of these is in progress. No significant diseases so far detected. Several parasites, one possibly incidental Vibrio isolate, and one lobster with gut necrosis / mummification syndrome have so far been seen.

King Island

Sampled January: 41 juvenile / undersized lobsters, carapace length 39-93 mm. One with minor shell erosion, which was confirmed histologically.

East Coast

Sampled February: 41 sampled, carapace length 34 - 108, 2 with gill barnacles, 2 with black gill spots (possibly related to barnacles.

Flinders Island

Sampled March: 26 lobsters only collected, as collection was abandoned due to weather conditions and concern for divers safety. Carapace length 60 - 93 mm, 1 lobster with gill chamber turbellarians plus eggs, 1 with black spots in gill. One lobster showed gut mummification and necrosis. Bacteria appear to be visible within the gut reaction, though it is uncertain if this is the primary infection. Bacterial necrosis and mummification hepatopancreas tubules has been seen in lobsters in New Zealand, which were possibly flagellate associated. (Diggles, pers com.) That case was associated with particular feed, as was a similar freshwater crayfish condition (WA). Similar findings have been rare and largely incidental.

Bruny Island

Sampled April / May: 42 samples, 1 with barnacle on tentacle, 1 with shell fouling by white polychaetes, 2 with muscle streaks (no significant findings on wet smears, histology to follow), 1 with gill detritus plus a nematode and sessile protozoa.

Low numbers Vibrio navarrensis cultured from one lobster. This is the only bacteria so far isolated, with no obvious significance or associated pathology.

Discussion / Interpretation

Results so far are promising, and not unexpected, given the very few diseases of lobsters so far defined. However there have so far been few studies of spiny lobster health. A pilot study which monitored health of juveniles and phyllosoma of this species in culture for two years has previously

been reported. (Handlinger et al, 2001) In that study, no major diseases or specific pathogens in either phyllosoma or juvenile age group were detected, though very few animals were examined, with no data on wild animals.

Losses related to environmental conditions and/or secondary infection with ubiquitous organisms. Very occasional unidentified parasites were seen. Diseases of juvenile lobsters known from Tasmania include external fouling, shell disease, bacterial enteritis, and food associated inappetence (not infectious, associated with one batch of mussels fed). Fouling with epibionts is common on gill, body and appendages, associated with filamentous Leucothrix-like bacteria, other bacteria and protozoa.

Heavy fouling may be associated with exoskeleton damage by non-Leucothrix-like bacteria, leading to sub-dermal granulomas, gill necrosis, and exoskeleton ulceration. This was associated with poor water, poor flow, high temperatures, and possibly contamination via the food. Shell disease with chitinoclastic bacteria and unidentified fungi was uncommon, associated with trauma. This has also been seen in wild Tasmanian stocks (not this survey). One past episode was associated with poor trap design. Bacterial enteritis has so far been seen in one group of newly caught juveniles from Tasmania and New Zealand , and is very common in phyllosoma.

Other potential threats include the exotic bacterial disease gaffkemia; and a recently discovered lobster virus, also regarded as exotic (Shields, J.D. and D.C. Behringer. (In preparation). Most other described lobster diseases are related to poor environments or involve ubiquitous pathogens. Unless additional diseases are found, the major risk to success of release could be the survival of released animals, not translocated pathogens. Whether release of cultured juveniles is the best strategy for population neutrality may also depend on more data, particularly with regard to differential puerulus survival at differing sites. Selective collection from poor survival sites could still be a possible future alternative if disease risks are discovered.

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J. Handlinger1*, J Carson1, A.J. Ritar2, B.J. Crear2, D.P. Taylor1 and D. Johnston3 (2001). Disease conditions of cultured phyllosoma larvae and juveniles of the southern rock lobster (*Jasus edwardsii*, Decapoda; Palinuridae). Proceedings of the International Symposium on Lobster Health Management, Adelaide 1999. L.H. Evans & J.B. Jones (Eds). Fisheries Research and Development Corporation, South Australian Research and Development Institute. (available at www.curtin.edu.au/curtin/muresk/lhm/index.htm)

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Progress in southern rock lobster reseeding research

Caleb Gardner[1], David Mills[1], Megan Oliver[2], Rob Stewart[2] and Alison Macdiarmid[2]

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[2] National Institute for Water & Atmospheric Research Ltd, Auckland, New Zealand

Introduction

"Reseeding" is the process of releasing southern rock lobster juveniles from tank culture back onto natural coastal reef. Research into this process of reseeding was originally initiated to provide information for the management of the harvest of puerulus for ongrowing in Tasmania. Permits for puerulus harvest were issued on the condition that a proportion of juveniles be released or reseeded after 1 year to compensate for the removal of puerulus from wild stocks. Issues that were identified for the management of this process included:

- the survival of juveniles released back onto reef;
- the implications of atypical behaviour in tank-reared animals on predation after release;
- the habitat of release;
- the method of release;
- the effect of altered juvenile density on survival in coastal reef;
- assessment of the risk of introduction of disease from aquaculture to the wild.

Although each of these research areas relate specifically to the release of juveniles in conjunction with the harvest of puerulus, they are equally applicable to gaining knowledge on the broader concept of enhancement. Each of these aspects has been addressed through projects funded by RLEAS. The first 5 are addressed by FRDC project 2000/185, the last, on disease, by project 2001/094.

Rock lobster aquaculture based on the harvest of puerulus has proceeded slowly in Tasmania and no reseeding has occurred to-date. Permits for the harvest of 350,000 puerulus were first issued in 2001, but there was insufficient time between issue of these permits and the settlement peak in August 2001 for the deployment and conditioning of collectors. As a result, no collection gear was successfully deployed in 2001.

The first opportunity to evaluate the commercial harvest of puerulus will occur in August-October 2002. Several harvest plans have now been submitted to the State Government and commercial collectors have been deployed. Commercial operations in this first year appear to be mainly of a "prospecting" nature – permit holders are evaluating sites and collector design.

The survival of juveniles released back onto reef

Short term survival

This component of the project relates to actually quantifying or measuring the survival of juveniles in large-scale releases over the first few weeks after release. Techniques for conducting this research were developed in an earlier project (RLEAS 1999/314). Large-scale releases are planned for late 2002 and 2003 using juveniles ongrown from puerulus collected on research collectors and salmon predator exclusion nets.

A key point to understand about the planned large scale releases is that they will provide us with estimates of survival of reseeded juveniles, but for only a limited number of sites. Although these experimental large-scale releases will provide a guide to the success of these operations on an industry scale, they will be few in number and thus have low-value for comparing survival between habitat types. Consequently, comparisons between release habitats will be assessed though different techniques, described later in this report.

Long term survival

We intend to augment data collected on short-term survival with longer term tracking of reseeded juveniles. This "longer term" survival could be over periods of several years or until the lobsters reach legal size. For this purpose, we require a tag that is both retained through moults, and is also small and benign so that it does not affect survival. Microwire tags (Fitz and Wiegert, 1991) are ideal and have been used in several other research projects on small crustaceans (Figure *1*).

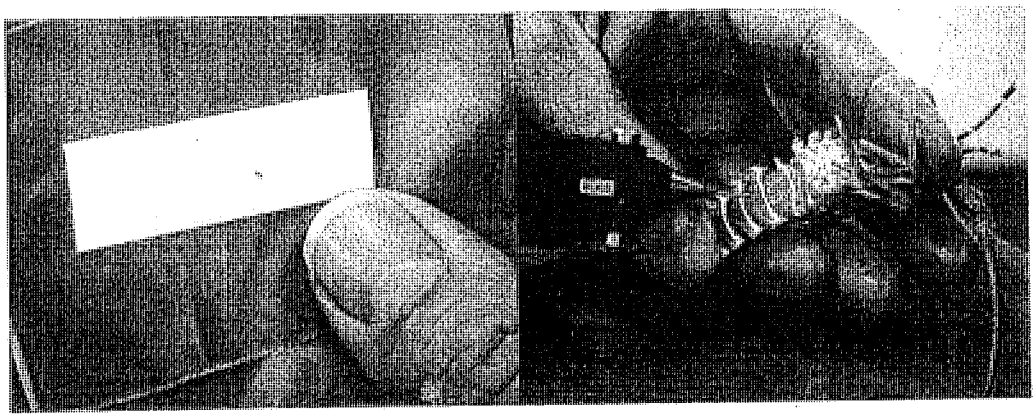

Figure 1. *Microwire tagging of juvenile southern rock lobsters. The tag is shown at left as a small wire fragment less than 1mm long. Implantation of a juvenile is shown on the right.*

Tagging reseeded animals so that they can be identified in future catches is only half of the solution to obtaining information about the fate of reseeded juveniles over longer time periods, such as several

years after release. We also need a mathematical method to relate tag-recapture data back to some measure of abundance of reseeded lobsters on the sea-floor.

We have recently evaluated a new technique for assessing lobster density from tag-recapture data, termed a trapping web design. This technique has been adapted from bird research methodology and no research has been published in applying it to crustaceans. It is an interesting technique for research on reseeded lobsters as it provides an estimate of the density of lobsters (numbers per square meter), rather than abundance (numbers). These differences are subtle, but given that experimental reseeding will target isolated patches of reef, a measure of density would be helpful.

The trapping web technique involves setting lobster traps in an array shaped like the radiating spokes on a wheel – or a spiders web (Figure 2). Traps are spaced evenly along the "spokes" so that they form concentric circles. The technique relies on the assumption that a lobster living towards the centre of the "web" is more likely to be captured as traps become clustered more closely.

The trapping web technique has two assumptions, which we tested in a pilot scale exercise at the Crayfish Point Scientific Reserve, Hobart. The first of these relates to the proportion of animals captured at the centre of the web – which we measured using underwater video cameras placed on the traps in the centre of the web. The second assumption relates to the scale of lobster foraging movement in relation to the spacing of the traps. We tested this by tagging some individual lobsters with acoustic tags; the position of these tags was then recorded every few minutes over 2 weeks using a "VRAP" triangulation system (Figure 3).

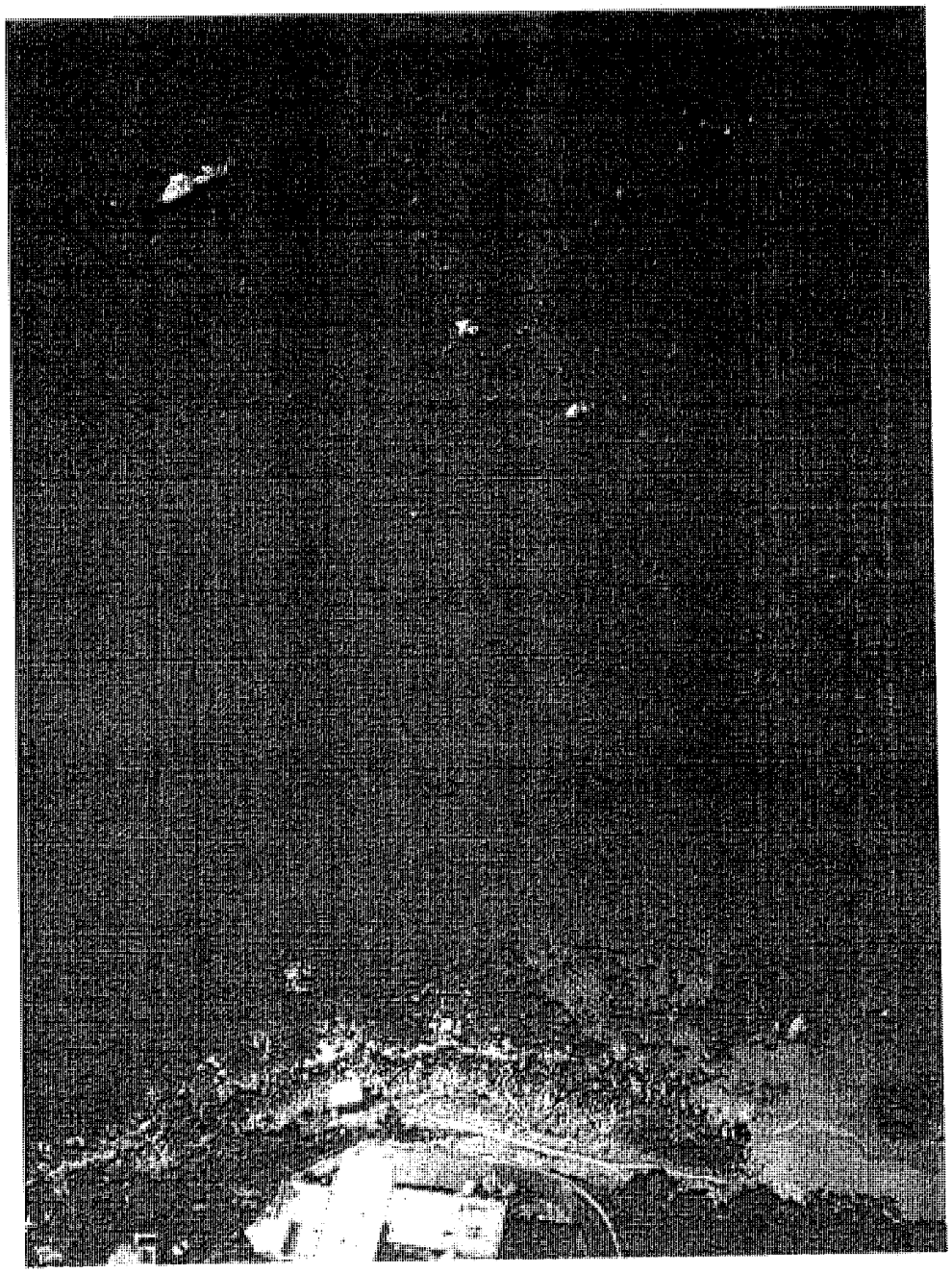

Figure 2. *Layout of the trapping web at Crayfish Point Scientific Reserve, Hobart. This method of estimating lobster abundance has promise for long-term surveys of the abundance of reseeded lobsters on patch reef. Lines of buoys attached to traps can be seen radiating outwards from a central point. Three vessels involved in checking the traps are also visible. Note that the proximity of traps increases towards the centre of the web, which implies increased probability of capture.*

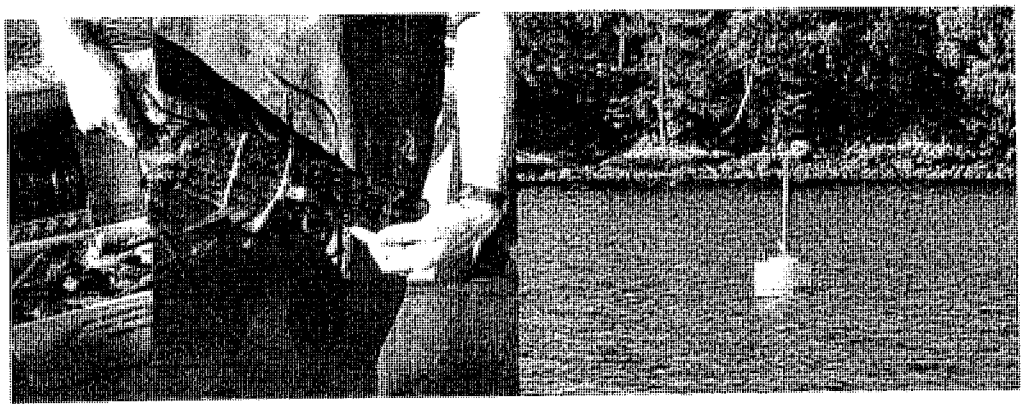

Figure 3. *VRAP sonic tracking used to evaluate lobster foraging range. The extent of the foraging range influences the size of the trapping web – a technique being evaluated for future surveys to measure the abundance of reseeded lobsters on patch reef. Sonic tags were placed on adult lobsters (left). These tags are detected every few minutes by hydrophone buoys (right) which than transmit the position data back to a shore-based receiving station.*

Results from the first trial of the trapping web are encouraging although it is clear that the assumption of 100% probability of capture of lobsters in the centre of the trapping web will be violated. Analysis of video recordings of the behaviour of animals attracted to the traps is underway in an honours project (Nikki Green). It appears that a large proportion of lobsters attracted to the traps do not enter. We are seeking to refine the trapping web technique by scaling our estimates of lobster density based on some measure of proportion tagged in the centre of the web.

The behaviour of tank-reared animals after release and the implications for predation

Juvenile lobsters ongrown in captivity develop daily cycles of emergence from shelter and foraging that are different from that of wild animals, with much more time spent away from shelters during daylight hours (Figure 4). This is of concern for reseeding operations as it suggests that reseeded lobsters may behave in a manner that increases their predation risk after release.

Several experiments have been conducted to address this issue including:

- developing methods to entrain cultured animals into natural cycles of emergence behaviour;
- conditioning cultured juveniles to predators prior to release to improve predator-avoidance responses;
- examining the emergence behaviour of reseeded juveniles after release – do they forage like wild juveniles?
- examining the predator response of cultured juveniles after release – do they exhibit normal predator avoidance/defence behaviour?

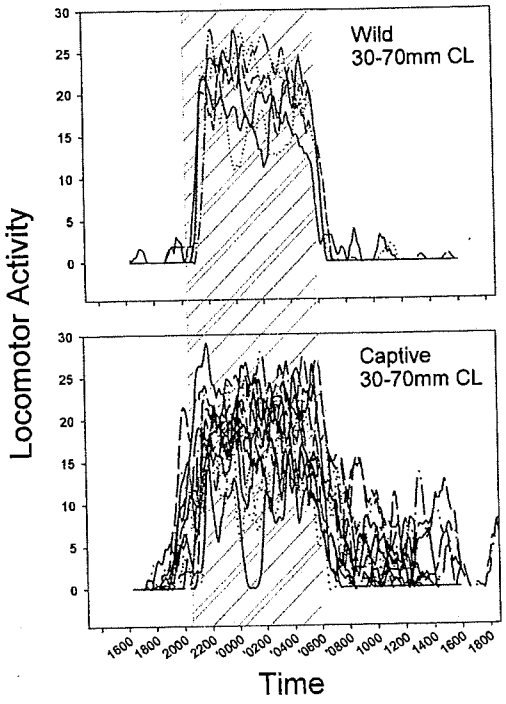

Figure 4. *Emergence times of recently captured wild and captive-reared juvenile Jasus edwardsii in a laboratory tank. The shaded area indicates the hours of darkness. Each line indicates a replicate trial of ten lobsters. Note that captive reared lobsters are more likely to emerge during daylight hours.*

Methods to entrain cultured animals into natural cycles of emergence behaviour

Experiments were conducted to entrain juvenile lobsters in culture into normal emergence patterns (ie sheltering during daylight hours and emerging from shelters at night). We found that it was relatively simple to reduce the incidence of emergence from shelters during daylight hours by either exposure to predators in tanks, or simply by only adding feed at dusk. These simple techniques could be used in culture to develop more normal emergence behaviour before release. However, as we explain below, this step may not be necessary, as reseeded juveniles appear to respond to predators appropriately, even without prior conditioning in tanks.

The shelter emergence behaviour of reseeded juveniles after release – do they forage like wild juveniles?

To test if cultured juvenile lobsters reverted to normal emergence behaviour after release onto coastal reef, infra-red (IR) video observations were made of juveniles in dens. It was found that duration of emergence from dens was the same for reseeded juveniles and wild controls. Also, the daily timing of emergence was the same with reseeded animals vacating dens mainly at night. This experiment indicated that reseeded lobsters will immediately revert to normal foraging behaviour after release – which is clearly encouraging for future releases.

The predator response of cultured juveniles after release – do they exhibit normal predator avoidance/defence behaviour?

IR video observations have been made on the response of juveniles reseeded in both Tasmania and New Zealand. Analyses are yet to be completed, although preliminary results suggest that lobsters reared without a predator exhibit appropriate anti-predator behaviour when they encounter a predator for the first time (Figure 5). Juveniles attempt to avoid predators by backing into their crevices or tail-flipping, and attempt to fend off predators with their antennae. As predicted by the initial experiment examining the behaviour of naïve lobsters on reef, these results reinforce the instinctive nature of anti-predator responses and suggest it may be unnecessary to deviate from existing captive rearing practices when on-growing juvenile lobsters for release into wild environments.

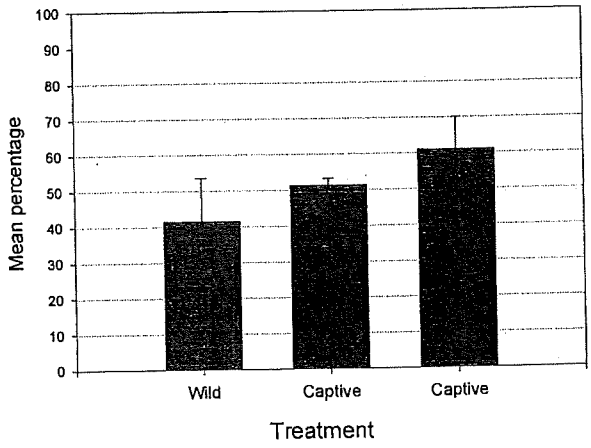

Figure 5. *Mean percentage of time (± SE) spent by juvenile Jasus edwardsii responding to the presence of predators on a shallow reef in New Zealand with high predator density. Three cameras were in operation, one trained on wild juveniles the other two on recently released captive juveniles. Captive reared lobsters clearly responded to predators when approached for the first time.*

Evaluation of the effect of habitat type on survival of released 1 year juveniles

We expect that the survival of reseeded juveniles will vary between habitat types, which is clearly important information for optimising the benefit of any large-scale release operation. Numerous observations of survival success in different habitat types is needed for investigation of this issue as "habitat-type" encompasses such a wide range of variables – eg rugosity, algal cover, wave exposure.

In practice, the only means of collecting such a broad range of observations of survival under different conditions is through "tethering" experiments. This experimental method has been widely applied in marine ecological research and involves attaching the animal to a weight by a cord or tether (Figure 6). The technique ensures that the animal can be recovered –either alive or dead – there is no uncertainty with animals that disappear (have they died or simply walked away?).

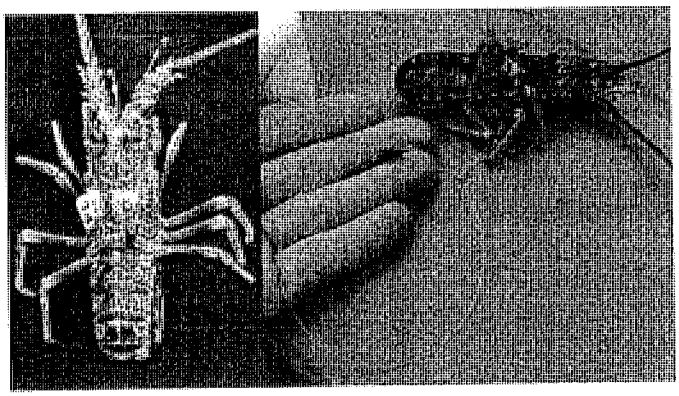

Figure 6. *Tethering of juvenile rock lobster for trials to compare predation rates between habitat types. A swivel is attached to the top of the carapace. A mono-filament tether is then run from the juvenile to a weight – and the animal released into a crevice on coastal reef.*

As with the large –scale releases of tagged (and un-tethered) juveniles that will occur in this project, results from tethering trials contain a series of assumptions that must be tested in separate trials. Problems inherent in tethering studies will be overcome by:

- mesocosm trials to quantify tethering artefacts (Figure 7);
- video observations of a proportion of animals to evaluate the nature of the bias of the tether (Figure 8)
- tethering trials will be conducted in the same locations as large scale releases – consistency of outcomes will provide a test of tethering results.

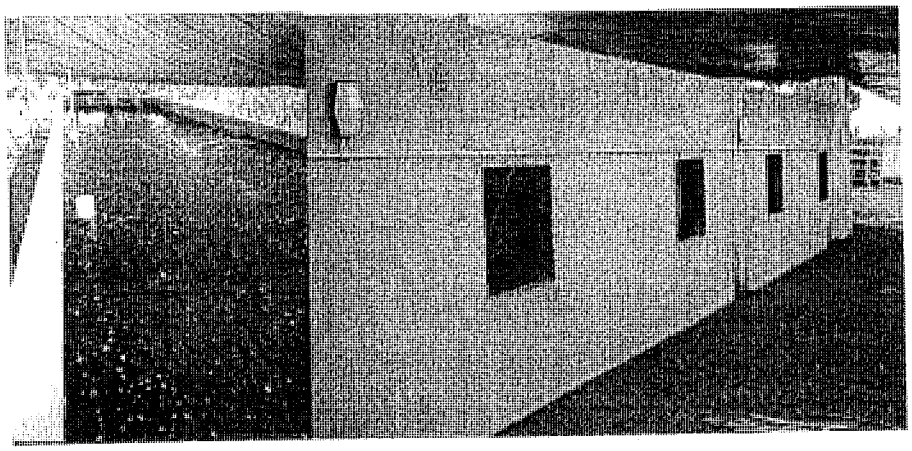

Figure 7. *Mesocosm to be used for testing assumptions of tethering experiments. This large tank (200 cubic meters) contains viewing windows so that predation of animals with and without tethers can be monitored in the presence and absence of different predators. Predators to be tested are those identified from video observations in field trials.*

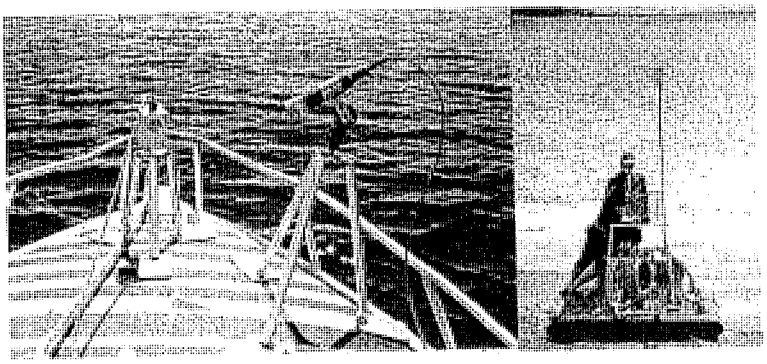

Figure 8. *Infra-red video equipment used to monitor the source of predation in tethering trials.*

Experimentation for these trials to assess the effect of habitat type on survival is to be conducted in both New Zealand and Tasmania. Experimentation will be repeated at the same sites in Tasmania to evaluate the influence of season on habitat type – it may be that predation is high in some habitats only in certain seasons (Figure 9). This work is still in progress although some preliminary results are given here. At this stage we are unable to detect any difference in survival between New Zealand sites, although predator abundance varied between sites (Figure 10 and Figure 11).

An automatic variable selection process was used to select which of the 16 environmental variables best explained survival in the New Zealand data set. The two variables with greatest influence, carapace length and abundance of juvenile algae, were then analysed using a multiple regression. There was no significant effect of carapace length on survival and although there was a significant effect of juvenile algae abundance, this was very small ($P = 0.031$). This effect is statistically significant but probably not biologically significant.

Video observation of the predation of tethered juveniles indicates that most daytime predation is by wrasse, while most night time predation is by larger lobsters. The effect of tethering bias on these observations requires further testing in mesocosm trials.

In conclusion, at this stage there are no clear indications of what habitat variables affect survival – the mortality of reseeded juveniles appears to be a largely random process – being in the wrong place at the wrong time. Note however that we are yet to pool Tasmanian and New Zealand data. Trends may emerge from the larger data set.

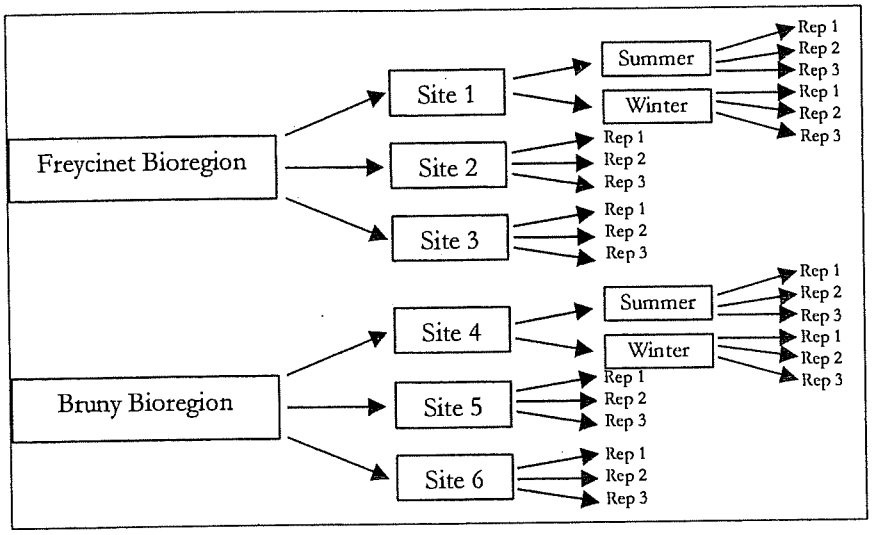

Figure 9. *Experimental design of experiment in Tasmania to assess influence of habitat type using tethered lobsters and video observation. Large scale releases of un-tethered lobsters will occur at some of these sites and this provides a means of testing the results from the tethering trials.*

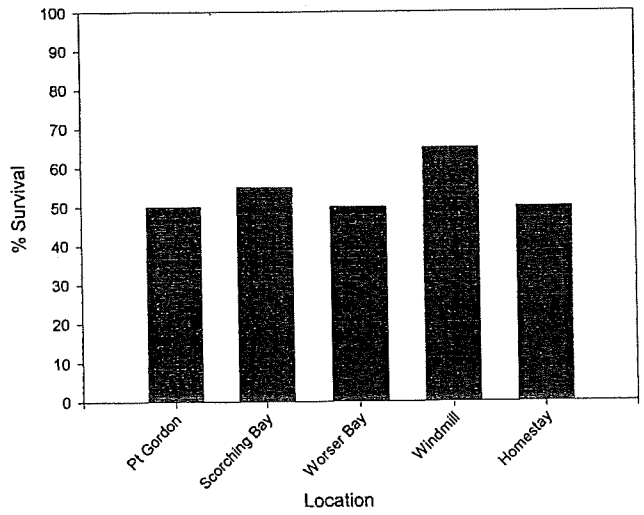

Figure 10. *Survival of tethered animals at 5 sites in New Zealand. Note that predator abundance and habitat type varied between each of these sites (see Figure 11).*

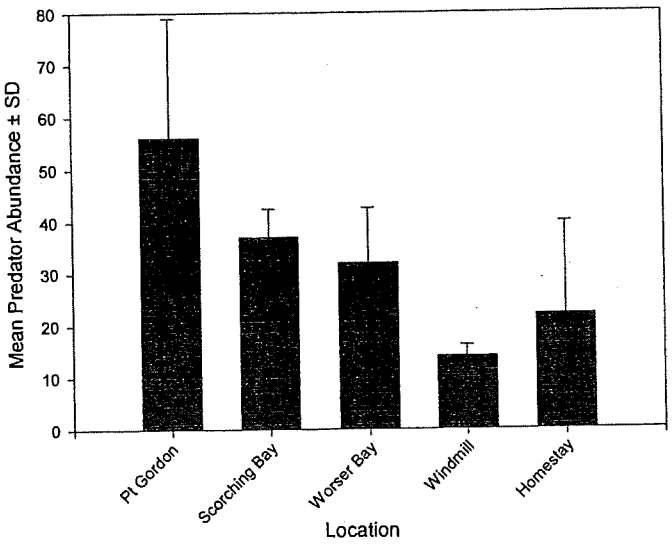

Figure 11. *Abundance of predators at each of the tethering sites in New Zealand.*

References

Fitz, H.C., and R.G. Wiegert. 1991. Tagging juvenile blue crabs, *Callinectes sapidus*, with microwire tags: retention, survival and growth through multiple molts. Journal of Crustacean Biology 11:229-235.

Propagation of rock lobsters: An overview

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FRDC project 2000/214 (The development of rock lobster propagation techniques for aquaculture in Australia) focuses on nutritional aspects of rock lobster larval rearing. The project has produced a significant amount of information (see major findings below) that will be extremely useful in the long-term goal of the successful rearing of large numbers of puerulus from eggs. However, in the short-term it has not resulted in a significant improvement in our ability to rear phyllosoma.

Major findings

- data from analyses of wild larvae and potential prey items, suggest that a diet containing a polar lipid-rich oil, high in docosahexaenoic acid (DHA), may be appropriate for *J. edwardsii* phyllosoma.
- nutritional studies have so far proven inconclusive regarding an appropriate *Artemia* enrichment to use.
- depletion of lipids during starvation of *J. edwardsii* phyllosoma was mainly due to a reduction in polar lipids, with minor changes in other lipid classes. This is a further indication that a diet containing a polar lipid-rich oil may be more appropriate.
- digestive enzyme production occurs post hatch in stage 1 *J. edwardsii* phyllosoma.
- total enzyme activity increased with development. This indicates that phyllosoma have a significant ability to digest a wide range of dietary components from an early stage of development.
- mouthpart and gut structure have outlined the feeding processes of phyllosoma. The results give an indication of the appropriate form of diets (both live and formulated) to provide to phyllosoma.
- Stage1 and 2 *J. verreauxii* phyllosomas rely on random encounters to capture prey but phyllosomas as young as Stage 3 are able to actively pursue and capture prey and will accept inert diets.
- formulated diets in various forms have been produced, although growth and survival of phyllosoma have been lower with formulated diets than with *Artemia*.
- *J. verreauxii* phyllosoma had an increased ability to handle and grow on formulated diets as they progress through the stages.
- phyllosoma appear to be attracted to formulated diets. Fresh mussels proved to be the most attractive dietary ingredient to *J. verreauxii* phyllosoma.
- formulated diets appear to cause increased fouling and microbial contamination in culture systems.

Much of the planned research has been made difficult, and the results in some cases made ambiguous, by poor survival during experiments. At this stage the low survival rates appear to be due to disease issues (probably due to bacterial contamination) rather than poor nutrition, although there would obviously be an interaction between these factors. Under appropriate conditions high rates of survival can be attained through the first 4-5 larval stages (spanning 30-45 days), indicating that lobster larvae have attributes amenable to culture conditions. Ensuring that we can consistently reproduce those high rates of survival over successive larval rearing runs will increase the efficacy of results when examining factors such as nutrition and system design.

The development of successful culture techniques for rock lobsters is a difficult goal that will require a long-term commitment. Some species may prove more adaptable to culture than others, but no particular species have been identified as yet. The issues hindering success appear similar between the species, so research effort put into one species should prove beneficial to others.

There has been considerable effort put into rock lobster culture over the last 3-4 years, which has resulted in the development of significant research capability. Several research organizations now have the facilities to undertake research on rock lobster propagation, as well as experienced personnel. This capability will ensure that future research efforts are optimised.

One of the major short-term goals (1-2 years) needs to be to develop the ability to achieve consistent high survival through the early phyllosoma stages. Unless this is being realised then high levels of success over a protracted larval cycle would be unlikely. I believe if we are to be successful in our future research efforts than an integrated approach, encompassing aspects of health, nutrition and system design will be required.

Propagation research in New Zealand

Dr Michael Bruce & Graeme Moss

National Institute for Water & Atmospheric Research Ltd, Auckland, New Zealand

Summary

- Manipulation of holding conditions for broodstock has meant that NIWA now has the theoretical capability to produce larvae as and when desired.
- Early stage phyllosoma as young as instar 3 will accept inert artificial diets, however, survival is best on those fed solely on *Artemia*.
- Later stage phyllosoma will readily take and feed effectively on artificial diets with survival becoming comparable to those fed solely on *Artemia*.
- The best performing artificial diets were those made using moist components (mussel, squid and *Artemia*) and bound using a food grade enzyme commonly used in human food products.
- Preliminary trials to investigate the use of beneficial bacteria to control the growth of harmful bacteria in the phyllosoma production system have provided promising results.

Introduction

Over several years now NIWA has endeavoured to complete the life cycle of the two species of rock lobster found in New Zealand, *Jasus edwardsii* and *Jasus verreauxi*. Latterly efforts have concentrated on *J. verreauxi* as its hardier nature and shorter life cycle make it a more attractive option for aquaculture development in New Zealand. So far culture efforts have succeeded in producing puerulus of *J. edwardsii* but the animals failed to reach the final stage of the larval life cycle and become settled juveniles. However, NIWA's efforts to complete the rock lobster life cycle have meant that a range of research topics has been covered. These include larval production and quality, studies on feeding behaviour and nutrition and other aspects important to the production cycle namely disease management and the development of rearing systems.

Propagation topics

Broodstock Management, Egg Production & Larval Quality

Any aquaculture operation needs a reliable high quality supply of eggs to stand any chance of being successful. Hence a priority at NIWA has been to investigate ways to optimise egg production through broodstock management. The first management tool thought worthy of development was the ability to accurately predict hatching and release of naupliosoma at any given temperature. To achieve this, researchers measured the appearance of several embryonic developmental stages against time, namely the median eye, eyespot, chromatophore and hatching carried out at various temperatures. This information was combined and used to calculate an eye index value. The eye index once calculated

and correlated to temperature can be used to very precisely predict when the eggs will hatch (Figure 1).

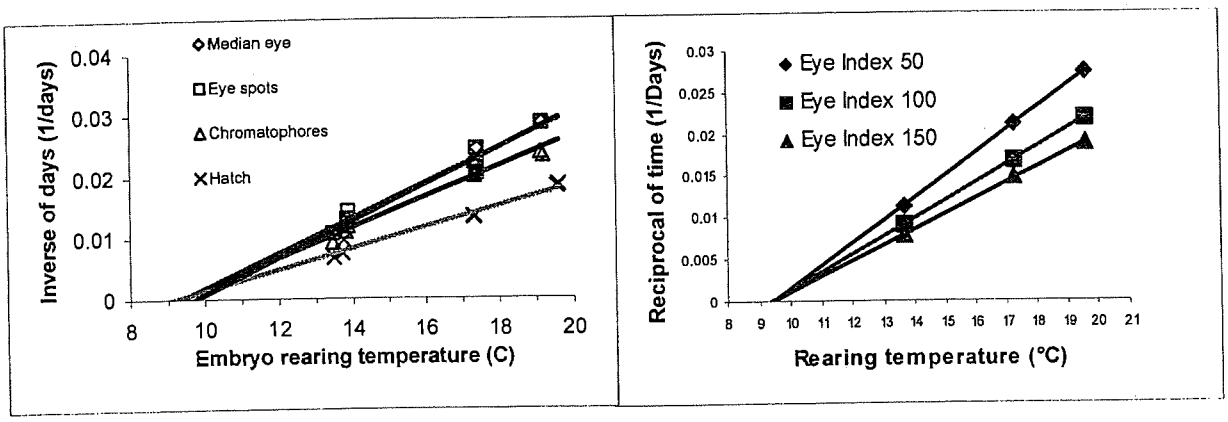


Figure 1. *Shows how larval development parameters are used to produce an eye index value used to predict hatching.*

In addition to being able to accurately predict hatching, NIWA has, over the last 3 years, sought to establish the optimum holding conditions needed to induce natural courtship and mating behaviour for *Jasus sp.* to produce the best quality larvae.

Figures 2 and 3 below summarise an experiment aimed at finding out the optimum temperature needed for embryogenesis for *Jasus verreauxi*. In this experiment the broodstock were held at three different temperatures 13, 17 and 21°C. Figure 2A presents the numbers of larval released plotted against the duration of larval release from the broodstock held at the three respective temperatures.

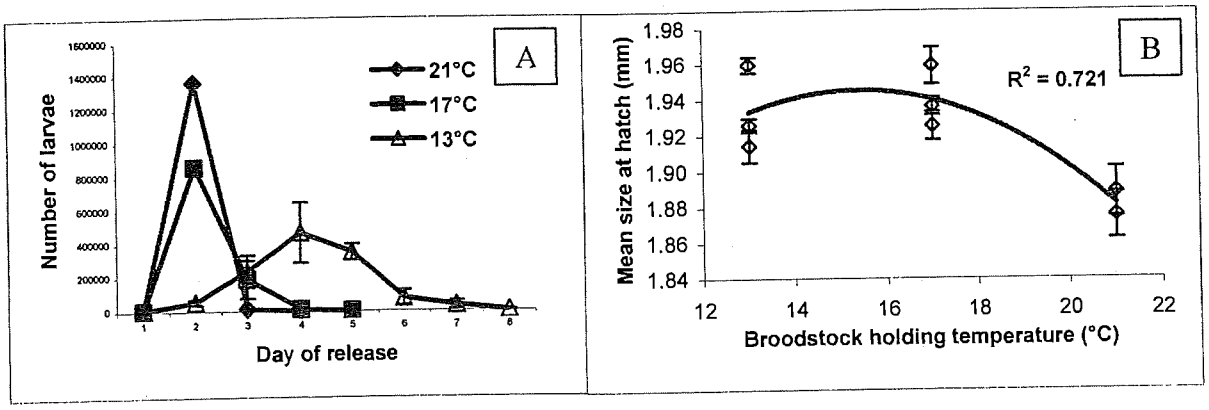


Figure 2. *Graph A details the number of larvae produced on each day of release at three broodstock holding temperatures. Graph B plots the mean larval size at hatch (mm) against the broodstock holding temperatures.*

Figure 2A shows that as broodstock holding temperature decreased, the duration of larval release increased. Figure 2B reveals that on the day of peak hatching when most larvae are released the largest larvae produced were derived from those broodstock held at 17°C.

In addition, Figure 3 clearly shows that increasing larval quality, in this case represented by the LD_{50} values, is strongly correlated to larval size. These results suggest that the optimum temperature for embryogenesis would be 17°C.

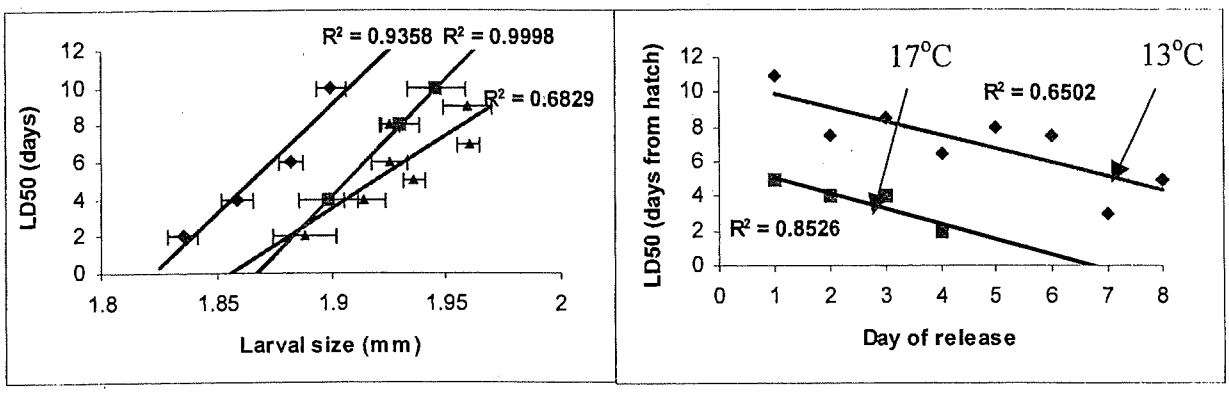


Figure 3. *LD_{50} plotted against larval size at hatching (A) and the day of release (B).*

However, Figure 3B plots the day of larval release against the LD_{50} for larvae derived from broodstock held at 13 and 17°C. The graph shows that the later the larvae are released the lower their quality. More importantly, larvae derived from 13°C broodstock on a day-to-day basis have the highest LD_{50}'s and are therefore better quality, although the numbers of larvae released with the highest LD_{50}'s for 13°C broodstock are low due to the spread of release.

At NIWA we think that a solution to this problem might be to use colder temperatures during embryogenesis, which would be around 13°C for *J. edwardsii* and around 15°C for *J. verreauxi*. Approximately 1week prior to the expected due date, the broodstock holding temperature could then begin to be gradually increased to about 20-21°C. The predicted outcome of such a manipulation would the be concentration of the larval release into one or maybe two days resulting in significantly better quality larvae. However, this is speculation based on the results and would need to be confirmed experimentally.

Larval Feeding Behaviour

The research on feeding behaviour was designed to answer two general questions. Firstly, identify the capture and orientation behaviour of early stage phyllosoma while feeding on *Artemia* and secondly identify what causes the initiation of feeding behaviour, both chemical and physical. The aim of this work was to produce information that would ultimately form part of the criteria for the construction and formulation of an artificial diet.

The results of the observational work were that the methods of prey capture, handling and manipulation by Instar I-III phyllosoma were remarkably similar between the two species, and

followed a characteristic sequence of events. In addition the work revealed that feeding behaviour of *J. edwardsii* and *J. verreauxi* phyllosoma was consistent with that exhibited by other crustaceans whereby, food is captured by the pereiopods and passed forward to the mouthparts.

Our conclusions from this work are that the early stages, particularly Instar I with their tight and tumbling feeding action, would seem to require large numbers of targets so small artificial diet particles or *Artemia* would seem to be most suitable during the early sages of the larval life cycle. However, later instars become more mobile hence more able to attack larger fleshier prey or attach to a large inert feed item.

During the next phase of our research, we assessed feeding induction and stimulation as a basis for identifying attractants that could be used in an artificial diet. Our first experiment examined a combination of single potential attractant chemicals identified from the literature and combined these in a inert base pellet which was then placed at one end of a laminar flow chamber. The relative success of the candidate attractant was gauged by timing the speed of movement of larvae from the opposite end of the chamber. The results showed that mussel and *Artemia* were the most successful attractants but proline, glycine and glucosamine showed promise.

The attractant work was further extended to consider what information on attractants might be gleaned from the associated zooplankton, or potential natural prey items, caught during trawls to catch and assess phyllosoma population dynamics carried out under NIWA's fisheries research programme. While it was difficult to assess what zooplankton the phyllosoma were feeding on in the wild, it was possible to split the zooplankton trawls into broad groups and from these derive extracts. The extracts were then tested during an experiment in which phyllosoma were tethered under a video microscope and exposed to the extracts. A standard *Artemia* extract was also tested. An increase in exopod beating was considered a positive response.

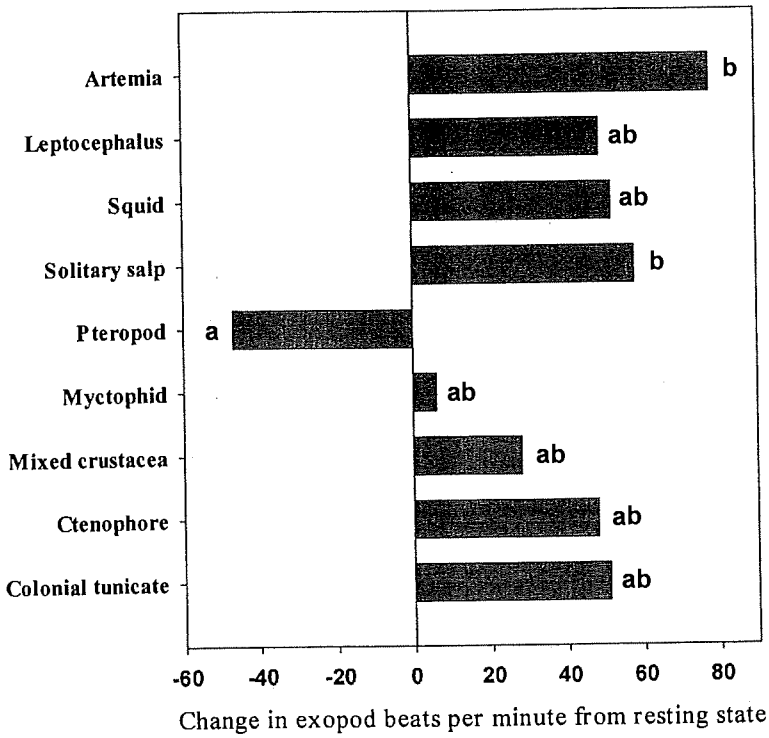


Figure 4. *Effectiveness of wild zooplankton extracts against an Artemia extract*

The information in Figure 4 shows that most of the prepared extracts elicited a positive response to varying degrees, however *Artemia*, performed best. Interestingly, pteropods produced a negative response, which could perhaps be a hard-wired predator response.

Larval Nutrition

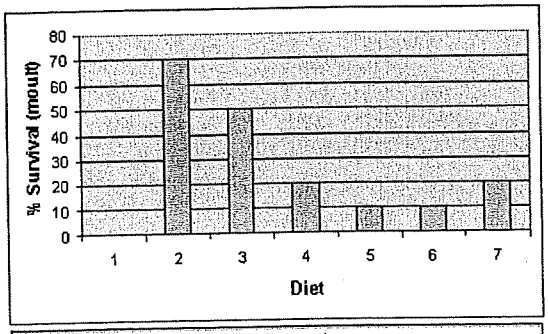
During the previous production season, staff at NIWA demonstrated that phyllosoma fed very enthusiastically on a so-called "feed station", based on minced mussel and gelatin. However, the format fell apart at the temperatures needed to culture *J. verreauxi*. Promising results have been obtained during the current season by making use of an enzyme commonly used in human foods called transglutaminase. This enzyme creates glutamate-lysine cross links and sets the mixtures to a stable, pliable mixture which can in certain formulations remain stable in seawater up to 30°C.

Throughout this season we have been trialing various artificial diets against free swimming *Artemia* using the new method of stabilising the diets. The conclusions to date are:

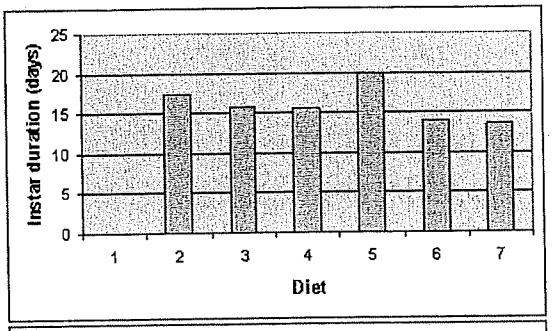
- Early stage phyllosoma as young as instar 3 (those that have been through 3 moult cycles) will accept inert artificial diets.
- Younger stages will take artificial diets, however, although early stage phyllosoma will feed on artificial diets, the survival is best on those fed solely on *Artemia*.
- Later stage phyllosoma will readily take and feed effectively on artificial diets with survival comparable to those feed solely on *Artemia*.

- The best performing artificial diets were those made using moist components (mussel, squid and enriched *Artemia*) and bound using a food grade enzyme commonly used in human food products.

Figure 5 below shows the actual results of the experiment where the mussel, squid and *Artemia* diet was tested for the first time.



1	Starved		
2	Artemia		
3	Fresh	Mussel:Squid:Artemia	1:1:1
4	Fresh	Mussel:Squid	1:1 + Free Artemia
5	Fresh	Mussel:Squid	1:1
6	Processed	Mussel:Krill	1:1+ DHA
7	Processed	Mussel:Krill	1:1+ DHA + Free Artemia



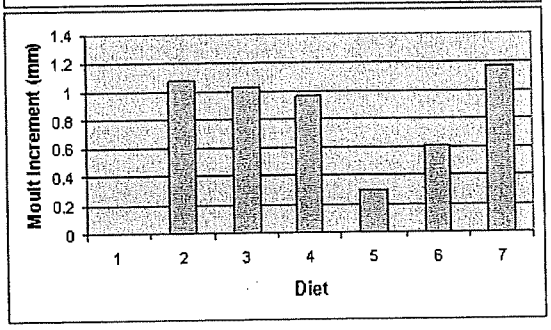


Figure 5. *Comparison of different diet regimes using starved, enriched Artemia and enriched*

Artemia/ feed station combinations.

One implication from the success of the mussel, squid and enriched *Artemia* diet is that it becomes unnecessary to on-grow or enrich the *Artemia*. The diet could theoretically include stage I *Artemia* nauplii with the enrichment products included in the final formulation.

Additional information:

- Work at NIWA has showed that it is possible to use 48hour enriched *Artemia* with no detrimental effects for Instar 1 and 2 and possibly 3 provided they are fed in high enough densities
- Preliminary trials with fish eggs and larvae indicate that this could also be made part of the overall protocol, but more likely as a nutrient source for the feed stations.

Larval Disease Management

Over the last 5 years, NIWA has been working on methods of disease management in culture systems as part of the biotechnology development programme encompassing a range of larval fish and shellfish species.

Within this programme, NIWA has identified several ways to address this issue. For instance, one approach would be to use alternative bacteria to achieve a favourable microbial balance either within the live feeds and/or the target larval species. Another method would promote larval health via immune stimulation through the use of bioactives with specific or non-specific activity, which are introduced to the animal using a number of means, but again primarily through the nutrition in a formulated feed or as part of a live feed enrichment.

Additionally, staff at NIWA have been testing green water/mature water systems although not as yet on phyllosoma.

So far during NIWA's disease management work, we have identified and isolated several bacteria with antagonistic activity *in vitro*. Currently, we are endeavouring to trial these bacteria *in vivo* within *Artemia* cultures to assess their effectiveness in modifying the bacterial flora of *Artemia* cultures, used to feed phyllosoma larvae. Our initial results with *Artemia* have shown that the bacteria significantly alters the microbial balance of the *Artemia* cultures infected with *Vibrio harveyii* a known pest in *Artemia* and phyllosoma systems.

Conclusions

The final section summarises knowledge gained at NIWA through our work and that of the Rock Lobster Sub-Programme team.

- The careful management of broodstock will yield high quality larvae.
- Feeding capability changes with age; this is revealed in both the physical and behavioural development of the animals and the results of feeding trials carried out to date.
- Feed stations begin to be effective from instar 5 but with some careful attention to detail, for instance tank design and feed format, it might be possible use the feed station format to feed the earlier stages thus inducing them to feed and moult successfully.
- The use of bacteria to achieve a favourable microbial balance shows great promise and may prove to be an effective tool in disease management of phyllosoma cultures.

How to proceed

- Green water culture methods could be revisited for the earlier instars.
- Continued development of feed station formats, using *Artemia* nauplii as a significant component but utilising products readily available in New Zealand, for instance mussel and squid meals.
- Continued development of bacteria to achieve a favourable microbial balance.
- Current rearing aquaculture systems need to be simplified by making use of technologies present in commercial systems, which have well developed methods for keeping delicate animals for long periods of time using cheap and effective laminar flow systems.

Digestive capabilities of Spiny Lobster *Jasus edwardsii* phyllosoma

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Abstract

The digestive enzymes of *Jasus edwardsii* phyllosoma larvae were examined at progressive stages of development (stage I: day 0, day 1, day 6; stages II, III, IV, V, VI, mid) to provide an indication of digestive function and nutritional requirements throughout larval culture. All digestive enzymes assayed (total protease, trypsin, α-glucosidase, chitinase and lipase) were present post-hatch which indicates that larvae have well developed digestive capabilities from an early age (at onset of feeding). Lipase activity was highest in all phyllosoma stages reflecting the importance of lipid in their diet. α-Glucosidase activity was negligible suggesting that phyllosoma may not be efficient at digesting carbohydrate in the early stages. This data needs to be verified by α- amylase assays. Developmental changes in enzyme profiles were evident indicating that they may be shifts in the importance of dietary components during the hatchery culture of phyllosoma. Proteolytic activity was highest post hatch and declined steadily to stage VI, whereas lipase activity was lowest post –hatch and increased during development to stage V.

Introduction

An understanding of basic digestive physiology is critical in investigations of nutritional requirements of marine crustaceans (Biesiot and Capuzzo, 1990). To date, very little research has been undertaken on the development of digestive function by early life history stages of crustaceans, with work concentrating on the digestive enzymes of larval prawns (Lovett and Felder, 1990a, b). It has been demonstrated that crustacean digestive physiology is adapted to different feeding strategies during larval development. Consequently an examination of digestive physiology provides an insight into the design of optimal feeds for commercial culture (Jones et al., 1997). As most larvae are dependent on enzymatic breakdown of ingested food, development of secretory digestive gland tissue dictates the type of diet which can be consumed. Research undertaken on the gut morphology of *J. edwardsii* phyllosoma to date (FRDC 99/315) has revealed that digestive function in early Stage phyllosoma (Stages I and II) is simple, with unspecialised digestive gland cells. However, by Stage V digestive gland epithelial cells appear to have differentiated, the F- (fibrillar) cells presumably being capable of enzyme production. This suggests that digestive capabilities vary between phyllosoma Stages and reflects a likely change in trophic level through development.

The digestive function of rock lobster phyllosoma has not yet been reported. Studies of gut ontogeny and digestive enzymes of clawed homarid and nephropid lobsters have shown that the larvae have limited enzymatic capacity compared to herbivorous crustacean larvae, but that trypsin-like proteases dominate. Lipases and amylases are also present but at much lower activities (Biesiot and Capuzzo, 1990; Kurmaly et al., 1990; Jones et al., 1997; Kumlu and Jones, 1997). This enzyme specificity, together with a comparatively slow gut evacuation rate, means that lobster larvae rely upon high-energy digestible live prey (most likely from released peptide/ amino acids initially, and subsequently protein and lipid) for their early nutrition. The digestive enzymes of rock lobster phyllosoma will be investigated to provide an indication of digestive function and nutritional requirements throughout larval culture. Specifically the types and concentrations of digestive enzymes will be documented and changes in digestive enzyme profiles highlighted during development. This study will establish whether phyllosoma larvae are capable of digesting prey immediately post hatch and are therefore capable of extracting essential nutrients with the onset of feeding. It will also establish whether their digestive capabilities differ (increase) with age. This information is critical for the provision of optimal diets throughout the hatchery period in order to provide the best ratios of nutrient inclusion in live and formulated diets.

Methods

Newly-hatched phyllosoma larvae were collected in July 2001 from a female held at the Marine Research Laboratories, Taroona for two years since capture. The female was on an altered phototherm regime to mate and hatch out of season. The temperature at the time of hatch was 17.5°C. After disinfection with 25 ppm formaldehyde in sea water for 30 min, phyllosoma were dispensed into 18 culture vessel (approximately 1,600 larvae vessel^{-1}; determined volumetrically). The system for culture of phyllosoma was described previously (Ritar, 2001), and consisted of sea water filtered to 1 µm, heated to 18°C and disinfected with ultraviolet irradiation before entering circular 35 l plastic vessels (containing 10 l water). Constant water flow via jets in the vessel assisted phyllosoma movement and mixed the *Artemia* through the water column. There was partial recirculation of water through the entire system at a rate of approximately six complete exchanges daily. *Artemia* ongrown to ≥1.5 mm and enriched for 6 h with DHA Selco (INVE, Belgium) and *Isochrysis* sp. (Tahitian strain) were fed daily at 3 ml^{-1}.

Stages of larval development were determined according to Lesser (1978). Mortalities were assessed in 3 culture vessels approximately 5 days after the peak of moulting at each stage. At this time, samples of larvae (n=15) were measured for length (from the anterior tip of the cephalic shield between the eyestalks to the posterior tip of the abdomen) and width (left and right extremes of the cephalic shield) on a Nikon 6C Profile Projector (Japan) before returning to the culture vessel.

For enzyme analyses, samples were taken in triplicate with the sample size ranging from 1000 for stage I to 100 for stage VI (see Table 1). Samples of 10 larvae were also collected at hatch and then at intervals thereafter (see Table 1) and fixed in glutaraldehyde for histology. Spectrophotometric enzyme assays were developed for phyllosoma using 200 ul microplate wells and associated enzyme kinetic software during 2001. The following key digestive enzymes: general protease, trypsin, lipase, alpha-glucosidase and chitinase, were chosen based on their importance in crustacean digestion. Pooled animals were homogenised for 5 min in 1 ml of chilled Tris buffer, the homogenate centrifuged at 18 000 g at 4°C and the supernatant stored at –4°C. General protease activity was measured using 1% casein as substrate and using a tyrosine standard curve. Trypsin activity was determined using N-α–benzoylarginine-ρ-nitroanalide (BAPnA) as substrate and monitoring the release of ρ-nitroanalide at $A_{400\text{-}410}$. α-Glucosidase activity was determined using the substrate ρ-nitrophenyl α-D-glucopyranoside and monitoring the release of ρ-nitrophenol at A_{400}. Lipase activity was determined using 4-nitrophenyl caproate (4-NPC) as substrate and monitoring the release of nitrophenol at $A_{400\text{-}410}$. Chitinase activity was determined using ρ-nitrophenyl β-D glucosaminide as substrate and monitoring the release of ρ-nitrophenol at $A_{400\text{-}410}$. Amylase was trialled but could not at this stage be applied as a microplate assays. An amylase kit (Sigma) is being investigated. Activity was recorded as specific activity in units.mg larval protein^{-1}. Larval protein was determined by the method of Bradford (1977) using bovine serum albumin (BSA) as standard. *Artemia* samples (n = 1000 x 3 replicates) were also assayed for enzyme activity during the trial to compare with larval activity.

Results and Preliminary Discussion

Growth and Survival

Larvae moulted to Stages II, III, IV, V and VI on d 11-13, d 21-23, d 32-37, d 45-51 and d 59-69, respectively. Survival and size of larvae are presented in Figure 1 and Table 1. Larval development and growth rates appeared normal and survival was within expected levels for hatchery culture (40-60% between stages).

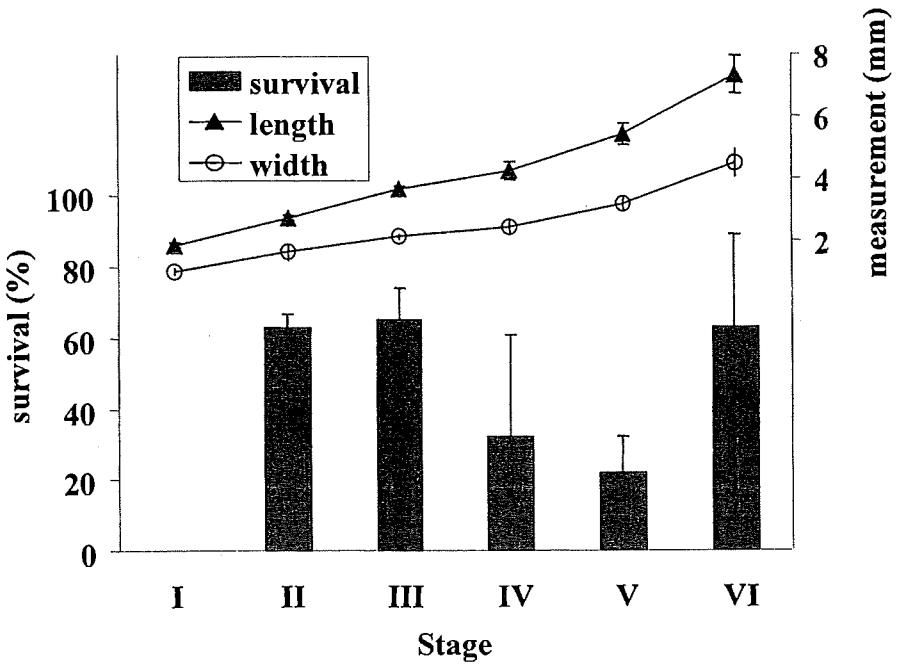

Figure 1. *Length and width of phyllosoma larvae and survival between stages of development. Data are presented as mean ± SD.*

Table 1: *Ontogenetic Changes in Digestive Enzymes and Histology -Calendar of Major Events*

Date	Day of Sample	Stage	Length & Width (mean±SD, n=45)	Survival % (mean±SD)	Larval Samples
31.7.01	0	hatch			3 x 1000 for enzymes
					1 x 10 for histology
1.8.01	1	1 (early)			3 x 1000 for enzymes (TK. 7,8,9)
					1 x 10 for histology
6.8.01	6	1 (mid)	L:1.85±0.08		3 x 1000 for enzymes (TK. 10,11,12)
			W:0.99±0.09		1 x 10 for histology
15.8.01	15	2 (mid)	L:2.73±0.08	To Stage 2	3 x 500 for enzymes (TK. 4,5,6)
			W:1.62±0.26	63.3±4%	1 x 10 for histology
29.8.01	29	3 (mid)	L:3.64±0.12	Stage 2-3	3 x 300 for enzymes (TK. 1,2,3)
			W:2.12±0.08	65±9%	1 x 10 for histology
10.9.01	41	4 (mid)	L:4.24±0.28	Stage 3-4	3 x 150 for enzymes (TK. 4)
			W:2.42±0.18	32±29%	1 x 10 for histology
24.9.01	55	5 (mid)	L:5.42±0.34	Stage 4-5	3 x 100 for enzymes (TK. 1,2,17,18)
			W:3.15±0.23	22±10%	1 x 10 for histology
12.10.01	73	6 (mid)	L:7.32±0.61	Stage 5-6	3 x 100 for enzymes (TK. 1,2,17,18)
			W:4.46±0.44	63±26%	1 x 10 for histology

Enzyme Activity

All digestive enzymes assayed were present in all phyllosoma stages (I-VI) indicating that *J. edwardsii* phyllosoma are producing and secreting enzymes post hatch. That is, they are capable of digesting a range of dietary components from an early age (stage I) (Figure 2 and 3). Negligible activity was recorded for □-Glucosidase suggesting that phyllosoma may be not be efficient at digesting carbohydrate in the early stages (Figure 2). This will be confirmed once an amylase assay kit has been trialled. Protease and trypsin activity were initially high at day 0 and day 1 but decreased significantly at day 6 and declined steadily through to stage 6 (Figure 3). This suggests that a high proportion of protein is utilised post-hatch but to a lesser extent during development. In contrast, lipase activity steadily increased from day 0 through to stage 5 and 6. This reflects the importance of lipid in phyllosoma nutrition and suggests that lipid is hydrolysed to a greater extent in mid-stage larvae than early-stage larvae. The specific activity of lipase is also considerably higher (ten-fold) than trypsin and chitinase, further indicating the importance of lipid in the diet of phyllosoma larvae (Figure 3). Chitinase activity is highest in stages 1, 5 and 6 larvae and lowest in stage 2, 3 and 4. The detection of chitinase is indicative of the ability of phyllosoma to digest chitin, which is present in its diet in the form of *Artemia* exoskeletons and suggests that phyllosoma enzyme profiles can shift according to diet (it is unlikely that *Artemia* are part of their natural diet). The trend in chitinase with development is difficult to explain at present.

Enzyme activity in *Artemia* samples were also recorded. Observations of feeding behaviour during culture indicate that only pieces of Artemia are ingested, not whole animals. Therefore it was concluded that activity detected in the phyllosoma were attributable only to larvae and not ingestion of whole *Artemia*.

Conclusions

- Digestive enzymes are present post–hatch indicating that phyllosoma have well developed digestive capabilities from an early age.
- Lipase activity appears to be highest in all stages of phyllosoma reflecting the importance of lipid in their diet.
- There are developmental changes in enzyme profiles indicating that they may be shifts in the importance of dietary components during the hatchery culture of phyllosoma.

*** More comprehensive discussion of this data will be made in a subsequent refereed publication.**

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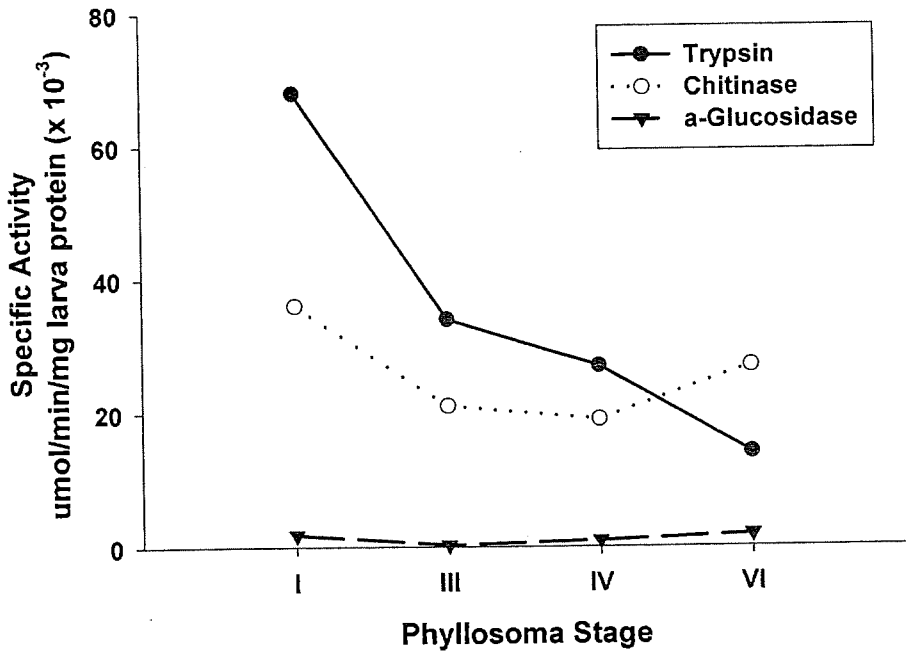
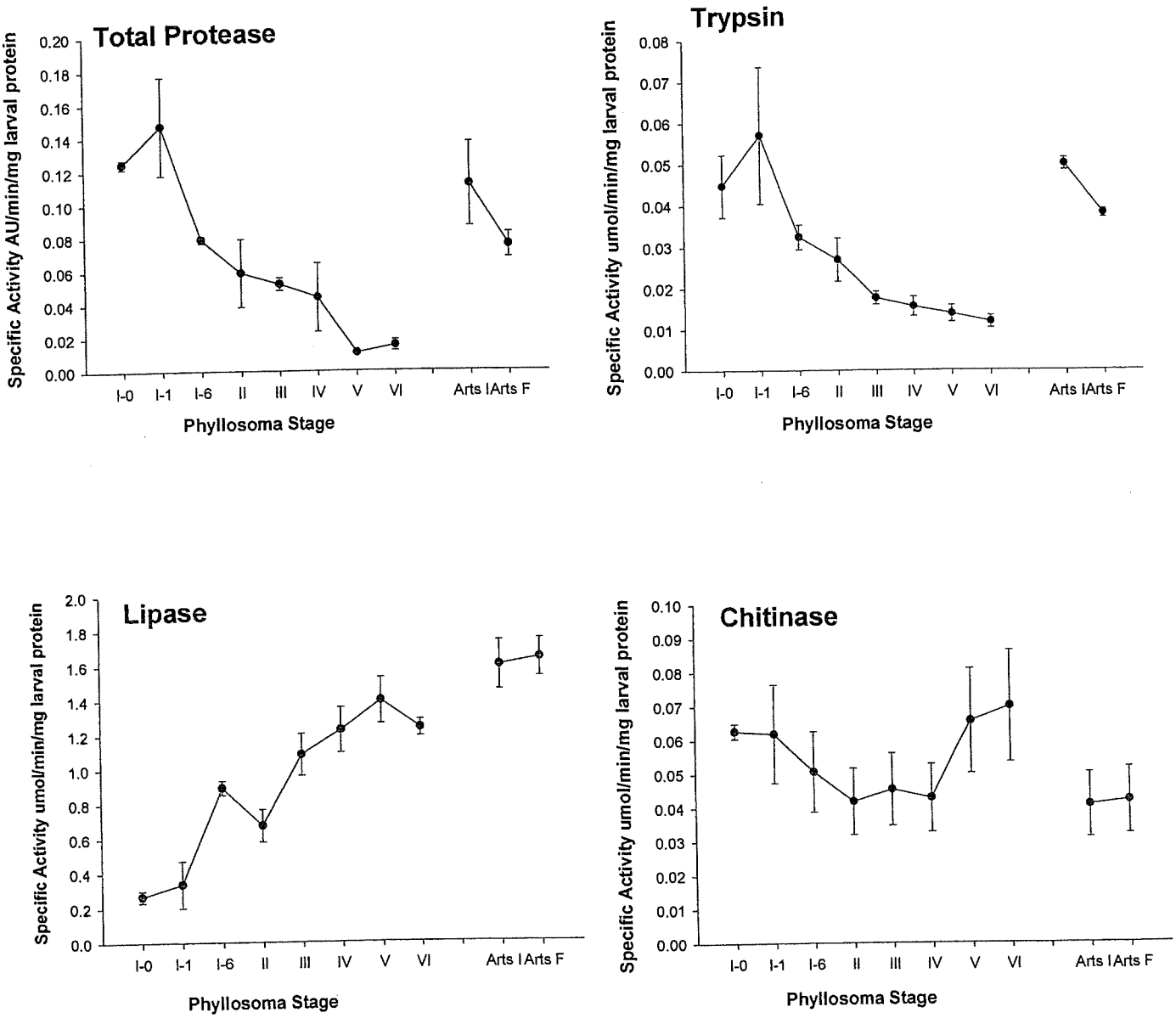


Figure 2: *Mean specific activities of 3 digestive enzymes during phyllosoma development of Jasus edwardsii. Data collected during assay development.*



I-0, I-1, I-6 = Stage I phyllosoma sampled at 0, 1 and 6 days
Arts I = Artemia sampled at beginning of trial
Arts F = Artemia sampled at end of trial

Figure 3. *Specific activities of digestive enzymes during phyllosoma development.*

Propagation of rock lobsters / Larval quality

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Introduction

It is important to use the best phyllosoma for larval rearing, but it is often difficult to visually assess larval viability at hatch. The use of compromised phyllsoma is a waste of time for long-term hatchery rearing while good larvae have high survival and grow rapidly. There is considerable variation in the quality of newly-hatched phyllosoma depending on broodstock history before mating and then during embryonic development to hatch. Therefore, broodstock and larval quality are intimately linked and we need to ensure that the best broodstock are used.

