Rock Lobster Enhancement and Aquaculture Subprogram Project 5: Determination of the Optimum Environmental and System Requirements for Juvenile and Adult Rock Lobster Holding and Grow-out

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Tasmanian Aquaculture & Fisheries Institute University of Tasmania



PROJECT No. 1998/305

Rock Lobster Enhancement and Aquaculture Subprogram Project 5: Determination of the Optimum Environmental and System Requirements for Juvenile and Adult Rock Lobster Holding and Grow-out

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1 Non-technical Summary

1998/305 Rock Lobster Enhancement and Aquaculture Subprogram Project 5: Determination of the Optimum Environmental and System Requirements for Juvenile and Adult Rock Lobster Holding and Grow-out

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OBJECTIVES:

- 1. Assess the effectiveness of different feeds in maintaining and improving condition and on the growth performance of adult southern rock lobsters in existing sea-based and land-based holding systems in different seasons.
- 2. Determine the effects of temperature, salinity and stocking density on the growth rate and survival of juvenile tropical rock lobsters in existing land-based holding systems.
- 3. Determine the effects of temperature, photoperiod, stocking density, and shelter on the growth rate and survival of juvenile southern rock lobsters in existing land-based holding systems.
- 4. Evaluate existing system design and management regimes for land-based captive grow out of juvenile rock lobsters and for sea-based and land-based holding of adult rock lobsters.

NON-TECHNICAL SUMMARY:

OUTCOMES ACHIEVED

Demonstrated long term holding of adult southern rock lobsters and developed a feed that maintains lobsters in good condition

Provided understanding of the likely weight gain at moult in adult southern rock lobsters

Shown the tolerance and high growth potential of the tropical rock lobster

Outlined the effects of photoperiod and temperature on growth and survival in juvenile southern rock lobsters

There is growing interest in Australia in the commercial potential for on-growing and culture of rock lobster. A major development initiative is currently being implemented in Tasmania for southern rock lobster (*Jasus edwardsii*) and similar plans are being developed for the tropical rock lobster (*Panulirus ornatus*) in Queensland. There is also a fledgling industry in South Australia based on value-adding through live-holding of fishery-caught adult *J. edwardsii*. For each of these areas to reach full commercialisation, it is necessary to determine the optimum environmental and system requirements for grow-out and liveholding. The present project aimed to address these areas and was comprised of three components: I. Live-holding of adult *J. edwardsii*.

Component I of the project investigated the effectiveness of different 'natural' and manufactured diets in maintaining/improving condition and in promoting growth at moult in adult *J. edwardsii* held in sea-based and land-based systems. Trials were run over "summer" (November 1998 to March 1999) and "winter" (April to November 1999) on industry facilities at Port Lincoln and Kangaroo Island. These provided information on diets and feeding regimes, comparison of holding systems, the growth, mortality and biology of lobsters under long-term liveholding and the product quality of live-held lobsters.

The various diets used, live mussel, octopus, and manufactured pellets were all successful in keeping lobsters alive, promoting growth at moult, and maintaining/improving the condition of lobsters. Diets were formulated in consultation with RLEAS Project 98-303 Lobster Nutrition. Diets were initially extruded and supplied as moist or dry pellets. In the later part of the Project, diets were steam pelleted and this method produced an easily stored and handled pellet that had good water stability and that was well accepted by the lobsters in seacages and raceways. Addition of mussel mince to pellets as a feeding stimulant did not increase survival or growth. Different inclusions of carophyll pink, a carotenoid necessary to maintain red colouration in lobsters, were trialled and the lower level of 0.07% proved sufficient to maintain and improve colour in sea-based and land-based live holding. Lobsters were fed to excess in these trials and further work on pellet dimension and feeding strategy needs to be undertaken. Overall natural feeds slightly outperformed pellet feeds, although in some cases survival was better on pellet diets. These results were pleasing as improvements in formulation, pellet production and food delivery can be expected.

Lobsters were held successfully in both sea-based cages and land-based raceways. The sea-based systems were purpose built for commercial industry use. They allow the holding of high densities of lobsters without water quality deterioration. They are difficult to service and it is difficult to follow the fate of food fed to the cages. Raceways offer advantages in management, however water quality and system failure are key issues to be addressed. In the present study sea-based cages performed better than the raceways, although the performance of both can be improved.

Lobsters were successfully held over both "summer" and "winter" periods in both raceways and seacages under varying temperature (12.3 to 24.7 °C), water quality and feeding conditions, including no feed provided for 30 weeks. This demonstrates that *J. edwardsii* is a robust species with substantial environmental tolerances. In summer only male lobsters were held and they showed high survival in all of the "Fed" treatments and up to 71% of lobsters moulted. Average individual weight gains were 4.7 to 8.0% in fed treatments. In winter both males and females

were held. All lobsters in the sea-cages at Port Lincoln and Kangaroo Island moulted between July and October. Males showed substantially greater growth at moult than females, mean of 8% for females and 17% for males. The winter moult increments were greater that those recorded in summer, 8% in summer and 18% in winter for lobsters fed live mussels. Over an extended winter holding period of 30 weeks duration, one experimental treatment had 100% survival, 100% moulting activity, and an average individual weight gain at moult of 17%. The biomass of male lobsters increased in the pellet fed winter trials at Kangaroo Island (approximately 16% biomass gain) and the pellet fed treatment in the Port Lincoln sea-cages (12% gain), where growth of individual lobsters more than compensated for weight loss via mortality. Where females were held separately but in cages adjacent to males, there was some spawning activity in the winter trial, resulting in small batches of infertile eggs on 13% of the females. The groups of lobsters at Port Lincoln that were held for 50 weeks showed good survival (80-95%) and substantial growth (ranging from 20-30% by weight) when fed on artificial diets over this long term demonstrating the success of this formulated diet.

Speckled/white lobsters that were fed on either octopus or manufactured pellet diets with inclusion of 0.07 to 0.25% carophyll pink changed to a more red colouration. Long-term live-held lobsters survived a simulated overseas export, and the taste of these lobsters was excellent. The one negative outcome of the project was that tail fan damage was found to be a major problem with live-held lobsters. Tail fan damage occurred in sea-based and land-based trials and across all diets without apparent pattern. In some lobsters, tail fan damage progressed to unacceptable condition within the first month on live holding. The condition was generally worse/more progressed in the summer-held lobsters. The causes and management of tail fan necrosis need to be addressed before a long term live holding industry can be developed

Component II of the project investigated the effects of temperature, salinity, and density/biomass on the growth and survival of juvenile P. ornatus. Results of the experiments completed and the experience of conducting the experiments and the associated collection, handling and processing of lobsters, collectively confirm the excellent aquaculture potential for ornate lobsters, P. ornatus, in land-based tank systems. Although many important biological and physico-chemical factors need to be addressed in further defining the most effective production technologies for this species, the basic protocols for tank-based culture have now been established. An optimal temperature range of 25 to 30°C was clearly defined and a moderate tolerance to reduced salinity was revealed. P. ornatus was shown to be a robust species, with a range of attributes that will suit commercial aquaculture circumstances, including resistance to compromised water quality, tolerance of frequent handling, low susceptibility to stress and health problems. Results of the density experiment provided an assessment of the species likely performance under commercial aquaculture conditions, in that the experiment covered a protracted period and a growth phase from small juvenile (3g) to moderate-size pre-adult (225g). Of the densities applied, none had any significant effect on survival or growth, and there is capacity to culture this species at higher levels, greater than 4 kg per square metre. Although survival (52%) was less than ideal, the mortality experienced was attributed to causes that can be overcome. Growth was excellent for the experimental period, and can be confidently extrapolated to permit growth to 1 kg within 18 months, at commercially relevant densities. There is a priority however on defining growth for the post-puerulus to 3g phase.

Component III of the project investigated the effects of temperature and photoperiod on the growth and survival of juvenile J. edwardsii. A temperature range of between 19 and 21°C is optimal for post-puerulus J. edwardsii in terms of survival, growth and feed conversion ratio. At higher temperatures the measured performance criteria were reduced and the upper thermal limits appeared to be 24°C. Culturists would need to consider the economic advantages of lobsters reaching market size in the shortest possible time against the increased costs associated with heating water. In addition, at higher temperatures lobsters have a greater respiratory requirement and excrete higher levels of ammonia. If lobsters are to be cultured at elevated temperatures in intensive recirculating aquaculture systems then there will be even greater reliance on water treatment, to ensure water quality is not limiting growth. Finally, if lobsters were to be grown in flow-through systems, or in cages in the sea these results indicate that it will be necessary to select sites where summer water temperatures do not rise above 22°C. Photoperiod appears to have only minimal influence on growth and survival compared to other factors, such as temperature and diet. It is possible to subject the lobsters to reasonably long light periods without affecting growth or survival. If J. edwardsii were to be cultured in an indoor system with artificial light regimes, then husbandry practices can be significantly simplified, as there will be no need to undertake those practices in the dark.

KEYWORDS: aquaculture, southern rock lobster, tropical rock lobster, live-holding, moulting, juvenile growth, survival, salinity, temperature, density, photoperiod

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3 Background

There is growing interest in Australia in the commercial potential for on-growing and culture of rock lobster. A major development initiative is currently being implemented in Tasmania for southern rock lobster and similar plans are being developed for the same and other species of rock lobster in other Australian states. There is also international interest in these developments with active research groups in New Zealand and Japan.

A priority setting process is outlined in the Rock Lobster Aquaculture Sub-Program Project 1. Based on these research priorities, a total of six research project submissions were presented to the FRDC in the 1998/99 funding round from six different research organisations. A comparison between the research objectives of these projects and the research priorities established above revealed considerable overlap between projects and a number of research gaps. In particular, gaps were identified in the high priority areas of puerulus nutrition and environmental manipulation, system design and handling associated with husbandry in adult holding systems, and the study of international markets and impacts of changes on existing markets under economics and marketing. Significant overlaps were identified in the areas of nutrition, health, and handling of adult rock lobsters, and health of juveniles.

Based on the overlaps and relatively common national focus associated with rock lobster aquaculture, the formation of a Rock Lobster Aquaculture Sub-Program has been recommended (see project 1). This approach will allow a highly coordinated and integrated program based on national and international collaborations to address critical research issues with the outcome being a viable rock lobster aquaculture industry that has minimal impact on the natural population and environment.

SUB-PROGRAM COMPONENTS

A planning meeting was held in South Australia in March, 1998 to discuss sub-program objectives and to develop a set of coordinated research applications. The following research projects were defined and form the basis of the sub-program:

Project 1: Facilitation, administration and promotion of the FRDC Rock Lobster Aquaculture Sub-Program.

Principal Investigator and Sub-Program Leader: Dr Robert van Barneveld (Barneveld Nutrition Pty Ltd)

Time frame: Start: 1 May, 1998 Finish: 30 June, 2001

Project 2: Towards establishing techniques for large-scale harvesting of pueruli and obtaining a better understanding of mortality rates.

Principal Investigator: Professor Bruce Phillips (Fisheries WA, Curtin University of Technology) Time frame: Start: 1 July, 1998 Finish: 30 June, 2001

Project 3: Feed development for rock lobster aquaculture Principal Investigator: Dr Kevin Williams (CSIRO) Time frame: Start: 1 September, 1998 Finish: 31 March, 2000 Project 4: Pilot study of disease conditions in all potential rock lobster aquaculture species at different growth stages.

Principal Investigator: Associate Professor Louis Evans (Curtin University of Technology) Time frame: Start: 1 July, 1998 Finish: 30 June, 1999

Project 5: Determination of the optimum environmental and system requirements for juvenile and adult rock lobster holding and grow-out. Principal Investigator: Associate Professor Michael Geddes (Adelaide University)

Time frame: Start: 1 July 1998 Finish: 30 June, 2000

An additional project that has been approved by the FRDC Board to commence in 1998/99 will form an integral part of the Rock Lobster Aquaculture Sub-program by developing a collaborative national project with international partners for the propagation of rock lobster (98/300). At this stage, viability of research in this area is uncertain, and this project will serve the sub-program by establishing research priorities and partnerships.

The subprogram commenced in July 1998 and was later re-named the Rock Lobster Enhancement and Aquaculture Sub-program (RLEAS).

PROJECT SPECIFIC BACKGROUND

Project 5 will be run as three parallel sets of experiments carried out on adult *J. edwardsii* in South Australia (Component I), on juvenile *P. ornatus* at the QDPI Northern Fisheries Lab at Cairns, Queensland (Component II), and on juvenile *J. edwardsii* at the TAFI labs in Hobart, Tasmania (Component III).

The principal investigator, Associate Professor Michael Geddes, will be responsible for overall project coodination and scientific integrity. The Co-investigators bring experience with aquaculture development (Mr Steven Clarke), knowledge of the live holding industry (Mr Andrew Ferguson), experience and a strong track record in establishing tropical crustacean aquaculture (Dr Clive Jones), current expertise in aquaculture of juvenile Southern Rock Lobsters (Dr Piers Hart) and expertise in shellfish fisheries biology and management (Dr Paul McShane).

SUMMARY

If rock lobster aquaculture systems are to be successfully developed in Australia, it is essential that all research groups and parties are involved in the development process. In addition, as many critical research issues need to be addressed in a short time period with a range of species in most states, the development of collaborative research projects is essential to ensure research objectives are met. This sub-program will deliver the mechanism for the required collaboration while efficiently addressing research priorities identified by industry.

4 Need

Rock lobster fisheries throughout the world are generally fully or over-exploited while market demand remains very high with this product positioned at the premium end of the crustacean market spectrum. The proposed research will assist in increasing supply of this valuable product in a sustainable way and will consequently decrease pressure on wild populations. System design and basic husbandry information must be completed in conjunction with health and nutrition research as these factors combine to influence the efficiency of production.

5 Objectives

5.1 Original Objectives

- 1. Assess the interactions between stocking density and feed delivery system on maintaining and improving condition and on the growth performance of adult rock lobsters in existing seabased holding systems in different seasons.
- 2. Determine the effects of temperature, salinity and photoperiod on the growth rate and survival of juvenile rock lobsters in existing land-based holding systems.
- 3. Determine the effects of stocking density and shelter on the growth rate and survival of juvenile rock lobsters in existing land-based holding systems.
- 4. Evaluate existing system design and management regimes for land-based captive grow out of juvenile rock lobsters and for sea-based holding of adult rock lobsters.

5.2 Amended Objectives

To best reflect the structure of the research being conducted for Project 1998/305, the four major objectives (with accompanying sub-objectives) have been re-arranged and re-stated as follows:

- 1. Assess the effectiveness of different feeds in maintaining and improving condition and on the growth performance of adult southern rock lobsters in existing sea-based and land-based holding systems in different seasons.
- 1.1 To evaluate pellet form, feeding strategy, feeding behaviour and cage design/operation.
- 1.2 To evaluate the effectiveness of two different prepared feeds and a 'natural' diet in maintaining condition and promoting growth at moult over the summer moult in male rock lobster.
- 1.3 To evaluate the effectiveness of different feeds in promoting moult and improving condition and colour <u>over the summer moult</u> in poor condition, "white" male rock lobsters.
- 1.4 To evaluate the effectiveness of different feeds in maintaining condition and promoting growth at moult over the winter moult in male rock lobster.
- 1.5 To evaluate the effectiveness of different diets in promoting moult and improving condition and colour <u>over the winter moult</u> in "white" female <u>and male</u> rock lobsters.

- 2. Determine the effects of temperature, salinity and stocking density on the growth rate and survival of juvenile tropical rock lobsters in existing land-based holding systems.
- 2.1 Determine growth rate and survival in relation to temperature.
- 2.2 Determine growth rate and survival in relation to salinity.
- 2.3 Determine growth rate and survival in relation to density/biomass.
- 3. Determine the effects of temperature, photoperiod, stocking density, and shelter on the growth rate and survival of juvenile southern rock lobsters in existing land-based holding systems.
- 3.1 Determine growth rate and survival in relation to temperature.
- 3.2 Conduct a literature review of southern rock lobster growout.
- 3.3 Determine growth rate and survival in relation to photoperiod.
- 4. Evaluate existing system design and management regimes for land-based captive grow out of juvenile rock lobsters and for sea-based and land-based holding of adult rock lobsters.
- 4.1 To provide a Code of Practice for sea-based live-holding of rock lobsters. This will cover the areas of lobster farming management and technologies and best practices.
- 4.2 Evaluate existing system design and management regimes for captive growout of tropical rock lobster.

This revised statement of objectives provides a better match with the three components of the Project and was endorsed at the RLEAS meetings in Geraldton (March 1999) and Hobart (February 2000). Objectives 1 and 4 have been further expanded here (as indicated by underlining) for clearer definition and to include additional research being conducted in land-based raceway holding systems with adult southern rock lobsters and in cage systems with "white" male southern rock lobsters during the winter.

6 Component I: Adult Sea-Based Live-Holding of Jasus edwardsii

6.1 Introduction

The southern rock lobster (*Jasus edwardsii*) fishery is South Australia's most valuable, landing approximately 2600 tonnes annually worth an estimated \$100m in export revenue. About 95% of the commercial catch is exported live to Asian markets. The fishery is harvested sustainably and there is limited scope for improvement of returns through higher catches. Live-holding of fisheries-caught southern rock lobster represents a means of value-adding to the commercial catch through strategic marketing and product enhancement. Based upon fluctuating market prices during the fishing season, a fledgling industry has developed in the Northern Zone commercial fishery of South Australia in which fishers live-hold lobsters for short periods (generally less than one month) in purpose-built sea-based facilities so that they can strategically market their catch when prices are optimal. Live-holding of lobsters also enables strategic marketing during the closed fishing season. In addition, live-holding may enable product enhancement through weight gain, damage repair, and the improvement of condition and colour in lower-priced "speckled/white" lobsters.

Weight gain and repair of damaged limbs in a live-holding system can only be achieved if lobsters are held through a 'moult' because the only way that crustaceans such as lobsters can grow and regenerate limbs is by periodically shedding or moulting their hard outer shell. As this occurs periodically during the lobster's life and the same sort of physiological events (e.g. accumulation of energy and tissue) occur between each moult, the sequence of events is called the "moult cycle", with the interval between moults called the "intermoult period." Moulting shows a seasonal pattern in South Australia that is not yet fully understood. Prescott et al. (1997) found two peaks in moulting activity per year in males of <120mm carapace length, with one in "summer" (between October to March) and a second in "winter" (between March to October). Larger males of >120mm carapace length generally moult only once per year either in "summer" or "winter." Small females of <90mm carapace length may moult twice per year, females up to 110mm carapace length moult once per year, and females >120mm carapace length may moult once per year but show only very small moult increments. Thus small female and small and medium sized male lobsters offer the best prospects for growth in live-holding systems. The likely moult increments and seasonality of moulting have been taken into account in the present project.

The colour of lobsters in South Australia is related to the depth from which they are caught, with "red" lobsters occurring in the shallower waters, "speckled" lobsters in deep waters, and pale "white" lobsters in the deepest waters (McGarvey *et al.*, 1999). The white lobsters are generally in poorer 'condition' than red lobsters and do not handle live export as well as red lobsters. In addition, a red colouration is preferred in the Asian markets. Consequently, white lobsters are generally lower priced than red lobsters. Therefore, the ability to improve both the condition and colour of white lobsters, represents a further means of product enhancement through live-holding. The ability to improve condition and colour in white lobsters is addressed in the present study.

Previous studies by Aquasearch (1996) and Lorkin *et al.* (1999) have explored the potential for long-term sea-based live-holding of adult *J. edwardsii*. However, while the results from these

studies were encouraging, the Aquasearch (1996) study was inconclusive and the Lorkin *et al.* (1999) study was conducted on a pilot scale. The present project aims to build on the results of these two studies and to investigate the effects of different feeds on the condition and growth of red and white lobsters over the summer and winter moult periods.

Growth at moult depends upon the extent of energy and tissue accumulation during the intermoult period, i.e. the condition of the lobster prior to moult. Maintaining and/or improving condition and accumulating tissue and energy for growth in live held *J. edwardsii* will require feeding and a key component of the proposed research will be to evaluate alternative feeds. There are several studies in which rock lobsters have been fed a natural diet (especially mussels), but very few using a manufactured diet. The present study will trial both natural and manufactured pellet diets developed through Project 3 (98/303) of the RLEAS.

Southern rock lobsters are recognized as a relatively robust and disease free species, but the health status of live-held lobsters still needs to be monitored. Health status of rock lobsters can be measured by the presence/absence of parasites, harmful bacteria and fungi and viruses (e.g. Evans, 1997). The present state of knowledge of spiny lobster diseases has been reviewed by Evans and Brock (1994). Health status surveys and pathology will be undertaken opportunistically in this project. Another major issue in the emerging live-holding industry in South Australia is the quality and market acceptability of lobsters that have been live-held for extended periods and either not fed or fed natural or manufactured diets. To this end, the external condition, live-shipping capabilities, and taste of live-held lobsters will all be assessed in the present project.

Work required for the completion of Component I of the present project was undertaken by the Department of Environmental Biology at Adelaide University, in collaboration with the South Australian Research and Development Institute (Aquatic Sciences), and industry members in South Australia. Component I was originally concerned only with <u>sea-based</u> live-holding of adult *J. edwardsii*, but through the involvement of the industry group 'Southern Australian Seafoods', the project was expanded to include <u>land-based</u> live-holding of adult *J. edwardsii*. In view of this change in scope, some of the objectives were modified slightly (Section 5.2). Additional work to that outlined in the original proposal was also conducted in which lobsters were held throughout the summer and winter periods. The following chapter is comprised of a general methods section (Section 6.2) detailing methods that were generic to the different experiments in Component I, five sections (Sections 6.3-6.7) detailing work that addressed Objectives 1.1–1.5, and a general discussion section (Section 6.8) on the results from Sections 6.3-6.7.

6.2 General Methods

6.2.1 Introduction

Objective 1.1 was aimed at providing information for the design and assessment of field trials. In order to address Objective 1.1, a combination of methods was employed, including developmental and observational work in the laboratory and field, and controlled experiments in the laboratory (see Section 6.3). In order to address Objectives 1.2 to 1.5, live-holding trials were conducted in the field (see Sections 6.4-6.7).

6.2.2 Laboratory work

All laboratory work was conducted at the South Australian Aquatic Sciences Centre, West Beach, South Australia. Lobsters for use in observational and experimental laboratory work were maintained in a flow-through outdoor tank at ambient temperature. The tank had a volume of 20000 litres and a flow rate of $\approx 15-20$ litres per minute. A modified cage based on the design of a single compartment from a sea-cage (see Section 6.2.3) was used to house the lobsters within the tank. Lobsters were fed regularly with a variety of pellet types used in the project (see Section 6.3.1.1). Lobsters were both males and females in the size range of 400-2000g.

6.2.3 Field work

In order to address Objectives 1.2 to 1.5, a total of nine live-holding field trials were conducted (Table 1; see Sections 6.4-6.7). Field trials were conducted at one of three locations: a sea-cage system in Boston Bay adjacent to the township of Port Lincoln on Eyre Peninsula; a land-based raceway system at the South Australian Mariculture site near Port Lincoln; and a sea-cage system in Nepean Bay adjacent to the township of Kingscote on Kangaroo Island. Three of the nine field trials were conducted in the Port Lincoln sea-cage system, another three trials were conducted in a raceway system, and three trials were conducted in the Kangaroo Island sea-cage system (Table 1). For each system there was a 'Summer', 'Summer/Winter', and 'Winter' Trial (Table 1). The three Summer Trials ran for about 17-18 weeks from November/December 1998 to March 1999 (Table 1). The three Summer/Winter Trials were extensions of the Summer Trials and ran for 49-50 weeks from November/December 1998 to November 1999 (Table 1). The three Winter Trials ran for 26-30 weeks from April 1999 to November 1999 (Table 1).

Table 1. Details of the nine live-holding field trials

Durations shown are for the majority of lobsters and do not include the replacement animals. PL, Port Lincoln; KI, Kangaroo Island; *, the lobsters from this trial that were used in the PL Cage Summer/Winter Trial were not assessed until 31 Mar 1999; **, the majority of lobsters in these trials died on 24 Oct 1999 and the durations have been adjusted to allow for this; -, no replacements.

Trial	Start	Replacements	Finish	Duration (weeks)	Related Objective
PL Cage Summer	19 Nov 1998	8 Dec 1998	23 Mar 1999*	18	1.2
PL Cage Summer/Winter	19 Nov 1998	8 Dec 1998	2 Nov 1999	50	1.4
PL Cage Winter	16 Apr 1999	23 Apr 1999	2 Nov 1999	29	1.4
Raceway Summer	18 Nov 1998	8 Dec 1998	23-24 Mar 1999	18	1.2
Raceway Summer/Winter	18 Nov 1998	8 Dec 1998	3 Nov 1999**	49	1.4
Raceway Winter	23 Apr 1999	12 May 1999	3 Nov 1999**	26	1.4
KI Cage Summer	2 Dec 1998	17-18 Dec 1998	31 Mar 1999	17	1.3
KI Cage Summer/Winter	2 Dec 1998	17-18 Dec 1998	17 Nov 1999	50	1.5
KI Cage Winter	21-22 Apr 1999	-	17 Nov 1999	30	1.5

The Port Lincoln and Raceway Trials were principally aimed at evaluating the effectiveness of different feeds in maintaining condition and promoting growth at moult in <u>red</u> lobsters (Objectives 1.2 and 1.4). The three Kangaroo Island Trials were principally aimed at evaluating the effectiveness of different feeds in promoting growth at moult and improving condition and colour in <u>white</u> lobsters (Objectives 1.3 and 1.5). However, in all trials it was difficult procuring lobsters of the desired colour. In the Port Lincoln and Raceway Trials the majority of lobsters were red but with some speckled animals included. In the Kangaroo Island Trials the lobsters were a mixture of speckled and white. Nonetheless the use of speckled lobsters was still important to investigate if colour change could be induced through diet.

The Port Lincoln sea-cage system is comprised of a purpose-built floating pontoon with holding bays for lobster cages and a gantry/winch system for maneuvering the cages. The pontoon is constructed of steel and measures 24m long, 8m wide and 3.5m deep (see Saunders and O'Sullivan, 1997/98, for description). Lobsters are held within purpose-built cages (~1m x ~1.7m x ~1.2m) which consist of four tiered compartments of ~1m x ~1.7m x ~0.3m. The cages are constructed of polyethylene tubular framing and lined with ~12mm x ~12mm polyethylene oyster mesh. All holding bays are covered with shadecloth-lined lids. The pontoon is moored to the bottom at one end ('the front') such that it swings around with the changing currents and always faces into the prevailing current. All experimental cages were placed in the back two holding bays away from strong wave action at the front. Lobsters were stocked in the Port Lincoln system at 20 per compartment. This equates to a density of 11.8 lobsters per m² of bottom compartment surface and 4.0 lobsters per m² of total compartment surface (the lobsters utilise all compartment surfaces).

The raceway system comprised eight independent sections of $1.2m \ge 0.45m \ge 0.4m$ with flowthrough water at a rate of ≈ 1 litre per second. Water depth in each section was 0.35m. Total water volume in each section was ≈ 190 litres. The system was constructed of marine plywood coated with fibreglass. Each section was covered with shadecloth. Lobsters were stocked in the raceway system at 10 per compartment. This equates to 18.5 lobsters per m² of bottom surface.

The Kangaroo Island sea-cage system is comprised of a purpose-built floating pontoon with holding bays for lobster cages and a crane/winch system for maneuvering the cages. The pontoon is constructed of steel and measures 16m long, 5m wide and 2m deep. Lobsters are held within the purpose-built cages ($\sim 1.5 \text{m x} \sim 1.5 \text{m x} \sim 1.5 \text{m}$) which consist of three tiered compartments of $\sim 1.5 \text{m x} \sim 0.5 \text{m}$. The cages are constructed of polyethylene tubular framing and lined with $\sim 12 \text{mm x} \sim 12 \text{mm}$ polyethylene oyster mesh. Each individual compartment is fitted with a vertically-oriented chute made of PVC which enables lobsters to be placed into cages without removing cages from the water. The pontoon is moored to the bottom at one end ('the front') such that it swings around with the changing currents and always faces into the prevailing current. All experimental cages were placed in the back four holding bays away from strong wave action at the front. Experimental holding bays were covered with shadecloth-lined lids. Lobsters were stocked in the Kangaroo Island system at 15 or 19 per compartment. This equates to densities of 6.7 or 8.4 lobsters per m² of bottom compartment surface and 2.0 or 2.5 lobsters per m² of total compartment surface.