At TAFI MRL, we investigated the environmental manipulation of broodstock of southern rock lobster *Jasus edwardsii* to extend the period of larval availability from the normal 2 months at ambient temperature to 5 months using a range of temperatures. We measured characteristics believed to be related to larval viability including size and development of larvae, and their biochemistry (lipids, fatty acids, Vitamin C). We also developed a quick diagnostic test to use before hatchery rearing as a predictor or indicator of larval viability, and related the performance of phyllosoma in the test to survival after feeding or starvation.

Temperature manipulation of broodstock

Mature females normally mate and extrude eggs in late autumn and hatch in mid-spring (October). When warm and cold temperature regimes were applied to broodstock in July (eggs extruded in May), embryonic development was accelerated or slowed, respectively, compared to those held at ambient (Figure 1). Hatching also occurred over a shorter period at warm temperatures and a longer period at colder temperature.

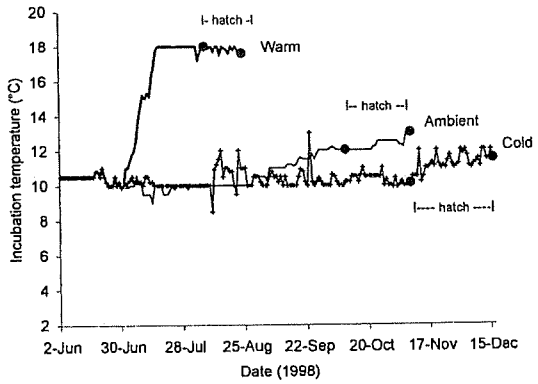

Figure 1. *Temperature during embryo development, hatch dates and hatch durations for Jasus edwardsii phyllosoma from broodstock in the warm, ambient and cold treatments* (adapted from Smith et al. 2002).

The consequence of the accelerated development at the warm temperature was that smaller larvae were produced whereas cold temperatures had no obvious effects on larval size (Table 1). The 5% reduction in larval length at warm temperature may translate to a loss of 10-15% in body mass which can have a major influence on larval viability. Ascorbic acid (AsA) was conserved in cold water. For the fatty acids, EPA was depleted in warm water which was reflected in reduced polyunsaturates accompanied by an elevation in monounsaturates. The main lipid class in phyllosoma is the polar lipid which did not vary between treatments, whereas sterols, which are used in the production of hormones for moulting, were depleted at the warm temperatures. These results suggest that excessively warm temperatures during embryonic development may produce poor larvae.

Table 1. *Selected measures in newly-hatched larvae from Jasus edwardsii broodstock held in warm (18 ℃), ambient and cold (11 ℃) water. Different letters indicate significant differences (P<0.05)* (adapted from Smith et al. 2002).

	Warm	Ambient	Cold
Larval body length (mm)	1.96±0.01 ^{a}	2.05±0.01 ^{b}	2.04±0.01 ^{b}
Ascorbic acid (AsA) (μg^{-1} dw)	134±11 ^{a}	139±11 ^{a}	204±20 ^{b}
% fatty acids			
Eicosapentaenoic acid (EPA)	13.4±0.4 ^{a}	15.0±0.5 ^{b}	15.1±0.5 ^{b}
Polyunsaturated	35.6±1.3 ^{a}	38.7±1.4 ^{b}	37.3±1.4 ^{b}
Monounsaturated	29.9±0.8 ^{a}	29.3±0.9 ^{ab}	28.2±0.8 ^{b}
Lipid class			
Polar	81.8±1.5	78.0±0.79	77.0±1.3
Sterols	8.7±0.3 ^{a}	9.4±0.7 ^{b}	10.9±0.6 ^{b}

When data were compiled for newly-hatched larvae from broodstock derived from various locations around Tasmania, we found that lipid content varied almost three-fold from 74.1-219.2 mg g^{-1} dry mass (Table 2). The predominant lipid class was polar lipid which varied considerably depending on broodstock origin. The largest variation was in wax esters which constituted 21.1% of total lipids in larvae from Bruny Island animals whereas there was only 0.7% in larvae from Port Davey origin. Considering that lipid is a crucial energy source, these large changes may represent important contributions to larval viability.

Table 2. *Selected measures in newly-hatched larvae from Jasus edwardsii broodstock collected from different locations around Tasmania* (adapted from Smith et al. 2002 and Smith et al. 2003a).

	Bruny Is. (east coast)	Maatsuyker Is. (west coast)	Triabunna (east coast)	Port Davey (west coast)
Total lipid (mg g^{-1} dry mass)	219.2	164.1	104.6	74.1
% lipid class				
Wax esters	21.1	2.1	6.0	0.7
Triacylglycerols	0.5	2.2	2.6	0.5
Free fatty acids	0.6	0.7	1.2	0.2
Sterols	6.5	7.6	9.4	9.2
Polar lipids	71.3	87.5	78.0	88.9

Rapid test for larval viability

In light of the large biochemical differences in newly-hatched phyllosoma, it would be useful to have a rapid test to discern larval quality before commencing hatchery rearing, to maximise the chance of survival and growth through the long larval phase. We subjected newly-hatched phyllosoma to:

1. 60-min activity test in a range of temperatures and salinities
2. 14-day culture without feeding
3. 42-day culture to Stage IV when fed *Artemia*

Newly-hatched phyllosoma (n = 20) from individual females were counted into 200 ml sample vials containing one of five salinities (10, 15, 35, 55, 60‰) in triplicate, then placed into one of three water baths (18, 23, 28°C). Phyllosoma in each vial were monitored at 3 min intervals with the number of animals prostrate on the vial floor not responding to light stimuli (halogen light, 7 µmol s^{-1} m^{-2}) with visible appendage movement counted as 'inactive'. Cumulative totals of inactive animals were obtained by adding the sub-totals recorded every 3 min for the 1 h duration of the test. Thus, a large cumulative total (inactive) indicated that animals had succumbed sooner to the effects of a particular temperature and salinity combination. The possible range of results was from 0, where there were no animals inactive during the test, to 400, where all animals were inactive within 3 min and thereafter for the duration of the test. Phyllosoma originated from broodstock (wild, captive) previously held at different temperatures (ambient, 17°C, 21°C) during embryonic incubation.

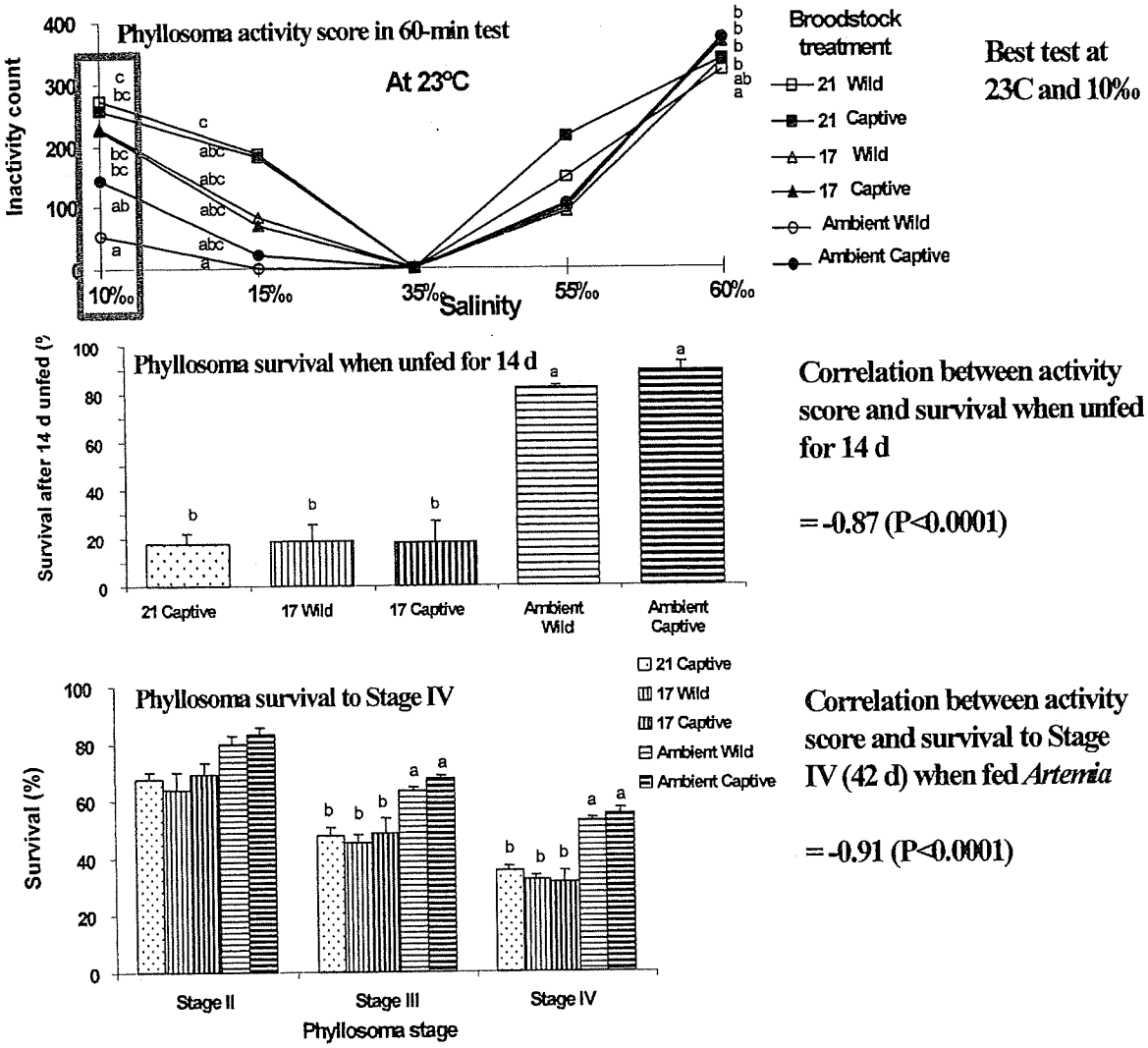

Figure 2. *Activity test (measuring the number of inactive newly-hatched Jasus edwardsii phyllosoma at 3 min intervals for a total of 1 h when subject to 23°C and 10- 60‰ salinity) and larval survival when unfed for 14 d or fed Artemia for 42 d to Stage IV. Phyllosoma were from different broodstock sources (wild and captive) and incubation histories (21°C, 17°C and ambient)* (adapted from Smith et al. 2003b).

An extract of the findings in Figure 2 shows that the best test (highlighted segment) was for larvae submitted to 23°C and 10‰ salinity which was closely correlated with survival of congenetics in culture (unfed 14 d, fed 42 d). The highest score was for poor quality larvae derived from broodstock previously held at 21°C, while conversely the lowest activity was for high quality larvae from broodstock held at ambient temperature during embryonic development. There was a high correlation between activity score at 23°C and 10‰ salinity with survival for larvae remaining unfed for 14 days (correlation = -0.87) and for larvae fed on *Artemia* to Stage IV at 42 days old (correlation = -0.91). Therefore, this combination of 23°C and 10‰ salinity was used as the preferred diagnostic test to determine larval viability for hatchery rearing.

Summary and future research

In conclusion, broodstock from the wild produce larvae of widely variable quality while warm temperature manipulation of ovigerous broodstock may reduce larval quality. We can measure larval quality by means of physical and biochemical characteristics but we now also have a rapid activity test which predicts larval survival to determine future rearing performance. However, this activity test needs to be validated for larvae from wild broodstock and broodstock subjected to different nutritional regimes.

Future research needs to address:

1. Improving nutrition to enhance larval quality. Therefore, diets need to be optimised (both live and pellet) in terms of protein and energy, but including the use of supplements (e.g. ascorbic acid, Vitamin E).
2. Effects of broodstock size on larvae. For *Jasus edwardsii,* females at least 900 g appear to produce large larvae.
3. Normal hatchery stress may be detrimental to reproductive performance, so this needs to be compared to animals held under minimal stress.
4. Out-of-season supply of larvae, requiring broodstock to be manipulated with light and temperature. The effect on larval viability also needs to be assessed.
5. Target sources of broodstock in the wild which produce optimum larvae.

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Molecular approaches for advancing larval rearing of rock lobsters

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Introduction

The Australian aquaculture industry set itself a vision at the National Aquaculture Workshop in Canberra in 1999 that the industry would achieve at least $2.5 billion in annual sales in 2010. Presently the vast majority of aquaculture value is accounted for by the tuna, pearl oyster, salmon, edible oyster and prawn sectors. However, there are a number of native Australia marine species that are potential aquaculture candidates. Based on the production value of the wild harvest rock lobster sector, between $400 to $550 million per year, with high value per kilogram and excellent export markets, these crustaceans represent highly valuable potential as an aquaculture candidate.

The propagation life cycle of any potential aquaculture candidate can be broken down into several components (Figure 1). It has been repeatedly demonstrated that suitable rock lobster broodstock can be collected from the wild and held for periods of years in captivity and be successfully bred. It is also known that post-planktonic juveniles can be readily grown out under culture conditions.

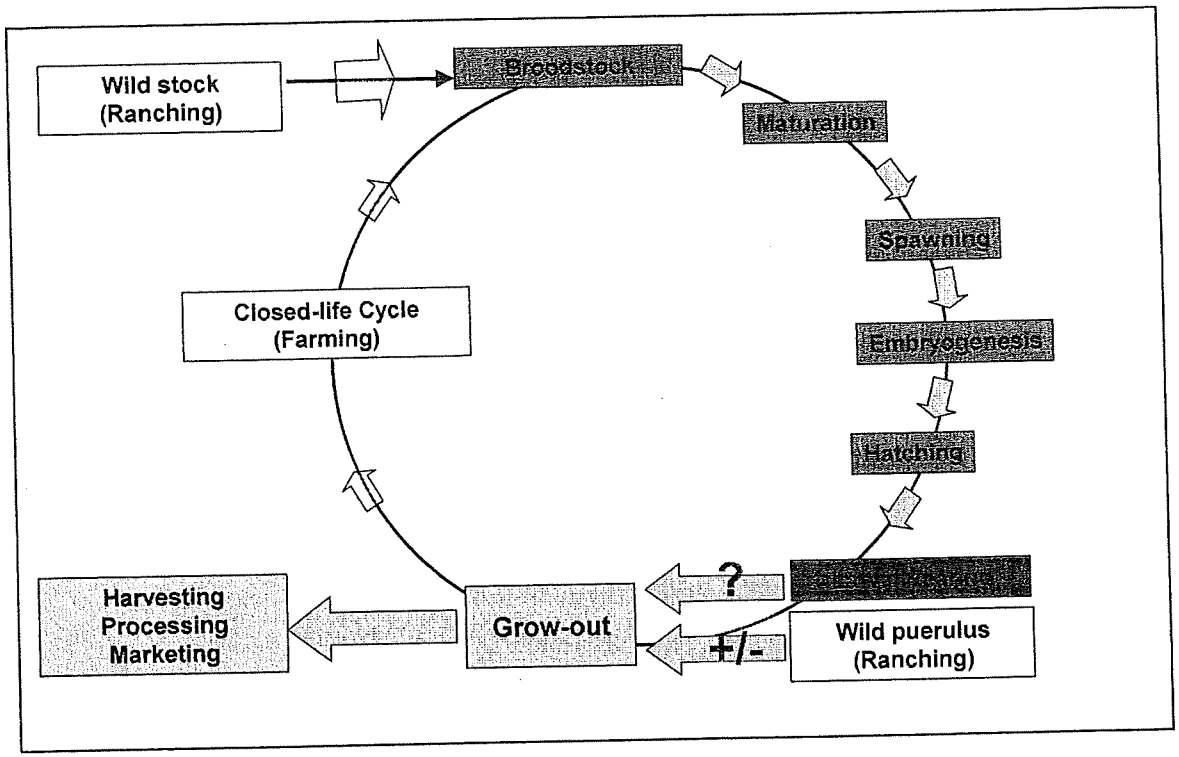


Figure 1. *The propagation cycle of aquaculture candidates and specifically rock lobsters.*

Rock Lobster Larval Rearing

Although the ranching of rock lobsters through the collection of post-planktonic larvae (puerulus or post-juveniles) is possible it is high risk for a commercial venture due to massive annual variation in recruitment and hence is incapable of guaranteeing a consistent supply of new stock into an aquaculture venture. It is generally thought that the complete closure of the life cycle of rock lobster is necessary for the development of a sustainable rock lobster aquaculture sector.

To date, the successful crustacean aquaculture sectors include those species which have either larval phases of a few weeks, i.e. penaeid prawns, some crabs, and homarid lobsters, or no planktonic phase, i.e. freshwater crayfish (Figure 2). The greatest challenge to closing the life cycle of rock lobsters is the shear length of the planktonic larval phase that, depending on the species, may extend for over a year. Not only must the technological difficulties of larval rearing for such extended periods be overcome but also the production of sufficient numbers of post-plankton juveniles to meet commercial demands.

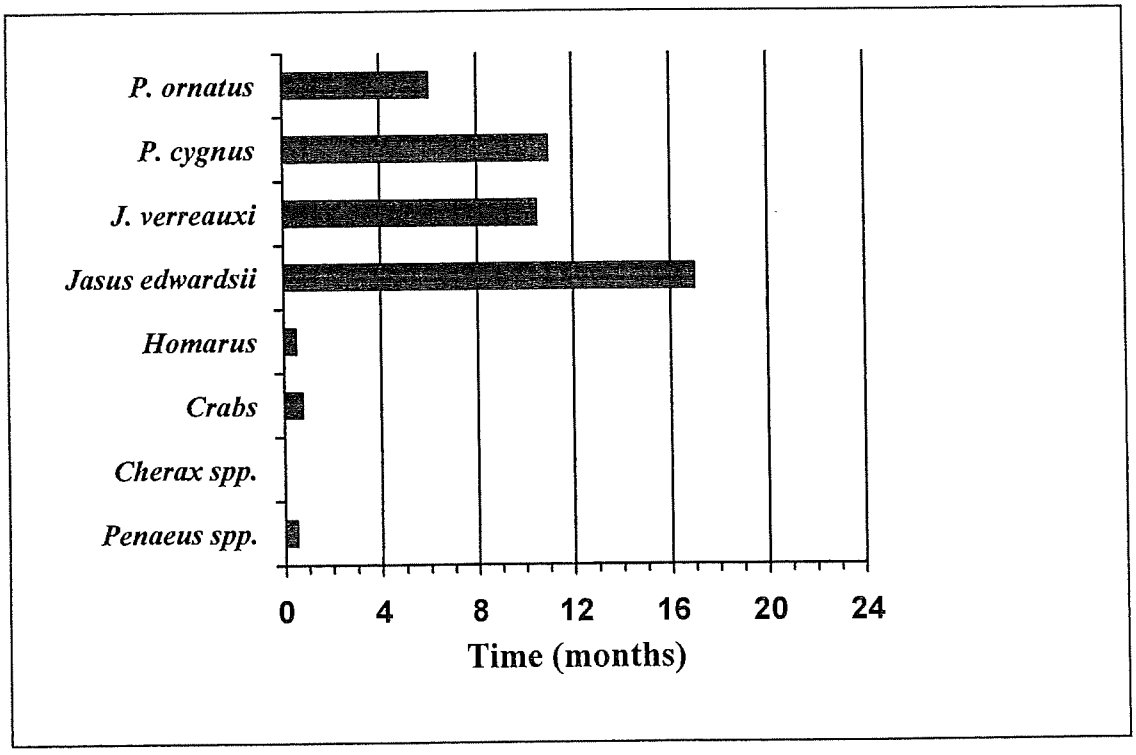


Figure 2. *The planktonic larval period of selected crustacea.*

Of the 9 species of rock lobsters native to Australia, 6 are sub-tropical to tropical and the other 3 are temperate, with the latter accounting for nearly the entire wild fishery value. However, of the Australian species, those with the shortest larval phase are the tropical ones and from an aquaculture perspective may be the best models, if not the most likely species, to be amendable to culturing if the length of the larval phase is the critical bottleneck.

Attempts to complete the larval cycle of rock lobster has spanned over half a century, with more intensive efforts over the last two decades (Tale 1). A few have successfully produced juveniles but survival is nearly always far less than 1%. Given that a significant amount of research effort has been spent on classical husbandry approaches it may be time to consider alternative approaches to understand the basic mechanisms underlying the potential problems preventing the successful and reliable larval rearing of large numbers of phyllosomas.

Species	Reference
P. japonicus	Oshima (1936), Inoue (1965, 1978), Kittaka and Ikegami (1988), Kittaka and Kimura (1990), Yamakawa et al. (1989), Souza et al. (1996), Sekine et al. (2000)
P. interruptus	Johnson (1956), Dexter (1972)
P. polyphagus	Saisho (1966), Sin (1967)
J. lalandii	Kittaka (1988)
J. edwardsii	Kittaka and Booth (2000), Igarashi et al. (1990), Illingworth et al (1997), Tong et al. (1997), Moss et al. (1999), Tong et al. (2000), Ritar (2001)
P. argus	Moe (1991)
P. homarus	Radhakrishnan and Vijayakumaran (1986), Radhakrishnan (1995)
J. verreauxi	Kittaka et al. (1997), Diggles et al. (2000)
P. elephas	Mercer et al. (1997), Kittaka et al. (2001)
P. longipes	Matsuda and Yamakawa (2000)
P. stimpsoni	Wei and Lai (2000), Chen et al. (2001)
J. frontalis	Dupre and Guisado (1996)

Table 1. *Rock lobster species, and selected references, for which captive larval rearing has been attempted.*

In the first instance it appears desirable to shorten the larval period as much as possible. In the 5 species of rock lobsters from which some puerulus have been produced the larval phase is typically reduced by about 1/3 from estimated periods of wild conspecifics. It is assumed that some of this may be due to a consistent food source under captive conditions compared to a potential variable one in the wild. However, it is also typical for the body size of captive reared larval to be smaller that the equivalent stage wild conspecifics, which may indicate the contrary. In addition, of the typical 11 phyllosoma stages, the inter moult period of early, mid, and late stages progressively increases from approximately 1 to 2 to 3 weeks per intermoult period. In addition, there can be several moults between stages, which may or may not be due to progressive nutritional inadequacies. Whatever the case it would be highly desirable to shorten the larval period if at all possible.

Molecular Approaches of the Larval Phase

Larval development, metamorphosis and moult are all hormonally driven events. In insects, especially the fruit fly Drosophila, a detail understanding and model of these processes are understood at a hormonal and molecular level. Based on such models we have examined the role of ecdysone and the nuclear hormone receptors (NHRs) that putatively drive larval developmental processes in rock lobster phyllosomas. We have isolated and identified 4 homologous NHRs genes based on those found in Drosophila: Panulirus ornatus hormone receptor-3 (PoHR3), fushi tarazu (Ftz) Factor-1 (PoßFTZ-F1), hormone receptor-78 (PoHR78) and the ultraspircacle receptor (PoUSP). All of these NHRs must be expressed in a specific manner to successfully complete each cycle of larval development. By using the quantitative polymerase chain reaction PCR (Q-PCR) we have been able to show that PoHR3 is only expressed transiently at the time of moult (Figure 3).

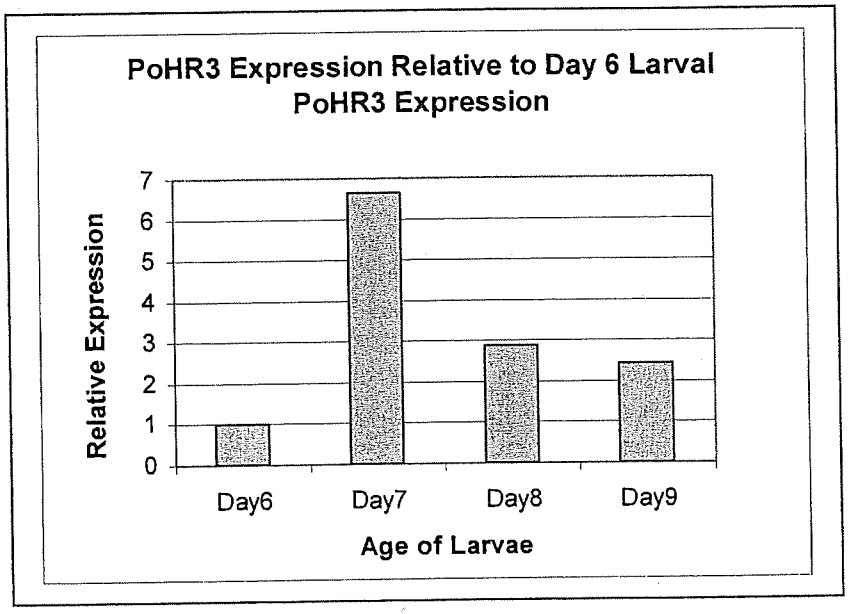


Figure 3. *Expression of PoHR3 during the transition from phyllosoma stage 1 to 2.*

The objectives are now to complete the temporal profiling of the PoFTZ-1 and PoHR78 NHR genes. We are presently isolating the ecdysone hormone receptor gene (PoEcR) to obtain the required knowledge which should allow the testing of the viability of using ecdysone as a dietary supplement to manipulate the timing of molt and metamorphosis, and hence the larval period, in rock lobster phyllosomas.

Molecular Approaches to Larval Nutrition

The natural diet of phyllosoma larval is unknown. In captive rearing attempts various food items have been trialled including various types of phytoplankton and zooplankton. Fish larvae appear to be a good nutritional item, especially for the mid and late phyllsoma stages. However, critical to the development of commercial hatchery production, larval feeds are required in reliable and consistent

supply. To date the most successful diets include Artemia for the early phyllosoma stages with chopped molluscs in the mid to late phyllosoma stages. Neither of these items can be part of the natural diet. Although it has been argued that other aquaculture species that have problematic larval stages, such as finfish, have been successfully rearing on non-natural live diets and therefore it is not essential to have information on the natural diet. In contrast, others have argued that an understanding of the natural diet allows the development of a 'knowledge' based diet and may lead to significant advancements in successful larval rearing husbandry.

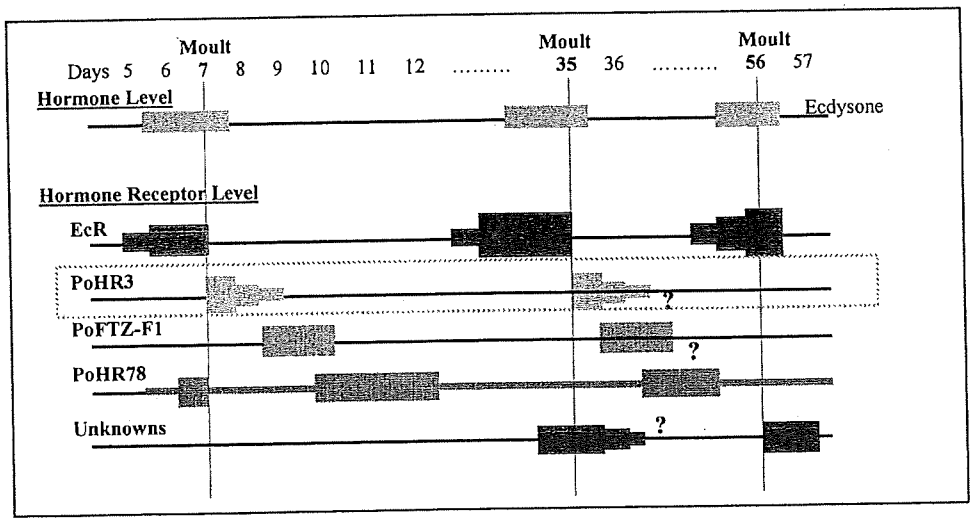


Figure 4. *The profiling of NHRs required to obtain the knowledge required for potential manipulation of the larval period in phyllosomas. Bars represent periods of expression of the respective NHRs. Note that all are only expressed transiently.*

In recent years it has proved possible to undertake molecular analysis of gut contents of insects and crustacea to identify prey species (Table 2). This has proved a particularly valuable tool in identifying prey items (even fluid diets such as those from blood feeding predators) that cannot be recognized using a more classical morphological approach.

Table 2. *Identification of prey species, by means of molecular identification, of predators whose prey were otherwise previously unknown.*

Predator	Target Genes	Prey	Reference
Dicyphus tamaninii (Heteroptera)	RAPD-PCR Isolated sequences (genomic)	Helicoverpa armigera (Lepidoptera) Trialeurodes vaporariorum (Homoptera)	Agusti et al. (1999) Agusti (2000)
Pterostichus cupreus (Coleoptera)	Esterase genes (nuclear)	Culex quinquefasciatus (Diptera)	Zaida et al. (1999)
Hippodamia convergens (Coleoptera)	COII (mtDNA)	Rhopalosiphum maidis (Homoptera)	Chen et al. (2000)
Chrysoperla plorabunda (Neuroptera)	COII (mtDNA)	5 aphid sp.	Chen et al. (2000)
Coelemegilla maculata (Coleoptera)	rRNA (nuclear)	Ostrinia nubilalis (Lepidoptera)	Hoogendoorn & Heimpel (2001)
Anthocoris tomentosus (Heteroptera)	COI (mtDNA)	Cacopsylla pyricola (Homoptera)	Agusti & Symondson (2001)

The phyllosoma larval form is found in both scyallarids and panulirid lobsters. They have particularly conserve morphology of body appendages and mouthparts. The phyllosoma larval form is found through the oceans of the world and it could be assumed that they have similar prey, possibly gelatinous zooplankton that would be difficult or impossible to recognise from gut samples. By adopting a molecular gut analysis approach of phyllosomas from several species it should prove possible to accurately identify the natural prey and develop nutritional balanced live diets or bioencapsulated diets suitable for phyllosoma larval rearing.

In addition a molecular analysis of the phyllosoma gut would also be capable of identifying the microbial fauna in the gut, which may be important from a probiotic point of view. Furthermore, a similar approach could also identify the range of gut enzymes which phyllosomas can produce, which would again provide useful information on what dietary items could be successfully digested by phyllosomas.

Molecular Approaches to Microbiology and Larval Health

During larval rearing a high mortality rate of phyllosomas can occur particularly around the time of moult. Epidemiological observations are indicative of such mortality being due to opportunistic microbial pathogens. Fundamental differences are experienced in water quality and the microbial community of aquaculture reared and wild type (oceanic) rock lobster phyllosomas. An understanding of the microbial flora of both environments may be required to improve the rearing success of these animals and develop a system for microbial control in the hatchery environment.

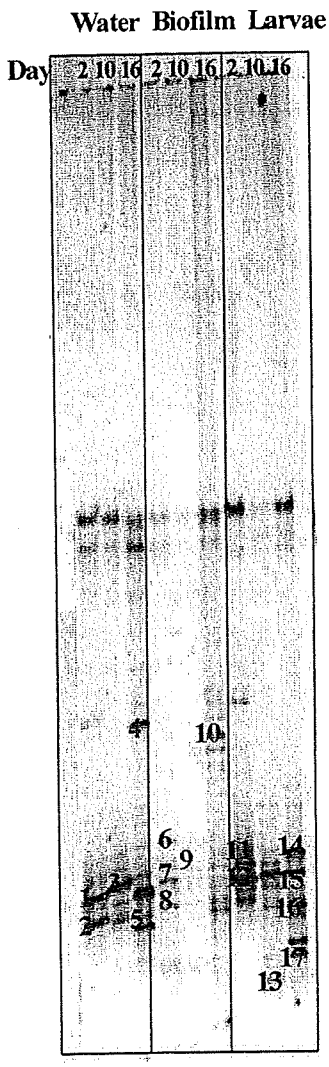
We have isolated bacteria from water column, biofilm and extracts of phyllosomas on marine agar and TCBS agar plates. Colonies were sub-sampled from plates when conditions during larval rearing indicated increase mortality bouts. These colonies were isolated and re-grown on fresh agar plates. Using PCR we have identified 7 Vibrio species in the phyllosoma hatchery environment (Table 3). Some of these Vibrios are known pathogens for other marine larvae.

The use of agar plates to identify marine bacteria is severely limited in that it is estimated that only 0.1% to 5% of bacteria presence in a sample will actually grow on semi-solid agar plates. To address this problem we have adopted a more robust methodology in revealing the presence of the actual bacterial population using denaturing gradient gel electrophoresis (DGGE). This method relies on the extraction of bacterial DNA from samples and the subsequent examination for sequence homology of extracted DNA with primers designed to react with specific forms of bacteria. Species identification is made by examining homology of sequence data with global microbial database searches. Species are only identified if they have already been isolated and identified by others and filed on the global

databases. Nevertheless, the DGGE approach is probably the best available to examine microbial biodiversity in extracts. This approach has revealed a different species profile from agar plating (cf Table 3 and Fig. 5). Of potential interest, is that one of the bacteria species revealed by DGGE, and not by agar plating, is a species known to either produce a toxin or to be associated with toxin producing organisms. The exact role of any of these microbes, and their relevance to rock lobster larval rearing, remains to be determined.

Table 3. *Summary of bacterial isolated from agar plates from the rock lobster larvae rearing system. Numbers in parenthesis: number of new colonies / number of colonies sampled.*

Water Column (7/9)	**Biofilm** (6/14)	**Phyllosomas** (11/28)
Vibrio sp. BV25Ex	*Vibrio sp.* BV25Ex	*Vibrio sp.* BV25Ex
Vibrio sp. NAP-4	---	*Vibrio sp.* NAP-4
Vibrio proteolyticus	Vibrio proteolyticus	Vibrio proteolyticus
Vibrio parahaemolyticus	Vibrio parahaemolyticus	Vibrio parahaemolyticus
---	*Vibrio tubiashi*	*Vibrio tubiashi*
---	---	*Vibrio sp.* NLEP - 1599
---	---	*Vibrio campbelli*
Pseudoalteromonas sp.	---	*Pseudoalteromonas sp.*
---	Pseudoalteromonas piscicida	--
Photobacterium leiognathi	---	---
---	---	*Paracoccus sp.*
---	---	*Alteromonas sp.*
---	*Marine gamma proteobacterium DC*	*Marine gamma proteobacterium DC*
Bacillus sp. YSS	---	---



Water Column

1	Vibrio shiloi (mediterranei)	99%
2	Marine bacterium ATAM 407_56 (toxic)	100%
3	Uncultured bacterium gamma Arctic 96A-12	94%
4	Uncultured bacterium clone CR98-35-21	97%
5	Sulfitobacter sp. GAI-37	97%

Biofilm

6	Uncultured bacterium ECS1	97%
7	Methylphaga thalassica	98%
8	Marine bacterium ATAM407 (toxic)	100%
9	Methylophaga sulfudorans	98%
10	Unculture crater lake bacterium CL500-55	96%

Phyllosoma

11	Vibrio shiloi	99%
12	Marine bacterium ATAM407 (toxic?)	100%
13	Desulfovibrio mediterraneus	93%
14	Uncultured Pirellula clone 6013	96%
15	Possible Thiothrix sp (poor sequence)	
16	Uncultured gamma proteobacterium Sva0862	95%
17	Uncultured gamma proteobacterium Sva0862	95%

Figure 5. *Bacterial species present in water column, biofilm and phyllosomas from day 2, 10 and 16 of larval rearing as revealed by DGGE analysis.*

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Technical feasibility of rock lobster propagation – Review of current research

Rodney Grove-Jones, Sagiv Kolkovski & Robert van Barneveld

The task of rearing of large numbers of rock lobster larvae to metamorphosis at will is undoubtedly one of the greatest challenges in aquaculture today. Success will only be achieved if there is an intense focus on achieving results that take the project closer to its final goal, if there is genuine collaboration between those involved and a great deal of innovation and willingness to explore new approaches. The following represents a summary of outcomes from a review of propagation research being undertaken within the Rock Lobster Enhancement and Aquaculture Subprogram. These outcomes have been incorporated into the RLEAS Strategic Directions 2002-2007.

The overall goal of propagation research within the RLEAS is to develop the technical ability to produce puerulus at will in any number required and each milestone of each project should contribute measurably to the achievement of this goal. It will likely take more than five years to reach the overall goal given a coordinated and determined effort and may yet prove too difficult at the current level of technical understanding of larval production systems. It is clear that new base survival diets (both for *Artemia* enrichment and for inclusion in a manufactured diet), improved larval husbandry techniques and systems that minimize the proliferation of bacteria will have to be developed. Investigators will need to demonstrate a high level of innovation and determination to overcome these obstacles.

The immediate goal for the propagation research program is to provide the technical ability to spawn adults during any month of the year, and to produce healthy and nutritionally balanced larvae to stage V. Completion of this goal will require the development and implementation of a dedicated broodstock conditioning program, the adoption of standardized *Artemia* husbandry techniques and the development of a base enrichment for *Artemia* that provides at least the minimum nutrition for small larvae, as well as the development of rearing systems and culture techniques that reduce bacterial proliferation in the culture tanks. These projects can run concurrently and should be achieved within three to five years.

Once the base survival enrichment formula has been developed and healthy larvae can be produced to stage V in large quantity, it will be necessary to develop a diet for larger larvae and refine the culture procedures. At present, the most likely candidate for late phyllosoma nutrition appears to be a manufactured diet with a composition based initially on the *Artemia* enrichment formula but progressively modified to meet the needs of larger larvae. Culture systems will be scaled up to commercial size and modified as necessary.

The results of any research undertaken will be evaluated according to their ability to contribute measurably to achieving the immediate goal.

The recent review of propagation research within the RLEAS has resulted in suggested revisions to the current propagation research program based on three distinct phases:

PHASE I

- Develop detailed project proposals and milestones consistent with revised goals.
- Primary focus on achieving high growth and survival through to phyllosoma stage V.

The suggested time frame for the completion of stage I is three years.

Goal 1 **Establish a reliable supply of stage one larvae at any time of year using the following suggested method:**

a) Manipulate photoperiod to control gonad maturation and timing of extrusion.

b) Manipulate incubation temperature to control the developmental period of embryos.

c) Produce larvae monthly.

d) Assess the effect of broodstock diet on phyllosoma quality.

Goal 2 **Develop a base Artemia enrichment diet that provides adequate nutrition to support growth at a minimum predetermined level from phyllosoma stages I through V using the following suggested method:**

a) Identify and prioritise key obstacles to developing a base enrichment diet.

b) Develop base *Artemia* enrichments incorporating knowledge gained to date.

c) Assess the effect of enrichments on growth of phyllosoma initially in static culture using anti microbial agents if necessary and report results.

d) Continually modify test diets according to results of trials until pre-determined growth and survival standards are met

Goal 3 **Develop a culture system that suppresses undesirable bacterial blooms and is suitable for use over periods of several months using the following suggested method:**

a) Review and standardise all aspects of *Artemia* husbandry including decapsulation, hatching, on-growing, enriching, and delivery with a view to reducing the bacterial load in *Artemia* and phyllosoma culture systems.

b) Review literature for recent developments in methods of bacterial control in marine larval rearing systems with an emphasis on low intervention techniques suitable for long culture periods.

c) Construct several prototype culture systems based on the review and assess the development of bacterial communities and numbers while culturing early stage phyllosomas. Parallel studies using the larvae of other species readily available and familiar to the investigator and for which the husbandry techniques and larval nutrition are known may also be beneficial.

d) Raise lobster larvae produced in goal 1 and fed *Artemia* enriched in goal 2 using a variety of bacterio-suppressant rearing techniques identified in part b) and developed in part c) of goal 3.

PHASE II

Commencement of stage II is contingent upon successful completion of the second year milestones in stage I. That is Stage II should start one year before the end of stage I. This is to ensure a smooth transition to stage three.

The primary focus of this stage is on the physical and chemical assessment of formulated diets ready for assessment with stage V+ phyllosoma.

Goal 1 **Commence preliminary work developing a formulated feed for stage V+ phyllosoma**

This goal should be addressed through a project with a 12 month duration and should be contingent upon the successful completion of goals to produce phyllosoma to stage V and above. The suggested methodology is:

a) Assess binders.

b) Produce a test diet based on the knowledge of ingredients gained from the development of *Artemia* enrichments but incorporated into an artificial pellet. The goal is to produce a base survival diet that provides adequate nutrition to support growth and survival at a level predetermined by the research team.

PHASE III

Diet optimisation studies and commercialisation of culture techniques:

Goal 1 **Upscale larval rearing systems developed in Stage I to semi-commercial scale and capability to produce tens of thousands of puerulus.**

Goal 2 **Optimise diet for Stage V+ phyllosoma.**

The research objectives and outcomes of the RLEAS propagation research program are summarised in the table below:

Goal	Impediments	Research Approach	Key performance indicators	Time frame
Technical ability to culture spiny lobster puerulus from eggs, at will and in any number.	1. Regular supply of larvae. 2. Larval nutrition. 3. Larval rearing systems. 4. Larval health.	1. Establish a reliable supply of stage I larvae through improved broodstock management. 2. Develop enriched *Artemia* diets to support phyllosoma growth from stages I-V. 3. Develop *Artemia* husbandry and phyllosoma culture systems to suppress undesirable bacterial blooms. 4. Develop manufactured diets to support phyllosoma stages V and above. 5. Initially focus on 1-2 rock lobster species.	1. Capacity to produce Stage I larvae throughout the year. 2. Enriched *Artemia* diets that support phyllosoma growth from stages I-V. 3. Culture systems that suppress bacterial blooms. 4. Capacity to produce healthy stage V larvae predictably and reliably. 5. Manufactured diets for rearing phyllosoma stages V+. 6. Capacity to produce puerulus at will and in any number.	2002-2007
Efficient production of spiny lobster puerulus from eggs, at will and in any number.	1. Larval nutrition. 2. Control of larval phases.	1. Optimisation of manufactured diets for phyllosoma stages V+. 2. Hormonal manipulation of larval phases.	1. Efficient production of puerulus at will and in any number using manufactured diets and *Artemia*. 2. Reduction in larval rearing time through manipulation of larval phases.	2005-2010
Commercial production of puerulus from eggs at will and in any number.	1. Larval rearing systems. 2. Larval health. 3. Larval nutrition.	1. Upscale research-scale production focusing on rearing systems, survival and the cost-effectiveness of supplying manufactured diets. 2. Expand the number of spiny lobster species cultured.	1. Economically-viable commercial production of a variety of species of spiny lobster puerulus from eggs at will and in any number.	2010+

Physiological studies on stress and morbidity during post-harvest handling of western rock lobsters

Prof Louis Evans

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Study aims

- To develop and validate a suite of stress assays based on hemolymph immune system parameters
- Use the assays in an investigation of stress responses in postharvest lobsters
- Use the assays and histopathology to study lobsters with poor health

What is Stress?

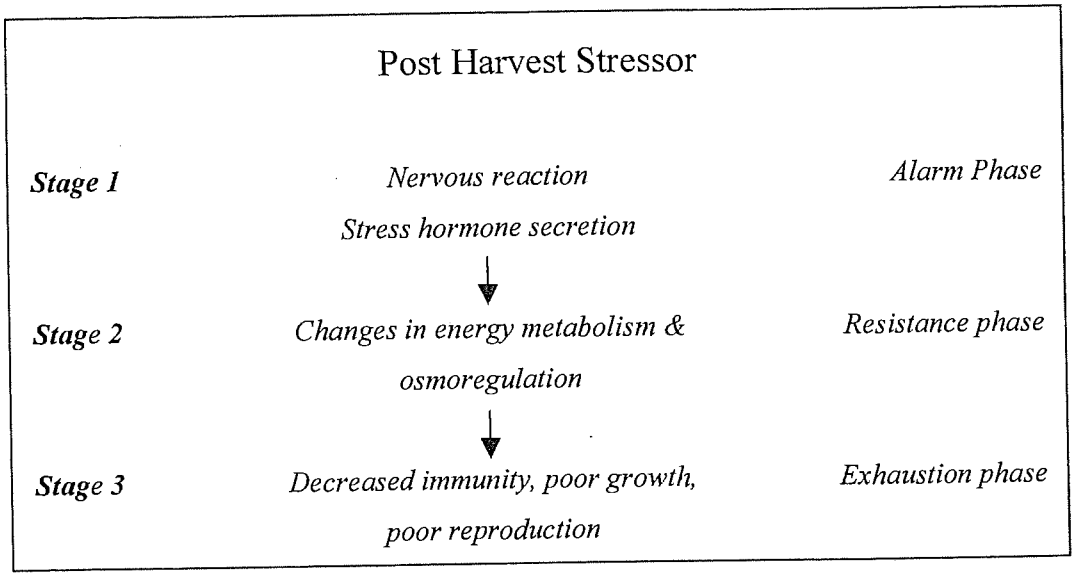

Figure 1. *Stress and how it relates to health*

How Does Stress Relate to Health?

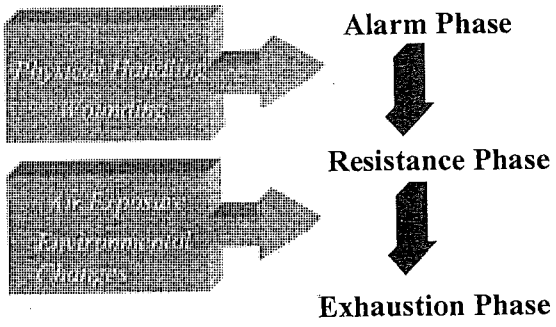

Figure 2. *Stressors in post-harvest handling of rock lobsters*

Studies performed

- Developed of suite of blood tests of stress and health status based on immune parameters
- Performed tests on lobsters from:
  - Laboratory
  - Boats
  - Trucks
  - Factories
- Performed blood tests and histopathology on healthy and unhealthy lobsters from factory holding tanks

Summary of Research Findings

Immune assay studies

- Eight different assays studied
- Main assays developed
  - THC
  - %Granular cells
  - %Bacteremia
  - Clotting time
- Developed approach to combine results into a single parameter – Immunity Web

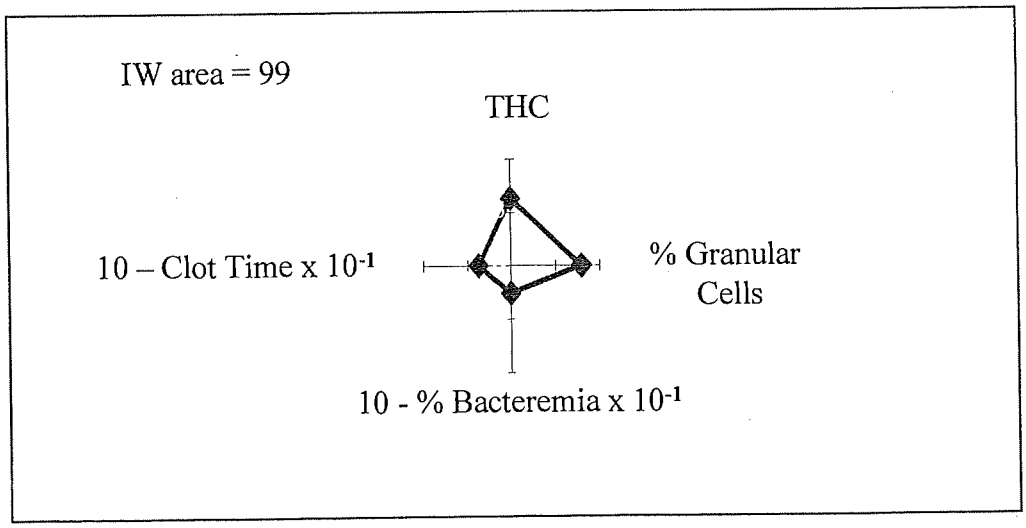

Figure 3. *Immunity Web (IW) for average immune parameter values*

Measurement of Lobster Health Status using Immune Parameters

Experimental approach

- Compared immune parameters in healthy and unhealthy (reject) lobsters
- Studied 5 groups of (6-13) reject lobsters with matching controls from the same tank
- Performed histopathology on body organs and quantified occurrence of inflammatory reactions

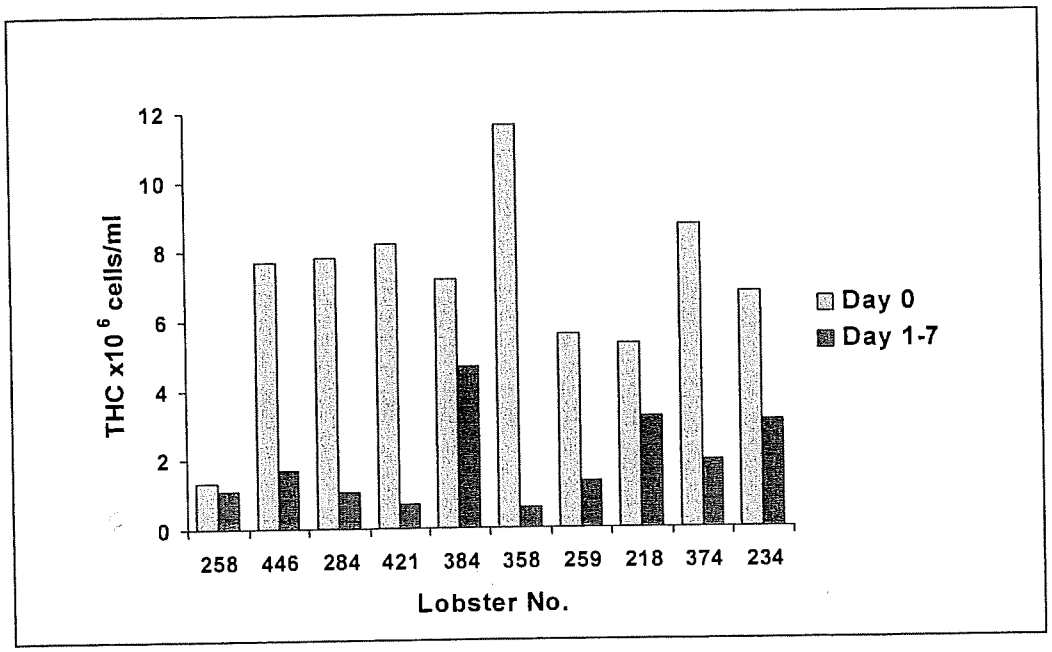

Figure 4. *THC in lobsters exhibiting deterioration in health status with time*

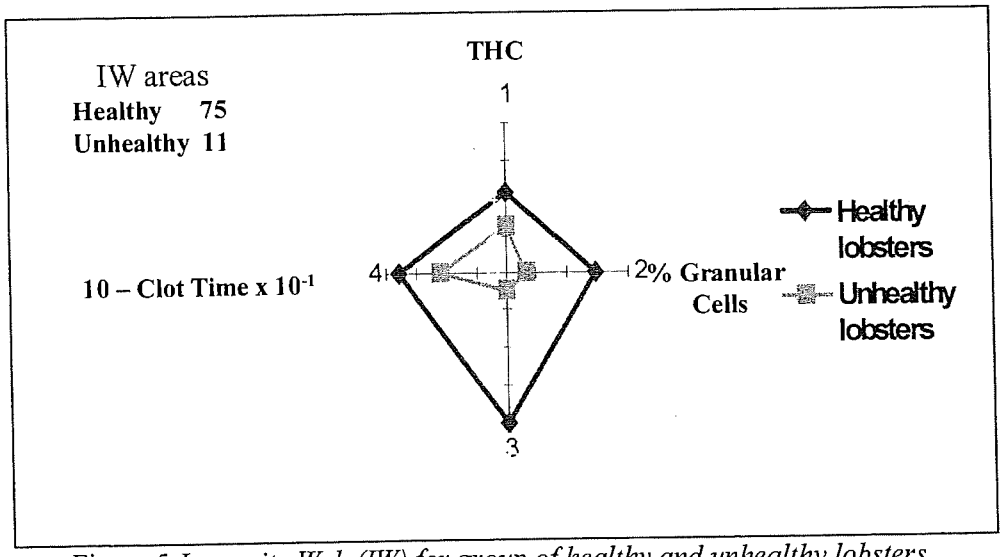

Figure 5.*Immunity Web (IW) for group of healthy and unhealthy lobsters*

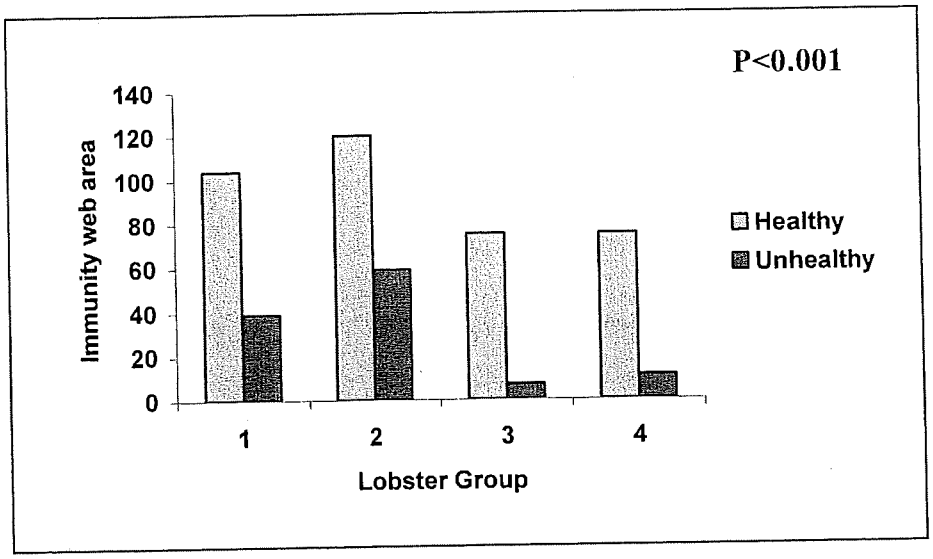

Figure 6. *Immunity Web areas for four groups of healthy and unhealthy lobsters*

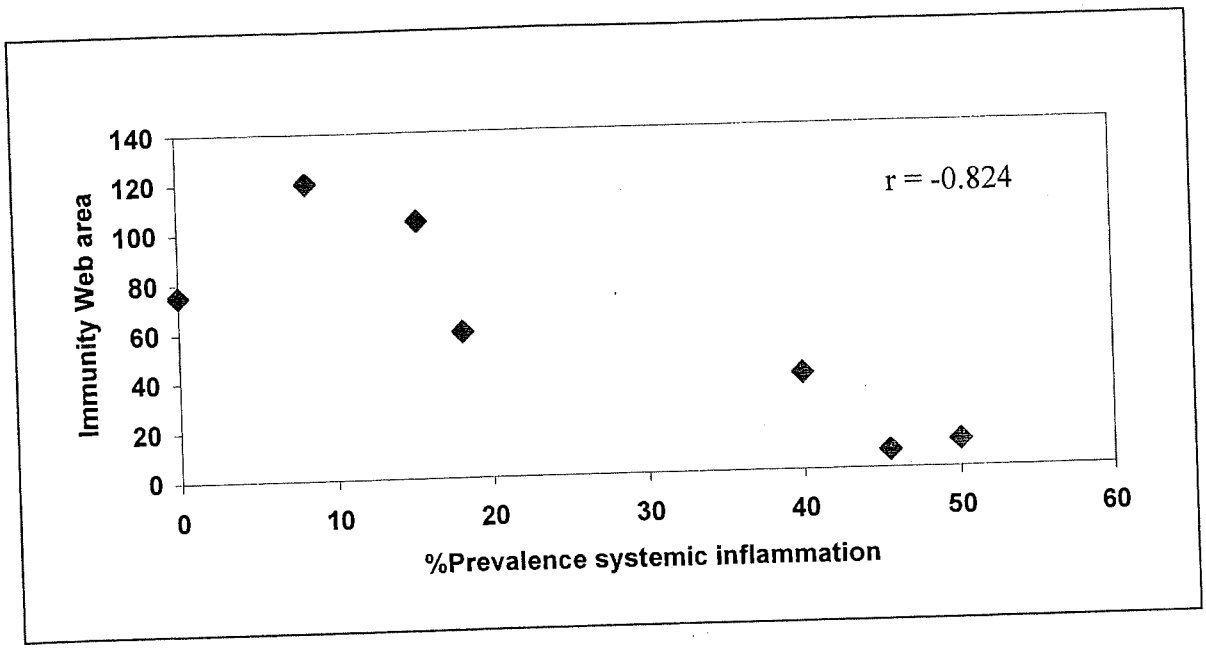

Figure 7. *Correlation between IW areas and inflammation*

Conclusions

- Immune parameters showed significant changes when lobster health status deteriorated
- Alterations best displayed using Immunity Webs
- Immunity Web areas provide quantitative measure of health in Panulirus Cygnus

Measurement of Lobster Stress Status using Immune Parameters

Laboratory trials

- Lobsters held in aquaria and then exposed to air, handling, exercise and wounding stressors
- Control groups kept in aquaria without any physical handling
- Observed responses at intervals from 1 min to 5 days after exposure

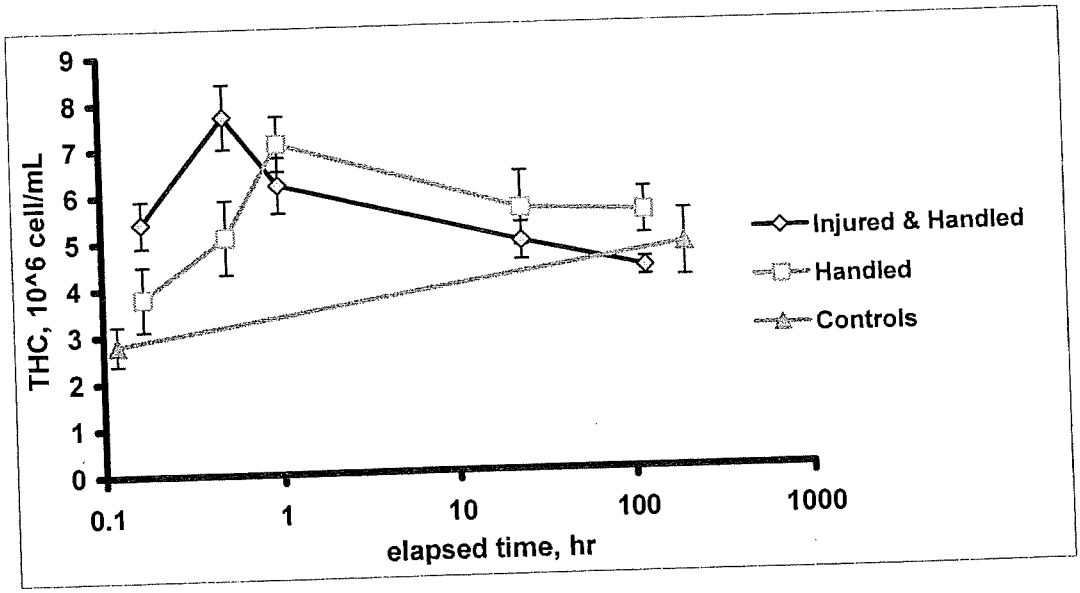

Figure 8. *THC response to physical handling, air exposure & injury*

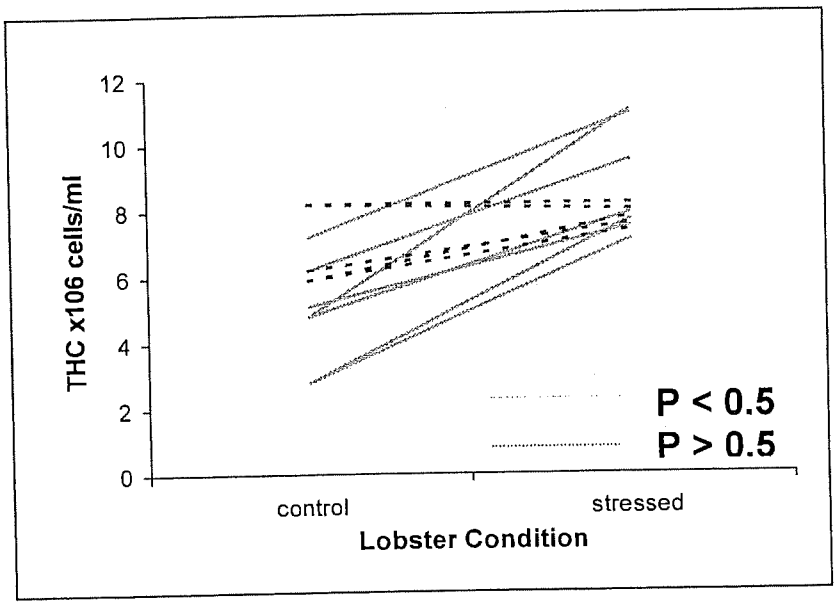

Figure 9. *Acute stress responses - Effect of physical handling on THC values*

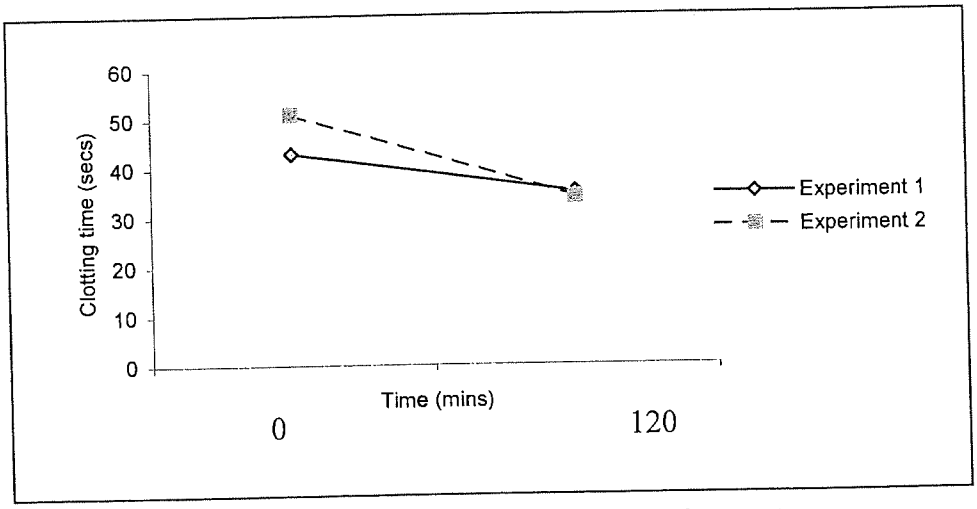

Figure 10. *Effect of physical handling on clotting time*

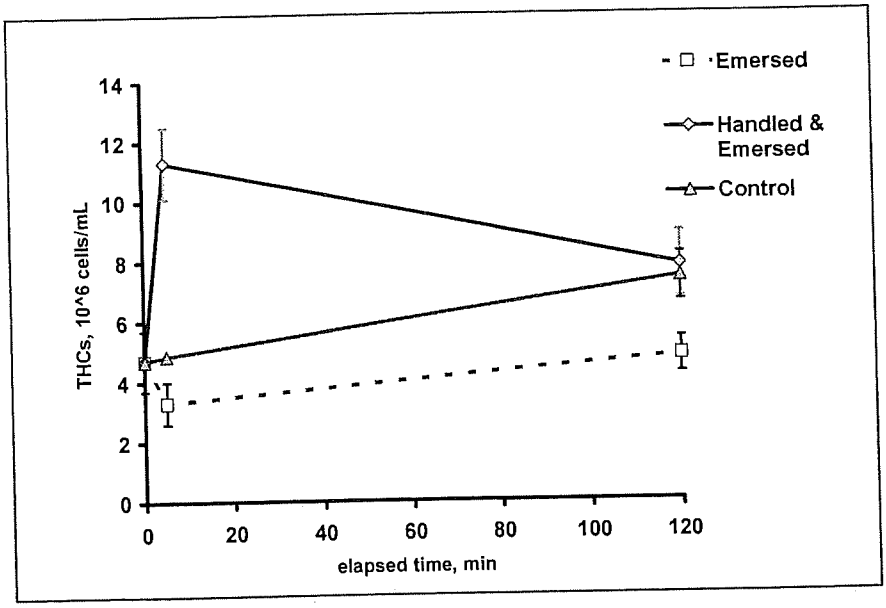

Figure 11. *THC response following air exposure with and without handling*

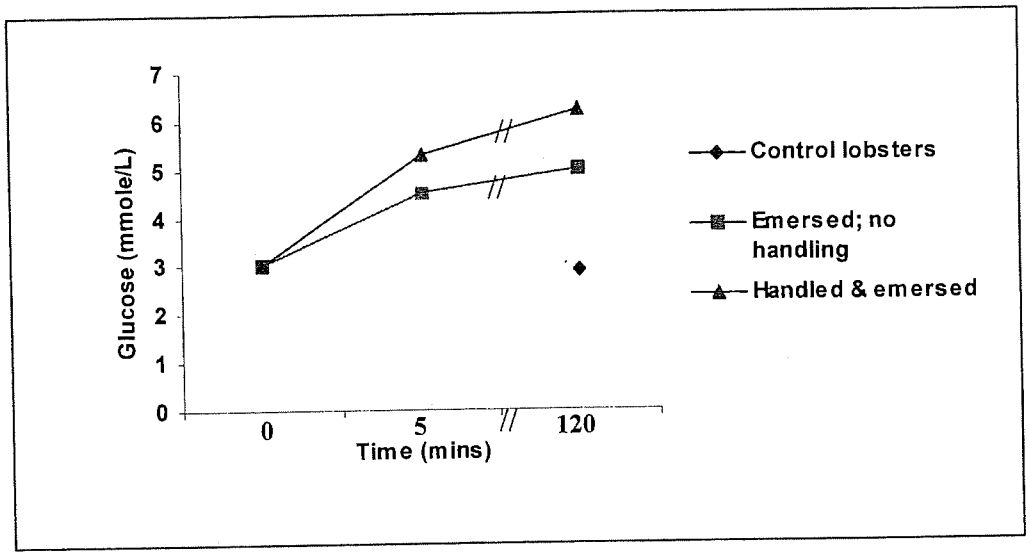

Figure 12. *Glucose response following air exposure with and without handling*

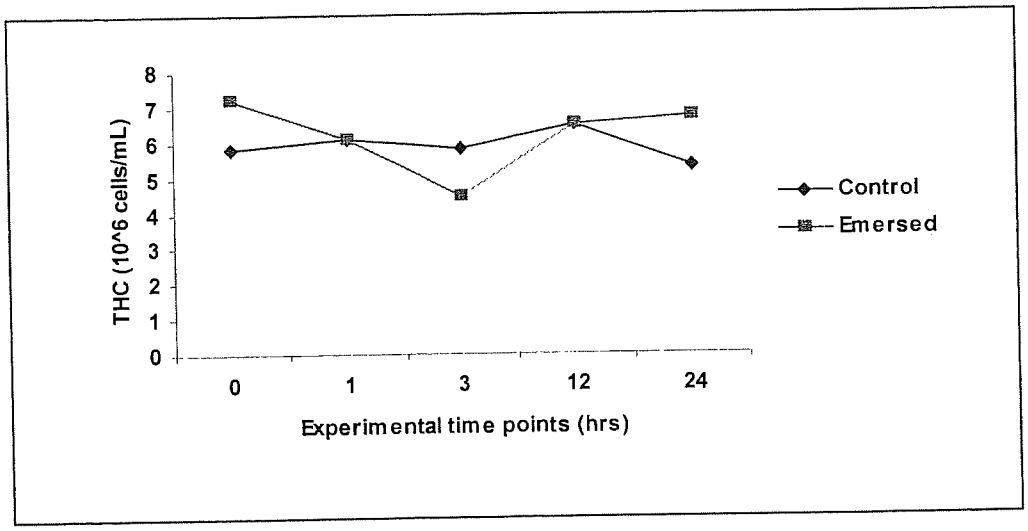

Figure 13. *THC in control and emersed lobsters*

Conclusions

- Immunity is not initially affected by emersion, even though emersion causes a stress response
- Lobsters have different types of stress responses
- Environmental factors (lack of oxygen, altered salinity, altered temperature) elicit a 'environmental ' stress response – glucose elevation only
- Behavioural factors (events that frighten or disturb lobsters) elicit a behavioural stress response – activated immunity + glucose elevation
- Air exposure is more detrimental to lobster health if behavioural stress response is superimposed on environmental stress

What Other Factors Cause a Behavioural Stress Response in Panulirus Cygnus?

Behavioural stress experiment - Environmental conditions

Room – Night	**Room – Day**
Low light	Intense light
Low visual	High visual disturbance
Low noise	High noise
	Air exposure
	Handling

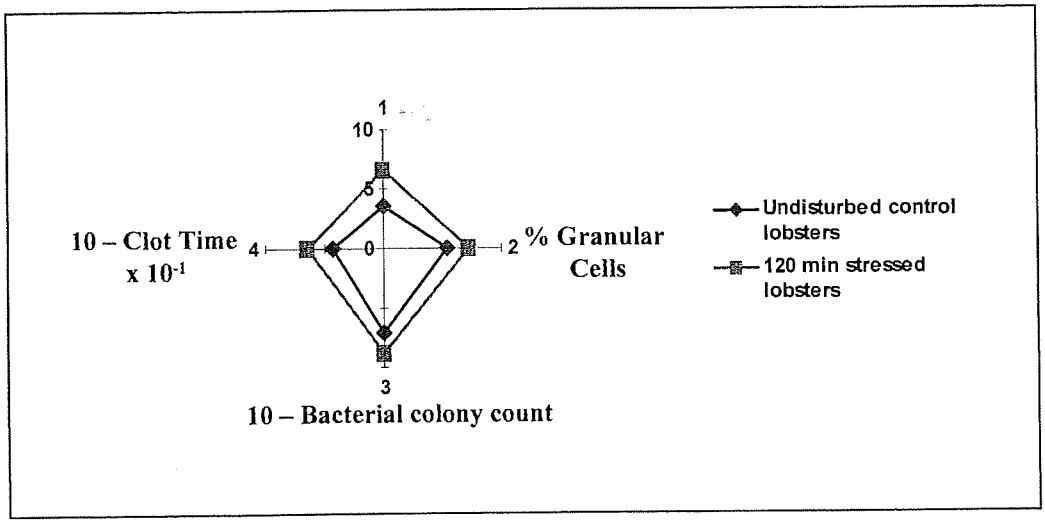

Figure14. *Comparison of IW areas for stressed and unstressed lobsters*

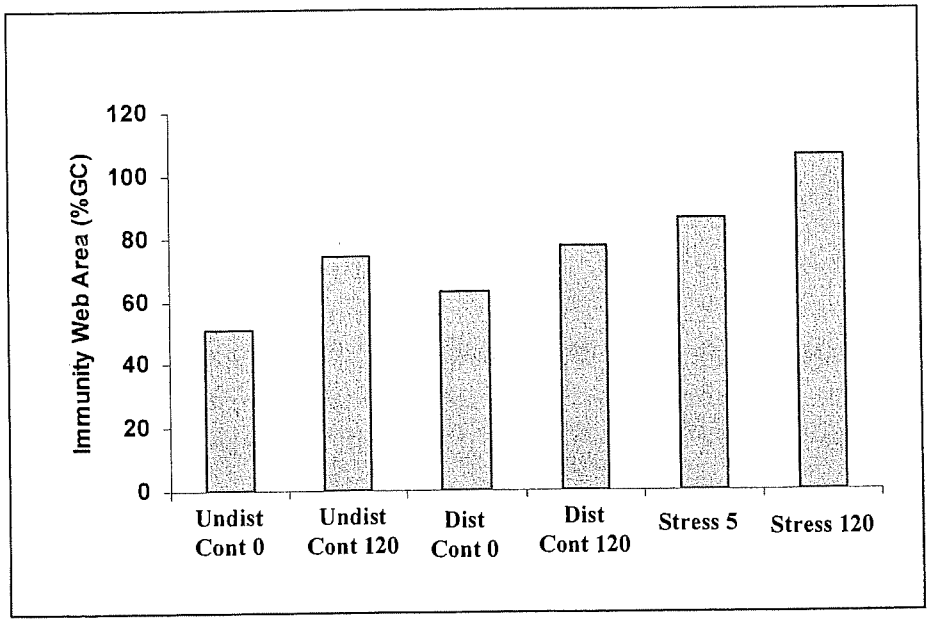

Figure 15. *Immunity Web areas for control and stressed lobsters (%granular cells)*

Conclusions

- Lobsters exhibit a behavioural stress response to environmental factors such as visual disturbance, physical handling
- Effect of noise and light still to be determined
- Behavioural stress response can be measured with THC, clotting time and other assays

Evidence of Behavioural Stress Response in Different Transport Conditions

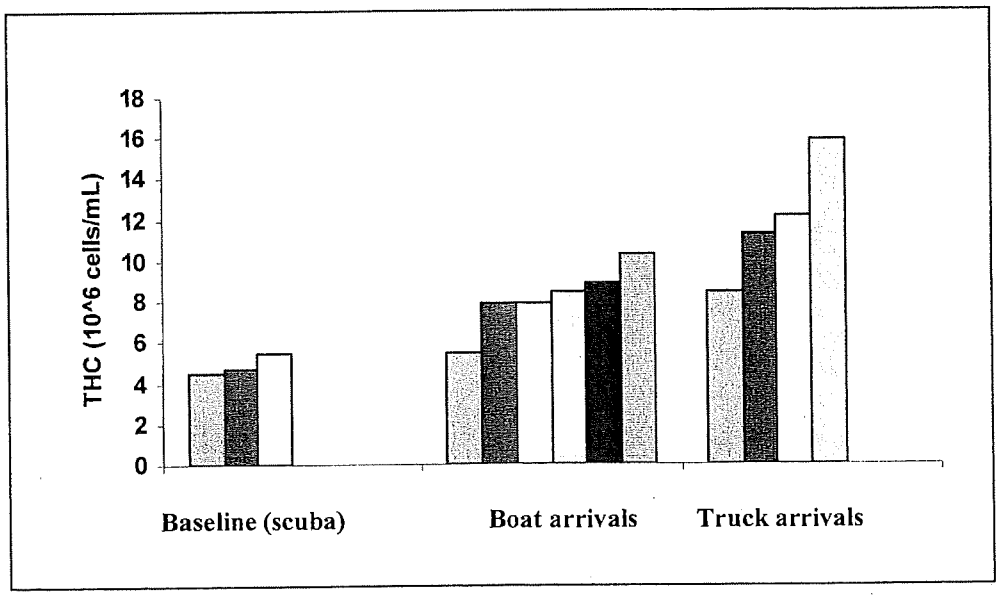

Figure 16. *THC in baseline lobsters and following transport to factory*

Conclusion

- Behavioural stress is higher in lobsters delivered to factory in trucks compared to delivery in boats
- The relative contribution of behavioural stress and environmental stress to deterioration in lobster health is unknown

Recommendations

- Extend the findings of this study to other spiny lobster species
- Validate the application of immunity analysis to health monitoring in aquaculture facilities
- Develop an Immunity Web specifically for behavioural stress studies
- Use this improved IW to evaluate effect of different behavioural stressors on lobster health in post-harvest handling

Acknowledgements

- FRDC and lobster processing companies
- Curtin research team – Japo Jussila, Seema Fotedar, Elena Tsvetnenko, Brian Jones

Cray potter & the indicator of doom - What do indicators of physiological stress tell us about responses of western rock lobsters to post-harvest handling?

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(1) Geraldton Fisherman's Cooperative, Geraldton, WA, Australia

Western rock lobsters (*Panulirus cygnus*) removed from factory tanks because they have weakened during live storage have probably been stressed too much. Factory-based trials in this project (FRDC 96/345) have shown, in conjunction with FRDC 96/344, that when changes were seen in certain physiological/immunological parameters in the blood of lobsters during a 6h storage treatment then they were more likely to die within the next week. The changes in the blood of lobsters in terms of basic blood electrolytes (Sodium, Chloride, Calcium etc) and biochemicals (glucose and lactic acid) examined during this study reflect the way that handling impacts on their well-being. Lobsters rely upon water movement over their gills, (thin feathery structures located in the "head" or cephalothorax) to obtain oxygen and excrete dissolved wastes. The key physiological indicators found here, lactic acid and magnesium, reinforce the point that storage in air at ambient temperature is highly detrimental to lobsters- even when seawater sprays are provided. These indicators are not simple enough for routine factory use, but they were still useful in monitoring the responses of lobsters to alternative storage/transport methods to ensure that the deleterious effects of out-of-water storage are minimised.

The word stress is used so frequently in a range of different contexts that it is worthwhile noting exactly what we mean by the term. Consider the lobster living within its normal environment. Put simply, its internal state will lie within a "normal" or "baseline" physiological range, bounded by the day to day or season to season biological factors such as feeding, exercise, moulting or reproduction. In contrast, the lobster is demonstrably "stressed" when its internal state moves beyond this normal range in response to an unusual external factor, the "stressor." The extent to which the lobster is "stressed," that is, how widely it deviates from normality, can of course mean the difference between whether it can recover upon withdrawal of the stressor or whether irrevocable harm has resulted.

Two things were accepted when we began our work, firstly that research shows that crustaceans such as lobsters are stressed during commercial post-harvest storage and secondly that different commercial handling storage methods for lobsters and crabs can show different levels of subsequent mortality. In order to vindicate the premise of this research, that stress during post-harvest handling was responsible for losses of western rock lobsters during storage in factory tanks, we drew together these threads of knowledge. We wanted to know that when a basket of live lobsters enters the factory, did the lobsters in that basket that later died differ in some particular way from the lobsters that survived.

Previous research has shown that crustaceans such as crabs and lobsters are stressed when they are handled commercially, and it is reasonable to expect this stress to have downstream effects in the factory. But that was the step that was actually missing until this research was conducted. While it was probably true that minimising stress will have a beneficial outcome, nobody knew how much the stress needed to be reduced. Just about all lobsters ARE going to be stressed by capture and handling- but it doesn't kill all of them. Why not? The answer is that few lobsters are stressed to the point that it will kill them.

Clearly the way that the lobsters in that basket are treated will have a major impact. We know that some methods of handling and transporting lobsters are more successful than others, and the implication is presumably that some treatments are more stressful than others. While comparing mortality rates in treatments allows you to chose the best method available, measuring the magnitude of the stress experienced by the lobsters in various treatments helps us to understand what is killing the lobsters. It tells you what needs to change to get a better outcome and why.

This study showed that during the post-harvest handling of rock lobsters, several physiological parameters deviated from baseline levels (established by sampling lobsters on the sea floor using SCUBA and by sampling captive acclimated lobsters). These changes parallelled the respiratory problems seen when rock lobsters were kept out of water in laboratory experiments. Using these findings as a basis, a series of factory-based experiments, some using alternative ambient temperature storage methods, were used to establish which of these physiological changes could be linked to later mortality in stressed lobsters.

Table 1. *Explanation of the treatments used in the factory based trials*

Treatments	Explanation
Flow subm.	lobsters submerged in continually replaced, flowing seawater
Rec. Subm.	lobsters submerged in the same seawater recirculated around the lobsters
Humid air	lobsters stored out of water in humid air
Flow spray	lobsters stored out of water, but sprayed with seawater from the factory supply
Rec. Spray	lobsters stored out of water, but sprayed with re-used, recirculated seawater (same volume of water as used in Recirc. Subm. above)

Immediately after the imposed stress, the lobsters had blood samples taken from then. They were then given a numbered tag and returned to tanks in the factory. When some lobsters died over the following week, we check through the results of the original blood tests and found that when originally taken from the treatments, the lobsters that die later were already significantly different from the survivors

with respect to some blood parameters (particularly lactate and magnesium concentration). We applied a particular kind of statistical method called discriminant analysis to the blood test results, and this way it was possible to correctly explain the fate of 80-90% of lobsters within that trial. However with each replicate of the study, a different set of blood tests were required, though there was some consistency from time to time, particularly regarding lactate's importance to the analysis. The real test of the stress indicators is to take the results obtained from stressing one group of lobsters and apply to the results of another trial (Figure 1).

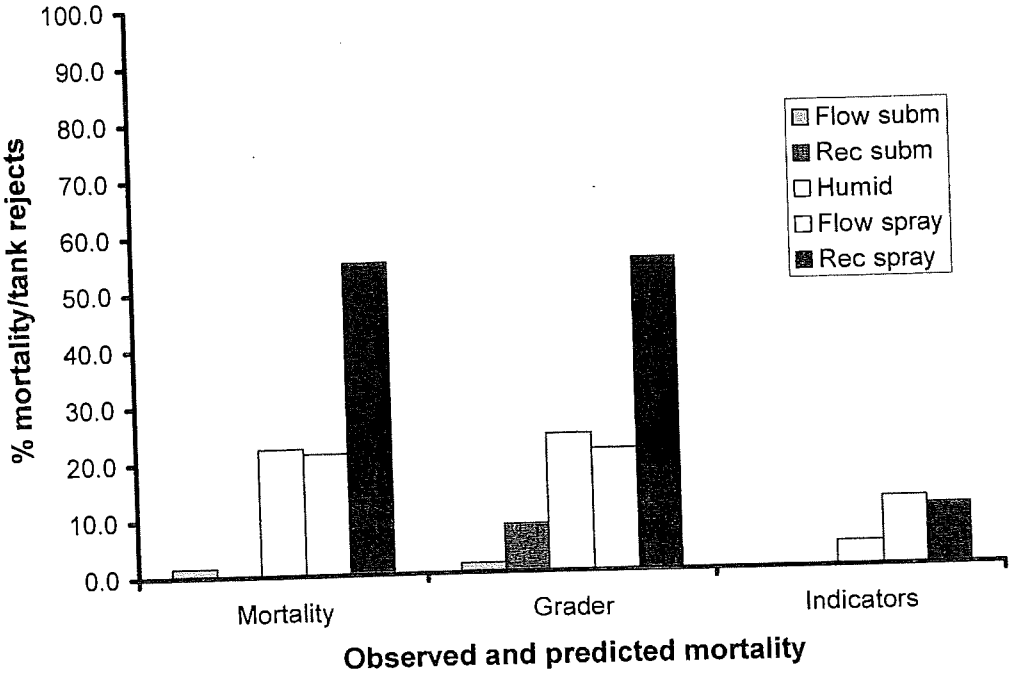

Figure 12. *Results of one of three controlled storage/transport trials conducted in a lobster factory at Geraldton (November 1998). showing the Mortality seen in the factory tanks after lobsters were subjected to a number of different treatments were 6h (26 C). Predictions of mortality by the Grader, and a stress Indicator based on blood tests of a different group of stressed lobsters.*

We showed that key indicators for commercially significant stress clearly existed, but none of them were simple enough nor rapid enough to apply in a factory context. When comparing the performance of lobsters in alternative storage methods, the most accurate method was clearly to count the actual mortality after a storage test, but the results of human grader collaborating in this study could also be used to rank treatments from best to worst (Figure 1). The advantage of stress indicators was that they showed why lobsters were dying and suggested the changes required to improve outcomes. Across all trials, the stress indicators consistently predicted correctly that submerged lobsters would survive, even lobsters submerged in a fixed volume of seawater within which water quality is deteriorating (eg. Figure 1).

The major recommendation arising from the study of controlled transport environments was that full submersion was recommended when of lobsters were transported or stored at ambient temperature for several hours. The results provided by the key stress indicators show that submersion of lobsters were the only way to avoid stressing them by asphyxiation. The storage environment trials also showed that the prognosis was excellent for lobsters stored at high densities in aerated, recirculated seawater for 6 hours. This work suggests that as long as aeration is maintained that water quality deterioration is not a major issue, and provision of biological filtration of the water does not seem to be a priority for short periods of exposure.

Of course, submerged transport will not always be possible or practical. These observations regarding lobster transport and storage are confined to ambient temperature and hence are strictly applicable to bulk transport of lobsters (eg. on carrier boats). Cooling the lobsters down (eg. truck transport) introduces another variable, that of reduced metabolic rate, which may of course improve the outcome.

Optimising water quality in rock lobster post-harvest processes

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Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Australia

Lobsters may be subjected to poor water quality at any stage of post-harvest handling. This can have a detrimental effect on lobster quality, and may result in a reduction of market value or at worst a complete loss of the product. The most important water quality variables are oxygen, temperature, salinity and ammonia. Ammonia is a product of normal metabolic processes in lobsters and bacterial decomposition of faeces, bait and dead animals in the tank. At high concentrations ammonia is toxic and at low concentrations it can inhibit normal physiological processes. However, there is a very limited understanding of these effects in lobsters.

In flow-through holding systems water exchange is generally more than adequate to prevent the build-up of ammonia. On the other hand ammonia can accumulate in holding or transport systems where recycled water is used. Peak ammonia levels in holding systems usually occur shortly after a fresh consignment of lobsters is put in the tanks, especially if they have been transported in air. Waste products that have been stored up in the bodies of the lobsters are excreted rapidly as soon as they are put into the holding tanks. For this reason ammonia concentration should be regularly monitored and when high corrective action (eg. water exchanges) undertaken.

Lobsters are stressed during post-harvest procedures due to handling and air exposure. Stress in turn may also affect the ability of lobsters to tolerate ammonia. In a study supported by the FRDC Rock Lobster Post Harvest Subprogram (Project 2000/252: Optimising water quality in rock lobster post-harvest processes) we quantified the acute toxicity of ammonia to adult southern, *Jasus edwardsii*, and western, *Panulirus cygnus*, and determined the affect of stress on that toxic level.

Methodology

Southern rock lobsters

Legal sized lobsters (mean weight of 703 g) were used for the experiments. After transport to the laboratory the lobsters were allowed to recover for at least 36 hours before the start of an experiment. Lobsters were unfed for the duration of the experiments.

A static-renewal bioassay system was used for the experiments. This consisted of fifteen 75 L experimental tanks maintained at 13°C. Aeration to each tank was provided by two airstones, maintaining an oxygen saturation level of greater than 70% at all times. Eight lobsters were placed into each treatment tank, with three replicates per treatment. The experiments were run over 96 hours.

The concentration which results in the mortality of 50% of the test animals over 96 hours (LC_{50}) is generally used to compare the ammonia tolerance of different species.

Based on a preliminary trial, five treatments were used, nominally containing 0, 40, 60, 90 and 135 mg L^{-1} of ammonia. The actual average ammonia concentrations in the treatments over the 4 days were 2, 41, 56, 82 and 122 mg L^{-1}. Lobsters in the stressed experiment were subjected to simulated truck transport conditions for 4 hours prior to the start of the experiment and were handled and exposed to air for 15 minutes every 6 hours during the 96 hour experiment. At the start of the 96 hour experimental period the tanks were drained and filled with 70 L of the appropriate treatment solution. Water in the experimental tanks was renewed every 24 hours from stock tanks containing the treatment solutions.

Western rock lobsters

The same methodology was used as for the southern rock lobsters apart from the following. There were 6 lobsters (mean weight of 424 g) per experimental tank and each tank contained 40 L of water. The water temperature was maintained between 19 and 20°C. The experiments were undertaken at a commercial holding facility in Geraldton, Western Australia.

Results

The study found that stress did not have an effect on the ammonia tolerance of either the western or the southern rock lobster. Figure 1 shows that southern rock lobsters have an LC_{50} of 83 mg L^{-1} and are reasonably tolerant of ammonia. Adjusting this figure to take account other water quality parameters resulted in a decrease of the LC_{50} to 35 mg L^{-1}, which is still high but lower than that found for other large lobsters such as the American clawed lobster.

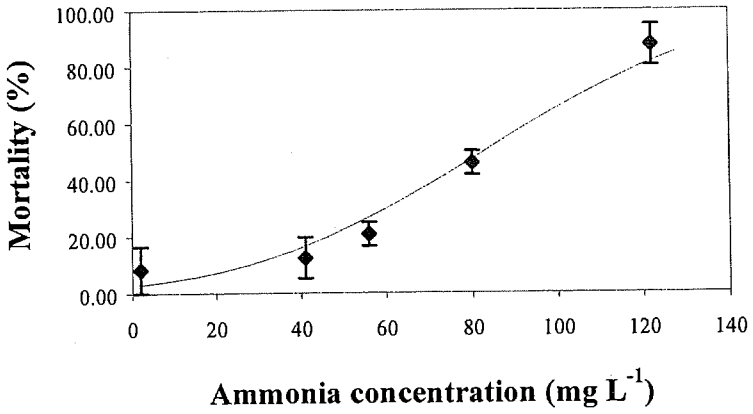

Figure 1. *The mortality of southern rock lobsters when exposed to various ammonia concentrations over a 96 hour period.*

Figure 2 shows that western rock lobsters have an LC_{50} of 61 mg L^{-1} and are also reasonably tolerant of ammonia. Adjusting this figure to take account other water quality parameters resulted in a decrease of the LC_{50} to 25 mg L^{-1}.

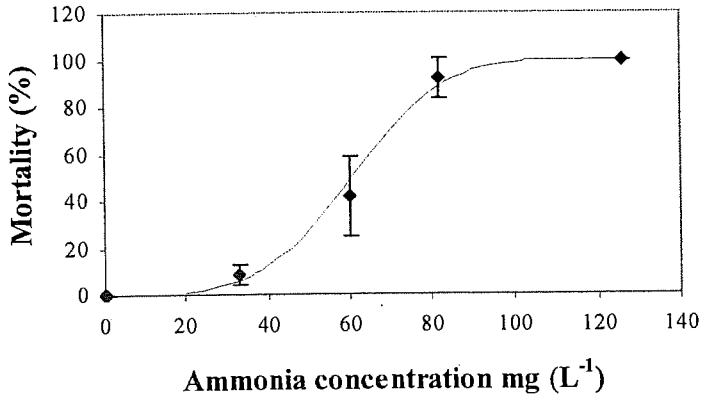

Figure 2. *The mortality of western rock lobsters when exposed to various ammonia concentrations over a 96 hour period.*

We found that ammonia concentration levels were generally low in holding systems, although levels of 30-50 mg L^{-1} were occasionally measured. While lobsters are tolerant to ammonia, mortalities occur even at low ammonia concentrations. In addition when lobsters were exposed to ammonia they became very aggressive - to each other and to researchers! The aggression resulted in physical damage, with a high incidence of chewed legs and antennae. Such damage obviously has implications for the market value.

Another finding was that the lobsters had higher respiratory rates when exposed to ammonia. The higher oxygen consumption rate could lead to a deterioration of the water quality in the holding tanks (low oxygen levels).

Practical outcomes

The tolerance of rock lobsters to ammonia provides options when transporting or holding them. For example, when entering harbours where poor quality water may be encountered (eg. low salinity), it can be avoided by stopping the inflow of water and keeping the lobsters submerged in the holding tanks. As lobsters would generally be held in this situation for a short period (a few hours) before being unloaded, it is unlikely that the ammonia level will rise to a dangerous level. It would be

extremely important to maintain an adequate oxygen supply to the lobsters (by aerating the water) during this time.

Based on the toxicity data and using a "safety factor" of 10%, a safe level of ammonia for adult southern rock lobsters held at 13°C would be about 3.5 mg L^{-1} and adult western rock lobsters at 19-20°C would be 2.5 mg L^{-1}. Concentrations up to that level could be tolerated for short periods. Other water quality parameters (such as higher temperature and low oxygen levels) are likely to result in a decrease of this "safety level". The presence of elevated levels of ammonia for short periods after a tank is stocked should not necessarily be a cause for concern. However, a continuously elevated level of ammonia is an indication that changes to the design or management of the system should be considered.

Development of a method for alleviating leg loss during post-harvest handling of rock lobsters

Glen Davidson[a] and Wayne Hosking[a]

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Introduction

The western rock lobster is exceptionally prone to autotomising legs during post-harvest handling and this is a significant problem in an industry where intact lobsters command premium prices.

The western rock lobster industry incurs costs from leg loss in the following ways:

1) **Loss of weight.** Industry estimates suggest that between 40-80 tonnes of legs are lost from the landed catch each season. At $25-30/kg this is worth between $1-3 million. This figure does not include any estimate for legs lost by caught and returned lobsters, such as undersized and breeding female lobsters. Given that some 16-20 million undersized lobsters are handled each year (Brown and Caputi, 1984), it is likely that the true figure is much higher than previously estimated.

2) **Loss of value/restricted marketing opportunities.** Lobsters with too many missing legs are downgraded from premium product forms, such as live export or whole cooked or raw, to lesser product forms, such as tails. Depending on the markets this often, but not always, results in reduced profitability.

3) **Increased mortality of damaged returned lobsters** (reproductive females and undersized). This is suggested by reduced recapture rates of damaged western rock lobsters in tag and release studies (Brown and Caputi, 1983).

4) **Reduced growth of damaged returned lobsters** (reproductive females and undersized). Brown and Caputi (1985) showed that for each additional missing leg, the moult increment in western rock lobsters was reduced. The slower-growing damaged lobsters take longer to reach minimum legal size. As a result more undersized lobsters will succumb to natural mortality before recruiting to the fishery.

5) **Reduced reproductive success of damaged returned mature females.** Damaged females divert energy to regenerating lost limbs rather than to egg production, potentially resulting in smaller broods or failure to reproduce at all. In addition, slow-growing damaged females mature at a smaller size with disproportionately reduced brood sizes.

The total value of the direct losses in 1) & 2) is estimated to exceed of $3 million per annum. While it is not possible at this stage to estimate losses resulting from 3), 4) and 5), these losses are also likely to be substantial. Development of a method for preventing handling-induced appendage loss would be of major benefit to the industry and to the fishery.

Methods

A commercial lobster boat operating out of Port Denison, 60 km south of Geraldton was used in the trials. The boat was fitted with a modified sorting (or cacka) box to allow controlled trials of cold water stunning and normal handling. The box had a refrigerated sea water bath on one side and a standard aluminium tray on the other (Fig. 1). The sea water bath was chilled using a 1.5 hp chiller unit below decks. Water in the bath was aerated constantly to prevent thermal stratification and to maintain high dissolved O_2 levels.

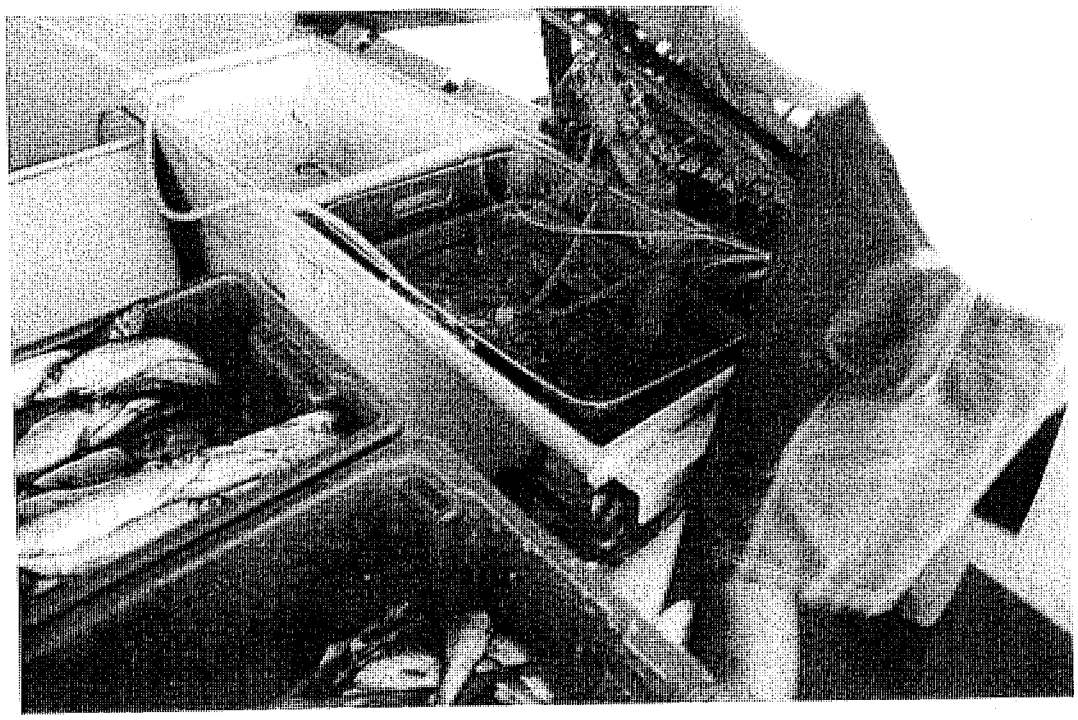

Fig. 1. *Lobsters being tipped into the experimental cacka box fitted on board a commercial lobster boat. The box is divided to allow controlled comparison of cold-stunning and normal handling.*

During trials, alternate pot catches were sorted using normal practice (control) or were subjected to cold-stunning prior to sorting (treatment). Control lobsters were emptied from the pot into the aluminium tray. The deckhands then removed the lobsters from the tray individually and checked them for size. Undersized lobsters and breeding females were immediately returned to the ocean. Legal-sized lobsters were checked for reproductive condition before being stored in below deck live tanks.

Lobsters from treatment pots were emptied into a basket in the seawater bath where they remained for a maximum of 5 sec. The stun period was started at the time the first lobster entered the bath. After 5 sec. the basket was removed from the bath and the lobsters were sorted. Legal-sized treatment lobsters were placed in a separate basket in the live tanks. In this way, the treatment and control lobsters were kept separate until they could be delivered to the factory.

The best measure of the commercial success of the treatment is the difference in leg loss between control and treatment lobsters at the time the processor purchases the catch from the fisher. Upon return to shore, the lobsters were transported 1.5 km in air by utility vehicle to a local depot where they were stored in a recirculating holding system at 17°C for between 1.5 and 24 hours. The lobsters were then transported to the live holding factory in Geraldton by truck. Upon arrival at the live factory, the catch was placed in a 10°C seawater bath for 20-60 minutes before all lobsters were inspected for old (>>24 h) and new (<24 h) autotomy wounds and missing antennae. The age of the autotomy wounds was estimated from the degree of melanisation of the limb stumps.

Results and Discussion

The sea trials were conducted over 44 days during the 2000/2001 season. During this time, data were collected from over 5200 pot lifts and nearly 24500 lobsters were handled.

Stun temperatures from 0 - 21°C (ambient) were tested. Temperatures between 0 – 10°C appeared to be equally effective for reducing on board leg loss. In this temperature range, the daily reduction in leg loss was 70-80%.

On the basis of factory experiments, we assumed the preferred temperature for use in the field is the highest effective temperature for preventing leg loss. Such a temperature will presumably allow the most rapid recovery in protected animals returned to the ocean. For this reason, and because a refrigeration system usually regulates temperature to within a couple of degrees either side of a set point, we chose to focus on the range of 5 - 10°C. The results presented below are for the days where stun temperatures in this range were used.

The daily average on board leg loss in control lobsters was 0.145 ± 0.014 legs/lobster (n = 32). A 5 sec. 5 - 10°C stun prior to on board sorting significantly reduced this to 0.023 ± 0.002 legs/lobster (paired t-test, t = -9.045, $P < 0.01$; n = 32). The average daily reduction in leg loss for these lobsters was 80.3 ± 2.2%.

The daily percentage reductions in leg loss in returned protected and retained legal-sized lobsters (81.4 ± 2.2% and 77.3 ± 4.0%, respectively) were similar (paired *t*-test, $t = 1.414$, $P > 0.05$, $n = 32$).

The results of the catch assessments at the Geraldton factory are presented in Table 1. Treatment lobsters showed significantly fewer new (< 24 h old) leg stump wounds and newly broken antennae (Table 1).

The catch was also assessed in terms of the proportions of lobsters suitable for different product forms. Lobsters with fewer than 1 missing leg + 1 missing antenna were considered fit for live export (FFL). Lobsters with up to 3 missing legs + missing antenna were considered fit for whole cooked or whole raw (FFW) and lobsters with more than 3 missing legs or more than 1 missing antenna were considered fit only for tailing (FFT). These assessments were made on the basis of damage only, no consideration was given to the vigour of the lobsters arriving at the factory. Treated lobsters showed significantly greater proportions of intact lobsters (i.e. no missing legs or antennae) and lobsters that were FFL and FFW and a smaller proportion of lobsters fit only for tailing.

Table 1. *Summary of factory assessment data for stunned (on board cold-stun 5-10 °C) and control lobsters consigned by the Windjana. Data are presented as the average (± 1 s.e.m.) daily proportions of intact lobsters and lobsters suitable for various product forms. n = 23 for all samples. The asterisks indicate significant differences between the 2 groups (*** = P < 0.01, ** = P < 0.05, * = P < 0.10).*

	Control	Stunned
New leg wounds (wounds/lobster)	0.547 ± 0.064***	0.311 ± 0.043
New Antenna wounds (wounds/lobster)	0.106 ± 0.012**	0.085 ± 0.010
% Intact	61.3 ± 2.8***	72.3 ± 2.6
% FFL	84.0 ± 1.9***	88.7 ± 1.7
% FFW	10.8 ± 1.4*	7.8 ± 1.2
% FFT	5.1 ± 0.9*	3.5 ± 0.7

Cold-stunning may have other benefits. For example, large numbers of undersized western rock lobsters are caught and returned annually and the effects of this repeated handling are seen as cumulative leg loss, antennal loss and tail fan necrosis (TFN). In the sea trials, cold-stunned lobsters showed significantly less post-harvest antennal loss ($P < 0.05$) than controls. Cold-stunning also reduces tail-flicking during handling and may therefore be beneficial for reducing TFN. This information, coupled with the recent interest in TFN in the southern rock lobster, *Jasus edwardsii*, suggests further investigation is warranted.

Conclusion

Cold water stunning shows excellent potential as a practical and cost-effective method for preventing post-harvest leg loss in western rock lobster. The potential economic benefit is considerable, as is the potential to minimise fishing-related damage and mortality of returned lobsters.

Further sea trials are being conducted in the Southern C zone of the fishery to determine the effectiveness of the method under the varied conditions that exist in this geographically widespread fishery. Experiments are also being conducted to determine the effect of cold water stunning on the survival of caught and returned undersized and breeding female lobsters.

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Hypo- and hypersaline-induced leg autonomy in western rock lobsters

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Introduction

It is estimated that post-harvest leg autotomy (or reflex leg loss) costs the western rock lobster (WRL) industry in excess of $3 million per annum (see previous article for details). FRDC 2000/251 was funded to investigate solutions for this substantial problem. One year into that project, a major discovery was made which has already revolutionised the way in which autotomy is understood and managed by lobster fishers and processors in Western Australia. Consequently this project, FRDC 2001/255, was commissioned to further investigate the phenomenon of hypo- and hypersaline-induced autotomy in WRL.

For reasons not fully understood, WRL can respond to contact with freshwater or concentrated seawater by autotomising one or more legs. The reaction to these stimuli can be almost instantaneous, and the stimuli are ubiquitous throughout the WRL industry. One kilogram of normal seawater contains approximately 36 grams of salt. The salinity level in the Indian Ocean, home to the WRL, is highly stable. However, due to evaporative concentration and standard industry processing techniques, captured lobsters regularly come into contact with waters of markedly different salt concentrations to that in which they have evolved.

Fishers and processors throughout the industry have long been aware of a correlation between hot, dry and windy conditions and high levels of autotomy, but no causal relationship had been established. It is precisely these weather conditions that lead to high evaporation levels from seawater films on boats, in factories, on gloves and even on the lobsters themselves. Evaporation removes only pure water. The salt remains behind, resulting in increasingly concentrated seawater. It is contact with these hypersaline films that induces lobsters to drop legs, i.e. to autotomise.

Approximately 7 500 tonnes of lobsters are processed for the frozen export market each season in Western Australia. Before they can be processed, they must first be killed by immersion in tap water. Initial observations suggested that exposure to tap water may also induce autotomy. Considering the high volumes processed, even a relatively low rate of autotomy at this stage may be economically important.

Given these observations, the main objectives of this project are to:

- Define the relationship between salt concentration and the magnitude of the autotomy response during either surface contact or full immersion

- Survey the prevalence of freshwater and concentrated seawater on surfaces with which lobsters come into contact during the post-harvest process
- Test and develop practical and cost-effective solutions for the identified risks
- Extend this information to industry so that the maximum benefit of the research is realised

Contact with hypersaline films

In this experiment, a range of contact films were prepared, ranging from freshwater through to nearly 500% concentrated seawater. The experiment was designed to mimic lobsters coming into contact with grading tables or weighing tubs. The results, summarized in Figure 1 below, show a clear relationship between increasing salinity of the film and increasing rates of leg loss. The highest autotomy rate was 2 legs per lobster at 180 grams of salt per kilogram. Lobsters coming into contact with normal seawater films did not suffer appreciable autotomy. Interestingly, contact with freshwater films did not induce increased levels of autotomy, although later experiments showed that immersion in freshwater does have an effect (see below).

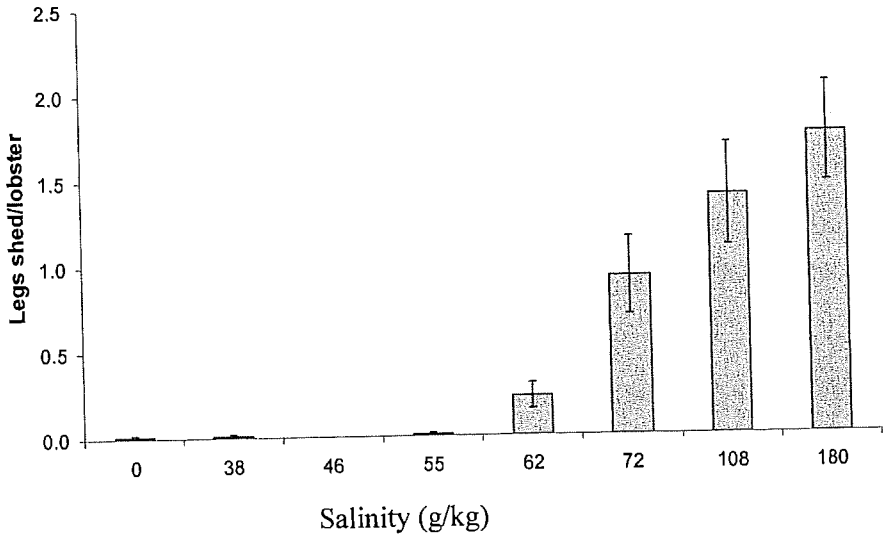

Figure 1: *Autotomy rates are directly proportional to salt concentrations of contact surfaces.*

Surveys of contact surfaces on boats and in factories have commenced to determine the prevalence of concentrated seawater films, and therefore the likely scope of the problem. Figure 2 summarises interim results of a factory survey. Surfaces sampled included grading tables, gloves, holding crates and weighing tubs. The results show that lobsters do come into contact with seawater films at concentrations that induce autotomy. The scale of this problem is likely to vary widely depending on prevailing weather conditions, factory design, and the degree to which the processor has already taken corrective measures. Similar surveys are being conducted on board fishing vessels.

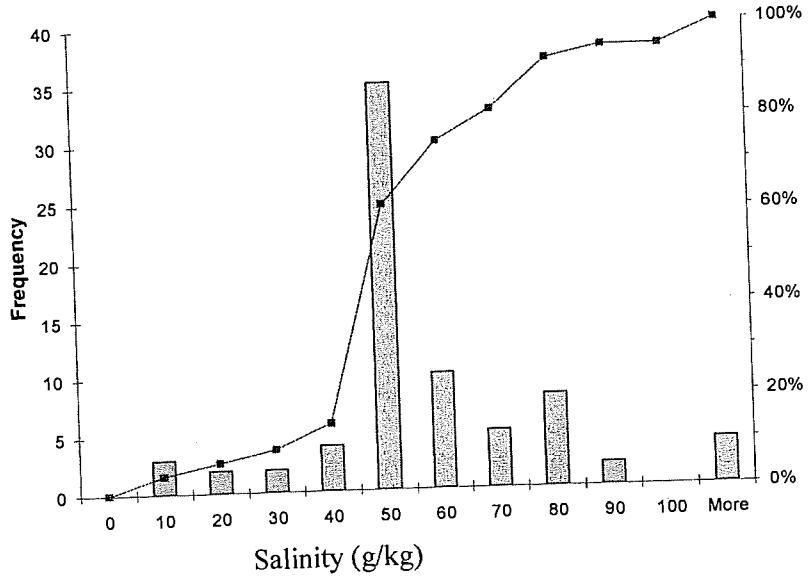

Figure 2: *In this example, 25% of factory surfaces sampled had salinities in the danger zone.*

Having now clearly identified the problem, what can be done about it? In most instances, the solution is obvious and straightforward. Reducing the potential for evaporation is the first step, and removing any build up of salt through a process of washing is usually simple (see Figure 3 below). The ability to measure the concentration of seawater films will enable a processor or fisher to monitor the success of any improvements, or the development of any problems. Reference to Figure 1 will show whether the approach has been successful, although in our experience, improvements are usually immediately obvious to staff and management.

Interestingly, a brief (~5 sec) cold water stun, correctly applied, largely prevents autotomy during contact with hypersaline films. On-board fishing vessels and elsewhere in the field, it may be impossible or impractical to prevent hyper-salinity, particularly on the lobster itself. In addition, contact with hypo- or hypersaline films is not the only trigger for autotomy in WRL. Therefore in many cases, there is currently no substitute for cold stunning.

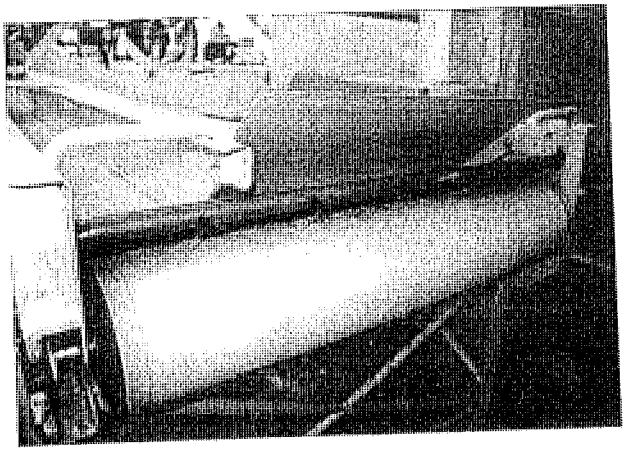

Figure 3: *For many of the problem areas, the solution to salt build up is obvious and simple. Here, seawater sprays constantly wash a revolving grading belt.*

Freshwater Immersion

Lobsters were graded according to size and sex, and then placed in tubs filled with ambient temperature (18.5-25.5°C) tap water, in a manner that ensured no autotomy occurred prior to immersion. After 50 minutes, all movement had ceased, the lobsters were carefully removed, and the amount of leg loss was recorded. The results are summarised below:

- A grade (350 to 500g): 9 legs per 100 lobsters
- G grade (1200 to 1500g): 6 legs per 100
- A grade males: 14 legs per 100
- A grade females: 21 legs per 100

A number of interesting trends are emerging from this ongoing work. The results suggest there may be a difference in autotomy rates between males and females, and small and large lobsters, although differences are not statistically significant. The key finding however is that immersion in freshwater does cause autotomy. More recent experiments suggest that even higher autotomy rates may occur at certain times, but even assuming a rate of loss of 10 legs per 100 lobsters, extrapolated across the industry, losses could be substantial. Initial indications are that most of this leg loss can be prevented by stunning lobsters in cold seawater prior to immersion in freshwater, and work is continuing in this area.

Conclusion

Exposure to hypo- and hypersaline seawater has been clearly defined as a substantial problem facing industry, and the later part of this project will focus on quantifying the benefits of potential solutions, and providing industry with the information it requires to make the necessary cost-effective changes. It should be noted that contact with hypo- and hypersaline seawater is not the only cause of autotomy, and removal of the offending films will not provide a complete remedy for all leg-loss. In many cases, there will likely be no substitute for cold-water stunning. Implementation of the techniques developed in this and the companion project are producing real benefits to industry through the prevention of post-harvest leg loss.

Striking a balance between melanosis and weight recoveries in western rock lobster (*Panulirus cygnus*)

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Introduction

Melanosis is the blackening of the tissues that occurs on thawing of frozen pre-cooked western rock lobster (*Panulirus cygnus*) and results in decreased consumer acceptance. It is currently believed that melanosis can be reduced by increasing cooking time however this may also result in reduced cooked weight recoveries and substantial financial losses to the processor. Johnson and Evans (1991) noted that melanosis in rock lobster is confined to those regions containing high concentrations of hemolymph, in particular the pericardial sinus and heart. Boon (1975) conducted research into the discolouration of canned crabmeat and noted that it was directly attributable to copper-containing enzymes found in the hemolymph. Further work by Söderhäll (1982) showed that the copper-containing enzymes found in the hemolymph of crustaceans are phenoloxidases.

The majority of the phenoloxidase present in the hemolymph is stored in the granulocytes as the inactive form, **prophenoloxidase (proPO)** (Söderhäll 1982). A low level of active phenoloxidase is maintained in the plasma of the hemolymph. It acts as a fast response reserve for the creature and is referred to as **baseline activity** (Smith & Söderhäll 1991). Smith & Söderhäll (1991) have also shown that the addition of trypsin will activate all of the stored prophenoloxidase allowing for the measurement of the maximum potential phenoloxidase activity i.e. **total activity.**

The inactive proPO is activated by many factors as an immune response. When activated, the phenoloxidase oxidizes phenolic compounds in the hemolymph to quinones. Quinones are highly reactive and undergo non-enzymatic oxidation and polymerization to produce melanin pigments (Söderhäll & Cerenius 1992). These pigments are used to encapsulate foreign matter or bacteria rendering them harmless to the creature. They also have a role in wound repair (Söderhäll & Cerenius 1992). Ashida and Söderhäll (1984) showed that non-physiological factors, such as heat, detergent and organic solvents, can also activate proPO, resulting in quinone production. The activity of phenoloxidases before and during cooking may have a considerable impact on the occurrence of melanosis in cooked rock lobster. For the industry to consistently achieve maximum cooked weight recoveries, whilst reliably controlling melanosis, a systematic investigation of the impact of processing on these factors is essential.

The aim of this paper is present the initial findings of the FRDC project 2001/235 which was established to undertake a comprehensive study to evaluate the impacts of post-harvest handling and processing on weight recovery and melanosis formation in cooked Western Rock Lobster. The information generated by this study will be used to define best processing practices in order to increase overall quality of the product and profitability of the industry.

Methods

1. Identification of the impact of temperature on PO activity

- *Heat trials: Steady state*

Hemolymph was mixed with pre-heated buffer in six test tubes and placed in the hot water bath at a set pre-determined temperature. After 5 minutes one tube was removed from the bath, plunged into a bed of ice and iced buffer was then added. Baseline PO activity and total PO activity were then determined spectrophotometrically by measuring the change in absorbance at 490 nm (Smith & Söderhäll 1991). The procedure was repeated at 5-minute intervals to a maximum time of 30 minutes. Initial PO activity and hemolymph protein content were determined using a sample held at room temperature. The tests were conducted at 40, 50, 60, 70, 80, 90 and 100 °C with 4 lobsters being used for each temperature series. Results were expressed as % of the initial baseline or total activity, respectively i.e. % Relative activity.

- *Heat trials: Unsteady state*

Hemolymph was added to buffer in nine test tubes held at room temperature. The tubes were mixed thoroughly using a vortex mixer and eight were placed in the water bath. The initial temperature was recorded and the water bath switched on. When the temperature in the tubes reached a predetermined set level (40,50,60,70,80,90 or 100 °C) one tube was removed from the bath and plunged into a bed of ice. Iced buffer was added and the contents of the tube mixed thoroughly. Baseline PO activity and total PO activity were then determined spectrophotometrically by measuring the change in absorbance at 490 nm (Smith & Söderhäll 1991). Initial PO activity and hemolymph protein content were determined using the remaining sample held at room temperature. A total of eight lobsters were used for the test series.

2. Thermal profile of cooking process

The aim of this phase of the study is to establish the thermal profile of current commercial practices. Three research trips were carried between November 2001 and April 2002. The work was conducted at the M.G. Kailis lobster processing facility in Dongara, WA.

Discussions with processors have revealed that considerable differences in the time-temperature profiles used to process whole lobsters exist throughout the season and between processors. Therefore,

for the purposes of this study a "representative" processing protocol was devised based on the typical practice observed in the industry.

Lobsters were drowned in fresh water at ambient temperature (22 to 24 °C) for 20 minutes. They were then packed into baskets to give an average load of 100 kg per cook. The cooker was brought to the boil. The baskets were then put into the cooker and heated for a total of twenty minutes from point of entry to removal. Upon removal the baskets were immediately plunged into a freshwater ice slurry bath and held there for 20 minutes prior to washing, draining, and packing. Each lobster was wrapped in cellophane prior to packing and freezing in a blast freezer (-40°C)

Fourteen rock lobsters were selected after drowning and the third leg on the right hand side of each lobster was snapped off at the joint closets to the body and a nickel cadmium thermocouple probe inserted. This positioned the probe in the flesh below and slightly posterior to the heart region. Previous trials have shown that this is the coldest part of the lobster during cooking. Another thermocouple was placed on the dorsal carapace between the supraorbital spines in order to describe the immediate thermal environment of each lobster during the cook. The thermocouples were secured with plastic cable ties.

The instrumented lobsters were packed into baskets in such a way as to give a three dimensional spatial array of temperature recording throughout the cook as shown in Figures 1 and 2. The probes remained *in situ* whilst the lobsters were cooled in ice slurries, but were removed immediately prior to freezing. The trials were repeated over 5 days and at 3 times during the season using Grade 'A' lobsters (max weight 460g) and over 3 days at 3 times throughout the season using Grade 'B' lobsters (weight range = 461-570g).

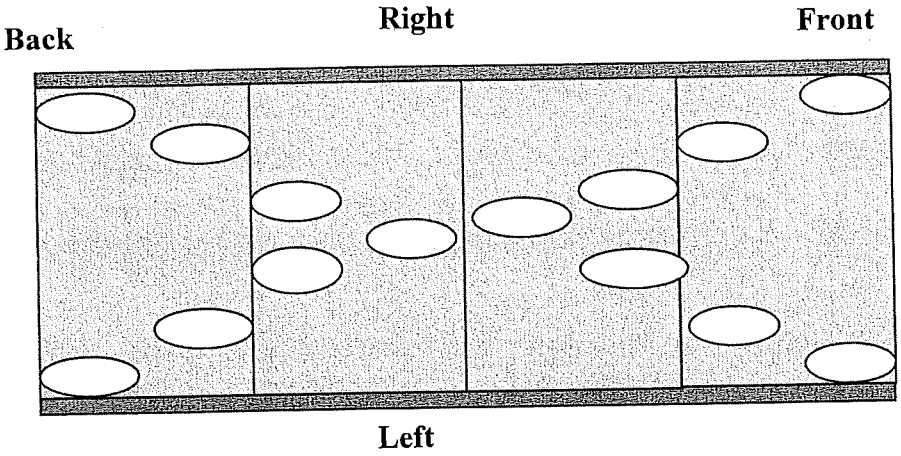

Figure 13: *3D array viewed from above*

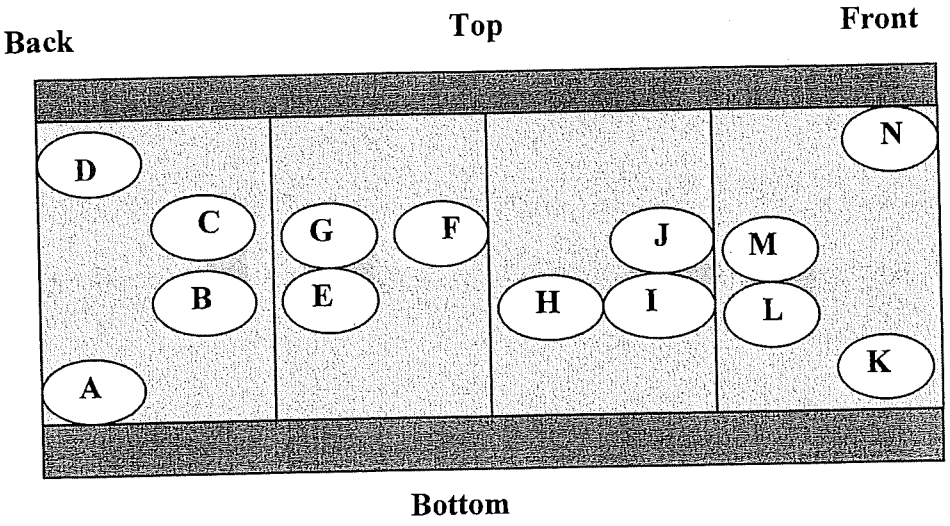

Figure 14: *3D array viewed from the side*

Results and Discussion

Total activity is defined as the maximal enzyme activity following trypsin mediated release. Baseline activity is the level of phenoloxidase activity that exists without trypsin treatment and corresponds to the level of activity that would occur during cooking. The two measures provide a profile of all the changes in enzyme availability and activity that occur under the experimental conditions.

1. Identification of the impact of temperature on PO activity

- *Heat trials: Steady state*

Baseline activity increased at 40°C, possibly due to activation and release of phenoloxidase from the hemocytes as the hemolymph coagulated. After five minutes at 50 and 60°C no baseline activity was detected. However after 20 minutes at 60 °C, baseline activity was detectable only at low levels. Significant increases in baseline activity occurred at 70 and 80 °C after five minutes. This may be attributed to heat activation of the enzyme and/or increasing disruption of the hemocytes. The activity then followed a downward trend throughout the rest of the heating period but was still significantly higher than the time zero value. There was no increase in activity at 90 °C and levels decreased steadily until there was no detectable activity after 20 minutes. No baseline activity was detected in samples exposed to 100 °C for 5 minutes.

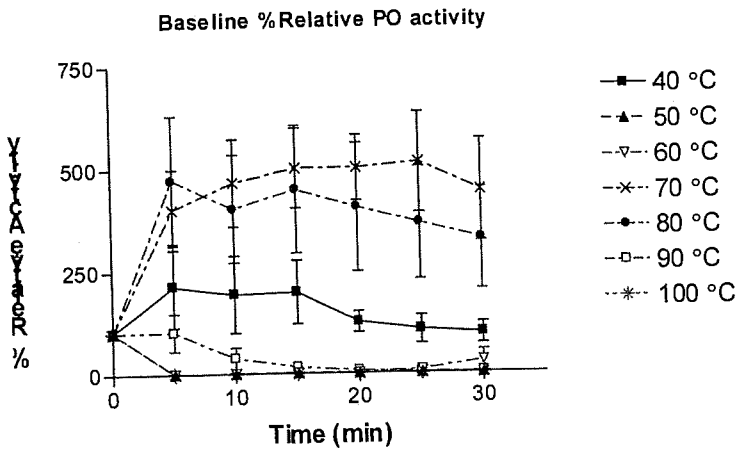

Figure 15: *Baseline relative activity over time under steady state heating conditions (mean±SEM)*

Total activity increased slightly over time at 40, 50 and 60 °C. Decreasing total activity over time was displayed at 70 and 80 °C. After 20 minutes at 90 °C total activity was approaching 0. After 5 minutes at 100 °C no activity was detected.

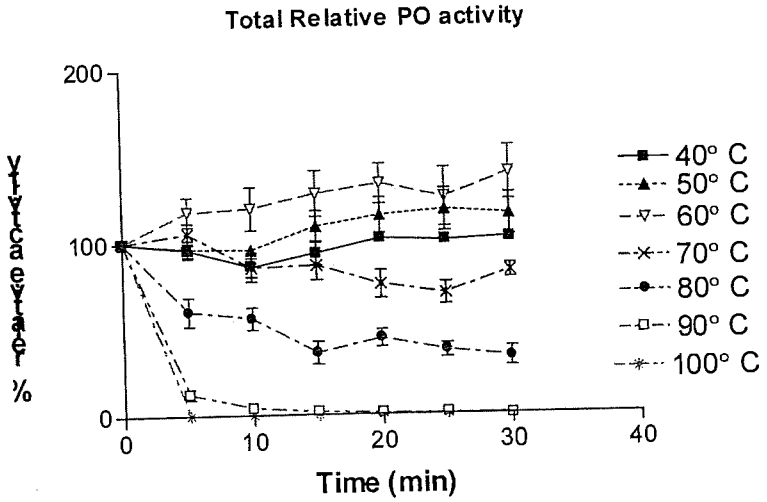

Figure 16: *Total relative PO activity over time at differing temperatures (mean±SEM)*

Under the conditions used, activation of the enzyme occurs between 60 and 80° C therefore prolonged exposure at this temperature is undesirable. Increasing the temperature beyond 80 °C results in a decreased time exposure required for deactivation of the enzyme. Similar results were obtained with unsteady state heating

- *Heat trials: Unsteady state*

Baseline activity followed a similar pattern to that shown in the steady state trials. Activity increased as the temperature approached 40 °C. This initial increase was followed by a sharp decease at 50 °C. No baseline activity was detected at 60 °C. However, significant increases in baseline activity occurred

as the temperature approached 70 °C. This may be attributed to heat activation of the enzyme and/or complete disruption of the hemocytes. The activity then decreased as temperature approached 90 °C and no baseline activity was detected in the samples by the time they reached 100 °C

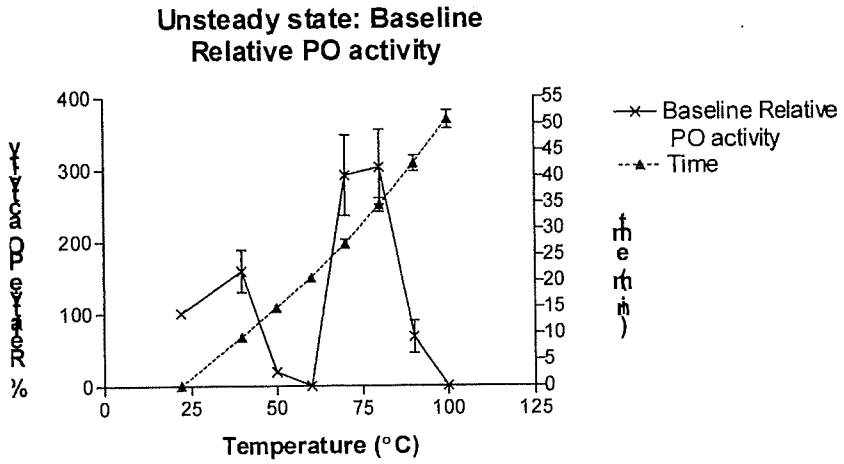

Figure 17: *Baseline relative PO activity under unsteady state heating conditions (mean±SEM)*

Total activity did not change significantly as the temperature approached 60 °C. Decreasing total activity was displayed as the temperature reached 80 °C. At 90 °C total activity was approaching zero and by the time the samples reached 100 °C no activity was detected.

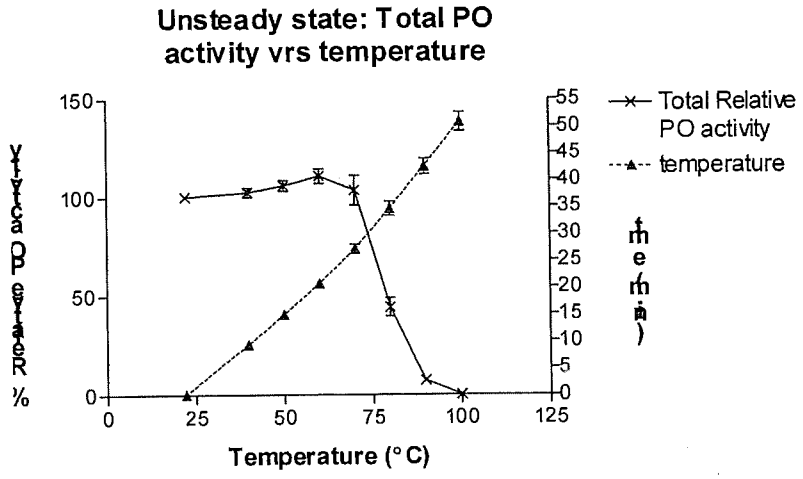

Figure 18: *Total relative PO activity under unsteady state heating conditions (mean±SEM)*

When the baseline values are compared to the total activity values at the same temperature it appears that at 70 °C, 50% of the total available enzyme has been converted to the active form and at 80 to 90 °C, 100% of the existing enzyme is in the active form. However, even at this point of maximum activation of the pro-enzyme, the level reached does not exceed 50% of the initial level of total enzyme activity. This leads to the conclusion that under actual cooking conditions the availability of

the active enzyme will be determined by a balance between temperature-induced activation of the pro-enzyme and temperature deactivation of the active enzyme form.

2. Thermal profile of cooking process

For each cook the change in temperature over time during cooking and cooling was plotted for the internal and external probes of each lobster. This gives a typical thermal curve as shown in Figure 7. At the beginning of the cooking period the temperature in the cooker is 100 °C. When the lobsters are put in the cooker the temperature drops between 3 and 5 degrees. There is a gradual increase until the external temperature for all positions in the cooker reached 100 °C again. A sharp drop in external temperature occurs when the baskets are removed from the cooker and plunged into the ice slurry. The internal temperature of the individual lobsters shows a gradual increase in the temperature throughout the cooking period and an equally gradual decrease in temperature during cooling. After 20 minutes of cooling, the average internal temperature of Grade A lobsters was 26 ± 13 °C and the average internal temperature of Grade B lobsters was 27 ± 10 °C.

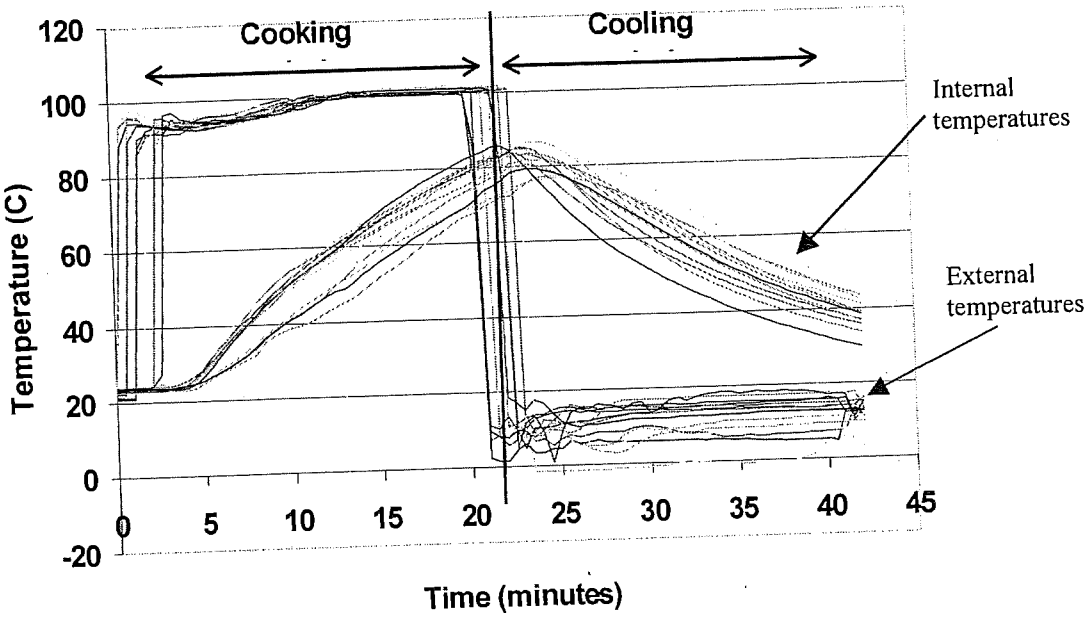

Figure 19: *Typical thermal profile of western rock lobster processing experiments*

The average temperature for each position during heating is shown in Figure 8. The greatest average difference between the two grades of lobster used occurred at position A, +4.2 °C (84.2±0.44 °C cf. 80.0±1.15 °C). The smallest difference occurred at position G, -0.15 °C (81.8±1.23 °C cf. 81.9 ± 0.78 °C).

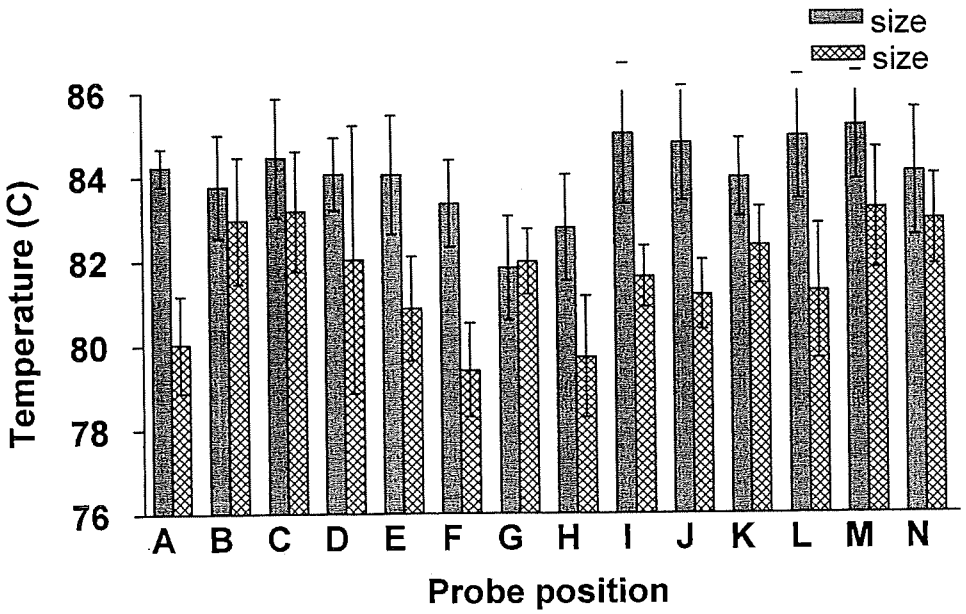

Figure 20: *Average maximum temperature reached during cooking (mean±SEM)*

The average temperature for each position during cooling is shown in Figure 9. The greatest average difference between the two grades of lobster used occurred at position C +3.7 °C (22.9 ± 1.7 °C cf. 26. 7 ± 2.39 °C). The smallest average difference occurred at position F, +0.5 °C (26.4. ± 2.21 °C cf. 26.7± 2.9 °C).

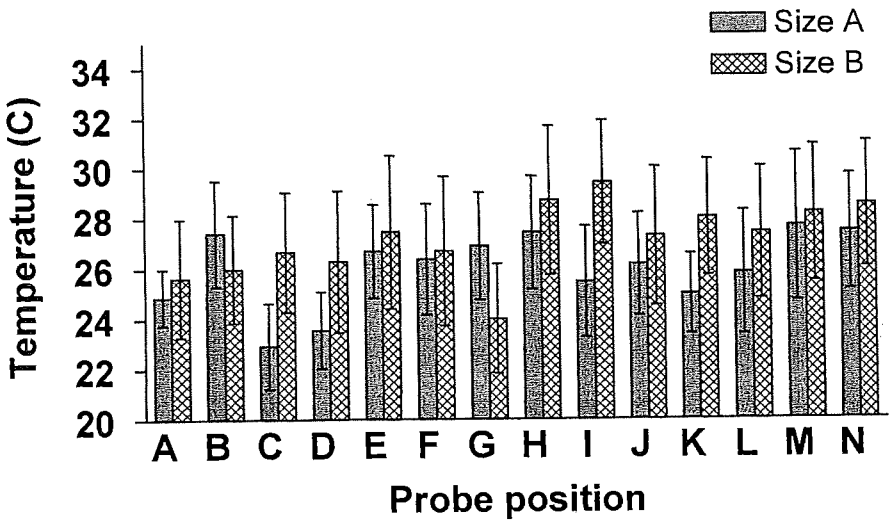

Figure 21: *Minimum temperature reached during cooling (mean±SEM)*

Whether the grade influences temperature exchange rates due to the weight-surface area ratio or due to the difference in packing density is unclear from these results. Further work is required with a larger size difference to elucidate this fully.

Examination of the cooking thermal profile shows that the maximum temperature reached did not exceed 84.0 ±1.22 °C for Grade A lobsters and 81.6± 1.3 °C for Grade B lobsters over all positions in the three dimensional array. Comparison of these values to the *in vitro* heating trials shows that they are insufficient to achieve significant deactivation of the enzyme. Each cook also spent 15.7 ± 0.77 minutes between 60 and 80 °C during cooking and cooling. Examination of the *in vitro* heating data shows that this is the zone were maximum activation of the enzyme occurs. From this it can be concluded that the heating profile used will result in high levels of melanosis occurrence after thawing of the lobsters. Further work to establish a modified profile that achieves a faster rate of heating and a higher end point must be undertaken. Examination of the utilization of alternative means of enzyme deactivation, such as using anti-browning agents, will also be valuable.

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Causes of tail fan necrosis in the southern rock lobster *(Jasus edwardsii)*

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Background

This project follows from Project 98-305 which investigated live holding of adult southern rock lobsters in sea pens and in raceways. One major outcome from that project was that live-held lobsters developed Tail fan infections. We have defined this condition as Tail fan Necrosis; a loss of tissue resulting from bacterial invasion of a physically damaged tail fam with progressive necrosis of the tail fan with associated melanisation of the wound. Tail Fan Necrosis(TFN) is a constrain on the advancement of the rock lobster live-holding industry..

This project aims to understand the effects of post-harvest handling , feeding frequency , density of holding and temperature on TFN. In particular a new post-harvest handling strategy of placing lobsters in mesh bags directly after capture and keeping them in those bags until they enter long term holding facilities will be trialled. This was termed bagging. Experiments were undertaken in the field and in the laboratory. An overview of the methods is given in the Fig below.

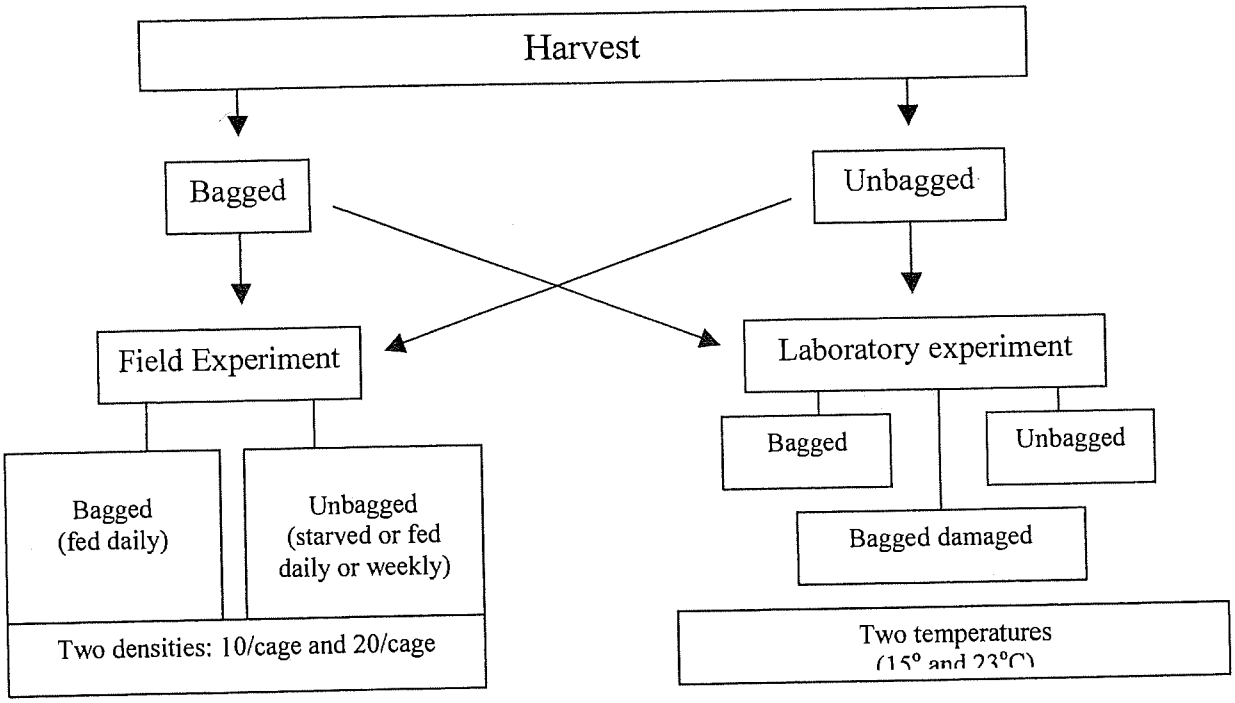

Figure 1. *Methods*

Field Studies

The field experiment was run in raceways at South Australian Seafoods aquaculture facility near Port Lincoln Lobsters were stocked on 27 November 2000 and harvested on 40 March 2001. Lobsters were stocked at two densities and under three feeding regimes; fed daily, fed weekly and unfed. The food was the rock lobster pellet developed in FRDC98-304 and 304. In addition one treatment involved lobsters that had been bagged at capture by lobster fishers. The lobsters were assessed at 0, 2 and 4 months for survival, moulting, condition and TFN. The results here relate just to TFN and especially compare the bagged and unbagged treatments.