6.2.4 General protocol for the field trials

6.2.4.1 Assessment of lobsters

Lobsters were obtained from commercial lobster fishers and held in processor tanks or sea-cage systems prior to use in field trials. The majority of lobsters used in all of the field trials were close to the Northern Zone minimum legal size of 102mm carapace length. These animals weighed between 450-650g in most cases. An initial sacrificial 'fishery' sample of 18-20 lobsters was randomly selected before the start of the trials for comparison with the experimental lobsters. Four of these fishery samples were collected: two at the start of the summer trials (red male, and speckled/white male), and two at the start of the winter trials (red male, and speckled/white female). At the commencement of a trial, each lobster from within a compartment group and the fishery group was individually marked by pleopod clipping (see Lorkin *et al.*, 1999). Pleopods were numbered ventrally from 1 to 8 with the left-side (anterior to posterior) as 1-4 and the right-side (anterior to posterior) as 5-8.

For all lobsters, the following details were recorded:

- <u>carapace length</u>: measured to the nearest 0.1mm from the antennal platform to the posterior dorsal mid-margin of the carapace using vernier callipers.
- <u>wet weight</u>: measured to the nearest 1 or 5g using an electronic balance.
- <u>sex</u>: male or female determined by pleopod structure.
- <u>reproductive condition</u>: for females only determined by the length of setal hairs on the endopodite of the pleopods and classified as short (=sexually immature) or long (=sexually mature), after Prescott *et al.* (1997).
- <u>colour</u>: determined by visual and photographic examination (see below) of the carapace and classified as red, speckled or white. Each of these categories relates to the extent and intensity of red pigmentation with an increase in pigmentation from white through speckled to red.
- <u>carapace hardness</u>: determined by physical examination and classified as hard or soft.
- <u>carapace fouling</u>: described by the visual presence of polychaete and/or algal fouling.
- <u>leg loss</u>: described by the absence of legs and whether the leg loss was old or new. Legs were numbered dorsally from anterior to posterior as left 1-5 and right 1-5.
- <u>tail fan damage</u>: described by the presence of raggedness, blistering, and/or erosion (see Lorkin *et al.*, 1999) on each of the five tail fans. Raggedness is the loss of sections of exoskeleton along the posterior margins of the tail fan. Blistering is the appearance of swollen areas of tissue under the surface of the tail fan. Erosion is the loss of sections of exoskeleton and tissue from the tail fan. Tail fans were numbered dorsally from left to right as 1-5. The absence of an entire fan was noted as 'missing.'
- <u>external lesions</u>: described by the presence and location of unusual lesions on the exoskeleton.
- <u>moult stage</u>: determined later in the laboratory using the technique of Musgrove (2000) on the number 1 or 5 pleopod (see below).

After assessment, sacrificial fishery samples were frozen whole for later condition analyses (see Section 6.2.4.5). After 1-2 weeks, the trials were checked for mortalities and these were replaced with live lobsters. Experimental lobsters were assessed approximately monthly during the

summer trials, and approximately bi-monthly during the winter trials and the second part of the summer/winter trials. Assessment involved recording all of the details listed above for the surviving lobsters, except for moult stage. The presence of re-growth on clipped pleopods was also noted, as this is an excellent indicator that a lobster has moulted (Lorkin *et al.*, 1999). Lobsters that had pleopod re-growth were re-clipped so that subsequent moults could be detected. At the completion of each trial, the usual assessment was undertaken. In addition, pleopod number 5 was removed from each animal for moult staging purposes. All lobsters were then frozen whole for later condition analyses (see Section 6.2.4.5).

For the three Kangaroo Island Cage Trials that were aimed at inducing colour change in lobsters, a colour photograph was taken of the entire dorsal view of each lobster at the beginning and end of the trials. Photographs were taken on a standardized background of grey-coloured polypropylene sheeting that had been roughened with sandpaper to reduce light reflections. Photographs were taken with a 35mm SLR camera and flash using Kodak Gold ISO 100/21° colour print film (CAT 402 2026). Photographs were used in assessing colour change in lobsters from the Kangaroo Island trials.

6.2.4.2 Environmental conditions during the trials

Water temperature was monitored during all trials with either a Hobo, Tinytag, or Tinytag Plus temperature datalogger (Gemini Data Loggers UK). Temperature was recorded every 2 hours. Temperature dataloggers were placed in the water adjacent to where lobsters were being held.

A water quality meter (YSI Model no. 3800) was used to measure diurnal changes in dissolved oxygen, salinity, temperature, and pH on one occasion in the Port Lincoln cage system and on one occasion in the Kangaroo Island sea cage system. Data were recorded every 30 minutes on these two occasions.

6.2.4.3 Survival and growth of lobsters

Growth increments were calculated only for those lobsters that had survived a trial, moulted during the trial, and were hard-shelled at the final assessment at the end of the trial. This is because the only significant weight gain in lobsters occurs after they have moulted and hardened their shell. A lobster was deemed to have moulted if it displayed one or more of the following features: pleopod re-growth, leg re-growth, the disappearance of fouling on the carapace, a change in carapace length of >2mm, and a substantial change in weight (see Lorkin et al., 1999). Pleopod re-growth was generally the most reliable indicator of moulting except in lobsters that had moulted soon after being pleopod clipped at the commencement of a trial (these lobsters generally showed no pleopod re-growth). Lobsters in the summer and winter trials moulted only once during the trials. For these lobsters the moult increment was taken as the difference in carapace length or weight between the initial assessment and the final assessment. Lobsters in the summer/winter trials moulted up to three times. For these lobsters moult increments were calculated for each moult by taking the difference in carapace length or weight between assessments when the lobsters had moulted and were hard-shelled. Multiple moults were labelled as moult 1, moult 2, and moult 3. Carapace length and weight increments were also calculated as percentage changes from the initial or previous measurement.

6.2.4.4 Feed conversion ratios

Feed conversion ratios were only calculated in those cases where there was an <u>increase</u> in total treatment weight (i.e. biomass) over the duration of a trial. Two feed conversion ratios were calculated:

Feed conversion ratio (wet) = Wet weight of feed used (kg) / Biomass gain (kg)

Feed conversion ratio (dry) = Dry weight of feed used (kg) / Biomass gain (kg)

6.2.4.5 Physiological condition of lobsters

The size and moisture content of the hepatopancreas and abdomen are regularly used as measures of physiological condition in decapod crustacea (e.g. Dall, 1974; Trendall and Prescott, 1989; McClain, 1995; Musgrove, 1998). A large hepatopancreas and/or abdomen with low moisture content is an indication of an animal in good condition. In order to standardize for the size of a lobster, the size of the hepatopancreas and abdomen can be presented in relation to the size of the animal as a condition index.

Frozen whole lobsters from the fishery and experimental groups were thawed in warm water and then dissected to remove the hepatopancreas and abdomen tissues. Tissues were weighed wet and then either placed in an oven for drying or re-frozen for later drying. Tissues were dried for 96 hours at 60°C and then re-weighed to obtain the dry weight. Moisture content was calculated as:

Moisture content (%) = 100 - [(Tissue dry weight / Tissue wet weight) x 100]

Dry weight index was calculated as:

Dry weight index (%) = (Tissue dry weight / Lobster wet weight) x 100

6.2.4.6 External condition of lobsters

Several measures of external condition relating to fouling, colour, legs, tail fan damage, and external lesions were calculated for the surviving lobsters from each of the Summer and Winter trials:

Incidence of fouling - the percentage of lobsters that had fouling at the end of a trial

Appearance of fouling - the percentage of lobsters that had no fouling at the start but had fouling at the end of a trial

Disappearance of fouling - the percentage of lobsters that had fouling at the start but had no fouling at the end of a trial

Colour maintenance – the percentage of lobsters that did not change in colour between the start and end of a trial

Colour enhancement – For the Kangaroo Island trials in which photographs were taken (see Section 6.2.4.1) colour enhancement was defined as the percentage of lobsters that increased in the extent and/or intensity of red pigmentation between the start and end of a trial. This was determined by visual examination of 'before' and 'after' photographs. For the Port Lincoln and Raceway trials in which colours were categorised (see Section 6.2.4.1) colour enhancement was defined as the percentage of lobsters that changed in colour from speckled to red between the start and end of a trial.

Colour loss – For the Kangaroo Island trials in which photographs were taken (see Section 6.2.4.1) colour loss was defined as the percentage of lobsters that decreased in the extent and/or intensity of red pigmentation between the start and end of a trial. This was determined by visual examination of 'before' and 'after' photographs. For the Port Lincoln and Raceway trials in which colours were categorised (see Section 6.2.4.1) colour loss was defined as the percentage of lobsters that changed in colour from red to speckled, or from speckled to white between the start and end of a trial.

Incidence of missing legs – the percentage of lobsters that had one or more <u>old</u> missing legs at the end of a trial. Only old leg loss was counted (and not legs that were lost during the final assessment).

Regeneration of missing legs – the percentage of lobsters that had one or more missing legs at the start of a trial and that had re-grown one or more of those legs by the end of the trial.

Loss of legs – the percentage of lobsters that had lost one or more legs during a trial. Only old leg loss was counted (and not legs that were lost during the final assessment) at the end of a trial.

Incidence of any type of tail fan damage – the percentage of lobsters that had tail fan raggedness, and/or blistering, and/or erosion at the end of a trial

Incidence of tail fan raggedness – the percentage of lobsters that had tail fan raggedness at the end of a trial

Incidence of tail fan blistering - the percentage of lobsters that had tail fan blistering at the end of a trial

Incidence of tail fan erosion - the percentage of lobsters that had tail fan erosion at the end of a trial

Development of any type of tail fan damage - the percentage of lobsters that had developed tail fan raggedness, and/or blistering, and/or erosion by the end of a trial

Development of tail fan raggedness – the percentage of lobsters that had no tail fan raggedness at the start of a trial but had tail fan raggedness at the end of a trial

Development of tail fan blistering - the percentage of lobsters that had no tail fan blistering at the start of a trial but had tail fan blistering at the end of a trial

Development of tail fan erosion - the percentage of lobsters that had no tail fan erosion at the start of a trial but had tail fan erosion at the end of a trial

Incidence of external lesions - the percentage of lobsters that displayed some type of visible external lesion at the end of trial

Development of external lesions - the percentage of lobsters that had no type of visible external lesion at the start of a trial but had some type of visible external lesion at the end of trial

6.2.4.7 Health condition of lobsters

Histopathological and microbiological analyses were performed on lobsters from selected treatments in the Port Lincoln Cage Summer Trial, the Raceway Summer Trial, the Port Lincoln Cage Winter Trial, and the Kangaroo Island Cage Winter Trial. Analyses were conducted using standard techniques by Veterinary Pathology Services, Adelaide. Tissues examined were heart, gill, hepatopancreas, tail muscle, antennal gland, and the exoskeleton and underlying muscle of the tail fan.

6.2.4.8 Statistical analyses

Statistical tests were used to compare the growth measures of percentage carapace length increment and percentage weight increment between the different feed treatments in each of the nine trials. Statistics were also used to compare the condition measures of hepatopancreas dry weight index, hepatopancreas moisture content, abdomen dry weight index, and abdomen moisture content between the different feed treatments and the sacrificial (fishery) sample in each of the nine trials. In each test, data from replicate compartments within treatments were pooled for greater statistical power. Data were tested for normality using the Shapiro-Wilk test and for homogeneity of variances using the Levene test. If data conformed to the assumptions of normality and homogeneity of variances then a parametric 1-way ANOVA or Student's t-test was performed to detect differences between group means. If the data did not meet the assumptions for parametric testing then a non-parametric 1-way Kruskal-Wallis test or Mann-Whitney U-test was used to detect differences between groups. The significance level was set at $\alpha = 0.05$. In tests where the result was significant, differences between groups were detected using Tukey's HSD test after an ANOVA, and using a multiple comparison testing procedure described by Zar (1984) after a Kruskal-Wallis test. For the lobsters that moulted twice in the summer/winter trials, the percentage weight increment of moults 1 and 2 were compared within treatments using a Paired *t*test. All statistical tests were performed using the software package 'JMP IN Version 3.2 for Windows' (SAS Institute Inc.).

6.3 <u>Objective 1.1</u> To evaluate pellet form, feeding strategy, feeding behaviour and cage design/operation

6.3.1 Developmental and observational work

6.3.1.1 Pellet form and feeding strategy

A variety of 'natural' and prepared feeds were trialed in Component I of the present study. These were blue mussels (*Mytilus edulis*), Maori octopus (*Octopus maorum*), and several types of manufactured pellet. The blue mussel is aquacultured in South Australia, and the Maori octopus is a common by-catch species in the southern rock lobster fishery of South Australia. Both species represent possible 'natural' food sources for live-held lobsters in South Australia (see Lorkin *et al.*, 1999). In the field trials, mussels were fed to lobsters live and unopened such that lobsters could feed whenever necessary, while octopus was fed to lobsters as skinned and chopped 'cubes' (~2-3cm) of tentacle that had been frozen prior to feeding. The manufactured pellets used in the present study were formulated and manufactured according to advice from Project 3 (98/303) within the RLEAS.

The form of manufactured pellets changed during the course of the project based upon their performance under field conditions and on recommendations from Project 3 (98/303) within the RLEAS. A total of 10 different manufactured pellets were used in the laboratory and field trials (Table 2). These pellets differed in one or more of the following aspects: the ingredient formulation, the die diameter used in manufacturing, and the manufacturing process itself (Table 2). Pellets 1-8 were 'dry' pellets and Pellets 9 and 10 were 'moist' pellets. Pellets 1-3 and 9-10 were used in the summer trials, while Pellets 4 and 6-8 were used in the winter trials and the second part of the summer/winter trials (see Sections 6.4-6.7 for further details). Pellet 5 was used solely in laboratory trials (see Section 6.3.2). The diameter of the pellets was dependent on the availability of dies for the different types of pellet manufacturing equipment. Pellets were generally 1-2 cm in length.

Two basal pellet formulations were developed within the RLEAS Project 3 (98/303) for use in the present study. The first, RL1.3 (Table 3), was formulated in October 1998 and that formulation was used in Pellets 1, 2, 3, 9 and 10 (Table 2) for the summer field trials. These pellets were produced by cold or hot extrusion technology and either maintained moist (M) or dried (D) (Table 2). In March 1999, a second basal formulation was developed for the winter trials; RL3.2 (Table 4). When it was produced commercially in South Australia, fish oil was substituted for squid oil and this resulted in RL3.3D (Table 4). Diets RL3.4D, RL3.5D and RL3.6D were manufactured for the field trials that investigated the effectiveness of elevated Carophyll Pink (i.e. carotenoid) levels in improving the colour of "white" lobsters and compared pellets with and without the inclusion of minced mussel flesh (Tables 2 and 4). Pellets 4-8 that utilised formulations RL3.3-3.6D were produced in a commercial steam pelleting machine at Eyre Peninsula Aquafeeds, Cummins, South Australia (Table 2). Pellets made using the steam pelleter were superior in stability and appearance than the extruded pellets.

Table 2. Pellets used in the Laboratory and Field Trials.

Formulation codes are those developed by CSIRO within the RLEAS. RL, rock lobster; D, dry pellet; M, moist pellet. The two values for moisture content of Pellet 9 are from two different batches.

Pellet no.	Formulation code	% moisture	Die diameter (mm)	Manufacturing process
1	RL1.3D	25	10	Cold extrusion, oven dried
2	RL1.3D	4	10	Cold extrusion, autoclaved
3	RL1.3D	10	6	Hot extrusion, oven dried
4	RL3.3D	7	4	Steam pelleted
5	RL3.3D	7	12	Steam pelleted
6	RL3.4D	7	4	Steam pelleted
7	RL3.5D	6	4	Steam pelleted
8	RL3.6D	6	4	Steam pelleted
				_
9	RL1.3M	37, 42	10	Cold extrusion, air cooled
10	RL1.3M	27	6	Hot extrusion, air cooled

Table 3. Formulation of diets RL1.3D and RL1.3M

Ingredient	Inclusion (%)
Fish meal	35.00
Wheat gluten	6.00
Wheat flour	10.00
Squid mince	20.00
Krill meal	8.00
Sodium alginate	4.00
Tetra-sodium pyro-phosphate	1.30
Vitamin pre-mix	0.85
Mineral pre-mix	1.00
Carophyll Pink	0.15
Cholesterol	0.30
Lecithin	0.60
Calcium chloride solution (10%)	11.80
Fish oil	1.00

In all field trials, lobsters were fed at a set rate of 2% (<u>dry</u> weight feed) per wet weight of lobster per day for manufactured pellets, and at a set rate of 2% (<u>wet</u> weight feed) per wet weight of lobster per day for live mussels and octopus. Values of 36% wet mussel flesh per whole mussel and 13.7g for the average weight of a whole mussel were used when calculating the number of live mussels required to maintain a 2% feeding rate. The feeding rate of live mussels actually equated to *ad libitum* in the field trials, i.e. they were always in excess.

Ingredient			Diet		
-	RL3.2D	RL3.3D	RL3.4D	RL3.5D	RL3.6D
Fish meal	43.40	43.40	43.40	43.40	43.40
Wheat gluten	6.00	6.00	6.00	6.00	6.00
Wheat flour	24.20	24.20	24.10	25.10	25.00
Mussel mince	1.00	1.00	1.00	-	-
Crustacean meal	20.00	20.00	20.00	20.00	20.00
Aquabind	3.00	3.00	3.00	3.00	3.00
Banox E	0.01	0.01	0.01	0.01	0.01
Vitamin pre-mix	0.20	0.20	0.20	0.20	0.20
Vitamin C	0.10	0.10	0.10	0.10	0.10
Carophyll Pink	0.07	0.07	0.15	0.15	0.25
Cholesterol	0.20	0.20	0.20	0.20	0.20
Lecithin	1.20	1.20	1.20	1.20	1.20
Squid oil	0.60	-	-	-	-
Fish oil		0.60	0.60	0.60	0.60

Table 4. Formulations for diets RL3.2D, RL3.3D, RL3.4D, RL3.5D, and RL3.6DValues are % inclusion.

The quantity of feed given to each feed treatment in the field trials was based on the feeding rates stated above and the total weight (or biomass) of lobsters in each treatment. Because the biomass of treatments changed during a trial due to mortality and individual weight changes at moult, the quantity of feed given was also adjusted after each assessment to compensate for this. Pellet and octopus feeds were pre-weighed and assigned into labelled individual bags after each trial assessment in order to save time during feeding.

In the cage systems of Port Lincoln and Kangaroo Island, lobsters were fed twice weekly, i.e. 7% per feed time. In the raceway system, lobsters were fed three times a week, i.e. 4.7% per feed time. Raceway lobsters were initially fed twice per week but due to potential water quality problems associated with large amounts of feed (7%) and small volumes of water in the raceways, the frequency was changed to three times a week (4.7%) after the first few weeks of the Raceway Summer Trial and was kept at this level for all subsequent raceway trials. At each feeding time in the raceway system, any uneaten pellets were siphoned out of the tanks before the next feed. The employed strategy of feeding large quantities of small pellets at each feeding time, rather than a few large pieces of food, was designed to discourage competitive and aggressive encounters between lobsters and to enable all lobsters access to some food.

6.3.1.2 Feeding behaviour

Contrary to the feeding behaviour reported by Fielder (1965) for *J. edwardsii* in which lobsters picked up food and then retreated before consuming it, laboratory observations in the present study revealed that lobsters were feeding on pellets where they located them. Therefore in order to prevent dominant lobsters from monopolising food in one specific area of a cage compartment, it was decided that food should be spread out across the compartment bottom in field trials. This was achieved by having a feeding chute on the ceiling of each compartment (see Section 6.3.1.3)

whereby pellets could disperse with cross currents as they entered a compartment and they would then be further spread across the bottom by the movements of water and lobsters. Having a small, separate feeding tray in one specific area of a compartment was not considered to be the best option.

Laboratory observations in the present study showed that lobsters were readily feeding during the day-time. This behaviour is contrary to that previously documented in the field (MacDiarmid *et al.*, 1991) and in the laboratory (Fielder, 1965). Based upon observations from the present study it was therefore felt that the time of feeding in the field trials was not critical. Consequently lobsters were fed at a range of times during the day-time at the discretion of the people who were hired to feed them. Subsequent *in situ* observations during the field trials revealed that lobsters were feeding readily on pellets during the day-time.

During June/July of the Winter Trials it was observed that the food consumption of lobsters had declined from previous levels. This was possibly related to the decrease in water temperature at the time and an associated decrease in lobster activity. Lobsters were also preparing for the winter moult and may have ceased feeding some time before moulting (e.g. Lipcius and Herrnkind, 1982). Due to the decline in feeding and in order to save on feeding costs associated with travelling out to the pontoons, the feeding frequency was changed from twice per week to once per week during July of the summer/winter and winter cage trials at Port Lincoln and Kangaroo Island (see Sections 6.6 and 6.7).

Laboratory observations of lobsters feeding on pellets revealed that lobsters were able to pick up and feed on pellets of varying sizes between 4 and 12mm diameter. In general their feeding behaviour could be described as 'messy.' This is because of the way that they use the mandibles to 'pinch' off pieces of pellet, especially if the pellet diameter is larger than the diameter of their oesophagus.

6.3.1.3 Cage design and operation

The bottom and lower 10cm of the insides of all experimental cage compartments were lined with 3mm oyster mesh for the field trials. This was designed to prevent the immediate loss of pellets through the existing cage mesh of ~12mm. The finer 3mm mesh does, however, still enable pellet and animal wastes to be washed out of the compartments. During the Kangaroo Island Cage Summer Trial it was noticed that despite the presence of the 10cm high strip of 3mm mesh on the lower sides of the cages, pellets were still being washed out through the rear end of the cages. This was not noted in the Port Lincoln cage system and was probably due to the stronger wave action on the cages in the Kangaroo Island pontoon. To remedy this problem, the height of the 3mm mesh was increased to 20cm on the rear end of all cage compartments for the Kangaroo Island Cage Winter Trial and the second part of the Kangaroo Island Cage Summer/Winter Trial.

To enable feeding of lobsters in cage compartments without removing cages from the water, all experimental cage compartments were fitted with individual 'feed chutes.' These chutes were made from sections of PVC pipe that ran from the upper outside of a cage to each compartmental level. To enable identification when feeding different experimental treatments in the field trials, each of the feed chutes was individually marked and cross-referenced with a particular compartment. For the Port Lincoln cages, 90mm diameter pipe was fitted in the centre of each

compartment. For the Kangaroo Island cages, each compartment had already been fitted with a piece of PVC pipe for industry use. These pipes had three different diameters within a cage such that lobsters could be size-graded and separated into the three different compartments. However they still performed the function of feeding during the field trials. The feed chutes on the Kangaroo Island cages were located on the sides of the cage, rather than in the centre. In order to minimise feed loss out through the sides of the cage when sending feed down a chute, and to enable the feed to spread across the bottom of each compartment via water currents, the Kangaroo Island cages were always positioned within the holding bays such that the feed chutes were facing the front of the pontoon, i.e. into the water current.

Laboratory observations of communally held lobsters during the day-time showed that they were rarely using the shelters provided, but instead they were congregating in the corners of the holding cage. This was unexpected because day-time is when *J. edwardsii* normally seeks shelter in the field (MacDiarmid *et al.*, 1991). The abnormal activity and feeding behaviours observed in the laboratory during the present study were probably related to the absence of predators and a subsequent change in behaviour by the lobsters. Lozano-Alvarez (1996) found that when the spiny lobster *Panulirus argus* was held in sea-cages in the absence of predators its behaviour changed over time, becoming progressively more active during the day-time and more aggressive. The nocturnal and communal behaviour of adult southern rock lobsters is apparently related largely to the avoidance of predators (e.g. MacDiarmid *et al.*, 1991, 1998/99). Due to the lack of shelter utilisation in the present laboratory study and the exclusion of predators in the cage and raceway systems, it was decided that shelters were unnecessary for the field trials.

6.3.2 Experimental laboratory work

6.3.2.1 Pellet Retention Trials – Pellet stability under simulated field conditions

6.3.2.1.1 Introduction

Two experiments ('Experiment 1' and 'Experiment 2') were conducted to compare the 'retention' of different types of manufactured pellet under simulated sea-cage conditions in the laboratory. Retention is defined here as pellets retaining their integrity in a cage in the presence of lobsters such that they are still available for consumption by lobsters. Experiment 1 compared two pellets of the same formulation but of differing size, while Experiment 2 compared two pellets of differing formulation but of similar size. These experiments were conducted during late October to December 1999. The results of the Pellet Retention Trials are useful for interpretation of the results from the field trials and for planning feeding regimes in future industry operations.

6.3.2.1.2 Methods

In order to conduct the trials under laboratory confines, a scaled-down version of a sea-cage compartment needed to be constructed. These so-called 'mini-cages' were constructed by joining a 'Modular Crate Base' (IH984) with a single 'Modular Crate' (IH976, Menzel Plastic Traders) to create an open-ended polypropylene box of \sim 70cm x \sim 50cm x \sim 25cm dimensions. The open-end of the box and the insides of the Modular Crate were then lined with 3mm oyster mesh to create a closed box or 'mini-cage.' The mini-cage was then inverted such that the mesh-lined half

(i.e. the Modular Crate) was the 'bottom.' These mini-cages were similar in height to a Port Lincoln sea-cage compartment (see Section 6.2.3). Ten mini-cages were constructed.

Three of the pellets listed in Table 2 were used in the Pellet Retention Trials to test pellet diameter and pellet formulation (Table 5). The pellets were significantly different in mean length and mean width (Table 5). However, Pellets 4 and 7 are simply referred to as 4mm pellets and Pellet 5 as a 12mm pellet. Experiment 1 utilised Pellets 4 (4mm) and 5 (12mm) to compare two pellets of the same formulation but of differing size. Experiment 2 utilised Pellets 4 (with mussel) and 7 (without mussel) to test whether the addition of mussel mince to the pellet formulation affected pellet retention.

Table 5. Length and width of a random sub-sample of the three types of pellets used in the PelletRetention Trials

Pellet numbers are cross-referenced with Table 2. Values are mean \pm standard error. n = 100 for each mean value. Different superscripts in the same column indicate statistically significant differences (P < 0.05).

Pellet no./type	Length (mm)	Width (mm)	
4 (4mm with mussel) 5 (12mm with mussel) 7 (4mm without mussel)	9.15 ± 0.30^{a} 19.41 ± 0.49^{b} 12.93 ± 0.35^{c}	$\begin{array}{c} 4.12 \pm 0.01 \ ^{a} \\ 13.00 \pm 0.02 \ ^{b} \\ 4.07 \pm 0.01 \ ^{c} \end{array}$	

For each of the experiments there were several experimental 'runs' in which pellets were exposed to simulated field conditions for differing time periods. Experiments were conducted in this manner due to logistical constraints. For Experiment 1 there were seven experimental runs of 1, 4, 12, 24, 48, 72, and 96 hours soak time. For Experiment 2 there were only two experimental runs of 24, and 72 hours.

For each experimental run, three hard-shelled individually-tagged lobsters (between 400-800g) were randomly assigned to each of the 10 mini-cages. The stocking density of 2.3 lobsters per m² of total cage surface was similar to that used in the field trials (see Section 6.2.3). Mini-cages were then placed in the outdoor holding tank at the South Australian Aquatic Sciences Centre (see Section 6.2.2). The mini-cages floated just below the water line and were attached to the side of the tank during an experimental run. The lid to the holding tank was closed during each experimental run such that lobsters were in dimmed light during the day and in darkness at night. Temperatures over the duration of the Pellet Retention Trials ranged between 17 and 22°C.

Lobsters were allowed to acclimate to the mini-cages for 2 hours before being fed. Lobsters were fed once at the start of each experimental run. The amount of food given was based on the feeding rate in the field trials, i.e. 7% (dry weight pellet) per wet weight of lobster. Five of the mini-cages were assigned to each of the two pellet types and pre-weighed amounts of pellets were placed into each of the mini-cages based on the total biomass of lobsters in each mini-cage. After a set time period the lobsters were removed from the mini-cages. The pellet that was retained on the 3mm oyster mesh bottom of each mini-cage was siphoned out with a plastic hose

and passed through two sieves of 250µm and 125µm mesh. Any foreign matter, shell fragments, or faeces were discarded. The contents of both sieves were rinsed into individual pre-weighed 500ml plastic containers. This procedure was repeated until all 10 mini-cages were cleaned. The plastic containers were then placed into an oven at 55°C until they achieved constant weight and dry matter values could be determined. This weight of pellet was defined as being retained and available for lobster feeding. Mean percentage values of the amount of dry matter retained for each pellet type were then calculated using data from the five replicate chambers. For Experiments 1 and 2, values of mean percentage pellet retained from the two different pellet types were compared for each time period using Student's t-test at $\alpha = 0.05$.