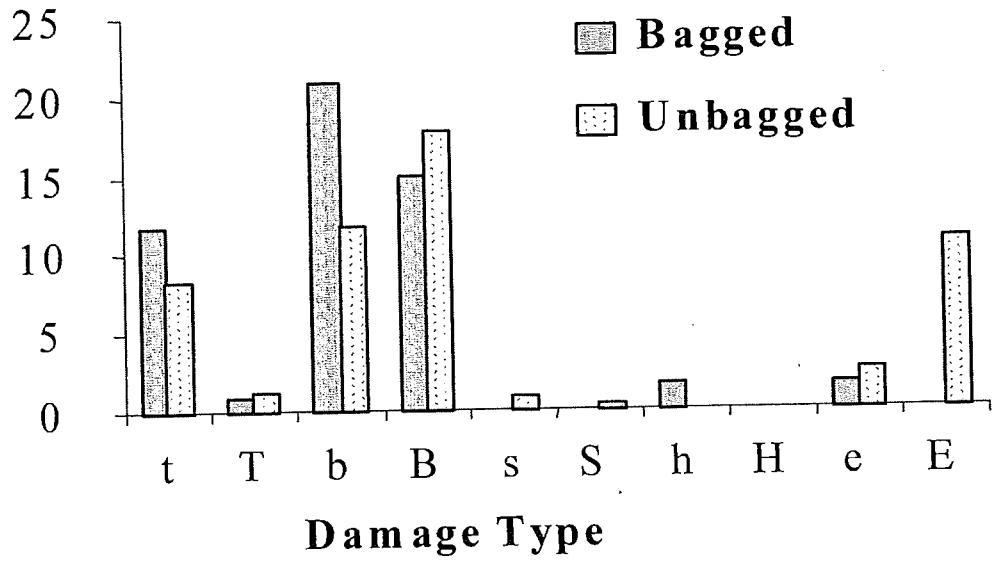

Figure 2. *Key Results: Field*

The lobsters were assessed for tail damage at time 0 with tears (t and large T) blisters, holes and erosion noted. The damage reflected the post harvest handling and holding damage. The Figure shows that bagged lobsters had low incidence of TFN, especially in the category of advanced erosion ,E After four months the bagged lobsters still showed substantially lower frequency of advanced erosion, greater than 25% of a tail limb eroded. The conclusions were that TFN increased significantly during the holding period and bagged treatments showed significantly less advanced erosion than unbagged treatments. The bacteria Vibrio and Aeromonas were identified in the TFN of these field held lobsters.

Laboratory studies

Laboratory studies were run at the SARDI Aquatic Sciences Laboratories in Adelaide. Lobsters were held individually in flow through tanks. Groups of 20 lobsters were collected from the fishery and either treated as normal post-harvest (unbagged) or placed in bags. The bagged lobsters were then

"damaged" with sterile implements by a whole punched in a tail limb or an incision. These unbagged, bagged and bagged/damaged lobsters were held at 15 or 23 degree C for 6 weeks. They were assessed for TFN each two weeks.

Again the initial results showed that bagged animals had less damage and tail erosion when entering the experiment than the unbagged lobsters. Analysis was based on the proportion of lobsters showing greater than 25% erosion of a tail limb. Temperature had an effect on the progression of TFN at the 2 week time, but at later times there were no significant differences. There was significantly less advanced erosion in the bagged lobsters. The lobsters damaged with sterile implements did not show TFN beyond that of the control lobsters. It seems that bacterial inoculation with a wound is necessary for TFN to proceed. The bacteria *Vibrio vulnificus*, *V. parahaemolyticus* and *V. alginolyticus* and *Aeromonas caviae* were isolated from the TFN in the laboratory.

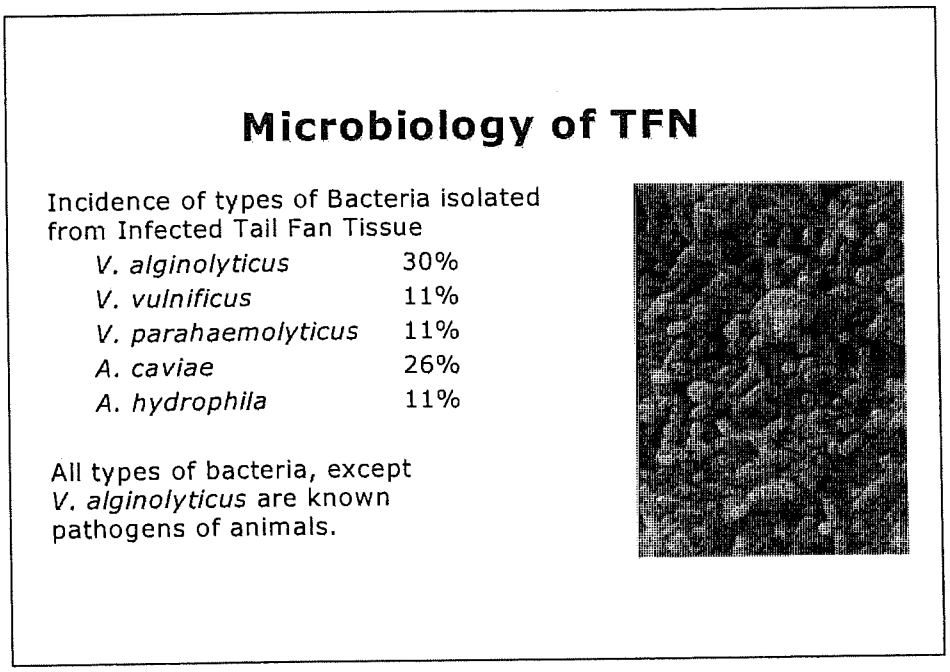

Figure 3. *Microbiology of TFN*

The major bacteria isolated from TFN infection are shown in Figure 3. To investigate whether these bacteria are the causal agents in TFN cultures of these bacteria were grown and lobster tail fan limbs were damaged by making a 2mm hole as in the previous experiment but with pure strains of the various bacteria on the implements used to inflict the damage. Sterile instruments were used as a control. The induction of TFN after innoculation of the various bacteria into the damage was assessed as the size of the hole around the damage, indicating that necrosis and loss of tissue had occurred. The outcome of these experiments is shown in Figure 4.

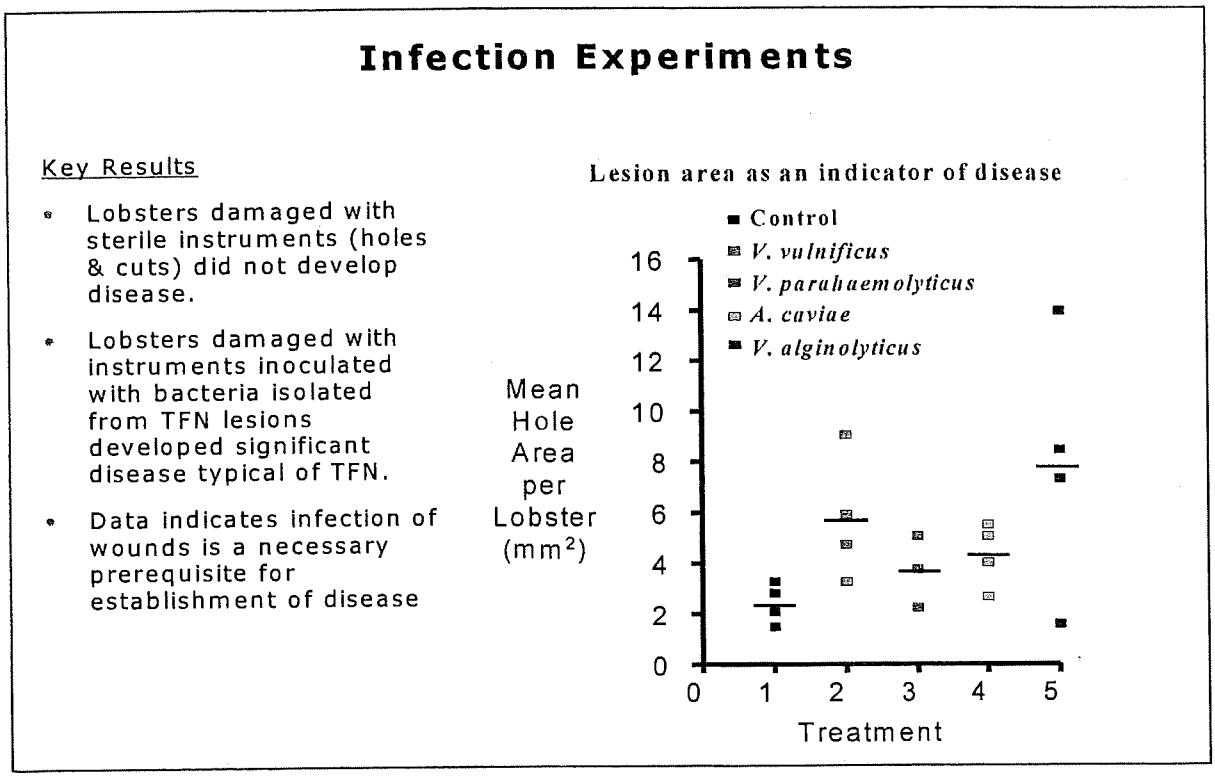

Figure 4. *Infection Experiments*

Each point is the result for a single lobster; the numbers are small with only four lobsters per treatment. The results show that the control lobsters showed little increase in the diameter of the 2mm hole, whereas all of the bacterial treatments showed necrosis and loss of tissue. Work is in progress to establish that the bacteria inoculated were later present in the TFN condition.

Conclusion

This study has shown that TFN is a condition that involves physical damage and bacterial inoculation. This wound is often inflicted on rock lobsters in the immediate post capture period. When lobsters are live-held at ambient temperatures the would progresses to TFN. TFN can be minimized by stopping the initial physical damage and bacterial inoculation, which presumably occurs when lobsters are handled post capture. Bagging offers a means on minimizing damage and limiting subsequent TFN. More study in needed on the methods of bagging, on the progression of TFN and on the microbiology and possible treatment of the condition.

Appendix I:

Workshop Agenda

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Agenda

Rock Lobster Post-Harvest Subprogram &

Rock Lobster Enhancement and Aquaculture Subprogram

4th Annual Workshop – Wednesday, 29th May, 2002

Session I. Chair: Dr Robert van Barneveld (Leader RLEAS)

9.00 Welcome and introduction: Mr Peter Dundas Smith (FRDC)
9.10 Official opening: Hon Henry Palaszczuk (Minister for Primary Industries)

Commercial perspectives of rock lobster aquaculture

9.30 Economics and marketing: Establishing models for rock lobster aquaculture: Mr Roger Edwards (SARLAC)
9.50 Commercial development of tropical rock lobster aquaculture systems: Mr James Fogarty (MG Kailis)
10.10 Commercial development of temperate rock lobster aquaculture systems: Mr Pheroze Jungalwalla (Tassal)
10.30 General Discussion
10.40 Morning tea

Session II: Chair: Mr Ian Finlay (WAFIC, RLEAS Steering Committee)

Enhancement

11.00 Assessing the possibilities of enhancing the natural settlement of western rock lobsters: Prof. Bruce Phillips (Curtin University of Technology, WA)
11.20 Health assurance for southern rock lobsters: Dr Judith Handlinger (TAFI)
11.40 Evaluating the release and survival of juvenile lobsters released for enhancement purposes: Megan Oliver (NIWA) & Dr Caleb Gardner (TAFI)

Propagation

11.55 Propagation of rock lobsters:

- Overview: Dr Bradley Crear (TAFI)
- Propagation research in New Zealand: Dr Michael Bruce (NIWA)
- Feeding and digestive capabilities of phyllosoma: Dr Danielle Johnston (TAFI)
- System design/larval quality: Dr Arthur Ritar (TAFI)

12.45 Molecular biology of lobster rearing: Dr Mike Hall (AIMS)
1.05 Technical Feasibility of rock lobster propagation – Review of current research: Mr Rodney Grove-Jones
1.25 General Discussion
1.30 Lunch

Session III: Chair: Prof Bruce Phillips (Leader RLPHS)

Post-harvest handling of lobsters

2.15 Physiological studies on stress and morbidity during post-harvest handling of western rock lobsters: Prof. Louis Evans (Curtin University of Technology, WA)
2.35 Physiological stress indicators: Dr Brian Paterson (Queensland Department of Primary Industries)
2.55 Optimising water quality for live holding of rock lobsters: Dr Bradley Crear (TAFI)
3.15 Afternoon tea

Optimising post-harvest product quality for aquaculture and market

3.30 Alleviating leg loss in western rock lobster: Dr Glen Davidson (Geraldton Fishermen's Cooperative)
3.45 Effects of hyper- and hyposaline seawater on leg loss: Wayne Hosking (Geraldton Fishermen's Cooperative)
4.00 Striking a balance between melanosis and weight recoveries in Western Rock Lobster: Dr Hannah Williams (Curtin University of Technology)
4.30 Tail fan necrosis in rock lobsters: Pathology, causes and minimisation strategies: Assoc. Prof Mike Geddes (Adelaide University)
4.50 General Discussion
5.00 Tours of QDPI research facilities (led by Dr Clive Jones, QDPI)

Appendix 4

Newsletter 1
Newsletter 2
Newsletter 3
Newsletter 4
Newsletter 5

ROCK LOBSTER POST-HARVEST SUBPROGRAM FRDC

THE LOBSTER NEWS

Volume 1, Issue 1 February 2001

From the Subprogram Leader

Welcome to the first issue of our bi-annual newsletter. We hope you find it informative and useful for keeping up to date with current projects and innovations in Rock Lobster Research.

The format may change over the next issue so please feel free to put forward any suggestions.

Just a reminder that if you have any photographs to illustrate projects or other data that you feel would be beneficial, please submit them to the editor - details on page 8.

I look forward to meeting up with or making contact with many of you in the coming months.

Bruce Phillips
Sub Program Leader.

inside this issue:

Establishment of the Rock Lobster Post-Harvest Subprogram

This Subprogram was established in 1996 to support the live section of the rock lobster industry. Its aim was to increase the percentage of lobsters delivered to the factory in a condition suitable for live export, and to examine if better methods could be identified for industry to use in identifying the lobsters selected for overseas shipment.

The Subprogram was independently reviewed in 1999. The review recommended the continuance of the Subprogram, with a requirement for industry to play a more dominant role in determining what, if any, research is carried out in the post-harvest sector.

A new Steering Committee for the Subprogram has been established, and it has adopted the following provisional Strategic Plan:

MISSION:

To conduct research to increase the value of the rock lobster catch for Australia through improvements in post-harvest practices.

PRIORITIES:

- Reduce appendage loss
- Improve long-term holding information
- Upgrade and expand Code of Practice
- Condition enhancement
- International transport
- Improve processing practices
- Condition indexes
- Information transfer

This is a living document and will be revised annually. During 2001 visits will be made to South Australia, Victoria, Tasmania and Queensland for discussion with industry of their needs in the post-harvest area, and for discussions with researchers in these States of research, which may provide the solutions.

Lobsters being stunned during sea trials on-board the "Windjana"

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Steering Committee Members

For further clarity on the priorities or any other information please contact one of the following:

MEMBER	TELEPHONE	FAX	EMAIL
Bruce Phillips Subprogram Leader	08 9266 7963 0417 189 956	08 9266 2495	rphillip01@alpha7.curtin.edu.au
Nick Polgeest	03 5237 6511	03 5237 6511	
Glenn O'Brien	08 9921 1022 0418 939 208	08 9921 8019	glenno@brolos.com.au
Kym Redman	08 8735 4241 0418 839 734	08 8735 4228	kredman@dove.net.au
Rodney Treloggen	03 6376 1796 0418 138 768	03 6376 1805	treloggen@bigpond.com.au
Stephen Hood	08 9239 9200 0418 901 048	08 9239 9222	stephenhood@kailis.com.au
Robert van Barneveld	08 8524 6477 0418 802 462	08 8524 6577	robvanb@dove.net.au
Richard Stevens	08 9244 2933 0419 195 510	08 9244 2934	r&d@wafic.org.au
Patrick Hone	02 6285 0412 0419 628 400	02 6285 4421	honep@frdc.com.au

Current Projects The following are the current projects within the Subprogram

94/134.02:
Code of Practice

Principal Investigator: Mr Richard Stevens
Abercromby Management Services P/L
21 Eckford Way
Duncraig W.A. 6023

PROJECT OBJECTIVES:
To produce a Code of Practice for the handling of rock lobster.

96/344:
Physiological studies of stress and morbidity during post-harvest handling and storage of Western Rock Lobster: 11. Standard autopsy techniques and immune system competency

Principal Investigator: Dr Louis Evans
Curtin University of Technology
Aquatic Science Research Unit
GPO Box U1987
Perth W.A. 6845

PROJECT OBJECTIVES:

1. To identify suitable immune system parameters which can be used to evaluate stress responses and health status in captive lobsters and to apply those parameters in a study of stress induced by post-harvest handling procedures.
2. To investigate the causes of mortality in captive lobsters held in processing factories. This study will focus on bacteriological and histopathological examinations and will result in the development of a standard protocol for the autopsy of lobsters.
3. To evaluate the influence of temperature change on immunological and physiological stress responses.
4. To study the influence of hormonal secretions on immunological and physiological stress responses.
5. To investigate innovative techniques which will boost immunocompetence but not adversely affect marketability of live product.

96/345:
Physiological studies of stress and morbidity during post-harvest handling and storage of Western Rock Lobster: 1 Physiological Stress Indicators

Principal Investigator: Dr Brian Patterson

PROJECT OBJECTIVES:

1. Identify key physiological stress parameters that either describe stress levels and/or predict likely further mortality in lobsters after harvest and apply these parameters in studies aimed at improving post-harvest handling practices.
2. Obtaining baseline measurements of physiological parameters in resting undisturbed lobsters, with reference to interactions between season and locality and the effects of moult stage and other biological variables.
3. Identifying physiological parameters, through field studies aimed at studying the effect of harvest and post-harvest handling on lobsters, which can be used to evaluate deviations from baseline values in captive lobsters.
4. Identifying physiological parameters through controlled laboratory experiments using identified stressors which can be used to evaluate deviation from baseline values in captive lobsters.
5. Develop simple methods of measuring one of the stress parameters identified in objectives 3 and 4 for use in lobster processing factories in the evaluation of stress levels in selected lobster shipments.
6. Apply the results and understanding of harvest and post-harvest handling gained from the field work in objective 3, and the stress parameters identified in objectives 3 and 4, in a study or studies of lobster post-harvest handling practices aimed at developing improved post-harvest procedures.
7. Use the findings of earlier sub-objectives to make recommendations for improvements in handling practices described in the recently published Code of Practice.
8. Use the findings to develop detailed knowledge and understanding of the physiological processes involved in the stress responses in lobsters which can be used by processing companies and fishers to devise improved methods of post-harvest handling and transport.

99/202:

Rock Lobster autopsy manual

Principal Investigator: Dr Louis Evans
Curtin University of Technology
Aquatic Science Research Unit
GPO Box U1987
Perth W.A. 6845

PROJECT OBJECTIVES:

The publication of an autopsy manual to be used in the lobster industry.

362/250:

Facilitation, administration and promotion of the post-harvest Subprogram

Principal Investigator: Dr Bruce Phillips
Curtin University of Technology
Muresk Institute of Agriculture
GPO Box U1987
Perth W.A. 6845

PROJECT OBJECTIVES:

1. Coordinate the FRDC rock lobster post-harvest Subprogram.
2. Conduct an annual research workshop to present outcomes from the Subprogram and to define research objectives for subsequent years.
3. Facilitate travel of the Subprogram project principal investigators, industry representatives and Subprogram leader to biannual scientific committee meetings.
4. Facilitate travel of the industry representatives, Subprogram leader of the Enhancement and Aquaculture Subprogram, and Subprogram leader to biannual Steering Committee meetings.
5. Coordinate the preparation of Subprogram media releases and workshop publications.
6. Integrate with other FRDC funded rock lobster research programs including the Enhancement and Aquaculture Subprogram.
7. Coordinate the preparation and distribution of a biannual Subprogram newsletter.
8. Develop and maintain a strategic plan for post-harvest rock lobster research.
9. Develop a strategic plan for the Subprogram.

362/251:

Development of a method for alleviating leg loss during post-harvest handling of Rock Lobsters

Principal Investigator: Dr Glen Davidson
Department of Zoology
University of Western Australia
Stirling Highway
Nedlands W.A. 6907

PROJECT OBJECTIVES:

1. To identify a cold-water immersion treatment that rapidly immobilises western rock lobsters, while allowing swift recovery from immobilisation upon return to ambient temperature seawater. To investigate the effect of season/acclimation temperature on effectiveness of cold stunning in western rock lobsters. To investigate the use of sea sprays vs immersion for cold stunning in western rock lobsters.

2. To investigate, in captivity, the effectiveness of the preferred treatment (identified in objective 1) for reducing leg loss in western rock lobsters.
3. To test the accuracy of factory grading of cold stunned western rock lobsters vs untreated controls.
4. To describe the occurrence of leg loss, morbidity and mortality of western rock lobsters subjected to cold stunning prior to episodes of handling during the post-harvest process (i.e. at the time of pot-pulling and sorting, prior to factory grading) and to compare these to the performance of animals handled using current methods.
5. To investigate the effects of multiple simulated pot capture and release events, either with or without cold stunning, on growth, leg loss and survival of undersized western rock lobsters.
6. To compare, in captivity, effects of handling, with and without cold stunning, on the reproductive success of setose, tar spot and ovigerous female western rock lobsters. To investigate the effects of limb loss on the reproductive success of female western rock lobsters.
7. To conduct a survey to determine the extent and nature of leg loss in the southern rock lobster fisheries of Tasmania and South Australia.

362/252:
Optimising water quality in Rock Lobster post-harvest

Principal Investigator: Dr Brad Crear
University of Tasmania
Tasmanian Aquaculture and Fisheries Institute
Marine Research Laboratories
Nubeena Crescent, Taroona
Tasmania, 7053

PROJECT OBJECTIVES:

1. Production of a manual on optimising the provision of oxygen during rock lobster post-harvest processes.
2. Determine the median lethal concentration (LC-50) of ammonia to adult southern and western rock lobsters (stressed and unstressed).
3. Determine the physiological consequences of exposing lobsters to sub-lethal ammonia concentrations, and the consequences of further exposing lobsters to acute post-harvest stressors.
4. Production of a manual on ammonia problems during rock lobster post-harvest processes.

Proposed new projects for funding in current round

Striking a balance between melanosis and weight recoveries in Western Rock Lobster (*Panulirus cygnus*)

Principal Investigator: Dr Hannah Williams
Curtin University of Technology
School of Public Health
GPO Box U1987
Perth W.A. 6845

PROJECT OBJECTIVES:

1. To establish the impact of temperature and food additives on the activity of *Panulirus cygnus* haemolymph phenol oxidase (PO) in vitro.
2. To establish the impact of current commercial practices on weight recovery and melanosis formation.
3. To establish the impact of post-harvest transportation on PO activity, weight recovery and melanosis formation.
4. To determine the effects of anti-browning agents on weight recovery and melanosis formation.
5. To validate the use of experimentally determined cooking profiles for improvement of cooked weight recoveries and prevention of melanosis.
6. To formulate recommendations and guidelines that will enable industry to apply the findings of the study.

Code of Practice

Principal Investigator: Mr Richard Stevens
Abercromby Management Services P/L
21 Eckford Way
Duncraig W.A. 6023

PROJECT OBJECTIVES:

To produce a Code of Practice for the handling of rock lobster.

363/251:

Rock Lobster post-harvest Subprogram: Development of a method for alleviating leg loss during post-harvest handling of Rock Lobsters

Principal Investigator: Dr Glen Davidson
Co-investigator: Wayne Hosking

PROGRESS SINCE LAST MILESTONE:

- The time/temperature matrix, designed to identify preferred stunning treatments for use prior to on board sorting and factory grading (objective 1), has been completed for A grade (~0.45 kg) lobsters acclimated to ambient sea temperatures of 18˚ and 22˚C. The latter encompassed the period of the whites in November 2000.
- A cursory look at the results suggests that the sensitivity of lobsters to stunning did not vary over this ambient temperature range.
- The matrix is due to be repeated in February 2001 using animals acclimated to highest annual water temperature (~24-26˚C).
- A prototype cold water stunning cacka box has been installed on the boat, Windjana, operating out of Port Denison. The sea trials have begun and the initial results are very promising. The trials started with the cacka box set at 0˚C. This proved to be very effective, significantly reducing onboard leg loss from 0.130 ± 0.020 legs/lobsters in control animals to 0.033 ± 0.009 legs/lobster in the treated animals ($P < 0.01$). Note that onboard leg loss rates are averaged over the entire catch, including retained and returned lobsters (i.e cackas, setose etc) and that a sizeable proportion of the total leg loss has been observed to occur when protected animals from the control group are thrown overboard.
- Each day the catch from the experimental boat was intercepted upon arrival at the factory. The numbers of legs in the baskets were counted along with the occurrence of old and new wounds. These observations revealed that the difference in leg loss between treated and control retained animals upon arrival at the factory was usually greater than the difference recorded by the onboard technician for the entire catch (i.e including protected animals). Upon receipt, differences of approximately 0.5 legs/lobster have been commonly recorded. All the experimental animals were transported from Dongara to the factory by truck showing that the benefits of onboard cold-stunning were not negated by intermediate handling and transportation steps.
- The results from the sea trials to date are all the more significant given that the prevailing weather conditions have been unseasonally mild, with temperatures in the low to mid 20s, relatively light winds and high humidities. Such mild conditions are typically associated with low levels of leg loss. Despite this, we have seen significantly lower rates of leg loss in cold-stunned lobsters compared to unstunned controls. We expect that, as we encounter more adverse weather (higher temperatures etc), the difference in leg loss between the controls and treated lobsters will be greater. In addition, atmospheric conditions recorded on the boat were often quite different to those recorded on land. This validates the approach we have taken of recording atmospheric conditions on board.
- After operating for several days at 0˚C, we have been progressively increasing the box temperature in order to identify the highest effective stun temperature. The highest effective temperature should alleviate leg loss whilst allowing for the shortest possible recovery times in returned protected animals. Thus far 5, 7.5 and 10˚C appear to be equally effective for reducing leg loss.
- Tenders for the survey work to be conducted in Tasmania and South Australia have negotiated with Stewart Frusher and Roger Edwards, respectively. Sub-contract agreements have been sent to TAFI and SARLAC. I have also been granted access to the catch assessment records from Southern Ocean Rock Lobster Ltd in Port Lincoln and I have Tasmanian Sea Life collecting data on the incidence of leg loss in lobsters being delivered to their factory.

PROJECT 2000/252:

Optimising water quality in Rock Lobster post-harvest processes

Principal Investigator: Dr Bradley Crear

Organisation: University of Tasmania
Tasmanian Aquaculture and Fisheries Institute,
Marine Research Laboratories
Nubeena Crescent, Taroona,
Tasmania, 7053

Collaborators: Dr Mark Powell
University of Tasmania
Tasmanian Aquaculture and Fisheries Institute,
School of Aquaculture, Launceston, Tasmania

SCOPE OF THIS REPORT

This Report outlines the research plan for the 2 year project and details the work that has been done since the Project commenced in July 2000.

PROGRESS

Staff

Mr Grant Allen was appointed as the Technical Officer to support the research at the TAFI Marine Research Laboratories in Hobart.

RESEARCH

The technician (Mr Grant Allen) was appointed to the project. Due to delays in receiving final contracts from FRDC his appointment was delayed and he did not commence work until October, 2000. The system for the experiments has been completed. The pamphlet on oxygen consumption is in the final stages of writing and a draft will be sent out by the end of January, 2001.

Progress of Research

Preliminary trial to evaluate the LC-50 (96 h) of ammonia to the Southern Rock Lobster, *Jasus edwardsii*

Little is known of the toxicity of ammonia to the southern rock lobster, *Jasus edwardsii*. The aim of this trial was to determine a range of ammonia concentrations to be trialed in a more detailed study.

MATERIALS AND METHODS

Based on the results of studies with other crustaceans, concentrations of 100, 200, 300, 400 and 500 mg/l of ammonium (NH_4^+) were used for the trial. This relates to 78, 155, 233, 310 and 388 mg/l total ammonia nitrogen (NH_3-N). A control treatment of lobsters held in the same system, but in flowing seawater was also included. Test solutions were made up using reagent grade ammonium chloride (Sigma). Each test solution was renewed daily, in accordance with the static renewal method for toxicity tests.

The experimental system consisted of eighteen 80l tanks which were filled with 70l of the test solution. All treatments were run in triplicate with eight lobsters in each tank. Lobsters (within the size range 150g to 300g) were removed at random from a main holding tank, drained to remove residual water, individually weighed and added to each of the treatment tanks. The lobsters were starved and rested in flowing seawater for 36 hours prior to the trial taking place; faeces was removed from the tanks. The water was maintained at ambient temperature (16°C) during the course of the experiment.

At the completion of the trial, a 1ml haemolymph sample was taken from each of three lobsters per replicate (9 per treatment in total) and placed into an Eppendorf tube. The pH and protein of each sample was measured and then 250µl of each sample was added to Eppendorf tubes containing 500 µL of 1M perchloric acid (PCA), to precipitate protein. Both samples were then frozen at -18°C for later analysis of blood ions, ammonia, lactate and glucose.

The lobsters were recovered in the tanks for a minimum of 30 minutes before their standard oxygen consumption was determined. Aeration was removed from the tanks and the lobsters were allowed to deplete the oxygen in the tanks over a 2 hour period. Microbial oxygen consumption was accounted for by taking a 1l subsample of the test water and measuring oxygen depletion over the same 2 hour period (without lobsters). The water was re-oxygenated and then the active oxygen consumption was measured; lobsters were induced to be active (by prodding with a stick) over a 25 min measuring period. After 30 min of recovery the lobsters were moved to tanks containing 20l of aerated fresh seawater (0 mg/l NH_3-N) to measure the ammonia excretion rate. The excretion rate was measured over 2 hours. Ammonia was analysed by the phenol-hypochoride method of Solarzano (1969).

RESULTS AND DISCUSSION

The animals stocked at 310 and 388 mg/l NH_3-N were clearly in poor condition after 1 hour in the treatments. Therefore, those treatments were terminated and the lobsters were removed from the tanks. All lobsters in the 233 mg/l NH_3-N treatment had died by hour 48. Only one lobster survived 96 hours in the 155 mg/l NH_3-N treatment, whilst only two lobsters died in the 78 mg/l NH_3-N treatment. No mortalities were recorded in the 0 mg/l treatment. The 96 hour mortality data is shown in Fig. 1. Using a Probit analysis, the calculated 96 hour LC-50 was112 mg/l NH_3-N with 95 % confidence limits of (lower) 98 mg/l NH_3-N and (upper) 128 mg/l NH_3-N.

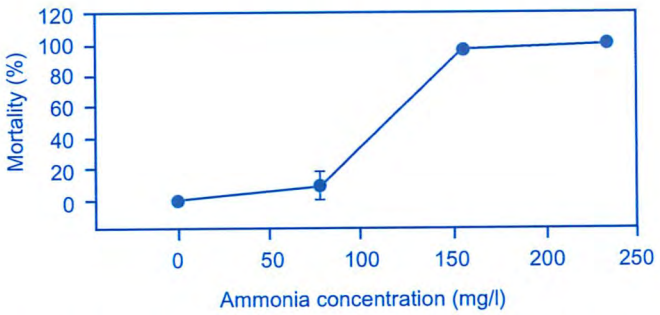

Fig. 1: The 96 hour mortality data for J. edwardsii held in a range of ammonia (NH_3-N) concentrations (mg/l).

At the end of the trial there were only sufficient animals in the 0 and 78 mg/l NH_3-N treatments to be able to measure oxygen consumption and ammonia excretion rates. The lobsters in the 78 mg/l NH_3-N treatment appeared to have a decreased ability to fund their metabolic processes when under stress. They had higher standard rates of oxygen consumption and lower active rates of oxygen consumption than those held at 0 mg/l NH_3-N (Fig. 2).

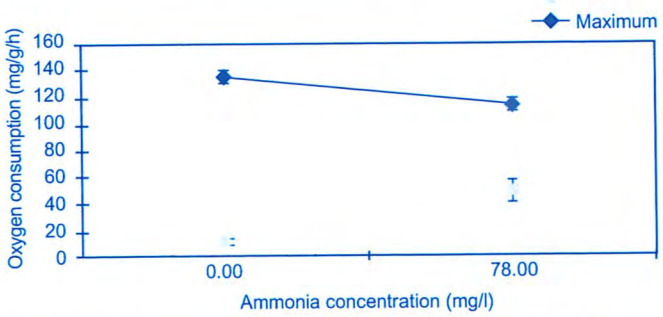

Fig. 2: Oxygen consumption (standard and active - mg/g/h) rates of lobsters after 96 hours in 0 or 78 mg/l ammonia (NH_3-N).

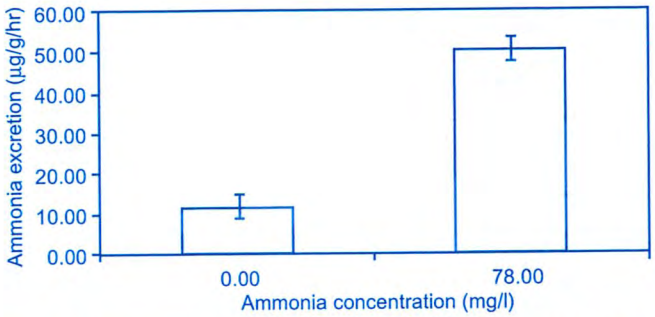

Fig. 3: Ammonia excretion (mg/g/h) rates of lobsters after 96 hours in 0 or 78 mg/l ammonia (NH_3-N).

When placed into fresh (0 mg/l NH_3-N) seawater, the ammonia excretion rate of lobsters in the 78 mg/l NH_3-N treatment was much higher than those in the 0 mg/l NH_3-N treatment (Fig. 3). This suggests that the lobsters in the 78 mg/l NH_3-N treatment had excess ammonia stored in their body due to an inability to excrete it across the concentration gradient when they were in a high external concentration of ammonia. Analysis of the blood ammonia in the two treatments showed that the level in the lobsters that were held in 78 mg/l ammonia was approximately three times higher than in the lobsters held at 0 mg/l.

It was apparent that lobsters, which had just moulted or were in the process of moulting, were more susceptible to increased ammonia concentrations. Therefore, in future studies lobsters will be moult staged to ensure that only intermoult lobsters are used in the experiments. Also lobsters in the treatments containing elevated levels of ammonia were observed to be aggressive and dying or dead animals were frequently cannibalised.

CONCLUSIONS

The results of this study show that *J. edwardsii* appear to be able to handle high levels of ammonia, with a preliminary 96 hour LC-50 of 112 mg/l NH_3-N being calculated. The results of this study will form the basis for determining the range of ammonia concentrations to be used in a 96 hour LC-50 experiment to be run early in 2001.

WATER QUALITY ANALYSES AT A HOLDING CENTRE FOR SOUTHERN ROCK LOBSTERS

The *J. edwardsii* fishing season commenced in Tasmania on the 12th November. Water quality in a rock lobster holding system in Hobart was monitored from the time of initial stocking. It was a recirculating system, with three separate tanks and biofilters. The tanks had water volumes of 50 m^3 (Tank A), 42 m^3 (Tank B) and 21 m^3 (Tank C). They were designed to hold 100 kg of lobsters/m^3, however the highest stocking density over the measurement period was only 60 kg/m^3 in Tank A. The system had only been recently constructed. Therefore, there was no acclimation period to allow the biofilter to start working properly prior to the introduction of large quantities of lobsters. The water quality became very poor in terms of ammonia loading during the measuring period (Table 1). The highest level of ammonia measured was 53 mg/l. Other water quality parameters were well within the levels generally regarded as optimal.

It would appear from the nitrite levels that the biofilters were not operating by the 23rd November, but had started working by the 1st December (no testing for nitrates or nitrites was done between 23/11/00 and 01/12/00). There was a high mortality of lobsters during the initial weeks of stocking. The results are being used in helping to design the ammonia toxicity trials.

Table 1: Water quality parameters and stocking details for a J. edwardsii holding facility in Hobart, Tasmania

DATE	TANK	STOCK (kg)	PH	SALINITY (‰)	TEMP (C)	OXYGEN (mg/l)	OXYGEN %	NH_3-N mg/l	NITRITE ppm	NITRATE ppm
15/11/00	A	0	-	-	15.5	-	?	0.45	-	-
15/11/00	A	431	-	-	13.3	-	-	0.9	-	-
16/11/00	A	1396	-	-	10.5	-	-	2.7	-	-
17/11/00	A	2308	-	-	10.2	-	-	5.16	-	-
19/11/00	No sample	3000	-	-	-	-	-	-	-	-
20/11/00	A	3000	7.72	36.5	9.4	9.12	100	5.76	-	-
23/11/00	A	200	-	-	-	-	-	47	< 1	40-80
28/11/00	A	200	8.03	36	11.8	6.99	80.6	30.3	-	-
01/12/00	A	200	8.05	36	12.3	-	93	43	10	40-80
06/12/00	A	20	-	-	-	-	-	7	10	40-80
08/12/00	A	150	7.99	36.5	12.5	-	94	6	7.5	40
18/11/00	C	1500	-	-	11.9	-	-	0.96	-	-
20/11/00	C	2000	7.81	36	11.5	8.74	100	6.48	-	-
23/11/00	C	0	-	-	-	-	-	36	< 1	40-80
28/11/00	C	0	7.89	36.5	11.5	7.89	90.4	29.6	-	-
01/12/00	C	0	8.1	36	12.1	-	95	47	10	40
06/12/00	C	0	-	-	-	-	-	48	7.5	40
08/12/00	C	0	7.95	36	13.0		93	53	7.5	40-80

Lobsters being stunned during sea trials on-board the "Windjana"

Photographs courtsey of Dr Glen Davidson to accompany the Research News 363/252

Lindsay McDonald (left), research assistant at the Geraldton Fishermen's Cooperative and Dr Glen Davidson of the University of Western Australia) conducting factory trials

MAILING LIST

This Newsletter is distributed widely. However, if you would like a personal copy mailed to you please contact the administrator Emma Phillips on 0417 980 801 or by email at emmaphil@ozemail.com.au.

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

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ROCK LOBSTER POST-HARVEST SUBPROGRAM FRDC

THE LOBSTER NEWS

Volume 1, Issue 2 May 2001

From the Subprogram Leader

During February 2001 visits were made by myself, accompanied by Richard Stevens, to South Australia, Victoria, Tasmania and Queensland.

In each state we presented the new priorities of the Subprogram to industry and discussed with them their needs in the post-harvest area.

These discussions have already led to additional dialogue with regard to suitable projects, methods of approach, and degree of industry involvement and support in projects.

In April I attended the Steering Committee Meeting of the Enhancement and Aquaculture Subprogram in Wellington in New Zealand. Wayne Hosking presented a report on the alleviation of leg loss project to the joint meeting with New Zealand's Fishing Industry and Scientists, titled "A sharing of Knowledge; Australasian Rock Lobster Research".

Bruce Phillips
Sub Program Leader.

Wayne Hosking presenting details of the alleviation of leg loss project to the meeting in New Zealand titled "A sharing of Knowledge; Australasian Rock Lobster Research", in April 2001.

inside this issue:

To conduct research to increase the value of the rock lobster catch for Australia through improvements in post-harvest practices.

Recently Completed Projects

94/134.02:

Code of Practice

Principal Investigator: Mr Richard Stevens
Abercromby Management Services P/L
21 Eckford Way
Duncraig, W.A. 6023

PROJECT OBJECTIVES:

To produce a Code of Practice for the handling of rock lobster.

The project achieved the following objectives. The industry was surveyed by questionnaires tailored to both fishermen and processors, and by a large number of individual conversations with industry participants. The code was produced in draft form and distributed widely for comment prior to publication in a loose-leaf format on tear-proof and waterproof paper. Over forty-five hours of videotapes were shot in Western Australia, South Australia, Victoria and Tasmania, and condensed into a fifteen-minute video. This too was disseminated widely for comment prior to its publication.

Copies of the code of practice were sent to each rock lobster licence holder in each of the above states under the auspices of their respective state peak industry bodies. Over one thousand six hundred copies were so distributed, with a further two hundred copies being sent to interested people in New South Wales, Queensland, the Northern Territory and New Zealand. The code became a text for lectures in seafood science at Curtin University, and the University of Hull in the UK. Additionally, the peak industry bodies in each of the four participating States, and elsewhere sold three hundred and sixty copies of the video.

The video not only services the commercial fishing industry but also the other major areas of commercial vessel operation such as charter vessels and recreational fishing. The production of the video provided a training resource that was previously not available to vessel owners and operators and allowed the video to be used at times convenient to them.

Delays to the completion of companion projects prevented the update of the Code within the life of the project. The results of those projects were to be published independently as industry pamphlets.

(Continued overleaf)

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

An independent study of the project, within the FRDC rock lobster post-harvest Subprogram, noted that:

> " The widespread use of the code of practice for handling live rock lobsters, combined with active on-board extension by processors, has reportedly led to improvements in post harvest handling practices and, consequently, in the proportion of 'fit for live' lobsters landed." (pp5) It continued "...only the Code of Practice...is considered by industry to be of value" (pp10). (McKoy & Sven 1999).

The video was reviewed by Dr Zoran Gacik, Instructional Media Producer at The Centre for Educational Advancement of the Curtin University of Technology. His comments were:

> "Technically, the video is well produced with a high standard of audio and video, as well as shots and visual structure. This may seem an obvious point, but in a video such as this the learner should be concentrating on the content and not be distracted by the technical aspects of production regardless of whether they are 'good' ("Wow, look at those effects"/Ha, Ha, that joke was really funny") or 'bad' ("I can't really hear what they're saying"). It is for these reasons, for example, that streaming media over the Internet is not an instructionally sound use of technology. Instructionally, the video has emphasised the 'motivational' aspects of learning quite well. For example, after viewing the video I was in no doubt that handling lobsters correctly was important - although the emphasis on prices may be more 'motivating' to a skipper than to a deckhand."

This is not the case as most deckhands are paid on a share of the catch, so the landed value directly affects their income (Z. Gazik, pers. Com.).

A revised edition of the Code of Practice is planned.

The standard of handling rock lobster in all of the four States in which the Code of Practice was distributed has risen significantly since. This improvement would possibly have occurred naturally over time, the major achievement of the Code of Practice is that it accelerated that improvement, and cemented good handling practice in the lobster fishing industry.

96/344:

Physiological studies on stress and morbidity during post-harvest handling and storage of western rock lobster Panulirus Cygnus: 11. Standard autopsy techniques and immune system competency

Principal Investigator: Assoc Prof Louis H. Evans
Curtin University of Technology
Aquatic Science Research Unit
GPO Box U1987
Perth W.A. 6845

This project has been completed, but due to illness the final report has not yet been submitted. However, to provide information as soon as possible, the following non-technical summary has been released.

1. PROJECT AIMS AND OBJECTIVES

The main aim of this project was to evaluate the application of immune system indicators in the assessment of the stress or health status of post-harvest lobsters. In this context, the term 'health status' refers to the likelihood of the lobster dying within one or two weeks. The immune system indicators investigated included total hemocyte (blood cell) counts, differential hemocyte counts (measures of the different types of blood cells), clotting time (the rate at which the hemolymph clots), antibacterial factor (antibacterial activity in lobster hemolymph), phagocytic capacity (the ability of hemocytes to sequester and destroy bacteria and other foreign agents), bacterial colony count (the number of live bacteria in the hemolymph) and quantitative histopathological measurements (quantification of occurrence of specific types of histological features seen in fixed sections of lobster tissues).

At the commencement of the research it was envisaged that the main outcome of the project would be the availability of a suite of simple stress tests which could be used by fishers or processors to measure stress and/or health status in a batch of lobsters. It was seen that this capability would be of value to fishers and processors in the improvement of post-harvest handling procedures and in maximising the proportion of the catch that are 'fit for live'.

The project was conducted in collaboration with another group of researchers who investigated physiological indicators of lobster stress status. At the commencement of the project it was anticipated that the final stress or health status indices would combine both immune and physiological indicators.

A secondary aim was to determine common causes of mortality of lobsters in live holding tanks. This aim was achieved by conducting autopsy investigations on lobsters from live holding tanks which showed behavioural and gross features indicating they were weak and unlikely to survive live export (reject lobsters).

2. ACHIEVEMENT OF PROJECT OBJECTIVES

The original project objectives and achievements with respect to these objectives were as follows:

2.1 To identify suitable immune system parameters which can be used to evaluate stress responses and health status in captive lobsters and to apply those parameters in a study of stress induced by post harvest handling procedures.

Six different immune system parameters have been identified, test procedures developed and responses to post-harvest stressors determined. All but one parameter (phagocytic capacity) have been analysed in both 'accepted' and 'reject'

lobsters and the variation from normal values which occurs in reject (unhealthy) lobsters evaluated. Significant findings were:

- Characteristic changes in the level of immune parameters occur when lobsters become weakened through poor post-harvest handling practices. This finding means that measurement of an appropriate suite of immune parameters in a batch of lobsters can provide a quantitative measure of the health (strength) of the lobsters
- Physical handling, air exposure, wounding and other minor post-harvest stressors cause reproducible changes in the levels of selected immune parameters in laboratory held lobsters. The degree of change appears to reflect the level of stress effect. These findings suggest that measurement of an appropriate suite of immune parameters can provide a measure of stress status of a batch of lobsters. However, this conclusion has yet to be confirmed through measurements of post-harvest lobsters on boats and in factories
- Factory based simulated truck and live shipment trials showed that a selected suite of immune parameters could accurately differentiate between holding conditions that resulted in high lobster survival (submerged storage) and those that resulted in high mortality (held in air with or without spray treatments at ambient temperatures). This finding means that these tests can be used to evaluate the efficacy of different post-harvest practices.
- In the same factory trials, the immune parameters successfully predicted the outcome (classification of lobsters into those that would survive the simulated truck transport and live shipment and those that would not) in 66% of lobsters (trial 1) and 79% of lobsters (trial 2). Addition of the physiological parameters (measured by an affiliated research team) to the analysis improved the discrimination to 77% (trial 1) and 81% (trial 2).
- Variations occurred in some of the parameters with the moult stage. Seasonal and/or diurnal variations may also occur but these were not studied. No differences in the parameters have been observed with the sex of the lobster but sex differences in each of the parameters, if any, have still to be fully evaluated. This means that normal values with respect to moult stage, time of year, time of day and lobster sex will have to be determined if these parameters are to be used by processing factories.

In summary, technologies for assessing lobster stress and health status based on immune parameters were developed and used in studies of stress induced by post-harvest handling procedures. The majority of these tests are simple to perform and could be carried out by trained factory staff.

Six tests, two more than specified in the project contract, were developed over the three year period. A health status index comprising selected immune parameters was identified. This index can be used by lobster processors to improve lobster post-harvest handling practices, in particular the long term holding of lobsters, selection of batches of lobsters for live export and transport of lobsters to factories in trucks.

2.2 To investigate the causes of mortality in captive lobsters held in processing factories. This study will focus on bacteriological and histopathological examinations and will result in the development of a standard protocol for autopsy of lobsters.

Four possible causes of mortality in captive lobsters were hypothesised:

- Cell injury and organ failure due to physiological disturbances - air exposure, rough handling and other stressors
- Opportunistic bacterial infections resulting from impaired immunity induced by above stressors
- Wounding - increased likelihood of bacterial infections
- Pre-existing disease conditions - weakens ability to resist stress

Autopsies were conducted on 135 lobsters, 49 reject lobsters and 86 accepted lobsters. Only a small proportion of lobsters exhibited pre-existing disease conditions, mainly parasite infections, thus eliminating pre-existing disease conditions as a main cause of post-harvest mortality. Wounding as a cause of mortality was not investigated apart from recording the occurrence of missing appendages and exoskeleton lesions in accepted and reject lobsters at autopsy.

The contribution of bacterial infection as a cause of mortality was investigated by both bacteriological and histopathological investigations. No evidence was obtained to suggest that the post-harvest lobsters were dying of an infection by a highly pathogenic bacterial species or strain, i.e. one that will cause disease in healthy lobsters. This observation confirms that bacterial infections in post-harvest western rock lobsters are most likely to be due to opportunistic infections, i.e. infections by bacteria which will not cause disease in healthy lobsters. 17% to 90% of the 'reject' lobsters had bacteria in their hemolymph and a high proportion of 'reject' lobsters had inflammatory reactions in their tissues, suggestive of bacterial infections. The level of bacteremia and inflammatory lesions in accepted lobsters was low. These observations suggest that bacteria could play a significant role in post-harvest mortality in those lobsters which have been weakened due to prior stressor exposure. Further studies on the development of bacteremia as stress reactions in lobsters, and the relationship between bacteremia and tissue injury in post-harvest lobsters should be conducted.

Recently Completed Projects Continued

The cause of death in lobsters dying in the simulated truck transport and live shipment trials was shown to be an infection in the bladder and antennal glands, presumably caused by either exposure to high levels of bacteria in the holding systems or to urinary stasis resulting from the cessation of urine output.

Approximately half of the 'reject' lobsters had no histo-pathological evidence of tissue injury so the precise cause of mortality in this group was not identified. Similar results have been obtained in studies of fish exposed to environmental stressors in which it was concluded that the cause of mortality was physiological failure caused by an irreversible loss of organ function. Whether the same conclusion can be applied to lobsters remains to be determined. A full analysis of biochemical parameters as well as immunological parameters in reject lobsters should resolve this question.

A standard protocol for autopsy for lobsters was developed and documented.

The results of this aspect of the study have made a significant contribution to the understanding of causes of mortality in post-harvest lobsters. These data and information will be of benefit to processors evaluating approaches to reduce post-harvest mortality. The findings will also assist in development of innovative approaches to long term holding of lobsters such as those based on probiotic treatments.

2.3 To evaluate the influence of temperature change on immunological and physiological stress responses

Following recommendations from the Steering Committee this objective was modified to a laboratory based study of the influence on immune stress parameters of handling with and without air exposure.

Air exposure and/or handling was shown to cause alterations in several immune system parameters. Of particular note was the finding that handling causes an alarm reaction in lobsters as evidenced by a rapid rise in total hemocyte counts (THC). A more delayed response in clotting time (decrease) was also observed as a reaction to physical handling. Air exposure without handling had no initial effect on THC but caused an elevation in this parameter after 24hr exposure.

These studies will contribute to the interpretation of results obtained in field studies and, hence, to improvement of postharvest handling procedures, particularly truck transport.

2.4 To study the influence of hormonal secretions on immunological and physiological stress responses.

Following recommendations from the Steering Committee this objective was changed to an investigation of the influence of holding conditions on immune stress parameters achieved through a series of simulated truck transport and live shipment trials conducted in a lobster processing factory (Geraldton Fishermen's Cooperative). Some studies on hormones produced by lobsters were commenced by an affiliated project team in 1998 but the factory trials were given precedence over hormone investigations and this work was discontinued.

2.5 To investigate innovative techniques which will boost immunocompetence but not adversely affect marketability of live product.

Following recommendations from the Steering Committee this objective was changed to an investigation of the influence of holding conditions on immune stress parameters achieved through a series of simulated truck transport and live shipment trials conducted in a lobster processing factory (Geraldton Fishermen's Cooperative). However, studies in this area are seen to be important and should be conducted in the future.

New Project Funded in Current Round

The following project, which has the strong support of industry, has been funded in the 2000/2001 round.

Striking a balance between melanosis and weight recoveries in western rock lobster (Panulirus cygnus)

Principal Investigator: Dr Hannah Williams
Curtin University of Technology, School of Public Health
GPO Box U1987, Perth W.A. 6845

PROJECT OBJECTIVES:

1. To establish the impact of temperature and food additives on the activity of Panulirus cygnus haemolymph phenol oxidase (PO) in vitro.
2. To establish the impact of current commercial practices on weight recovery and melanosis formation.
3. To establish the impact of post-harvest transportation on PO activity, weight recovery and melanosis formation.
4. To determine the effects of anti-browning agents on weight recovery and melanosis formation.
5. To validate the use of experimentally determined cooking profiles for improvement of cooked weight recoveries and prevention of melanosis.
6. To formulate recommendations and guidelines that will enable industry to apply the findings of the study.

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

The following project has been found to be of considerable interest to industry, and although targeted mainly on the western rock lobster, includes components of examining leg loss of southern rock lobsters in South Australia and Tasmania.
As part of the annual workshop of the FRDC Enhancement and Aquaculture Subprogram held this year in New Zealand in April 2001, Wayne Hosking presented the project to the Workshop, demonstrating the close links between the two Subprograms.

363/251:

Rock lobster post-harvest Subprogram: Development of a method for alleviating leg loss during post-harvest handling of rock lobsters

Principal Investigator: Dr Glen Davidson
Co-investigator: Wayne Hosking

Milestone Report 28/2/01

Completion of experiments addressing Objective 1.

To identify a cold-water immersion treatment that rapidly immobilises western rock lobsters, while allowing swift recovery from immobilisation upon return to ambient temperature seawater. To investigate the effect of season/acclimation temperature on effectiveness of cold stunning in western rock lobsters. To investigate the use of sea sprays vs immersion for cold stunning in western rock lobsters.

- The time/temperature matrix, designed to identify preferred stunning treatments for use prior to on board sorting and factory grading (Objective 1), has been completed for A grade (~0.45 kg) lobsters acclimated to ambient sea temperatures of 18˚ and 22˚C. The latter encompassed the period of the whites migration in November 2000.
- A cursory look at the results suggests that the sensitivity of lobsters to stunning did not vary appreciably over this ambient temperature range.
- The matrix was to be repeated in February 2001 using animals acclimated to peak annual water temperatures (~24-26˚C). However, the sea temperature this season has remained unusually cool and to date has not exceeded 23˚C. Because of this, it has not been possible to complete the matrix using lobsters acclimated to ~24-26˚C.
- We will continue to monitor sea temperature for the remainder of the season and if it rises above 24˚C we will conduct the third run of the matrix and report the completion of this part of Objective 1 at the next milestone (31/07/01). If the sea temperature does not exceed 24˚C this season we will complete the matrix in the 2001/2002 season and report the completion of this part of Objective 1 at the next milestone (31/07/02).
- To date, tail flip activity and recovery time have been used to assess the effects of the various treatments in the matrix experiments. Whilst informative, these measures tell us nothing about the usefulness of treatments for alleviating leg loss. To complicate matters, out of the nearly 3000 lobsters handled in these experiments, we have recorded only 37 autotomised legs. This rate of leg loss is much lower than recorded in the sea trials (see below). We have been trying to develop a reliable method for inducing leg loss in captive lobsters so we can assess directly in the factory the effects of the experimental treatments on leg loss. To this end we have recently identified exposure to hyper saline water as an effective inducer of autotomy (see below). Preliminary experiments also indicate that cold water stunning is effective for alleviating leg loss induced by exposure to hyper saline water.
- Part C of Objective 1 was to test the effectiveness of cold water applied as a spray rather than as an immersion bath. This work has been delayed while we have been trying to develop a reliable inducer of autotomy to apply to the treatment groups. This will enable us to directly assess the efficacy of sprays for alleviating leg loss. We will report the completion of this part of Objective 1 at the next milestone (31/07/02).

Additional

- Prior to the start of the 2000/2001 season a prototype cold water stunning cacka box was installed on the boat, "LFB Windjana", operating out of Port Denison. Since the start of the season, 31 of the proposed 50 days of sea trials (Objective 4) have been completed and the initial results are very promising.
- The trial started with the cacka box set at 0˚C. This proved to be very effective, significantly reducing onboard leg loss from 0.130 legs/lobsters in control animals to 0.033 legs/lobster in the treated animals ($P < 0.01$). Note that these onboard leg loss rates are averaged over the entire catch, including both retained and returned lobsters (i.e cackas, setose etc) and that a sizeable proportion of the total leg loss in the control group has been observed to occur when returns are thrown overboard. This phenomenon has not been previously described and is especially significant given the negative impacts of leg loss on the survival, growth and reproduction of returns. In addition, a sizeable proportion of the leg loss incurred by cackas prior to Feb 1 can be regarded as an immediate loss to the fishery since many of the 76 mm lobster will be caught again shortly after Feb 1, before they have had a chance to moult and regenerate the missing legs.
- After operating for several days at 0˚C and in order to identify the highest effective stun temperature, the stun tank temperature was increased progressively. The highest effective temperature should alleviate leg loss whilst allowing for the shortest possible recovery times

and 10°C appeared to be equally effective for reducing leg loss ($P > 0.05$) preventing about 80% of on board leg loss (Table 1). Interestingly 15°C appears to have some benefit, but does not appear to be as effective as 10°C or lower.

- Many fishermen report that a small amount of sea water in the bottom of the cacka box is effective for reducing leg loss. To test this idea the stun tank was run at ambient temperatures for two days only. The results show that leg loss in the ambient sea water group was worse than in the control group (Table 1). This may be due to differences in the design of the two sides of the experimental cacka box.
- The results to date are all the more significant given that the prevailing weather conditions have been very mild, with temperatures in the low to mid twenties (Table 1), relatively light winds and high humidity. We expect that, as we encounter more adverse weather (higher temperatures etc), the difference in leg loss between the controls and treated lobsters will be greater.
- The low sea temperatures during the trials have been to our advantage in that we know the stun treatments tested so far are likely to be effective when the sea temperature is at, or above 21°C; this covers most of the season. However, we still need to test the effectiveness of the warmer stun treatments (e.g. 7.5 and 10°C) during the cooler periods of the season when the sea temp is at its minimum (16-17°C).
- As of December 15, each day's catch was intercepted upon arrival at the factory. In a sub-sample of the catch, the numbers of old and new limb wounds on the lobsters were counted along with the numbers of loose legs in the baskets. These observations revealed that the difference in leg loss between treatment and control legal-sized animals upon arrival at the factory was usually greater than the difference recorded by the onboard technician. Upon receipt at the factory, differences of approximately 0.3 legs/lobster have been recorded on average (Table 1). This suggests some residual effect on leg loss of the on board stunning and indicates the net benefit of on board cold-stunning.
- All the experimental animals were transported from Dongara to the factory by truck, showing that the benefits of onboard cold-stunning were not negated by intermediate handling and transportation steps.
- In the course of the research we have identified two previously unrecognised causes of leg loss:
  - ✓ The sea trial data show that lobsters transported in partially filled baskets show a much higher rate of leg loss than lobsters transported in "full" baskets (in this case "full" means containing 40 or more lobsters) (Fig. 1).
  - ✓ Additional experiments have revealed that exposure to concentrated sea water causes lobsters to autotomise legs. Preliminary salinity measurements at points in the post-harvest chain revealed that lobsters may indeed be exposed to concentrated sea water. This finding supports anecdotal observations that leg loss is correlated with hot, dry, windy days. Under such conditions, high evaporation rates would concentrate any sea water on surfaces with which lobsters come into contact (cacka boxes, gloves, sorting tables etc) in addition to concentrating any sea water films on the lobsters themselves.
- It is likely that post-harvest leg loss can be further reduced by developing strategies to circumvent these effects. We will be preparing submissions seeking additional funding to further investigate these effects.
- Recently we spent time recording the incidence of leg loss in 76 mm and ≥ 77 mm lobsters following the gauge change on Feb 1. As expected, the incidence of old leg loss was higher in 76 mm than ≥ 77 mm lobsters. This clearly reflects the damage 76 mm animals suffer during their repeated capture and release between Nov 15 and Feb 1. If cold-stunning becomes a standard industry practice, the bulk of this leg loss could be prevented.
  If we can estimate the average amount of damage caused during a single capture event, we can calculate the number of times 76 mm lobsters are caught and returned before they recruit to the fishery on Feb 1. This information will be used in subsequent experiments to determine the impact of repeated capture with cold-stunning on the survival and growth of returned lobsters.
- The first field survey of leg loss in South Australia (Objective 7) has been successfully completed. The subcontractors have prepared a report of the findings which awaits more detailed analysis.
- The first field survey of leg loss in Tasmania is in progress.

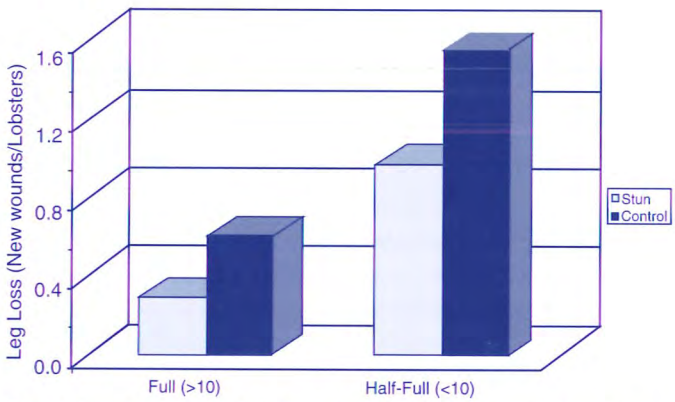


Fig. 1: Leg loss recorded from treatment and control lobsters transported in full (≥ 40 lobster/basket) and half-full baskets (> 40 lobsters/basket)

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

News Continued

Table 1) Summary of sea trial data as of 7/2/01

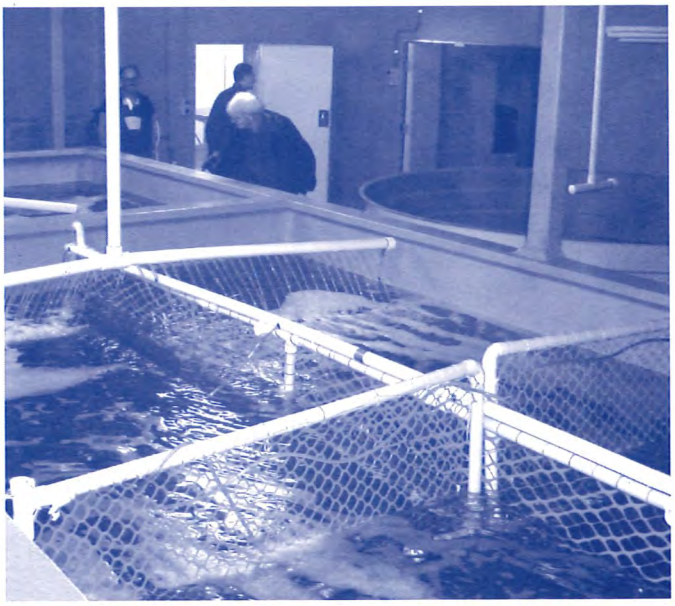
STUN TEMP	AIR TEMP	SEA TEMP	NO. OF DAYS TESTED	LEG LOSS/SIZE OF LOBSTER LEG (ON BOARD)		LOSS/SIZE OF LOBSTER (FACTORY)	
				Control	Treatment	Control	Treatment
0	21-22	18-23	5	0.151	0.027	-	-
5	19-25	21	8	0.156	0.018	-	-
7.5	20-24	-	3	0.140	0.028	0.513	0.212
10	20-23	21-22	7	0.164	0.031	0.673	0.383
15	21-28	22	3	0.087	0.044	0.858	0.514
Ambient	18-25	22	2	0.103	0.262	1.238	1.436

Special

A Post-Harvest Subprogram project conducted by Brad Crear and Nigel Forteath which was concluded in 1998 looked at methods of improving survival after capture in both southern and western rock lobsters.

The results have been presented to FRDC in a comprehensive report (Project 94/134.03) and will be part of a pamphlet on holding techniques which is being prepared. However, interest in the best temperatures and oxygen levels to hold the lobsters, which were identified during this study are of special interest to those holding live lobster. The following table presents a very brief summary of these data:

In addition the studies indicated that spray systems, which were commonly used in trucks and other transport systems for the western rock lobsters at that time, caused "secondary" health problems, unless temperature can be controlled. Because of these studies and other industry studies, which supported this conclusion, wherever possible live western rock lobsters intended for export are now transported fully submerged. This is confirmed by the design of the latest carrier boats transporting live lobsters from the Abrolhos Islands to the mainland.



Some of the Australian participants at the meeting in New Zealand titled "A sharing of Knowledge; Australasian Rock Lobster Research", in April 2001, inspecting New Zealand red rock lobsters being held prior to export. Research News

SPECIES	TEMPERATURE(S) EXAMINED	OXYGEN LEVELS REQUIRED
Western Rock Lobster	23 Degrees Centigrade, although capable of handling a wide range of temperatures (16-27) without impacting their physiological capability	>70%, preferably 80%
Southern Rock Lobster	9-13 Degrees Centigrade	>60%, preferably 80%

For further clarity on the priorities or any other information please contact one of the following:

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MAILING LIST

This Newsletter is distributed widely. However, if you would like a personal copy mailed to you please contact the Subprogram Secretary Emma Phillips by email at emmaphil@ozemail.com.au.

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Editor: Bruce Phillips, Subprogram leader Tel: 08 9266 7963 Fax: 08 9266 2495 Mobile: 0417 189 956
Email: rphillip01@alpha7.curtin.edu.au

Publications

A number of publications have arisen from studies conducted within the Subprogram or the Enhancement and Aquaculture Subprogram. The following are some recent papers, which will be of interest:

1. In the August September Edition of *Fishing Today* the "FRDC Rock Lobster Post-Harvest Subprogram" was described.
2. In the February March Edition of *Fishing Today* the project by Brad Crear and Mark Powell on "Optimising water quality in rock lobster post-harvest processes", is described.
3. A final report of a project "Application of health indices of dietary regimes and live transport stressors in the southern rock lobster, *Jasus edwardsii*." is available. This has been submitted to FRDC by Dr Louis Evans and colleagues.
4. Several papers presented at the Lobster Health Management Symposium in Adelaide are to be published in 2001 by the Aquatic Science Research Unit, Muresk Institute of Agriculture, Curtin University of Technology. Enquires should be sent to this Unit.

ROCK LOBSTER POST-HARVEST SUBPROGRAM FRDC

THE LOBSTER NEWS

Volume 3, February 2002

rom the ubprogram eader

uring the year FRDC approved a new roject for the Subprogram, and in eptember 2001 the Steering Committee et in Geelong and examined a series of re-proposals of possible new projects.

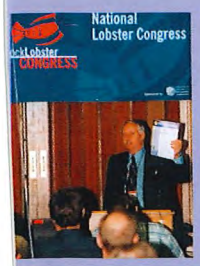
ive projects have been recommended to FRDC for consideration in the current ound a further project has been deferred ntil next year.

During April, I will visit South Australia, Victoria and Tasmania for discussions re industry needs and development of proposals for the next round of applications. I will be in Queensland in May and will discuss with the Queensland lobster industry their specific problems, again with a view to finding solutions to their problems.

The annual public Workshop (which is a combined event with the Enhancement and Aquaculture Subprogram) is to be held in Cairns on 29 May 2002. Please contact me if you would like further information on the Workshop.

Bruce Phillips

Sub Program Leader.



The Subprogram Leader Bruce Phillips presenting details of the Rock Lobster Post-Harvest Subprogram to the National Lobster Congress in Geelong in September 2001.

nside this issue:

To conduct research to increase the value of the rock lobster catch for Australia through improvements in post-harvest practices.

Recently Completed Projects

96/345:

Physiological studies of stress and morbidity during post harvest handling of western rock lobsters (Panulirus cygnus)

I. Physiological stress indicators

Principal Investigator: Dr Brian Paterson
Centre for Food Technology,
Department of Primary Industries
19 Hercules St Hamilton QLD 4007

SUMMARY

Lobsters removed from factory tanks because they have weakened during live storage have probably been stressed too much. Factory-based trials in this project have shown, in conjunction with FRDC 344, that when changes were seen in certain physiological/immunological parameters in the blood of lobsters during a 6h storage treatment then they were more likely to die within the next week. The key physiological indicators found here, lactate and magnesium, reinforce the point that storage in air at ambient temperature is highly detrimental to lobsters - even when seawater sprays are provided. These indicators were not simple enough for routine factory use, but they were still useful in monitoring the responses of lobsters to alternative storage/transport methods to ensure that the deleterious effects of out-of-water storage are minimised.

These findings vindicate the initial premise of this research, that stress during post-harvest handling was responsible for losses of rock lobsters during storage in factory tanks. The objective of this study was to find indicators of that stress so that they could be either used by some factories to grade live product for export, or used in studies of alternative handling practices so that fewer lobsters were stressed.

The study showed that during the post-harvest handling of rock lobsters, several physiological parameters deviated from baseline levels (established by sampling lobsters on the sea floor using SCUBA and by sampling captive acclimated lobsters). These changes parallelled the respiratory problems seen when rock lobsters were kept out of water in laboratory experiments. Using these findings as a basis, a series of factory-based experiments, some using alternative ambient temperature storage methods, were used to establish which of these physiological changes could be linked to later mortality in stressed lobsters. Immediately after an imposed stress, the lobsters that eventually died over the following week were significantly different from future survivors with respect to some blood parameters (particularly lactate and magnesium concentration). Using discriminant analysis of several blood test results, it was possible to correctly classify the fate of 80-90% of lobsters. However, with each replicate of the study, a different set of blood tests were required, though there was some consistency from time to time, particularly regarding lactate's importance to the analysis.

(Continued overleaf)

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Recently Completed Projects Continued

Key indicators for commercially significant stress clearly existed, but none of these were simple enough to apply in a factory context. When comparing the performance of lobsters in alternative storage methods, the most accurate method was clearly to count the actual mortality, but the results of human grader collaborating in this study could also be used to rank treatments from best to worst. The advantage of stress indicators was that they showed why lobsters were dying and suggested the changes required to improve outcomes.

If reduced deviations in these key indicators was used as the proviso for choosing storage/transport environments, then this criterion would continue to emphasise the need for submerged storage/transport of lobsters. The storage environment trials also showed that the prognosis was excellent for lobsters stored in recirculated seawater for 6 hours.

Changes to the code of practice would seem warranted. In recommending submerged transport as the best method for short-term movement of lobsters, this work suggests that as long as aeration is maintained that water quality deterioration is not a major issue, and provision of biological filtration of the water does not seem to be a priority.

Submerged transport will not always be possible or practical. This research shows that contrary to what you might first expect, that spraying seawater onto lobsters in air at ambient temperature serve no apparent benefit in terms of lobster condition over and above that provided by simple humid air. Further work may be required, if there is a call for it, to establish exactly why sprays fail to benefit lobsters in air.

These observations regarding lobster transport and storage are confined to ambient temperature and hence are strictly applicable to bulk transport of lobsters (eg. on carrier boats). Cooling the lobsters down (eg. truck transport) introduces another variable, which of reduced metabolic rate, which may alter the lobster's responses.