6.3.2.1.3 Results

At the 1 hour time period of Experiment 1 there was no significant difference in the amount of pellet retained (Figure 1). However, at the 4, 12, 24, 48, and 72 hour time periods, significantly more of the 12mm pellet had been retained than the 4mm pellet (Figure 1). By the 96 hour time period very little of either pellet had been retained and there was no significant difference between the two (Figure 1). Thus, the 12mm pellet had greater retention than the 4mm pellet. For example, after 24 hours there was about 55% of the 12mm pellet retained but only about 10% of the 4mm pellet retained (Figure 1).





12mm, Pellet 5; 4mm, Pellet 4. Values are mean \pm standard error. n = 5 for each mean value. Different superscripts for the same time period indicate statistically significant differences (P < 0.05) between the 2 pellets.

In Experiment 2 there was a significantly greater amount of Pellet 7 (without mussel mince) retained after 1 day (Table 6). At the 3 day time period very little of either pellet had been retained and there was no significant difference between the two (Table 6). Thus the retention of the pellet without mussel was superior to that of the pellet with mussel.

Table 6. Amount of pellet retained in the mini-cages for two different pellet types at two time periods after feeding in Experiment 2 of the Pellet Retention Trials

Values are mean \pm standard error. n = 5 for each mean value. Different superscripts within the same column indicate statistically significant differences (P < 0.05).

Dallat no /typa	% reta	ined
reliet li0./type	1 day	3 days
4 (with mussel) 7 (without mussel)	5.59 ± 2.38 ^a 23.00 ± 1.49 ^b	1.09 ± 0.86 ^a 3.43 ± 1.48 ^a

6.3.2.1.4 Discussion

While the relative contributions of consumption and leaching to the disappearance of pellets from the mini-cages in Experiments 1 and 2 are unknown, based upon consumption rates (see Section 6.8.6) and the fact that leaching rates are usually low, it is likely that wastage (i.e. pellet falling through the mesh bottom) was the main factor related to pellet loss from the cages. Pellet loss could be related to lobsters handling, feeding, and walking on the pellets, and water movement and diffusion of the pellets. Therefore the results of Experiments 1 and 2 are related to differences in the stability of the different pellets under simulated field conditions. The results of Experiments 1 and 2 make intuitive sense because a larger pellet would be expected to take longer to break down than a smaller pellet, and the pellet with mussel appeared more susceptible to breakdown due to its poorer binding quality. The results of Experiments 1 and 2 clearly demonstrate that size and formulation can have a significant effect on the retention of pellets under field conditions.

Based upon the results of Experiments 1 and 2, it is suggested that in a sea-cage situation of infrequent feeding that is similar to that used in the field trials of the present study, a 12mm diameter pellet should be favoured over a 4mm diameter pellet. However, the amount of time that a pellet remains attractive and will still be eaten by a lobster is unknown, i.e. even though the larger pellet has superior retention quality, the remaining pellet might not attract consumption by the lobsters. The relationship between soakage time and consumption is one area that deserves further research so that optimum feeding regimes can be determined for the field situation.

6.3.2.2 Pellet Wastage Trial – Handling wastage of pellets under simulated field conditions

6.3.2.2.1 Introduction

In the Pellet Retention Trials (see Section 6.3.2.1) food retained on the feeding mesh was collected but the amount of pellet that fell through the 3mm mesh during a given time period was not quantified. This quantity of food can be termed as wastage as it is no longer available for consumption by the lobsters. In the experiment described here ('Pellet Wastage Trial'), the relative wastage of two pellets (of the same composition but of different sizes) was compared. Of particular interest in the Pellet Wastage Trial was the amount of wastage that occurs when a lobster actually handles and consumes fresh pellets over a limited time period rather than the amount of wastage that occurs over extended periods due to physical breakdown by water and lobsters. The Pellet Wastage Trial focussed on examining whether there were differences in 'messiness' of feeding when different diameter pellets (4mm and 12mm) were used. The Pellet Wastage Trial was conducted during February 2000.

6.3.2.2.2 Methods

Ten individual feeding chambers were constructed using 50cm x 40cm x 25cm polypropylene containers fitted with 3mm oyster mesh platforms suspended 5cm off the bottom. This design allowed the lobster to sit and feed on the platform where uneaten feed was retained on the mesh but wastage fell through the mesh and gathered below the platform on the bottom of the chamber. Chambers were fitted with solid lids to dim the light and to prevent escape. Each chamber was connected to a flow-through system at ambient temperature with a flow rate of ≈ 1 litre per minute. Aeration was supplied to each chamber with an air stone. Total water volume in each chamber was \approx 45 litres. Water temperatures ranged from about 22 to 25°C during the Pellet Wastage Trial.

Due to the small number of feeding chambers, four experimental runs were conducted to provide 20 replicates of each pellet treatment. At the commencement of an experimental run, a single hard-shelled individually-tagged lobster (between 400-800g) was randomly placed into each one of the 10 feeding chambers. These lobsters had not been fed for between three and four days prior to experimentation. After a 24 hour acclimation period, five of the chambers were randomly allocated to be fed with Pellet 4 (4mm diameter) and the other five chambers with Pellet 5 (12mm diameter). A single, 12mm pellet of average length (see Table 5) was weighed and allocated to each of the Pellet 5 replicate chambers. An equivalent weight of 4mm pellets was then allocated to each of the Pellet 4 chambers. These pellets were within \pm one standard error of the average length for Pellet 4 and the number totaled between 15 and 20 individual pellets per chamber. Aeration was removed from each container. Pellets were then randomly spread across the centre of the platform in each chamber. Lobsters were then left undisturbed for two hours to feed.

After the two hour feeding period, all of the lobsters were removed from their feeding chambers. A plastic hose was then used to siphon the remaining pellet from the mesh platform through a $125\mu m$ sieve. The contents of the sieve were washed with distilled water into a pre-weighed plastic container. The platform was then removed and any suspended particles allowed to settle.

The 'waste' pellet on the bottom of the chamber was then removed with the siphon and 125μ m sieve and washed into a separate pre-weighed plastic container. This procedure was repeated until all 10 feeding chambers were emptied. The plastic containers were placed into an oven at 55°C until they achieved constant weight and dry matter values could be determined. This provided values for pellet retention and for pellet wastage. Mean percentage values were then calculated for the amount of dry matter remaining on the mesh, the amount of dry matter that had fallen through the mesh, and (by default) the amount of dry matter that was unaccounted for. Values were calculated by combining data from the five replicate chambers for each of the four experimental runs.

6.3.2.2.3 Results

It was anticipated that the lobsters would attempt to consume most of the pellets offered. Unfortunately, feeding activity was lower than expected and there was still a considerable amount of both pellet types remaining on the mesh after the two hour period (Table 7). There was no significant difference between the two pellet types in the amount of pellet that was retained on the mesh, the amount of pellet that fell through the mesh, or the amount of pellet that was unaccountable (Table 7). The unaccountable percentage of pellet can be attributed to consumption by lobsters and leaching into the water. As leaching over the two hour period might be expected to be less than 10%, most of the pellet that was unaccountable had probably been consumed by the lobster. Assuming that the 'unaccountable' portion reflected consumption, an additional measure was created to determine wastage during feed handling. This was termed 'handling wastage' and was calculated as the amount of pellet that fell through the mesh as a percentage of the amount of pellet that was unaccountable (i.e. assigned as consumed). Handling wastage was higher for 12mm pellets than for 4mm pellets, but there was no significant difference between the two pellet types (Table 8). However, in the individual instances in which none of the pellet was retained on the mesh (i.e. feeding activity was high), there was 0% handling wastage for the 4mm pellet (n = 3) and an average of 18.6% handling wastage for the 12mm pellet (n = 6).

Table 7. Fate of two types of pellet after 2 hours in the Pellet Wastage Trial

Values are mean \pm standard error. n = 20 for each mean value. Different superscripts within the same column indicate statistically significant differences (P < 0.05).

Pellet type	% retained on mesh	% fallen through mesh	% unaccountable
12mm	53.63 ± 9.14 ^a	5.61 ± 2.50^{a}	40.76 ± 8.13^{a}
4mm	44.97 ± 6.16 ^a	3.72 ± 1.55^{a}	51.31 ± 6.16^{a}
Table 8. Handling wastage of two types of pellet after 2 hours in the Pellet Wastage Trial Values are mean \pm standard error. n = 20 for each mean value. Different superscripts within the same column indicate statistically significant differences (P < 0.05).

Pellet type	% handling wastage
12mm	13.05 ± 5.89 ^a
4mm	8.88 ± 3.96 ^a

6.3.2.2.4 Discussion

Based upon the diameter of the oesophagus of a lobster it was expected that the 4mm pellet would be consumed with less handling wastage than the 12mm pellet (David Smith, personal communication). This was the basis for choosing 4mm pellets in the winter field trials (see Table 2). In fact the 12mm pellet did have a higher handling wastage than the 4mm pellet in the Pellet Wastage Trial, but there was no significant difference between the two values (Table 8). It may be that while the 12mm pellet is broken up when being handled in feeding (confirmed by video observations), the unconsumed pieces of the 12mm pellet are larger than the 3mm oyster mesh lining the cage. Whether these pieces are later consumed is unknown (see Section 6.3.2.1.4). Data on individual lobsters within the experiment suggest that feeding on larger pellets does create more waste.

6.4 Objective 1.2 To evaluate the effectiveness of two different prepared feeds and a 'natural' diet in maintaining condition and promoting growth at moult over the summer moult in male rock lobster

6.4.1 Introduction

Objective 1.2 was addressed by two field trials; the Port Lincoln Cage Summer Trial, and the Raceway Summer Trial (see Table 1). Two different prepared feeds (a dry pellet, and a moist pellet) and one natural diet (live mussel) were evaluated during these trials for their ability to maintain condition and promote growth at moult over the summer moult in male lobsters.

6.4.2 Port Lincoln Cage Summer Trial

6.4.2.1 Methods

6.4.2.1.1 Experimental design

Four feed treatments (Table 9) were stocked with red, male lobsters. Each of the three 'fed' treatments (Dry Pellet, Moist Pellet, and Live Mussel) was comprised of two replicate compartments containing 20 lobsters per compartment. The No Feed treatment comprised only one compartment of 20 lobsters. The seven compartments from the four treatments were spread across four separate cages. Several batches of pellets were manufactured during the course of the trial; the formulation was constant (RL1.3) but batches varied in moisture content and pellet diameter (Table 9, and see Table 2).

Table 9. Experimental design for the Port Lincoln Cage Summer Trial

Feed Treatment	No. lobsters per treatment	Pellet number and weeks of usage in parentheses
No Feed Dry Pellet Moist Pellet Live Mussel	20 40 40 40	- 1(4), 2(4), 3(10) 9(8), 10(10)

Pellet numbers are cross-referenced with Table 2.

6.4.2.2 Results

6.4.2.2.1 Environmental conditions during the trial

Temperature data were recorded from 25/11/98 - 21/1/99; there were marked fluctuations in temperature but with a general increase over time during this part of the trial (Figure 2). Temperatures averaged 20.3°C with a minimum of 18.2°C on 25/11/98 and a maximum of 24.2°C on 9/1/99. Based upon temperature records from a nearby abalone farm where the Raceway Summer Trial was being conducted at the same time (see Section 6.4.3.2.1 and Figure 4), it is likely that temperatures remained between 19 and 24°C for the latter part of the Port Lincoln Cage Summer Trial (i.e. 22/1/99 - 23/3/99).



Figure 2. Seasonal temperature changes during the Port Lincoln Summer, Summer/Winter, and Winter Cage Trials.

Data are missing for 18-26 November 1998 and 19 January-20 April 1999.

Diurnal abiotic conditions were logged on 9-10/2/99 and there was little variation in salinity, temperature or pH during the 23 hour period (Figure 3). There was however considerable variation in dissolved oxygen (%) with a maximum of 114.7% at 6 PM and a minimum of 92% at 11.30 PM and again at 7.30AM (Figure 3). Dissolved oxygen (%) had a mean of 103.7% for the 23 hour period and was higher during the day than at night.

6.4.2.2.2 Survival and growth of lobsters

Survival was greatest in the three fed treatments (95%) and lowest in the No Feed treatment (75%, Table 10). Moulting activity was greater in the three fed treatments than the No Feed treatment with a maximum of 71% moulted in the Live Mussel treatment. Carapace length and weight percentage increments were also larger in the three fed treatments than the No Feed treatment, with the Live Mussel treatment having the highest mean values (Table 10). While none of these differences were statistically significant, the tests on these data lacked power because of the unequal sample sizes and the very small sample size in the No Feed treatment.



Time (9th-10th Feb, 1999)

Figure 3. Diurnal changes in dissolved oxygen, salinity, temperature, and pH during a 23 hour period of the Port Lincoln Cage Summer Trial

6.4.2.2.3 Feed conversion ratios during the trial

There were no biomass gains in any of the treatments (Table 11). However, while the three fed treatments (Dry Pellet, Moist Pellet, and Live Mussels) almost maintained biomass, the No Feed treatment lost a considerable 25.1% (Table 11). Large amounts of feed were used during the course of the trial (Table 11). Due to the biomass loss in each treatment, feed conversion ratios were not calculated.

6.4.2.2.4 Physiological condition of lobsters

High values for hepatopancreas and abdomen dry weight indices and low values for hepatopancreas and abdomen moisture contents are indicators of good physiological condition. Values for these four condition measures show that the No Feed treatment lobsters were in significantly poorer condition than the fed treatment (Dry Pellet, Moist Pellet, and Live Mussel) lobsters at the completion of the trial (Table 12). This was due to a general increase in condition of the fed lobsters (even though only the measure of hepatopancreas moisture content for the Live Mussel treatment was significantly different to the Fishery sample) and to a decline in condition of the No Feed lobsters during the trial (both of the abdomen measures for the No Feed treatment were significantly different to the Fishery sample).

Table 10. Survival and growth data for lobsters fed on different diets during the Port Lincoln CageSummer Trial.

Moulters (%) are surviving lobsters that had moulted during the trial. Carapace length (CL) and weight data are mean \pm standard error and were calculated from surviving lobsters that had moulted and were hard-shelled at the completion of the trial. Different superscripts within the same row indicate statistically significant differences (*P* < 0.05).

	No Feed	Dry Pellet	Moist Pellet	Live Mussel
Initial no. lobsters	20	40	40	40
Final no. lobsters	15	38	38	38
Survival (%)	75	95	95	95
Number moulted	6	21	25	27
Moulted (%)	40	55	66	71
Initial CL (mm)	105.12 ± 0.73	105.86 ± 0.58	105.54 ± 0.59	106.18 ± 0.54
CL increment (mm)	0.94 ± 0.49	1.90 ± 0.34	2.03 ± 0.39	2.37 ± 0.37
CL increment (%)	0.90 ± 0.46 $^{\rm a}$	$1.79 \pm 0.32^{\rm \ a}$	$1.93\pm0.37^{\mathrm{a}}$	2.25 ± 0.36^{a}
n	5	21	25	24
Initial weight (g)	565.0 ± 14.7	571.2 ± 7.7	568.0 ± 9.9	570.8 ± 6.8
Weight increment (g)	11.0 ± 12.6	26.7 ± 6.7	39.0 ± 7.7	45.6 ± 6.3
Weight increment (%)	1.94 ± 2.13^{a}	4.66 ± 1.17^{a}	6.92 ± 1.42^{a}	$8.03\pm1.11^{\rm \ a}$
n	5	21	25	24

Table 11. Biomass returns, feed usage, and feed conversion ratios for lobsters fed on different diets during the Port Lincoln Cage Summer Trial.

-, not applicable. Feed conversion ratios are wet weight of feed used: wet weight of lobster gained (wet), dry weight of feed used: wet weight of lobster gained (dry).

	No Feed	Dry Pellet	Moist Pellet	Live Mussel
Initial biomass (kg)	11.235	22.650	22.520	22.555
Final biomass (kg)	8.420	22.115	22.455	22.390
Change in biomass (kg)	-2.815	-0.535	-0.065	-0.165
Change in biomass (%)	-25.1	-2.4	-0.3	-0.7
Wet weight of feed used (kg)	-	65.7	85.5	41.8
Dry weight of feed used (kg)	-	57.2	57.2	6.2
Whole wet weight mussels (kg)	-	-	-	143.3
Feed conversion ratio (wet)	-	-	-	-
Feed conversion ratio (dry)	-	-	-	-

Table 12. Physiological condition measures for fishery-caught lobsters and live-held lobsters fed on different diets during the Port Lincoln Cage Summer Trial.

	No Feed	Dry Pellet	Moist Pellet	Live Mussel	Fishery
		1.40 + 0.00 h	154 0 11 h	1.51 + 0.11 h	1 07 + 0 00 sh
Hepatopancreas dry weight index (%)	0.90 ± 0.11^{a}	1.40 ± 0.09^{-6}	1.54 ± 0.11^{-6}	1.51 ± 0.11^{-6}	1.27 ± 0.09 at
Hepatopancreas moisture content (%)	72.35 ± 2.18 $^{\rm a}$	60.04 ± 1.61 bc	61.87 ± 1.89 ^{bc}	59.17 ± 1.73 ^c	66.38 ± 1.69 ^{ab}
Abdomen dry weight index (%)	7.54 ± 0.27 $^{\rm a}$	8.59 ± 0.13 $^{\rm b}$	8.78 ± 0.12 $^{\rm b}$	$8.93\pm0.09~^{\rm b}$	8.59 ± 0.16 ^b
Abdomen moisture content (%)	76.30 ± 0.62 $^{\rm a}$	74.00 ± 0.28 $^{\rm b}$	74.03 ± 0.29 $^{\rm b}$	$73.52\pm0.34~^{\rm b}$	73.48 ± 0.37 ^b
n	15	18	18	20	20

Values are mean \pm standard error. Different superscripts within the same row indicate statistically significant differences (P < 0.05).

6.4.2.2.5 External condition of lobsters

The incidence of carapace fouling at the completion of the trial was low in all four treatments (Table 13). A few lobsters developed fouling during the trial, and of the few lobsters that had fouling at the beginning of the trial, most of these lost it (Table 13). In all cases the degree of fouling was minimal and would not have represented a marketing problem.

The majority of lobsters maintained colouration in all four treatments during the trial (Table 13). Of the few speckled lobsters included at the start of the trial, some of these also enhanced in colouration (Table 13). None of the lobsters lost colouration (Table 13).

There was a moderate incidence of missing legs amongst the four treatments at the completion of the trial (Table 13). This was due to a combination of leg loss during the trial (Table 13) and to lobsters already having missing legs at the beginning of the trial. Of the lobsters that already had missing legs, some regenerated at least one missing leg during the trial (Table 13). The Dry Pellet treatment had the highest frequency for leg loss during the trial (Table 13).

There was a high incidence of lobsters with one or more types of tail fan damage at the completion of the trial (Table 13). Within each treatment, tail fan raggedness was the most prevalent type of damage, followed by erosion, and then blistering (Table 13). By comparing the incidence and development values for tail fan damage, it is apparent that the majority of each type of damage occurred during the trial (Table 13). The Dry Pellet treatment had the highest frequency for development of tail fan erosion during the trial (Table 13).

A small number of lobsters exhibited external lesions at the completion of the trial (Table 13). These lesions occurred on the dorsal surfaces of the carapace and abdomen and all of them developed during the trial (Table 13).

	No Feed	Dry Pellet	Moist Pellet	Live Mussel
Incidence of fouling (%)	7	9	16	16
Appearance of fouling (%)	7	6	19	18
Disappearance of fouling (%)	-	50	100	100
Colour maintenance (%)	93	95	97	97
Colour enhancement (%)	7	5	3	3
Colour loss (%)	0	0	0	0
Incidence of missing legs (%)	27	45	29	39
Regeneration of missing legs (%)	33	29	42	44
Loss of legs (%)	13	32	21	16
Incidence of any type of tail fan damage (%)	67	82	61	61
Incidence of tail fan raggedness (%)	47	47	32	37
Incidence of tail fan blistering (%)	13	21	11	29
Incidence of tail fan erosion (%)	27	45	29	29
Development of any type of tail fan damage (%)	67	66	47	45
Development of tail fan raggedness (%)	47	47	29	34
Development of tail fan blistering (%)	13	16	11	29
Development of tail fan erosion (%)	27	45	29	29
Incidence of external lesions (%)	0	5	3	0
Development of external lesions (%)	0	5	3	0

Table 13. External condition measures for lobsters fed on different diets during the Port Lincoln Cage Summer Trial

6.4.2.2.6 Health condition of lobsters

Histopathological examination showed that the lobsters were generally healthy and that there were no striking differences in health status between any of the experimental groups (No Feed Cage, Dry Pellet Cage, Live Mussel Cage) or the Fishery group (Table 14). Microscopic examination revealed varying degrees of inflammation and aggregation of haemocytes in the heart in all groups, with gill, muscle, hepatopancreas and antennal gland relatively normal in most animals. Generalised inflammation was seen in one sample (#1) in the Fishery group and one sample (#34) from the Live Mussel group. Crystals were seen in the antennal gland of several animals in each group, including the Fishery group. Their significance is unknown at this time. Examination of tail fan damage samples showed a range of symptoms including cracks and fissures in the chitin layer, erosion of tissue, haemorrhaging, thrombosis, and inflammation of the underlying tissue (Table 14). Microbiological examination of cultured samples of tail fan damage (viz. erosion) from four lobsters in the Live Mussel treatment group revealed the presence of the bacteria species *Vibrio alginolyticus* in all four samples and *Plesiomonas shigelloides* in one sample. No significant fungi were isolated from these samples.

Table 14. Health assessment of Fishery lobsters and live-held lobsters fed on different diets from the Port Lincoln Cage Summer Trial and the Raceway Summer Trial

NVL, no visible lesions; INF, inflammation; -, not examined; TH, thrombosis; CR, crystals; AGG, aggregation of haemocytes, F, tail cracks/fissures; E, erosion, H, haemorrhage. Grading: +, occasional; ++, moderate; +++, numerous.

Group	Sample no.	Heart	Gill	Muscle	Antennal gland	Hepato- pancreas	Tail	Other
Eichony	1	INIE	ACC	NIVI	TIL	TIL		
Fishery	1	IINF++ NVI	AUU+		I H++		-	Diaddar INE
	2	NVL	NVL	NVL	NVL	NVL	-	Diauter INT+
	1	INF	NVI	NVL			-	
	+ 5	INF+	NVL	NVL		NVI		
	6	INF+	NVL	NVL.	CR+	NVL.	_	
	7	NVL	NVL	NVL.	INF+ CR+++	NVL.	_	
	8	NVL	NVL	NVL	NVL	NVL	-	Bladder INF+ AGG+
	9	NVL	NVL	NVL	NVL	NVL	-	bladder in the Proof
	10	NVL	NVL	NVL	NVL	NVL	-	Bladder INF+ AGG+
No	11	INF+	AGG+	NVL	NVL	NVL	_	
Feed	12	NVL	NVL	NVL	NVL	NVL	-	
Cage	13	NVL	NVL	NVL	NVL	NVL	-	
cuge	14	NVL	AGG+	NVL	AGG+	NVL	-	
	15	NVL	NVL	NVL	NVL	NVL	-	
	16	NVL	NVL	NVL	AGG++	NVL	F, E, H, TH	
	17	AGG+	NVL	NVL	NVL	AGG+	-	
	18	NVL	NVL	NVL	CR+++	NVL	F, E, H, INF	
	19	AGG+	NVL	NVL	NVL	NVL	-	
	20	NVL	NVL	NVL	-	NVL	-	
Drv	21	NVL	NVL	NVL	NVL	NVL	-	
Pellet	22	NVL	NVL	NVL	NVL	NVL	-	
Cage	23	NVL	NVL	NVL	NVL	NVL	-	
U	24	NVL	NVL	NVL	NVL	INF+	INF++, TH	
	25	NVL	NVL	NVL	-	NVL	-	
	26	INF+	NVL	NVL	NVL	NVL	-	
	27	INF++	NVL	NVL	CR++	NVL	F	
	28	AGG+	NVL	NVL	CR+++	NVL	-	
	29	INF++	NVL	NVL	NVL	NVL	-	
	30	NVL	NVL	NVL	NVL	NVL	-	
Live	31	NVL	NVL	NVL	NVL	NVL	F, H, INF+++	
Mussel	32	NVL	AGG+	NVL	NVL	NVL	-	
Cage	33	AGG+	NVL	NVL	NVL	NVL	-	
	34	INF+++	AGG++	INF++	INF+++	INF++	-	
	35	NVL	NVL	NVL	NVL	NVL	-	
	36	AGG+	NVL	INF+	NVL	INF+	F, E, H	
	37	INF++	NVL	NVL	CR+++	NVL	-	
	38	NVL	NVL	NVL	NVL	NVL	-	
	39	INF+	NVL	NVL	CR++ INF+	INF++	-	
	40	NVL	NVL	NVL	CR+	NVL	-	
Dry	41	NVL	NVL	NVL	NVL	NVL	-	
Pellet	42	NVL	NVL	NVL	NVL	NVL	-	
Raceway	43	NVL	NVL	NVL	CR++	NVL	-	
	44	NVL	NVL	NVL	NVL	NVL	-	
	45	NVL	NVL	NVL	CR+	NVL	-	
	46	INF+++	NVL	NVL	AGG+	NVL	-	
	47	NVL	NVL	NVL	CR++	NVL	-	
	48	NVL		NVL	NVL	NVL	-	
	49	IN V L			IINF+		-	
	50	INVL	INVL	INVL	INVL	INVL	-	

6.4.3 Raceway Summer Trial

6.4.3.1 Methods

6.4.3.1.1 Experimental design

Two feed treatments (Table 15) were stocked with red, male lobsters. Each of these 'fed' treatments (Dry Pellet, and Moist Pellet) was comprised of four replicate compartments containing 10 lobsters per compartment. Different batches of pellets were manufactured during the course of the trial (Table 15, and see Table 2).

Table 15. Experimental design for the Raceway Summer Trial

Feed Treatment	No. lobsters per treatment	Pellet number and weeks of usage in parentheses
Dry Pellet	40	1(4), 2(4), 3(10)
Moist Pellet	40	9(8), 10(10)

Pellet numbers are cross-referenced with Table 2.

6.4.3.2 Results

6.4.3.2.1 Environmental conditions during the trial

For the period during which temperature data were recorded (i.e. 23/12/98 to 23/3/99) there were marked fluctuations in temperature but with a general increase and then decrease during the trial (Figure 4). During this period, temperatures averaged 21.6° C with a minimum of 19.4° C on 25/11/98 and again on 23/3/99. The maximum of 23.7° C occurred on 12-13/2/99.

6.4.3.2.2 Survival and growth of lobsters

Survival was similar in both the Dry and Moist Pellet treatments at around 80% (Table 16). Moulting activity in the Moist Pellet treatment was about double that of the Dry Pellet treatment at 52% (Table 16). However, there were no significant differences in carapace length or weight percentage increments between the two treatments (Table 16).



Figure 4. Seasonal temperature changes during the Raceway Summer, Summer/Winter, and Winter Trials

Data are missing for 18 November – 22 December 1998.

6.4.3.2.3 Feed conversion ratios during the trial

Both treatments had a large biomass loss of around 20% (Table 17). Large amounts of feed were used during the course of the trial (Table 17). Due to the biomass loss in each treatment, feed conversion ratios were not calculated.

6.4.3.2.4 Physiological condition of lobsters

Values for the hepatopancreas dry weight index, abdomen dry weight index, and abdomen moisture content indicated that lobsters from both treatments maintained physiological condition during the trial (Table 18). Values for the hepatopancreas moisture content indicated that lobsters from both treatments improved in physiological condition (Table 18).

 Table 16. Survival and growth data for lobsters fed on different diets during the Raceway Summer

 Trial

Details as for Table 10.

	Dry Pellet	Moist Pellet
Initial no. lobsters	40	40
Final no. lobsters	33	31
Survival (%)	83	78
NT 1/1	0	1.6
No. moulted	9	16
Moulted (%)	27	52
Initial CL (mm)	104.02 ± 1.00	106.20 ± 0.59
	104.92 ± 1.00	100.20 ± 0.38
CL increment (mm)	1.59 ± 0.43	1.23 ± 0.33
CL increment (%)	$1.53\pm0.42^{\mathrm{a}}$	$1.16\pm0.31^{\rm a}$
n	9	15
Initial weight (g)	5717+152	5667+89
Weight increment (g)	371.7 ± 13.2	300.7 ± 0.7
weight increment (g)	25.0 ± 4.0	30.0 ± 5.6
Weight increment (%)	$4.51 \pm 0.84^{\text{ a}}$	5.35 ± 1.00^{a}
n	9	15
Initial weight (g) Weight increment (g) Weight increment (%) <i>n</i>	571.7 ± 15.2 25.6 ± 4.6 4.51 ± 0.84^{a} 9	566.7 ± 8.9 30.0 ± 5.6 5.35 ± 1.00^{a} 15

Table 17.	Biomass returns, feed usage, and feed	l conversion ratio	os for lobsters fed or	n different diets
during the	e Raceway Summer Trial			
Details as	for Table 11.			

	Dry Pellet	Moist Pellet
Initial biomass (kg)	22.875	22.520
Final biomass (kg)	18.855	17.820
Change in biomass (kg)	-4.020	-4.700
Change in biomass (%)	-17.6	-20.9
Wet weight of feed used (kg)	64.2	84.2
Dry weight of feed used (kg)	56.1	56.2
Feed conversion ratio (wet)	-	-
Feed conversion ratio (dry)	-	-

Table 18. Physiological condition measures for fishery-caught lobsters and live-held lobsters fed on different diets during the Raceway Summer Trial

	Feed Tre	Feed Treatment	
	Dry Pellet	Moist Pellet	Fishery
Hepatopancreas dry weight index (%)	$1.54\pm0.10^{\text{ a}}$	1.65 ± 0.16^{a}	$1.27\pm0.09^{\mathrm{~a}}$
Hepatopancreas moisture content (%)	57.32 ± 2.06^{a}	55.42 ± 3.14 a	66.38 ± 1.69^{b}
Abdomen dry weight index (%)	8.75 ± 0.12 $^{\rm a}$	$8.62\pm0.31~^{\rm a}$	$8.59\pm0.16^{\rm a}$
Abdomen moisture content (%)	73.26 ± 0.32 a	73.94 ± 0.61 a	$73.48\pm0.37^{\text{ a}}$
n	18	16	20

Values are mean \pm standard error. Different superscripts within the same row indicate statistically significant differences (*P* < 0.05).

6.4.3.2.5 External condition of lobsters

The incidence of carapace fouling at the completion of the trial was low in both treatments (Table 19). A few lobsters developed fouling during the trial, and of the few lobsters that had fouling at the beginning of the trial, a moderate proportion of these lost it during live-holding (Table 19). In all cases the degree of fouling was minimal and would not have been a marketing problem.

The majority of lobsters in both treatments maintained colouration during the trial (Table 19). Of the few speckled lobsters included at the start of the trial, some of these also enhanced in colouration (Table 19). One of the lobsters lost colouration in the Moist Pellet treatment (Table 19).

There was a moderate incidence of missing legs in the two treatments at the completion of the trial with the Dry Pellet treatment having a greater value than the Moist Pellet treatment (Table 19). This was due to a higher level of leg loss during the trial in the Dry Pellet treatment than the Moist Pellet treatment (Table 19). Of the lobsters that already had missing legs at the start of the trial, some regenerated at least one missing leg during the trial (Table 19).

There was a moderate incidence (around 50%) of any type of tail fan damage at the completion of the trial (Table 19). The prevalence of each type of damage was different between the two treatments with raggedness the most prevalent in the Dry Pellet treatment and erosion the most common in the Moist Pellet treatment (Table 19). By comparing the incidence and development values for tail fan damage, it is apparent that almost all damage occurred during the trial (Table 19). The Moist Pellet treatment had a higher frequency of tail fan erosion development than did the Dry Pellet treatment (Table 19). This is in contrast to the Port Lincoln Cage Summer Trial (Table 13).

A small number of lobsters in the Dry Pellet treatment exhibited external lesions at the completion of the trial (Table 19). These lesions occurred on the abdomen and they all developed during the trial (Table 19).

	Dry Pellet	Moist Pellet
Incidence of fouling (%)	21	16
Appearance of fouling (%)	20	15
Disappearance of fouling (%)	67	67
Colour maintenance (%)	94	84
Colour enhancement (%)	6	13
Colour loss (%)	0	3
Incidence of missing legs (%)	48	29
Regeneration of missing legs (%)	50	25
Loss of legs (%)	48	23
Incidence of any type of tail fan damage (%)	58	48
Incidence of tail fan raggedness (%)	24	16
Incidence of tail fan blistering (%)	15	6
Incidence of tail fan erosion (%)	21	39
Development of any type of tail fan damage (%)	36	39
Development of tail fan raggedness (%)	24	16
Development of tail fan blistering (%)	12	6
Development of tail fan erosion (%)	21	39
Incidence of external lesions (%)	9	0
Development of external lesions (%)	9	0

Table 19. External condition measures for lobsters fed on different diets during the Raceway Summer Trial

6.4.3.2.6 Health condition of lobsters

Histopathological examination revealed that the Dry Pellet Raceway group was generally healthy and that there was no difference in health status between this group and any of the other groups (Table 14; see Section 6.4.2.2.6).

6.5 <u>Objective 1.3</u> To evaluate the effectiveness of different feeds in promoting moult and improving condition and colour over the summer moult in poor condition, "white" male rock lobsters

6.5.1 Introduction

Objective 1.3 was addressed by the Kangaroo Island Cage Summer Trial (see Table 1). Two different prepared feeds (a dry pellet, and a moist pellet) and one natural diet (octopus) were evaluated during the trial for their ability to improve condition and colour and to promote growth at moult over the summer moult in "white" male lobsters.

6.5.2 Kangaroo Island Cage Summer Trial

6.5.2.1 Methods

6.5.2.1.1 Experimental design

Three feed treatments (Table 20) were stocked with speckled/white, male lobsters. Each of these three 'fed' treatments (Dry Pellet, Moist Pellet, and Octopus) was comprised of two replicate compartments containing 19 lobsters per compartment. The six compartments from the three treatments were spread across four separate cages. Several batches of pellets were manufactured during the course of the trial; the formulation was constant (RL1.3) but batches varied in moisture content and pellet diameter (Table 20, and see Table 2).

Due to difficulties in obtaining speckled/white lobsters, the majority of lobsters used in this trial were captured from the Southern Zone fishery in South Australia. These lobsters were trucked from the south-east of South Australia to Adelaide where they were held in processor tanks. They were then trucked to Kingscote on Kangaroo Island and placed in processor tanks before finally being assessed and then transported to the holding pontoon for the trial. There was substantial mortality in the processor tanks at Kingscote; the survivors were used in the trial. It was apparent then that the majority of lobsters used in the trial had experienced major handling stress prior to the trial.

Table 20. Experimental design for the Kangaroo Island Cage Summer Trial Pellet numbers are cross-referenced with Table 2

Feed Treatment	No. lobsters per treatment	Pellet number and weeks of usage in parentheses
Dry Pellet Moist Pellet Octopus	38 38 38	1(2), 2(4), 3(11) 9(6), 10(11)

6.5.2.2 Results

6.5.2.2.1 Environmental conditions during the trial

Temperature data were recorded from 2/12/98-13/1/99 and 17/2/99-31/3/99. There were marked fluctuations in temperature but with a general increase and then decrease during the trial (Figure 5). During these periods, temperature averaged 20.2°C with a minimum of 15.8°C on 31/3/99 and a maximum of 24.7°C on 10/1/99.

Diurnal abiotic conditions were logged on 12-13/1/99 and there was little variation in salinity or pH over the 19 hour period (Figure 6). There was however considerable variation in dissolved oxygen (%) with a maximum of 121.7% at 7.30 PM and a minimum of 100.6% at 4.30 PM (Figure 6). Dissolved oxygen (%) had a mean of 111.8% for the 19 hour period. There was also some diurnal variation in temperature with a maximum of 22.5°C from 5-6.30 PM and a minimum of 20.9°C at 10AM.



Figure 5. Seasonal temperature changes during the Kangaroo Island Cage Summer, Summer/Winter, and Winter Trials Data are missing for 14 January – 16 February 1999.



Figure 6. Diurnal changes in dissolved oxygen, salinity, temperature, and pH during a 19 hour period of the Kangaroo Island Cage Summer Trial

6.5.2.2.2 Survival and growth of lobsters

Survival was similar in all three treatments at around 75-80% (Table 21). Moulting activity was also similar in the three treatments with around 20% of lobsters moulting during the trial (Table 21). Carapace length and weight increments were greater in the Octopus treatment than the Dry Pellet and Moist Pellet treatments, however, these differences were not statistically significant (Table 21). The lack of significance was probably due to the small sample sizes in each treatment (Table 21).

6.5.2.2.3 Feed conversion ratios during the trial

All three treatments had a large biomass loss of around 20-25% (Table 22). Large amounts of feed were used during the course of the trial (Table 22). Due to the biomass loss in each treatment, feed conversion ratios were not calculated.

6.5.2.2.4 Physiological condition of lobsters

Values for the four condition measures showed mixed results. The hepatopancreas dry weight index indicated that the Dry Pellet and Moist Pellet treatments significantly improved in condition, while the Octopus fed lobsters maintained condition (Table 23). Hepatopancreas moisture content showed that only the Moist Pellet lobsters significantly improved in condition while the Dry Pellet and Octopus treatments maintained condition (Table 23). In contrast, the abdomen dry weight index and abdomen moisture content values indicated that the Octopus fed

lobsters significantly improved in condition while the two pellet fed treatments simply maintained condition (Table 23). Overall, the three treatments showed a significant improvement in condition when compared to the 'initial' Fishery sample (Table 23).

Table 21. Survival and growth data for lobsters fed on different diets during the Kangaroo Island Cage Summer Trial

Details as for Table 10.

	Dry Pellet	Moist Pellet	Octopus
Initial no. lobsters	38	38	38
Final no. lobsters	30	30	28
Survival (%)	79	79	74
No. moulted	7	5	7
Moulted (%)	23	17	25
Initial CL (mm)	100.35 ± 1.81	100.88 ± 1.68	100.80 ± 3.54
CL increment (mm)	1.22 ± 0.42	1.98 ± 0.59	2.30 ± 0.64
CL increment (%)	1.24 ± 0.44 a	1.99 ± 0.62 a	$2.34\pm0.69^{\text{ a}}$
n	6	5	6
Initial weight (g)	487.3 ± 35.1	495.0 ± 20.6	499.7 ± 48.4
Weight increment (g)	11.8 ± 7.3	9.6 ± 9.4	32.7 ± 10.3
Weight increment (%)	2.74 ± 1.58 $^{\mathrm{a}}$	2.04 ± 2.01 a	7.06 ± 2.37 $^{\rm a}$
n	6	5	6

Table 22. Biomass returns, feed usage, and feed conversion ratios for lobsters fed on different diets **during the Kangaroo Island Cage Summer Trial** Details as for Table 11.

	Dry Pellet	Moist Pellet	Octopus
Initial biomass (kg)	21.229	22.351	21.437
Final biomass (kg)	16.677	17.257	16.168
Change in biomass (kg)	-4.552	-5.094	-5.269
Change in biomass (%)	-21.4	-22.8	-24.6
Wet weight of feed used (kg)	49.8	71.5	46.2
Dry weight of feed used (kg)	44.4	48.0	9.2
Feed conversion ratio (wet)	-	-	-
Feed conversion ratio (dry)	-	-	-

Table 23. Physiological condition measures for fishery-caught lobsters and live-held lobsters fed on different diets during the Kangaroo Island Cage Summer Trial

		Feed Treatment		
	Dry Pellet	Moist Pellet	Octopus	Fishery
Hepatopancreas dry weight index (%)	1.96 ± 0.16 ab	2.14 ± 0.19 a	$1.51\pm0.11^{\rm\ bc}$	1.41 ± 0.10 °
Hepatopancreas moisture content (%)	54.39 ± 2.95^{ab}	48.85 ± 3.20^{a}	$60.11 \pm 1.82^{\text{ b}}$	61.69 ± 1.68^{b}
Abdomen dry weight index (%)	$8.90\pm0.18^{\text{ b}}$	$9.11\pm0.17^{\text{ ab}}$	9.64 ± 0.16^{a}	$8.70 \pm 0.16^{\text{ b}}$
Abdomen moisture content (%)	73.57 ± 0.41 ^b	$73.16\pm0.37^{\text{ ab}}$	72.00 ± 0.27 ^a	73.36 ± 0.35 ^b
n	15	16	15	18

Values are mean \pm standard error. Different superscripts within the same row indicate statistically significant differences (*P* < 0.05).

6.5.2.2.5 External condition of lobsters

The incidence of carapace fouling at the completion of the trial was moderate in the Dry Pellet and Moist Pellet treatments and high in the Octopus treatment (Table 24). This was due to lobsters developing fouling during the trial (Table 24). In fact fouling was such a problem that modifications were made to the holding structure during the trial to reduce incident light and the subsequent growth of algae. Of the few lobsters that had fouling at the beginning of the trial some of these lost it when they moulted during the trial (Table 24). The overall degree of fouling seen in this trial may have caused a marketing problem.

Despite the occurrence of fouling, the majority of lobsters in each treatment enhanced in colouration during the trial (Table 24). The remainder of lobsters maintained their colouration (Table 24). The octopus treatment had the highest proportion of lobsters that enhanced in colouration (Table 24). Several of the Octopus fed lobsters developed a distinctive purple hue during the trial.

There was a moderate incidence of missing legs (around 60%) in the three treatments at the completion of the trial (Table 24). This was due to leg loss during the trial (Table 24). Of the lobsters that already had missing legs at the start of the trial, very few regenerated legs during the trial (Table 24). This was due in part to the low level of moulting activity (Table 21).

There was a moderate incidence (around 65%) of any type of tail fan damage in each of the treatments at the completion of the trial (Table 24). Within each treatment, tail fan raggedness was the most prevalent type of damage, followed by erosion, and then blistering (Table 24). By comparing the incidence and development values for tail fan damage, it is apparent that the majority of each type of damage occurred during the trial (Table 24). The Moist Pellet treatment had the highest frequency for development of tail fan erosion during the trial (Table 24). No external lesions were observed in this trial (Table 24).

	Dry Pellet	Moist Pellet	Octopus
Incidence of fouling (%)	37	43	79
Appearance of fouling (%)	39	38	81
Disappearance of fouling (%)	100	50	0
Colour maintenance (%)	27	30	14
Colour enhancement (%)	73	70	86
Colour loss (%)	0	0	0
Incidence of missing legs (%)	57	63	61
Regeneration of missing legs (%)	8	0	22
Loss of legs (%)	40	43	50
Incidence of any type of tail fan damage (%)	63	67	68
Incidence of tail fan raggedness (%)	40	40	57
Incidence of tail fan blistering (%)	13	13	14
Incidence of tail fan erosion (%)	20	30	18
Development of any type of tail fan damage (%)	37	20	43
Development of tail fan raggedness (%)	30	27	50
Development of tail fan blistering (%)	13	10	14
Development of tail fan erosion (%)	20	30	18
Incidence of external lesions (%)	0	0	0
Development of external lesions (%)	0	0	0

Table 24. External condition measures for lobsters fed on different diets during the KangarooIsland Cage Summer Trial

6.6 <u>Objective 1.4</u> To evaluate the effectiveness of different feeds in maintaining condition and promoting growth at moult over the winter moult in male rock lobster

6.6.1 Introduction

Objective 1.4 was addressed by four field trials; the Port Lincoln Cage Winter Trial, the Raceway Winter Trial, the Port Lincoln Cage Summer/Winter Trial, and the Raceway Summer/Winter Trial (see Table 1). Two different prepared pellets and one natural diet (live mussel) were evaluated during the four trials for their ability to maintain condition and promote growth at moult over the winter moult in male lobsters. At the completion of the Port Lincoln Cage Winter Trial an assessment was also made of the live-shipping capabilities and taste of the experimental lobsters versus fishery lobsters. The live-shipping trial was designed to simulate the conditions experienced by lobsters from the time that they arrive in a processing factory in Australia to the time that they are available for sale in an Asian market. The taste trial was designed by Ruello & Associates P/L to enable an unbiased taste comparison of lobsters from the field trials with lobsters direct from the fishery.

6.6.2 Port Lincoln Cage Winter Trial

6.6.2.1 Methods

6.6.2.1.1 Experimental design

Four feed treatments (Table 25) were stocked with red, male lobsters. Each of the four treatments (No Feed, Dry Pellet, Dry Pellet + M, and Live Mussels) was comprised of two replicate compartments containing 20 lobsters per compartment. The eight compartments from the four treatments were spread across four separate cages. The formulation of the pellets used in the Dry Pellet + M treatment changed slightly during the course of the trial (Table 25, and see Table 2), due to unavailability of some ingredients needed to make Pellet 6 (Table 25). The main ingredient variation that was being compared between the Dry Pellet and Dry Pellet + M treatments in this trial (viz. minced mussel) was present in both Pellets 4 and 6 of the Dry Pellet + M treatment (Tables 2 and 4). The feeding frequency during this trial was also changed to once per week for two weeks during July.

6.6.2.1.2 Live-shipping assessment of lobsters

At the completion of the live-holding trial, all surviving lobsters from one compartment within each of the Live Mussel (n = 15), Dry Pellet (n = 19), and No Feed (n = 13) treatments were kept alive for a live-shipping trial. These lobsters were placed in the holding tanks at a lobster processing factory and maintained at 12.5°C for a six day recovery period prior to 'export.' 15 fishery-caught lobsters were also placed into these holding tanks two days prior to 'export.' The four treatments were kept separate throughout the trial. Immediately prior to the live-shipping trial all lobsters were assessed for vigour based upon tail position and movement, appendage movement, and eyestalk response. Three categories for assessment were used: healthy, unhealthy,

and dead. Healthy lobsters were those that displayed strong tail and appendage movements (especially 'tail flipping'). Unhealthy lobsters were those in which the tail was hanging flaccidly and the appendage movements were weak. Dead lobsters were those that had no tail or appendage movements and which did not respond to eyestalk squeezing.

After the initial recovery period, lobsters were dip-chilled in 11°C water for approximately five minutes and then packed into four separate foam boxes each containing wood wool and a 1kg frozen gel pack. Small holes were punched in the sides of the boxes for aeration. Lobsters remained emmersed within the boxes for 28 hours. During this time the boxes were kept in a warm, noisy part of the processing factory and were periodically shaken. This was meant to simulate conditions during air freight. Lobsters were handled by industry processors during the export part of the trial. At the completion of the export, lobsters were returned to the processor holding tanks for a final recovery period of two days. This period was designed to simulate a recovery period in Asia prior to sale. After this recovery period, all lobsters were again assessed for vigour.

Table 25. Experimental design for the Port Lincoln Cage Winter Trial

Pellet numbers are cross-referenced with Table 2. + M, signifies the addition of minced mussel used in Pellets 4 and 6 in comparison to Pellet 7 (see Tables 2 and 4).

Feed Treatment	No. lobsters per treatment	Pellet number and weeks of usage in parentheses
No Feed Dry Pellet Dry Pellet + M Live Mussel	40 40 40 40	- 7(27) 4(16), 6(11)

6.6.2.1.3 Taste assessment of lobsters

At the completion of the live-shipping trial (see Section 6.6.2.1.2 above), five healthy lobsters from each of the Dry Pellet and Live Mussel treatments, and 10 healthy lobsters from the Fishery-caught treatment were selected for use in a tasting trial. Lobsters from each of the three treatments were simultaneously drowned in freshwater for approximately 25 minutes, cooked in boiling salted water for nine minutes, and then placed in an ice slurry (freshwater) for 30 minutes. Lobsters were prepared for the tasting trial according to a method devised by Ruello & Associates P/L. This involved cutting the abdomen off at the posterior end of the carapace and then cutting off the tail fan. The exoskeleton and intestine were then removed and the abdomen cut into two halves down the midline. Each of these halves was then cut transversely into five equal portions, giving a total of 10 pieces per lobster. Abdominal meat from within the carapace was not used in this exercise.

The tasting trial was conducted at the Lincoln Marine Science Centre at Port Lincoln between 2.30-5.00PM on 11/11/99. A total of 45 people of differing backgrounds attended the trial. These included lobster processors, lobster fishers, scientists, students, and aquaculturists. Tasters had no prior knowledge of why they were tasting lobster and the samples were tasted 'blind.' Tasting was conducted according to a method designed by Ruello & Associates P/L. The trial was divided into Part A and Part B. Part A compared the Dry Pellet and Fishery treatment lobsters, while Part B compared the Live Mussel and Fishery treatment lobsters. Each person was given a piece of paper with the following instructions and questions:

Name
Date Time
PART A
There are two samples of lobster meat, X & Y. Please examine them carefully and then taste them.
Is there any difference, Yes or No ? (Circle one)
If so, which do you prefer, X or Y ?
Why do you prefer it ? (eg flavour/texture/appearance/aroma etc)
PART B
There are now another two samples of lobster meat, X & Z. Please examine them carefully and then taste them.
Is there any difference, Yes or No? (Circle one)
If so, which do you prefer, X or Z ?
Why do you prefer it ? (eg flavour/texture/appearance/aroma etc)

Each person was then given a cardboard plate labelled X with two pieces of pellet-fed lobster on it and a second cardboard plate labelled Y with two pieces of fishery-caught lobster on it. Once they had filled in Part A, they were then given a cardboard plate labelled X with two pieces of mussel-fed lobster on it and a second cardboard plate labelled Z with two pieces of fishery-caught lobster on it. People were offered tooth picks for picking up the lobster meat and water for

cleansing their palate. Talking amongst people was disallowed during tasting. Upon completion of the test, if people were interested then they were told the identity of the samples.

The results of Part A were collated as the number of people that found no difference between the samples, and (of those that did find a difference), the number of people that preferred the fishery sample and the number of people that preferred the pellet-fed sample. The results of Part B were collated as the number of people that found no difference between the samples, and (of those that did find a difference), the number of people that preferred the fishery sample and the number of people that preferred the samples.

6.6.2.2 Results

6.6.2.2.1 Environmental conditions during the trial

Temperatures showed a gradual decrease and then increase over the duration of the trial (Figure 2). The average temperature was 15.2°C with a minimum of 12.9°C on 13/8/99 and a maximum of 19.0°C on 18/4/99.

6.6.2.2.2 Survival and growth of lobsters

Survival was very high in the Dry Pellet (95%) and Moist Pellet+M (98%) treatments, slightly lower in the Live Mussel treatment (83%) and lowest in the No Feed treatment (65%; Table 26). All surviving lobsters in each treatment moulted during the trial (Table 26). All three of the fed treatments had significantly greater carapace length and weight percentage increments than the No Feed treatment (Table 26). The Live Mussel treatment had the largest mean carapace length (5.14%) and mean weight (18.49%) increments. Percentage increments of carapace length and weight for the Live Mussel lobsters were significantly greater than those of the Dry Pellet+M treatment but not of the Dry Pellet treatment (Table 26).

6.6.2.2.3 Feed conversion ratios during the trial

The No Feed treatment had a large loss of biomass (33.1%) while the Live Mussel treatment had a small loss of biomass (3.7%; Table 27). The Dry Pellet and Dry Pellet+M treatments had biomass gains of 10 and 12%, respectively (Table 27). Large amounts of feed were used in each of the three fed treatments (Table 27). Consequently, the feed conversion ratios for the two pellet treatments were very high (Table 27).

6.6.2.2.4 Physiological condition of lobsters

Hepatopancreas dry weight index, hepatopancreas moisture content, and abdomen dry weight index values all indicate that the No Feed lobsters had a significant decline in condition while the three fed treatments all maintained condition in comparison to the Fishery sample (Table 28). Abdomen moisture content values show the same trend except that the Live Mussel treatment was in significantly better condition than all other treatments and the Fishery sample (Table 28).

Overall, the No Feed lobsters declined in condition while the fed lobsters (Dry Pellet, Dry Pellet+M, Live Mussel) maintained or improved condition.

Table 26. Survival and growth data for lobsters fed on different diets during the Port Lincoln Cage Winter Trial

Details as for Table 10.

	No Feed	Dry Pellet	Dry Pellet + M	Live Mussel
Initial no. lobsters	40	40	40	40
Final no. lobsters	26	38	39	33
Survival (%)	65	95	98	83
No. moulted	26	38	39	33
Moulted (%)	100	100	100	100
Initial CL (mm)	104.35 ± 0.34	105.32 ± 0.39	105.49 ± 0.44	104.21 ± 0.42
CL increment (mm)	1.92 ± 0.24	4.45 ± 0.29	4.40 ± 0.28	5.35 ± 0.26
CL increment (%)	$1.84\pm0.23^{\rm a}$	4.21 ± 0.26^{bc}	4.17 ± 0.26^{b}	$5.14\pm0.25^{\mathrm{c}}$
n	26	38	39	32
Initial weight (g)	538.2 ± 6.0	551.9 ± 6.5	561.6 ± 6.8	535.5 ± 6.8
Weight increment (g)	17.8 ± 5.0	87.2 ± 5.1	82.8 ± 5.3	98.3 ± 5.3
Weight increment (%)	3.32 ± 0.96^{a}	15.75 ± 0.83^{bc}	14.80 ± 0.94^{b}	$18.49 \pm 1.02^{\circ}$
n	26	38	39	32

Table 27. Biomass returns, feed usage, and feed conversion ratios for lobsters fed on different diets during the Port Lincoln Cage Winter Trial Details as for Table 11.

	No Feed	Dry Pellet	Dry Pellet + M	Live Mussel
Initial biomass (kg)	21.614	22.076	22.441	21.611
Final biomass (kg)	14.455	24.288	25.131	20.808
Change in biomass (kg)	-7.159	2.212	2.690	-0.803
Change in biomass (%)	-33.1	10.0	12.0	-3.7
Wet weight of feed used (kg)	-	89.3	92.7	76.8
Dry weight of feed used (kg)	-	84.0	86.2	11.3
Whole wet weight mussels (kg)	-	-	-	263.0
Feed conversion ratio (wet)	-	40.4	34.5	-
Feed conversion ratio (dry)	-	38.0	32.0	-

Table 28. Physiological condition measures for fishery-caught lobsters and live-held lobsters fed on different diets during the Port Lincoln Cage Winter Trial

	Feed Treatment				
	No Feed	Dry Pellet	Dry Pellet+M	Live Mussel	Fishery
Hepatopancreas dry weight index (%)	0.31 ± 0.02 $^{\rm a}$	$1.25\pm0.06^{\text{ b}}$	$1.45\pm0.06^{\text{ b}}$	$1.47\pm0.08^{\rm\ b}$	$1.17 \pm 0.10^{\mathrm{b}}$
Hepatopancreas moisture content (%)	$84.83\pm0.55^{\text{ a}}$	$66.04 \pm 1.24^{\text{ b}}$	63.68 ± 1.21 ^b	$60.28\pm1.46^{\text{ b}}$	66.43 ± 2.11 ^b
Abdomen dry weight index (%)	7.10 ± 0.11 $^{\rm a}$	$8.31\pm0.09^{\text{ b}}$	$8.34\pm0.09^{\text{ bc}}$	$8.72\pm0.13^{\mathrm{c}}$	$8.56\pm0.15^{\text{ bc}}$
Abdomen moisture content (%)	77.31 ± 0.25 ^a	73.80 ± 0.19^{b}	73.78 ± 0.19^{b}	$72.54 \pm 0.35^{\rm c}$	73.81 ± 0.36^{b}
n	26	33	39	28	20
14	20	55	57	20	20

Values are mean \pm standard error. Different superscripts within the same row indicate statistically significant differences (P < 0.05).

6.6.2.2.5 External condition of lobsters

The incidence of fouling was low in the three fed treatments (between 5 and 18%) but much higher in the No Feed treatment (46%; Table 29). This was due mainly to the appearance of fouling during the trial in the No Feed treatment (Table 29). Of the few lobsters that had fouling at the start of the trial, most of these had lost it by completion of the trial (Table 29).

The majority of lobsters (around 70%) in each of the four treatments maintained colouration during the trial (Table 29). The remainder of these enhanced in colouration from speckled to red (Table 29). There was a low incidence of missing legs (between 12-18%) at the completion of the trial. This was due to a high percentage of animals regenerating legs and a low percentage of animals losing legs during the trial (Table 29).