New Project Funded during the year

New Project 255/2001

Funded by Geraldton Fishermen's Cooperative, Western Rock Lobster Development Association, Development and Better Interest Fund, Industry Development Unit of WAFIC and FRDC.

Quantifying and controlling hyper- and hyposaline-induced post-harvest leg autotomy in the western rock lobster.

Principal investigator: Wayne Hosking
Co-Investigator Dr Glen Davidson
Both of Geraldton Fishermen's Cooperative

Project Objectives

1. Survey salinity concentrations of surface films on individual lobsters and on relevant contact surfaces on boats and within factories.
2. Describe the relationship between autotomy and exposure to seawater of various salinities for lobsters of various sizes and moult stages.
3. Quantify leg loss during industry standard freshwater "drowning" procedures.
4. Compare responses to ionic and non-ionic solutions to elucidate the potential role of other contaminants, and the possible nature of the receptors and stimuli.
5. Investigate the relationship between daily environmental conditions and levels of post-harvest leg loss.
6. Field-test practical solutions for hyper/hyposaline-induced autotomy and make recommendations to industry.

BACKGROUND

There is substantial anecdotal evidence suggesting that, under certain environmental conditions (e.g. hot, easterly winds), western rock lobsters become especially sensitive to handling and many animals shed legs spontaneously (Tod et al, 1990). Until recently, the precise reason for this phenomenon was unclear. However, in the course of research work for FRDC project 2000/251, a method for reliably inducing autotomy through the application of moderately hypersaline seawater has been developed. Subsequently, preliminary investigations have suggested that seawater with salt concentrations capable of inducing this phenomenon may be widespread throughout the industry, and lobsters probably come into contact with such water on a daily basis both on fishing vessels and within processing facilities. It now appears probable that there is in fact a link between environmental conditions, evaporation rates, the formation of hypersaline seawater and autotomy in *P. cygnus.*

Dr Brad Crear of the Tasmanian Aquaculture and Fisheries Institute (TAFI) has conducted experiments under our direction to test the susceptibility of the southern rock lobster, Jasus edwardsii, to hypersaline-induced autotomy. Results showed that *J. edwardsii* do not autotomise in response to hypersaline seawater, and it would appear that no further work is required on this species.

It is proposed to investigate hyper- and hyposaline induced autotomy and the extent to which it can occur throughout industry. Methods will be developed for avoiding or controlling this phenomenon, and these will be widely publicised and demonstrated so that the maximum benefits of this research can be realised by industry.

2001/235:

Striking a balance between melanosis and weight recovery in western rock lobster

Principal Investigator: Hannah Williams
School of Public Health,
Curtin University of Technology

Progress Report to December 2001

The Communication/extension action plan has been submitted to the FRDC as required.

Considerable variation in phenoloxidase (PO) activity and hemolymph protein levels exists between different lobsters,(n=58). However, analysis of the data does not show a correlation between hemolymph protein levels and PO activity.

Freezer trials of buffered and unbuffered hemolymph showed that PO activity dropped after two days and then held steady for the twenty-day duration of testing. The ratio between total activity and baseline is maintained through out, however the sharp drop from the initial levels renders it undesirable for samples of fresh hemolymph to be frozen and held over for later testing.

A series of tests were conducted to establish the best position for the measurement of the core temperature of lobsters during cooking. The aim was to insert the thermocouple in such a way as to reduce water ingress and disruption of the normal internal state whilst measuring the core temperature at the point of slowest temperature increase.

When these initial studies were completed work began on Objective one. Objective one consists of two parts with the overall aim of establishing the impact of temperature and food additives on the activity of *P. cygnus* hemolymph phenol oxidase (PO) in vitro.

Objective 1: Phase 1:

To establish the impact of heating on the enzyme under steady state and unsteady state conditions;

1. Heat trials: Steady state

Hemolymph was mixed with pre-heated buffer in six test tubes and placed in the hot water bath at a set predetermined temperature. After 5 minutes a tube was removed from the bath and plunged into a bed of ice and iced buffer was added. Baseline PO activity and total PO activity were then determined. The procedure was repeated at 5 minute intervals to a maximum time of 30 minutes. Initial PO activity and hemolymph protein content were determined using a sample held at room temperature. The tests were conducted at 40, 50, 60, 70, 80, 90 and 100°C with 4 lobsters being used for each temperature series. Results were expressed as a % of the initial baseline or total activity respectively i.e. % Relative activity.

Discussion

- Baseline activity increased at 40°C, possibly due to release from the hemocytes as the hemolymph coagulated. After five minutes at 50 and 60°C no baseline activity was detected. However after 20 minutes at 60°C baseline activity made a slight comeback. Significant increases in baseline activity occurred at 70 and 80°C after five minutes. This may be attributed to heat activation of the enzyme and/or increasing disruption of the hemocytes. The activity then decreased slightly over the time span but was still significantly higher than the time zero value. There was no increase in activity at 90°C and levels decreased steadily until baseline activity was not detectable in the majority of samples after 20 minutes. 1 sample however showed a very low level of activity for the entire time span. No baseline activity was detected in samples exposed to 100°C after 5 minutes.

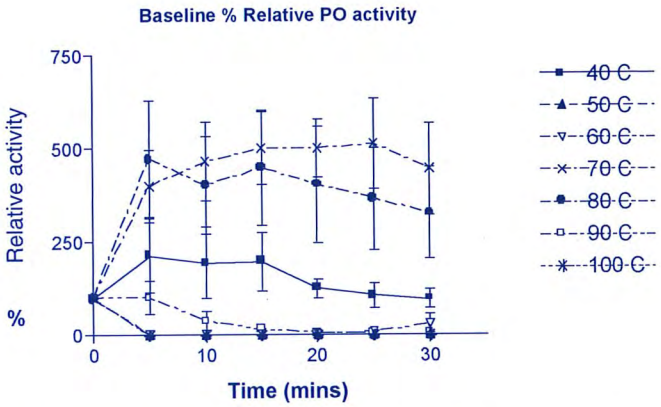

Fig 1: Baseline relative activity over time under steady state conditions

- Total activity increased slightly over time at 40, 50 and 60°C. Decreasing total activity over time was displayed at 70 and 80°C. After 20 minutes at 90°C total activity was approaching 0. After 5 minutes at 100°C no activity was detected.

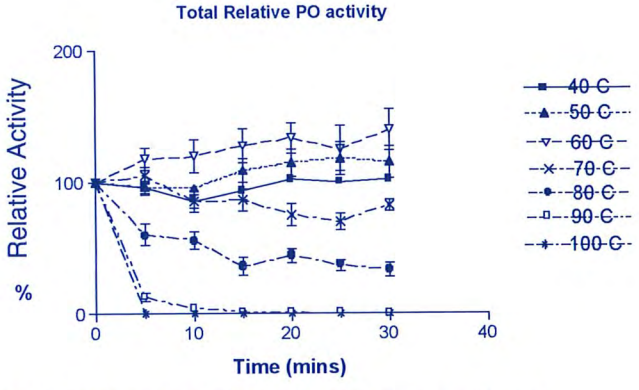

Fig 2: Total relative PO activity over time at differing temperatures

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2. Heat trials: Unsteady state

Hemolymph was mixed with buffer in each of nine test tubes at room temperature. The tubes were mixed thoroughly using a vortex mixer and eight were placed in the water bath. The initial temperature was recorded and the water bath switched on. When the temperature in the tubes reached a predetermined set level (40,50,60,70,80,90 or 100°C) one tube was removed from the bath and plunged into a bed of ice. Iced buffer was added and the contents of the tube mixed thoroughly. Baseline PO activity and total PO activity were then determined. Initial PO activity and hemolymph protein content were determined using the remaining sample held at room temperature. A total of eight lobsters were used for the test series.

Discussion

- Baseline activity followed a similar pattern to that shown in the steady state trials. Activity increased as the temperature approached 40°C followed by a sharp decease at 50°C. No baseline activity was detected at 60°C. However, significant increases in baseline activity occurred as the temperature approached 70°C. This may be attributed to heat activation of the enzyme and/or complete disruption of the hemocytes. The activity then decreased as temperature moved towards 90°C and no baseline activity was detected in the samples by the time they reached 100°C .

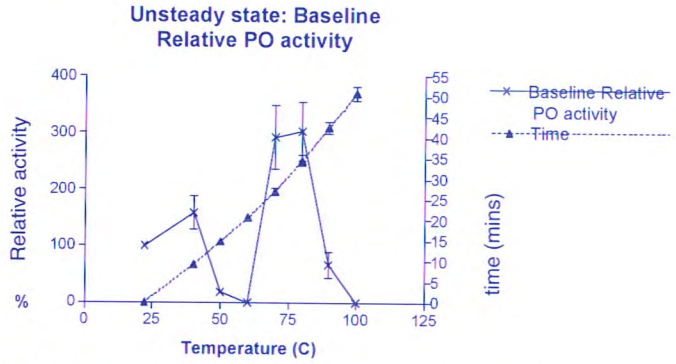

Fig 3: Baseline relative PO activity under unsteady state conditions

- Total activity did not change significantly as the temperature approached 60°C. Decreasing total activity was displayed as the temperature reached 80°C. At 90°C total activity was approaching 0 and by the time the samples reached 100°C no activity was detected.

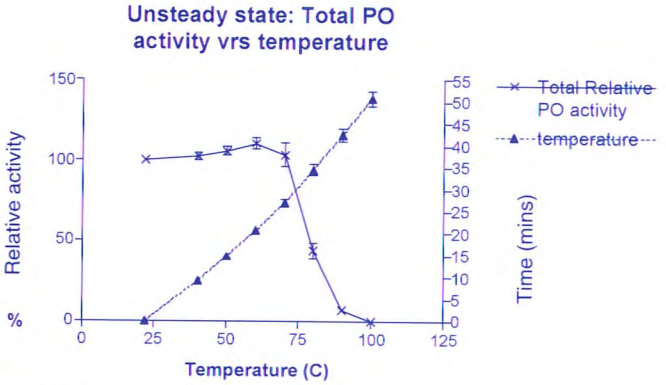

Fig 4: Total relative PO activity under unsteady state conditions

When the baseline values are compared to the total activity values at the same temperature it appears that at 70°C, 50% of the total available enzyme has been converted to the active form and at 80 to 90°C, 100% of the existing enzyme is in the active form. However, even at this point of maximum activation of the pro-enzyme, the level reached does not exceed 50% of the initial level of total enzyme activity. This leads to the conclusion that under actual cooking conditions the availability of the active enzyme will be determined by a balance between temperature-induced activation of the pro-enzyme and temperature deactivation of the active enzyme form.

Objective 1: Phase 2:

To establish the impact of heating on the enzyme under steady state and unsteady state conditions in the presence of a range of antibrowning agents.

Work has begun on this phase of the project. However, information generated in Phase 1 has shown that to achieve this second phase will take considerably more time and effort than originally envisaged. Therefore a modification of the project has been requested and approved. The experiments for Phase 2 of Objective One will be run concurrently with Objectives 2 and 3 of the project with a milestone date of 31st December 2002. There would be no further cost incurred as the funds can be transferred from the monies received for Objective 1.

Objective 2

The aim of this objective is to establish the impact of current commercial practices on weight recovery and melanosis formation.

Three field trips will be run over the current season to measure the thermal profile of the cooking process and to evaluate the impact of processing on melanosis and weight recovery. The work will be carried out at the M.G. Kailis factory in Dongara following a standardized protocol. The first field trip was carried out in mid December and analysis of the results is now underway.

Time line

- Anti-browning agents to be completed by 31st December 2002.
- Objective 2 will be completed by 30th June 2002.

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FRDC 2000/251:

Rock Lobster Post-Harvest Sub-program: Development of a Method for Alleviating Leg Loss during Post-Harvest Handling of Rock Lobsters.

Principal Investigator: Dr Glen Davidson
Geraldton Fishermen's Cooperative Ltd.

PROGRESS REPORT TO 31 DECEMBER

- Questionnaires on the occurrence of post-harvest appendage loss have been sent to license holders in the southern rock lobster fisheries in South Australia and Tasmania. In conjunction with these questionnaires, SARLAC and TAFI subcontracted to undertake 2 periods of sampling (nominally "summer" and "winter") aboard commercial lobster vessels in South Australia and Tasmania, respectively (Objective 7). The purpose of these sampling periods was to provide precise information on the nature and occurrence of post-harvest appendage loss. Sampling in both states has been completed. A cursory analysis of the data collected in South Australia indicated that very little post-harvest appendage loss occurs in the South Australian fishery (Fig. 1). This finding is consistent with the initial responses to the questionnaires.

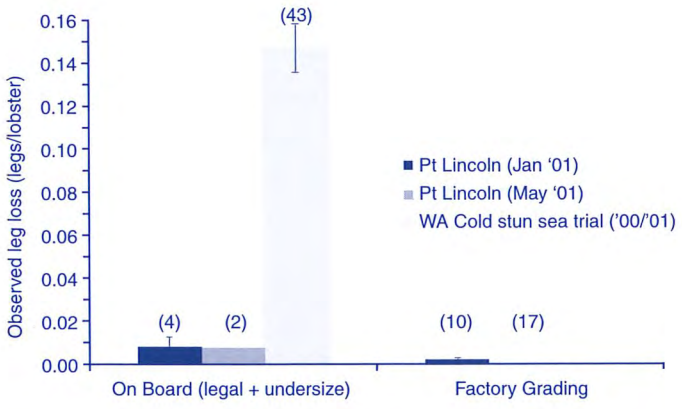


Fig 1) Rates of observed leg loss during on board sorting and factory grading of daily catches in Port Lincoln, South Australia were very low. For comparison, data collected during sea trials of on board cold-stunning in Western Australia are included. Numbers in brackets indicate days of fishing. Data presented are means ± 1 s.e.m.

- Under the conditions of recent field trials conducted in Zone B of the Western Australian fishery, cold water stunning reduced on board leg loss by 80%. However, operating conditions in different zones of the fishery vary widely, which may limit the usefulness of cold-stunning in some areas. For example, most fishers in Zone B fit their pots with gates and use cacka boxes to sort the catch. In contrast, Zone C fishers generally use pots without gates and skin the pot directly through the pot neck. In order for the industry to reap maximum benefit from this project, technologies and recommendations arising from the research must be implemented as widely as possible. Field trials of cold water stunning under unique local conditions are the most effective means for demonstrating the usefulness of the technique. With this in mind, we requested additional funds from FRDC to extend the field trials of cold-stunning to other zones of the fishery. The request for the additional funds was granted and the commercial lobster vessel *Shark Raider*, skippered by Glenn Byass and operating out of Mindarie Keys, has been fitted with a cold-stunning cacka box (Fig. 2) in preparation for sea trials this 2001/'02 season. These trials will compare leg loss rates in lobsters sorted after cold-stunning to those in lobsters sorted after being skinned through the pot neck.

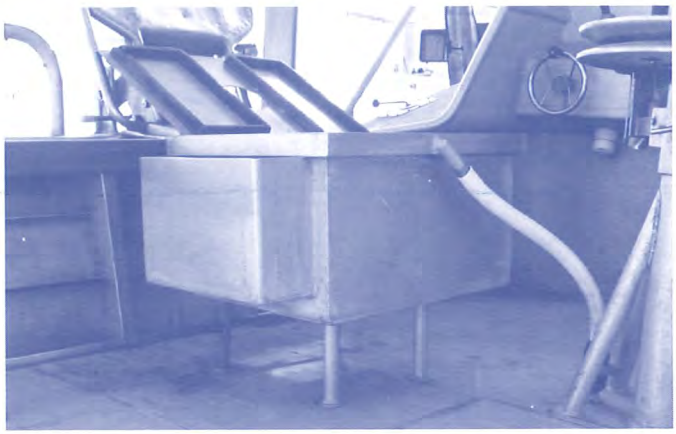


Fig. 2) The cold water stun tank fitted aboard the Shark Raider. For this set up an existing day tank was modified for use as the stun tank.

- A live holding facility has been established at a site in Jurien Bay, W.A. Experiments to determine the effects of cold water stunning on the viability of eggs carried by ovigerous females (Objective 6) and on the survival, growth, and appendage loss of caught and returned undersized lobsters (Objective 5) will be conducted at this site over the next 12-15 months.
- A paper was presented at the 2nd National Rock Lobster Congress in Geelong in September 2001. Commercial fishers and fisheries scientists and managers from New Zealand and Australia attended the meeting.
- Presentations were made to fishers and fisheries scientists and managers attending meetings on the annual Rock Lobster Industry Advisory Committee, Coastal Tour. Presentations were given in Fremantle, Dongara and Jurien Bay. The presentations were well received and generated much interest.

New Projects

Submitted to FRDC for consideration for funding in the current round

2002/237

A code of Practice for Handling Rock Lobster

Principal Investigator: Richard Stevens

Project Objective

To produce a Code of Practice for the handling of rock lobster

2002/238

Quantification of shell hardness in southern rock lobster

Principal Investigator: Dr Caleb Gardner

Project Objectives

1 To calibrate the rate of change in shell hardness before and after the moult of southern rock lobsters relative to lobster size, sex, region and temperature.

2 To identify the region of the exoskeleton that is most suited for measuring hardness.

2002/239

The effect of on board cold water stunning on the survival and growth of caught and returned western rock lobsters (Panulirus cygnus).

Principal Investigator: Wayne Hosking

Project Objectives

1 To determine the effect of commercial capture with or without cold-stunning on the survival and growth of returned protected western rock lobsters.

2 To observe and film in the wild the behaviour of western roc lobsters caught and returned with or without cold-water stunning.

2002/240

Optimal selective fishing strategies for southern rock lobster

Principal Investigator: Dr Linda Eaton

Project Objectives

1 To determine the viability of adopting a selective fishing strategy given the economic and biological structure of the fishery.

2 To determine the impact on the fishery dynamics and stock composition of the fishery if a change in fishing strategy is made to one of selectively landing only the best quality fish.

241/2002

Aetiology of the pink flesh syndrome in the western rock lobster, Panulirus cygnus

Principal Investigator: Dr Patrick Spanogue

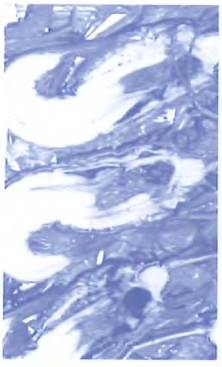
Project Objectives

1 To investigate the prevalence and aetiology of pink flesh syndrome in lobsters collected from processing facilities

2 To investigate the influence of endogenous factors (e.g. gender, shell colour, moult stage, key enzyme activity), opportunistic pathogens (e.g. vibrio sp), environmental factors (e.g. water quality, temperature, moon phase) and processing techniques (e.g. type of tank, crowding and period of holding) on the development pf pink flesh syndrome in lobsters in tanks under industry conditions.

3 To provide rock lobster processors with clear recommendations for strategies to reduce or eliminate losses due to this syndrome.

Hannah Williams - Principal Investigator for Project 2001/235, Page 3



Melanosis 1: Project 2001/235, Page 3

Alan Snow

Seafood Services Australia –
Technical Information and Advice
Snowa@dpi.qld.gov.au

Considering that rock lobster is our most valuable fishery, little research is available in the public on rock lobster post-harvest handling and processing. In a reflection of the prevailing demands of the export market, most recent post-harvest research has focussed on survival and live transport. For example, the only two reports available through Seafood Services Australia are *"Reducing post-harvest mortality when storing tropical rock lobsters for live export"* and *"Airfreight of live seafood: An improved packaging system for live western rock lobster"*. A further project *"Evaluation of the commercial benefits derived from steam cooking Western Rock Lobster"* should be completed in the near future.

This is not to say that there is no information available. A number of post-harvest studies were done in the 1950's, 60's, and 70's by the then CSIRO Division of Food Research, and there is also a considerable volume of material available from overseas sources. At SSA we still get requests for information packages on such topics as freezing, cooking and packaging systems for lobsters. We handle these with information from Japan, the US, Cuba and Europe. Obviously this is not an ideal situation, but at least it supplies some leads to our clients. Here is a list of some of the requests for information that we have provided information.

- Live Packing of Lobsters for export
- Prevention of blackspot in Lobsters
- Cooking of lobster using microwave techniques
- Phosphate levels in Lobsters
- Prevention of mortality of tropical rock lobsters
- Processing cooked, fresh chilled, and frozen cooked Lobsters
- Meat yields form lobster
- Techniques for processing and preservation of lobster
- Post harvest handling of bugs and lobsters
- Handling and air transport of lobsters
- Handling and transport of southern rock lobsters
- Utilisation of lobster waste

Members of the Subprogram Steering, which met in Geelong in September, thereby allowing the members to participate in the National Lobster Congress.

From the left, Steve Hood, Kym Redman, Nick Polgeest, Robert Van Barneveld, Richard Stevens, Patrick Hone, Rodney Treloggen, Bruce Phillips. Glen O'Brien was unable to attend

For further clarity on the priorities or any other information please contact one of the following:

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This Newsletter is distributed widely. However, if you would like a personal copy mailed to you please contact the Subprogram Secretary Emma Phillips by email at emmaphil@ozemail.com.au.

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

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Publications

A number of publications have arisen from studies conducted within the Subprogram. The following are some recent papers, which will be of interest:

1 Jussila, J., McBride S., Jago, J. and Evans, L. H. (2001) Hemolymph clotting time as an indicator of stress in western rock lobster (*Panulirus cygnus* George). Aquaculture 199, 185-193.

2 Paterson, D. B., Davidson, G.W. and Spanogue, P. T. (2001) Identifying stress when western rock lobsters are stored out of water: The average and individual blood lactate concentrations. Contact Aquatic Science Research Unit, Curtin University of Technology, Perth.

3 Paterson, D. B., Davidson, G.W. and Spanogue, P. T. (2001) Measuring total protein concentration in blood of the western rock lobster (*Panulirus cygnus* George). Contact Aquatic Science Research Unit, Curtin University of Technology, Perth.

4 Powell, M., Crear, B. and Allen, G. (2001) Lobsters in the toilet: Acid-base effects of amonia exposure in the spiny lobster *Jasus edwardsii* . Presented at the Australian and New Zealand Society of Comparative Physiology and Biochemistry , Adelaide, December.

The Lobster News is funded by the Fisheries Research and Development Corporation.

POST-HARVEST SUBPROGRAM
ROCK LOBSTER PROGRAM
FRDC

THE LOBSTER NEWS

Volume 4, September 2002

rom the ubprogram eader

ree new projects were approved by e FRDC in the current round. etails are given later in the Newsletter.

ne Subprogram now has a full revised eb Site. You can access it through the RDC site or direct at ww.frdc.com.au/research/programs/rlph/

ne annual Workshop (which was a mbined event with the Enhancement nd Aquaculture Subprogram) was held Cairns on 29 May 2002. It was a reat success with 77 attendees. Please ontact me if you would like a copy of he Proceedings of the Workshop.

Cost: $22 inc GST plus $8 Postage)

Bruce Phillips
Subprogram Leader.

losephine Walker measuring shell hardness on a male southern rock lobster prior to the moult.

nside this issue:

To conduct research to increase the value of the rock lobster catch for Australia through improvements in post-harvest practices.

Research News

2001/235:

Striking a balance between melanosis and weight recovery in Western Rock Lobster

Principal Investigator: Hannah Williams
School of Public Health,
Curtin University of Technology

PROGRESS REPORT

Objective 1a
To establish the impact of heating on the enzyme under steady state and unsteady state conditions. Report submitted to FRDC January 2002

Objective 1b
To establish the impact of heating on the enzyme under steady state and unsteady state conditions in the presence of a range of antibrowning agents. To be completed by 31st December 2002.

Objective 2
The aim of this objective is to establish the impact of current commercial practices on weight recovery and melanosis formation.

This phase of the project can be subdivided into three studies that will be carried out in parallel:

a) Measurement of the temperature-time profile of standardized commercial processing

b) Determination of the heat penetration characteristics of different sized lobster.

c) Determination of PO activity, and substrate levels throughout the processing chain

Three research trips were carried between November 2001 and April 2002. The work was conducted at the MG Kailis Lobster Processing facility in Dongara WA.

a) Thermal profile of the standardized commercial processing
Discussions with processors have revealed that considerable differences in the time-temperature profiles used to process whole lobsters exist throughout the season and between processors. Therefore, for the purposes of this study a "representative" processing protocol was devised based on the average practice observed in the industry.

Lobsters were drowned in fresh water at ambient temperature for 20 minutes. They were then packed into baskets to give an average load of 100 kg per cook. The cooker was brought to the boil. The cook (100 kg of lobsters) was put into the cooker and cooked for a total of twenty minutes from point of entry to removal. Upon removal they were immediately plunged into an ice slurry bath and held there for another 20 minutes prior to washing, draining and packing. Each lobster was wrapped prior to packing and freezing to -40ºC.

Continued overleaf

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Temperature sensors were inserted into fourteen rock lobsters prior to cooking. Temperature probes were also attached to the outsides of each instrumented lobster in order to describe the immediate thermal environment of each lobster during the cook. The instrumented lobsters were packed into baskets in such away as to give a three dimensional array of temperature recording through out the cook. The probes remained in situ whilst the lobsters were cooled in ice slurries, but were removed immediately prior to freezing. The trials were repeated over 5 days and at 3 times during the season using size 'A' lobster.

Weight on receipt, after drowning and after cooking was recorded for each lobster. Also physical damage, sex, moult stage and initial phenol oxidase enzyme activity data was collected. Analysis of this data is on-going.

b) Determination of the heat penetration characteristics of different sized lobster

Cooks of size 'B' lobsters were profiled in the same manner as in part a as single cooks on each of 3 days at 3 times throughout the season. Weight on receipt, after drowning and after cooking was recorded for each lobster. Also physical damage, sex, moult stage and initial phenol oxidase enzyme activity data was collected. Analysis of this data is on-going.

c) Determination of PO activity, and substrate levels throughout the processing chain

One hundred rock lobsters were taken from a single catch. Haemolymph was collected from different groups of 20 lobsters upon receival, after holding for 48 hours, and before and after drowning. The hemolymph was analysed for baseline and total phenoloxidase activity, and total substrate concentrations (total phenols). Twenty lobsters were collected and held after cooking. Physical damage, sex, moult stage, weight on receipt, weight after drowning and weight after cooking was recorded for every lobster used. The experiment was repeated at 3 times throughout a season in an attempt to describe any seasonal variation occurring.

The aim of this experiment is to determine if steps in the processing chain may increase the risk of melanosis formation by inducing changes in levels of these parameters in lobsters prior to cooking. Analysis of this data is on-going.

Follow on work

In order to characterise the development of melanosis, and measure variations between treatments, digital image analysis will be used to objectively determine the rate and intensity of melanosis development occurring in all the lobsters used in the trials for Objective Two. This work is currently underway.

Time line

- Objective 1b (Anti-browning agents *in vitro*) to be completed by 31st December 2002.
- Objective 2 (Thermal profile of commercial cooking) should be completed by 30th June 2002.
- Objective 3 (impact of transportation) to commence November 2002

FRDC 2000/251:

Development of a Method for Alleviating Leg Loss during Post-Harvest Handling of Rock Lobsters.

Principal Investigator: Dr Glen Davidson
Co-Investigator: Wayne Hosking
Geraldton Fishermen's Cooperative Ltd.

Progress Report to the Steering Commitee

- Southern C zone sea trials of cold-stunning have begun aboard Shark Raider, operating out of Mindarie Keys and skippered by Glenn Byass. Initial results will be presented. Some technical difficulties were experienced during installation of the refrigeration unit and associated hardware. This experience has proved useful for making recommendations regarding the preferred installation aboard commercial vessels.
- Access to the CSIRO Marmion Marine Laboratory to carry out experiments investigating the effects of cold water stunning on female reproductive success and egg viability and growth and mortality of undersized lobsters was denied. An alternative site located at Jurien Bay was identified for this work. An experimental live holding system was built at the site in early 2002.
- With the assistance of a local commercial fisherman, 60 tar spot females were collected in February 2002 and were installed at the Jurien site. A small number of these animals proceeded to go to the "berry" stage. Most of these shed the eggs and one animal moulted, discarding the egg mass with the old shell. Since that time many of remaining females have moulted and shed their tar spots. Despite this, we continued to hold and feed the animals in the hope that some useful provisional information might be gathered. This has not occurred and no animals have successfully incubated eggs. At the time of writing this brief, out of 60 lobsters collected 8 have died and 42 others have moulted. The researchers will seek advice from the Steering Committee as to how to proceed.
- 225 undersized lobsters were also collected in early February 2002 and taken to the Jurien Bay site. A mass synchronous moult is generally believed to occur in February and the idea was to collect the animals and allow them to moult before commencing the experiment. In these types of experiments, the first moult after capture shows signs of being affected by capture stress, etc (ie. reduced moult increment, etc.). By allowing the animals to moult before commencing the experiment, the growth is effectively "re-set" to a more "normal" pattern. Moulting of these undersized began in March and has continued through to the present time. There has been some indication that it may have ceased in the last week. The animals have shown a high mortality rate that clearly coincides with the period of moulting activity. Gross observations of dead animals suggests that they are dying in the process of moulting – similar to the moult death syndrome reported in many culture situations. Cannibalism is not the cause of the mortalities.

The high mortality rate of these animals is of concern. Western rock lobsters are a very hardy species and the investigators have extensive experience with and success at maintaining them in captivity for extended periods.

The mortalities do not appear to be related to diet. Their diet has been comprised mainly of mussels, with some octopus and fish included. Mussels are generally regarded to be the best feed for rock lobsters in captivity.

Water quality is also unlikely to be the cause. The site is supplied from a seawater bore. When the site was first identified the owners supplied us with water quality data indicating the water was of high quality. In addition, the site is used for the culture of finfish and crustacean species. According to the owners, sensitive larval stages of fish species have been successfully reared at the site. Further water quality analyses are being carried out at present. The investigators will be seeking advice from the Steering Committee concerning these issues.

- The investigators have received a number of inquiries from boat builders, refrigeration engineers and other interested parties seeking advice on the development and construction of on board cold-stunning systems. The investigators have made every effort to advise interested parties accordingly. On the 2001 RLIAC Coastal Tour, the RLIAC Executive Officer, Tim Bray, stated that cold water stunning was not illegal under current legislation. The situation now exists where fishermen may freely implement on board cold-stunning. It is critical therefore, that we develop a coordinated approach to extending the results of our research to interested parties to prevent the adoption of inappropriate or ineffective practices. On the basis of the results from the first season's sea trials and upon our experiences from the current trials, we are confident we can provide accurate information. We propose to conduct a seminar for these interested parties, in particular targeting boat builders.
- Leg loss surveys have been conducted in South Australia and Tasmania. The survey in SA targeted fisher/processors. The survey in Tasmania was sent to all licensed fishers. The initial response was poor and Rodney Treloggen has conducted a follow-up on the survey.
- TAFI has submitted a final report for the leg loss survey work conducted by Stewart Frusher. This has been accepted.
- An application to FRDC to conduct cold-stunning tag and recapture study was approved in the 2002 round. The Midwest College of TAFE research and training vessel *Lady TAFE* was identified in the proposal as the vessel to be used in the research. TAFE is in the process of building a new vessel that is due to be commissioned in early 2002. We would like to use this new vessel for a number of reasons,1) the Lady TAFE is due to be sold and 2) TAFE have agreed to install a cold-stunning unit on the vessel during construction. The first period of tagging was planned for November 2002, we would like to discuss with the Steering Committee the possibility of delaying the start of the research, so that the first period of tagging will occur at the Abrolhos Islands in March 2003.

2001/255:

Quantifying and controlling hyper- and hyposaline-induced post-harvest leg autotomy in the western rock lobster.

Principal investigator: Wayne Hosking
Co-Investigator Dr Glen Davidson
Both of Geraldton Fishermen's Co-operative Ltd.

Project Objectives

1. Survey salinity concentrations of surface films on individual lobsters and on relevant contact surfaces on boats and within factories.
2. Describe the relationship between autotomy and exposure to seawater of various salinities for lobsters of various sizes and moult stages.
3. Quantify leg loss during industry standard freshwater "drowning" procedures.
4. Compare responses to ionic and non-ionic solutions to elucidate the potential role of other contaminants, and the possible nature of the receptors and stimuli.
5. Investigate the relationship between daily environmental conditions and levels of post-harvest leg loss.
6. Field-test practical solutions for hyper/hyposaline-induced autotomy and make recommendations to industry.

PROGRESS REPORT

It is estimated that post-harvest leg autotomy (or reflex leg loss) costs the western rock lobster (WRL) industry in excess of $3 million per annum (see previous article for details). FRDC 2000/251 was funded to investigate solutions for this substantial problem. One year into that project, a major discovery was made which has already revolutionised the way in which autotomy is understood and managed by lobster fishers and processors in Western Australia. Consequently this project, FRDC 2001/255, was commissioned to further investigate the phenomenon of hypo- and hypersaline-induced autotomy in WRL.

For reasons not fully understood, WRL can respond to contact with freshwater or concentrated seawater by autotomising one or more legs. The reaction to these stimuli can be almost instantaneous, and the stimuli are ubiquitous throughout the WRL industry. One kilogram of normal seawater contains approximately 36 grams of salt. The salinity level in the Indian Ocean, home to the WRL, is highly stable. However, due to evaporative concentration and standard industry processing techniques, captured lobsters regularly come into contact with waters of markedly different salt concentrations to that in which they have evolved.

Fishers and processors throughout the industry have long been aware of a correlation between hot, dry and windy conditions and high levels of autotomy, but no causal relationship had been established. It is precisely these weather conditions that lead to high evaporation levels from seawater films on boats, in

factories, on gloves and even on the lobsters themselves. Evaporation removes only pure water. The salt remains behind, resulting in increasingly concentrated seawater. It is contact with these hypersaline films that induces lobsters to drop legs, i.e. to autotomise.

Approximately 7 500 tonnes of lobsters are processed for the frozen export market each season in Western Australia. Before they can be processed, they must first be killed by immersion in tap water. Initial observations suggested that exposure to tap water may also induce autotomy. Considering the high volumes processed, even a relatively low rate of autotomy at this stage may be economically important.

Given these observations, the main objectives of this project are to:

- Define the relationship between salt concentration and the magnitude of the autotomy response during either surface contact or full immersion
- Survey the prevalence of freshwater and concentrated seawater on surfaces with which lobsters come into contact during the post-harvest process
- Test and develop practical and cost-effective solutions for the identified risks
- Extend this information to industry so that the maximum benefit of the research is realised

CONTACT WITH HYPERSALINE FILMS

In this experiment, a range of contact films were prepared, ranging from freshwater through to nearly 500% concentrated seawater. The experiment was designed to mimic lobsters coming into contact with grading tables or weighing tubs. The results, summarized in Figure 1 below, show a clear relationship between increasing salinity of the film and increasing rates of leg loss. The highest autotomy rate was 2 legs per lobster at 180 grams of salt per kilogram. Lobsters coming into contact with normal seawater films did not suffer appreciable autotomy. Interestingly, contact with freshwater films did not induce increased levels of autotomy, although later experiments showed that immersion in freshwater does have an effect (see below).

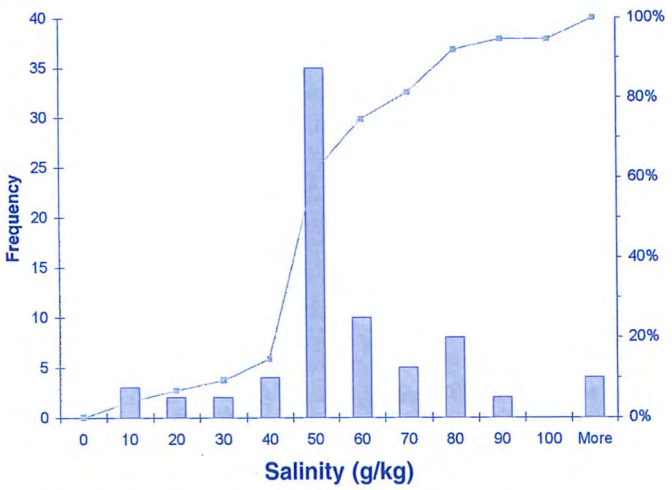


Figure 1: Autotomy rates are directly proportional to salt concentrations of contact surfaces.

Surveys of contact surfaces on boats and in factories have commenced to determine the prevalence of concentrated seawater films, and therefore the likely scope of the problem. Figure 2 summarises interim results of a factory survey. Surfaces sampled included grading tables, gloves, holding crates and weighing tubs. The results show that lobsters do come into contact with seawater films at concentrations that induce autotomy. The scale of this problem is likely to vary widely depending on prevailing weather conditions, factory design, and the degree to which the processor has already taken corrective measures. Similar surveys are being conducted on board fishing vessels.

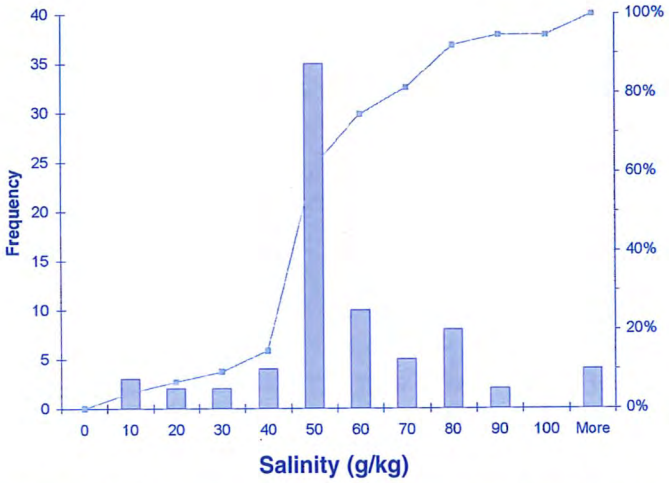


Figure 2: In this example, 25% of factory surfaces sampled had salinities in the danger zone.

Having now clearly identified the problem, what can be done about it? In most instances, the solution is obvious and straightforward. Reducing the potential for evaporation is the first step, and removing any build up of salt through a process of washing is usually simple (see Figure 3 below). The ability to measure the concentration of seawater films will enable a processor or fisher to monitor the success of any improvements, or the development of any problems. Reference to Figure 1 will show whether the approach has been successful, although in our experience, improvements are usually immediately obvious to staff and management.

Interestingly, a brief (~5 sec) cold water stun, correctly applied, largely prevents autotomy during contact with hypersaline films. On-board fishing vessels and elsewhere in the field, it may be impossible or impractical to prevent hyper-salinity, particularly on the lobster itself. In addition, contact with hypo- or hypersaline films is not the only trigger for autotomy in WRL. Therefore in many cases, there is currently no substitute for cold stunning.

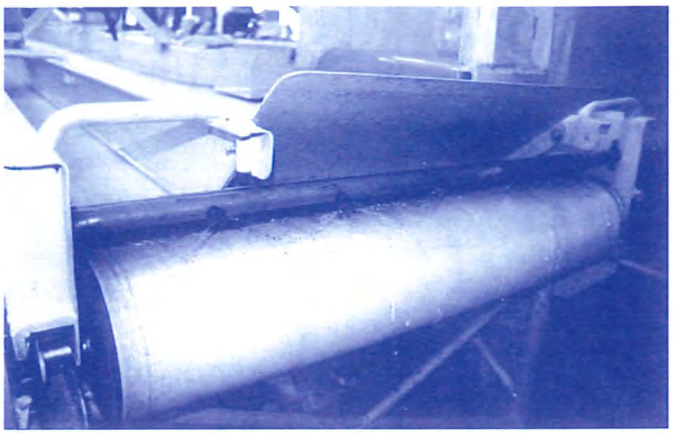


Figure 3: For many of the problem areas, the solution to salt build up is obvious and simple.

Here, seawater sprays constantly wash a revolving grading belt.

FRESHWATER IMMERSION

Lobsters were graded according to size and sex, and then placed in tubs filled with ambient temperature (18.5-25.5∞C) tap water, in a manner that ensured no autotomy occurred prior to immersion. After 50 minutes, all movement had ceased, the lobsters were carefully removed, and the amount of leg loss was recorded. The results are summarised below:

- A grade (350 to 500g): 9 legs per 100 lobsters
- G grade (1200 to 1500g): 6 legs per 100
- A grade males: 14 legs per 100
- A grade females: 21 legs per 100

A number of interesting trends are emerging from this ongoing work. The results suggest there may be a difference in autotomy rates between males and females, and small and large lobsters, although differences are not statistically significant. The key finding however is that immersion in freshwater does cause autotomy. More recent experiments suggest that even higher autotomy rates may occur at certain times, but even assuming a rate of loss of 10 legs per 100 lobsters, extrapolated across the industry, losses could be substantial. Initial indications are that most of this leg loss can be prevented by stunning lobsters in cold seawater prior to immersion in freshwater, and work is continuing in this area.

CONCLUSIONS

Exposure to hypo- and hypersaline seawater has been clearly defined as a substantial problem facing industry, and the later part of this project will focus on quantifying the benefits of potential solutions, and providing industry with the information it requires to make the necessary cost-effective changes. It should be noted that contact with hypo- and hypersaline seawater is not the only cause of autotomy, and removal of the offending films will not provide a complete remedy for all leg-loss. In many cases, there will likely be no substitute for cold-water stunning. Implementation of the techniques developed in this and the companion project are producing real benefits to industry through the prevention of post-harvest leg loss.

2002/237:

A code of Practice for Handling Rock Lobster

Principal Investigator: Richard Stevens
Address: WAFIC, PO Box 55, Mt Hawthorn WA 6915
Project Objective
To produce a Code of Practice for the handling of rock lobster

PROGRESS REPORT

The rock lobster handling Code of Practice project has started, with consultation on the script. At their request, copies of the script of the previous video were sent to Tasmanian, South and Western Australian stakeholders.

The stakeholders now have an idea of how a script is constructed, and how shot selection follows the script. They will be able to say exactly what they want in the script, following the logical sequence of the previous video.

As there was no video of handling tropical rock lobster, fishermen from far-North Queensland have requested that the PI spend some time with them on the fishing grounds, to work out how the video can best be constructed. This will happen during the full moon in late August.

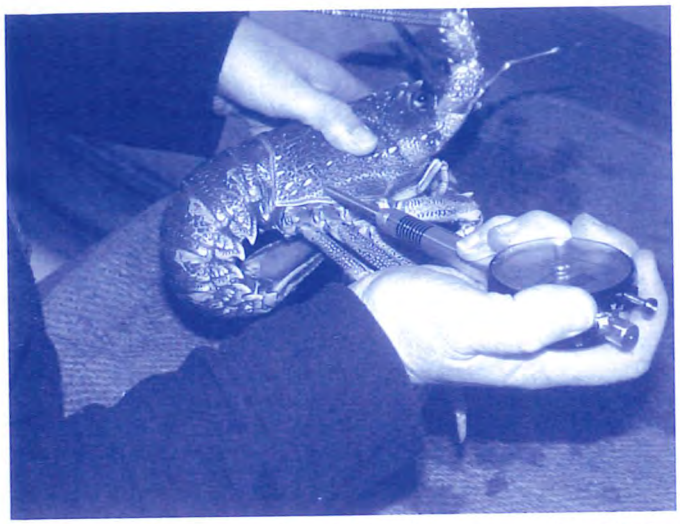
The videos will demonstrate only best practice, and will be useful as a marketing, as well as a training tool. There is steady international interest in Australian rock lobster, but strong competition from countries such as Cuba and Brasil.
If the video gives the industry an advantage then every opportunity to exploit that advantage needs to be taken. An example of how this can be done is shown in the photograph below. The next time will be at Brussels, during the European Seafood Exposition (6-8 May 2003). Australia, led by WA, is making strong representation to remove the 15% import tariff on rock lobster in this market, and tools such as the MSC certification, and the Rock Lobster Code are essential to underpin this, and deflect the tendency to replace tariffs with non-tariff trade barriers.

Ian Finlay, WAFIC Chairman and former rock lobster fisherman, explaining on Japanese television the world first environmental certification of the Western Rock Lobster Fishery by the internationally recognised Marine Stewardship Council. The interview took place at the 4th International Tokyo Seafood Expo, in July.

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

New Projects

Funded by FRDC



Josephine Walker measuring shell hardness on a male southern rock lobster prior to the moult.

2002/238:

Quantification of shell hardness in southern rock lobster

Principal Investigator: Dr Caleb Gardner

Address: Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Marine Research Laboratories
Co-Investigator Dr Richard Musgrove

Project Objectives

1 To calibrate the rate of change in shell hardness before and after the moult of southern rock lobsters relative to lobster size, sex, region and temperature.

2 To identify the region of the exoskeleton that is most suited for measuring hardness.

PROGRESS REPORT

Moulting in commercial crustaceans is an important biological process and often influences the decisions we make in designing research projects, or managing fisheries. The behaviour and catchability of animals can change around the moult, as can their marketability. For these reasons, the moult stage of individual animals is often recorded in catch sampling projects with rock lobsters, although the methods we use are generally quite rough. For instance, researchers may measure the amount of fouling on the shell or test the flexibility of the carapace with their hands. This sort of measure is quite subjective and we frequently encounter problems with this data because of inconsistencies between individuals – what one person calls hard-shelled, another may call soft-shelled. This sort of problem has recently hindered research in Tasmania on the effect of extending the fishing season into September, when there is a lot of moulting activity in males from some regions. Processors and exporters of lobsters in both Tasmania and South Australia are concerned about the effect of export of soft-shelled lobsters on their markets during these moulting periods.

We are aiming to develop tools for rapidly measuring the shell hardness and moult stage of lobsters, for both research and industry application. We are focussing on the use of "durometers" tools designed for measuring the hardness of plastics. We will be testing different configurations of durometers and relating our measures of shell hardness to the moult stage of animals of different sizes and from different regions.
An important part of the project will be "calibrating" our durometer measures against the level of shell hardness that processors consider acceptable for export.

The project is only just commencing. At this stage we are beginning to obtain animals and construct holding systems. One interesting development is that the suppliers of our "durometers" are working in with the project and are custom-building a "southern rock lobster durometer" with a working range designed specifically around the hardness of the shell of a legal-sized lobster.

2002/239:

The effect of on board cold water stunning on the survival and growth of caught and returned western rock lobsters *(Panulirus cygnus)*.

Principal Investigator: Wayne Hosking
Geraldton Fishermen's Cooperative Ltd

Project Objectives

1 To determine the effect of commercial capture with or without cold-stunning on the survival and growth of returned protected western rock lobsters.

2 To observe and film in the wild the behaviour of western rock lobsters caught and returned with or without cold-water stunning.

The application to FRDC for funds to conduct a tag and recapture study to investigate the effect of on board cold-stunning on the survival of commercially caught and returned breeding female and undersized rock lobsters was successful. The Project Agreements have been exchanged. Planning has begun for this work which is due to start in early November, prior to the opening of the new season.

Special Note

Relevance and Clarity of Research Results

Two recently completed Final Reports have raised questions about the relevance of their findings to actual industry practices; and their research results may be unintentionally confusing.

The two projects were 96/ 344 by Professor Louis Evans and 96/345 by Drs B. D. Paterson, G. D. Davidson and P. T. Spanogue. There two linked projects were both made under the title of "Physiological studies of stress and morbidity during post-harvest handling of western rock lobster, *Panulirus cygnus*." However, project 96/344 concentrated on standard autopsy techniques and immune system competency and project 96/345 on physiological stress indicators.

Both of these projects were initially set up to try to assist the export trade in live rock lobsters. Their basic aim was try to identify what factors caused stress in rock lobsters, and then to try to determine both methods of measuring this stress, and methods of alleviating it where ever possible. This was eventually more simply defined as to attempt to have as many as possible of the lobsters delivered to the factory in a condition fit for export as live lobsters. By this is meant, whole (no limbs or other appendages lost) and with sufficient vigour to be classified as suitable for packing to be exported. Not all of the lobsters in this condition might be exported. This would be a decision by the factory management. Some lobsters might be sold in other forms than live. However, it was recognised that a lobster in "good condition" yields a better weight percentage return, e.g. after cooking for the "whole frozen lobster" export market.

The projects had specific objectives defined at the commencement, and the Final Reports provide ample evidence of the researchers efforts to obtain the answers to these questions. However, in some cases events have moved on, in others the results, which are communicated on a regular basis as the project progresses, have already been adopted or been taken account of by industry. In addition there is sometimes a communication problem. The results may not presented in a manner that is relevant to or understood by industry.

Stress in lobsters is a complex process. In fact Professor Evans has pointed out in a presentation to the Workshop in Cairns in May 2002, that as a result of her project findings there appear to be two kinds of stress! The studies, which were conducted, were lengthy and comprehensive. However, not all of the results were unequivocal. As pointed out by Paterson and his co-workers, using discriminate analysis of several blood test results, it was possible to correctly classify the fate of 80-90% of lobsters". However none of the stress indicators that they examined "were simple enough to apply in a factory context". Industry finds such statements unclear. It really needs a simple conclusive statement that the tests are not useful as predictive tools.

Scientists extrapolate from the results of their studies to provide what they hope is useful advice to industry. In this case they drew attention to possible changes that might be made to the "code of practice", and made a specific reference to transport of lobsters on carrier boats. What they had found was that lobsters transported while submerged in water were likely to be the least stressed on arrival at the factory. The recommendations were based on clear results. During the studies, virtually all of the lobsters that died in the factory tanks came from non-submerged treatments, i.e. held in air with or without spray treatments. In particular, the spray treatments used during transport of some lobsters produced high mortality and levels of rejection when the lobsters were first graded (for export), and high levels of mortality occurred in these lobsters during later submerged storage in the factory.

Their recommendations were quite reasonable. The results had already been accepted; and some carrier boats have been fitted with aerated flow-through tanks to ensure submerged transport of the lobsters under excellent conditions. However, the final statement,"sprays fail to benefit lobster in air", has been pointed out to be incorrect. There were situations on some carrier boats that were unknown to the scientists. In Western Australia during the summer the air temperatures can reach 48°C, and this is often accompanied by hot dry winds. Seawater sprays under these conditions have been found to be beneficial for temperature control. The recommendations were made in good faith, and it is always necessary for industry to interpret research results and apply them in the most appropriate manner for their operations.

Overall the studies by Professor Evans have shown that those lobsters that do die in the factories often have bacterial infections, which cause organ failure. The source of the bacterial infections is unknown but was not related to a pre-existing condition in the lobsters prior to capture; hence it is the result of handling etc. of the lobster after capture on their way to, in the factory.

Bruce Phillips

For further clarity on the priorities or any other information please contact one of the following:

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FISHERIES RESEARCH & DEVELOPMENT CORPORATION

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Publications

The following are the papers presented by members of the Subprogram at the Workshop in Cairns in May 2002:

Physiological studies on stress and morbidity during post-harvest handling of western rock lobsters: *Prof Louis Evans (Curtin University of Technology, WA)*

Physiological stress indicators: *Dr Brian Paterson (Queensland Department of Primary Industries)*

Optimising water quality for live holding of rock lobsters: *Dr Bradley Crear (TAFI)*

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Optimising water quality for live holding of rock lobsters: *Dr Bradley Crear (TAFI)*

The Lobster News is funded by the Fisheries Research and Development Corporation.

Haemolymph pCO_2 and totalCO_2 concentrations did not recover to those seen in unexposed animals within 72h of recovery. This suggested that the carbonate/bicarbonate buffering is not a critical element of the acid-base buffering in lobsters.

Exposure of lobsters to 60 mg/L NH_4Cl in conjunction with 10 mg/L NaNO2 for 24 h then recovery in clean water resulted in an elevation in haemolymph pH which then recovered to pre exposure levels. Both pCO2 and total CO2 did not decline during recovery but were lower than those seen in unexposed lobsters. Thus indicating a strong respiratory component in the acid base control of lobsters during ammonia exposure. Exposure of lobsters to hypoxia (50% air saturation) concurrently for 3h prior to recovery from a 24h exposure to 60 mg/L NH_4Cl resulted in a response similar to that for lobsters exposed to $NaNO_3$ as well as NH_4Cl.

Analaysis of haemolymph ammonia concentrations and urea concentrations are essential for assessing the interaction of the acid-base effects of ammonia exposure. These are currently being analysed and the data compiled. In a separate study, we have discovered that the southern rock lobster can produce significant amounts of urea and switch to its excretion when environmental pH and ammonia concentrations are unfavourable for excretion by the usual NH4+ excretion pathways. Moreover, this process of urea excretion appears to be labile and can be switched on and off depending upon the internal/external ammonia concentration of ammonia, and internal/external pH. We are currently analysing the tissues and haemolymph samples from the previous study to examine the internal ammonia and urea concentrations of lobsters exposed to 60 mg/L NH_4Cl, with $NaNO_2$ and in response to hypoxia. This additional work will give us an excellent understanding of not only what concentrations are toxic to lobsters, but also how environmental parameters interact to enhance or reduce ammonia toxicity in lobsters. This will significantly advance our understanding of lobster health particularly under conditions of reduced environmental water quality.

VARIATIONS TO PROJECT:

A 12-month extension for the completion of this project is requested. The project was to have been completed in September 2002 but has been delayed by the departure of the PI. The final report will be available in time for the workshop in Perth on the 15th September 2003.

FRDC 2002/238:

Rock lobster post-harvest subprogram: quantification of shell hardness in southern rock lobster.

Principal Investigator: Caleb Gardner

Overall Project progress:

The project has proceeded as planned.

Experiment 1, to identify the **location** on the exoskeleton most suited for measuring shell hardness, has been completed.

Experiment 2, to quantify the effect of **temperature** on changes in shell hardness, has been completed for males with the work on females to be conducted over the next few months.

Experiment 3, to quantify the effect of **region** on changes in shell hardness, has been completed for males with the work on females to be conducted over the next few months.

Experiment 4, to quantify the effect of **sex** on changes in shell hardness, has been completed for males with the work on females to be conducted over the next few months.

Experiment 5, to correlate quantitative measures of lobster hardness with commercial **processor standards**, has been completed.

Overall, durometers appear to provide a useful tool for applications where the shell hardness of a population needs to be assessed. This is typically the case for research uses. The information that has been collected is already of value in describing the time taken for the shell to harden and the effect of region and temperature on this process. This provides a useful guide in the planning of closed seasons.

However, there appears to be substantial variation, which limits the use of durometers for screening of individual animals, which is required for extension to industry. Reducing variation in readings is essentially an engineering problem. In addition, some performance problems have been identified in industry trials. Internal components tend to become clogged by repeated exposure to salt water. South Australian fishermen and processors have also expressed concern about the time taken to make each measurement and the extreme precision required to minimise variation between measurements. The tool is also not robust enough for use by fishermen on lobster boats, reducing the probability that this will be taken up by industry.

However, through the course of the research work we have identified an alternative system for measuring shell hardness across a broad area of the shell, rather than just the region in contact with the base of the durometer. A gauge to suit this application would have to be custom built. One option that we are investigating is to involve the engineering schools of the Universities of Tasmania and South Australia. We have supervisors interested in the project so this looks set to proceed. This development may shift the IP status to potentially commercial.

POST-HARVEST SUBPROGRAM
ROCK LOBSTER
FRDC

THE LOBSTER NEWS

Volume 5, 2003

rom the ubprogram eader

has been a busy year with grant pplications occupying quite a lot of eoples time.

he good news is the Subprogram has een renewed for three years from 1 July 003, subject to an external review at the nd of the first year.

new project on southern rock lobsters as been added to the Subprogram and ve welcome John Carragher and Michael Brooks to the Subprogram and Richard Musgrove on his return.

The big events for 2003 are in Perth. The oint Rock Lobster Enhancement and Aquaculture and Post-Harvest Annual Workshop will be held on 16 September, followed by the Rock Lobster Congress 3, and the Seafood Directions Conference on 17 and 18 September.

Bruce Phillips
Subprogram Leader.

Egg-bearing Western Rock Lobster female housed in bucket fitted with filter mesh "windows" for collecting larvae.

inside this issue:

To conduct research to increase the value of the rock lobster catch for Australia through improvements in post-harvest practices.

Research News

2000/252:

Optimising water quality in rock lobster post-harvest processes

Principal Investigator: The FRDC Board has approved the change from Dr Brad Crear to Dr Stephen Battaglene. Dr Stephen Battaglene is currently working on the ammonia booklet and we look forward to its release at the congress in September 2003.

ORIGINAL MILESTONE DATE AND TITLE:

Milestone 1, 30 June 2002.
Pamphlet on ammonia produced (Objective 4).

REVISED MILESTONE DATE AND TITLE:

Milestone 1, 15 September 2003.
Pamphlet on ammonia produced (Objective 4).

PROGRESS AGAINST MILESTONE:

The PI for this project Dr Brafley Crear resigned from TAFI on the 30 th October to take up a position in WA with the Geraldton Fisherman's Co-operative. Work on this milestone is in progress. The pamphlet is being designed in the same form as the oxygen booklet (Objective 1), which is currently in publication. A delay in the finalising of that booklet has meant a delay in the writing of the booklet (pamphlet) on ammonia. A draft of the ammonia booklet is being circulated for comment.

FINAL REPORT

The final report for the project is in production. All objectives have been met with the exception of the investigations into the physiological consequences of exposing lobsters to ammonia (OBJECTIVE 3). This research was undertaken by Drs Crear and Powell.

To date we have examined physiological acid-base responses of Southern rock lobsters (*Jasus edwardsii*) during and following acute exposure to ammonia in the form of ammonium chloride. Exposure of lobsters to a range of concentrations of NH_4Cl for 24h showed that exposure of up to 40 mg/L resulted in a maintenance of haemolymph pH and low levels of haemolymph CO_2 both as HCO_3^- and pCO_2. Concentrations above 40mg/L, there was a significant acidosis attributable to increases in pCO_2 suggesting that the response to high levels of ammonia was likely a reduction in respiratory ventilation.

Exposure of lobsters to 60 mg/L for 24 h then placement into clean (ammonia free) water resulted in a rapid decline in haemolymph pCO_2 and total CO_2 below that measured in unexposed lobsters. This was consistent with the maintenance of blood pH.

Continued overleaf

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

PROGRESS AGAINST MILESTONE:

Trials with males involving comparisons between regions, size and temperature completed. Each animal was repeatedly sampled at around 10 day intervals for: (a) shell hardness – with 2 durometer types x 5 locations on the exoskeleton; (b) blood serum protein; (c) blood colour.

Results from durometer trials were contrasted with classification of shell hardness by commercial processors (Experiment 5, Figs. 1-3). This experiment showed that even with extensive experience in grading lobsters by hand, there was a great deal of variation in processor classifications and that these processor grades were a poor indicator of time before/after moult. Overlap was greatest for lobsters pre-moult where there was no significance difference in time to moult between "hard -" and "soft-shelled" groups (P>0.3).

Analysis of the remaining data has not been completed, as this requires additional data from females. Preliminary results for all animals grouped are shown below. These results include data from all Tasmanian animals from all sites so some of the scatter may be due to treatment effects such as site and size. By incorporating these factors into analyses, scatter should be reduced and a clearer picture may emerge. Very similar trends are visible for South Australian lobsters (graphs not shown).

Even at this coarse level of analysis it can be seen that the level of variation of durometer readings is similar to that obtained from the other measures. That is, the durometer measure appears to provide a similar level of precision for estimation of the moult cycle. When used in conjunction with the other measures, precision is increased, as the shape of the function around the moult cycle is different for each index.

Patterns in change in shell hardness detected with the durometer appear to be of value in tracking change within a population. For instance, information shown on the time taken to harden post-moult is of value for setting closed seasons designed to exclude soft-shellers from market. As this is a quantitative measurement, the effect of site and size on hardening rates can also be examined.

Although the technique appears promising for population level analyses, variation from all measures is too great to permit conclusions to be drawn on individual animals, a level of discrimination required for grading of animals for export. Upcoming work will extend the durometer analyses to females, but we have also recognised the need to investigate alternative engineering solutions and are pursuing this.

Fig. 1. Industry acceptable levels of lobster hardness were estimated with the help of processors. Ken Smith of A. Garth Seafoods Pty. Ltd and Cindy Yuen of Soareast Pty. Ltd classify animals by hand into "soft" and "hard" shell categories. Only those classed as "hard" would normally be exported.

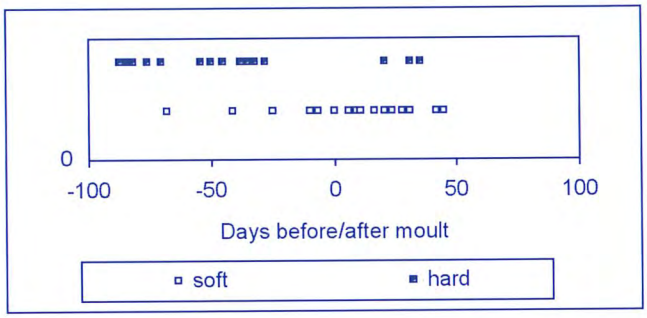

Fig. 2. Shell hardness classification by a commercial processor in relation to time before and after the moult. These animals were tested by squeezing the carapace and tail by hand. Note that there was considerable overlap between soft- and hard-shelled grades.

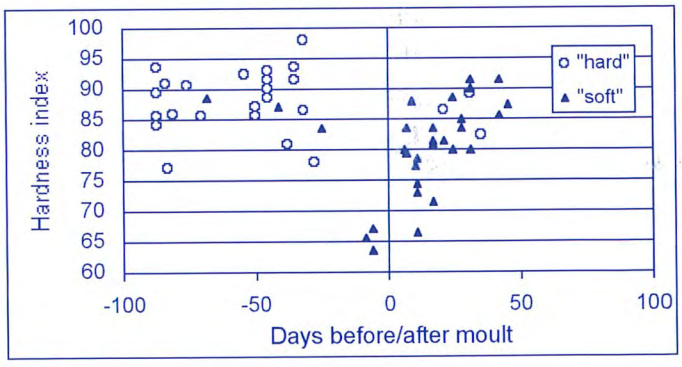

Fig. 3. Comparison between hardness measurements taken by durometer (hardness index) and the grades given by a processor using manual "squeeze" tests of the carapace and tail (soft and hard classes).

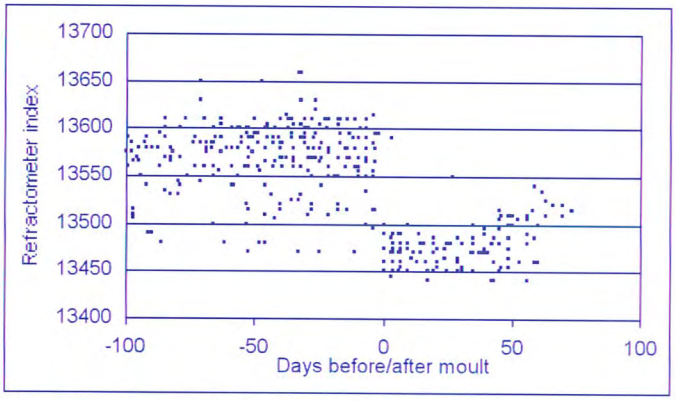

Fig. 4. Change in blood colour index through the moult cycle.

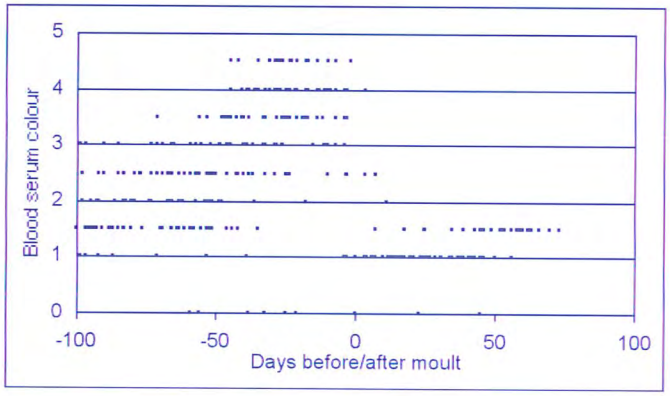

Fig. 5. Change in blood serum protein (refractive) index through the moult cycle.

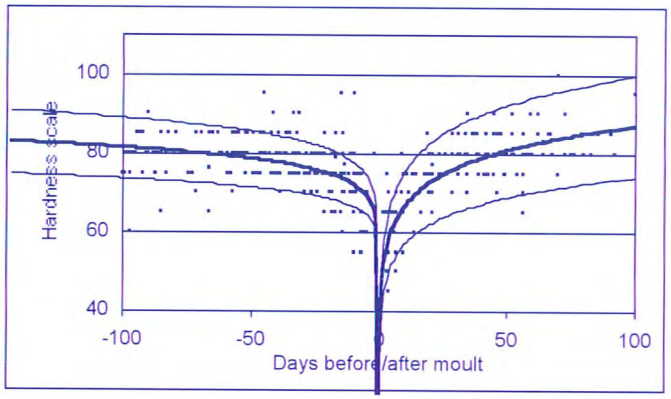

Fig. 6. Change in shell hardness at site 3 (side of carapace) through the moult cycle with fitted log curves and 99% confidence limits

FRDC: 2001/235

Striking a balance between melanosis and weight recovery in Western Rock Lobster.

Principal investigator: Mrs. Hannah Williams

OVERALL PROJECT PROGRESS:

- Milestone 1b: Experiments addressing Objective 1B have been completed.
- Milestone 3: Fieldwork for Objective 3 has been completed. Laboratory analysis continues. Analysis of the results is ongoing.

PROGRESS AGAINST MILESTONE:

Objective 1b

- To establish the impact of heating on the enzyme in the presence of a range of antibrowning agents.

Antibrowning agents are compounds that interact with the enzyme or its substrates and/or the products of reaction to prevent the formation of the pigments responsible for melanosis. When selecting the antibrowning agent to be used several constraints must be considered. The agent must be:

- Non-toxic, and permissible for food use
- Ideally be perceived as "Natural"
- Cheap
- Relatively easy to apply
- Have no negative impacts on organoleptic characteristics of the food.
- And above all effective.

Having taken these factors into consideration, four anti-browning agents were selected based on their mode of action:

- Ascorbic acid – Reducing agent, oxygen scavenger
- Citric acid – chelating agent, acidulant
- 4-Hexylresorcinol – enzyme inhibitor
- Carbon dioxide (CO2) – inactivator

When determining the concentration of antibrowning agent to be used several factors were considered:

- recommended dosage rates from similar studies
- Legal constraints such as allowable residue limits

Previous work has shown that a low level of the active enzyme was present at all times in the lobster hemolymph. Throughout this study, this residual activity is designated as the **baseline activity**. Addition of trypsin to the reaction mixture has been shown to result in activation of the inactive pro-enzyme form (Smith & Söderhäll 1991). Throughout this study, this has been designated as the **total activity**.

For each concentration of antibrowning agent, hemolymph was collected from a lobster into a chilled tube, and held on ice. A sub-sample was taken to determine initial PO activity levels. Hemolymph was then mixed with a set concentration of anti-browning agent (in buffer) in each of four test tubes. A control series without antibrowning agent was run in parallel. The tubes were mixed thoroughly using a vortex mixer and placed a preheated water bath at 70°C for 30 minutes. One tube was removed from the bath at each time interval and plunged into a bed of ice. Iced buffer was added and the contents of the tube mixed thoroughly. Baseline PO activity and total PO activity were then determined spectrophotometrically.
Every concentration of each antibrowning agent was evaluated in triplicate.

Solutions were heated at 70°C as previous work (Objective 1) had shown that at this temperature heat activation of the enzyme would occur in the first five minutes. It was also apparent that heating for 30 minutes at 70∞C is not enough

to ensure significant deactivation of the enzyme therefore any deactivation effect noticed will be due to the impact of the antibrowning agent on the enzyme.

A modification of the method was used in the evaluation of CO_2. Firstly CO_2 was bubbled through the buffer solution for 30 minutes at a rate of 50ml/minute. The solution was held on ice for this period and pH was measured at the beginning and end of testing. Hemolymph was added and the solution sub-sampled. A control series was run in parallel without CO_2 addition. Baseline PO activity and total PO activity were determined spectrophotometrically. The trials were repeated with the hemolymph being added to the buffer and then CO_2 bubbled through for 30 minutes while the solution was held on ice. A control series was run in parallel without CO_2 addition. Baseline PO activity and total PO activity were determined spectrophotometrically before and after the CO_2 addition. The solution was then divided between four tubes and heated at 70°C for thirty minutes. One tube was removed for each time interval and baseline PO activity and total PO activity were determined.

ASCORBIC ACID

Ascorbic acid and its derivatives are leading GRAS (Generally Recognised As Safe) antioxidants for use in food products. However there are some problems with its use in that;

1. It has been reported that low concentrations of ascorbic acid may activate the PO enzyme (McEvily & Iyengar 1992);
2. Ascorbic acid may become depleted by the action of PO since ascorbic acid is irreversibly oxidized during the process and activity can recommence once the ascorbic acid is used up (Kim & Marshall 2000); and
3. Ascorbic acid may also be destroyed by increasing temperatures over time since it is quite heat sensitive.

However these factors must be balanced against the fact that ascorbic acid has wide consumer appeal due to its vitamin functionality (Vitamin C), it is also relatively cheap and readily obtainable. The maximum residual concentration of ascorbic acid allowed by law in seafood is 400ppm (0.4 mg/ml) (Australian New Zealand Food Authority 2000). Therefore the effectiveness of ascorbic acid was tested at this concentration and at one-quarter that strength (100ppm or 0.1 mg/ml).

The control showed initial heat activation up to 10 minutes followed by a gradual reduction in activity as time increased. At 400ppm, ascorbic acid inhibited the baseline activity of the enzyme completely.

Comparison of total activity in the control series with that shown by the 100ppm and 400ppm solutions at the same temperature clearly shows that the addition of ascorbic acid does not have a significant (P=0.05) impact on total PO activity.

From the data, it appears that ascorbic acid does not significantly affect the activity of polyphenoloxidase in the western rock lobster. Since the inactive form of the PO enzyme, prophenoloxidase, is still present in the product and could be activated by non-enzymatic mechanisms at either concentration it is concluded that ascorbic acid is not a suitable antibrowning agent for use by the western rock lobster industry.

CITRIC ACID

Citric acid possesses dual activity against PO as an acidulant and a chelator. As a chelator it binds with the copper ions at the active site of the enzyme rendering it inactive. The acidulant effect requires the use of a concentration high enough to drop the pH below 3 (Kim & Marshall 2000). Citric acid has high consumer appeal due to its perceived natural origin, is very cheap and readily available. Typical dosage rates in antibrowning treatments range from 2mg/ml to 10mg/ml so a wide range of concentrations was tested.

After heating, activity was detected in solutions with concentrations less than 1mg/ml. Solutions of higher concentration showed no activity. At 0.2 mg/ml there was no significant difference (P= 0.05) between the control and the citric acid solution for total PO activity, however baseline activity was significantly (P= 0.05) reduced. The pH of all solutions were measured and were not below 4 with the exception of the highest concentration, pH = 3.4. Previous work has shown that pH greater than 3 will not impact on PO activity (Kim & Marshall 2000), therefore any effect noted can be attributed to the chelation of the copper ions by citric acid. Temperature had no impact on the effectiveness of the agent.

From these results it can be concluded that citric acid is effective against PO in rock lobster hemolymph and maybe effective in prevention of melanosis in western rock lobster.