There was a high incidence of any type of tail fan damage in each of the four treatments at the completion of the trial (Table 29). Within each treatment (except Dry Pellet), tail fan raggedness was the most prevalent type of damage, followed by erosion, and then blistering (Table 29). By comparing the incidence and development values for tail fan damage, it is apparent that the majority of each type of damage occurred during the trial although there was already some damage at the beginning of the trial (Table 29). No external lesions were observed at the completion of the trial (Table 29).

6.6.2.2.6 Health condition of lobsters

Histopathological examination of experimental lobsters revealed no striking differences between the three fed treatments (Dry Pellet + M, Live Mussel, and Dry Pellet) and nothing to suggest that they were unhealthy (Table 30). Aggregations of haemocytes dominated the heart samples and were seen in lower numbers in the other organs (Table 30). Generalised inflammation was seen in two animals (Dry Pellet + M no. 4, and Live Mussel no. 1) but was relatively mild. Small numbers of crystals were present in the antennal glands of only four animals from the three groups. Examination of nine tail fan damage samples showed a range of symptoms including cracks and fissures in the chitin layer, erosion of tissue, haemorrhaging, and inflammation of the underlying tissue (Table 30). Collections of bacteria were seen in five of these samples, with ciliates in two samples, and fungi in one sample (Table 30). Microbiological examination of cultured samples of tail fan damage (viz. erosion) from four lobsters in the Live Mussel treatment group revealed the presence of the bacteria species *Vibrio alginolyticus* and *Pseudomonas stutzeri* in all four samples. No significant fungi were isolated from these samples.

	No Feed	Dry Pellet	Dry Pellet + M	Live Mussel
Incidence of fouling (%)	46	5	15	18
Appearance of fouling (%)	48	6	13	18
Disappearance of fouling (%)	60	100	71	-
Colour maintenance (%)	73	74	74	70
Colour enhancement (%)	27	26	26	30
Colour loss (%)	0	0	0	0
Incidence of missing legs (%)	12	16	18	15
Regeneration of missing legs (%)	100	100	92	93
Loss of legs (%)	8	16	15	15
Incidence of any type of tail fan damage (%)	77	76	87	94
Incidence of tail fan raggedness (%)	69	71	77	70
Incidence of tail fan blistering (%)	12	37	15	27
Incidence of tail fan erosion (%)	38	16	31	48
Development of any type of tail fan damage (%)	38	47	51	36
Development of tail fan raggedness (%)	58	58	56	45
Development of tail fan blistering (%)	8	24	8	15
Development of tail fan erosion (%)	38	16	31	33
Incidence of external lesions (%)	0	0	0	0
Development of external lesions (%)	0	0	0	0

Table 29. External condition measures for lobsters fed on different diets during the Port Lincoln Cage Winter Trial

6.6.2.2.7 Live-shipping assessment of lobsters

Lobsters in each of the four treatments were all healthy prior to the live-shipping trial. At the completion of the trial, the majority of lobsters across all four treatments were still healthy (Table 31). One lobster in the Dry Pellet treatment died and one lobster in the No Feed treatment was unhealthy (Table 31). However, overall there were no real differences in vigour between the four treatments.

Table 30. Health assessment of experimental lobsters fed on different diets from the Port Lincoln Cage Winter Trial

NVL, no visible lesions; INF, inflammation; -, not examined; TH, thrombosis; CR, crystals; AGG, aggregation of haemocytes, F, tail cracks/fissures; E, erosion, H, haemorrhage. Grading: +, occasional; ++, moderate; +++, numerous.

Dry 1 - 4 Pellet 3 - + M 4 INF++ 4 6 - 4	AGG+ NVL AGG+ AGG+ NVL	NVL NVL NVL	INF+ NVL	INF++	-	
Dry 1 - 4 Pellet 3 - + M 4 INF++ 4 6 - 4	AGG+ NVL AGG+ AGG+ NVL	NVL NVL NVL	INF+ NVL	INF++	-	
Pellet 3 - + M 4 INF++ 4 6 - 11 NVI	NVL AGG+ AGG+ NVL	NVL NVL	NVL	100		
+ M 4 INF++ 4 6 - 4	AGG+ AGG+ NVL	NVL		AGG+	-	
6 - 4	AGG+ NVL	NTX /T	AGG+	INF+	INF, E, F, H	
11 NVI	NVL	NVL	CR++	AGG+	-	
		NVL	NVL	NVL	-	
12 NVL	NVL	NVL	NVL	NVL	-	
13 - 4	AGG+	NVL	NVL	AGG+	-	
14 AGG+++	NVL	NVL	NVL	NVL	INF, F	
17 INF++	NVL	NVL	-	NVL	INF, E, H, AGG	++ bacteria, ciliates
18 NVL	NVL	NVL	AGG+	NVL	-	
Live 1 INF++	INF+	NVL	INF+	INF+	INF, H, F	++ bacteria, fungi, ciliates
Mussel 2 NVL	NVL	NVL	INF+	NVL	-	
5 INF+	NVL	NVL	AGG+	NVL	-	
6 AGG+	NVL	NVL	CR++	NVL	-	
7 AGG+ AG	G+, INF+	NVL	AGG+	AGG+	-	
11 AGG+	NVL	NVL	AGG+	AGG+	INF+++, F	
13 AGG+	NVL	NVL	CR++	AGG+	-	
16 AGG+	NVL	NVL	NVL	NVL	-	
18 AGG+++	NVL	NVL	NVL	NVL	INF+++, F	Bacteria
19 AGG+++	NVL	NVL	AGG+	NVL	INF+++, E	Bacteria
Drv 1 AGG+ AG	G+. INF+	NVL	_	AGG+	_	Bacteria
Pellet 5 AGG+	NVL	NVL	AGG+	AGG+	INF. F. AGG+	
8 INF+++	NVL	NVL	AGG+	NVL	-	
10 AGG+	NVL	NVL	AGG++	AGG+	-	
11 AGG++	AGG+	NVL	AGG+	NVL	INF+++, F	
13 AGG+	NVL	NVL	AGG+	AGG+	-	
16 AGG+	NVL	NVL	CR+	NVL	-	
18 AGG+	NVL	NVL	NVL	NVL	-	
20 AGG+++	NVL	NVL	NVL	NVL	-	

6.6.2.2.8 Taste assessment of lobsters

The majority of people detected a difference between the fishery-caught and pellet-fed samples in Part A of the tasting trial (Table 32). However, there was no real difference in preference for either of the samples (Table 32). Part B of the tasting trial comparing fishery-caught versus mussel-fed samples showed a similar result to Part A with the majority of people detecting a difference but having no distinct preference for either of the samples (Table 33).

Table 31. Vigour assessment of lobsters from a fishery sample and from three different feed treatments in the Port Lincoln Cage Winter Trial upon completion of a live-shipping trial

No. lobste	ters in each vigour catego		
Healthy	Unhealthy	Dead	
15	0	0	
18	0	1	
12	1	0	
15	0	0	
	No. lobste Healthy 15 18 12 15	No. lobsters in each vigouHealthyUnhealthy150180121150	

Table 32.	Response of people to Part	A of the tasting tri	al comparing	Fishery-caught	and Pellet-Fed
lobster sa	mples				

Response	No. people
No difference between samples	4
Preferred fishery-caught sample	21
Preferred pellet-fed sample	20

 Table 33. Response of people to Part B of the tasting trial comparing Fishery-caught and Mussel

 Fed lobster samples

Response	No. people
No difference between samples Preferred fishery-caught sample Preferred mussel-fed sample	11 17 17
Total no. people	45

6.6.3 Raceway Winter Trial

6.6.3.1 Methods

6.6.3.1.1 Experimental design

Two feed treatments (Table 34) were stocked with red, male lobsters. Each of these 'fed' treatments (Dry Pellet, and Dry Pellet + M) was comprised of two replicate compartments containing 10 lobsters per compartment. As with the Port Lincoln Cage Winter Trial (see Section 6.6.2), the manufacture of the pellets used in the Dry Pellet + M treatment changed during the course of the trial (Table 34, and see Table 2).

Table 34. Experimental design for the Raceway Winter Trial

Pellet numbers are cross-referenced with Table 2. + M, signifies the addition of minced mussel used in Pellets 4 and 6 in comparison to Pellet 7 (see Tables 2 and 4).

Feed Treatment	No. lobsters per treatment	Pellet number and weeks of usage in parentheses
Dry Pellet	20	7(26)
Dry Pellet + M	20	4(15), 6(11)

On 24/10/99, just one week before this trial was due for completion, a system failure caused the death of almost all lobsters. However, these lobsters were quickly retrieved and frozen for later assessment. Consequently, all of these lobsters were treated as being alive at the end of the trial and were included in all of the usual calculations. A few of the dead lobsters were, however, partially eaten by other lobsters before they could be retrieved. As this may have affected some of the calculations involving accurate body weight measurement (i.e. weight increment, hepatopancreas dry weight index, and abdomen dry weight index), they were omitted from these calculations.

6.6.3.2 Results

6.6.3.2.1 Environmental conditions during the trial

Temperatures showed a gradual decrease and then increase over the duration of the trial (Figure 4). The average temperature was 15.2°C with a minimum of 12.3°C on 3/8/99 and a maximum of 18.4°C on several days during October and on 2/11/99.

6.6.3.2.2 Survival and growth of lobsters

Survival was very similar in the two treatments at 75-80% (Table 35). Moulting activity of surviving lobsters was high in both treatments with 80% in the Dry Pellet and 94% in the Dry

Pellet + M (Table 35). Carapace length and weight percentage increments were higher in the Dry Pellet + M treatment than the Dry Pellet treatment (Table 35). However, these differences were not statistically significant (Table 35).

Table 35. Survival and growth data for lobsters fed on different diets during the Raceway Winter Trial

Details as for Table 10.

	Dry Pellet	Dry Pellet + M
Initial no. lobsters	20	20
Final no. lobsters	15	16
Survival (%)	75	80
No. moulted	12	15
Moulted (%)	80	94
Initial CL (mm)	104.13 ± 0.63	103.59 ± 0.50
CL increment (mm)	3.24 ± 0.30	4.28 ± 0.45
CL increment (%)	$3.12\pm0.31^{\rm \ a}$	$4.14\pm0.43^{\mathrm{a}}$
n	12	15
Initial weight (g)	530.4 ± 10.8	542.9 ± 9.3
Weight increment (g)	41.3 ± 6.0	56.7 ± 7.1
Weight increment (%)	$7.84 \pm 1.10^{\mathrm{a}}$	$10.49\pm1.27^{\rm a}$
n	10	15

6.6.3.2.3 Feed conversion ratios during the trial

There were considerable biomass losses in each of the treatments (Table 36). Large amounts of feed were used in each of the treatments (Table 36). Due to the losses of biomass, feed conversion ratios were not calculated (Table 36).

6.6.3.2.4 Physiological condition of lobsters

All four condition measures indicate that lobsters in each of the treatments maintained condition during the trial (Table 37).

Table 36. Biomass returns, feed usage, and feed conversion ratios for lobsters fed on different diets during the Raceway Winter Trial

Details as for Table 11.

	Dry Pellet	Dry Pellet + M
Initial biomass (kg)	10.573	10.855
Final biomass (kg)	8.309	9.582
Change in biomass (kg)	-2.264	-1.273
Change in biomass (%)	-21.4	-11.7
Wet weight of feed used (kg)	40.4	42.6
Dry weight of feed used (kg)	38.0	39.7
Feed conversion ratio (wet)	-	-
Feed conversion ratio (dry)	-	-

Table 37. Physiological condition measures for fishery-caught lobsters and live-held lobsters fed on different diets during the Raceway Winter Trial

Values are mean \pm standard error. Different superscripts within the same row indicate statistically significant differences (P < 0.05).

	Feed Treatment		
	Dry Pellet	Dry Pellet+M	Fishery
Hepatopancreas dry weight index (%)	1.25 ± 0.15 $^{\rm a}$	$1.47\pm0.08^{\rm\ a}$	$1.17\pm0.10^{\mathrm{a}}$
Abdomen dry weight index (%)	$8.34\pm0.43^{\rm \ a}$	8.83 ± 0.13 a	8.56 ± 0.15 a
n	12	16	20
Hepatopancreas moisture content (%)	66.31 ± 1.99^{a}	62.95 ± 1.20^{a}	66.43 ± 2.11 ª
Abdomen moisture content (%)	$74.93\pm0.64^{\rm \ a}$	74.06 ± 0.23 a	73.81 ± 0.36^{a}
n	15	16	20

6.6.3.2.5 External condition of lobsters

There was no incidence or appearance of fouling in this trial (Table 38). Of the few animals that had fouling at the beginning of the trial, all of these lost it during the trial (Table 38). The majority of lobsters in both treatments maintained colouration, while the remainder enhanced in colouration from speckled to red (Table 38).

The incidence of missing legs was low in the Dry Pellet treatment (13%) and slightly higher in the Dry Pellet + M treatment (38%; Table 38). These levels were due mainly to loss of legs during the trial (Table 38). Of the few lobsters that had missing legs at the start of the trial, all of these regenerated at least one leg during the trial (Table 38).

There were high incidence levels for any type of tail fan damage in both of the treatments (Table 38). Within each treatment, tail fan raggedness was the most prevalent type of damage, followed by erosion, and then blistering (Table 38). By comparing the incidence and development values for tail fan damage, it is apparent that the majority of each type of damage occurred during the trial, although there was already some damage at the beginning of the trial (Table 38). One lobster in the Dry Pellet + M treatment developed an external lesion on the ventral surface of the tail during the trial (Table 38).

	Dry Pellet	Dry Pellet + M
Incidence of fouling (%)	0	0
Appearance of fouling (%)	0	0
Disappearance of fouling (%)	100	100
Colour maintenance (%)	73	56
Colour enhancement (%)	27	44
Colour loss (%)	0	0
Incidence of missing legs (%)	13	38
Regeneration of missing legs (%)	100	100
Loss of legs (%)	13	31
Incidence of any type of tail fan damage (%)	92	80
Incidence of tail fan raggedness (%)	85	80
Incidence of tail fan blistering (%)	15	7
Incidence of tail fan erosion (%)	31	13
Development of any type of tail fan damage (%)	46	27
Development of tail fan raggedness (%)	69	67
Development of tail fan blistering (%)	8	0
Development of tail fan erosion (%)	31	13
Incidence of external lesions (%)	0	6
Development of external lesions (%)	0	6

 Table 38. External condition measures for lobsters fed on different diets during the Raceway

 Winter Trial

6.6.4 Port Lincoln Cage Summer/Winter Trial

6.6.4.1 Methods

6.6.4.1.1 Experimental design

This trial was an extension of the Port Lincoln Cage Summer Trial whereby single compartments from each of the three fed treatments (Dry Pellet, Moist Pellet, Live Mussel) were not terminated at the completion of the Port Lincoln Cage Summer Trial but were continued on through the winter in conjunction with the Port Lincoln Cage Winter Trial (see Section 6.6.2). In the two pellet treatments the pellet was changed during the winter period to Pellet 7; the improved steam pelleted form (Table 39 and see Tables 2 and 4). The treatments were then named Moist/Dry Pellet, and Dry Pellet treatments (Table 39 and see Tables 2 and 4). Feeding frequency during the trial was reduced to once per week for two weeks during July.

Table 39.	Experimental de	sign for the	Port Lincoln	Cage Summer/V	Winter Trial
Pellet num	bers are cross-refe	erenced with	Table 2.		

Feed Treatment	No. lobsters per treatment	Pellet number and weeks of usage in parentheses
Dry Pellet Moist/Dry Pellet Live Mussels	20 20 20	1(4), 2(4), 3(16), 7(25) 9(8), 10(16), 7(25)

6.6.4.2 Results

6.6.4.2.1 Environmental conditions during the trial

The seasonal pattern of temperature change is shown in Figure 2, with highest temperatures during summer and lowest temperatures during winter. For the period when data were logged, temperatures ranged from 24.2° C on 9/1/99 to 12.9° C on 13/8/99, with an average of 16.1° C.

6.6.4.2.2 Survival and growth of lobsters

Survival was high in all three treatments with the Moist/Dry Pellet treatment having the highest survival at 95% and the Dry Pellet treatment the lowest at 80% (Table 40). All lobsters in each treatment moulted at least once with the majority moulting twice (Table 40). The Live Mussel treatment had the highest percentage of lobsters that moulted twice (83%). One animal in the Dry Pellet treatment moulted three times (Table 40). For moult 1, the Live Mussel treatment had the largest carapace length and weight percentage increments, followed by the Moist/Dry Pellet treatment, and then the Dry Pellet treatment (Table 40). However none of these differences were statistically significant (Table 40). For moult 2, the Live Mussel treatment again had the largest

carapace length and weight percentage increments, followed by the Dry Pellet treatment, and then the Moist/Dry Pellet treatment (Table 40). In this case there was a significant difference between the Live Mussel and Moist/Dry Pellet treatments (Table 40) but not with the Dry Pellet treatment.

In all three treatments the carapace length and weight percentage increments were larger for moult 2 than moult 1 (Table 40). Indeed, for those lobsters that moulted twice, a statistical comparison of mean percentage weight increment revealed that moult 2 was significantly larger than moult 1 for each of the three treatments (Table 41).

6.6.4.2.3 Feed conversion ratios during the trial

The Moist/Dry Pellet and Live Mussel treatments both had biomass gains while the Dry Pellet treatment had a biomass loss (Table 42). Substantial amounts of feed were used in each of the treatments (Table 42). Feed conversion ratios were accordingly high for the Moist/Dry Pellet and Live Mussel treatments, although the <u>dry</u> feed conversion ratio for the Live Mussel treatment was relatively low (Table 42).

6.6.4.2.4 Physiological condition of lobsters

The four condition measures indicate that lobsters in the Dry Pellet and Moist/Dry Pellet treatments maintained condition during the trial while the Live Mussel lobsters improved in condition during the trial (Table 43).

Table 40. Survival and growth data for lobsters fed on different diets during the Port Lincoln Cage Summer/Winter Trial

Details as for Table 10.

	Dry Pellet	Moist Pellet / Dry Pellet	Live Musse
Initial no. lobsters	20	20	20
Final no. lobsters	16	19	18
Survival (%)	80	95	90
No. moulted once	5	5	3
No. moulted twice	10	14	15
No. moulted thrice	1		
Moulted once (%)	31	26	17
Moulted twice (%)	63	74	83
Moulted thrice (%)	6		
Initial CL (mm)	106.41 ± 0.51	106.13 ± 0.75	105.72 ± 0.53
CL increment 1 (mm)	1.81 ± 0.37	2.19 ± 0.51	2.78 ± 0.47
CL increment 2 (mm)	4.82 ± 0.57	4.57 ± 0.55	6.57 ± 0.52
CL increment 3 (mm)	3.20		
CL increment 1 (%)	1.69 ± 0.35 $^{\rm a}$	$2.08\pm0.49^{\text{ a}}$	2.64 ± 0.45
CL increment 2 (%)	$4.45\pm0.51~^{ab}$	$4.23\pm0.50^{\rm \ a}$	6.09 ± 0.49^{10}
CL increment 3 (%)	2.90		
n Initial CL	16	19	18
n CL increment 1	16	19	18
n CL increment 2	11	14	15
n CL increment 3	1		
Initial weight (g)	571.9 ± 7.2	573.7 ± 12.4	561.1 ± 6.3
Weight increment 1 (g)	31.6 ± 7.3	47.1 ± 9.0	55.6 ± 8.6
Weight increment 2 (g)	92.7 ± 11.8	71.1 ± 10.1	118.7 ± 11.6
Weight increment 3 (g)	30.0		
Weight increment 1 (%)	$5.53\pm1.29^{\rm \ a}$	8.36 ± 1.64^{a}	$9.98 \pm 1.60^{\circ}$
Weight increment 2 (%)	$15.50\pm1.91^{\text{ ab}}$	$11.39\pm1.47^{\mathrm{a}}$	19.49 ± 1.96^{11}
Weight increment 3 (%)	4.55		
n Initial weight	16	19	18
n Weight increment 1	16	19	18
n Weight increment 2	11	14	15
n Weight increment 3	1		

Table 41. Results of Paired *t*-tests comparing mean percentage weight increment of moult 1 versus moult 2 for those lobsters that moulted twice in the Port Lincoln Cage Summer/Winter Trial *, significant at α =0.05 level; df, degrees of freedom

Treatment	t	Р	df
Dry Pellet	4.89928*	0.000624	10
Moist Pellet / Dry Pellet	2.42877*	0.030401	13
Live Mussel	4.71275*	0.000333	14

Table 42. Biomass returns, feed usage, and feed conversion ratios for lobsters fed on different diets **during the Port Lincoln Cage Summer/Winter Trial** Details as for Table 11.

	Dry Pellet	Moist Pellet / Dry Pellet	Live Mussel	
Initial biomass (kg)	11.385	11.450	11.285	
Final biomass (kg)	10.705	12.790	12.880	
Change in biomass (kg)	-0.680	1.340	1.595	
Change in biomass (%)	-6.0	11.7	14.1	
Wet weight of feed used (kg)	86.5	103.6	65.7	
Dry weight of feed used (kg)	78.6	82.9	9.7	
Whole wet weight mussels (kg)	-	-	225.1	
Feed conversion ratio (wet)	-	77.3	41.2	
Feed conversion ratio (dry)	-	61.9	6.1	

 Table 43. Physiological condition measures for fishery-caught lobsters and live-held lobsters fed on different diets during the Port Lincoln Cage Summer/Winter Trial

Values are mean \pm standard error. Different superscripts within the same row indicate statistically significant differences (*P* < 0.05).

Feed Treatment			
Dry Pellet	Moist Pellet / Dry Pellet	Live Mussel	Fishery
1.58 ± 0.07 $^{\rm a}$	1.29 ± 0.11 $^{\rm a}$	1.97 ± 0.12^{b}	$1.27\pm0.09^{\text{ a}}$
62.21 ± 1.04 ^a	67.06 ± 1.96^{a}	$61.30\pm2.09^{\text{ a}}$	$66.38\pm1.69^{\text{ a}}$
8.55 ± 0.12 a	8.23 ± 0.20^{a}	9.22 ± 0.13^{b}	$8.59\pm0.16^{\rm a}$
$73.43 \pm 0.29^{\text{ a}}$	74.31 ± 0.54 ^a	$71.61 \pm 0.32^{\text{ b}}$	73.48 ± 0.37 a
16	19	18	20
	Dry Pellet 1.58 ± 0.07^{a} 62.21 ± 1.04^{a} 8.55 ± 0.12^{a} 73.43 ± 0.29^{a} 16	$\begin{tabular}{ c c c c c } \hline Feed Treatment \\ \hline Dry Pellet & Moist Pellet \\ / Dry Pellet \\\hline 1.58 \pm 0.07 & 1.29 \pm 0.11 & a \\ 62.21 \pm 1.04 & 67.06 \pm 1.96 & a \\ 8.55 \pm 0.12 & 8.23 \pm 0.20 & a \\ 73.43 \pm 0.29 & 74.31 \pm 0.54 & a \\ 16 & 19 \\\hline \end{tabular}$	$\begin{tabular}{ c c c c c } \hline Feed Treatment \\ \hline Dry Pellet & Moist Pellet \\ / Dry Pellet & Live Mussel \\ \hline 1.58 \pm 0.07 & 1.29 \pm 0.11 & 1.97 \pm 0.12 & 0.12 & 0.12 & 0.12 & 0.12 & 0.13 & 0.1$
6.6.5 Raceway Summer/Winter Trial

6.6.5.1 Methods

6.6.5.1.1 Experimental design

This trial was an extension of the Raceway Summer Trial whereby four compartments from the two treatments (Dry Pellet, and Moist Pellet) were not terminated at the completion of the Raceway Summer Trial but were continued on through the winter in conjunction with the Raceway Winter Trial (see Section 6.6.3). In both treatments the pellet was changed during the winter period to Pellet 7; the improved steam pelleted form (Table 44 and see Tables 2 and 4). The treatments were then named Moist/Dry Pellet, and Dry Pellet treatments (Table 44 and see Tables 2 and 4).

Pellet numbers are cross-referenced with Table 2.						
	Feed Treatment	No. lobsters	Pellet number and week			

Table 44. Experimental design for the Raceway Summer/Winter Trial

Feed Treatment	No. lobsters per treatment	Pellet number and weeks of usage in parentheses
Dry Pellet	20	1(4), 2(4), 3(16), 7(25)
Moist/Dry Pellet	20	9(8), 10(16), 7(25)

On 24/10/99, just one week before this trial was due for completion, a system failure caused the death of almost all lobsters. However, these lobsters were quickly retrieved and frozen for later assessment. Consequently, all of these lobsters were treated as being alive at the end of the trial and were included in all of the usual calculations.

6.6.5.2 Results

6.6.5.2.1 Environmental conditions during the trial

The seasonal pattern of temperature change during this trial is clearly evident from Figure 4, with maximum temperatures during summer and minimum temperatures during winter. During the period of data logging, temperatures averaged 17.4°C with a maximum of 23.7°C on 12-13/2/00 and a minimum of 12.3°C on 3/8/99.

6.6.5.2.2 Survival and growth of lobsters

Survival was low in this trial at 50% for the Dry Pellet treatment and 35% for the Moist/Dry Pellet treatment (Table 45). In the Dry Pellet treatment the majority of lobsters moulted only once while in the Moist/Dry Pellet treatment the majority moulted twice (Table 45). There were no

significant differences between the two treatments for weight and carapace length percentage increments in either moult 1 or moult 2 (Table 45). For those lobsters that moulted twice, there was no significant difference for percentage weight increment between moult 1 and moult 2 within the two treatments (Table 46). However, sample sizes were very small for these tests (Table 46) and consequently the tests had low power.

Table 45. Survival and growth data for lobsters fed on different diets during the RacewaySummer/Winter Trial

Details as for Table 10.

	Dry Pellet	Moist Pellet / Dry Pellet
Initial no. lobsters	20	20
Final no. lobsters	10	7
Survival (%)	50	35
No. moulted once	7	3
No. moulted twice	3	4
Moulted once (%)	70	43
Moulted twice (%)	30	57
Initial CL (mm)	106.44 ± 1.12	105.70 ± 0.88
CL increment 1 (mm)	3.00 ± 0.73	1.51 ± 0.69
CL increment 2 (mm)	3.33 ± 0.64	3.33 ± 0.79
CL increment 1 (%)	2.81 ± 0.67 a	1.44 ± 0.66 a
CL increment 2 (%)	$3.07\pm0.52^{\text{ a}}$	$3.09\pm0.73^{\rm \ a}$
n Initial CL	10	7
n CL increment 1	10	7
n CL increment 2	3	4
Initial weight (g)	579.5 ± 11.7	565.0 ± 14.3
Weight increment 1 (g)	47.0 ± 10.9	27.1 ± 9.7
Weight increment 2 (g)	41.7 ± 17.4	26.3 ± 16.6
Weight increment 1 (%)	8.15 ± 1.88^{a}	4.86 ± 1.74 a
Weight increment 2 (%)	6.48 ± 2.55 a	4.09 ± 2.83 a
n Initial weight	10	7
<i>n</i> Weight increment 1	10	7
<i>n</i> Weight increment 2	3	4

Table 46. Results of Paired *t*-tests comparing mean percentage weight increment of moult 1 versus moult 2 for those lobsters that moulted twice in the Raceway Summer/Winter Trial *, significant at α =0.05 level; df, degrees of freedom

Treatment	t	Р	df
Dry Pellet	1.881470	0.200635	2
Moist Pellet / Dry Pellet	0.410116	0.709250	3

6.6.5.2.3 Feed conversion ratios during the trial

There were very large biomass losses in both treatments of this trial (Table 47). This is despite the substantial amounts of feed that were used during the trial (Table 47) and reflects the low survival values and small moult increments of surviving lobsters in this trial (Table 45).

Table 47.	Biomass returns, feed usage, and feed conversion ratios for lobsters fed on different diets
during the	e Raceway Summer/Winter Trial
Details as	for Table 11

Details as for Table 11.

	Dry Pellet	Moist Pellet / Dry Pellet
Initial biomass (kg)	11.435	11.385
Final biomass (kg)	6.390	4.250
Change in biomass (kg)	-5.045	-7.135
Change in biomass (%)	-44.1	-62.7
Wet weight of feed used (kg)	67.8	82.6
Dry weight of feed used (kg)	61.3	64.0
Feed conversion ratio (wet)	-	-
Feed conversion ratio (dry)	-	-

6.6.5.2.4 Physiological condition of lobsters

Despite the generally poor survival and growth performance of lobsters in this trial, the hepatopancreas measures indicate that lobsters in the Dry Pellet treatment actually improved in condition during the trial (Table 48). The hepatopancreas and abdomen values show that the Moist/Dry Pellet lobsters maintained condition (Table 48).

Table 48. Physiological condition measures for fishery-caught lobsters and live-held lobsters fed on different diets during the Raceway Summer/Winter Trial

	Feed Tr		
	Dry Pellet	Moist Pellet / Dry Pellet	Fishery
Hepatopancreas dry weight index (%)	1.69 ± 0.13 $^{\rm a}$	$1.56\pm0.14^{\text{ ab}}$	$1.27\pm0.09^{\text{ b}}$
Hepatopancreas moisture content (%)	$59.35\pm2.09^{\text{ a}}$	$61.11\pm2.62^{\text{ ab}}$	$66.38 \pm 1.69^{\mathrm{b}}$
Abdomen dry weight index (%)	$8.83\pm0.17^{\text{ a}}$	9.07 ± 0.18 a	8.59 ± 0.16^{a}
Abdomen moisture content (%)	73.38 ± 0.34 a	73.32 ± 0.32 a	73.48 ± 0.37 a
n	10	7	20

Values are mean \pm standard error. Different superscripts within the same row indicate statistically significant differences (*P* < 0.05).

6.7 <u>Objective 1.5</u> To evaluate the effectiveness of different diets in promoting moult and improving condition and colour over the winter moult in "white" female and male rock lobsters

6.7.1 Introduction

Objective 1.5 was addressed by two field trials; the Kangaroo Island Cage Winter Trial, and the Kangaroo Island Cage Summer/Winter Trial (see Table 1). Different prepared pellets and a natural diet (octopus) were evaluated during the trials for their ability to improve condition and colour and to promote growth at moult over the winter moult in "white" male and female lobsters. At the completion of the Kangaroo Island Cage Winter Trial an assessment was also made of the live-shipping capabilities of the long-term live-held experimental lobsters. The live-shipping trial was designed to simulate the conditions experienced by lobsters from the time that they arrive in a processing factory to the time that they are available for sale in a Chinese market.

6.7.2 Kangaroo Island Cage Winter Trial

6.7.2.1 Methods

6.7.2.1.1 Experimental design

Five treatments were compared in this trial (Table 49). Three of the treatments were stocked with speckled/white, female lobsters, and the other two treatments with speckled/white, male lobsters. Each of the female and male 'fed' treatments (Female-Dry Pellet, Female-Dry Pellet + C, Male-Dry Pellet, and Male-Dry Pellet + C) was comprised of two replicate compartments containing 15 lobsters per compartment. The 'No Feed' treatment was comprised of a single compartment containing 15 lobsters. The nine compartments from the five treatments were spread across four separate cages. The feeding frequency during this trial was changed to once per week for three weeks during July.

Table 49. Experimental design for the Kangaroo Island Cage Winter Trial

Pellet numbers are cross-referenced with Table 2. *, one lobster was lost during transit to the holding system for this treatment and was not replaced. + C, signifies the higher carotenoid level used in Pellet 8 in comparison to Pellet 7 (see Tables 2 and 4).

No. lobsters per treatment	Pellet number and weeks of usage in parentheses
15	
29*	7(28)
30	8(28)
30	7(28)
30	8(28)
	No. lobsters per treatment 15 29* 30 30 30

6.7.2.1.2 Live shipping assessment of lobsters

At the completion of the live-holding trial, all surviving lobsters (except three females that were carrying eggs on their pleopods) from one compartment within each of the Male Dry Pellet (n = 15), Female Dry Pellet (n = 11), and Female No Feed (n = 12) treatments were kept alive for a live-shipping trial. The three treatments were kept separate throughout the trial. Each of the lobsters was assessed for vigour (see Section 6.6.2.1.2) and then placed into foam boxes with wood wool and immediately air-freighted to Adelaide. Lobsters were placed into lobster processor tanks in Adelaide within 6 hours of their removal from the Kangaroo Island sea-cage facility. Lobsters were maintained in the holding tanks at 11°C for an initial recovery period of four days prior to 'export.'

After the initial recovery period, lobsters were dip-chilled in 7°C water for approximately five minutes and then packed into three separate foam boxes each containing wood wool and two 1kg frozen gel packs. Small holes were punched in the sides of the boxes for aeration. After 'packout' the boxes were driven in a car and shaken periodically for 1 hour before being stored in a darkened room. The boxes were then shaken 4 hours later to simulate their arrival at and departure from Sydney airport. At 28 hours after the initial 'packout' the boxes were shaken again to simulate their arrival in China. One hour after this the boxes were driven in a car for 30 minutes and taken back to the lobster processing factory and unpacked. Lobsters were emmersed within the boxes for 30 hours in total. Lobsters were handled by industry processors during packout and unpacking. At the completion of the export, lobsters were returned to the processor holding tanks for a final recovery period of three days. This period was designed to simulate a recovery period in China prior to sale. After this recovery period, all lobsters were again assessed for vigour.

6.7.2.2 Results

6.7.2.2.1 Environmental conditions during the trial

Temperatures fluctuated considerably during the trial but showed a marked seasonal pattern with lowest temperatures during winter and maximum temperatures during October/November at the end of the trial (Figure 5). The average temperature was 15.4°C with a minimum of 12.3°C on 15/6/99 and a maximum of 19.8°C on several days during October 1999.

6.7.2.2.2 Survival and growth of lobsters

Survival was very high (93-100%) in all four of the fed treatments (Female Dry Pellet, Female Dry Pellet + C, Male Dry Pellet, and Male Dry Pellet + C) and lowest in the Female No Feed treatment at 80% (Table 50). All surviving lobsters in each treatment moulted during the trial (Table 50). Carapace length and weight increments were smallest in the Female No Feed treatment and largest in the two male treatments (Table 50). In fact the No Feed treatment had a negative value for mean percentage weight increment (Table 50). The two fed female treatments had significantly larger percentage weight increments than the No Feed female treatment (Table

50). The two male treatments had significantly larger carapace length and weight percentage increments than all three of the Female treatments (Table 50).

Table 50. Survival and growth data for lobsters fed on different diets during the Kangaroo IslandCage Winter Trial

Details as for Table 10.

	Female			Ma	ale
	No Feed	Dry Pellet	Dry Pellet +C	Dry Pellet	Dry Pellet + C
Initial no. lobsters	15	29	30	30	30
Final no. lobsters	12	27	29	30	30
Survival (%)	80	93	97	100	100
No. moulted	12	27	29	30	30
Moulted (%)	100	100	100	100	100
Initial CL (mm)	98.04 ± 0.47	106.89 ± 1.30	104.16 ± 1.01	104.62 ± 0.94	104.87 ± 0.61
CL increment (mm)	0.84 ± 0.25	2.41 ± 0.23	2.34 ± 0.32	4.52 ± 0.40	5.60 ± 0.39
CL increment (%)	0.87 ± 0.26 a	$2.31\pm0.24^{\rm \ a}$	2.27 ± 0.31 $^{\rm a}$	4.36 ± 0.40^{b}	5.35 ± 0.38^{b}
n	12	27	29	30	30
Initial weight (g)	489.7 ± 8.1	621.3 ± 20.4	573.0 ± 16.9	546.9 ± 16.6	552.9 ± 10.8
Weight increment (g)	-6.1 ± 6.9	43.9 ± 5.6	42.2 ± 6.1	83.8 ± 6.9	93.7 ± 7.1
Weight increment (%)	$-1.08 \pm 1.40^{\text{ a}}$	$7.67 \pm 1.01^{\text{ b}}$	$7.78 \pm 1.13^{\text{ b}}$	15.82 ± 1.33 °	17.11 ± 1.35 °
n	12	27	29	30	30

6.7.2.2.3 Feed conversion ratios during the trial

The Female No Feed treatment had a substantial loss of biomass while the two Female Pellet treatments more or less maintained biomass (Table 51). The two Male Pellet treatments had substantial increases in biomass (Table 51). Large amounts of feed were used in each of the Pellet treatments and as such the feed conversion ratios for the two male treatments were high (Table 51).

6.7.2.2.4 Physiological condition of lobsters

The physiological condition of the female and male treatments were analysed separately (Tables 52 and 53). For the female lobsters, the condition measures indicate that the No Feed animals significantly declined in condition while the Dry Pellet and Dry Pellet + C lobsters significantly improved in condition (Table 52). For the male lobsters, the condition measures indicate that the Dry Pellet and Dry Pellet + C lobsters significantly improved in condition (Table 52). For the male lobsters, the condition measures indicate that the Dry Pellet and Dry Pellet + C lobsters significantly improved in condition (Table 53). In one instance there was also a significant difference between the two male pellet treatments (Table 53).

Table 51. Biomass returns, feed usage, and feed conversion ratios for lobsters fed on different diets during the Kangaroo Island Cage Winter Trial

Details as for Table 11.

Female			Male	
No Feed	Dry Pellet	Dry Pellet +C	Dry Pellet	Dry Pellet + C
7.375	17.950	17.131	16.408	16.588
5.803	17.961	17.841	18.923	19.399
-1.572	0.011	0.710	2.515	2.811
-21.3	0.1	4.1	15.3	16.9
-	74.1	71.0	70.7	70.2
-	69.6	66.8	66.4	65.9
-	-	-	28.1	25.0
-	-	-	26.4	23.4
	No Feed 7.375 5.803 -1.572 -21.3 - -	Female No Feed Dry Pellet 7.375 17.950 5.803 17.961 -1.572 0.011 -21.3 0.1 - 74.1 - 69.6 - - - -	Female No Feed Dry Pellet Dry Pellet +C 7.375 17.950 17.131 5.803 17.961 17.841 -1.572 0.011 0.710 -21.3 0.1 4.1 - 74.1 71.0 - 69.6 66.8 - - - - - -	Female M No Feed Dry Pellet Dry Pellet +C Dry Pellet 7.375 17.950 17.131 16.408 5.803 17.961 17.841 18.923 -1.572 0.011 0.710 2.515 -21.3 0.1 4.1 15.3 - 74.1 71.0 70.7 - 69.6 66.8 66.4 - - - 28.1 - - - 26.4

Table 52. Physiological condition measures for female fishery-caught lobsters and female live-held lobsters fed on different diets during the Kangaroo Island Cage Winter Trial

Values are mean \pm standard error. Different superscripts within the same row indicate statistically significant differences (*P* < 0.05).

	Feed Treatment			
	No Feed	Dry Pellet	Dry Pellet+C	Fishery
Hepatopancreas dry weight index (%)	0.41 ± 0.03 $^{\rm a}$	$1.86\pm0.07^{\text{ b}}$	2.00 ± 0.09^{b}	$1.28\pm0.05^{\rmc}$
Hepatopancreas moisture content (%)	82.15 ± 0.74 a	54.89 ± 1.18^{b}	55.05 ± 1.42^{b}	62.26 ± 1.14 ^c
Abdomen dry weight index (%)	7.00 ± 0.13 a	$9.33\pm0.09^{\text{ b}}$	$9.21 \pm 0.11^{\text{ b}}$	9.37 ± 0.12^{b}
Abdomen moisture content (%)	76.91 ± 0.27 a	71.83 ± 0.20^{b}	72.03 ± 0.26^{b}	72.88 ± 0.22 °
n	12	27	29	20

6.7.2.2.5 External condition of lobsters

There was zero incidence and appearance of fouling in this trial (Table 54). The vast majority of lobsters in all five treatments enhanced in colouration (Table 54). There was a moderate incidence of missing legs in the four fed treatments (Female Dry Pellet, Female Dry Pellet+C, Male Dry Pellet, and Male Dry Pellet+C) and a very high incidence of 83% in the Female No Feed treatment (Table 54). These levels were reflected by the levels of leg loss in each of the five treatments, despite the high levels of leg regeneration (Table 54).

There was a very high incidence of any type of tail fan damage in each of the treatments at the end of the trial (Table 54). Within each treatment, tail fan raggedness was the most prevalent type

of damage, followed by erosion, and then blistering (Table 54). By comparing the incidence and development values for tail fan damage, it is apparent that the majority of each type of damage occurred during the trial although there was already some damage at the beginning of the trial (Table 54). No was no incidence of external lesions at the completion of this trial (Table 54). Some of the females in the Dry Pellet and Dry Pellet+C treatments had egg masses at the end of the trial (Table 54).

Table 53. Physiological condition measures for male fishery-caught lobsters and male live-held lobsters fed on different diets during the Kangaroo Island Cage Winter Trial

	Feed Tr	eatment	
	Dry Pellet Dry Pellet+C		Fishery
Hepatopancreas dry weight index (%)	1.70 ± 0.09 $^{\rm a}$	$2.04\pm0.08^{\:b}$	1.17 ± 0.10
Hepatopancreas moisture content (%)	59.61 ± 1.50^{a}	57.24 ± 1.10^{a}	66.43 ± 2.11
Abdomen dry weight index (%)	8.67 ± 0.13 a	$8.86\pm0.08^{\rm \ a}$	8.56 ± 0.15
Abdomen moisture content (%)	72.34 ± 0.25 a	72.40 ± 0.19^{a}	73.81 ± 0.36
n	30	30	20

Different superscripts within the same row indicate statistically significant differences (P < 0.05). The Fishery sample was the same as that used in the Port Lincoln Cage Winter Trial.

6.7.2.2.6 Health condition of lobsters

Microbiological examination of cultured samples of tail fan damage (viz. erosion) from five lobsters across three treatment groups (Female No Feed, Male Dry Pellet, Female Dry Pellet) revealed the presence of the bacteria species *Vibrio alginolyticus* and the fungal species *Fusarium solani* in all five samples.

6.7.2.2.7 Live shipping assessment of lobsters

All lobsters were healthy at the commencement of the live-shipping trial. At the completion of the trial all of these lobsters were still healthy (Table 55). Thus there was no difference in vigour between the treatments.

	Female			Male		
	No Feed	Dry Pellet	Dry Pellet + C	Dry Pellet	Dry Pellet + C	
Incidence of fouling (%)	0	0	0	0	0	
Appearance of fouling (%)	0	0	0	0	0	
Disappearance of fouling (%)	-	-	-	-	-	
Colour maintenance (%)	0	4	10	0	7	
Colour enhancement (%)	100	96	90	100	93	
Colour loss (%)	0	0	0	0	0	
Incidence of missing legs (%)	83	37	45	27	33	
Regeneration of missing legs (%)	100	83	86	83	88	
Loss of legs (%)	83	37	41	27	33	
Incidence of any type of tail fan damage (%)	100	93	90	93	90	
Incidence of tail fan raggedness (%)	83	70	76	73	70	
Incidence of tail fan blistering (%)	25	30	14	23	23	
Incidence of tail fan erosion (%)	33	41	38	43	43	
Development of any type of tail fan damage (%)	42	41	41	43	47	
Development of tail fan raggedness (%)	75	63	62	67	63	
Development of tail fan blistering (%)	8	11	3	13	10	
Development of tail fan erosion (%)	33	19	38	37	43	
Incidence of external lesions (%)	0	0	0	0	0	
Development of external lesions (%)	0	0	0	0	0	
Incidence of egg mass (%)	0	11	14	-	-	

Table 54. External condition measures for lobsters fed on different diets during the Kangaroo Island Cage Winter Trial

Table 55. Vigour assessment of lobsters from three different feed treatments in the Kangaroo Island Cage Winter Trial after completion of a live-shipping trial

Food Trootmont	No. lobste	No. lobsters in each vigour category					
reed freatment	Healthy	Unhealthy	Dead				
Male Dry Pellet	15	0	0				
Female Dry Pellet	11	0	0				
Female No Feed	12	0	0				

6.7.3 Kangaroo Island Cage Summer/Winter Trial

6.7.3.1 Methods

6.7.3.1.1 Experimental design

This trial was an extension of the Kangaroo Island Cage Summer Trial whereby single compartments from each of the three treatments (Dry Pellet, Moist Pellet, and Octopus) were not terminated at the completion of the Kangaroo Island Cage Summer Trial but were continued on through the winter in conjunction with the Kangaroo Island Cage Winter Trial (see Section 6.7.2). In the two pellet treatments the pellet was changed during the winter period to Pellet 7; the improved steam pelleted form (Table 56 and see Tables 2 and 4). The treatments were then named Moist/Dry Pellet, and Dry Pellet treatments (Table 56 and see Tables 2 and 4). Feeding frequency during the trial was reduced to once per week for three weeks during July.

Feed Treatment	No. lobsters per treatment	Pellet number and weeks of usage in parentheses
Dry Pellet Moist/Dry Pellet Octopus	19 19 19	1(2), 2(4), 3(17), 7(26) 9(6), 10(17), 7(26)

Table 56. Experimental design for the Kangaroo Island Cage Summer/Winter TrialPellet numbers are cross-referenced with Table 2.

6.7.3.2 Results

6.7.3.2.1 Environmental conditions during the trial

The seasonal pattern of temperature change is evident from Figure 5, with highest temperatures during summer and lowest temperatures during winter. For the period when data were logged, temperatures ranged from 24.7°C on 10/1/99 to 12.3°C on 15/6/99, with an average of 16.8°C.

6.7.3.2.2 Survival and growth of lobsters

Survival was moderate in this trial (Table 57). The Dry Pellet treatment had the highest survival at 74% and the Octopus treatment the lowest survival at just 53% (Table 57). Despite the low survival, of those that did survive, the majority moulted twice in all three treatments (Table 57). Individual lobsters in both the Moist/Dry Pellet and Octopus treatments moulted three times

(Table 57). Carapace length and weight increments for moult 1 and moult 2 were all larger in the Octopus treatment than in the two Pellet treatments (Table 57). However, only for moult 2 were these values statistically significant (Table 57).

Carapace length and weight increments were larger for moult 2 than moult 1 within all three of the treatments (Table 57). For those lobsters that moulted twice, a statistical comparison of mean percentage weight increment revealed that moult 2 was significantly larger than moult 1 for each of the three treatments (Table 58).

6.7.3.2.3 Feed conversion ratios during the trial

There were substantial losses of biomass in all three treatments with the Octopus treatment having the worst loss of 35.1% (Table 59). Large amounts of feed were used in each of the treatments (Table 59). Due to the biomass losses, feed conversion ratios were not calculated.

6.7.3.2.4 Physiological condition of lobsters

Overall, the four condition measures indicate that the lobsters in all three treatments maintained condition when compared to the Fishery sample (Table 60). The only case of a significant difference was the hepatopancreas dry weight index for the Dry Pellet treatment which showed an improvement in physiological condition when compared to the Fishery sample (Table 60).

Table 57. Survival and growth data for lobsters fed on different diets during the Kangaroo IslandCage Summer/Winter TrialDetails as for Table 10.

	Dry Pellet	Moist Pellet / Dry Pellet	Octopus
Initial no. lobsters	19	19	19
Final no. lobsters	14	12	10
Survival (%)	74	63	53
No. moulted once	3	4	3
No. moulted twice	11	7	6
No. moulted thrice		1	1
Moulted once (%)	21	33	30
Moulted twice (%)	79	58	60
Moulted thrice (%)		8	10
Initial CL (mm)	104.61 ± 1.52	109.09 ± 2.07	102.96 ± 2.48
CL increment 1 (mm)	2.22 ± 0.37	2.23 ± 0.48	2.66 ± 0.39
CL increment 2 (mm)	4.35 ± 0.28	4.94 ± 0.46	7.50 ± 0.49
CL increment 3 (mm)		5.10	7.30
CL increment 1 (%)	2.14 ± 0.36^{a}	$2.05\pm0.44^{\rm \ a}$	$2.58\pm0.38^{\mathrm{a}}$
CL increment 2 (%)	4.13 ± 0.26^{a}	$4.49\pm0.37^{\text{ a}}$	7.43 ± 0.56^{b}
CL increment 3 (%)		4.64	7.29
n Initial CL	14	12	10
n CL increment 1	14	12	10
n CL increment 2	11	8	7
n CL increment 3		1	1
1 . 1 . 1 . 1 . (.)	525 4 22 6		510.0 + 22.0
Initial weight (g)	535.4 ± 22.6	620.8 ± 38.3	519.2 ± 33.9
Weight increment 1 (g)	35.0 ± 7.2	27.0 ± 10.1	53.1 ± 10.9
Weight increment 2 (g)	71.1 ± 4.7	79.9 ± 13.0	121.3 ± 9.1
Weight increment 3 (g)		82.0	92.0
Weight increment 1 (%)	6.67 ± 1.39^{a}	$4.45 \pm 1.54^{\text{ a}}$	10.08 ± 1.81 ^a
Weight increment 2 (%)	13.04 ± 0.92^{a}	$12.75 \pm 1.40^{\text{ a}}$	24.25 ± 1.89^{b}
Weight increment 3 (%)		13.78	19.53
n Initial weight	14	12	10
n Weight increment 1	14	12	10
n Weight increment 2	11	8	7
n Weight increment 3		1	1

Table 58. Results of Paired *t*-tests comparing mean percentage weight increment of moult 1 versus moult 2 for those lobsters that moulted twice in the Kangaroo Island Cage Summer/Winter Trial *, significant at α =0.05 level; df, degrees of freedom

Treatment	t	Р	df
Dry Pellet	3.30753*	0.007912	10
Moist Pellet / Dry Pellet	3.45971*	0.010552	7
Octopus	4.48704*	0.004161	6

Table 59. Biomass returns, feed usage, and feed conversion ratios for lobsters fed on different diets during the Kangaroo Island Cage Summer/Winter Trial Details as for Table 11.

	Dry Pellet	Moist Pellet / Dry Pellet	Octopus
Initial biomass (kg)	10.091	11.973	10.282
Final biomass (kg)	8.768	8.494	6.664
Change in biomass (kg)	-1.323	-3.479	-3.618
Change in biomass (%)	-13.1	-29.1	-35.1
Wet weight of feed used (kg)	63.8	78.8	56.9
Dry weight of feed used (kg)	58.5	62.3	11.4
Feed conversion ratio (wet)	-	-	-
Feed conversion ratio (dry)	-	-	-

 Table 60. Physiological condition measures for fishery-caught lobsters and live-held lobsters fed on different diets during the Kangaroo Island Cage Summer/Winter Trial

Values are mean \pm standard error. Different superscripts within the same row indicate statistically significant differences (*P* < 0.05).

		Feed Treatment				
	Dry Pellet	Moist Pellet / Dry Pellet	Octopus	Fishery		
Hepatopancreas dry weight index (%)	$1.88\pm0.13^{\rm \ a}$	$1.50\pm0.15^{\text{ ab}}$	$1.38\pm0.11^{\text{ ab}}$	1.41 ± 0.10^{b}		
Hepatopancreas moisture content (%)	59.47 ± 2.20^{a}	65.17 ± 2.24 ^a	60.73 ± 2.09^{a}	61.69 ± 1.68^{a}		
Abdomen dry weight index (%)	8.37 ± 0.21 a	8.32 ± 0.21 a	8.92 ± 0.22 a	8.70 ± 0.16 a		
Abdomen moisture content (%)	73.26 ± 0.41 a	$73.72 \pm 0.40^{\text{ a}}$	72.26 ± 0.64 a	73.36 ± 0.35 a		
n	14	12	10	18		

6.8 General discussion

6.8.1 Introduction

This section is primarily a comparison of the different live-holding field trials so that general patterns can be determined relating to the effects of diet (natural versus prepared), season (summer versus winter), system (sea-cage versus raceway), colour (red versus speckled/white), and sex (male versus female) on the survival, moulting activity, growth at moult, biomass returns, physiological condition, and external condition of lobsters. To this end Figures 7-10 summarise the survival, moulting activity, weight gain at moult, and biomass returns for the three summer and three winter trials. To enable direct comparisons in Figures 7-10, treatments have been labeled under five categories of No Feed, Pellet, Live Mussel, Octopus, and Female.

6.8.2 Environmental conditions during live-holding

Temperature is one of the most important factors affecting the survival and growth of live-held lobsters (see Sections 7 and 8). In the present study, temperatures varied widely during each of the field trials. The maximum temperatures of $24-25^{\circ}$ C that lobsters were exposed to during the summer period were far higher than any temperatures that lobsters of that size-class would normally be exposed to in their natural habitat in South Australia. For this reason there is concern within the South Australian rock lobster industry that summer water temperatures in and around Boston Bay and Nepean Bay (where the two live-holding facilities are presently located) are too high for safe live-holding. Indeed, Hooker *et al.* (1997) documented highest mortalities for juvenile captive fed *J .edwardsii* when seasonal temperatures peaked at 23.3° C, and in Component III of the present study (see Section 8) it was found that exposure to an increased temperature of 24° C caused decreased survival in juvenile *J. edwardsii*. However, despite the high temperatures in the summer trials of Component I, survival rates were very high in some of the treatments (see Section 6.8.3 below), demonstrating that such temperatures (although probably stressful) can be tolerated by adult *J. edwardsii*.

Other environmental factors that are important for the live-holding of lobsters include dissolved oxygen and ammonia (Crear and Forteath, 1998). Due to the high water exchange rates associated with the sea-cage and raceway systems, neither of these factors should have presented problems in the present study. On the two occasions when dissolved oxygen was monitored on the two sea-cage systems, it did not fall below 92%. This level is well above that which may cause problems for *J. edwardsii* (Crear and Forteath 1998). It is anticipated that oxygen levels in the Port Lincoln and Kangaroo Island sea-cage systems would remain relatively high at all times due to tidal exchange and wave action. Likewise, it is expected that due to high water movements, ammonia levels (although not tested during this study) would not reach dangerous levels in the sea-cage systems.

The high water exchange rate in the raceway system would have maintained good water quality. However, on two occasions there were mechanical failures with this system that resulted in mortalities during the Raceway Winter and Raceway Summer/Winter Trials. Four days after the start of the Raceway Winter Trial a system failure resulted in the death of all 10 lobsters in one of the compartments. These animals were subsequently replaced on 12 May 1999 (Table 1).

Approximately one week before the Raceway Winter and Raceway Summer/Winter Trials were due for completion another system failure resulted in the death of almost all lobsters (see Sections 6.6.3.2.2 and 6.6.5.2.2). The reason for these system failures and the ultimate death of the lobsters remains unclear but the system failure probably was due to a prolonged blockage of the water supply leading to a decline in dissolved oxygen to levels that were lethal. Such system failures highlight the potential problems associated with land-based systems that rely on a mechanical supply of water, as opposed to sea-based cage systems.

6.8.3 Survival

Survival of lobsters during the field trials would have been affected by a number factors including their handling prior to the trials, and environmental conditions, nutrition, and cannibalism during the trials. A general trend across the Port Lincoln Cage Summer, Port Lincoln Cage Winter, and Kangaroo Island Cage Winter Trials is that survival was always lower in the un-fed (No Feed) treatments than in the fed (Pellet and Live Mussel) treatments (Figure 7). This can be attributed primarily to a lack of nutrition in the un-fed lobsters that resulted in decreased physiological condition (see section 6.8.7). These weakened lobsters may have died directly from a depletion of energy reserves (i.e. starvation) or through cannibalism by other hungry lobsters (see Lorkin *et al.*, 1999), especially if they were soft-shelled after moulting. Lorkin *et al.* (1999) also found that survival was lower in unfed lobsters than in fed lobsters. Nonetheless, in the present study the survival of >60% of lobsters in each of the No Feed treatments after extended periods of not being directly fed, demonstrates the incredible resilience of adult *J. edwardsii* to conditions of poor nutrition.



Figure 7. Survival (%) in all treatments from the summer and winter live-holding field trials N, no feed; P, pellet; M, live mussel; O, octopus; F, female; PL, Port Lincoln Cage; R, Raceway; KI, Kangaroo Island Cage. Data are taken from Tables 10, 16, 21, 26, 35, and 50. See Sections 6.4.2, 6.4.3, 6.5.2, 6.6.2, 6.6.3, and 6.7.2 for details.

In those trials where natural and prepared (pellet) feeds were compared, there was a trend towards higher survival on the pellets (Figure 7). The reason for this is unclear. When comparing systems, survival in the pellet treatments of the summer trials was greatest in the Port Lincoln cage system and lowest in the raceway and Kangaroo Island cage systems (Figure 7). However, in the winter trials, survival in the pellet treatments was highest in the Kangaroo Island cage system and lowest in the raceway system (Figure 7). The consistently lower survival in the raceway system than the Port Lincoln cage system was possibly related to undetected system failures (see Section 6.8.2). It is also possible that cannibalism of soft-shelled post-moult lobsters was more of a problem in the raceway system where stocking density (per total surface area) was far higher than in the cage systems (see Section 6.2.3).