4-HEXYLRESORCINOL

4-hexylresorcinol was originally derived from figs. It has many uses in the cosmetics industry and is a GRAS substance. It has been shown to be a very effective PO inhibitor at low concentrations and is widely used in some branches of the seafood industry as a component of an antibrowning solution called "Everfresh" which is 5% 4-hexylresorcinol. At the recommended dose this equates to a dose rate of 87mg/l or 87ppm of 4-hexylresorcinol. While 4-hexylresorcinol is relatively expensive the low dosage rates and potentially low residual rates favour its use.

Several concentrations were screened to determine the lowest concentration that effectively inhibited PO activity starting with the dosage rate recommended for Everfresh. All concentrations were effective against the baseline PO activity however it appeared that residual total PO activity existed at concentrations less than 20 ppm. The level of activity was at the limit of detection (5 Units) and therefore may not be significant.

From these results it maybe concluded that hexylresorcinol should be effective against PO activity during western rock lobster processing.

CARBON DIOXIDE CO_2

Carbon dioxide CO_2 is a colourless, odourless gas. It is very cheap, readily available and leaves no residue in the tissues of treated foods. The use of CO_2 has a high degree of acceptability, as it would require no special labelling on packaging and poses no health threat to any individual. It has been suggested that elevated levels of CO_2 increase the susceptibility of PO to the impact of heat thus enabling reduced cook times (Chen et al. 1993). This idea has been untested until this project.

Both methods of exposure to CO_2 resulted in complete inhibition of both forms of PO activity. Measurement of pH before and after carbonation showed that in all carbonated samples pH dropped to 5.5 ± 0.3. Previous work has shown that this drop is insufficient for pH to impact on PO activity (Kim & Marshall 2000) therefore the reduction in PO activity was due to the inhibitory effects of CO_2. No pH drop occurred in control samples. The control samples also showed the normal patterns of heat-induced activation and reduction in PO activity over the same time frame.

From the results obtained it can be concluded that carbon dioxide possesses very good potential for use as an antibrowning agent in the western rock lobster processing industry.

OUTCOMES

The aim of these experiments was to determine the minimum concentrations of the various agents required to achieve inhibition of the PO enzyme. The identified concentrations for Citric acid, CO2 and 4-hexylresorcinol will be used as target concentrations in experiments addressing Objective 4 – Use of antibrowning agents in processing. Expected concentrations obtainable within lobsters post-drowning will be calculated based on water uptake figures obtained in Objective 2. These values will then be compared to actual uptake of agents achieved in drowned lobsters and the effectiveness of the achieved levels for the prevention of melanosis. Melanosis formation will be monitored using comparison to control samples and digital analysis.

PROGRESS AGAINST MILESTONE:

The planned programme for this objective called for three field trips to be run spanning the season to identify any seasonal effects, however the trials in December had to be aborted due to problems with the setting up of the pilot plant and simulations. Therefore modification of the experimental plan was required, as the trials would not span the season as previously intended. Alternatively four repeats of the simulations were conducted over a 4-day period

Large tanks capable of holding two prawn baskets were set up to simulate one of three transportation methods (Fig. 1).

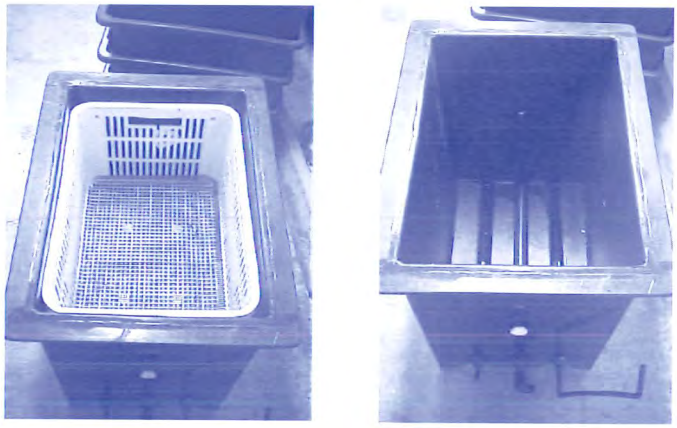

Figure 1: Tanks used in transportation simulations

The tank simulations used were:

- Submerged in flow through ambient temperature seawater with aeration (live tank on boat)
- Spray ambient temperature seawater, flow through at 11 litres/minute (carrier boat); and
- Recirculating cooled (15°C) spray at 11 litres/minute (spray truck)

A fourth simulation was set up using a commercial truck system to provide still air, cooled to 15°C (dry truck).

Two hundred lobsters from the previous day's catch were randomly subdivided into subgroups of 50. Blood samples were taken from 20 lobsters in each group, which were tagged and then randomly distributed throughout a basket, and the lid clamped on. As soon as basket was ready it was placed in one of the four treatments for five hours. Water quality (ammonia, oxygen saturation and pH) and temperature were monitored throughout the trials. Humidity was also logged in the still air treatment. On completion of the treatment the same lobsters from each group were bled and then all the lobsters were processed using the standardised processing protocol and frozen. The blood samples were analysed for total and basal PO activity immediately after bleeding and sub-samples frozen for later ammonia and total phenol determination. Analysis of the blood samples and data collected is continuing at present.

For references to any of these materials please contact Hannah Williams on Hwillams@curtin.edu.au

Updates/ New Projects

2003/242

Rock Lobster Post-Harvest Sub-program: Determining flesh quality attributes of under-valued large Southern Rock Lobsters.

Principal Investigator: Dr John Carragher

The FRDC is helping to fund a new initiative to identify the flesh characteristics of large lobsters. Currently, 17% of SA's harvested lobsters are over 1.5kg equating to 470 tonnes a year. Large lobsters are usually sold for less under split beach prices. It is likely that the lack of information on fundamental flesh quality characteristics was limiting the development of alternative high value products. This project will quantify flesh quality attributes of different sizes and shell colours throughout the year and from different locations. It will also investigate variations in flesh quality from lobsters held in commercial holding tanks over time and fed control diets. Aspects of tissue biochemistry, product taste, texture, colour, smell and shelf life will be used in this project to identify changes in flesh quality. These indicators will be correlated with feedback from a sensory panel to gauge significant changes in flesh quality.

The principal investigator of this project is Dr. John Carragher who is currently working with the Tuna industry to refine husbandry methods to increase the quality of their sashimi product. The co-investigators are Dr. Richard Musgrove (SARDI), Rodney Treloggen (TRLFA) and Kym Redman (SARLAC). Michael Roberts (photographed) will be working on the project full time and will use the research as a basis for his PhD at Flinders University. This project is also funded with in-kind contributions by Ferguson Fisheries Pty Ltd based in Adelaide.

2002/237

A code of Practice for Handling Rock Lobster

Principal Investigator: Richard Stevens

By the end of April, video footage had been taken in Tasmania, Apollo Bay, Victoria; Port Lincoln, South Australia, and in the Abrolhos islands, Geraldton, and Dongara in Western Australia. Further footage will be shot in Adelaide and Fremantle before the two best practice videos are compiled. Video footage contains a large number of interviews with skippers and deckhands, which will be used extensively in the final version of the video. The written code will now include a schedule fro Good Manufacturing practice, based on the Hygiene Certificate produced by the Western Rock Lobster Council and Western Rock Lobster Development Association in conjunction with AQIS.

Adherence to the GMP will ensure that vessels meet their obligations under the Primary Production and Processing Standard, currently under development. The Hygiene Certificate complies with EU legislation.

For further clarity on the priorities or any other information please contact one of the following:

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SEPTEMBER 2003 EVENTS

FRDC Rock Lobster Post-Harvest Subprogram

Rock Lobster Congress 3 | Seafood Directions 2003

MONDAY 15TH

RLPHS/RLEAS COMBINED ANNUAL WORKSHOP

Esplanade Hotel, Fremantle (Island Suite) | 9am – 5.30pm | Info: emmaphil@ozemail.com.au

ROCK LOBSTER 2003 CONGRESS WELCOME RECEPTION

Esplanade Hotel, Fremantle (Poolside) | 6pm – 8pm | Info: rocklobster.wafic.com

TUESDAY 16TH

ROCK LOBSTER 2003 CONGRESS

Esplanade Hotel, Fremantle | 7.30am – 5.30pm | Info: www.rocklobster.wafic.com

SEAFOOD DIRECTIONS WELCOME DRINKS

Maritime Museum, Fremantle | 5.30pm – 7.30pm | Info: www.asic.org.au

THE GREAT ROCK LOBSTER TASTING DINNER

Fremantle Sailing Club | 7.30pm – 12am | Info: www.rocklobster.wafic.com

WEDNESDAY 17TH

SEAFOOD DIRECTIONS CONFERENCE

Hyatt Regency, Perth | 8.45am – 5pm | Info: www.asic.org.au

THURSDAY 18TH

SEAFOOD DIRECTIONS CONFERENCE

Hyatt Regency, Perth | 9am – 4.30pm | Info: www.asic.org.au

2003 AUSTRALIAN SEAFOOD INDUSTRY AWARDS DINNER

Hyatt Regency, Perth | 7pm – 11.30pm | Info: www.asic.org.au

MAILING LIST

This Newsletter is distributed widely. However, if you would like a personal copy mailed to you please contact the Subprogram Secretary Emma Phillips by email at emmaphil@ozemail.com.au.

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

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Appendix 5

ANNUAL OPERATING PLAN – 2002

Rock Lobster Post-Harvest Subprogram

Dr Bruce Phillips

A) ACTIVITY DESCRIPTION FOR LAST 12 MONTHS

i) SECTOR PROGRESS

The demand for live lobsters to overseas markets continues to be maintained/or increased.

The demand for all lobsters to be delivered to the factory in a condition suitable for export continues, because such lobsters provide higher yields, and therefore increased profits.

ii) MAJOR RESEARCH OUTPUTS OF THE SUBPROGRAM

The annual workshop, which is usually planned to be a combined event with the Enhancement and Aquaculture Subprogram, was somewhat different in 2001 because it was held in Wellington in New Zealand. It was decided not to hold the Steering Committee meeting associated with the workshop in April. However, Wayne Hosking presented details of the project to reduce appendage loss to the workshop on behalf of the Subprogram, and Richard Stevens and the Subprogram Leader also participated in the workshop.

A booklet on water quality arising from project 2000/252 will be released at the workshop in May 2002. It has already been issued in a provisional form to industry and was well received.

iii) RELATED PROJECTS AND RESEARCH LINKAGES

There is a direct link to the Rock Lobster Aquaculture and Enhancement Subprogram, as the Subprogram Leaders sit on both Steering Committees. Projects 1998/301-305 in the Aquaculture and Enhancement Subprogram will benefit from the results of some of the studies of the Post-harvest Subprogram.

The project 2000/252 will be of direct benefit to industry and scientists holding lobsters in tanks, and was initiated after discussions between the two Subprograms.

The Subprogram leader attended both of the Steering Committee meetings of the Enhancement and Aquaculture Subprogram, and the Leader of the Enhancement and Aquaculture Subprogram attended the meting of this Steering Committee in Geelong in September 2001.

iv) ROLE THE SUBPROGRAM HAS PLAYED IN INDUSTRY DEVELOPMENT

1994/134.02 Code of practice for rock lobster The Code of practice is seen as a major method of industry development and it has been well adopted by industry. A revision of the Code, (2000/237) incorporating sections on, reducing appendage loss,

holding of lobsters in display tanks, methods of humane killing, truck transport, and water quality for holding post harvest lobsters, is an endorsed project application in the current round of applications. It will have a significant effect on industry development.

New methods of presentation to be used to disseminate the revised Code, including the development of a page on the Internet, will improve impact of the Code.

v) OPERATING PROCEDURES

During February 2001, Richard Stevens and the Subprogram Leader visited South Australia, Victoria, Tasmania and Queensland, on behalf of the Subprogram. Discussions were held with industry and industry groups, seeking to identify industry post-harvest problems, with a view to solving these problems.

The five new projects submitted in the current round to FRDC by this Subprogram arise directly from these activities. Another project, which is a joint New Zealand/Australian research activity, is already in development for submission in the following year.

A significant increase was made of email during the year as a method of communication within the Subprogram, thereby increasing the amount of Steering Committee interaction in the Subprogram on a whole range of issues.

vi) MEETINGS AND WORKSHOPS:

As mentioned above, we participated in the Workshop in New Zealand. Steering Committee meetings were held in Adelaide in June 2001 and in Geelong in September 2001.

Members of the Subprogram Steering, met in Adelaide in April 2001. They met again in Geelong in September 2001, thereby allowing the members to participate in the National Lobster Congress. The Subprogram leader presented the work of the Subprogram to the Congress.

vi) SUMMARY OF CURRENT PROJECT STATUS

1994/134.02 Code of practice for rock lobster **PI Richard Stevens** This project has been in a holding situation, pending the results of information becoming available from completed projects. A professional review of the material and presentation methods used in the last version of the code was undertaken. The final report of this project was lodged during 2001. A new project has been developed to bring effect to important revisions to the Code.
1996/345 Physiological studies of stress and morbidity during post harvest handling of western rock lobsters (*Panulirus cygnus*), I Physiological Stress

Indicators

PI Dr Brian Patterson

The final report of this project was lodged during 2001.

The study aimed to identify key physiological stress levels and/ or predict likely further mortality in lobsters after harvest and apply these parameters in studies aimed at improving post – harvest handling practices.

Outcomes from this study will be a revision of the code of practice for handling rock lobsters, and summaries of the results in non-scientific language in the Subprogram Newsletter in January 2002.

1996/344 Physiological studies of stress and morbidity during post harvest handling of western rock lobsters (*Panulirus cygnus*), II Immune Studies

PI Dr Louis Evans

Unfortunately the Principal Investigator has a life-threatening disease, and no firm date can be currently set for receipt of the final report of this project.

However, the non-technical summary of the project was published in the results in non-scientific language in the Subprogram Newsletter in May 2001.

1999/202 Rock lobster autopsy manual

PI Dr Louis Evans

This project arose out of developments and discussions in the Aquaculture and Enhancement Subprogram.
There is a need for methods of determining the cause of death of animals found dead or dying in holding tanks.

The outputs will be in two forms 1) A relatively simple autopsy guide and a set of photographs of known diseases. This will be useful for first examinations of dead and dying lobsters. 2) A comprehensive text with more detailed autopsy information, details of diseases and also photographs and references to other related literature. This will be utilized for more detailed examinations were they are needed to confirm results, or identify unknown causes.

The Principal Investigator has a life-threatening disease, and no firm date can de currently set for receipt of the final report of this project.

A book containing a chapter on lobster diseases (*Spiny Lobster: Fisheries and Culture*) was published in November 2000. This contains some of this information, which will be included in the final report.

2000/250 Project I : Facilitation Administration and Promotion

PI Professor Bruce Phillips

This project was set up to ensure that post-harvest research and development is

coordinated, and to make the most efficient use of resources, and ensure that effective and efficient communication is maintained between the industry, the researchers and FRDC.

Lack of uptake of research results is commonly the result of poor communication of the results and there presentation in forms not suitable for industry uptake

The results of this project are seen in better project design and coordination, increased industry involvement in project initiation and direction to yield practical results and better communication of the outputs of the research.

During the last year the Subprogram has developed cohesiveness and attempted to identify the needs of industry, and to develop projects to address these needs.

A number of new projects have been examined and endorsed by the Steering Committee, including two projects on southern rock lobsters.

363/251 Development of a method for alleviating appendage loss during post-harvest handling of rock lobsters.

Dr Glen Davidson

Leg loss causes significant financial loss to the industry, by way of weight, value, and increased mortality to undersized and reproductive animals released back into the sea.

The project aims to develop methods of alleviation appendage loss both during handling at sea, and in the factory.

This has been a very successful project, which has had considerable press coverage over the last 12 months.

2000/252 Optimizing water quality in rock lobster post-harvest processes

Dr Brad Crear

Studies of oxygen and temperature have already been made in an earlier project, reported above. This study extends our understanding to the toxicity of ammonia as part of the attempts to achieve high water quality.

The final report of this project was received during 2001.

The results will be incorporated into the code of practice, and also released is a booklet on maintaining water quality. The booklet and the code are being designed to be part of a series.

2001/235 Striking a balance between melanosis and weight recoveries in western rock lobster (*Panulirus cygnus*)

Dr Hannah Williams

This was a new project funded in the last round. It is progressing well.

It involves strong industry collaboration. A modification to the original objectives

was approved in January 2002 to make the final results more applicable and useful to industry. There was no change in the budget.

2001/255 Quantifying and controlling hyper- and hyposaline-induced post-harvest leg autotomy in the western rock lobster.

Principal investigator: Wayne Hosking
Co-Investigator Dr Glen Davidson
Both of the Geraldton Fishermen's Cooperative

Project Objectives

1. Survey salinity concentrations of surface films on individual lobsters and on relevant contact surfaces on boats and within factories.
2. Describe the relationship between autotomy and exposure to seawater of various salinities for lobsters of various sizes and moult stages.
3. Quantify leg loss during industry standard freshwater "drowning" procedures.
4. Compare responses to ionic and non-ionic solutions to elucidate the potential role of other contaminants, and the possible nature of the receptors and stimuli.
5. Investigate the relationship between daily environmental conditions and levels of post-harvest leg loss.
6. Field-test practical solutions for hyper/hyposaline-induced autotomy and make recommendations to industry.

This is a new project funded during 2001 with support from Geraldton Fishermen's Cooperative, Western Rock Lobster Development Association, Development and Better Interest Fund, Industry Development Unit of WAFIC.

SUMMARY OF STRATEGIC PLAN OR DIRECTIONS

A strategic plan for the Subprogram was developed at the Steering Committee meeting in Adelaide in July 2000 and was endorsed at the April 2001 meeting.

MISSION

To conduct research to increase the value of the rock lobster catch for Australia through improvements in post-harvest practices.

PRIORITIES

Reduce Appendage Loss
International Transport

Improve Long-Term Holding Information
Improve Processing Practices
Upgrade and Expand Code of Practice
Condition Indexes
Condition Enhancement

Information Transfer

The strategic plan is being expanded to ensure a full understanding of the objectives of the plan. It may also be expanded to include the need of stakeholders in Queensland, who are not currently represented on the Steering Committee.

COMMUNICATION AND TECHNOLOGY TRANSFER ACTIVITIES

Two Subprogram Newsletters (February and May) were issued. Theses were well received and the Steering Committee has directed that future issues be forwarded to all rock lobster endorsement holders in WA. SA, VIC and TAS.

PROPOSED NEW RESEARCH

The following projects have been examined by the Steering Committee and are recommended to the FRDC for consideration for funding.

Title	PI	Amount requested	Total Cost	Priority for Funding
2002/237 A code of practice for handling rock lobster	Richard Stevens	$114,377	$180.377	1
2002/238 Quantification of shell hardness in southern rock lobster	Dr Caleb Gardner	$80,405	$189,850	2
2002/239 The effect of on board cold-water stunning on the survival and growth of caught and returned western rock lobsters	Wayne Hosking	$122,496	$186,906	2
2002/240 Optimal selective fishing strategies for southern rock lobster	Dr Linda Eaton	$172,577	$509,106	3
2002/241 Aetology of pink flesh syndrome in the western	Dr Patrick Spanogue	$119,578	$174,478	4

rock lobster				

I will be submitting a separate and more detailed letter to FRDC in support of the projects.

WORK PLAN FOR NEXT 12 MONTHS

Development and completion of the Subprogram page on the FRDC Internet site is a top priority for 2002.

Work plans for the individual projects are set out in their milestones. I will be following these up with each PI. A Technical Subcommittee meeting is planned for Perth in February or March 2002.

During April, the Subprogram Leader will visit South Australia, Victoria and Tasmania for discussion re industry need and development of proposals for the next round of applications. The Subprogram Leader will be in Queensland in May for the Workshop and will discuss with the lobster industry their specific problems, with a view to finding solutions to their problems.

Steering Committee meetings are planned for May (Cairns) and September 2002. A Scientific Committee meeting will be held in March.

Two Newsletters will be issued in 2002. The first will be issued in January and the second about July.

F) BUDGET

The budget appears to be adequate, and is on track. The major expenses in year 2 are in the second half of the year.

The annual workshop (which is a combined event with the Enhancement and Aquaculture Subprogram) is to be held in Cairns in May 2002. A meeting of the Steering Committee will be held associated with the workshop. This will be an expensive undertaking because of the high cost of airfares, etc.

Another increased cost is the wider distribution of the Newsletter. It has been well received and the Steering Committee has directed that it should be sent to all rock lobster endorsement holders in Victoria, South Australia, Tasmania and Western Australia.

At this stage I believe these increased expenses can be met within the budget. However, I will reassess this in December 2002.

G) RECOMMENDATIONS/VARIATIONS

To further improve linkage between "FRAB's" and Subprograms perhaps FRDC could suggest that Subprogram Leaders be invited to join the "FRAB" in the state in which they reside. Some developments along these lines were initiated in most States in 2001, but not in WA.

However, since Richard Stevens, who is the secretary to the Ward in WA, is a member of the Steering Committee of this Subprogram, there is no lack of communication between the Ward and the Subprogram.

Appendix 6

Strategic Plan

Rock Lobster Post-Harvest Research in Australia. Strategic Directions 2002-2007

Prepared by

Professor Bruce Phillips
Leader, Rock Lobster Post-Harvest Subprogram

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Mission

To ensure that Australia obtains the maximum value for its rock lobster catch

Introduction

The catches of Australia's rock lobster fisheries are at or near their maximum level. However, adding value to the rock lobster catch will ensure continuing and improved profits for industry. This can be achieved by way of enshrining maximum quality on delivery to the processing factories, maximum survival of live lobsters shipped to overseas destinations, perfect cooking regimes for the product processed for this market, the maximum recovery during processing, a continuous maintenance and upgrading of handling conditions, maintaining and improving health and safety conditions, and having respect for community welfare concerns.

A major objective of the Subprogram has been to ensure delivery to the processing factories of rock lobsters that are alive, intact (no limbs missing) healthy and strong, so that they are in a condition suitable for live export. Not all such lobsters are exported live. However, this has provided the processor/marketer with the greatest choice, and lobsters cooked and frozen that are in this condition, provide greater percentages of flesh recovery. The studies conducted under the Subprogram have shown that rock lobsters at the time of capture are healthy and vigorous and that all of the reductions in condition are the result of less than perfect handling and transport conditions on the way to the factory.

The markets for rock lobsters and the forms in which they are sold constantly change. There has been a dramatic change from frozen products to live marketing of a large portion of the catch. Due to these changes industry faces new challenges to retain, maintain and expand markets and profitability.

The purpose of the Subprogram is to work with industry to identify the problems that the industry faces in meeting these challenges. It then seeks to support research studies to provide answers to the problems in a cost effective and timely manner. The outcomes of the research are rapidly given to industry in a form that allows industry to capture the benefits of the research for the Australian industry.

PRIORITIES

Reduce appendage loss

Improve International transport techniques

Improve long-term holding

Improve processing practices

Upgrade and expand the Code of Practice

Develop condition indexes

Develop methods for condition enhancement

Enhance Information Transfer

Reduce appendage loss

Loss of legs and antennae is a major problem, especially for western rock lobsters although it also occurs to a lesser degree in southern rock lobsters.

Losses in the order of millions of dollars occur through the delivery of incomplete lobsters to both the processors and the market.

Several projects developed by the Subprogram are underway to solve this problem. Spectacular success has been achieved in these studies, the results of which have already been widely adopted, and major reductions in appendage loss will follow.

Improve international transport techniques

At the initiation of this subprogram, several projects that were both FRDC and industry funded were directed towards improving processes during the transport of lobsters from point of capture to the processing factories.

The best practice conditions for the transport of the rock lobsters were identified, and industry has now implemented these where possible. The Subprogram believes that sufficient studies have been conducted in this area to permit industry to develop efficient local transport practices.

However, the markets for rock lobsters are expanding, and industry is looking at live shipment into the European Union and directly into mainland China.

This is an area of interest in which the Subprogram does not have any projects. However, we would be pleased to hear from scientists, engineers and industry, of possible projects examining this topic.

Improve long-term holding

A number of companies have shown interest in holding legal sized rock lobsters after the end of the season, to make sales at increased prices during the closed season. The RLEAS Subprogram also has interests in this area and has conducted some studies in South Australia.

The Subprogram has supported studies to provide data on oxygen levels suitable for holding southern rock lobsters and also western rock lobsters, and a booklet giving these data is available from the Subprogram Leader. A similar booklet providing data on ammonia levels will be available late in 2002.

Improve processing practices

This heading could include a whole range of topics for research, and it is one in which in which the industry still faces considerable challenges. At present a project is examining the causes of melanosis in cooked western rock lobsters and attempting to balance the cooking temperatures and times against weight recovery. The Subprogram would welcome further ideas for projects in this area.

Upgrade and expand the Code of Practice

The code of practice has been a major method of dissemination to industry of the results of research conducted under the Subprogram.

A project to produce three separate 'Best Practice' Codes for the western, southern and tropical rock lobsters was approved in 2002. These codes will

include clear information on best practice for handling rock lobsters, as well as covering health and safety and other issues for industry.

The codes are to be issued as videos, DVD and hard copy text. It is planned to place them on the Subprogram website, which is linked to industry websites in individual States so that information and video footage relevant to each State can be accessed through their State website.

Develop condition indexes

Two projects established under the Subprogram studied the physiological effects of stress and morbidity during post-harvest handling and storage of Western rock lobsters. One of the objectives of these studies was to try to develop a condition index for use in processing factories, which would be an improvement on the current visual method used to select rock lobsters for overseas export. This did not prove possible but such a technique is highly desirable.

Develop methods for condition enhancement

Rock lobsters that have been stressed by handling or processing can usually be rejuvenated if they are held in tanks with good water quality. This is certainly the case with Southern rock lobsters. However, western rock lobsters are not as hardy, and often do not regain their former vigour after being stressed, and may die.

Defining the most effective techniques for condition enhancement is an important objective.

Enhance information transfer

The Subprogram is keen to see the results of the research adopted by industry. All techniques to achieve this are considered, especially the latest technology such as the web, use of videos, etc.

Rock Lobster Post-Harvest Subprogram

The Fisheries Research and Development Corporation established the Rock Lobster Post-Harvest Subprogram (RLPHS) in 1996. Following an external review in 1999 it was renewed.

A summary of the Subprogram projects and activities since 1999 is presented below:

	00-01	01-02	02-03	03-04	04-05
94/132.02 – Code of Practice. PI Richard Stevens.					
96/344 – Physiological studies of stress and morbidity during post-harvest handling and storage of western rock lobster: 11 Standard autopsy techniques and immune system competency. PI Professor Louis Evans					
96/345 - Physiological studies of stress and					

morbidity during post-harvest handling and storage of western rock lobster: 1 Physiological Stress Indicators. PI Dr Brian Paterson					
99/202 – Rock Lobster Autopsy Manual. PI Professor Louis Evans					
2000/250 – Facilitation, administration and promotion of the FRDC Rock Lobster Post-Harvest Subprogram. PI Professor Bruce Phillips					
2000/251 – Development of a method for alleviating leg loss during post-harvest handling of rock lobsters. PI Dr Glen Davidson					
2000/252 – Optimising water quality in rock lobster post-harvest process. PI Dr Brad Crear					
2001/235 – Striking a balance between melanosis and weight recovery in western rock lobsters. PI Hannah Williams					
2001/255 – Quantifying and controlling hyper- and hypo-saline- induced post-harvest leg autotomy in the western rock lobster. PI Wayne Hosking					
2002/237 – A code of Practice for Handling Rock Lobster. PI Richard Stevens					
2002/ 238 – Quantification of shell hardness in the southern rock lobster. PI Dr Caleb Gardiner					
2002/239 – The effect of on-board cold water stunning on the survival and growth of caught and returned western rock lobsters. PI Dr Glen Davidson					

Publications

A number of publications have arisen from studies conducted within the Subprogram. The following are some recent papers, which will be of interest:

1 Jussila, J., McBride S., Jago, J. and Evans, L. H. (2001) Hemolymph clotting time as an indicator of stress in western rock lobster (*Panulirus cygnus* George). Aquaculture 199, 185-193.

2 Paterson, D. B., Davidson, G.W. and Spanogue, P. T. (2001) Identifying stress when western rock lobsters are stored out of water: The average and individual blood lactate concentrations. Contact Aquatic Science Research Unit, Curtin University of Technology, Perth.

3 Paterson, D. B., Davidson, G.W. and Spanogue, P. T. (2001) Measuring total protein concentration in blood of the western rock lobster (*Panulirus cygnus* George). Contact Aquatic Science Research Unit, Curtin University of Technology, Perth.

4 Powell, M., Crear, B. and Allen, G. (2001) Lobsters in the toilet: Acid-base effects of ammonia exposure in the spiny lobster *Jasus edwardsii* . Presented at the Australian and New Zealand Society of Comparative Physiology and Biochemistry , Adelaide, December.

Annual Workshop

The following are the papers presented by members of the Subprogram at the Workshop in Cairns in May 2002:

Physiological studies on stress and morbidity during post-harvest handling of western rock lobsters: Prof Louis Evans (Curtin University of Technology, WA)

Physiological stress indicators: Dr Brian Paterson (Queensland Department of Primary Industries)

Optimising water quality for live holding of rock lobsters: Dr Bradley Crear (TAFI, Tasmania)

Alleviating leg loss in western rock lobsters: Dr Glen Davidson (Geraldton Fishermen's Cooperative)

Effects of hyper and hyposaline seawater on leg loss: Mr Wayne Hosking (Geraldton Fishermen's Cooperative)

Striking a balance between melanosis and weight recoveries in western rock lobsters: Ms Hannah Williams (Curtin University of Technology)

Selecting Research Projects

With the help of the FRDC the rock lobster industry throughout Australia has the opportunity to solve its problems and increase its profitability.

This Subprogram examines projects through a Steering Committee composed of industry members. Therefore projects considered worthy of support go to FRDC with a solid industry backing. FRDC values this assessment process in its annual examination of the projects to be considered for funding.

To make this process effective, the Subprogram makes an annual call for pre-proposals of projects to be sent to it for examination. This occurs in July each year. These should also be sent to the State FRDC advisory bodies that provide an examination of them for suitability, value to industry in that State, the level of funding sought, and the type of work to be undertaken.

An electronic form is available to streamline this process. Copies can be obtained from the individual State FRDC advisory bodies. A list of the FRABs may be found on the FRDC website www.frdc.com.au The Steering

Committee meets twice a year, and during these meetings examines projects for possible support by the Subprogram.

Applications must be submitted to the FRDC on 1 December each year. However, those projects recommended by the Subprogram go to the FRDC with strong industry backing and support.

Ideas for projects can be discussed with the Subprogram Leader at any time of the year. This is encouraged, as it usually takes some months to develop a project to a state sufficient for submission to the FRDC.

Further Information

Additional information on the Rock Lobster Post-Harvest Subprogram including all of the newsletters can be accessed by visiting the web-site www.frdc.com.au/research/programs/rlph/index.htm or by contacting the Subprogram Leader or the Subprogram Secretary, Emma Phillips [emmaphil@ozemail.com.au].

Appendix 7

Subprogram Publications

The Subprogram has now released the following publications:

Optimising Water Quality, Dr Bradley Crear and Dr Grant Allen

Recirculating Systems $NH_{3,\ Dr}$ Bradley Crear, Dr Jennifer Cobcroft and Dr Stephen Battaglene

Rock Lobster Health and Diseases: A Guide for the lobster industry, Dr Frances Stephens, Seema Fotedar and Professor Louis Evans

Best Practice in the Western Australian Lobster Industry, WAFIC, Richard Stevens

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CORPORATION

POST-HARVEST SUBPROGRAM
ROCK LOBSTER
FRDC

OPTIMISING WATER QUALITY

BRADLEY CREAR AND GRANT ALLEN

GUIDE FOR THE ROCK LOBSTER INDUSTRY No.1

Tasmanian Aquaculture
& Fisheries Institute
University of Tasmania

National Library of Australia Cataloguing-in-Publication Entry

Crear, B. (Bradley), 1963-

Allen, Grant, 1968-

Guide for the rock lobster industry. No. 1. Optimising water quality - oxygen.

Bibliography

ISBN 1-86295-032-6

Rock lobsters-oxygen. 2. Rock Lobster industry. I. Crear, B. (Bradley), 1963 -.
II. Tasmanian Aquaculture and Fisheries Institute. III. Title: FRDC Project no. 2000/252.
(Series: Technical report series (Tasmanian Aquaculture and Fisheries Institute); no. 9).

597.5609946

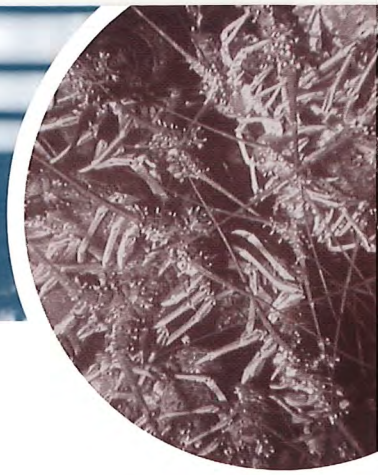
Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, PO Box 252-49, TAS 7000.

Booklet designed by Vanessa Tucker.

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

ROCK LOBSTER POST-HARVEST SUBPROGRAM FRDC

Tasmanian Aquaculture & Fisheries Institute
University of Tasmania



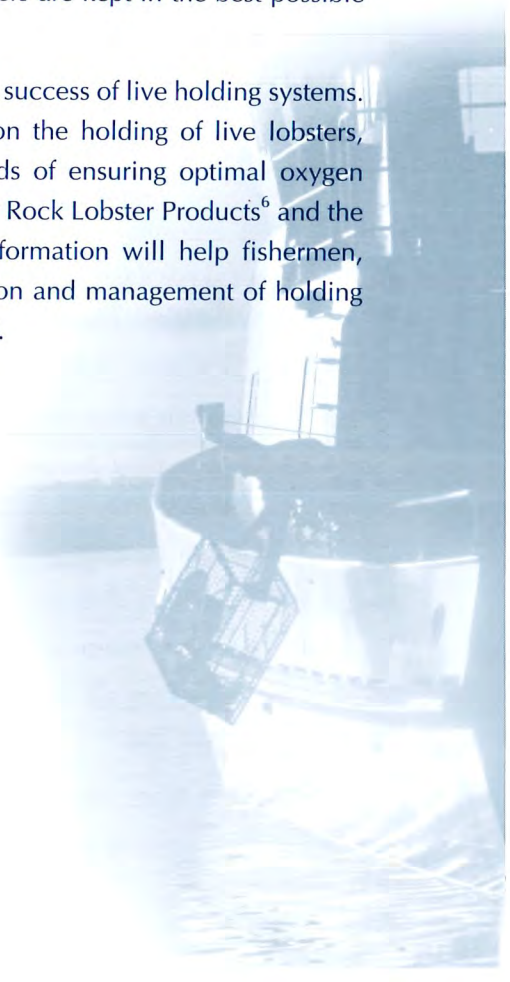
CONTENTS

INTRODUCTION 1

The primary aim of capturing, holding and transporting live lobsters is to deliver them to markets in the best possible condition. Lobsters will be exposed to some level of stress during all or part of the process. Stress can be defined as any factor (either external or internal) causing a physiological disturbance to the lobsters. In the live lobster industry these factors include capture and handling, poor water quality, strong sunlight, air exposure, and physical damage. Lobsters are generally able to recover from such stresses, however if any or a combination of those stresses are sufficiently intense, then poor quality or dead lobsters will result. Thus, transport and holding systems need to ensure lobsters are held in conditions that keep stress to a minimum.

The design of transport and holding systems is governed by a number of factors, with economics being a major driving factor. Systems also need to be practical to use and manage, and designed to suit the biological requirements of the animal. For a number of lobster species there is now a range of biological information that can be practically used in the design of systems. How the information is adopted will depend on the type of system being designed i.e. on boat, on shore, flow through or recirculating. For example, on a fishing vessel, ratios of kilograms of lobster to litres of water can exceed 1:1; space, weight and time are at a premium; the lobsters are in an extremely active state and the ambient seawater can be very warm and very low in oxygen. Some operators must store lobsters under these conditions for extended periods (days to weeks) before they are delivered to a processing facility. Given these factors, a range of innovative design features are required to ensure lobsters are kept in the best possible condition[7].

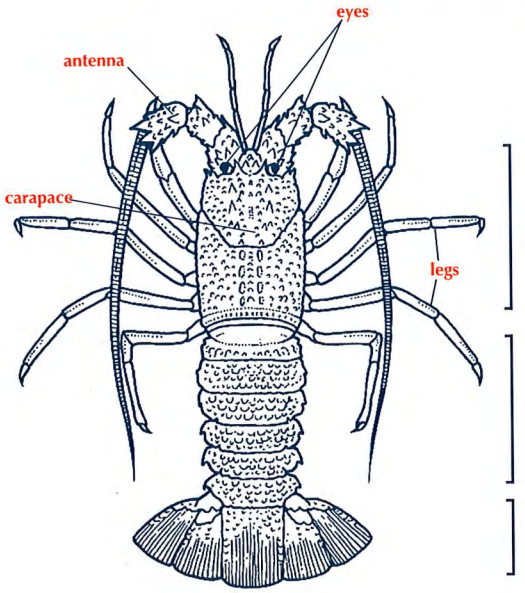
Oxygen is generally the major water quality variable limiting the success of live holding systems. This guide provides information to the rock lobster industry on the holding of live lobsters, focussing on the oxygen requirements of lobsters and methods of ensuring optimal oxygen supply. It has drawn from the New Zealand Code of Practice for Rock Lobster Products[6] and the Australian Code of Practice for Live Rock Lobster[11]. This information will help fishermen, processors and boat builders in the practical design, construction and management of holding systems for live rock lobsters (both on board boats and on land).



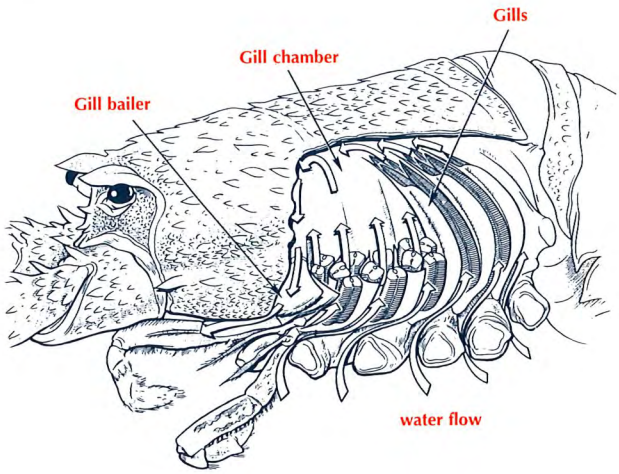
LOBSTER RESPIRATION BIOLOGY AND THE ENVIRONMENT 2

2.1 RESPIRATION BIOLOGY

Rock or spiny lobsters belong to a large class of invertebrate animals called Crustacea. In Australia, four species of lobsters support significant commercial and recreational fisheries[10]. The western rock lobster (*Panulirus cygnus*) is found off the lower western Australian coast, the tropical rock lobster (*Panulirus ornatus*) in northern Australia, particularly the Torres Strait and far north Queensland, the eastern rock lobster (*Jasus verreauxi*) off the central eastern Australian coast and the southern rock lobster (*Jasus edwardsii*) off the southern Australian coast.



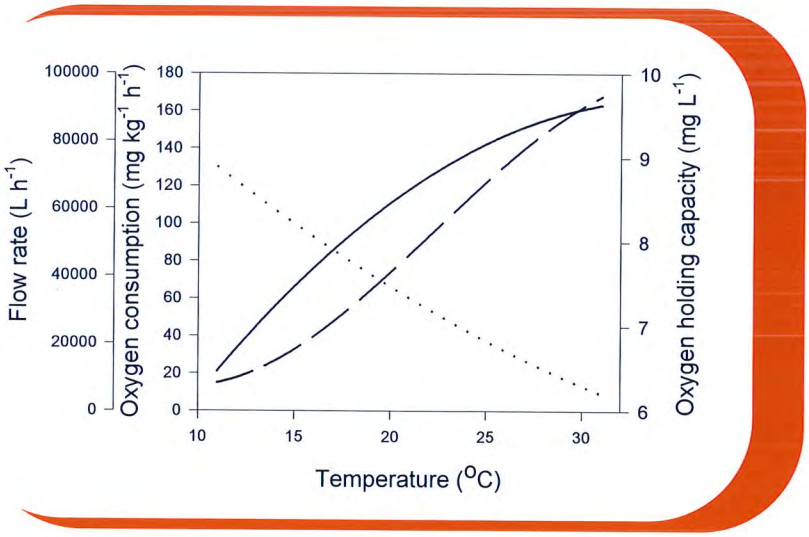
The main external features of lobsters are shown in the diagram left. The body of crustaceans is typically divided into three sections: head, thorax and abdomen (or tail). In lobsters, a carapace covers the head and thorax effectively forming a single section termed the cephalothorax (commonly called the head). Within the head are the major organs, including the heart, stomach, digestive gland and gills. The gills, situated in the gill chambers on either side of the head under the carapace, are used to extract oxygen from seawater. There are generally 21 gills on each side. Each gill is composed of numerous threadlike gill filaments containing capillaries enclosed in a thin membrane; oxygen is absorbed from the passing water and carbon dioxide is discharged. Water is drawn through the gill chambers, from back to front, and over the gills, by the beating of a special organ called the gill bailer, which is situated at the front of the chamber. See diagram below[12].



2.2 OXYGEN CONSUMPTION

Lobsters require oxygen in order to survive and they obtain this from seawater. Recent studies have shown that lobsters must be stored in water with dissolved oxygen concentrations greater than 70% saturation to ensure they are in the best possible condition[5]. Although maintaining dissolved oxygen levels > 70% at all times seems a relatively simple task, it can be anything but this in practice. There are many factors that affect the rate of oxygen consumption by lobsters, with water temperature, lobster weight and level of activity and/or stress being the major ones. There is a maximum level of oxygen consumption that lobsters can achieve; this is the level that needs to be used in the practical design of holding systems. Lobsters need to be provided with sufficient oxygen to ensure maximum consumption rates are possible at all times.

Factors that affect the oxygen requirements of lobsters can also alter the availability of oxygen. For example, as temperature increases, the lobster's demand for oxygen increases but the solubility of oxygen in water, and therefore it's availability to the lobsters, decreases. As a result the water flow required at higher temperatures increases markedly. The relationship is illustrated in the figure below. Salinity also affects the availability of oxygen to lobsters (Appendix A).



Oxygen consumption (solid line – mg kg^{-1} h^{-1}) of active western rock lobsters at various temperatures compared with the oxygen holding capacity (dashed line – mg L^{-1}) of water at those temperatures. The resultant water flow requirements (dotted line – L h^{-1}) for western rock lobsters at the temperatures are also shown[5].

The maximum solubility of oxygen in water at normal atmospheric pressure is termed 100% saturation. As the amount of oxygen in water varies with temperature it is much more convenient to express oxygen in terms of % saturation rather than as mg per litre (mg L^{-1}). The following example illustrates why. A generally recommended minimum oxygen level is 6 mg L^{-1}. In seawater at 27°C, 6 mg L^{-1} of oxygen equates to 92% saturation, whereas at 10°C it is 66% saturation. Thus, at certain temperatures, using 6mg L^{-1} as the minimum oxygen level can result in lower oxygen levels than the recommended minimum level of 70% saturation. At higher temperatures obtaining an oxygen level of 6 mg L^{-1} would be difficult to achieve using standard aeration equipment.

2.2.1 EFFECT OF EXPOSURE TO AIR

Lobsters are aquatic animals that are not naturally exposed to air (emersed). However, they do have a limited ability to extract oxygen from air and can survive for extended periods when exposed to air. It is this capacity that makes transport of live lobsters to markets around the world possible. Lobsters held in deoxygenated water suffocate quickly due to rapid loss of their available oxygen. For this reason, most lobster holding facilities are designed to be self-draining in the event of a systems failure that would prevent the circulation of oxygenated water.

Indicators of stress are evident when lobsters are exposed to air. Generally these changes are quickly corrected when lobsters are submerged. Thus, as long as lobsters are held under suitable conditions (appropriate temperature and humidity, with minimal disturbance) there appears to be no long-term effects due to short-term (<36 h) air exposure. The transport of lobsters to markets, which are further afield (eg. Europe) is likely to require longer exposure times. Exposure periods of up to 48 hours appear feasible, but require strict control of environmental parameters, which can be difficult.

NOTE: THE USE OF PURE OXYGEN TO AID THE OXYGEN UPTAKE OF LOBSTERS EXPOSED TO AIR IS NOT RECOMMENDED. MOST CRUSTACEANS DIE QUICKER WHEN EXPOSED TO A HIGH LEVEL OF OXYGEN IN AIR, AS IT RESULTS IN HARMFUL PHYSIOLOGICAL CHANGES.

2.3 WATER QUALITY

To maintain lobsters in prime condition they need to be provided with an environment that satisfies their physiological and behavioural requirements. Water quality has to be maintained at optimum condition for the lobsters, the most important being oxygen, temperature and salinity. Movement of any of those parameters outside the range tolerated by lobsters will result in mortalities within a short period of time (minutes to hours). There are other parameters that need to be monitored and controlled (e.g. ammonia, pH) although variations in these parameters will generally only result in mortalities over longer periods of time (hours to days). The table below outlines the water quality parameters regarded as being within the tolerance limits of rock lobsters.

Tolerance limits for various water quality parameters for the southern rock lobster (SRL) and the western rock lobster (WRL)

PARAMETER	TOLERANCE LIMITS
Temperature	SRL: 8 to 23°C (OHT^ 9-13°C)
	WRL: 12 to 31°C (OHT 17-23°C)
Dissolved Oxygen (% saturation)	Min. 70%, preferably >80%
Salinity (g kg^{-1} or ppt)	30 to 38
Ammonia (mg L^{-1})	<2
Nitrate (mg L^{-1})	<5
Nitrate (mg L^{-1})	<100
pH	7.8 to 8.4
Hardness (ppm)	100-200

^OHT - Optimum Holding Temperature.

HOLDING SYSTEMS 3

3.1 DESIGN AND OPERATION OF THE OXYGEN SUPPLY IN HOLDING SYSTEMS

Oxygen is supplied to lobsters via the sea water pumped through a holding system and/or via supplementary aeration. Flow through systems need to be designed so that under normal circumstances sufficient oxygen is supplied via the incoming water. However, aeration also should be incorporated into the system to provide for those times when there is insufficient oxygen being provided via the incoming water e.g. pump breakdown, restricted flow (seaweed at the intake), or low levels of oxygen in the incoming water. Aeration can also increase the carrying capacity of a system, provided all other water quality parameters are acceptable[8]. Aeration is currently the most effective means of increasing dissolved oxygen availability[3].

The design and operation of systems which provide oxygen to lobsters is based on:

- the amount of oxygen needed per unit of time.
- the minimum tolerable dissolved oxygen concentration required by the lobsters.

3.2 BOAT HOLDING FACILITIES

The only reliable method of maintaining lobsters in prime condition on board boats is to place them into live storage tanks where they are fully submerged in good quality water. Three types of holding systems are generally found on board boats – wells, below deck tanks and above deck tanks. Any of these methods are equally acceptable as long they are set up correctly.

One of the problems of holding lobsters on board boats, especially for extended periods, is the need to drive either water pumps and/or aeration equipment (either directly or via batteries). There is a range of aeration equipment now available, which should minimise any inconveniences, such as power usage and/or noise. The benefits to the fisherman should serve to counteract those inconveniences. For example, with aeration in a well tank it would be possible to move into a sheltered bay where water movement is minimal. Thus, the fishermen can be physically comfortable whilst knowing that the lobsters will still be maintained in good condition.

3.2.1 WELL TANKS

In well tanks it is difficult to control the flow of water, both in volume and in direction, through the tanks. Water flow through the well tank relies solely on the movement of the vessel or a reasonable current flow to force water into the well. Where there is a lack of boat movement or current flow, the transfer of water to the wells and hence the supply of oxygen to the lobsters can be restricted. Operators must be aware of this and move position if such conditions occur. Additionally, when lobsters are stored in large

quantities and at high densities in wells, lobsters in the centre can suffer from lack of water flow. Aeration should be an essential component of well tanks. The addition of aeration into the wells would largely prevent problems associated with this method of holding lobsters. It would increase the level of oxygen in the water and the movement of water (and thus distribution of oxygen and removal of waste).

When coming into estuaries or areas of poor quality water well tank inlets/outlets are usually blocked, and a pump is used to circulate water within the well. This prevents the lobsters coming into contact with external sources of poor quality water, but can result in the oxygen becoming depleted within the well. The addition of aeration would ensure that the lobsters in the static well tank remain in reasonably good quality water. Over a 2-3 h period it is unlikely that other water quality parameters would decrease to a harmful state.

3.2.2 BELOW DECK TANKS

Below deck tanks are perceived to be the best method of holding lobsters on board boats. Even so, they need to be designed properly to ensure they are effective. A dedicated pumping system is required to produce a constant flow of water to the tanks. This needs to pump sufficient water to supply the oxygen requirements of the lobsters. It is also necessary to check the water flow rate regularly or have a flow alarm. Below deck tanks face similar problems to the well tanks when coming into estuaries or areas of poor water quality. Similar strategies should be used to overcome those problems.

3.2.3 ABOVE DECK TANKS

Above deck tanks can be a very effective means of holding lobsters. However, when using above deck tanks it is important to consider the possible additional stresses which the lobsters can be subjected to: sunlight; increased temperature; and movement of people and objects across the deck of the vessel. Lobsters should be protected from these factors as much a possible by keeping tank lids in place. A shaded area for the tanks would also be good, however, it is not feasible on board many boats. One of the major problems in the use of above deck tanks can be the lack of a dedicated water source, with water flow being provided by the deck hose. The pump for the deck hose is often run directly off the main engine. Thus, if the deck hose is used for other purposes or the engine is not going fast (e.g. whilst idling at pot lifts) the water flow is stopped or reduced. As for below deck tanks, a dedicated pump that provides a constant flow of water via dedicated piping should be used.

3.3 ON SHORE HOLDING FACILITIES

3.3.1 FLOW THROUGH VERSUS RECIRCULATING

In both the flow through and recirculating systems oxygen is being stripped from the water and waste materials added. This is acceptable in a flow through system when oxygen levels are sufficient to maintain the lobsters' requirements and the used water goes to waste. In a recirculating system the water is re-used. Oxygen needs to be replaced and the waste material removed before the water is returned to the lobsters. The easiest way to achieve this is to aerate the water and have a physical and biological filter to remove the waste.

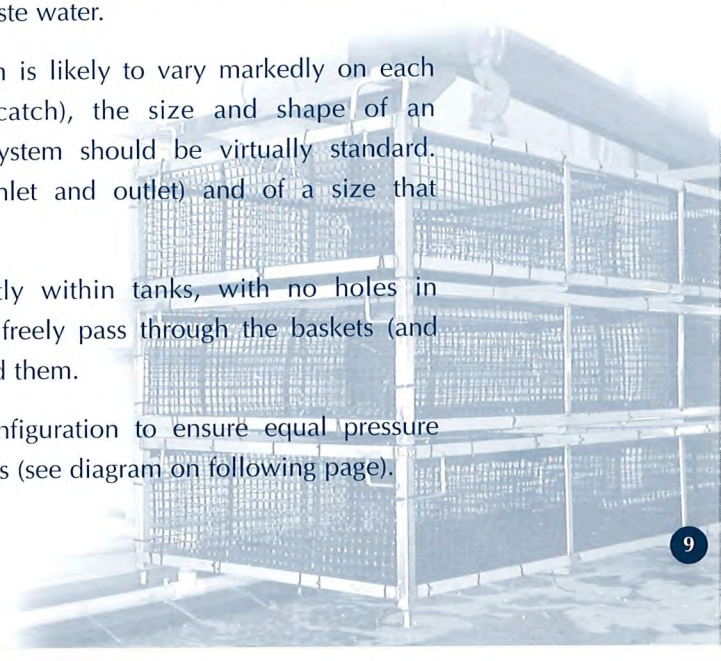
Recirculation systems are best used in areas where: external seawater quality is not guaranteed (e.g. estuaries); where pumping costs from the sea are excessive (inshore holding facilities); where specific control over temperature or other environmental parameters is required; or where environmental controls are in place to reduce nutrients in effluent water. One major down side to recirculation systems is that water quality parameters must be regularly monitored to ensure optimal water quality for the lobsters. These include temperature, pH, salinity, ammonia, nitrite and nitrate.

3.4 FACTORS THAT AFFECT HOLDING SYSTEM PERFORMANCE

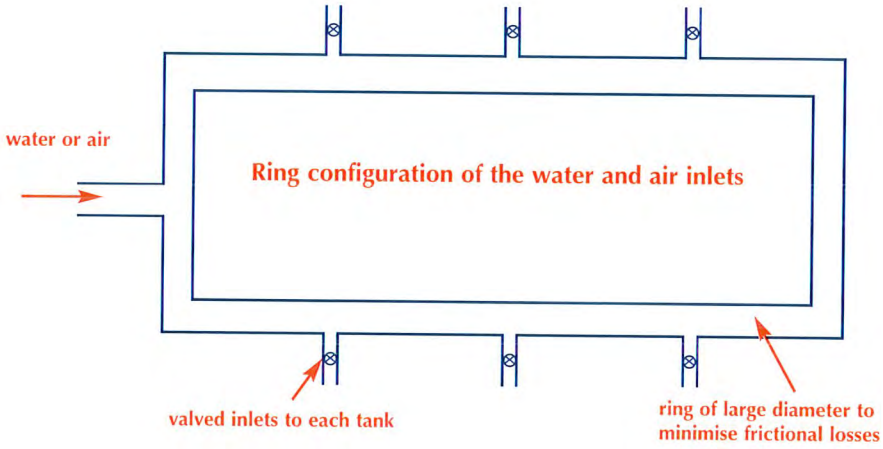
Outlined below are some general factors that should be considered when designing and/or using a live holding system. Water quality is the major factor that affects the performance of a holding system.

3.4.1 WATER FLOW

- When designing a holding system, water inlets and outlets should be situated at the greatest distance from each other i.e, inlets at the bottom and outlets at the top, or vice versa. Flow from the bottom to the top is preferable. This increases the distribution of water within the holding tank, minimises the formation of 'dead spots', and maximises the removal of waste water.
- Although the overall size of a holding system is likely to vary markedly on each boat (dependent on expected maximum catch), the size and shape of an individual tank in a below deck storage system should be virtually standard. Each tank should be self-contained (own inlet and outlet) and of a size that matches the baskets being used.
- Baskets containing lobsters should fit tightly within tanks, with no holes in baffles between tanks. Air and water must freely pass through the baskets (and hence the lobsters) and not short circuit around them.
- Plumbing of water should be in a ring configuration to ensure equal pressure and therefore equal delivery to all tank sections (see diagram on following page).



- The main outlet lines from the pump (ie. the ring configuration) should be of as large a size as practical to minimise frictional losses of the flow rate (see diagram below). The flow to each tank should be able to be controlled (each tank should have a valved pipe branching off the main ring configuration) so that water flow can be regulated according to tank size and stocking biomass.



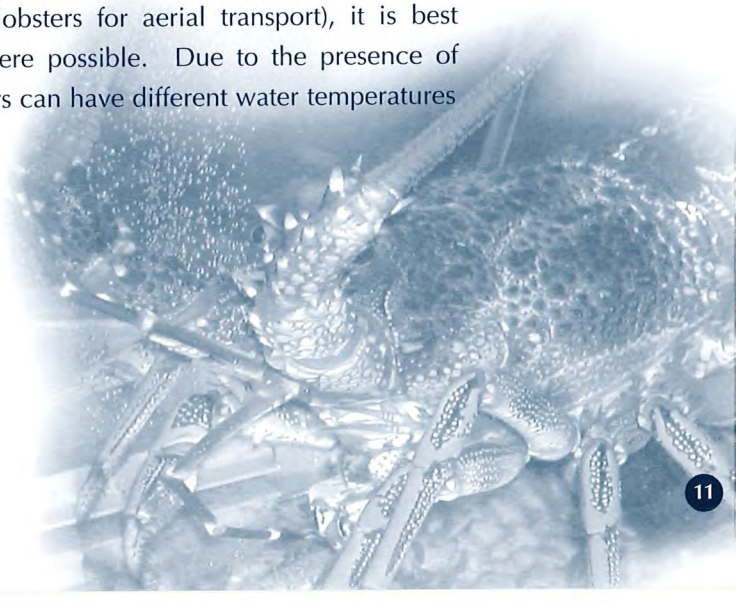
- Similarly, each tank should have its own water outlet to ensure tanks drain at the same rate as filling.
- How to measure water flow rate? Calculate the volume of the top 10 cm of tank. Then calculate the flow based on the time to refill this portion of the tank. This method accounts for effects of increasing head pressure with water depth. Another method is to simply fill a bucket of known volume with the outflow from a tank, and time how long it takes to fill. If the bucket is 20 L and it takes 10 seconds to fill, then there is a flow of 120L min^{-1} (= 60sec ÷ 10sec) x 20 L).
- If a flow alarm is fitted set it to 75% of water flow (for partial blockages of intake/strainers) and/or have a flow gauge.
- Ensure there are no suction side leaks and that the water intake is always below the water line, as air intake to the water pump can result in gas supersaturated water. Signs of suction leak: milky coloured water due to microbubbles.
- If the water quality parameters in a particular area (eg. estuary) are thought to be sufficiently different from the oceanic water from which the lobsters were caught, then it is best to shut off the flow through system and recirculate the water, ensuring appropriate aeration. Alternatively, the holding tanks should be completely drained and the lobsters kept emersed. Lobsters can survive much longer out of water than in de-oxygenated water.

3.4.2 AERATION

- Aeration should be fitted as a standard accessory to any vessel catching lobsters.
- Plumbing of airlines should be in a ring configuration to ensure equal pressure and therefore equal delivery to all tank sections.
- Each tank should have its own adjustable air valve so that air flow can be regulated. If a tank is empty and air flow is on, air will short circuit to the atmosphere, effectively cutting air flow to full tanks.
- Air diffusers (airstones, leaky pipe etc.) need to be replaced frequently as they become clogged and lose their efficiency. Also lobsters have a tendency to chew them. Therefore, they should be simple to replace (eg. at the end of season service). Some air diffusers are able to be cleaned and thus should be on a regular basis. Air diffusers should not be left submerged in water when not in use; tanks should drain to below the level of the air lines.
- The air pump should be located above the tank water line to prevent back siphoning if the power fails (or use stainless check valves).

3.4.3 ENVIRONMENT

- Estuarine and harbour water can vary greatly from oceanic water, with particular regard to temperature, salinity, sediment load and pollution. Therefore, care must be taken when entering estuaries and harbours.
- **Temperature** – lobsters can handle a wide range of temperatures; the recommended Optimum Holding Temperature (OHT) ranges are well within the tolerable temperature limits (see Section 2). Cooler temperatures tend to reduce activity, oxygen consumption, waste excretion and aggressive behaviour of lobsters. Therefore, the maintenance of the health of lobsters is easier when held at temperatures towards the lower end of the OHT. Although lobsters can generally tolerate large and/or rapid changes in temperature without any adverse effects (e.g. chilling is commonly used to prepare lobsters for aerial transport), it is best to avoid exposing them to such changes where possible. Due to the presence of shallow sand / mud flats, estuaries and harbours can have different water temperatures



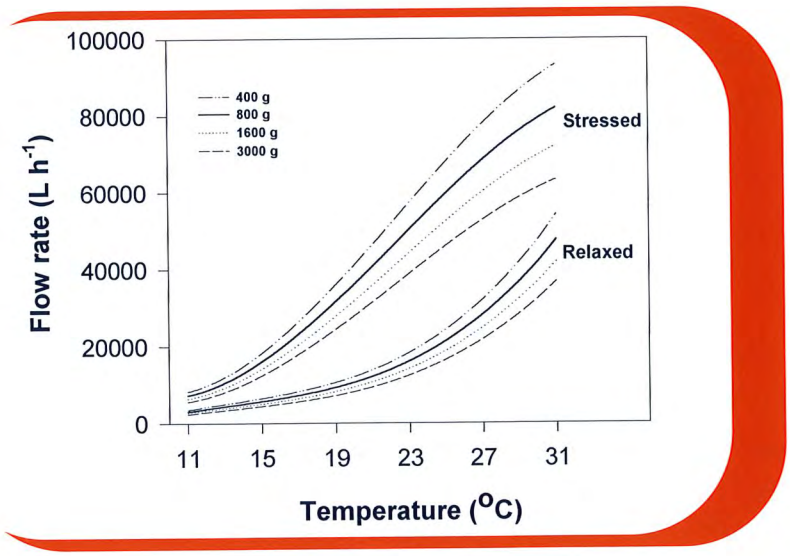
(usually warmer) from the ocean. Increased water temperature should be avoided as it leads to increased activity thereby increasing oxygen consumption and waste production.

- **Salinity** – fresh water is not as heavy as salt water and therefore floats on the surface. In an estuary, harbour or river after heavy rain the salinity of the surface water can be very low. Continued operation of a flow through system in this instance will begin to stress the lobsters.
- **Sediment** – storms and heavy rain can stir up sediment, which can result in clogging of lobster gills and make extraction of oxygen from the water more difficult.
- **Pollution** – estuaries and harbours can have heavy vessel traffic, industrial discharge and storm water run off. Any resulting pollution may have a detrimental effect on the lobsters.
- **Sunlight, wind** – in most situations the exposure of lobsters to direct sunlight and wind should be avoided. Both are stressful as they can quickly dry the lobsters out. Strong sunlight is also likely to damage the eyes of lobsters.

4 WATER FLOW AND AERATION

4.1 WATER FLOW REQUIREMENTS

Water flow requirements for lobsters vary in relation to animal size, temperature and stress. This is illustrated for the western rock lobster in the figure below. Thus, 1 tonne of relaxed 400g lobsters being held at 19°C will require 10,811 L of fully saturated seawater per hour. However, if that same 1 tonne of lobsters is moved into 23°C water and during the process are stressed, they will require 61,951 L of fully saturated seawater per hour, or almost 6 times as much.



The water flow (L h^{-1}) requirements of 1 tonne of western rock lobsters at various combinations of temperature, body weight and stress. Calculations are based on the incoming water being fully oxygen saturated and the maintenance of 70% oxygen saturation in the outgoing water[5].

4.1.1 CALCULATING FLOW RATES

From the above figure and Appendix B we can calculate the required water flow to maintain through a holding tank. Let us assume that western rock lobsters are stressed, the water temperature is 27°C and the incoming water is oxygen saturated. The flow rate required to provide the oxygen requirements of 1 tonne of 400g lobsters, whilst maintaining 70% saturation at the outlet would be 74539 L h^{-1}. This works out to be approximately 1.2 L kg^{-1} $minute^{-1}$ (= 74539 ÷ (60 sec x 1000 kg). However, if the incoming seawater was not fully oxygen saturated, then a flow of 1.2 L kg^{-1} min^{-1} water would not provide the necessary minimum of 70% saturation at the outlet. Thus, aeration of the water becomes necessary. The required water flow rate of lobsters under various conditions is outlined in the table on the following page.

The water flow (L h^{-1}) requirements of southern and western rock lobsters at various temperatures. Calculations are based on the incoming water being fully oxygen saturated and the maintenance of 70% oxygen saturation in the outgoing water.

	Southern rock lobster *J. edwardsii*			Western rock lobster *P. cygnus*	
Temp.(°C)	Flow rate		Temp.(°C)	Flow rate	
	L $tonne^{-1}$ h^{-1}	L kg^{-1} min^{-1}		L $tonne^{-1}$ h^{-1}	L kg^{-1} min^{-1}
5	4607	0.08	11	8384	0.14
9	16251	0.27	15	18755	0.31
13	29909	0.50	19	33081	0.55
17	33717	0.56	23	61951	1.03
21	40000	0.67	27	74539	1.24
			31	94053	1.57

Tropical Lobsters – there is little information available for tropical lobsters, although some is available for the Caribbean lobster, *P. argus*[1]. From that data it would appear that the recommendations for the sub-tropical species *P. cygnus* would be applicable. At higher temperatures the data would need to be extrapolated. That is at 35°C a flow rate of 1.96 L kg^{-1} min^{-1} (117,000 L $tonne^{-1}$ h^{-1}) would be required.

4.2 AERATION REQUIREMENTS

Aeration is required where the incoming water is low in dissolved oxygen or where the water is being recirculated. It can also be used to increase the carrying capacity within a particular body of water. There are many different methods of aerating water. Unfortunately, in general there is a poor understanding of aeration fundamentals and as a result aeration performance is often less than optimal.

In the case of a water pump breakdown all of the oxygen requirements of the lobsters will need to be supplied by aeration. Lobsters will survive for an extended period in a static tank as long as the oxygen is maintained at an appropriate level. For example, in a 10,000 L tank of water at a temperature of 23°C, stocked with 1 tonne of western rock lobsters (stocking density 100 kg m^{-3}), with no water flow or aeration, declining oxygen levels will cause significant mortalities after 1-2 hours (dependent on activity levels of the lobsters). However, if adequate aeration is available, the ammonia (the next critical water quality parameter) level will reach approximately 5 mg L^{-1} after 24 hours. This concentration

would result in few, if any, mortalities. Aeration systems therefore provide a large buffer time in the event of a problem with water flow.

If the pump breakdown is caused by a problem with the power supply, then it may also be affecting the aeration system. In such a case it is important that the tanks are self-draining as lobsters will survive significantly longer in humid air than in stagnant deoxygenated water. In a recirculating system, aerating the water is the only means of replenishing oxygen levels.

Aeration is also useful where water flow is purposely turned off to ensure that no problems are caused by poor water quality (pollution / low salinity), such as when a boat enters a harbour / estuary.

NOTE: WHEN THE FLOW IS TURNED OFF IT IS IMPORTANT TO REMEMBER THE WATER TEMPERATURE MAY QUICKLY RISE. THE RATE OF RISE WOULD DEPEND ON THE PROXIMITY TO HEAT SOURCES (ENGINES/SUNLIGHT) AND THE OUTSIDE AIR TEMPERATURE. IF THE CONDITIONS ARE UNFAVOURABLE AND THE TANKS NEED TO BE STATIC FOR A CONSIDERABLE TIME THEN THE TEMPERATURE MAY INCREASE TO UNACCEPTABLE LEVELS

4.2.1 DETERMINING THE AERATION REQUIREMENTS

As stated earlier, the design of aeration equipment is based on the amount of oxygen needed and the minimum tolerable dissolved oxygen concentration. As this information is available (Appendix B provides information on the oxygen consumption of lobsters under different conditions), it should just be a matter of using it to determine the appropriate aeration equipment. Unfortunately, it is not that simple. As a first step you need to be guided by what you wish to achieve. Ideally you should have an aeration system that provides all of the oxygen required by the lobsters at the maximum rate of oxygen consumption (Example 1a). However, that may not be practical due to cost or power restrictions (eg. on board a boat). Therefore, you may wish to install an aerator that provides supplemental aeration to that provided by the flow through water supply. This would be sized so that it provides the oxygen requirements of relaxed lobsters (Example 1b). Thus, even if the water flow is stopped the lobsters will still be provided with sufficient oxygen as long as they remain unstressed. Lobsters will generally return to relaxed levels of oxygen consumption within a few hours of being stressed (eg. due to capture or sorting).

Example 1. Aerator calculations

(a) Calculate the size of the aerator required to provide sufficient oxygen to 1 tonne of active and stressed 400 g western rock lobsters at 23°C.

The lobsters require 130g of oxygen h^{-1} (Appendix B). Under ideal (standard) conditions a 250 W ($^1/_3$HP) aerator with medium sized (3mm) bubble diffusers will transfer between 250 and 400g of oxygen to water per hour[4]. For this example we will assume 300g h^{-1} is being transferred; thus there would appear to be sufficient oxygen to meet the lobsters' requirements. However, that rate of transfer is only achievable under optimum conditions (i.e. if the oxygen level of the water is low then the transfer efficiency is high). Since there is a need to keep the oxygen level relatively high (above 70% saturation at all times), the efficiency of transfer of oxygen to the water is decreased to approximately 20% of the maximum rate. Thus, there are only 60g (20% of 300g) of oxygen available to the lobsters per hour: a bigger aerator would be required. A 560 W ($^3/_4$HP) pump would deliver sufficient oxygen (135g per hour) under those conditions.

(b) Calculate the size of the aerator required to provide sufficient oxygen to 1 tonne of relaxed 400 g western rock lobsters at 23°C

The oxygen requirements of relaxed lobsters is much less (~40 g h^{-1})(Appendix B). Thus, the 250 W aerator would more than adequately provide their oxygen requirements.

Another complicating factor is that the efficiency of transfer of oxygen from aeration systems to the water (transfer efficiency) is dependent on a number of factors, which include: depth of water, temperature, concentration of oxygen in the water, size of air piping, type of diffuser, bubble size, air flow rate and type of aerator. The ability of aeration equipment to transfer oxygen to water (standard oxygen transfer rates) is developed under standard conditions (including low oxygen saturation levels). Manufacturers of aeration equipment should be able to supply this data. Under field conditions the oxygen transfer rate can be much less. Typically in aquaculture or holding systems, where there is already quite a high oxygen saturation level in the water, the oxygen transfer rate is generally less than 25% of that under standard conditions.

As there are so many factors to consider, the design of an aeration system can be difficult and should be discussed with an experienced person. Appendix B outlines the airflow required to provide sufficient oxygen to lobsters under various conditions. However, due to differences between systems resulting in very different rates of aeration efficiency, these should be used as a guide only.

4.2.2 COST OF AERATION

Aeration is an insurance against water quality problems and can lead to increased survival and vigour of the lobsters. It also gives increased flexibility in how lobsters can be stored. Even though the cost of installing and running an aeration system is easy to justify, it is still worthwhile ensuring the costs are minimised. For example, aerator prices and running costs increase with size therefore obtaining one of the appropriate minimum size will result in lower overall costs.

Using Appendix B we can calculate the approximate cost of supplying aeration to 1 tonne of lobsters. First you must determine the size (wattage) of motor required to deliver the amount of air necessary for your given aeration demand. This will need to be discussed with an air pump supplier. When a unit cost of electricity is applied to the required wattage ratings for your motor, a cost per hour can be generated (see Example 2). Your local electricity supply company will be able to provide you with this unit cost.