There were few readily observable patterns for survival when comparing the relevant treatments and trials between summer and winter (Figure 7). The most obvious difference in survival was between the male pellet treatments from the Kangaroo Island Cage Summer and Kangaroo Island Cage Winter Trials (Figure 7). However, it is believed that the lower survival of the Kangaroo Island Summer Cage Trial lobsters was due largely to their initial poor handling (see Section 6.5.2.1.1) and the differences between the summer and winter trials are therefore not seen as a direct effect of the different seasons.

6.8.4 Moulting activity

Moulting activity of lobsters during the field trials could have been affected by a number of factors including their handling prior to the trials, their moult stage upon entry to the trials, and environmental conditions and nutrition during the trials. Several trends in moulting activity are apparent from the summer and winter field trials (Figure 8). Moulting activity was far higher during the winter trials than the summer trials for the equivalent feed treatments in all three of the holding systems (Figure 8). This could be due to a number of reasons. The most obvious explanation is that lobsters were held for a longer duration in the winter trials than the summer trials (see Table 1) and so they simply had more time to moult. This was quite possibly the case as moult-staging at the completion of the summer trials revealed that several lobsters were ready to moult (data not shown) and they would probably have moulted if the trials had been extended. Indeed all of the lobsters from the summer trials that were retained and survived through to the end of the summer/winter trials did eventually moult. However there appear to be other factors beside holding duration that were associated with the low level of moulting activity in the summer trials and the almost universal moulting of animals in the winter trials.

When comparing the timing of moulting during the holding period it is apparent that there were differences between the cage trials (Table 61). In the Port Lincoln Cage Summer Trial, moulting activity was evenly spread over the holding period while in the Kangaroo Island Summer Trial there were peaks in moulting activity at the start and end of the trial (Table 61). In contrast, in the two winter trials the majority of moulting occurred during the middle and end parts of the trial (Table 61). These differences reflect a number of factors. Firstly, in the two summer trials there was a mixture of pre-moult and inter-moult lobsters at the start of the trial (data not shown). This meant that some lobsters moulted soon after entry to the trial while others did not moult until much later. However, in the winter trial almost all lobsters were in inter-moult at the start of the trial (data not shown). This meant that they did not moult until later in the trial. A natural seasonal pattern which synchronises moulting in the late winter/early spring probably also

affected the timing of moulting in the winter trial animals. Decreased water temperatures and shorter day length in June may have signalled a decrease in feeding and a preparation for moulting. It appears that many animals moulted in August (Table 61) which was reflected by an increase in feeding rate. It is likely that many of the other 'winter' moults were in September, but unfortunately lobsters were not assessed frequently enough to demonstrate the extent of synchrony of the winter moult. Aquasearch (1996) also recorded high levels of moulting activity during winter for *J. edwardsii* held in sea-cages in Boston Bay. High water temperatures during the summer trials may have provided a stress (see Section 6.8.2) causing decreased moulting. Very low levels of moulting were also observed in a pilot study by Lorkin *et al.* (1999) which trialed live-held adult *J. edwardsii* during summer in the Port Lincoln sea-cage system in Boston Bay.



Figure 8. Moulting activity (%) in all treatments from the summer and winter live-holding field trials

N, no feed; P, pellet; M, live mussel; O, octopus; F, female; PL, Port Lincoln Cage; R, Raceway; KI, Kangaroo Island Cage. Data are taken from Tables 10, 16, 21, 26, 35, and 50. See Sections 6.4.2, 6.4.3, 6.5.2, 6.6.2, 6.6.3, and 6.7.2 for details.

Moulting activity may be influenced by nutrition; as energy reserves need to be stored prior to a moult. However, in the Port Lincoln and Kangaroo Island Cage Winter Trials all lobsters in the No Feed treatments moulted (Table 8). In addition, these No Feed lobsters did not moult any later than lobsters in the fed treatments (data not shown). Thus there also appears to be a strong seasonal factor in the moulting pattern such that lobsters of this size always undergo a winter moult.

The final factor that may have influenced moulting activity was the handling of lobsters prior to the trials. As detailed earlier (see Section 6.5.2.1.1), the Kangaroo Island Cage Summer Trial

lobsters were handled poorly prior to the trial. The stress of this handling may have contributed to their low moulting activity through a delayment of moulting.

Cage Trial	% of total moulting activity for each time interval between assessments						
Port Lincoln	18 Nov 1998 – 8 Jan 1999	8 Jan 1999 – 10 Feb 1999	10 Feb 1999 – 23 Mar 1999				
Summer	38	34	28				
Kangaroo Island	1 Dec 1998 – 12 Jan 1999	12 Jan 1999 – 16 Feb 1999	16 Feb 1999 – 31 Mar 1999				
Summer	47	5	47				
Port Lincoln	16 Apr 1999 – 16 Jun 1999	16 Jun 1999 – 25 Aug 1999	25 Aug 1999 – 2 Nov 1999				
Winter	2	53	45				
Kangaroo Island	20 Apr 1999 – 9 Jun 1999	9 Jun 1999 – 1 Sep 1999	1 Sep 1999 – 18 Nov 1999				
Winter	1	49	50				

Table 61. Timing of moulting over the duration of the summer and winter cage field trials

Overall it seems that male lobsters may moult at any time over an extended period in 'summer', November to March (no data are available for female summer moulting), while both male and female lobsters moult seasonally in 'winter', August to October.

6.8.5 Growth at moult

Growth at moult of lobsters during the field trials could have been affected by a number of factors including their handling prior to the trials, their moult stage upon entry to the trials, and environmental conditions and nutrition during the trials. Several general trends in growth at moult are evident from the summer and winter live-holding field trials (Figure 9). Growth at moult was far lower in the No Feed treatments than the feed treatments in the Port Lincoln Cage Summer, Port Lincoln Cage Winter, and Kangaroo Island Cage Winter Trials (Figure 9). This can be directly attributed to a lack of nutrition prior to and after moulting in the un-fed animals. Nonetheless, lobsters in the No Feed treatments could still have gained some nutrition by consuming the abundant bio-fouling organisms from the holding cage surfaces. This feeding behaviour was evidenced by the mesh surrounding the No Feed compartments which was always cleaner than that surrounding the fed compartments. Similar behaviour has also been documented for un-fed *Jasus lalandii* kept in sea-cages (Barkai and Branch, 1988). The No Feed lobsters in the present study could also have gained some nutrition through cannibalism.

Growth at moult was higher in the natural feed treatments (Live Mussel, Octopus) than in the comparable prepared (Pellet) feed treatments in the Port Lincoln Cage Summer, Kangaroo Island Cage Summer, and the Port Lincoln Cage Winter Trials (Figure 9). These results are clearly related to nutrition. However, it is unclear whether they are due to an increased availability of the natural feeds (live mussels were always available, while octopus was far more stable in water than pellets) and/or to superior nutritional qualities of the natural feeds. For example, mussels have been shown to be superior to prepared pellet feeds in growth trials of juvenile *J. edwardsii* (Crear *et al.*, 1999). While the differences in growth at moult between the natural and prepared feeds were not always statistically significant in the summer and winter trials, the beneficial long-term effects of the natural feeds was emphasised during the second moult in the summer/winter trials (see Sections 6.6.4 and 6.7.3).



Figure 9. Individual weight gains at moult (%) in all treatments from the summer and winter liveholding field trials

N, no feed; P, pellet; M, live mussel; O, octopus; F, female; PL, Port Lincoln Cage; R, Raceway; KI, Kangaroo Island Cage. Data are taken from Tables 10, 16, 21, 26, 35, and 50. See Sections 6.4.2, 6.4.3, 6.5.2, 6.6.2, 6.6.3, and 6.7.2 for details.

Growth at moult for the pellet-fed treatments was higher in the Port Lincoln cage system than in the raceway system during both summer and winter (Figure 9). This may have been related to stress and the suspected water flow problems in the raceway system (see Section 6.8.2). Growth at moult for the pellet-fed treatments was also higher in the Port Lincoln cage system than in the Kangaroo Island cage system during summer (Figure 9). However, growth at moult for these two systems was equivalent during winter (Figure 9). Therefore the summer result does not appear to be related to red (Port Lincoln) versus speckled/white (Kangaroo Island) lobsters. It is likely that the poor performance of the Kangaroo Island lobsters during the summer trial was related to their

poor handling prior to the trial (see Section 6.5.2.1.1) and white lobsters apparently have the same growth potential as red lobsters.

Growth at moult was far greater in the comparative Pellet and Live Mussel treatments during winter than summer in all three holding systems (Figure 9). The improved performance of the Pellet treatments during winter may be partly attributable to the improved pellet used in the winter trials (see Section 6.3.1.1), however, nutrition cannot explain the markedly better growth of the Port Lincoln Live Mussel treatment during winter (Figure 9). It may be that the high summer temperatures caused stress (see Section 6.8.2) and this resulted in reduced growth during the summer trials. In support of this theory is that in the summer/winter trials, the second moult (which mainly occurred during "winter") was consistently larger than the first moult (which mainly occurred during "summer") (see Sections 6.6.4 and 6.7.3). It may also be that the seasonal pattern of the winter moult allows for more extensive energy storage and weight gain at that moult.

Growth at moult for the pellet treatments in the Kangaroo Island Cage Winter Trial was far greater in males than females (Figure 9). The majority of females used in the Kangaroo Island Cage Winter Trial were sexually mature. Growth rate declines in *J. edwardsii* females when they become sexually mature (Prescott *et al.*, 1997). It is possible that immature females might attain similar moult increments to males of a similar size. This warrants further investigation.

Consistent differences in growth at moult were not apparent for lobsters fed on dry versus moist pellets or for the lobsters fed mussel-mince enhanced pellets. However, lobsters moulted only once in the summer and winter trials, and growth differences related to these different pellets may not become apparent until two or more moults. Minor differences in pellet formulation may not be particularly important for live-holding operations in which lobsters moult only once. The effects of pellet formulation and nutrition are more likely to be observed in juvenile grow-out where animals are moulting regularly.

Prescott *et al.* (1997) recorded an average carapace length moult increment of around 8mm for wild male *J. edwardsii* in the carapace length size classes of 80-89.9mm and 120-129.9mm. In the present study the maximum average carapace length moult increment for males around 100-110mm carapace length was about 4-5mm. Thus the full growth potential of *J. edwardsii* was not achieved in the live-holding field trials of the present study. Based upon regression estimates from the present study, if an increase from 5mm to 8mm in carapace length moult increment were achieved (i.e. up to the 'field potential'), this would add a further 50g (or 10%) in weight gain at moult for the live held lobsters. This would be a substantial improvement in growth to what was achieved in the present study.

Growth of 100mm carapace length *J. edwardsii* has been found to be lower for speckled/white lobsters captured from deep waters than for red lobsters taken from shallower waters (McGarvey *et al.*, 1999). This difference could be due to differences in diet/nutrition associated with depth (McGarvey *et al.*, 1999). It is therefore of significance that the growth at moult of speckled/white male and red male lobsters was equivalent when fed on the same pellet diets in the Port Lincoln Cage Winter and Kangaroo Island Cage Winter Trials of the present study (Figure 9). This suggests that differences in field growth between speckled/white and red lobsters are environmental rather than genetic.

6.8.6 Biomass returns and feed conversion ratios

Biomass returns in the present study were a reflection of survival, moulting activity, and weight gain at moult. Several patterns in biomass returns are observable from the summer and winter trials (Figure 10). Firstly, biomass returns were negative and much lower in the No Feed treatments than in the feed treatments for the Port Lincoln Cage Summer, Port Lincoln Cage Winter, and Kangaroo Island Cage Winter Trials (Figure 10). This was a direct result of the lowered survival and growth at moult in the No Feed treatments (see Sections 6.8.3 and 6.8.5).

Biomass returns for the male pellet treatments of the Port Lincoln and Kangaroo Island cage systems were negative in the summer and positive in the winter, while biomass returns in the raceway system were negative in both summer and winter (Figure 10). The improved results during winter for the cage systems are a reflection of improved survival, greater moulting activity, and larger growth at moult during winter (see Figures 7-9). The two pellet treatments using speckled/white male lobsters in the Kangaroo Island Cage Winter Trial had the highest biomass returns out of all the trials (Figure 10). These two treatments had 100% survival, 100% moulting activity, and substantial growth at moult (see Figures 7-9). Despite the high growth at moult in the Live Mussel treatment of the Port Lincoln Cage Winter Trial (Figure 9), this treatment had a negative biomass return (Figure 10). This was due to the lowered survival in this treatment (Figure 7), and it emphasises the need for a high survival rate in a live-holding operation based on weight gain for profit.



Figure 10. Biomass returns (%) in all treatments from the summer and winter live-holding field trials

N, no feed; P, pellet; M, live mussel; O, octopus; F, female; PL, Port Lincoln Cage; R, Raceway; KI, Kangaroo Island Cage. Data are taken from Tables 11, 17, 22, 27, 36, and 51. See Sections 6.4.2, 6.4.3, 6.5.2, 6.6.2, 6.6.3, and 6.7.2 for details.

Feed conversion ratios were extremely high for the pellet-fed treatments in the field cage trials. The main reasons for this were the high feeding rates and the breakdown and loss of pellets after feeding (see Section 6.3.2). Experimental work within Project 3 (98/303) of the RLEAS found that adult J. edwardsii of around 470g consumed only about 2-3g dry weight of feed per day, i.e. 0.4-0.6% of their body weight per day. The results of that work also demonstrated a cyclical nature to daily feed intake. In the present study, lobsters were fed at a rate of 2% of their body weight per day and due to the feeding frequency of twice per week in the sea-cage systems this rate was adjusted to 7% of their body weight per feed time (see Section 6.3.1.1). Clearly this amount of feed is far greater than that which the lobsters could have eaten in one day, and even if the pellets remained in the cages for two to three days after feeding (see results of Section 6.3.2.1), the amount of feed is still greater than what the lobsters could have consumed. Therefore there must have been a large amount of pellet that was uneaten after each feeding time (this is supported by personal observations) and which ultimately would have fallen through the cage mesh as waste. In order to reduce feed conversion ratios for lobsters being fed on pellets in seacage systems, the feeding rate, feeding frequency, and size of pellet all need to be optimised. This requires further investigation.

6.8.7 Physiological condition of lobsters

One function of the hepatopancreas is as an energy storage organ that can be utilised by lobsters in times of starvation, poor nutrition, and high metabolic demand. Studies have shown that the size and moisture content of the hepatopancreas can change markedly during times of starvation and poor nutrition (Stewart et al., 1967; Dall, 1974; Trendall and Prescott, 1989; McClain, 1995; Musgrove, 1998). The abdomen can also be used as an energy source if required, and the size and moisture content of this tissue also change during times of starvation and poor nutrition (Stewart et al., 1967; Dall, 1974; Trendall and Prescott, 1989; McClain, 1995; Musgrove, 1998). Four different measures for the two tissues were used in the present study to define the physiological condition of lobsters (see Section 6.2.4.5). The focus of the present study was on the ability of different feed treatments to maintain and/or improve physiological condition in live-held lobsters. While the size and moisture content of the hepatopancreas and abdomen do change through the moult cycle in J. edwardsii (Musgrove, unpublished data), this study did not investigate the effect of the moult cycle on these parameters. This was because the majority of lobsters analysed were in the inter-moult stage. Based upon the results of the live-holding field trials in the present study, two main conclusions can be drawn. Firstly, the physiological condition of lobsters declines markedly without feeding. Secondly, both the natural and prepared feeds trialed in this study were adequate in maintaining and/or improving the physiological condition of lobsters. Live mussels did tend to improve the condition of lobsters more so than pellets.

Objectives 1.3 and 1.5 were designed to examine the ability of different diets in improving the condition of <u>poor</u> condition "white" lobsters (see Sections 6.5 and 6.7). However, a statistical comparison of the four different initial fishery samples collected during this study (i.e. Kangaroo Island summer <u>speckled/white</u> male, Port Lincoln summer <u>red</u> male, Kangaroo Island winter <u>speckled/white</u> female, and Port Lincoln winter <u>red</u> male), revealed no significant difference between these samples for three out of the four physiological condition measures. Therefore the speckled/white fishery lobsters are not in poor physiological condition in the parameters

measured in the present study. The one statistically significant comparison of abdomen dry weight index between the Kangaroo Island winter speckled/white female sample and the other three male samples simply indicates that these females had slightly larger abdomens than the males.

While white lobsters have been labeled by industry as being in poor condition due to their lower survival rates in processor tanks and during live-shipping, results from the present study suggest that this 'poor condition' is not reflected in poor physiological condition as measured by the size and moisture content of the hepatopancreas and abdomen. Furthermore, results of the live-shipping trials showed that lobsters that had not been fed for 29-30 weeks (and which were in very poor physiological condition) were still able to survive extended periods of emersion during simulated live-shipping (see Sections 6.6.2.2.7 and 6.7.2.2.7). It is likely therefore that the higher mortality of white lobsters during processor holding and subsequent live-shipping is related to an acute stress. As white lobsters are predominantly found in deeper waters, it is possible that the removal of white lobsters from deep water causes physiological stresses not seen in red lobsters removed from shallower water. The condition and survivability of white lobsters is an area that needs further investigation.

6.8.8 External condition of lobsters

For acceptance in the Asian live lobster market the preferred attributes of a lobster are that it is clean (little or no fouling on shell), that it has little or no damage (preferably all legs intact), and that it is red in colouration. With respect to most of these criteria (but not tail fan damage – see below) the majority of lobsters at the end of the field trials in the present study were acceptable for export. While some lobsters did have considerable carapace fouling by algae in one of the trials, the cause of this was identified and rectified by providing adequate shading from sunlight. Likewise, while the incidence of missing legs was high in some instances, the majority of the leg loss probably occurred during the periodic experimental assessments. With respect to colour, the colour of red lobsters was maintained during the trials and the colour of speckled/white lobsters was generally improved during the trials toward the more desirable red colouration (see below).

In the present study, the ability both to maintain colouration in red lobsters and to improve colouration in speckled/white lobsters was important. Colour maintenance of red lobsters was achieved in live mussel-fed, pellet-fed, and un-fed treatments. Colour improvement of speckled/white lobsters was achieved in octopus-fed, pellet-fed, and un-fed treatments. Colour change over time in the exoskeleton of captive decapod crustaceans has been reported on several occasions (e.g. D'Abramo *et al.*, 1983; Howell and Matthews, 1991; Menasveta *et al.*, 1993; James and Tong, 1997; Lim *et al.*, 1997). These changes can usually be attributed to the level of carotenoids in the diet as carotenoids are essential for the pigmentation of the exoskeleton. In this respect it is interesting that in the present study, <u>un-fed</u> speckled/white lobsters changed in colouration. It would appear then that diet may not be the only factor responsible for colour change in adult *J. edwardsii*. Alternatively, it is possible that the un-fed lobsters were deriving carotenoids through consumption of biofouling organisms from the cage surfaces (see Section 6.8.5).

While the colour of speckled/white lobsters was improved towards a red colour in the present study, it is uncertain whether the amount of colour change was sufficient to improve their market

value. In addition, the existing red pigmentation in some lobsters (especially those in the octopusfed treatments) turned a distinct purple colouration. It is unknown as to why this change occurred and whether it would be acceptable for live markets. It is also unclear as to what role moulting plays in the ability to change colour. In summary, there is a definite need for further research into the mechanism and factors influencing colour regulation and colour change in adult *J. edwardsii*.

As the possession of ovigerous female lobsters in South Australia is illegal, there are concerns about live-holding females and the possibility that they will spawn during holding. Spawning was certainly prevalent in the study conducted by Aquasearch (1996) on sea-based live-holding of *J. edwardsii* in Boston Bay, Port Lincoln. However, males and females were not separated during the Aquasearch (1996) study and their trials were also conducted over winter/spring when *J. edwardsii* naturally mates and spawns. In the present study, females were held in only one trial (the Kangaroo Island Cage Winter Trial) and were separated from males to prevent mating. Despite this separation, some females showed some spawning activity (Table 54). While the egg batches from these females were abnormally small and the eggs were almost certainly infertile, these females were still technically ovigerous and therefore illegal to possess. It is apparent then that live-holding of sexually mature females during winter can present problems with spawning as it seems some females will spawn small batches of infertile eggs without the stimulus of mating.

The greatest problem with the external condition of lobsters in the present study was that of tail fan damage. In many cases the damage was so extreme that it made the lobsters very unsightly and probably unmarketable. Tail fan damage (raggedness, blistering, and erosion) was also a major problem in the pilot study of sea-based live-holding of *J. edwardsii* conducted by Lorkin *et al.* (1999). Recent discussions indicate that people from all countries, working with at least four species of crustaceans, are seeing a similar syndrome of blistering and progressive damage to the tail fans (Reuter, R, personal communication). A specific cause has not as yet been identified and there has been no definite correlation with a variety of factors, including season and location. Possible causes of tail fan damage in live-held *J. edwardsii* were discussed by Lorkin *et al.* (1999). Clearly, for a live-holding industry to proceed in South Australia, an investigation into the progression and cause of tail fan damage in live-held adult *J. edwardsii* requires immediate attention.

6.8.9 Health condition of lobsters

Spiny lobsters generally display low rates of debilitating disease and parasite infestation, and this was the case with the live-held lobsters in the present study. The only major infections were those associated with tail fan damage. As with the Lorkin *et al.* (1999) study, the species of bacteria that were isolated from damaged tail fans in the present study were all species that are naturally present in water and were most likely secondary opportunistic invaders, rather than the initial cause of the damage. Fungal infection was also detected in some damaged tail fans in the present study.

6.8.10 Live-shipping of lobsters

The ability of long-term live-held lobsters to survive live-shipping to Asian markets is crucial to the success of a live-holding industry in South Australia. In the present study, two live-shipping

trials suggested that lobsters that have been held for extended periods in sea-cage systems could survive live-shipping overseas. Even lobsters that had been un-fed for extended periods of liveholding were still able to survive simulated live-shipping in the present study. Nonetheless, simulated live-shipping exercises do not always reflect the real situation and real live-shipping trials to Asia are required to properly assess this aspect of a live-holding industry.

6.8.11 Taste of lobsters

There are general concerns among the public that the taste of aquacultured products may differ from that of wild products. It is often believed that manufactured aquaculture diets are responsible for this perceived difference. Certainly the flavour of captive lobsters and aquacultured prawns can be affected by their diet (Donahue *et al.*, 1997; Whitfield *et al.*, 1997). However, in the present study there was no evidence to suggest that the taste of long-term liveheld adult *J. edwardsii* had been adversely affected by feeding them with manufactured pellets or live mussels. Indeed, of the people who detected a difference between samples in the Taste Trial (see Section 6.6.2.2.8), approximately half of them preferred the live-held product to the wild product. This result is not particularly surprising considering that the pellets contained a substantial amount of marine-based animal products (viz. fish and crustaceans, Table 4).

6.8.12 Future work

The only significant weight gain that occurs in lobsters is after moulting when they take in water, harden-up their exoskeleton, and subsequently during intermoult replace the water with tissue. Moulting and growth offer the opportunity for profit to be made through weight gain in a liveholding industry. Based upon the results of their large-scale tag-recapture study in South Australia, Prescott *et al.* (1997) predicted that *J. edwardsii* of a similar size to those used in the present study moult twice a year; once in summer and once in winter. This low moulting frequency means that adult *J. edwardsii* must be held for long periods of time before they gain any weight. This was certainly the case in the present study and it has important consequences for a lobster live-holding industry that relies on weight gain for profit. Some of these consequences are the costs associated with holding and feeding, and the risk of mortality during the long intermoult period. Therefore possible ways of decreasing the holding period before moult, increasing weight gain at moult, and increasing survival during holding, all deserve further investigation.

Seven key areas have been identified for further research. Four of these areas relate to smarter selection of lobsters for use in live-holding and involve consideration of stage, source, size, and sex in selecting lobsters. The other three areas are tail fan damage, systems design/husbandry, and economics. The <u>stage</u> of lobsters relates to the moult stage of lobsters when they are placed into holding facilities and the influence that this has on subsequent growth at moult. The <u>source</u> of lobsters is concerned with where the lobsters are collected from prior to live-holding; this includes shallow water (red lobsters) versus deep water (speckled lobsters) versus deepest water (white lobsters), and also regions of the fishery with different productivities and different lobster growth rates (see McGarvey *et al.*, 1999). <u>Size</u> and <u>sex</u> are simply concerned with differences in growth at moult between different sized lobsters and between male and female lobsters, respectively. <u>Tail fan damage</u> is concerned with the unsightly external damage that occurs in live-held lobsters, while <u>systems design/husbandry</u> is concerned with improving sea-based and land-

based holding systems and husbandry practices. The area of <u>economics</u> is concerned with assessing the economic feasibility of live-holding.

The effect of moult stage upon entry to a holding system on the subsequent growth at moult was not analysed in the present study. Most of the lobsters were in inter-moult at the start of each trial and there were insufficient numbers of lobsters in other moult classes to do meaningful analyses. However, from the few pre-moult lobsters that were included at the start of the summer trials, there was some evidence to suggest that the growth at moult in these lobsters (which moulted early on in the trials) was greater than that of lobsters that were in inter-moult (and which moulted later on during the trials). This aspect of live-holding deserves further attention as the ability to select pre-moult lobsters for live-holding could result not only in greater growth but it would also reduce the amount of holding time required before weight gain was achieved. Selection of pre-moult lobsters could be based upon the external colour of the ventral surface of the tail (see Musgrove, 1998) or on blood colour (Musgrove, unpublished data).

The effect of lobster source (i.e. factors such as shallow versus deep, and also different regions within the fishery) on the survival and growth of lobsters during live-holding was not compared directly under the same conditions in the present study. Comparisons of separate trials in the present study did, however, suggest that speckled/white lobsters are comparable in survival and growth to red lobsters. This conclusion requires clarification. Growth at moult of lobsters sourced from different regions should also be evaluated.

Only one size range of lobster (\approx 450-650g) was used in the present study. Based upon field growth (see Prescott *et al.*, 1997), this size of lobster should have the maximum <u>percentage</u> moult increment (i.e. relative to its own body size) for lobsters that are larger than the legal minimum length. This was the rationale for using the smallest possible legal-sized lobsters in the present study. However, even though the percentage moult increment of larger lobsters may be smaller, the absolute weight gain of these larger lobsters may be greater than that of small lobsters. It would therefore be useful to directly compare the growth at moult of different sized lobsters. The ability for industry to live-hold a range of lobster sizes would allow greater flexibility when selecting lobsters and would therefore be advantageous.

The effect of sex on the growth and survival of lobsters was investigated in only one trial in the present study. Results from this trial showed that males had significantly greater growth at moult than females. Therefore male lobsters appear the best prospect for live holding. However, most of the females used in the trial were sexually mature and the growth of immature females should be further investigated.

Tail fan damage is presently the single greatest problem with long-term live-holding of adult *Jasus edwardsii*. In view of this, FRDC has funded a project on tail fan damage for 2000/2001. This new project will investigate the progression and potential causes of tail fan damage. Lorkin *et al.* (1999) discussed some potential causes of tail fan damage, but did not mention physical damage during capture and post-capture handling. It is possible that small breaks in the protective exoskeleton surface of the tail fan could occur when lobsters vigourously tail flip or when they are placed into bins and come into contact with the sharp carapace and tail spines of other lobsters. These small breaks in the exoskeleton may then lead to raggedness, blistering, and erosion, and they may also allow the entry of bacteria. This might explain why tail fan damage is

only seen occasionally in processor tanks where lobsters are held short-term but it was very common in holding trials during the present study in which lobsters were held long-term. It is possible that the initial damage (i.e. a break in the exoskeleton) has already occurred when lobsters arrive at the processors but because they are not held for extended periods in these facilities, the severe symptoms of raggedness, blistering, and erosion do not have time to manifest themselves. The influence of capture and post-capture handling on tail fan damage requires further investigation.

There are several areas of systems design and husbandry that could be modified and that might result in improved survival and growth at moult of live-held lobsters. The re-location of seabased cage systems to areas with cooler annual temperatures is one possible option that might reduce temperature-related stress and result in improved performance of lobsters (see Section 6.8.2). Even though survival rates were generally high in the present study, the provision of shelters might be useful in further reducing mortalities as shelters would offer protection from cannibalism for soft post-moult lobsters. A study on grow-out of juvenile *J. edwardsii* found that the majority of deaths occurred due to cannibalism after moulting and that the provision of shelters significantly increased survival rates (Thomas *et al.*, 1999). There were problems in the present study with the land-based raceway system and future research needs to investigate ways of preventing system failures and also in improving the design of land-based systems. A system with automatic feeders and which is self-cleaning needs to be developed. Finally there is also potential for improvement in pellet feeds and feeding regimes that result in maximum ingestion rates by lobsters. An FRDC project has been funded for 2000/2001 to address feeding issues in relation to live-held adult *J. edwardsii*.