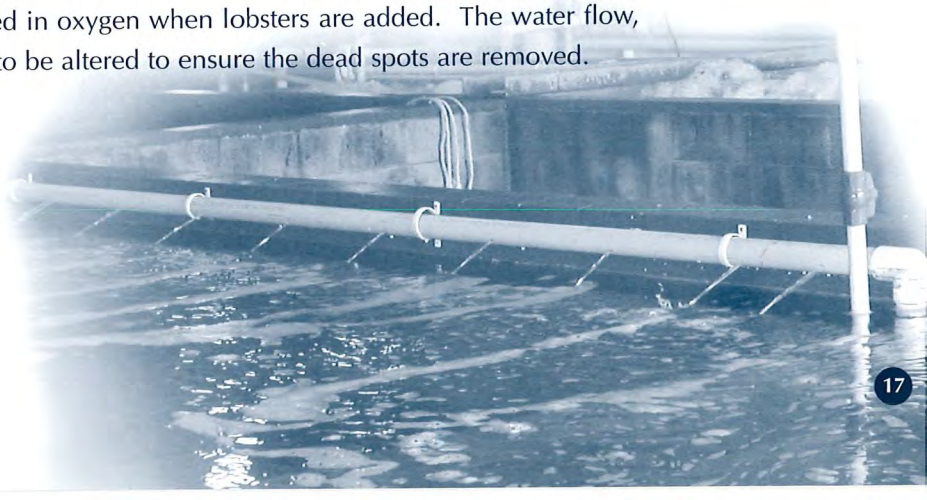
Example 2. Aeration running costs

If you are holding 1 tonne of stressed western rock lobsters at 23°C, the approximate wattage requirement of a motor to run an appropriate air pump is 600 W. If a unit cost of \$0.20 per kW h^{-1} is charged, then the cost to aerate your tank for an hour will be \$0.12 (0.6 kW x \$0.20).

4.3 WATER CIRCULATION

Although oxygenation is the most important function of aeration, water circulation caused by aeration is also beneficial. Good circulation ensures there are no dead spots in tanks, moves oxygenated water to the lobsters and helps maintain high oxygen transfer efficiencies.

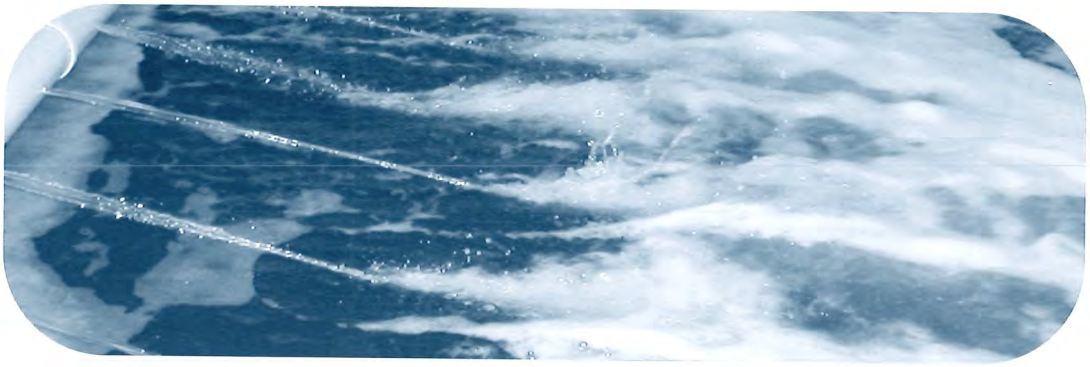
The circulation of water (and oxygen) within a tank can be easily tested using a dye (e.g. food colouring). Before stocking, add the dye to the inlet water and observe its' dispersal through the tank. Any dead spots will not receive coloured water; these represent areas of the tank that may become depleted in oxygen when lobsters are added. The water flow, aeration or tank design will need to be altered to ensure the dead spots are removed.



4.5 PURE OXYGEN AERATION

For applications that have very high oxygen demands or requirements for oxygen concentrations greater than 95% saturation, pure oxygen aeration systems should be considered[8]. The design and installation of pure oxygen aeration systems should to be discussed with an experienced person. The cost of pure oxygen aeration needs to be considered when examining the benefits. Although there may be some advantages in maintaining oxygen saturation levels close to or above 100% under certain conditions when holding live lobsters (e.g. during recovery of stressed lobsters[5]), the benefits are probably not sufficient to warrant the use of pure oxygen aeration. Correctly designed aeration systems using air should more than adequately meet most systems requirements.

Pure oxygen (in oxygen bottles) can be used as emergency aeration when the water and air pumps fail (e.g. mains power failure). The advantages of keeping a supply of oxygen on hand would depend on the type of system and the other backup equipment that is in place e.g. a generator could be used to provide power in the event of mains power failure. The ability of lobsters to survive extended periods out of water (under the right conditions) also limits the advantages of a pure oxygen backup.



RELATED ISSUES 5

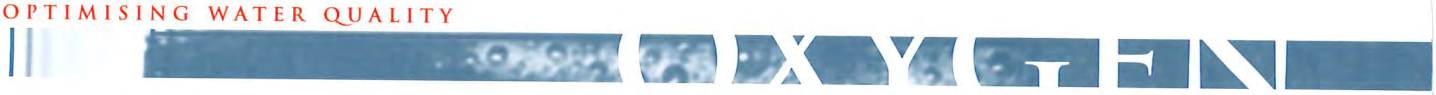
5.1 TOTAL GAS SUPERSATURATION

Gas supersaturation occurs when water contains more than the "natural" amount of a particular gas or gases. The sum of all the gasses dissolved in the water is called the total dissolved gas pressure (TDGP) of the water. Under normal conditions this is 100% and is equivalent to atmospheric pressure. Under some conditions however, TDGP can increase above 100%. This is calculated from the difference between atmospheric pressure and TDGP and is represented in terms of % Saturation or Δp = change in pressure (% Saturation = TGDP / atmospheric pressure x 100), (Δp = TDGP – atmospheric pressure). Δp is usually expressed in terms of mmHg.

Nitrogen and oxygen are the primary gasses in air. Nitrogen can cause health problems (gas bubble disease) at anything greater than 100% of its "natural" water concentration of around 79% of TDGP, whereas oxygen can be safe up to and over 200% of its "natural" water concentration of around 20% of TDGP. The main cause of supersaturation is air leakage on the intake side of water pumps. The air readily dissolves in the pressurised water. When the water leaves the pressurised pipe and enters the storage tank, it is suddenly back to normal atmospheric pressure. All the extra gas in the water is now in excess to the natural amount that the water should hold and the water becomes supersaturated. Nitrogen can only escape out of the water through the water surface and a reduction from 110% to 100% can take up to several hours.

A tank with supersaturated water can sometimes be detected by observing bubbles forming on tank surfaces and any items put in the water. Often however these signs are lacking as the saturation level is not high enough. Meters to measure excessive saturation levels (tensionometers or saturometers) are available, however, they are not common and thus may not be readily available. Although dissolved oxygen concentration is not a good measure of total gas supersaturation[8], it is useful as an indicator that supersaturation may be occurring. If an oxygen level of over 100% saturation is measured, and there is no pure oxygen being used anywhere in the system, then it is reasonable to assume that gas supersaturation is present. It is important to double-check the calibration of the oxygen meter if an oxygen level of over 100% saturation is measured.

Levels of concern for holding facilities either on shore or on board vessels for long term exposure would be over 103% nitrogen saturation (Δp = 25) or for short term exposure levels in excess of 105% (Δp = 40). These levels allow the dissolved nitrogen to exceed its "natural" level in water resulting in an imbalance with the physiology of the lobster that can eventually lead to gas bubble disease.



5.2 HEAVY METALS IN HOLDING SYSTEMS

Seawater contains a wide range of dissolved inorganic salts, in particular heavy metals, at levels that are generally not harmful to aquatic animals. However, in holding systems there is the opportunity for many of these to increase to harmful levels. This is especially so in recirculating systems through the use of inappropriate materials. Copper, iron, zinc and aluminium are metals that are most likely to reach high levels in holding systems, because of their use in chilling units, building materials (e.g. galvanised iron) and some pumps. The utmost care should be taken to remove or isolate all metals with the potential to contaminate water. High quality stainless steel and titanium are materials that can be used safely. Fibreglass, rendered concrete with an epoxy coating, plastics and PVC are usually the most suitable materials for pipe and tank construction. Even tanks made with those materials should be flushed several times prior to being used. Organometals such as tributyl tin (from antifouling paints) can also contribute to the heavy metal loads causing contamination of some waters, especially estuaries and harbours.

If mortalities occur in a system and no other obvious cause is found it may be worthwhile to have the water tested for heavy metals. However, very little is known of the concentrations of heavy metals that are harmful to lobsters. The table over the page shows the range of concentration of heavy metals normally found in seawater. It also shows the 95% trigger values at which remedial action must be taken. The 95% trigger value is a calculation based on available toxicity data of a number of marine species[2,9]. The value represents a probable point where 95% of species are protected. If your water contains any metals in these concentrations it is strongly recommended that an overhaul of the system be undertaken to identify possible causes of contamination.

The concentration of some metals in seawater and the concentration at which remedial action should be undertaken.

COMPOUND	NORMAL SEAWATER RANGE ($\mu g L^{-1}$)	95% TRIGGER VALUES ($\mu g\ L^{-1}$)
Copper (Cu)	0.025 – 0.38	1.3
(Fe)	0.006 – 0.14	ID
Zinc (Zn)	0.022 – 0.10	15
Aluminium (Al)	0.0 – 0.7	ND
Lead (Pb)	0.006 – 0.03	4.4
Nickel (Ni)	0.13 – 0.5	70
Mercury (Hg)	0.0007 – 0.003	0.4
Chromium (Cr)	0.062 – 0.10	4.4 (CrVI)
Tributyl tin (TBT)	ND	0.006

ID = Insufficient Data, ND = No Data.

WATER SAMPLING EQUIPMENT 6

It is important that the water quality in holding systems can be measured. Therefore, the appropriate sampling equipment needs to be available. Sampling equipment can be expensive, but considering the risks they are a necessary outlay. Sampling equipment is varied but can be basically broken into two groups: a) test kits or b) meters. Each has their advantages and disadvantages.

a) Test kits are usually simple to use however, each test is usually less accurate than can be obtained with meters. Although they are generally relatively cheap compared to meters, they tend to be more expensive per sample. If a lot of testing were to be under taken it would probably be cheaper to buy a meter.

b) Meters on the other hand are generally more difficult to use, requiring some technical knowledge about their operation and maintenance. They also generally require a larger initial capital outlay. Most importantly, they require calibration on a regular basis to ensure that accurate results are obtained. Some meters allow more than one parameter to be tested at the same time e.g. oxygen, salinity, pH and temperature can all be performed using a single instrument.

6.1 OXYGEN

MONITOR WEEKLY OR IF SYSTEM CHANGES

Oxygen meters are an expensive capital outlay, varying from just under $1000 to many thousands of dollars for highly complex integrated systems. Oxygen test kits, which generally cost several hundred dollars and do around 100 tests, are also available.

The oxygen concentration in new systems should be checked regularly (every 2-3 h) for a few days to ensure that there is a good understanding of the normal oxygen concentrations (over a range of stocking densities). Provided appropriate stocking densities, flow rates and aeration are maintained the oxygen concentration should not vary greatly. Thus, it is probably only necessary to measure the oxygen concentration on a weekly basis. If there were changes to the system (e.g. temperature change, increased stocking density, pump or aeration problems) then regular checking the oxygen concentration is vital. Oxygen probes that provide a permanent display and alarm capability are available, and would be worthwhile considering in some systems.

6.2 TEMPERATURE

MONITOR CONTINUOUSLY

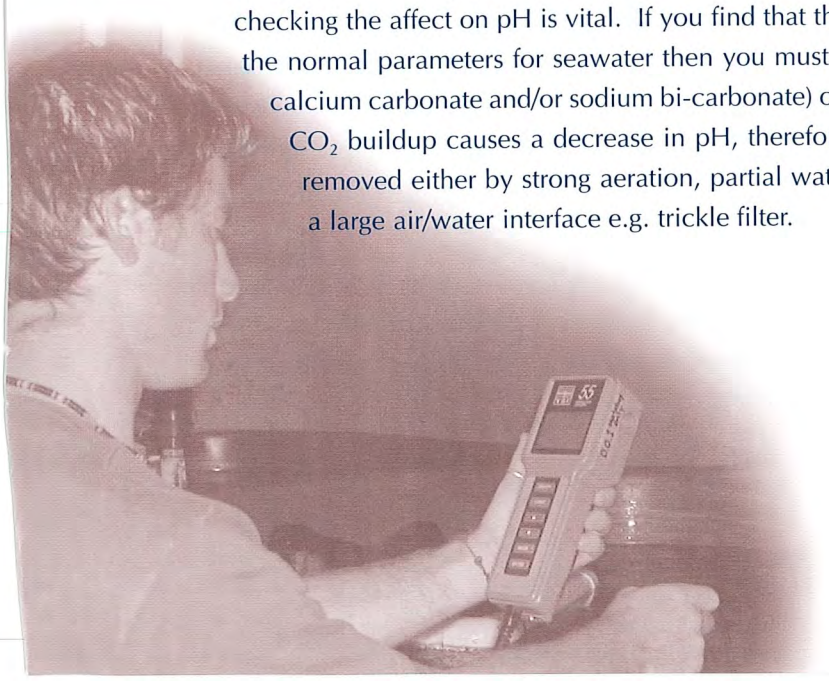
Temperature monitoring is relatively easy, and cheap, with many types of thermometers to choose from. It is up to individual preference as to whether to use a glass thermometer or a thermocouple with digital readout. Prices range from a few dollars to several hundred dollars. Potential contamination of lobsters from mercury and broken glass are an important consideration in the use of glass and mercury thermometers. A reasonably good quality digital thermometer should be able to be purchased for less than $50. A thermocouple thermometer placed in the tank with a permanent digital display that is clearly visible is the best option. Ideally the system should be set up so that an alarm is activated if the temperature moves outside the optimal set range.

6.3 pH

MONITOR WEEKLY OR IF SYSTEM CHANGES

pH is the measure of how acidic or alkaline a product is and is usually measured between 0 and 14. Seawater is always slightly alkaline with a pH between 7.8 and 8.4. As lobsters are marine animals, they require their holding water to be of the same pH range. To determine the level of pH in your holding system you can use a pH meter (most accurate method) or test strips (give a general estimate). pH meters range in cost from around $150 to several thousand dollars. Test kits can be purchased for around $20 to $100 and will generally do between 50 and 300 tests.

Flow through systems, either on land or on a vessel, generally do not need to monitor pH as it will remain constant with the seawater. Partial or full recirculation systems should test pH in conjunction with water hardness. If there are changes to the system (e.g. temperature change, increased stocking density, pump or aeration problems) then checking the affect on pH is vital. If you find that the pH of your holding system is below the normal parameters for seawater then you must take action to buffer the water (using calcium carbonate and/or sodium bi-carbonate) or make more regular water exchanges. CO_2 buildup causes a decrease in pH, therefore it is necessary to ensure the CO_2 is removed either by strong aeration, partial water change or via a device that creates a large air/water interface e.g. trickle filter.



6.4 SALINITY

MONITOR WEEKLY

The level of salt in seawater, salinity, can be measured with a refractometer, hydrometer or salinity meter. Seawater generally has a salinity of 33 to 35 g kg^{-1}. Levels as low as 28 g kg^{-1} or as high as 38 g kg^{-1} do occur and depend on rainfall, ocean currents, evaporation and many other factors. As seawater is heavier than fresh water, inlets for flow through systems should be located well below the water level. Salinity may need to be measured more often than weekly if flow through systems are subject to changes due to rainfall or currents.

A refractometer measures the degree to which the path of light is changed (refracted) as it passes through a thin layer of water. The amount of refraction is directly proportional to the amount of salt in the water, the more refraction the more saline the water. Refractometers can be purchased for around $200 to $300.

A hydrometer is used to determine the specific gravity (SG) of water and consists of a glass bulb with a weight at one end and a closed thin tube at the other. The greater the salinity the higher in the water the hydrometer will float. It is simply a matter of reading the salinity off the scale printed on the side of the tube (or converting the SG to g kg^{-1}). A reasonable quality hydrometer should not cost more than $50.

A salinity meter measures conductivity: the amount of electrical current that can be carried by seawater. The capacity of the water to conduct electricity is directly proportional to the level of salt in the water, the greater the current the more saline the water. Salinity or conductivity meters can be purchased for between $500 and $1000.

6.5 AMMONIA, NITRITE, NITRATE, ALKALINITY AND HARDNESS

MONITOR WEEKLY OR IF CONDITIONS CHANGE

There is a wide range of kits available on the market to test these parameters, the choice of which to use is up to individual preference. Most have simple to follow instructions and can be completed within a matter of minutes. Generally a water sample is treated with reagents to produce a colour, which is then compared against colour standards. They are usually available for under $50 and will perform between 25 and 200 tests.

Testing for ammonia, nitrite, nitrate, alkalinity and hardness is really only necessary for partial or full recirculation systems. However, it is good to know in a fully flow through system that the incoming water is of good quality or to check to ensure that flow is sufficient to prevent a build up of ammonia in the tanks.

SUMMARY 7

To ensure that live lobsters are maintained in prime condition after capture, the following key points summarise their requirements in terms of the provision of oxygen:

- Oxygen is generally the major water quality variable limiting the success of the live holding of lobsters.
- Oxygen levels should be maintained at or above 70% saturation (preferably >80%).
- Aeration should be a standard accessory in holding systems.
- It is important to ensure water (and thus oxygen) is distributed evenly to all parts of a holding tank (no dead spots).
- The maintenance of water temperature within an optimal range will increase the ease with which oxygen can be provided to the lobsters.

APPENDICES 8

8.1 APPENDIX A – SALINITY AND OXYGEN SOLUBILITY

The effect of temperature and salinity on the solubility of oxygen in water (mg L^{-1}).

TEMPERATURE °C	SALINITY	
	30	35
10	9.32	9.03
11	9.12	8.83
12	8.92	8.65
13	8.73	8.47
14	8.55	8.29
15	8.38	8.13
16	8.21	7.97
17	8.05	7.81
18	7.90	7.66
19	7.75	7.52
20	7.60	7.38
21	7.46	7.25
22	7.33	7.12
23	7.20	6.99
24	7.07	6.87
25	6.95	6.75
26	6.83	6.64
27	6.72	6.53
28	6.61	6.42
29	6.50	6.32
30	6.39	6.22

8.2 APPENDIX B – OXYGEN CONSUMPTION AND PROVISION

The amount of oxygen consumed (g h^{-1}) (top number), and the associated water flow (L h^{-1}) (middle number) requirements and air flow (L h^{-1}) (bottom number) requirements of 1 tonne of western rock lobsters (*Panulirus cygnus*) at various combinations of temperature, body weight and stress. Calculations are based on the incoming water being fully oxygen saturated and the maintenance of 70% oxygen saturation in the outgoing water.

Note: The air flow requirements assume an absorption efficiency (percent of oxygen transferred from the air to the water) of 2%. The efficiency is variable, therefore, the air flow rates should be used as a guide only.

WESTERN ROCK LOBSTER

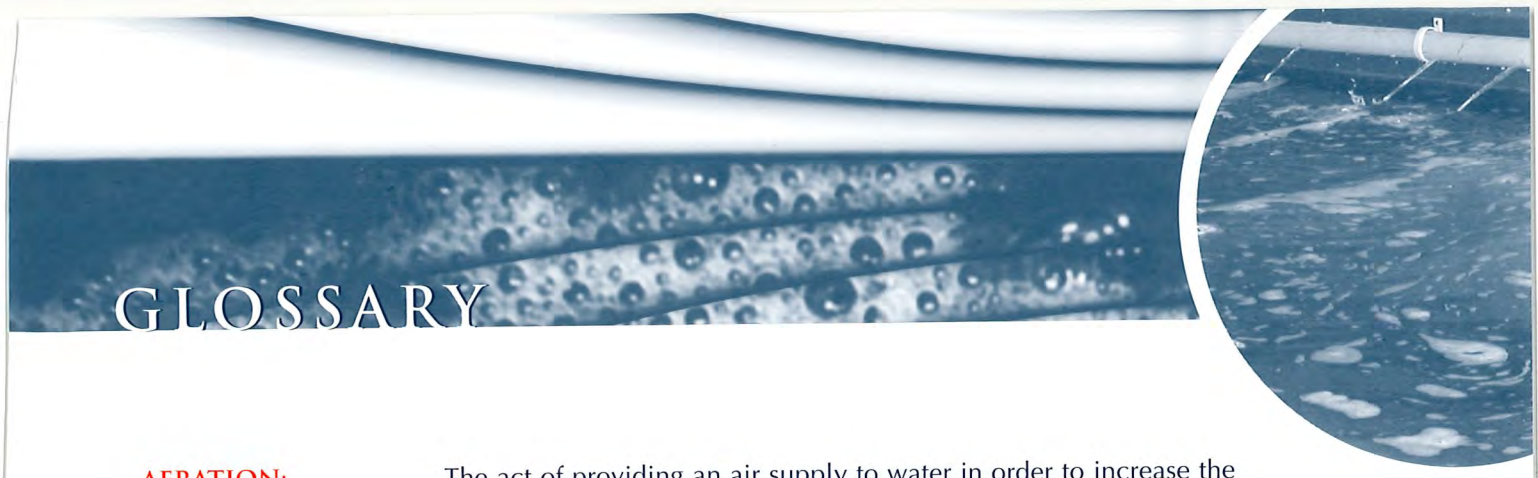
	TEMP. (°C)	400 g	800 g	1600 g	3200 g
RELAXED LOBSTERS	11	9.5	8.4	7.4	6.5
		3598	3163	2781	2444
		1833	1611	1417	1245
	15	15.2	13.4	11.8	10.4
		6252	5495	4831	4246
		2932	2577	2266	1992
	19	24.4	21.4	18.8	16.6
		10811	9503	8354	7343
		4690	4123	3624	3186
	23	39.0	34.3	30.1	26.5
		18604	16354	14375	12637
		7502	6595	5797	5096
	27	62.4	54.9	48.2	42.4
		31854	28001	24614	21637
		12000	10549	9273	8151
	31	100.1	88.0	77.4	68.0
		54366	47790	42009	36928
		19258	16929	14881	13081
STRESSED LOBSTERS	11	22.2	19.5	17.2	15.1
		8384	7370	6479	5695
		4271	3754	3301	2901
	15	45.7	40.2	35.3	31.1
		18755	16486	14492	12739
		8797	7733	6797	5975
	19	74.6	65.6	57.7	50.7
		33081	29080	25562	22470
		14352	12616	11090	9749
	23	129.9	114.2	100.4	88.2
		61951	54457	47870	42080
		24983	21961	19304	16970
	27	146.0	128.4	112.8	99.2
		74539	65523	57597	50630
		28081	24685	21699	19074
	31	146.0	128.4	112.8	99.2
		94053	82677	72676	63885
		33316	29287	25744	22630

The amount of oxygen consumed (g h^{-1}), and the associated water flow (L h^{-1}) and air flow (L h^{-1}) requirements of 1 tonne of 600 g southern rock lobsters (*Jasus edwardsii*) at various combinations of temperature and activity. Water flow calculations are based on the incoming water being fully oxygen saturated and the maintenance of 70% oxygen saturation in the outgoing water.

Note: The air flow requirements assume an absorption efficiency (percent of oxygen transferred from the air to the water) of 2%. The efficiency is variable, therefore, the air flow rates should be used as a guide only.

SOUTHERN ROCK LOBSTER

	TEMP. (°C)	OXYGEN CONSUMPTION (g h^{-1})	WATER FLOW REQUIRED (L h^{-1})	AIR FLOW REQUIRED (L h^{-1})
UNSTRESSED LOBSTERS	5	9.1	2985	1740
	9	16.0	5778	3075
	13	25.0	9839	4808
	17	37.1	15792	7127
	21	52.1	23908	10017
ACTIVE LOBSTERS	5	14.0	4607	2686
	9	45.0	16251	8647
	13	76.0	29909	14615
	17	79.1	33717	15218
	21	87.1	40000	16758



GLOSSARY

AERATION: The act of providing an air supply to water in order to increase the oxygen content of that water.

AMMONIA: Ammonia is the major end product of protein metabolism in most aquatic animals. Ammonia is toxic to lobsters and therefore must be prevented from building up in holding systems. Ammonia is present in two forms in water (ionised NH_4^+ and un-ionised NH_3), the higher the pH and temperature, the higher the percentage of the toxic fraction (un-ionised).

ALKALINITY: Alkalinity is basically a measure of the carbonate content of water. It gives a measure of the capacity of the water to accept acidity (i.e. it's buffering capacity). Alkalinity is usually measured as either mg L^{-1} (milligrams per litre) $CaCO_3$ (calcium carbonate) or meq (milli-equivalents). 1 meq = 50 mg/l $CaCO_3$.

BIOLOGICAL FILTER (BIOFILTER): A filter providing a large surface area on which denitrifying bacteria grow; used to remove waste (particularly ammonia and nitirite) from recirculating systems.

BIOMASS: Total weight (kilograms) of organisms in a system. Calculated as individual weight multiplied by total number.

EMERSION: Removal from water to a dry environment.

FLOW THROUGH: Single use water. Water enters a system, passes through the system and goes to waste.

IMMERSION: Submersing into water.

NITRATE: Formed as a result of the breakdown of ammonia to nitrite and then to nitrate by bacteria in biofilters. Generally not toxic at the levels found in recirculating systems. Chemical symbol NO_3.

NITRITE: Toxic chemical formed during the oxidation of ammonia to nitrate by bacteria in a biofilter. Most of the nitrite is converted to nitrate before the water exits the biofilter, therefore it is not generally found at toxic concentrations. Chemical symbol NO_2.

OXYGENATION: The addition of pure or very high purity oxygen to water in order to increase the dissolved oxygen concentration of the water.

pH: A measure of acidity of a solution. It is in effect a measure of the amount of hydrogen ions. The normal pH of seawater ranges from 7.9-8.2.

RECIRCULATION: The process of taking water from a holding system which would otherwise be discarded from the system and reintroducing it to the same system. Prior to being reintroduced, the water is often treated to remove some of the wastes so that the water quality is maintained at a sufficient high level that it remains suitable for the culture animals. Recirculation systems can be operated as 100% recirculation (no new water added) or may have partial replacement water added.

SALINITY: The term used for the measurement of the total amount of dissolved salts in the water. Full strength seawater has salinity in the region of 34-36 g kg^{-1}.

SUPERSATURATION: The term given to a body of water which contains more than the normal amount of a particular gas or gases. The sum of all the gasses dissolved in the water is called the total gas pressure of the water; under normal conditions this is 100%. Supersaturated water can cause problems in aquatic animals (gas bubble disease) although nitrogen supersaturation is far more dangerous than oxygen supersaturation. Oxygen can be safe up to and over 200% saturation.

VENTURI EFFECT: The act of drawing air into a water system via a small tube or crack in a pipe. As the water passes through a restriction in a pipe, it forms a vacuum at the end of the restriction. A hole bored into the pipe at the point where this vacuum occurs will cause air to be drawn into the main flow. Although efficient at mixing chemicals and gasses into water, the operational costs of a venturi is high due to the cost of the increased pumping pressure required for the unit to operate. Venturis have their applications in some systems where there is more pressure available than is required by the rest of the system components.

WATER HARDNESS: The amount of cations (positively charged ions) of the earth metals (mainly calcium and magnesium) in the water. In most waters, the hardness is similar to that of alkalinity, as calcium and magnesium are usually bound to the main alkalinity bases (bicarbonate and carbonate). Alkalinity tends to be used more as a measurement than hardness.

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NOTES

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ROCK LOBSTER POST HARVEST SUB-PROGRAM FRDC

RECIRCULATING SYSTEMS NH_3

BRADLEY CREAR
JENNIFER COBCROFT
STEPHEN BATTAGLENE

GUIDE FOR THE ROCK LOBSTER INDUSTRY No.2

Tasmanian Aquaculture & Fisheries Institute
University of Tasmania

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ROCK LOBSTER POST-HARVEST SUBPROGRAM
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CONTENTS

INTRODUCTION 1

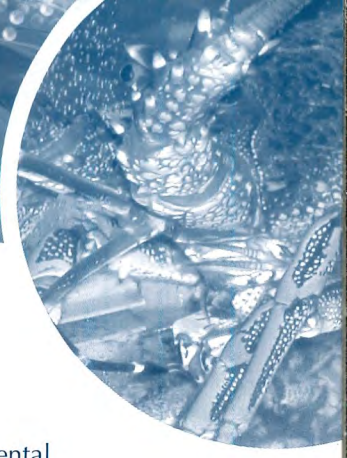
Two main systems are currently being used for holding lobsters: flow-through and recirculating. In flow-through systems, the water that is pumped into a tank is only used once. After passing through a tank all the water goes to waste, meaning that waste products should not build up to high levels. In recirculating systems, the majority of the water is re-used after each pass through the tanks, first being treated to remove waste products before being returned to the tanks. As a result recirculating systems have a greatly reduced water demand.

Flow-through systems for holding lobsters are generally easy to design and operate however, there is little control over the quality of the water. It is dependent on the point source and may not be of constant optimal quality. Even though initial set up costs may be higher, there is an increasing interest in and movement towards the use of recirculating holding systems. Experience has shown that lobsters can be successfully held in recirculating systems.

Recirculation systems have practical application to a range of situations, including: where seawater of optimal quality is not guaranteed (e.g. estuaries); where pumping costs from the sea are excessive (inshore holding facilities); where specific control over temperature or other environmental parameters is required; where lobsters are being held outside their normal geographical range; and where environmental controls are in place to reduce nutrients in effluent water. As lobsters are generally not fed whilst in captivity and as they possess a relatively good tolerance of poor water quality, the systems can be relatively simple.

This publication describes the principles of recirculating systems as they apply to the holding of rock lobsters. It covers all aspects of such systems, from design to daily operation. There are a number of reference manuals/books on recirculating systems, some of which deal in much more detail with aspects mentioned in this manual and the reader is referred to them if they require further information. This publication provides information specifically for lobster holding systems based on recently obtained data on ammonia excretion rates and ammonia tolerance of lobsters.

BASIC PRINCIPLES 2



Holding systems must provide a suitable environment for the lobsters. Critical environmental parameters include the concentrations of dissolved oxygen, ammonia, nitrite and carbon dioxide (Losordo et al., 1998). Nitrate concentration, pH, salinity and alkalinity levels within the system are also important. In flow-through systems the main limiting water quality parameter is dissolved oxygen. Sufficient water needs to be pumped through or the water needs to be aerated to ensure lobsters are supplied with sufficient oxygen. As the water is only used once and the turnover time is rapid then other water quality parameters should not reach toxic levels. That is assuming that the incoming water is of good quality in the first place.

In recirculating systems the main limiting factor is still dissolved oxygen, however the unionised ammonia (NH_3) concentration becomes increasingly important, and is probably the next limiting factor. Ammonia must be removed from the system at a rate equal to the rate of production to maintain a safe concentration (Losordo et al., 1998). Controlling the concentration of unionised ammonia in the tanks is the primary objective of recirculating treatment system design.

All recirculating systems remove waste solids, oxidise ammonia and nitrite, remove carbon dioxide, and aerate the water before returning it to the holding tanks. Waste solids are generally removed via some form of mechanical filtration, ammonia and nitrite via biological filtration, and carbon dioxide by the provision of an air/water interface. Aeration of the water is also achieved across the same air/water interface. In simple systems, such as glass aquaria, all these processes are done within the holding tank. In more complex systems, most if not all of the processes are undertaken external to the holding tank.

2.1 ADVANTAGES AND DISADVANTAGES OF RECIRCULATING SYSTEMS

2.1.1 ADVANTAGES

Low water requirements

A properly designed and operated recirculating system requires a minimum daily input of water, just enough to clean particulate waste filters and to replace water lost to evaporation. This permits the construction of holding systems at considerable distances from a seawater supply, thus allowing them to be situated in more convenient positions, such as close to airports and markets.

Control of water quality

The water quality in flow-through systems is largely dependent on the quality of the source water. At times that can be less than ideal: rain can cause decreased salinity, algal blooms can cause low oxygen levels, storms/wave action can cause increased sediment loads.

Good water quality can be managed in recirculating systems through effective operation of the system components and by avoiding water exchanges / collection in poor water quality events.

Control of water temperature

Good control over the water temperature is one of the major advantages of recirculating systems. The optimal water temperature for holding can be much more easily and cheaply maintained in recirculating systems than in flow-through systems. Also species can be held in geographical areas outside their normal range e.g. temperate water lobsters can be marketed in tropical areas. The temperature is normally maintained towards the lower end of the temperature range that a particular species is able to tolerate. At lower temperatures lobsters are less active, and thus require less oxygen and excrete lower levels of waste products, and are less aggressive. Lobsters can also be chilled down within the holding tanks, meaning there is less need for handling of the lobsters (i.e. moving them to a separate chilling tank) just prior to transport. This may increase the ability of lobsters to handle transport conditions and thus extend the life of the lobsters during transit.

Increased ability to control biofouling

Biofouling, both within pipework and within the tanks, is a significant problem within seawater systems. Biofouling in the pipework will lead to decreased flows and higher pumping costs. The piping will need to be cleaned on a regular basis, which can be expensive. Biofouling in the tanks also needs to be removed on a regular basis; the cleaning process can result in damage to the tanks. As the amount of water entering recirculating systems is relatively small, it is easier to prevent the growth of biofouling organisms. Water can be treated either chemically (e.g. chorinate/dechlorinate a header or storage tank) or mechanically (filtration). Filtration to 100 µm will remove the larvae and eggs of most biofouling organisms, such as mussels and fan worms.

2.1.2 DISADVANTAGES

Increased complexity

Recirculating systems are an intricate balance of bacterial populations, engineering equipment and seawater. The system operator must understand how the balance is created, maintained and most importantly, what to do when that balance is upset. Experience in the management of recirculating systems is a key component of their operational success. This is probably one of the principal reasons why recirculation systems for holding lobsters are not more commonly used.

Higher set up costs

As more equipment is required the setup costs can be higher. However, in some instances the use of recirculating systems can be cost-effective. The costs of pumps and electricity to provide water for flow-through systems can be large and filtration equipment can be required to prevent the flow of debris (silt/sediment/sand) into the tanks. A key to the successful use of recirculating systems is the use of cost-effective water treatment system components (see Section 3).

SYSTEM COMPONENTS 3

The major components of a recirculating system are the water supply, holding tanks, solids filter, biological filter, temperature control system, oxygen and carbon dioxide control system and sump tank (Fig. 1). Other more sophisticated components, such as ozonation and ultraviolet filtration, can be added, but are generally not required in lobster holding systems. A reservoir of fresh seawater is also a necessity but not shown in Fig. 1.

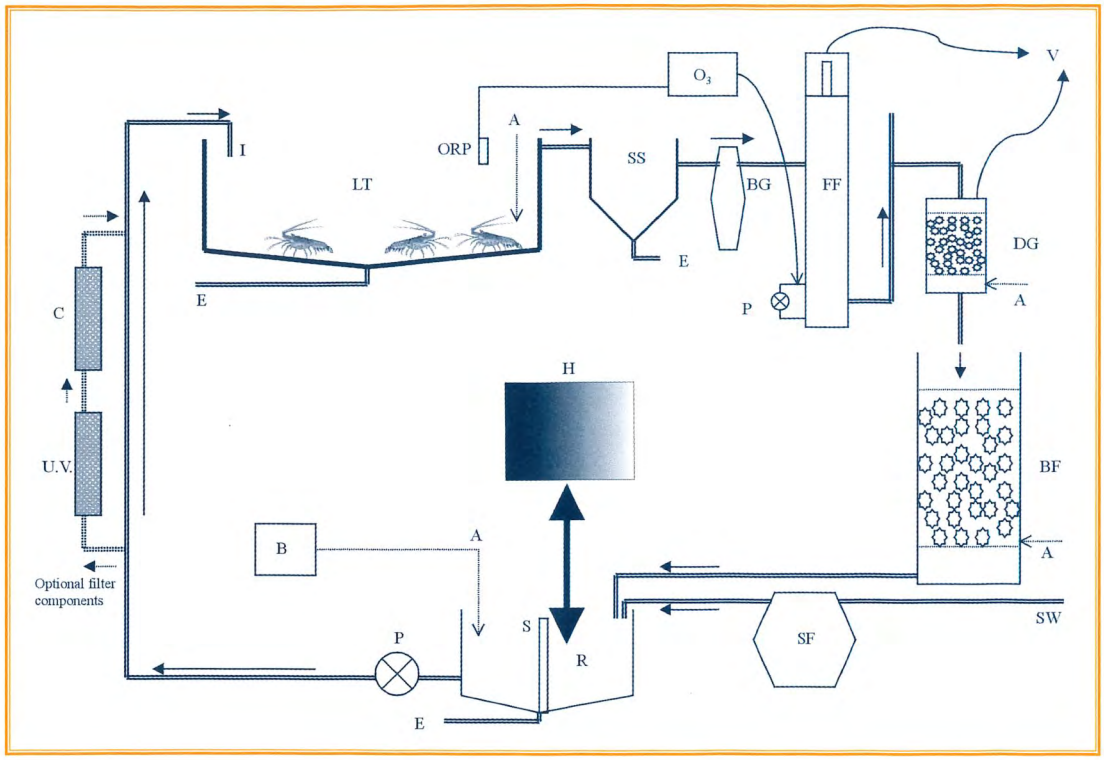


Figure 1. Schematic diagram of a recirculating system for holding lobsters showing all possible components. Abbreviations, A = Airline from blower (Note: not all connections to blower shown); B = Blower; BG = Bag filter; BF = Biofilter; C = carbon filter; DG = Degassing of residual ozone and CO_2 (in a packed column); E = Effluent discharge; FF = Foam fractionator; H = Heat-exchange unit; I = Inlet; LT = Lobster holding tank; O_3= Ozone generator; ORP = Redox probe (controls ozone generator); P = Pump; R = Reservoir (Sump tank); S = Standpipe; SF = Rapid sand filter; SS = Swirl separator (or other solids filter); SW = Fresh seawater supply; U.V. = Ultraviolet light steriliser; V = Venting off ozone gas and CO_2, outside of building.

3.1 WATER SUPPLY

3.1.1 INCOMING WATER

Water to add to the recirculating system would generally come either directly from the source (sea/estuary) via pipe or be transported to the system by truck. Either way it is important that it is obtained from a source of good quality water. If you are reliant on pumping from the sea/estuary you cannot always be sure that the water quality is going to be of suitable quality when it is required. It is because of this that a water reservoir (see Section 3.1.2) is an important component of a recirculating system.

3.1.2 WATER RESERVOIR

A reservoir of fresh seawater is a necessity. The reservoir provides backup if the quality of the water in the recirculating system decreases and a water exchange is necessary. It is not always possible to obtain suitable quality replacement water when it is required, especially if the holding system is situated a long way from a reliable seawater supply. It is also useful to have the ability to easily add water to the system to make up for losses associated with general day-to-day operations (backflushing, siphoning). Ideally the volume of water maintained as a reservoir should be similar to that in the system and it should be maintained at a similar temperature to that in the holding tanks.

3.2 HOLDING TANKS

Broadly, two methods are being used to hold lobsters: the tank or the stacked bin method. Each method offers advantages and disadvantages (adapted from Harvie, 1993).

	ADVANTAGE	DISADVANTAGE
TANK	Freedom of movement of lobsters Easy to see weak/dead lobsters	Take up a lot of space Extra handling of lobsters involved in transferring between tanks
STACKED BINS	Space efficient Reduced handling (especially just prior to live export)	Difficult to see weak/dead lobsters

A combination of the two methods may be the most appropriate. Tanks can be used for the initial stages of holding when the health of the lobsters needs to be regularly checked. Lobsters that are to be live exported can then be moved to the bin system in appropriate sized lots where they can be slowly chilled to the export temperature prior to packout. The major advantage of this system is that the lobsters do not undergo any handling stress just prior to the packout. Therefore, they should be in the optimal physiological condition to handle the stresses of live export. Tank systems can also be set up to achieve this advantage by having the ability to decrease the temperature in a tank where the correct number of lobsters have been previously stored in baskets. Extra chilling capacity may be required on the "live export preparation" tank to achieve this.

3.2.1 TANK DESIGN

There are two major design requirements for the holding of lobsters in tanks. First, they need to supply the lobsters with the appropriate conditions to maintain them in optimal condition. Second, to achieve an efficient processing operation the handling of lobsters, both into and out of the tanks, needs to be a simple process.

Commonly used tank designs include long narrow raceways or troughs, square or rectangular tanks, and circular tanks (Tetzlaff and Heidinger, 1990). Tank construction materials are usually constrained by budget. Common construction materials are concrete, block and mortar, fibreglass and plastic. Movable fibreglass or plastic tanks give some flexibility in the overall design of the holding system.

Raceways have many features that make them the most suitable tank design. If they are designed so that two baskets fit across the width of the raceways, the baskets can be easily accessed from the sides. Aeration would be required along the length of the raceway to ensure water quality, e.g. oxygen levels need to be maintained, and to ensure that no dead spots (areas of low flow) develop. There is likely to be some buildup of the ammonia level at the outlet end of the tank but it should not be excessive as long as there is a good water turnover rate. If the baskets used for holding lobsters are large and not easy to manually handle, some sort of mechanical lifting device (e.g. a gantry system) would need to incorporated into the design of the system.

If lobsters are to be held in tanks they should be placed within baskets. Lobsters should be size-graded and selected when they first enter the holding system. Lobsters judged as being suitable for live holding should be placed into the storage baskets and these placed into the tanks as soon as possible after grading.

3.3 WASTE SOLIDS REMOVAL

Waste solids add significantly to the load on a recirculating system. The breaking down of the waste solids by bacteria results in the consumption of oxygen and generation of ammonia. This organic matter can also clog the biofilter, interfering with the flow of the

water through, and the balance of, the biofilter. Thus, waste solids should be removed from the system as quickly as possible (Losordo et al., 1998). Numerous methods are available to remove this waste; an efficient system should remove as much of the waste as possible and concentrate it for easy removal, while using minimal amounts of water and energy (Tetzlaff and Heidinger, 1990). Waste solids can be classified into several categories: settleable, suspended (which includes floating) and dissolved. Specific methods are required to deal with each of the different forms.

3.3.1 SETTLEABLE SOLIDS

Large amounts of settleable solids material (faeces, regurgitated feed, seaweed, sand) enter the system when new lobsters are added. Due to the design of most holding tanks much of this waste settles out in the holding tanks. This should not be seen as a significant problem as it is better for the material to be in the holding tanks, where it can be more easily and regularly cleaned out, than for it to move into the biofilter. Lobsters should be separated from the waste material and the easiest way to achieve that is by keeping them in baskets supported off the floor of the tanks. Unless the tanks are specifically designed to allow easy removal of this waste material (e.g. V-shaped bottom) then regular siphoning of the tanks will be required. The loss of water associated with the siphoning can be substantial and it will need to be replaced or reused after the waste material is filtered from it. Another option would be to have the capacity to store the water from the recirculating system in a separate tank and then clean out the settled material approximately every week or after a tank is emptied of stock. To be able to easily clean a tank it is important that tanks have a good sized drain outlet at the bottom.

Another way of dealing with the large influx of waste solids is to have a settlement or purging tank, where lobsters are purged for 24 hours prior to being moved into the main recirculating tanks. Faecal production and high rates of ammonia excretion usually cease within 24 hours and thus most material will remain in the settlement tank when the lobsters are moved on. However, this method adds another handling step, adding further complexity to the process.

3.3.2 SUSPENDED SOLIDS

Other waste material does not settle out (suspended or floating solids) and specific mechanical filtration equipment is required to remove it. These filters catch and hold large and small particles from water flows for removal or *in situ* decay (Moe, 1992). The filtration is usually incorporated in the system between the tanks and the biofilter. This filtration can be as simple as the addition of mat filters placed over the top of the biofilter. Although they can serve a useful purpose, simple filtration units are not generally successful mainly because they are improperly designed. Also they tend to be awkward to clean, meaning cleaning is not undertaken as regularly as required. Poorly designed or cleaned filter mats result in water short circuiting the filter mats thus making them ineffective. Thus, it is important to have a properly designed mechanical filter in place.

There is a range of mechanical filters to choose from. The ones most applicable to lobster holding systems are filter sumps, sand filters, filter bags, canister type filters and microscreens:

- Filter sumps incorporate the material used for mat filters (as discussed above) into specially designed sumps, and waste is collected passively as the water flows through them.
- Pressurised sand filters are commonly used in many aquaculture applications. They consist of an enclosed vessel that is typically half to two thirds full with sand. Water is pumped into the top of the filter under pressure and is forced through the sand. The sand acts as the filter and the size of particle that can be filtered is largely dependent on the size of the sand grains. To clean sand filters the water flow is reversed and they are backflushed. Their limitations in recirculation systems are that, in addition to reasonably high operational costs, they use a lot of water for backflushing.
- Filter bags are cloth materials (usually nylon or polypropylene) in the shape of a bag (Huguenin and Colt, 2002) which are attached to the end of a pipe. The pipe usually discharges over or within an open tank and suspended solids are filtered out as the water passes through the bag. Head loss through the bags is small when they are clean. They need to be cleaned regularly. Filter bags are simple to set up and operate and are useful filters in the appropriate situation.
- Canister type filters are enclosed vessels designed for the removal of fine solids and operate under pressure. A filter material (e.g. mesh bag, cartridge) is inserted in the vessel and water passes from the inside to the outside. Filtration to less than 1 µm is possible with such filters although the cost of replacement of the filter material, and the cost of pumping is high. They require regular manual cleaning, usually once per day, but more frequently if the particulate content of the water is high.
- Microscreens are available in a wide variety of materials, configurations, and (Huguenin and Colt, 2002). Drum filters, disc filters, conveyer/belt filters are some of the different types. They are automatically cleaned filters where water jets are used to clean the screen. The screen material is constantly moving and is cleaned as it rotates. Such filters are capable of removing solids as small as 6 µm. These types of filters have not been used much in lobster holding facilities. They have the advantage that they have a very low head loss (typically 10-70 mm) which reduces the pumping costs in recirculation systems.

3.3.3 FINE AND DISSOLVED SOLIDS

Much of the waste material will be in the form of fine or dissolved solids (< 30 µm) which cannot be easily removed by sedimentation or mechanical filtration. Foam fractionation (see Section 3.7.1) is the most practical method of removing these waste materials, and thus foam fractionators are very important components of recirculating systems.

3.4 BIOLOGICAL FILTRATION

Ammonia exists in two forms in water – unionised (NH_3) and ionised (NH_4^+). Together these make up the total ammonia level which can be measured in water. NH_3 is much more toxic than NH_4^+. The percentage of ammonia present as NH_3 is dependent on the temperature and pH of the water, increasing with both parameters. At the temperature and pH normally found in lobster holding systems NH_3 represents 1-5% of the total ammonia. While the lethal concentration of ammonia has been established for many species, including lobsters, the sub-lethal effects of nitrogen are relatively unknown. There are a number of methods of removing ammonia from the water and the most widely used method is biological filtration.

3.4.1 THE NITROGEN CYCLE AND BIOLOGICAL FILTRATION

Biological filtration is the most common method of preventing the build-up of toxic levels of ammonia in recirculating systems. Basically, a biofilter is simply a surface on which bacteria grow. These bacteria convert the toxic ammonia to the relatively non-toxic nitrate via the natural nitrogen cycle.

NH_3 (ammonia) > NO_2 (nitrite) > NO_3 (nitrate)

Ammonia is oxidised to nitrite by a group of chemautotrophic (chemotrophic = chemical eaters) bacteria, of which the *Nitrosomonas* bacteria are the most well known genus. They need a substrate to live on (the biofilter material) and a source of ammonia and oxygen in a damp or aquatic environment to grow and form colonies (Moe, 1992). Nitrite is oxidised to nitrate by another group of chemoautotrophic bacteria, of which the *Nitrobacter* bacteria are the most well known genus. These bacteria need a source of nitrate and similar environments to *Nitrosomonas* to grow and form colonies. Bacteria of both groups are present in soils, freshwater and marine waters throughout the world (Moe, 1992).

Nitrate accumulates in the system until it is removed; in most systems this is generally achieved via a water change. Other bacteria can convert the nitrate to nitrogen gas but this process is unlikely to happen in most recirculating systems.

3.5 AERATION AND DEGASSING

The addition of oxygen to water and the release of excess carbon dioxide can be achieved through a variety of devices such as air diffusers, spray bars or packed columns. Maintaining oxygen concentrations of greater than 80% saturation is highly recommended in rock lobster holding systems, therefore aeration into the tanks and into the biofilter is a necessity. Aeration methods for rock lobster holding systems are outlined in a companion guide to industry by Crear and Allen (2002). Generally, increases in carbon dioxide concentration are the primary reason for decreases in the pH of recirculating systems for holding lobsters. Packed columns (also referred to as degassers), which have a large air to water ratio, are a

simple and efficient method of removing carbon dioxide from the water. Such columns are also very good at removing supersaturated gases, which can have a detrimental effect on the health of the lobsters, and thus provide a safety net within the system.

The use of pure oxygen is generally not necessary, as correctly designed aeration systems (i.e. adding air, which is 20% oxygen) should more than adequately meet most systems requirements.

3.6 TEMPERATURE CONTROL

Cooler temperatures tend to reduce activity, oxygen consumption, waste excretion and aggressive behaviour of lobsters. There are many sources of heat which act to increase the water temperature; pumps, aeration and external air temperature are the largest influences. Heat exchangers are the most common method of controlling the water temperature. Some chilling units contain copper coils. Copper can be detrimental to the health of lobsters at even quite low concentrations. Although the copper coils can be coated with plastic to prevent contact with the seawater, it is preferable to use units that contain titanium coils. The appropriate size unit for controlling temperature will be dependent on many factors, including the required water temperature, water volume and air temperature.

Chilling of water is often achieved through chilling the air. This is an inefficient way of chilling water, however there may need to be some sort of air temperature control maintained because if the air temperature is high the transfer of heat to the water may result in the heat exchangers have trouble maintaining the right temperature.

3.7 OTHER COMPONENTS

3.7.1 FOAM FRACTIONATOR

Foam fractionators (also referred to as protein skimmers) are used to remove fine and dissolved solids from the water. Foam fractionation is a process of introducing air bubbles at the bottom of a closed column of water. As the bubbles rise through the water column, solid particles attach to the bubbles' surfaces, forming the foam at the top of the column. The foam build-up is then channelled out of the fractionation unit to a waste collection tank. The process can be used to significantly reduce water turbidity and oxygen demand of the system (Losordo et al., 1998). Although they are not always incorporated into systems, correctly functioning foam fractionators are one of the most important components to include in a recirculating system. They can be purchased from aquaculture equipment suppliers, and are available in a range of sizes to suit the system demands. The size of fractionator is principally based on the total volume of water in the system.

3.7.2 OZONE

Ozone is a colourless gas (O_3) that is commonly used as a steriliser. Ozone is generated by passing oxygen (either as air or as pure oxygen) through an electric discharge. It is a very strong oxidising agent, highly toxic to all forms of life and corrosive to many materials. In recirculation systems ozone has several beneficial effects such as the breakdown (through oxidisation) of long chain molecules into simpler forms which can then be broken down further in the biological filter. It is through this process that ozone eliminates the yellow/brown colourations, that build up in recirculation systems. It is generally used in conjunction with a foam fractionator, with the ozone being introduced into the fractionating column via a venturi inlet. Control is essential, as over-dosing can result in concentrations harmful to both the lobsters in the tanks and to humans (through off-gassing of ozone into the air). The Redox level (which changes with the amount of ozone in the water) is the commonly used method of determining the amount of ozone in the water. Although a very useful tool in many situations, the use of ozone in recirculating systems for holding lobsters is usually unnecessary.

3.7.3 ACTIVATED CARBON

Activated carbon is available as a powder or in granular form, with the granular form being the most practical for commercial use. It has the ability to absorb organic and inorganic molecules to its surface. Carbon is often used in addition to a biofilter as a polishing stage. Again although a very useful tool in many situations, the use of activated carbon in recirculating systems for holding lobsters is usually unnecessary. However, it is an essential component if ozone is used as it removes toxic by-products.

3.7.4 ULTRAVIOLET DISINFECTION

Ultraviolet (or UV) light radiation is probably the most commonly used disinfection process with seawater. The effectiveness of the light depends on a number of factors including bulb wattage, age, cleanliness, the distance between the bulb and the organism you are trying to kill, the species you are trying to kill, the duration and intensity of light, and the clarity of the water. Again, although it is a very useful tool in many situations, the use of ultraviolet lights in recirculating systems for holding lobsters is usually unnecessary.

3.7.5 LIGHTS

Lights generally need to be kept on 24 hours per day. Lobsters are naturally active at night, and the increased activity will lead to greater oxygen consumption and may result in lobsters crawling out of tanks. Only a low level of light is required to stop the activity. When work is undertaken the lights should not be bright, only sufficient to work safely and efficiently.

SETTING UP AND OPERATING BIOFILTERS 4

A biofilter is the heart of a recirculating system.

4.1 BIOFILTER DESIGN

The two most commonly used biofilter designs in lobster systems are the submerged bed biofilter and the packed column (or trickle) biofilter.

Submerged bed biofilters are characterised by having a fixed (non-moving) medium that is constantly under water; the water may flow either upward (up-flow) or downward (down-flow). The biofilter medium used in these filters is highly diverse and includes gravel (blue metal), calcareous gravel (e.g. oyster shell, crushed coral, dolomite), plastic beads and extruded or high surface area plastic media. The gravel substrates are low cost, buffer the seawater (at least the calcareous ones), however they have higher frictional head losses and are more prone to clogging with solids and short-circuiting than plastic media (Huguenin and Colt, 2002).

A packed column is basically a vessel, open to the atmosphere, containing a media, over which water falls by gravity. Packed columns are used for degassing, dechlorination and are the basis for trickle biological filters. In a properly designed trickling filter the water cascades over the medium in a thin film (Tetzlaff and Heidinger, 1990). This provides a large interface between the air and the water allowing the efficient transfer of gases into and out of the water. The media used in trickle filters is typically high surface area plastic with a large void space to minimise clogging. Air can be added to the base of the vessel to improve the efficiency of CO_2 removal. Constant aeration prevents CO_2 levels from increasing in the air inside the packed column.

4.2 BIOFILTER SIZE

The size of the biofilter required to service a holding system is largely dependent on two factors: the amount of ammonia being added to the system and the nitrifying capacity of the biofilter.

Most of the ammonia added to systems is excreted by the lobsters, although the bacterial degradation of organic material (e.g. uneaten and regurgitated feed, algae) would also add to the ammonia load. Data is now available on the ammonia output of lobsters under a range of conditions. Feeding has the greatest effect on the ammonia output of lobsters (Crear and Forteath, 2002). A purging tank (see Section 3.3.1) would minimise the influence of placing recently caught lobsters into a system, as the effect of feeding on ammonia excretion rate only lasts approximately 24 hours.

The nitrifying capacity of the biofilter is largely dependent on the water temperature and the surface area of the media. Nitrifying capacity increases with water temperature. Biofilter media with a large surface area to volume ratio will have a high capacity to undertake nitrification as higher numbers of bacteria can grow.

The following outlines the calculations to determine the appropriate volume of a biological filter in a recirculating system that will hold 1000 kg of *J. edwardsii* at 13°C. Lobsters are generally not fed when they are held in recirculating systems, therefore, the endogenous rate of ammonia excretion was used. The ammonia excretion rate of a 500 g *J. edwardsii* at 13°C is 1 μg g^{-1} h^{-1}; 1000 kg of 500 g lobsters will excrete 24g of ammonia per day. The specific nitrification surface area (SSA) refers to the total exposed surface area of the substrate in the filter or the area on which the bacteria can grow. The SSA is calculated by the following formula:

SSA = TAN excretion rate / nitrification rate

TAN is the total ammonia nitrogen. The nitrification rates of biofilters used in aquaculture range from 0.15-1.0 g ammonia m^{-2} day^{-1} (Losordo and Hobbs, 2000). At 13°C, the rate would be expected to be towards the low end of that range. Therefore, a rate of 0.2 g ammonia m^{-2} day^{-1} is presumed. The SSA based on the above data is 120 m^2 (i.e. 24/0.2). This allows the volume of substrate required to give the SSA to be calculated.

Required biofilter volume = SSA / Specific surface area of filter medium

It is assumed that the specific surface area of the filter medium is 200 m^2 m^{-3}. Therefore, the required biofilter volume is 0.6 m^3 (i.e. 120/200). However, this calculation does not take into account the contribution of urea to the ammonia nitrogen. If all of the urea was oxidised to ammonia then there would be ~ 20% more ammonia in the system. Thus, a biofilter of 0.72 m^3 would be required. If the lobsters were to be fed in such a system the biofilter would be far too small to handle the ammonia load because of the large increase in ammonia excretion associated with feeding.

4.3 BIOFILTER CONDITIONING

Nitrifying bacteria form a living film over the surface area of the filter media. These films of living bacteria consume and convert the ammonia produced by the lobsters. However, there is a lag period between the starting up of the recirculating system and the establishment of a suitable population of bacteria (Fig. 2). When starting up a biofilter it is important to constantly monitor the ammonia and nitrite concentrations.

Under normal start up procedures the lag period can be several weeks. There are several ways to decrease the start up time.

- Bacterial inoculums can be added to the system. Commercially concentrated bacteria are readily available. It is important that the bacteria used are grown in similar environmental conditions (e.g. temperature, seawater not freshwater) to that present in the biofilter.
- The system can be run at a slightly higher temperature than normal to increase the rate of bacterial growth.
- Add media from an already operating biofilter. It is suggested that 10-30 percent of the new biofilter volume should be made up from the operating biofilter material. It is important that both biofilters are operating under similar environmental conditions.

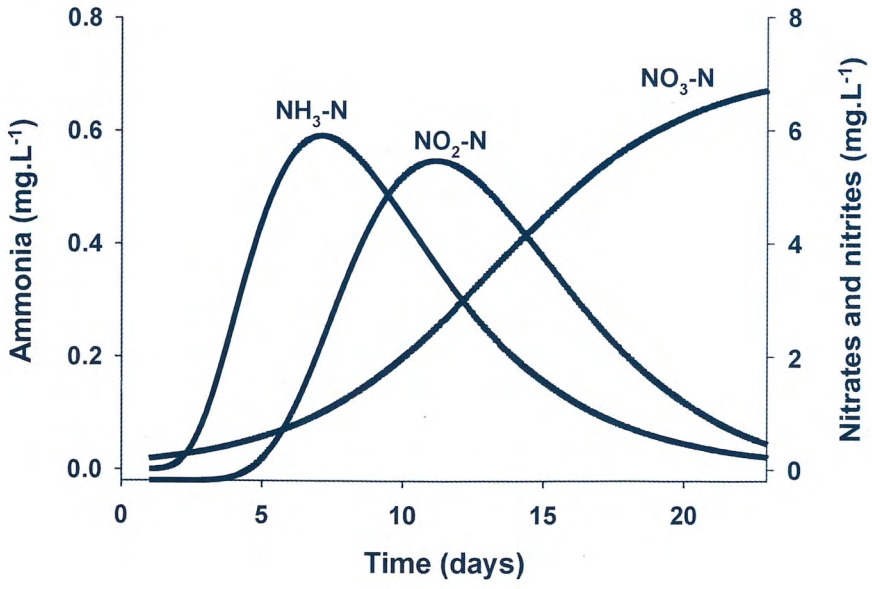


Figure 2. Representative diagram of biofilter start up over time.

4.4 SHOCK LOADING

Shock loading of a recirculating system occurs when there is a large increase in the ammonia load, resulting in an imbalance in the system. The bacterial population in the biofilter will take time to handle the extra ammonia load, meaning the ammonia levels will be maintained above the desirable level for a significant period of time. Success depends on the ability of the system to restabilise as quickly as possible (Harvie, 1993). The holding of lobsters is characterised by the rapid turnover of stock and thus variable stocking densities within tanks. The main cause of shock loading is the introduction of lobsters. The introduction of greater than 10% of the biomass already stocked into the system is likely to result in significant increases in ammonia. There are a variety of methods used to overcome and/or prevent shock loading.

- Extra biomass can be added slowly over a number of days. Unfortunately, this is unlikely to be possible in most cases as the time of delivery and quantity of lobsters coming into a holding system is generally variable.
- Extra water changes can be employed whilst the system is acclimating to the increased load. Large quantities of suitable quality water need to be available.
- The maximum biofiltration capacity of the system can be maintained by artificially feeding the bacteria in the filter during times of low stocking. Therefore, if a system can handle four tonnes of lobsters and only one tonne is in it, then 'artificial' ammonia equivalent to that produced by three tonnes of lobsters can be added to the system daily. This should be added slowly over the course of the day so that the addition of the artificial ammonia does not result in ammonia peaks.
- Some biofilter material can be maintained fully operational (by artificial feeding with ammonia) in a separate system and moved into the biofilter for the stocked tanks as required.
- Appropriate quantities of bacterial inoculums can be added to the biofilter when the extra stock is added to the system. Commercially concentrated bacteria are readily available.
- New stock can be spread out across a number of completely separate systems so that the load into any particular system is not large. This may make it difficult to trace stock.

It is under the conditions of shock loading that the experience of the operator becomes important. Shock loading does not necessarily need any action at all. Lobsters are reasonably tolerant of ammonia and if all other water quality parameters are kept optimal then they can handle moderate levels of ammonia for a couple of days. It is essential that the operator is able to determine when action is required to ensure that both the lobsters and the biofilter are maintained in optimal condition.

WATER QUALITY PARAMETERS AND MONITORING 5

Once a recirculating system is operating it is important that the appropriate water quality parameters are monitored regularly. Operators need to be trained in water quality management and need to understand the physiological needs of the organisms, which include those in the biological filter. This Section outlines the important parameters to measure and the frequency that they need to be monitored.

To be able to accurately measure the parameters suitable sampling equipment needs to be available. This section also outlines some of the sampling equipment choices. Sampling equipment can be expensive, but considering the risks they are a necessary outlay. Sampling equipment is varied but can be basically broken into two groups: a) test kits or b) meters. Each has their advantages and disadvantages.

a) Test kits are typically simple to use, however, each test is usually less accurate than can be obtained with meters. Although they are generally relatively cheap compared to meters, they tend to be more expensive per sample. If a lot of testing were to be undertaken it would usually be cheaper to buy a meter.

b) Meters on the other hand are generally more difficult to use, requiring some technical knowledge about their operation and maintenance. They also generally require a larger initial capital outlay. Most importantly, they require calibration on a regular basis to ensure that accurate results are obtained. Some meters are capable of measuring more than one parameter e.g. oxygen, salinity, pH and temperature.

Tolerance limits for various water quality parameters for the southern rock lobster (SRL) and the western rock lobster (WRL) (modified from Crear and Allen, 2002)

PARAMETER	TOLERANCE LIMITS
Temperature	SRL: 8 to 23°C (OHT[A] 9-13°C)
	WRL: 12 to 31°C (OHT 17-23°C)
Dissolved Oxygen (% saturation)	Min. 70%, preferably >80%
Salinity (g kg^{-1} or ppt)	30 to 38
Ammonia (mg L^{-1})	< 2
Nitrite (mg L^{-1})	< 5
Nitrate (mg L^{-1})	< 100
pH	7.8 to 8.4
Redox (mV)	200 - 350
Hardness (ppm)	100 - 200

[A]OHT – Optimum Holding Temperature

5.1 DISSOLVED GAS

5.1.1 OXYGEN

Monitor weekly or after system changes

The oxygen concentration in new systems should be checked regularly (every 2-3 h) for a few days to ensure that there is a good understanding of the normal oxygen concentrations, over a range of stocking densities. Provided appropriate stocking densities, flow rates and aeration are maintained the oxygen concentration should not vary greatly. Thus, in the longer term it is probably only necessary to measure the oxygen concentration on a weekly basis. If there were changes to the system (e.g. temperature change, increased stocking density, pump or aeration problems) then regular checking of the oxygen concentration is vital. It is also important to get an understanding of the oxygen concentrations at a range of points throughout the system e.g. at the influent and effluent of the biofilter, and at the top and the bottom of the tanks.

Oxygen meters are an expensive capital outlay, varying from just under $1000 to many thousands of dollars for highly complex integrated systems. Oxygen test kits, which generally cost several hundred dollars and do around 100 tests, are also available. Oxygen probes that provide a permanent display and alarm capability are available, and would be worthwhile considering in some systems.

5.1.2 CARBON DIOXIDE

Carbon dioxide (CO_2) is a by-product of lobster and bacteria respiration and it can accumulate within recirculating systems. Although carbon dioxide itself may not be toxic to lobsters at the concentrations normally found in holding systems, increased levels of carbon dioxide result in a lowering of the pH.

It is reasonably easy to determine if a drop in pH is due to an accumulation of CO_2. Take a sample (500 mL or greater is best) of the tank water and place it into a container. Measure the pH and then place an airstone into the container and aerate the water vigorously. The water in the container should be maintained at the same temperature, therefore place the container in the main tank and use it as a water bath. After 1 hour check the pH again. If the pH has increased noticeably (>0.1 of a pH point) then CO_2 is accumulating and an increase in the degassing ability of the system is necessary.

5.2 TEMPERATURE

Monitor continuously

Temperature monitoring is relatively easy, and cheap, with many types of thermometers to choose from. It is up to individual preference whether a glass thermometer or a thermocouple with digital readout. Prices range from a few dollars to several hundred

dollars. Potential contamination of lobsters from mercury and broken glass are an important consideration against the use of glass and mercury thermometers. A reasonably good quality digital thermometer should be able to be purchased for less than $50. A thermocouple thermometer placed in the tank with a permanent digital display that is clearly visible is the best option. Ideally the system should be set up so that an alarm is activated if the temperature moves outside the optimal set range.

5.3 pH

Monitor weekly or if system changes

pH is the measure of how acidic or alkaline a product is and is usually measured between 0 and 14. Seawater is always slightly alkaline with a pH between 7.8 and 8.4. As lobsters are marine animals, they require their holding water to be of the same pH range. In partial or full recirculation systems, pH should be tested in conjunction with water hardness. If there are changes to the system (e.g. temperature change, increased stocking density, pump or aeration problems) then checking the effect on pH is vital. If you find that the pH of your holding system is below the normal parameters for seawater then you must take action to buffer the water (using calcium carbonate and/or sodium bicarbonate) or make more regular water exchanges. CO_2 build-up causes a decrease in pH, therefore it is necessary to ensure the CO_2 is removed either by strong aeration, partial water change or via a device that creates a large air/water interface e.g. trickle filter.

To determine the level of pH in your holding system you can use a pH meter (most accurate method) or test strips (give a general estimate). pH meters range in cost from around $150 to several thousand dollars. Test kits can be purchased for around $20 to $100 and will generally do between 50 and 300 tests. pH probes that provide a permanent display and alarm capability are available, and would be worthwhile considering in some systems.

5.4 SALINITY

Monitor weekly

Salinity is the term used for the measurement of the total amount of salts in seawater. It can be measured with a refractometer, hydrometer or salinity meter. Seawater generally has a salinity of 33 to 35 g kg^{-1} (g L^{-1}). Levels outside this range occur as the result of factors such as rainfall, ocean currents and evaporation. As seawater is heavier than fresh water, water for topping up recirculating systems should be sourced from well below the water surface. It is preferable that water is sourced from as oceanic a site as possible (i.e. not from an estuary). Even so, the salinity of the water should be checked prior to it being added to the recirculating system.

Evaporation, resulting in elevated salinity levels, can be a problem in recirculating systems. Freshwater should be used to decrease the salinity. This needs to be done very carefully as a forgotten freshwater hose running into a recirculating system can lead to disastrous consequences. Add the freshwater in small amounts and check the salinity regularly. If a system contains 10 tonnes of water and the salinity is 40 g L^{-1}, to reduce the salinity to 35 g L^{-1} you would need to add approximately 1100 L of freshwater. This should be added in 3-4 batches (over 2-3 days), ensuring the water is well mixed between each addition.

A refractometer measures the degree to which the path of light is changed (refracted) as it passes through a thin layer of water. The amount of refraction is directly proportional to the amount of salt in the water, the more refraction the more saline the water. Refractometers can be purchased for around $200 to $300.

A hydrometer is used to determine the specific gravity (SG) of water and consists of a glass bulb with a weight at one end and a closed thin tube at the other. The greater the salinity the higher in the water the hydrometer will float. It is simply a matter of reading the salinity off the scale printed on the side of the tube (or converting the SG to g kg^{-1}). A reasonable quality hydrometer should not cost more than $50.

A salinity meter measures conductivity: the amount of electrical current that can be carried by seawater. The capacity of the water to conduct electricity is directly proportional to the level of salt in the water, the greater the current the more saline the water. Salinity or conductivity meters can be purchased for between $500 and $1000.

5.5 NITROGEN - AMMONIA, NITRITE AND NITRATE

Monitor daily

5.5.1 AMMONIA

Ammonia results from the normal metabolic processes of lobsters and from the bacterial decomposition of faeces, bait and dead animals. The unionised form of ammonia is the toxic part, and its concentration is dependent on the pH and temperature of the water. Ammonia will build up rapidly if lobsters are fed or if the biofilter's nitrifying bacteria *Nitrosomonas* are not adequately conditioned to handle a shock loading.

5.5.2 NITRITE

Nitrite is the intermediate step in the nitrification process. An increase in nitrite indicates that the *Nitrobacter* populations are not functioning well. Common causes of high nitrite are low oxygen in the biofilter and a surge of high ammonia (e.g. shock loading) (Tetzlaff and Heidinger, 1990).

5.5.3 NITRATE

Nitrate is the end product of the nitrification process and is generally regarded as having very low toxicity, thus it does not need to be monitored daily.

There is a wide range of kits available on the market to test these parameters, the choice of which to use is up to individual preference. Most have simple to follow instructions and can be completed within a matter of minutes. Generally a water sample is treated with reagents to produce a colour, which is then compared against colour standards. They are usually available for under $50 and will perform between 25 and 200 tests.

5.6 ALKALINITY AND HARDNESS

Monitor weekly or if system changes

5.6.1 ALKALINITY

Alkalinity (or carbonate hardness) of seawater gives an indication of its' buffering capacity i.e. the ability to prevent fluctuations in pH. It is a measure of the capacity of the water to accept acidity. Alkalinity is usually measured as either mg L^{-1} (milligrams per litre) $CaCO_3$ (calcium carbonate) or meq L^{-1} (milli-equivalents per litre), 1 meq L^{-1} = 50 mg L^{-1} $CaCO_3$. Ideally alkalinity should be greater than 100 mg L^{-1}. Commonly, calcareous biofilter materials such as coral gravel or oyster shells are used as a carbonate source. Even with such material the alkalinity can still decrease and it needs to be maintained with the addition of bases. Some bases commonly used, include hydrated lime or calcium hydroxide ($Ca(OH)_2$), quick or slaked lime (CaO), sodium bicarbonate ($NaHCO_3$), sodium carbonate (Na_2CO_3), calcium carbonate ($CaCO_3$), magnesium carbonate ($MgCO_3$).

5.6.2 HARDNESS

Hardness is a general term to describe the total amount of cations of the earth metals (mainly calcium and magnesium) in the water. In most waters, the hardness is similar to that of alkalinity, as calcium and magnesium are usually bound to the main alkalinity bases (bicarbonate and carbonate). Alkalinity tends to be used more as a measurement than hardness.

There is a wide range of kits available on the market to test these parameters, similar to those used for nitrogen, the choice of which to use is up to individual preference. Most have simple to follow instructions and cost and perform in a similar manner to nitrogen kits.

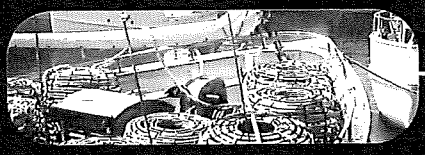
5.7 REDOX POTENTIAL or ORP

Redox is a word derived from a combination of the words Reduction and Oxidation. The redox potential or ORP (oxidation reduction potential) is a measure of the potential of the water for oxidation or reduction processes. The higher the reading, the higher the availability of oxidising agents in the water. Optimum levels of oxidising and reduction agents occur at around 300 mV and this is regarded to be the approximate ORP level of very good quality water. ORP levels in excess of 500 mV may prove toxic to life over prolonged periods, and ORP levels of over 600 mV are often maintained in systems where oxidising agents (such as ozone) are used to disinfect water. A low ORP level is a sign of poor water quality as the amount of oxidising compounds in the water is low, which limits the breakdown of organic matter.

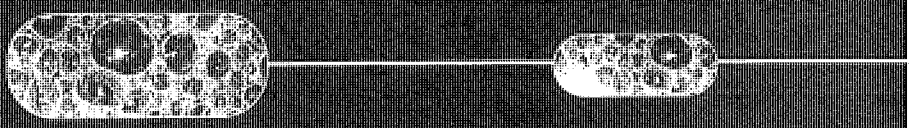
The measurement of ORP is usually undertaken in recirculating systems when ozone is being used in the system. ORP meters are available for about $200. ORP controllers measure and control the ORP to within desired levels by regulating the input of ozone into the system. ORP controllers are available for about $500.

OPTIMISING WATER QUALITY

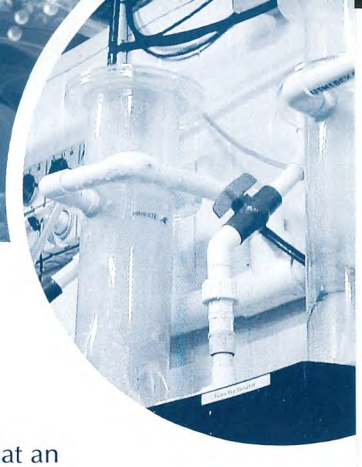
O_2



GUIDE FOR THE ROCK LOBSTER INDUSTRY No 1



APPENDIX 6



The calculation of the water flow rate required to ensure ammonia levels are maintained at an acceptable level in a flow-through system is largely dependent on two factors: the amount of ammonia being added to the system and the specified safe level of ammonia to be maintained. It is assumed that aeration is in place and thus the only requirement of the flow-through water is to ensure ammonia levels do not get too high. It is also assumed that the incoming seawater is good quality, with negligible ammonia.

As discussed under Biofilter size (Section 4.2), lobsters excrete most of the ammonia added to systems, although the bacterial degradation of organic material (e.g. uneaten and regurgitated feed, algae) would also add to the ammonia load. We have used the data available on the ammonia output of lobsters under a range of conditions (Crear and Forteath, 2002) and recent experiments to calculate flow rates required for unfed and for fed or purging lobsters. The example used is a flow-through system that will hold 1000 kg of *J. edwardsii* at 13°C.

The required flow rate (FR) is calculated by the following formula:

FR (L h^{-1}) = TAN excretion rate (g h^{-1}) / Specified "safe" level TAN (g L^{-1})

The safe level of ammonia for holding lobsters is <2 mg L^{-1}

UNFED LOBSTERS

The ammonia excretion rate of an unfed 500 g *J. edwardsii* at 13°C is 1 µg g^{-1} h^{-1}; 1000 kg of lobsters will excrete 1 g of ammonia per hour and 24 g per day. If you have larger animals the amount of ammonia excreted per unit weight of lobster decreases; *J. edwardsii* of around 700 g excrete 0.63 µg g^{-1} h^{-1}.

For 1000 kg of 500 g lobsters FR (L h^{-1}) = 1 g h^{-1} / 0.002 g L^{-1}
= 500 L h^{-1}

For 1000 kg of 700 g lobsters FR (L h^{-1}) = 0.63 g h^{-1} / 0.002 g L^{-1}
= 320 L h^{-1}

If lobsters are stocked at 100 kg m^{-3} of water (total water volume = 10 m^3), then this flow rate equates to a water turnover rate of once every 20 to 30 hours. This shows that the water flow does not need to be very high to ensure ammonia build-up is kept under control.

FED OR PURGING LOBSTERS

The maximum ammonia excretion rate of a fed 500 g *J. edwardsii* at 13°C is 7.5 μg g^{-1} h^{-1}; 1000 kg of 500 g fed lobsters will excrete 7.5 g of ammonia per hour and 180 g per day.

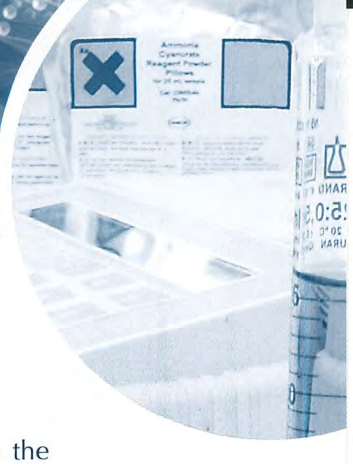
FR (L h^{-1}) = 7.5 g h^{-1} / 0.002 g L^{-1}
= 3750 L h^{-1}

These calculations do not take into account the contribution of urea to the ammonia nitrogen. If all of the urea was oxidised to ammonia then there would be ~ 20% more ammonia in the system, and a 20% increase in flow rate is required, i.e. 600 L h^{-1} for unfed and 4500 L h^{-1} for fed 500 g animals.

The basal level of ammonia excretion in a 500 g unfed *P. cygnus* at 23°C, is around 2 μg g^{-1} h^{-1}, which is double that for *J. edwardsii* at 13°C, although the peak in ammonia excretion following feeding is the same at 7.5 μg g^{-1} h^{-1}. More recent trials at lower temperatures of 19 to 20°C found that 442 g *P. cygnus* had excretion rates of 0.97 μg g^{-1} h^{-1}. In general, flow rate calculations must be adjusted according to the species held, size of animals, and holding temperature.

NB. IT IS IMPORTANT TO ENSURE OXYGEN REQUIREMENTS OF THE LOBSTERS ARE MET. SIGNIFICANTLY HIGHER FLOW RATES THAN THOSE CALCULATED HERE WILL BE REQUIRED IF AERATION IS NOT PROVIDED (SEE CREAR AND ALLEN, 2002).

GLOSSARY



AERATION The act of providing an air supply to water in order to increase the oxygen content of that water.

AMMONIA Ammonia is the major end product of protein metabolism in most aquatic animals. Ammonia is toxic to lobsters and therefore must be prevented from building up in holding systems. Ammonia is present in two forms in water (ionised – NH_4^+ and unionised – NH_3), the higher the pH and temperature, the higher the percentage of the toxic fraction (unionised).

ALKALINITY Alkalinity is basically a measure of the carbonate content of water. It gives a measure of the capacity of the water to accept acidity (i.e. its buffering capacity). Alkalinity is usually measured as either mg L^{-1} (milligrams per litre) $CaCO_3$ (calcium carbonate) or meq (milli-equivalents). 1 meq = 50 mg L^{-1} $CaCO_3$.

BIOLOGICAL FILTER (BIOFILTER) A filter providing a large surface area on which denitrifying bacteria grow; used to remove waste (particularly ammonia and nitirite) from recirculating systems.

BIOMASS Total weight (kilograms) of organisms in a system.

FLOW-THROUGH Single use water. Water enters a system, passes through the system and goes to waste.

NITRATE Formed as a result of the breakdown of ammonia to nitrite and then to nitrate by bacteria in biofilters. Generally not toxic at the levels found in recirculating systems. Chemical symbol: NO_3.

NITRITE Toxic chemical formed during the oxidation of ammonia to nitrate by bacteria in a biofilter. Most of the nitrite is converted to nitrate before the water exits the biofilter, therefore it is not generally found at toxic concentrations. Chemical symbol: NO_2.

OXYGENATION The addition of pure or very high purity oxygen to water in order to increase the dissolved oxygen concentration of the water.

pH A measure of acidity of a solution. It is in effect a measure of the amount of hydrogen ions. The normal pH of seawater ranges from 7.8-8.4.

RECIRCULATION The process of taking water from a holding system which would otherwise be discarded from the system and reintroducing it to the same system. Prior to being reintroduced, the water is often treated to remove some of the wastes so that the water quality is maintained at a sufficient high level that it remains suitable for the held animals. Recirculation systems can be operated as 100% recirculation (no new water added) or may have partial replacement water added.

REDOX Redox is a word derived from a combination of the words reduction and oxidation. The redox potential or ORP (oxidation reduction potential) is a measure of the potential of the water for oxidation or reduction processes.

SALINITY The term used for the measurement of the total amount of dissolved salts in the water. Full strength seawater has salinity in the region of 33-35 g kg^{-1} (grams per kilogram).

TAN The term given to total ammonia nitrogen, which is all nitrogen involved in the equilibrium between unionised ammonia and ammonium ions, as follows:

$$NH_3 + H^+ \leftrightarrow NH_4^+$$

VENTURI EFFECT The act of drawing air into a water system via a small tube or crack in a pipe. As the water passes through a restriction in a pipe, it forms a vacuum at the end of the restriction. A hole bored into the pipe at the point where this vacuum occurs will cause air to be drawn into the main flow. Although efficient at mixing chemicals and gasses into water, the operational costs of a venturi is high due to the cost of the increased pumping pressure required for the unit to operate. Venturis have their applications in some systems where there is more pressure available than is required by the rest of the system components.

WATER HARDNESS The amount of cations of the earth metals (mainly calcium and magnesium) in the water. In most waters, the hardness is similar to that of alkalinity, as calcium and magnesium are usually bound to the main alkalinity bases (bicarbonate and carbonate). Alkalinity tends to be used more as a measurement than hardness.

LIST OF SYMBOLS

WEIGHT

µg	microgram	10^{-6} grams; 1,000,000 µg = 1 g
mg	milligram	10^{-3} grams; 1,000 mg = 1 g
g	gram	
kg	kilogram	1000 grams; 1 kg = 1000 g

LENGTH

µm	micrometre (also called microns)	10^{-6} metres; 1,000,000 µm = 1 m
mm	millimetre	10^{-3} metres; 1,000 mm = 1 m

VOLUME

mL	millilitre	10^{-3} litres; 1,000 mL = 1 L
L	litre	
m^3	cubic metre	1 m^3 = 1,000 L

TIME

h	hour

TEMPERATURE

°C	degrees Celsius

ELECTRIC POTENTIAL

mV	millivolts

DERIVED UNITS

Defined in the context of this booklet

$g\ h^{-1}$	grams per hour
$\mu g\ g^{-1}\ h^{-1}$	micrograms (of ammonia) per gram (of lobsters) per hour = ammonia excretion rate
g ammonia $m^{-2}\ day^{-1}$	grams of ammonia per square metre per day
$m^2\ m^{-3}$	square metres (surface area) per cubic metre (filter medium)
$g\ L^{-1}$	grams per litre
$mg\ L^{-1}$	milligrams per litre
$L\ h^{-1}$	litres per hour = flow rate
$kg\ m^{-3}$	kilograms per cubic metre = lobster stocking density
ppm	parts per million

SALINITY

$g\ kg^{-1}$	grams (salts) per kilogram (water)
equivalent to $g\ L^{-1}$	grams (salts) per litre (water)
equivalent to ppt	parts (salts) per thousand parts (water)

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NOTES

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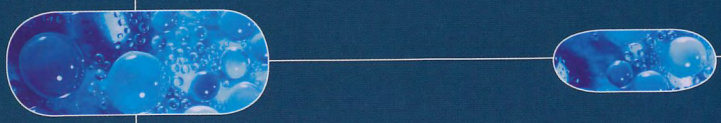
VANESSA TUCKER



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Rock Lobster Health and Diseases:

A Guide for the Lobster Industry

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Curtin University of Technology

ROCK LOBSTER POST-HARVEST SUBPROGRAM FRDC

Curtin UNIVERSITY OF TECHNOLOGY

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CONTENTS

Points of special importance are highlighted in the text

ACKNOWLEDGEMENTS

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INTRODUCTION

Rock lobsters inhabit coral or rocky reefs and ledges and eat a variety of food including molluscs, sea urchins and crustaceans. Their organ systems are similar to those of other decapods, a subgroup of Crustacea that includes prawns, crabs, crayfish and lobsters.

Rock lobsters have a spiny, hard exoskeleton or shell that is shed regularly (moulting), allowing the animal to grow. The calcium needed to produce the shell is obtained from salts in the water and from food. Beneath the exoskeleton and in and around the major organs there is a network of thin-walled vessels and sinuses containing blood (haemolymph). About 30% of a lobster's weight is blood and it is this almost colourless liquid that runs from the live lobster and clots on surfaces when a lobster is cut or injured.

The blood contains a pale bluish pigment called haemocyanin that contains copper and has a role in oxygen transport. The blood also contains cells, called haemocytes, and other chemicals and salts that have a role in ensuring that the lobster's organ systems can function normally. The blood also has a role in minimising the effects of injury and disease. When lobsters are stressed or diseased, the properties of their blood and the number and type of circulating haemocytes can vary. Examining the blood is one method of assessing the health of lobsters.

The quality of lobsters is judged by the number of missing legs or antennae and by the condition of the lobster. Stress and disease reduce the quality and value of harvested lobsters. Assuming that lobsters in the wild are relatively healthy when they enter the trap at harvesting, the development of weakness or disease and/or death must result from physical damage occurring during the processes of capture and post-capture processing, or from physiological responses to post harvest stressors. These adverse physiological reactions occur through exposure of lobsters to environmental stressors that either alarm the lobsters, initiating an acute stress response, or cause a marked alteration in a physiological process such as oxygen uptake, nitrogen metabolism or ion regulation and water balance.

Physical damage can be prevented by avoiding rough handling and using 'lobster friendly' gear. Minimizing stress, on the other hand, is achieved by reducing exposure of lobsters to environmental conditions that cause stress reactions. Whilst the detrimental effects of conditions such as air exposure, temperature extremes and poor water quality are well known to fishermen, the physiological reactions to these conditions are less well understood. One of the aims of this booklet is to explain, in simple terms, the underlying physiological responses that occur when a lobster is exposed to post-harvest environmental stressors and the effects that this may have on the health of the lobster. Some common abnormalities and diseases are also described.

CHAPTER 1

HOW TO PERFORM A ROCK LOBSTER AUTOPSY

How to collect lobsters for dissection

Live lobsters should be used if meaningful results are to be obtained from an autopsy. The usefulness of examinations of dead lobsters will depend on the type of disease process occurring in the lobster, the time delay between death and preservation (fixation) or collection of samples and the method that is used to preserve the lobster tissues. The laboratories listed in Appendix 2 can suggest alternative procedures if live lobster are not available for autopsy.

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.

How to collect haemolymph?

Lobster haemolymph might need to be collected if immune function tests are to be conducted. Haemolymph should be collected from the base of the fifth walking leg. Swab the area with 70% ethanol. Collect haemolymph with a sterile syringe and a 24 gauge needle, both of which should be stored on ice or kept in the refrigerator prior to use. Some tests require the use of an anticoagulant when collecting haemolymph. For details of haemolymph sampling and tests refer to FRDC 1999/202[1].

What observations and measurements should be made?

Prior to collection of haemolymph sample and dissection, the following measurements and observations should be recorded on the data sheet (see Appendix 1):

- **Carapace length and weight**

The carapace length is the length from between the horns to the end of the carapace. Weight is the wet weight to the nearest gram.

- **Sex**

The male and female lobsters can be distinguished by looking for the position of the genital openings on base of the walking legs. Females display a pair of pores at the base of the 3rd pair of walking legs whereas in males the pores appear as protrusions at the base of the 5th pair.

• **Vigour index**

Hold the lobster around the middle of the thoracic region and assess for the responses below.

Table 1. Determination of vigour index

RESPONSE	CLASSES					
	0(d)	1(m)	2(w)	3(h)	4(vh)	5(a)
Defensive horn response	-	-	-	-	-	+
Vigorous tail flip	-	-	-	-	+	+
Appendage 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

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

• **Moult stage**

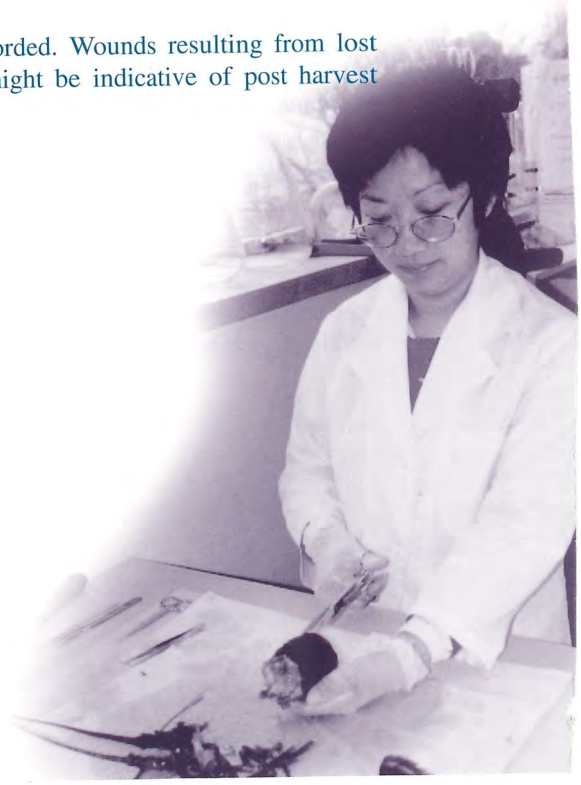
Moult stage is determined by examining the tip of the swimming appendage (pleopod) under the microscope[2]. Cut a small piece of the tip of the pleopod, preserve in 10% seawater formalin and forward to the diagnostic lab.

• **Presence of lesions**

The external surfaces of the animal should be examined for any damage or lesions and these should be recorded as they might aid in diagnostic analysis.

• **Appendage loss**

The number and location of lost appendages should be recorded. Wounds resulting from lost appendages could be a site of infection. Lost appendages might be indicative of post harvest maltreatment.



How to dissect

(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®).

The following dissection procedure is recommended:

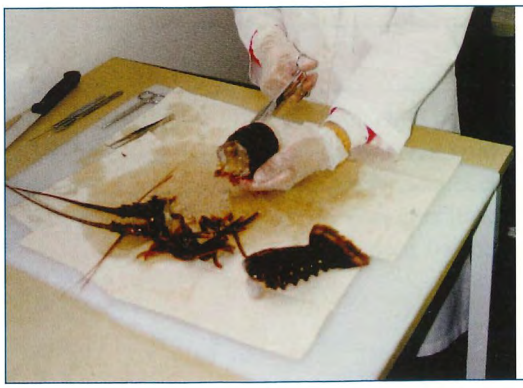
Step 1. Set up for dissection. You will need a dissection board, large knife, pair of scissors, pair of forceps and jars for collection of tissue samples.

Step 2. Remove the lobster from the aquarium and place it on the dissection board.

Step 3. Using a sharp knife remove the proximal area of the carapace just behind the antennae. (This will kill the animal, but some twitching may continue if the lobster was not cooled adequately).

Step 4. Dissect away all walking legs.

Step 5. Cut along the junction of the carapace and the abdomen to remove the tail. The specimen is now ready for removal of body tissues.



Step 6. Make two longitudinal cuts along the carapace, about 1cm from the centre. Carefully separate the carapace from the underlying tissues.

Figure 1

Tissue sample collection

Once the carapace has been removed and underlying organs exposed, the removal of tissues should be performed as rapidly as possible to avoid autolysis. The most sensitive tissues with respect to autolysis are the hepatopancreas and the antennal glands. These should be the first to be removed.

Tissue samples should be removed from the following organs and immediately placed in the fixative:

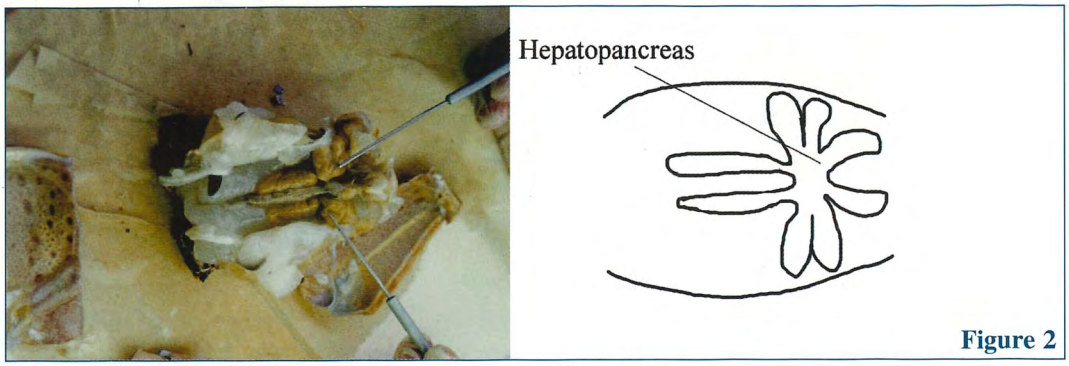


Figure 2

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)(Fig 2)

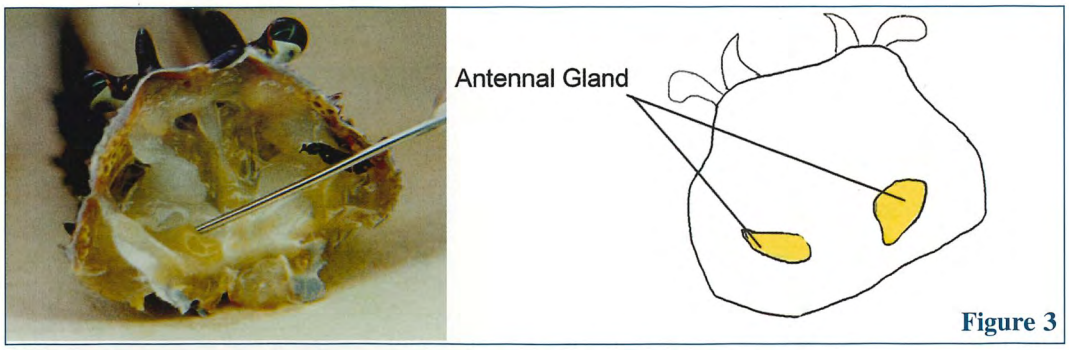


Figure 3

Antennal gland (two samples, comprising one half of each gland)(Fig 3)

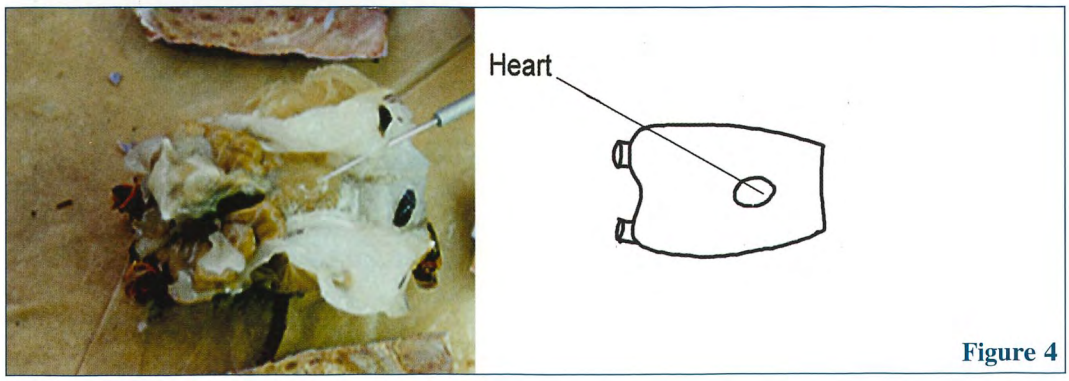


Figure 4

Heart (two samples, obtained by cutting the heart in half and then taking one half of each section)(Fig 4)

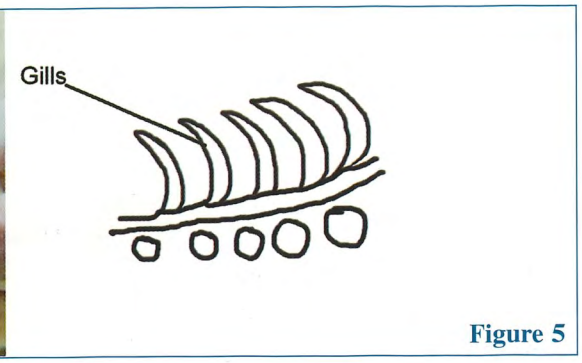


Figure 5

Gills (one piece from each side) (Fig 5).

Samples may also be taken of: 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) (fig. 6a).

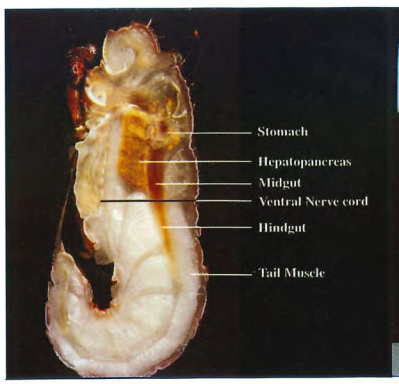


Figure 6a

Photo courtesy B. Paterson, CFT

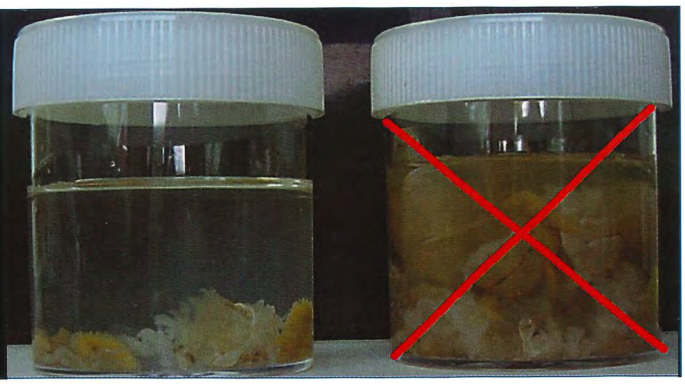


Figure 6b

Each tissue piece should be approximately 0.5 - 1cm³. Seawater formalin (see glossary) is used as fixative. The volume of fixative to tissue should be at least 20:1 (Fig. 6b).

Transfer tissues into 70% ethanol after 24h fixation and forward to a diagnostic lab for histopathological analysis (see Appendix 2 for list of diagnostic labs.)

CHAPTER 2

CAUSES OF DISEASE AND PRE-DISPOSING FACTORS

What is disease

The term 'health' describes a physiological state of an animal. A 'healthy' animal is one in which the physiological processes underpinning growth, maintenance, disease defence and reproduction are functioning normally. In this context the term 'normal' refers to a state that is appropriate and adequate for the animal at that point in its development. Simply put, the animal is in a state of 'ease'.

A 'diseased' animal, on the other hand, is in a state of 'dis-ease'. It is experiencing some form of injury or threat to its survival, which results in damage to body tissues and/or abnormal physiological function. The injury or threat may be in the form of a sudden environmental change, for example, exposing a fish to air or to a chemical toxin, or the invasion of body tissues by an infectious organism. Exposure to disease agents stimulates a suite of physiological processes – the host defence and immune processes – to counteract the threat and to repair the damaged tissue.

A healthy animal can withstand the challenge of a disease agent better than one in poor health, unless the agent has the capacity to cause disease regardless of health status. Some viruses and highly virulent bacteria (primary pathogens), and some forms of nutrient deficiencies or chemical exposures, fall in the latter category.

Infectious disease agents are primary or opportunistic pathogens that fall into five main categories – viral, bacterial, fungal, and those caused by protists (single cell organisms) and metazoans (multi-cell organisms). Non-infectious diseases or health problems are caused by nutritional deficiencies, genetic disorders, immune diseases and exposure of lobsters to toxins. There have been very few reports of non-infectious diseases of spiny lobsters. However, with the growth of aquaculture it is likely that more information on these types of conditions will become available.

More detailed information on diseases of rock lobster, lobster immune defence mechanisms and methods of assessing the health of lobsters can be found in 'A review of lobster diseases, their investigation and pre-disposing factors', produced as part of FRDC Project 1999/202[1].

How does a lobster fight disease?

Lobsters have a number of processes by which they fight disease. They include changes in the levels of circulating haemocytes, aggregation of haemocytes at sites of tissue injury and around foreign material, clotting of blood, phagocytic capacity in which lobster haemocytes take in foreign material and antibacterial activity in the blood and other tissues.

Haemocytes are intimately involved in most host defence responses of the lobster. The number of haemocytes found in lobster blood varies with the stage of the moult cycle and with the stress and nutritional status of the lobster. Weak lobsters have been shown to have reduced numbers of haemocytes.

The shell of the lobster is an effective barrier to most infectious agents. It impedes the entry of bacteria and other pathogens and also protects the underlying soft tissues from mechanical damage. A population of non-pathogenic bacteria permanently resides on the external shell of a lobster, limiting the growth of other harmful bacteria and playing an important role in host defence.

When the shell is damaged, as occurs during wounding or when a leg or antennae is lost, infectious agents such as bacteria may gain entry to lobster tissues. Furthermore, wounding leads to loss of blood, which clots and darkens upon exposure to air. Blood loss weakens lobsters and predisposes them to disease. Excessive bleeding leads to the presence of large amounts of dark, translucent fluid in boxes or containers of post-harvest lobsters and is a sign of poor handling practices.

What is a stress reaction?

A stress reaction is a physiological response to an environmental stressor. The reaction follows a set pattern, commencing with nervous responses to the stressor and culminating in either a return to the normal physiological state or a change to a new state – either ill health or, in the extreme, death.

How can a stress reaction kill a lobster?

Lobsters, like any animal, are exposed to changes in environmental conditions all the time. The external environment is constantly changing (e.g. changes in temperature, pressure, light, sound, food intake, air or water composition), so a lobster's physiology is geared to minimize the effect of these external changes on the state and composition of its internal environment – the cellular environment. Specialised cells within the body (mostly nerve cells) detect changes in the internal environment resulting from external environmental variations and initiate physiological responses to counteract these changes. This process is called homeostasis.

Blood components, and other physiological parameters, show similar patterns of oscillation to that of the internal temperature of a refrigerator. Minor changes in the external environment cause fluctuations in the levels of the component or physiological process. Nerves and hormones respond to these fluctuations, just like the temperature sensors and the compressor in a refrigerator, and initiate reactions, which result in the maintenance of the parameter within homeostatic levels.

If the animal is exposed to a larger than usual change in an environmental condition, the changes in the internal environment exceed those usually seen in a normal homeostatic variation. This excessive deviation in the physiological parameter initiates a *stress reaction* aimed at counteracting the internal environmental change. Two outcomes are then possible – if the deviation is not too extreme, the physiological parameter will, in time, return to the normal value. On the other hand, if the deviation is extreme, or if there are multiple environmental stressors superimposed, cell damage occurs.

Following the occurrence of cell damage two outcomes are likely. If cell damage is minimal, a new, sub-optimal steady state develops. In this new state the damaged cells, while remaining alive, cease to function at an optimal level. In this state the health of the animal is compromised and the lobster is said to be in poor health. Immunocompetence is affected and the lobsters are susceptible to disease. Death could follow, particularly if the lobster is exposed to further stress. Alternatively, if cell damage is extreme, the cells die (a process called necrosis). This can lead directly to organ failure or, if the nerves are affected, indirect organ failure due to nerve dysfunction. The end result is the death of the lobster.

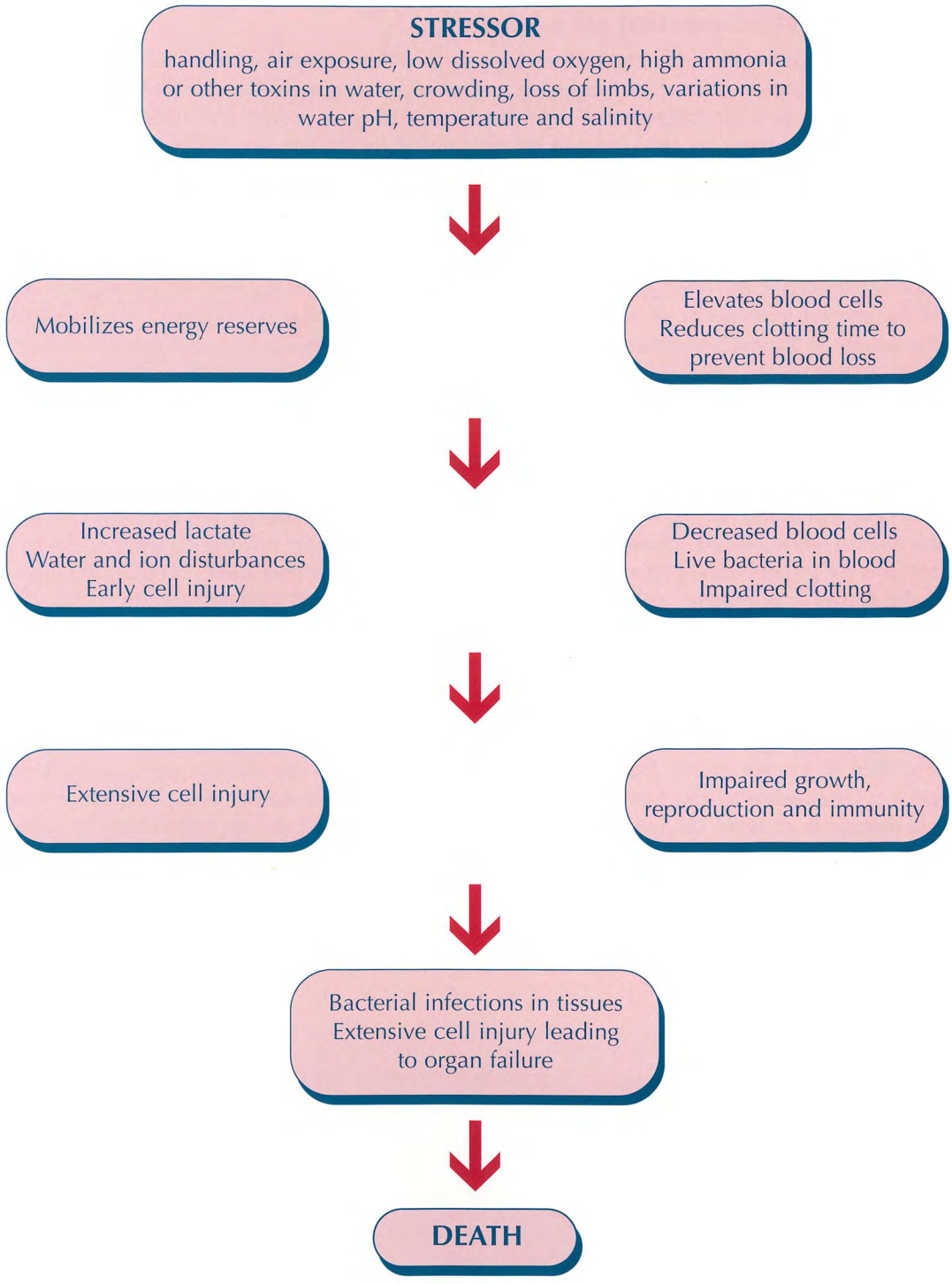


Figure 7.
The sequence of events that are triggered by stress factors are shown in the above diagram.

What does air exposure do to a lobster?

During respiration, oxygen is extracted from water at the gills and transported to the body cells via the blood. The cells use the oxygen to generate energy and produce carbon dioxide (CO_2) as a waste product. The carbon dioxide is transported back to the gills where it diffuses into the surrounding water.

It is important that CO_2 is removed from the body tissues as it can be harmful if allowed to accumulate. Increased CO_2 concentration causes an increase in acidity due to the following chemical reaction:

$$CO_2 + H_20 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

Changes in pH affect enzyme reactions and can adversely affect physiological processes. If the pH becomes very acidic, cellular damage and ultimately, cell death can occur.

When a lobster is removed from the water, the transfer of oxygen across the gills becomes less efficient. Similarly, the removal of CO_2 from the blood is impeded. As respiration still continues, the O_2 level falls and CO_2 rises. The lack of oxygen results in a change in the way cells generate energy. A different physiological process, called anaerobic respiration, is used[3,4]. The same change to anaerobic respiration occurs when the lobster flaps its tail. Anaerobic respiration results in the accumulation of lactic acid in the muscle and in the blood. The overall result is a fall in blood pH. This process is called acidosis.

Acidosis leads to lethargy in lobsters. The tail becomes limp, and leg and antennae movement is reduced. The term 'weak' is given to a lobster affected by acidosis. The condition is reversible providing the lobster is returned to the water before irreversible cell damage occurs. Once returned to the water the lobster is able to remove the excess acid from its tissues and normal aerobic respiration is restored. The length of time it takes for the lobster's physiology to return to normal depends on the degree of acidosis. This, in turn, is affected by the time period of air exposure and by how much exercise (tail flapping) took place during the air exposure.

What happens when a lobster is exposed to high or low temperatures?

Physiological processes are governed by enzyme reactions. Enzyme reactions occur at different rates depending on the temperature. All animals have an optimal temperature range within which their enzyme reactions and, hence, physiological processes occur at optimum levels. If the temperature rises or falls outside this range the enzyme reactions, and the body functions they control, are adversely affected.

High temperatures are generally more detrimental than low temperatures. Prolonged exposure to high temperatures in fish (i.e. those that exceed the normal range but not high enough to cause immediate death) can lead to malfunction of the immune system, disease outbreaks and general poor health. The same processes probably occur in lobsters. If the temperature is returned to normal the lobsters are likely to continue in a poor health status for some time.

Low temperatures slow the enzyme reactions down, particularly those involved in muscle contraction, and the lobster becomes comatose[3]. However, if the temperature returns to normal, high health status is quickly restored.

What effect does a high level of dissolved ammonia have on a lobster?

Ammonia (NH_3) is a toxin and can damage cells and their components. The precise nature of the damage is not well understood. When ammonia is dissolved in water, ammonium ions (NH_4^+) are formed according to the equation:

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

Ammonium is not as harmful as ammonia but is a toxin, nonetheless. Ammonia is produced in lobster tissues as a result of a breakdown of proteins. It is a normal waste product and will accumulate in the blood and in the water in which the lobsters are held. Bacteria in the water and in filtration systems convert ammonia to nitrite (NO_2^-) which is then converted to nitrate (NO_3^-). Nitrite is also a mild toxin but nitrate is only toxic in high concentrations. Collectively these three compounds are called nitrogenous wastes.

The levels of nitrogenous wastes must be kept low in lobster holding systems. In flow-through systems the rate of water replacement should be sufficient to prevent build-up of these toxins while in re-circulating systems the biological filter should have sufficient capacity to maintain ammonia levels below tolerance limits.

Lobsters can tolerate transient exposure to high ammonia levels but prolonged exposure will cause the lobsters to die[3]. Mortality is presumably due to injury of cells in vital organs or in the nervous system.

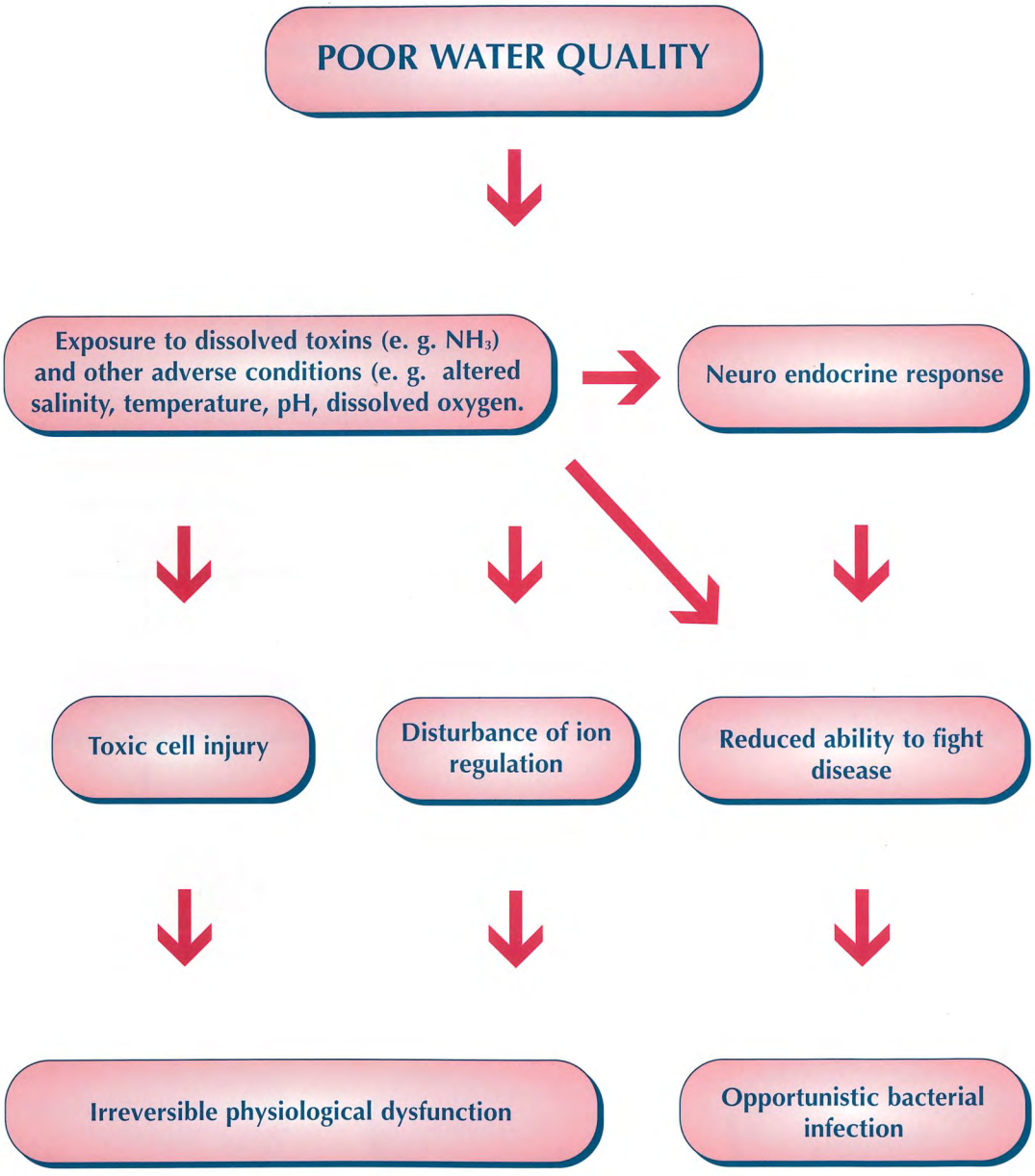


Figure 8.
Poor water quality may result in the above events in lobster and may result in disease.

CHAPTER 3

DISEASES OF ROCK LOBSTERS IN AUSTRALIA

Australian rock lobsters do not suffer from any of the serious diseases that have been reported in rock lobsters and clawed lobsters in many other parts of the world. The prevalence of diseased rock lobsters in the wild is very low, and the presence of disease usually indicates that the lobsters have been exposed to some type of stress. Following stress or injury, many bacteria or fungi that are present in the lobster's environment and do not normally harm lobsters, can invade lobster tissues causing disease. They are called opportunistic pathogens, rather than primary pathogens (Fig. 9). There are no species specific diseases of rock lobsters in Australia, but rather a range of health problems that can occur under certain conditions. For this reason, factors that pre-dispose lobsters to developing disease should always be considered as part of a strategy to manage disease.

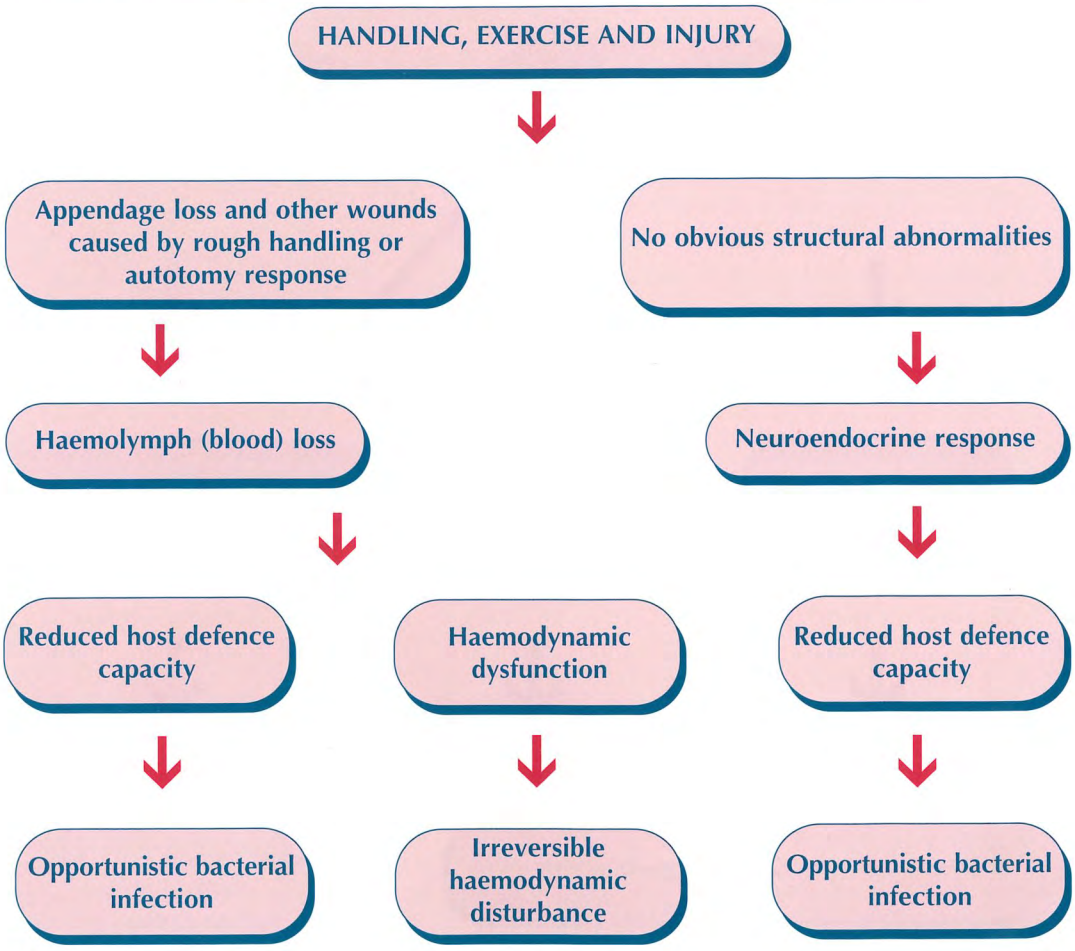


Figure 9.
Handling, exercise, exposure to air and injury trigger a series of responses in lobster. In some cases these responses successfully protect the lobster from further harm, but in other instances the end result may be death of the lobster due to opportunistic infections or physiological disturbances.

What causes limp, weak lobsters?

Lobsters become weak and have a slow or weak tail flap response when they are suffering from disease, lactic acidosis following exercise or handling, or when their water is contaminated with toxins, including those secreted by the lobster as end products of respiration and nitrogen metabolism.

Bacterial septicaemia is a common cause of weakness in lobsters. In this condition bacteria multiply in tissues within the lobster, including the blood. It occurs when the host response mechanisms of the lobster have been unable to control the spread and multiplication of the bacteria. The end result is serious disruption of cell structure and function and the physiological processes of the tissues. Affected lobsters often die even though they may show no external signs of disease or injury. *Vibrio* bacteria that are normal inhabitants of seawater and the lobster exoskeleton are often opportunistic pathogens that cause this condition in Australia.

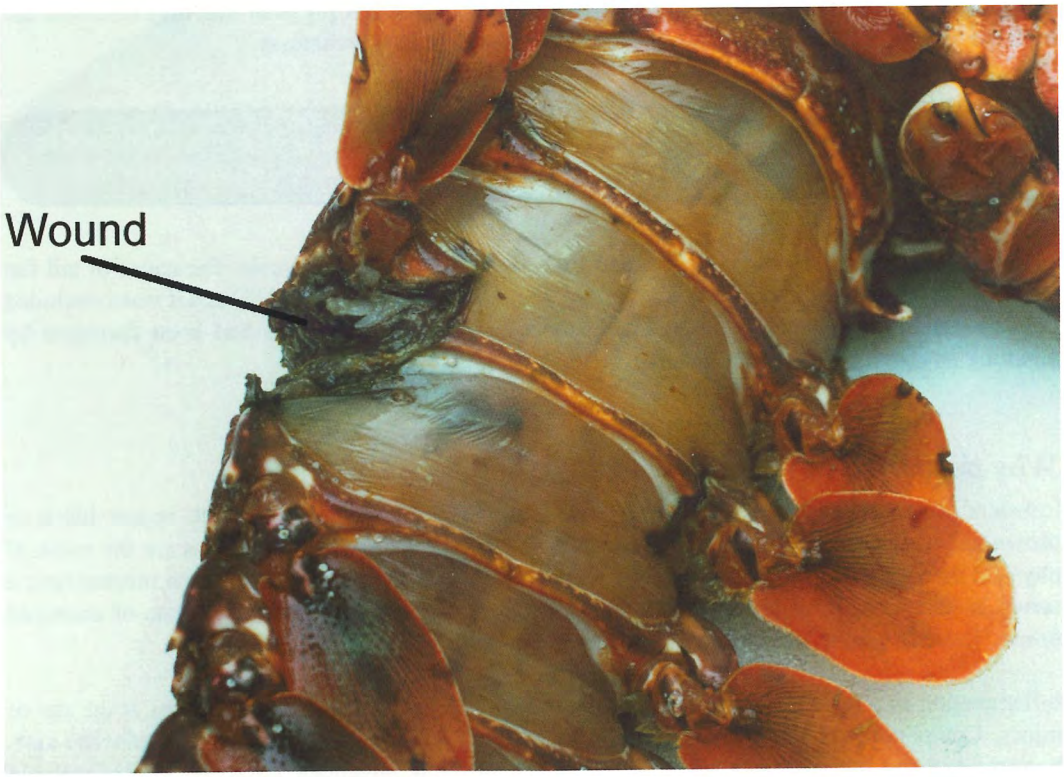


Figure 10.
Wounding with associated inflammation. A western rock lobster, *Panulirus cygnus*, with a lesion on the lateral side of the tail (arrow). Penetrating wounds or infections such as the one on this lobster, can result in bacterial septicaemia, loss of large amounts of blood and death.

What causes ulcerated lesions on lobster's shells?

Bacteria and/or fungi are usually present in ulcerated, brown or black areas of the lobster tail or shell. Many bacteria produce chemicals that dissolve the hard chitin found in the lobster's exoskeleton. This damages the shell, allowing bacteria, fungi or other disease agents to enter the lobster. It may result in loss of blood followed by clotting or coagulation of blood, a host defence mechanism that helps to limit the influx of foreign material and the exit of blood. Blood clotting causes dark colouration of the damaged area. Melanin, an end result of one aspect of the host defence response, is also produced, resulting in black or brown pigmentation of the damaged area. Shell lesions have been linked with poor water quality, injury and stress in studies overseas and in Australia. Such predisposing factors should be investigated and, if possible, eliminated when shell lesions are a problem.

Bacteria, often *Vibrio* species, are the most common cause of shell lesions, however, the fungus, *Fusarium solani*, was responsible for an outbreak of disease in western rock lobster in the wild fishery in 1979 (Fig. 11). This fungus is common in the environment and has been reported in diseased decopods in other areas of the world. It produces a black pigment that may resemble the melanin that is produced by lobster as part of the host defence mechanism.

The infectious agents involved in production of the shell lesion can only be identified by a diagnostic laboratory as the lesions look similar although the pathogens may differ.

The ulcerated lesions are sometimes called shell disease or tail fan necrosis. The cause of tail fan necrosis of southern rock lobster was investigated in FRDC Project 2000/211[5] and it was concluded that the lesions were caused by bacterial infection of appendages that had been damaged by handling or contact with other lobsters.

Why does a lobster get black or brown areas on its shell?

Lobsters often get lesions in their shell, particularly in the tail fan area, which appear black or brown and which are usually associated with tissue erosion (Fig. 12). The lesions are the result of physical damage or an infectious process. When cells are injured by either of these mechanisms a series of reactions occur which prevent further tissue damage and repair the areas of damaged tissue. These reactions are collectively referred to as inflammation and repair.

Inflammation in a lobster involves the accumulation of large numbers of haemocytes at the site of injury. Components of the blood produce melanin, a dark coloured pigment. Melanin has anti-bacterial and anti-fungal properties and is thought to help limit infections. Large amounts of melanin are deposited in the area of tissue damage causing blackening of the wound or infection area.

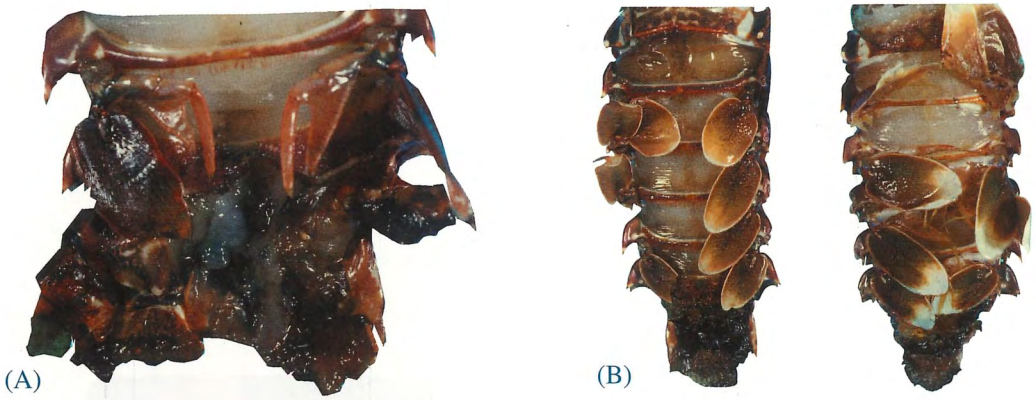


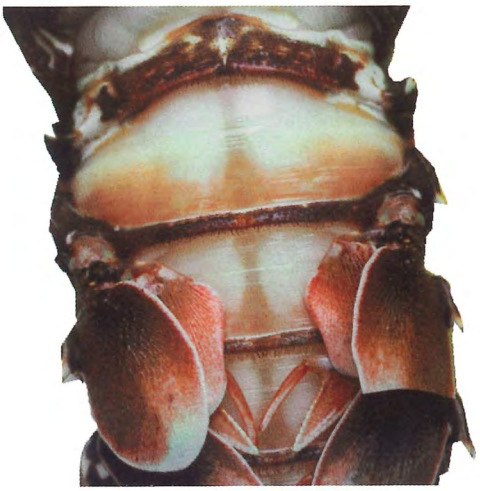
Figure 11.
Fusarium solani infection in a lobster.
A and **B:** Western rock lobster, *Panulirus cygnus*, infected with the fungus, *Fusarium solani.* The telson and uropods are severely damaged and the black pigment, melanin, produced by the lobster's host defence mechanism is visible in the affected areas.
(Photographs courtesy of School of Veterinary and Biomedical Sciences, Murdoch University)

Figure 12.
Tail necrosis in a western rock lobster, *Panulirus cygnus*. Note the loss of tissue as a result of necrosis (death and disappearance of tissue) of the uropod tissues. Bacteria, including several species of *Vibrio*, produce enzymes that dissolve chitin and are usually found in such lesions.

Why does the tail of a lobster turn white?

White tailed lobsters are occasionally seen by fishermen and processors. The live lobsters look as if they have already been cooked.

The whitening of the lobster flesh is caused by a parasite called a microsporidian[1, 6]. The parasite invades the muscle tissue and forms large numbers of microscopic spores within the muscle bundles. The presence of the spores and the consequent tissue damage affect the way light is reflected and absorbed by the muscle tissue and results in the white appearance of the tail.



White tail

Normal tail

Figure 13.
Microsporidiosis or 'white tail' in a western rock lobster, *Panulirus cygnus*. The disease occurs in decapods worldwide but may be caused by several species of Microsporidia.

What causes discoloured flesh in lobsters?

Both pink flesh and grey or black flesh have been observed in the western rock lobster. Lobster blood often has a pink or orange colour at moulting. The pigment astaxanthin, which is present in reserve cells, is the likely cause of this condition because reserve cells are present in high numbers in the haemolymph at moulting.

Western rock lobsters sometimes have pink flesh that is associated with an unpleasant taste and weakness in the lobster. This condition is most often seen in lobsters that have been held in live-holding facilities but has also been reported by fishermen. Factors other than moulting may contribute to this condition, but as yet, other possible causes have not been thoroughly investigated. Pink blood and flesh have been associated with infection with certain micro-organisms in other decapod species.

Another relatively common deviation from normal is the development of black or grey discolouration of flesh in cooked lobsters. This is caused by formation of the pigment, melanin, produced as a product of the prophenoloxidase cascade. This enzyme system is a component of the lobster's immune system, and has been described earlier in this booklet. The control of melanosis in cooked lobster is currently being investigated in FRDC Project 2001/235.

Figure 14.
A western rock lobster *Panulirus cygnus* with pink flesh (left), a condition that may occur at moulting and from other poorly understood factors.

Photograph courtesy P. Spanoghe.

What causes swelling in lobsters?

Two types of swelling are commonly seen in lobsters. The first type is characterised by a puffing-out of the soft membranes, particularly the membrane at the junction of the cephalothorax and the tail. The other form of swelling is seen as a blister in the tail fan involving part or all of the tail fan.

A. Swelling of whole lobster. This swelling has been reported in western rock lobsters, southern rock lobsters and tropical rock lobsters and is caused by an abnormal intake of water. The tissues become waterlogged and pressure builds up under the hard, inflexible shell or exoskeleton. This type of swelling is seen in several situations in post-harvest lobsters. It is caused by drowning lobsters in fresh water and, occasionally after lobsters are held in water of poor quality.

The accumulation of water in lobster tissues in fresh or low salinity water results from a process called osmosis. Osmosis occurs when two solutions of differing concentration are separated by a semi-permeable membrane. Since the solute cannot pass through the membrane, water diffuses across the membrane, until the two concentrations are the same. The net result is a movement of water from the area of low solute concentration to one of high solute concentration. The two solutions are said to have different osmotic pressures.

The solute concentration in lobster blood is similar to that of seawater. When the lobster is placed in low salinity water, or in freshwater, the differences in osmotic pressure leads to a movement of water into lobster tissues. This process causes the tissues to become waterlogged and the membranes to bulge. If the lobster is returned to normal salinity it will completely recover, providing the period of exposure has not been too long.

Figure 15.
A tropical rock lobster, *Panulirus ornatus*, affected by turgid lobster syndrome. Note the swelling causing protrusion of the membrane between the head and abdomen.

B. Blisters on the lobster shell. Another, less well understood, condition is that in which the tissues become waterlogged but there is no history of exposure to low salinity water. This condition is occasionally observed in batches of lobsters in processing tanks or it may be seen in only one lobster in the tank. The exact cause of this condition is not known but it is often seen in lobsters with shell disease[1, 5].

The processes resulting in blisters on the tail may be similar to those causing blisters in the skin of humans. In the latter case, local cell damage resulting from an injury or exposure to a bacterial toxin or some other toxic agent leads to a release of chemicals which affect the pattern of blood flow in the surrounding tissues. There is an increased blood flow to the area and a leakage of plasma into the tissue spaces. This leads to a localised accumulation of tissue water to form a blister.

Figure 16.
A blister can be seen on the tail of this western rock lobster, *Panulirus cygnus* (arrow).

What causes deformities in lobster?

Deformities in lobster are usually the result of defects in production of the exoskeleton, injuries to the exoskeleton that occur while the new shell if still soft after moulting or from diseases that prevent complete shedding of the old shell at moulting. The causes may be nutritional in origin or may be the result of pathogens such as bacteria or fungi causing damage beneath the exoskeleton that later interferes with production and shedding of the shell. In some cases the failure of moulting results in death of lobster. In other instances it results in loss of limbs or deformed appendages.

Figure 17.
A western rock lobster, *Panulirus cygnus*, with a deformed antenna. This type of deformity is likely to result from incomplete separation of the old and new exoskeleton at moulting. It may be caused by the cellular response of lobster to infection with bacteria, protozoan parasites or fungi.

Why do the lobster's shell and gills become fouled?

The shell and gills of lobster can become 'fouled' by the growth of other living organisms or by inorganic debris[1,7]. Fouling usually indicates that the lobster is weak and lethargic and is not adequately cleaning its shell. Affected lobsters are often stressed and have been held in less than optimal water conditions that has allowed the multiplication of free-living invertebrates such as nematode worms, protozoans, filamentous bacteria and algae. There may also be accumulations of material such as solid wastes from lobster food. The stress of the poor water quality may predispose the lobster to infection with opportunistic pathogens such as bacteria. In some cases the fouling agents are visible to the naked eye and in others they are may be seen as discoloured areas on the gill or shell and are identified as fouling agents following examination of tissues using a light microscope.

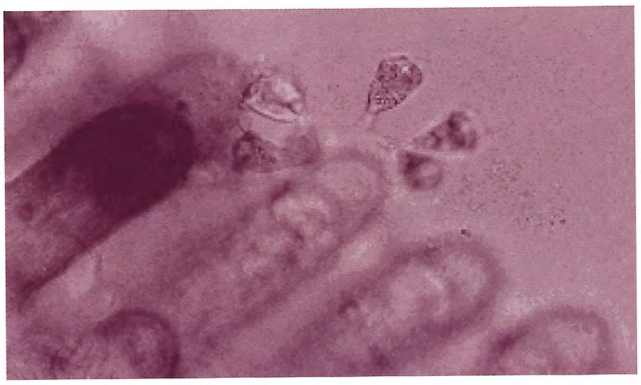


Figure18.
Sessile ciliate (*Carchesium sp.*) attached to juvenile *Jasus edwardsii* gill cuticle.Wet preparation, 200 x magnification.
Photograph B. Diggles.

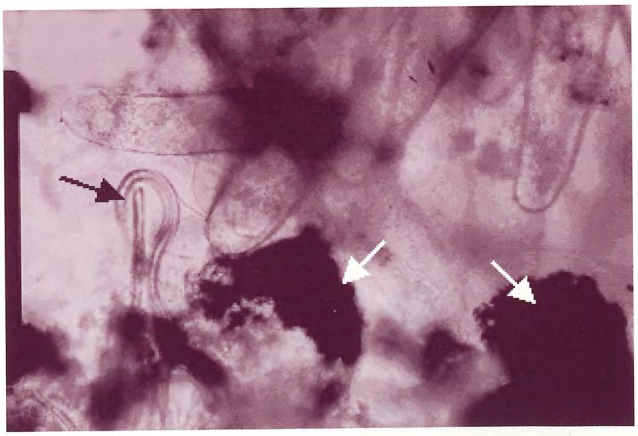


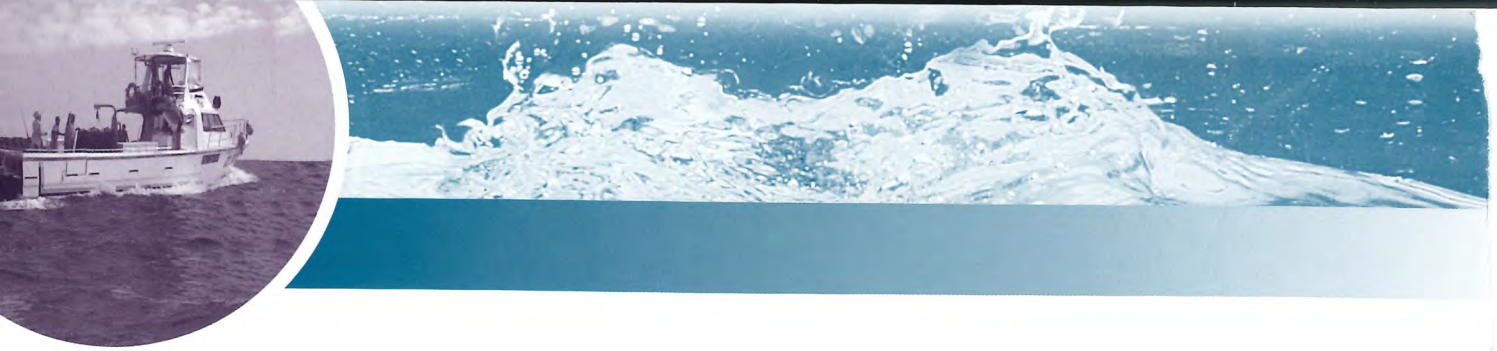
Figure 19.
Fouling of the gills of juvenile *Jasus edwardsii*. Organic detritus (white arrows), free living nematode (black arrow) and moderate *Leucothrix*-like infestation, wet preparation, 100 x magnification.
Photograph B. Diggles.

How can lobster health problems be managed?

The first step in controlling health problems in rock lobsters is to determine the cause or causes of the problem. In many instances there will be pre-disposing factors that have damaged or stressed the lobsters and made them more susceptible to disease. These factors include water quality problems, handling, injury or overcrowding. Once these pre-disposing factors have been identified and corrected, the resultant health problem will often resolve because many disease agents are opportunistic pathogens that only affect lobsters already in a weakened condition.

Serious outbreaks of disease may require additional treatment or control measures.There are no preparations specifically registered for the treatment of lobsters in Australia. and any treatments should be used only on the recommendation of a veterinarian so as to avoid potential toxicity to lobsters, residues in lobster meat, harm to the environment and harm to humans applying the treatment or handling lobsters.

This guide presents information on some of the more common diseases and health problems seen in rock lobsters in Australia. It is not intended to be a comprehensive guide that enables the diagnosis of conditions from their appearance, but aims to provide information on the more basic processes that may contribute to abnormal appearance or activity in rock lobsters. Further investigation of the causes of disease or ill thrift should be undertaken by a diagnostic service. Some government departments and laboratories providing this service are listed, by state, in Appendix 2. There are also private veterinarians offering a similar service.



GLOSSARY

Autolysis	A process by which the enzymes in dead cells continue to function after death, resulting in decomposition of the tissue.
Bacteria	Small, single celled organisms that are just visible on a light microscope.
Ethanol (70%)	Prepared by mixing 70ml of 100% (absolute) ethanol with 30ml of distilled water.
Fungus	A group of infectious agents that usually produce hyphae and spores. The hyphae are sometimes visible to the naked eye.
Haemolymph	The equivalent of blood in lobsters and other invertebrates.
Haemocyte	Cells in the haemolymph that have a role in host immune defence mechanisms of lobster.
Immunocompetence	The ability of an animal to fight pathogens.
Lesion	An area of tissue that is affected by a disease. It is usually different in size, colour and/or texture from unaffected tissue.
Necrosis	Death of tissue. This is the end result of many disease processes.
Opportunistic pathogen	An infectious agent that only causes disease in animals that have been pre-disposed to disease by unrelated factors such as injury or stress.
Pathogen	An infectious agent such as a bacteria, fungus, protozoan or virus that can cause disease.
Physiological response	The physical and chemical processes that occur in tissues to ensure that each organ performs its functions to the optimal degree despite changes in the external and internal environment of the lobster.
Seawater formalin	Prepared by mixing 10ml of formalin (saturated aqueous solution, 37-39% formaldehyde) with 90ml of filtered seawater.
Virus	A small pathogen that requires living cells to reproduce.

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The FRDC reports can also be accessed at the following
http//:www.frdc.com.au/research/program/rlph/index.html

APPENDIX 1

CODE No._____ **SOURCE**_____
DATE _____ **TIME** _____ **CONDITION**_____

Carapace Length mm		Weight gm	
Sex M F		Moult Stage	
Haemolymph colour		Vigour Index	

Gross Observations:

Appendage Loss	Yes/No	No. and Site	
Tail Blistering	Yes/No	No. of uropods/telson affected	
Tail Erosion	Yes/No	No. of uropods/telson affected	
Underskin formed?		Histology taken Yes/No	
Tail Weight	gm		
Muscle total wet weight gm	gm	Muscle total dry weight	gm
Hepatopancreas total wet weight	gm		
Hepato. sample wet wt	gm	Hepato. sample dry wt.	gm

APPENDIX 2

ORGANISATIONS THAT CAN HELP WITH DISEASE INVESTIGATIONS OF ROCK LOBSTERS IN AUSTRALIA

New South Wales

NSW Fisheries/Veterinary Officer
(Aquatic Animal Health)
Regional Veterinary Laboratory
Bruxner Highway
Wollongbar NSW 2477
Telephone: **(02) 66 261 293**
Mob: 0428 698 112
Fax: (02) 66 261 276

Northern Territory

Berrimah Veterinary Laboratory
Berrimah Farm
Strath Rd
Berrimah NT
Telephone: **(08) 8999 2249**
Fax: (08) 8999 2024

Queensland

Telephone: **13 25 23**

Oonoonba Veterinary Laboratory
PO Box 1085
Townsville QLD 4810
Yeerongpilly Veterinary Laboratory
6645 Fairfield Road
Yeerongpilly QLD 4105

South Australia

PIRSA Aquatic Animal Health Unit
14th Floor, 25 Grenfell St.
Adelaide SA
GPO Box 1625 Adelaide SA 5001
Telephone: **08 8226 0314**
Fax: 08 8226 0330

Idexx Laboratory
33 Flemington Street
Glenside SA 5065
Telephone: **08 8372 3700**
Fax: 08 8372 3777

Tasmania

Fish Health Unit
Animal Health Laboratories
Mt Pleasant Laboratories
Kings Meadows, Tasmania 7249
Telephone: **(03) 6336 5389**
Fax: (03) 6344 3085

Victoria

Department of Veterinary
Investigations
Victorian Institute of Animal
Science
475 Mickleham Road
Attwood Vic 3049
Telephone: **03 9217 4200**
Fax: 03 9217 4399

Western Australia

Fish Health Laboratory
C/- Animal Health Laboratories
Department of Agriculture
3 Baron-Hay Court
South Perth WA
Telephone: **(08) 9368 3351**
Fax: (08) 9474 1881

Aquatic Science Courses at Curtin University of Technology

The Aquatic Science Research Unit (ASRU) at Curtin University in Perth, Western Australia, has been delivering tertiary courses in the aquatic sciences for over ten years. Founded by Professor Louis Evans, ASRU has now expanded to offer units in Aquaculture, Seafood Science, Aquatic Resources, Fisheries Management, and Agribusiness. ASRU's growing number of post-graduate students specialise in fields like Ecotoxicology, Mine Lake Aquaculture, Aquatic Polyculture, Inland Saline Aquaculture, Aquaponics, Rock Lobster Nutrition and Stress Physiology and a number of other fields within the aquatic sciences. Students can study part-time or full-time with all undergraduate programmes being complemented with an Honours degree for high achievers. Courses combine practical and theoretical units to give students a wide range of carreer opportunities, including Fisheries Management, Aquatic Science Research, Environmental Protection, Commercial Aquaculture, Seafood Processing, and Conservation Management. Further information is available at the Curtin website: www.curtin.edu.au; or via Student Services: tel: 08 9266 4400.

Curtin
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Aquatic Science Research Unit
Curtin University of Technology
GPO Box U1987 PerthWestern Australia 6845

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