The present study has provided biological and husbandry information on potential survival rates, growth at moult, and conditioning and feeding of live-held lobsters. Such information is useful for industry to make better informed decisions and it has demonstrated that long-term live-holding of adult *Jasus edwardsii* is technically feasible. However, an economic analysis incorporating this information with different holding scenarios is still required to assess the economic feasibility of long-term live-holding of adult *Jasus edwardsii*. Such an analysis would be the next logical step for commercialisation of the results from the present study.

7 Component II: Determination of Optimum Environmental Requirements for Juvenile Tropical Rock Lobster (*Panulirus ornatus*) Grow-out

7.1 Introduction

Development of production technology for the captive growout of tropical rock lobsters (*Panulirus ornatus*) will necessitate a sound understanding of the environmental and biological requirements of the species and identification of optimal conditions, which maximise growth and survival. To this end, this study examined the effects of temperature, salinity, and density on growth and survival.

Temperature and salinity are likely to be two of the more important environmental factors, and each was examined through separate experiments to determine optimal levels and tolerances. For salinity in particular, identification of a capacity to withstand less than fully marine salinities may provide a significant expansion of potential sites and system requirements for commercial cultivation.

Economics of production will necessitate that reasonably high densities be maintained in culture systems that do not compromise good growth and high survival. Given that culture of rock lobster will likely involve growth from post-larval stage to in excess of 1kg, density must be considered in the context of biomass and not just numerically. In this study a combination of density and biomass values were factored into the experimental design which were considered to represent a range from low to high for the type of culture system employed.

7.2 General Materials and Methods

These experiments were performed at the aquaculture facilities of the Northern Fisheries Centre, Cairns. Experimental lobsters were sourced from the wild either by hand collecting from wharf pylons in Trinity Inlet, Cairns, or from artificial shelters deployed at various sites along the coast in the vicinity of Cairns. All stocks were acclimated to captive conditions on flow through seawater for a minimum of 7 days prior to their allocation to specific experiments.

7.3 <u>Objective 2.1</u> Determine growth rate and survival in relation to temperature

7.3.1 Temperature Experiment

7.3.1.1 Materials and Methods

A 5 x 5 factorial design was applied to assess the effect of temperature on growth and survival of juvenile rock lobster. Five temperature treatments were applied: 19, 22, 25, 28 and 31°C, each to an individual recirculation system containing five replicate tanks.

Experimental tanks were rectangular (1000mm x 1000mm x 400mm depth) and constructed of 6mm glass. Water was maintained at a depth of 250mm, thereby providing a volume of 250l. Each recirculation system flowed by gravity to a sump where a filter mat provided first stage removal of solids, then to cartridge filter (Rainbow Lifeguard), and a fluidised bed filter (Aquasonic). Water from the filters was supplied to each tank from a continuous loop at a flow rate of 60l per minute per tank.

Water temperature was maintained by a combination of controlled air temperature and water heating. An air conditioning unit (Fujitsu) kept room air temperature at 18°C. The sump of each recirculation system was equipped with a 3kw titanium heater (Stokes), controlled by thermostat to achieve the treatment temperature.

Each tank was equipped with a shelter consisting of a 300mm x 300mm PVC sheet supported on 75mm legs. Each tank was insulated on each side, and top and bottom with 10mm styrofoam sheet. A thin layer of silicon sprinkled with coarse sand was applied to the floor of each tank to provide a roughened surface for grip.

Six lobsters of uniform size were randomly selected from the pool of available stock and allocated to each tank. Lobsters were acclimated for a minimum of 24 hours at each intermediate temperature prior to being stocked to the treatment temperature. Individual weight, carapace length and sex were recorded at stocking. Lobsters were tagged with a 5mm diameter disc of white water-proof paper, super-glued to the dorsal carapace between the orbital horns, to distinguish moults.

Each recirculation system was filled with filtered seawater, and operated at its treatment temperature for 14 days prior to the experiment starting. Periodic replacement of water was made as determined from water quality monitoring, and was consistently applied to all treatments.

Chopped prawns were provided once per day after 3pm at a rate of 5% of biomass per day, adjusted according to observation. Rate was consistent for all tanks, and uneaten food was removed each morning. Supplementary feeding of kuruma prawn pellets was made on weekends. Quantity was consistent for all tanks, and was recorded.

Exuviae were measured for carapace length and returned to their tank. Moulted lobsters were identified daily and re-tagged after their weight, carapace length and sex had been recorded.

The experiment was run for 120 days. Statistical analyses were performed using Microsoft Excel and Genstat 4.1. Analysis of variance was applied to the size data, although due consideration was given to the lack of independence of individual tanks within each temperature treatment. LSD was applied to compare treatment means.

Specific growth rate (SGR) was calculated as an index of daily growth for the period of the experiment.

 $SGR = (Ln Wt_f - Ln Wt_i)/t x 100$

where Ln Wt_f is the natural log of final weight, Ln Wt_i is the natural log of initial weight, t is the period of growth in days

7.3.1.2 Results

Water quality remained within acceptable limits for the duration of the experiment. Minimum, maximum and mean values for pH, salinity and temperature of each treatment system are presented in Table 62.

System	Treatment Temperature	рН		Salinity (ppt)			Temperature (°C)			
		min	max	mean	min	max	mean	min	max	mean
А	25	6.1	8.2	7.7	30.9	34.5	32.6	24.8	26.0	25.1
В	28	7.2	8.2	7.8	31.2	34.8	33.1	27.7	29.8	28.2
С	22	7.5	8.3	7.9	30.8	35.5	33.1	21.6	24.3	22.5
D	19	7.7	8.3	8.0	30.9	34.2	32.0	18.6	19.8	19.2
Е	31	7.0	8.2	7.7	30.8	34.9	33.2	30.1	32.2	30.8

Table 62. Summary statistics for water quality in each experimental system

A summary of experimental results is presented in Table 63. Survival data indicated temperature above 28°C led to increased mortality. Temperature had a significant effect (p < 0.01) on growth as measured by carapace length at 120 days (Table 63). Size for 19°C and 22°C treatments were each significantly less than at 25°C to 31°C.

Table 63. Summary statistics (\pm SE) for lobsters under controlled temperature conditions, grown for 120 days. Final carapace length (CL) values with different superscript letters are significantly different (p < 0.05).

Temperature	19°C	22°C	25°C	28°C	31°C
Mean initial CL (mm)	32.71±0.22	32.37±0.29	32.98±0.15	32.52±0.32	32.96±0.09
Mean initial weight (g)	40.58±0.97	38.37±1.50	41.05±0.69	39.33±1.18	41.95±0.63
Mean final CL (mm)	34.38±0.50 ^a	41.65±0.39 ^b	47.37±0.98 °	46.84±0.64 °	46.18±0.74 °
Mean final weight (g)	45.23±1.60	74.74±2.32	106.42±6.15	105.05 ± 5.63	98.67±3.54
Mean survival (%)	100.0	100.0	96.7	70.0	60.0

Size at harvest for each temperature treatment is illustrated in Figure 11. Size was greatest for the 25°C treatment, but a temperature range of 25° to 28°C would appear to be optimal. The growth of lobsters was uniform and linear within each treatment as shown in Figure 12. Each temperature generated appreciable growth, with the exception of 19°C for which almost no growth occurred.

Intermoult period and moult increment data for each treatment are presented in Figure 13. They clearly mirror the growth data, but provide a more discrete measure of optimal temperature in terms of minimum intermoult period and maximum increment.



Figure 11. Carapace length of lobsters after 120 days culture at five temperatures (°C)



Figure 12. Weight (g) of lobsters over 120 days of culture at five temperatures (°C)



Figure 13. Mean moult increment and intermoult period for lobsters grown at different temperatures

Food intake varied significantly (p < 0.01) between treatments over the experiment period (Figure 14) and was positively correlated to temperature. Examination of food intake for the first 60 days of the experiment relative to the second 60 days indicated food intake increased at all temperatures except 19°C. Food conversion ratios (Figure 15) showed that the most efficient use of food was achieved at 25°C. Although food consumption at 31°C was the highest (Figure 14), its conversion to growth was inefficient as suggested by Figure 15.



Figure 14. Average daily food intake (g) per tank for the first 60 days and last 60 days of lobsters grown at five temperatures



Figure 15. Food conversion ratio (FCR) of lobsters fed prawns and prawn pellets over 120 days, at five temperatures (°C)

7.4 <u>Objective 2.2</u> Determine growth rate and survival in relation to salinity

7.4.1 Salinity Experiment

7.4.1.1 Materials and Methods

A 4 x 4 factorial design was applied to assess the effect of salinity on growth and survival of juvenile rock lobster. Four salinity treatments were applied: 20, 25, 30, and 35 ppt, each to an individual recirculation system containing four replicate tanks.

Experimental tanks were rectangular (1000mm x 1000mm x 400mm depth) and constructed of 6mm glass. Water was maintained at a depth of 250mm, thereby providing a volume of 250l. Each recirculation system flowed by gravity to a sump where a filter mat provided first stage removal of solids, then to cartridge filter (Rainbow Lifeguard), and a fluidised bed filter (Aquasonic). Water from the filters was supplied to each tank from a continuous loop at a flow rate of 60l per minute per tank.

Water temperature was maintained at 26°C by a combination of controlled air temperature and water heating. An air conditioning unit (Fujitsu) kept room air temperature at 24°C. The sump of each recirculation system was equipped with a 3kw titanium heater (Stokes), controlled by thermostat.

Each tank was equipped with a shelter consisting of an opaque plastic sheet (300mm x 300mm), supported on 75mm legs. Each tank was insulated on each side, and top and bottom with 10mm styrofoam sheet. A thin layer of silicon sprinkled with coarse sand was applied to the floor of each tank to provide a roughened surface for grip.

Ten lobsters of mean weight 11g were allocated to each tank. Lobsters were acclimated for a minimum of 24 hours at each intermediate salinity prior to being stocked to the treatment salinity. Individual weight, carapace length and sex were recorded at stocking. Lobsters were tagged with a 5mm diameter disc of white water-proof paper, super-glued to the dorsal carapace between the orbital horns, to distinguish moults.

Each recirculation system was filled with filtered seawater, and then diluted at 5 ppt day⁻¹ with aged town water until the treatment salinity was achieved. Periodic replacement of water was made by mixing the treatment salinity in a reservoir tank and flushing the recirculation system with approximately 50% of its volume. This was done at fortnightly intervals for each treatment.

Prawn flesh was provided once per day after 3pm at a rate of 5% of biomass per day, adjusted according to observation. Rate was consistent for all tanks, and recorded daily.

Exuviae were measured for carapace length and returned to their tank. Moulted lobsters were identified daily and re-tagged after their weight, carapace length and sex had been recorded.

The experiment was run for 91 days. Statistical analyses were as declared for the temperature experiment (see Section 7.3.1.1).

7.4.1.2 Results

Water quality generally remained within acceptable limits for the duration of the trial, with the exception of ammonia levels for system B (35ppt salinity treatment) (Table 64). Figure 16 indicates that ammonia for this system was generally equivalent to that of the others except for 21/12/99 when a level of 1.39 mg/l was recorded. Temperature averaged 25.9 ± 0.03 °C for all systems for the duration of the experiment.

	Table 64.	Summary of	water quality	data for ea	ach system of	the lobster s	salinity experime
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System	Α	В	С	D
Mean salinity (ppt)(±SE)	30.2±0.08	34.7±0.27	20.2±0.03	25.0±0.06
Mean pH	8.1	8.1	8.2	8.1
Mean ammonia (mg/l)	0.07	0.15	0.02	0.05
Maximum ammonia (mg/l)	0.23	1.39	0.06	0.14
Mean nitrite (mg/l)	0.04	0.03	0.02	0.05
Maximum nitrite (mg/l)	0.11	0.06	0.04	0.09
Mean nitrate (mg/l)	41.2	47.1	29.1	46.4
Maximum nitrate (mg/l)	100.0	90.0	54.0	90.0
Mean Calcium carbonate (mg/l)	125	133	131	138



Figure 16. Ammonia levels (mg/l) for each of four recirculation systems operated at different salinities

Summary statistics for lobster size and survival at the beginning and end of the experiment are presented in Table 65. Survival was significantly less (p<0.05) for lobsters at the 35ppt salinity treatment than at the other three salinities. Fifty two percent of lobsters at 35ppt survived the 91
day experiment, while in excess of 78% survived at the 20, 25 and 30ppt salinity treatments (Figure 17).

Table 65. Summary statistics (\pm SE) for lobsters under controlled salinity conditions, grown for 91 days. Final carapace length (CL) and survival values with different superscript letters are significantly different (p < 0.05)

Salinity	20ppt	25ppt	30ppt	35ppt
Mean initial CL (mm)	19.45±0.33	20.77±0.59	20.06±0.50	20.77±0.62
Mean initial weight (g)	9.62±0.39	11.88±0.84	11.01±0.76	12.17±0.81
Mean final CL (mm)	23.49±031ª	32.26±1.01 b	33.57±0.82 ^b	37.30±1.73 °
Mean final weight (g)	14.99±0.71	37.04±2.66	42.93±2.18	57.56±7.20
Mean survival (%)	88 ^a	90 ^a	78 ^a	52 ^b

Growth was also significantly affected (p<0.01) by salinity. Means comparisons (Table 65) indicated that lobsters grew most at 35ppt, and that lobsters grown at 25 and 30ppt were significantly larger than those grown at 20ppt salinities. Figure 18 shows carapace length at the end of the experiment. On the basis of weight after 91 days, lobsters grown at 35ppt were 44% larger than those grown at 25 and 30ppt, and 284% larger than those grown at 20ppt (Figure 19).



Figure 17. Survival (±SE) of lobsters grown at 4 salinities over 91 days



Figure 18. Mean carapace length (mm)(±SE) of lobsters after 91 days culture at four salinities



Figure 19. Weight (g) of lobsters at 30 day intervals over 91 days, grown at four salinities

7.5 <u>Objective 2.3</u> Determine growth rate and survival in relation to density/biomass

7.5.1 Density / Biomass Experiment

7.5.1.1 Materials and Methods

A 3 x 4 randomised block design was applied to assess the effect of density on growth and survival of juvenile rock lobsters.

Three densities (14, 29, 43 /m²) were applied to separate compartments in each of four fibreglass tanks (4500mm x 1750mm x 400mm), each holding 3000l of water. Tanks were supplied with flow-through seawater, pumped directly from Trinity Inlet filtered through a sand filter and supplied to each tank at 50l per minute. Each tank was divided across its width into 3 compartments 2500, 1200 and 800mm in length and 4.38, 2.10 and 1.40m² in area respectively. The barriers were made of 4mm polyethylene mesh mounted in an aluminium frame. Each compartment was equipped with a shelter consisting of rectangular polyethylene platform (600mm x 600mm) elevated above the tank floor on 4 x 100mm legs. A plastic tray of coral rubble was placed in each tank.

Each tank compartment was stocked with 60 lobsters of a mean size of $3.24g (\pm 0.21)$ randomly selected from the pool of available stock. Individual weight and carapace length were recorded at stocking.

Kuruma prawn pellets were provided from approximately 4pm until 4am by 12 hour belt feeders each day at an initial rate of 4% of biomass per day, adjusted according to observation. Feeding rate remained consistent for each density, and was recorded daily. Supplementary feeding of prawns 5 days per week was applied consistently across all compartments.

The experiment was run for 272 days (October 1999 to June 2000).

7.5.1.2 **Results**

A summary of yield statistics is presented in Table 66. After 9 months (272 days), neither survival nor growth were significantly different between densities. Mean survival at the three densities ranged from 58.3% to 44.6%, and was negatively correlated to density. Mortalities occurred consistently through the experimental period (Figure 20), although the reasons for them are likely to have changed as the lobsters grew. Growth was also uniform over the experimental period (Figure 21). Final weight averaged 225.3g across all densities, representing a specific growth rate of 1.56% per day. An examination of the size frequency of lobsters at each density (Figure 22) suggests that there were more larger individuals for the low density, and that a significant difference in mean weight may have developed if the experiment had continued for a longer period.

Table 66. Size, biomass and survival statistics for *P. ornatus* at stocking and harvest, cultured at three densities over 272 days

Density	Treatment	Low	Medium	High
Initial	Density (#/m ²)	14	29	43
	Mean weight (g)	3.25	3.32	3.15
	Mean carapace length (mm)	13.78	13.86	13.71
Final	Mean density (#/m ²)	7.95	15.60	19.11
	Mean weight (g)	235.1	225.9	214.7
	Mean carapace length (mm)	62.78	62.03	60.63
	Biomass (kg/m ²)	1.85	3.50	4.05
	Survival	58.3	54.6	44.6



Figure 20. Mean mortality of lobsters at three densities over 272 days



Figure 21. Mean weight (g) of lobsters at three densities cultured over 272 days

There was no significant difference in weight between male and female lobsters at harvest, although mean weight of males $(231.2 \pm 6.5g)$ was greater than that of females $(219.0 \pm 6.7g)$. Size frequency distribution of male and female lobsters at harvest is presented in Figure 23.



Figure 22. Size frequency (weight) of lobsters grown at three densities for 272 days



Figure 23. Size frequency of male and female lobsters after 272 days of growth

7.6 General Discussion

The three experiments completed provide important information on the optimal conditions necessary to grow tropical rock lobsters in tank systems, and confirm the excellent aquaculture potential for this species (Linton, 1998). Clear results in both the temperature and salinity experiments indicate that growth and survival of lobsters will be maximised if they are grown at 25 to 28°C temperature and full strength seawater (35ppt). Tropical rock lobsters are clearly tolerant of high density conditions, and grew well at all the densities applied (maximum 43/m²) and the biomasses generated (maximum 4.7kg/m²).

Notwithstanding the impact of occasional water quality deterioration, survival in the temperature and salinity experiments was good, even at the extremes of the treatment variables. This indicates the robustness of this species. Survival in the density trial was however less than had been anticipated, and was not strongly influenced by density. Although survival averaged 52% for the 272 days of the experiment, the absolute density of lobsters at the end was relatively high at between 8 and 19 lobsters per m². This is considerably higher than densities suggested as optimal for other Palinurids (Rahman and Srikrishnadhas, 1994; Booth and Kittaka, 1994). The mortality that occurred can in large part be attributed to two non-experimental factors in particular feeding strategy and shelter.

It was clear from general observation that *P. ornatus* displays a diel activity/ foraging pattern similar to most large benthic marine crustaceans, with dusk and dawn peaks. Automatic belt feeders were employed to extend the availability of food through the night, however, as the feeders had a maximum operation time of 12 hours, and were generally set prior to 5pm, feeding ceased before 5am when foraging would have been significant. This is likely to have contributed to greater cannibalism than might otherwise have occurred if food had been available between 5am and 8am (i.e. dawn).

Shelter was provided to each experimental unit in a manner to ensure it did not differentially influence the lobsters at different densities. At the outset, when experimental stock were small, the shelter appeared to be ample, however, once lobsters exceeded 100g mean weight, crowding was evident. Close proximity of individual lobsters may not be very significant for this species as it displays gregarious behaviour in nature. What is likely to be more significant is the unavailability of suitable refuge sites for moulting individuals. Pre-moult crustaceans often seek out sites distant from their con-specifics, to complete ecdysis. Once their shell is sufficiently hard to enable mobility, they move back to normal sites amongst other intermoult individuals. The absence of such refuges within the experiment may have exacerbated cannibalism.

Growth of lobsters in all experiments was good. The density experiment in particular provided an opportunity to gauge the commercial potential for growout as it encompassed growth from a very small size (3g) and covered a lengthy period of 272 days. The specific growth rate achieved for that experiment indicates that growth to 1kg may be achievable in less than 18 months. For a high value species such as *P. ornatus*, this is likely to be economically viable.

The good growth generated in the experiments was based on a mixed diet of natural feed (shrimp) and kuruma prawn pellets. The acceptance of the pellets and growth outcomes suggest that development of a complete formulated pellet diet for *P. ornatus* should not be problematic.

For the density experiment, in excess of 75% (on a dry weight basis) of the food provided was pellets, and this generated a specific growth rate of 1.56%.

Water quality and experimental design were both influential factors in the temperature and salinity experiments. The limitations of recirculation systems were evident for this type of experimentation where the treatment applied is integral to the water of the system. As each treatment was necessarily applied within a system, consisting of several tanks, replication was not truly independent. This weakness could be overcome with independent recirculation systems for each tank or with replication through time and reallocation of treatments to systems. Nevertheless, the results achieved are biologically valid and robust, and provide clear definition of optimal conditions for the culture of this species.

Water quality problems occurred on only a few occasions, however, because relatively small numbers of lobsters were used in these experiments, any mortalities resulted in a substantial impact on survival. The flow-on effect to density may have affected growth, independent to the treatment effect, and this has been carefully considered in interpretation of results. The harvest size of lobsters in the 35ppt treatment of the salinity trial would likely have been less if survival had been equivalent to the other treatments. Nevertheless, Figure 19 indicates that growth at 35ppt was greater to the other treatments prior to the impact of high ammonia and subsequent mortality.

These experiments also provided an opportunity of comparing the performance of *P. ornatus* in flow through and recirculation systems. Good growth and survival were achieved in both, and both may have application to commercial production systems. Difficulties experienced with water quality in the recirculation systems used in these experiments are attributable to design weaknesses that can be easily overcome with increased expenditure. The systems employed were necessarily primitive, and could be greatly enhanced with the addition of various off-the-shelf technologies.

8 Component III: Determination of Optimum Environmental Requirements for Juvenile Southern Rock Lobster (*Jasus edwardsii*) Grow-out

8.1 Introduction

The southern rock lobster, *Jasus edwardsii*, forms the basis of an important fishery in southern Australia and New Zealand. Research has shown the Australian and New Zealand stocks are the same (Booth *et al.*, 1990). *J. edwardsii* commands a high market price, identifying them as a potential aquaculture species. Typical of most spiny lobsters, they have a long and complex early life history. The planktonic phyllosoma stage is spent dispersed by oceanic currents for a period of 8-16 months before settling as puerulus in shallow coastal waters (Kennedy, 1990). Presently, it is not feasible to commercially produce puerulus in hatcheries; therefore, initial industry development is focussing on the collection and ongrowing of recently settled puerulus. Determining the optimum environmental requirements for the culture of *J. edwardsii* is an important focus during the early part of research into its aquaculture potential.

8.2 <u>Objective 3.1</u> Determine growth rate and survival in relation to temperature

8.2.1 The effect of elevated temperature on survival, growth, feeding and metabolic activity of the southern rock lobster, *Jasus edwardsii*.

8.2.1.1 Introduction

Temperature is one of the major environmental factors affecting the growth of crustaceans (Hartnoll, 1982). Growth has been shown to increase with increasing temperature to a maximum, before declining at the upper thermal limits (Chittleborough, 1975). Elevated temperature could potentially reduce the culture period, which would be important for the economic viability of J. edwardsii culture (Hooker et al., 1997). The optimum temperature range suggested for culture of J. edwardsii is between 18-20°C (Booth and Kittaka, 1994). However, considerable variation in results has been obtained at similar temperatures. It is unclear whether this variation is a result of different husbandry practices or from intraspecific variations (Sastry and Vargo, 1977). For example, Bunter and Westaway (1993) found reduced survival of juvenile lobsters at 18°C compared to lower ambient temperatures (10-16°C) in Tasmania. However, Manuel (1991) found 18°C did not adversely effect survival of the same species collected in southern New Zealand. Previous studies also indicated that growth rate was reduced at 22°C (Hollings, 1988; Manuel, 1991); while Hooker et al. (1997) reported increased mortalities associated with water temperatures of 23.3°C. Defining the upper thermal limits will be important for site selection and the most appropriate culture temperature. In addition, few data are available on the influence of temperature on feed consumption, food conversion ratio, oxygen consumption or ammonia excretion of juvenile J. edwardsii. This will have important implications for culture in regards to feeding costs and systems management.

The aim of this trial was to determine the effect of elevated temperature on survival, growth, feeding, oxygen consumption and ammonia excretion of post-puerulus *J. edwardsii*, and to identify the upper thermal limit and the most efficient temperature for culture.

8.2.1.2 Materials and Methods

Pueruli of *J. edwardsii* were collected from experimental collectors located at Bicheno on the east coast of Tasmania. They were maintained in a recirculating system at 18°C prior to the commencement of the trials.

Trials were conducted in a flow through system consisting of 4 x 280-l reservoir tanks and 12 x 33-l black fibreglass tanks (diameter 430mm, depth 300mm). Incoming water was filtered to 400 μ m prior to entering the reservoir tanks. The reservoir tanks were maintained at precise temperatures of 18, 20, 22 or 24°C using 1.2-2.4 kW titanium heaters. Vemco minilog data loggers (TR data loggers) monitored temperatures in the tanks at 10-min intervals. Temperatures were also manually checked each day. Each heated reservoir tank was connected to 3 replicate experimental tanks. The flow to each tank enabled a complete water exchange every 30-40 minutes. An 8-h light:16-h dark photoperiod was maintained with light intensity of 1 μ mol s⁻¹ m⁻² at the water surface.

Thirteen post-pueruli (initial mean weight \pm SE = 0.99 \pm 0.01 g) were stocked into each 33-l tank and were temperature-acclimatised from 18°C over 5 days prior to the first weight measurement. During the first week after the initial weight measurement, mortalities were replaced with temperature-acclimatised animals of similar weight. These mortalities were assumed to be due to stresses associated with handling and not included in the final mortality data.

Lobsters were fed once daily with either freshly opened cultured mussels (*Mytilus edulis planulatus*) or dry growout pellets for penaeids (*Penaeus japonicus*). Previous studies had indicated that fast growth and high survival could be achieved on a mixed diet of fresh mussels and dry pellet (Crear *et al.*, 1999). An estimation of daily food consumption was obtained via a visual estimate of food remaining in the tanks prior to the morning cleaning. Food was provided slightly in excess and was based on the previous days' consumption. Feed consumption was calculated on dry matter consumed per wet weight biomass and expressed as a percentage of wet lobster body weight per day. Samples of mussels and the dry diet were dried to give an estimate of moisture content. To take into account mortalities the number of lobster days of feeding was calculated (based on survivors at each weighing) and used to calculate daily weight gain and feed intake for each lobster. Food conversion ratios (FCR) were calculated as the estimated dry weight of feed consumed per day (g) per lobster wet weight increase per day (g).

Lobsters were weighed to the nearest 0.01 g at days 0, 29, 59 and 92. Animals were dried on absorbent paper for 30 s to remove excess water prior to weight measurement. Specific growth rate (SGR), percentage weight gain (%WG), moult increment and moult interval were used to evaluate growth. Moult interval and moult increment calculations were based on data collected between the second and third weight measurements as there was no mortalities during that period. These parameters were defined as: SGR = (ln Final weight -ln Initial weight) *100 / number of days; % WG = (final weight - initial weight) * 100 / initial weight; moult increment = mean % weight gain / mean moults per lobster; moult interval = number of days / moults per lobster.

At the completion of the growth trial, lobsters were starved for a period of 60 h prior to oxygen consumption trials at the experimental temperatures. The experimental system described above was supplied with 0.2 μ m filtered seawater. A total of 48 animals (12 per treatment) in the intermoult stage were selected and weighed and placed into brown glass BOD bottles (755 ml). Animal weights ranged between 2.4 and 7.8 g. Twelve respirometers (three per treatment) had no animals and served as controls to estimate the oxygen consumption of microorganisms and the oxygen electrode.

The respirometers were immersed in the experimental tanks, which acted as temperature baths. High oxygen saturation levels within the respirometers were maintained during the 16-h acclimatisation period by providing flow through filtered seawater. An initial oxygen concentration measurement was recorded before the respirometers were sealed. Oxygen concentration was recorded after 60 minutes. The respirometers were then provided with flow through water for 3 hours to re-oxygenate the water before the experimental procedure was repeated. During the measuring period the oxygen concentration did not fall below 4.0 mg/l. Standard oxygen consumption ($M_{O2} \ \mu g \ min^{-1}$) was determined by the equation:

$$M_{O_2} = \frac{(P_{O2}i - P_{O2f}) * V}{T}$$

where P_{02f} and P_{02i} are final and initial oxygen levels, respectively (mg/l), V is volume of water in respirometer (μ l) and T is elapsed time in minutes.

At the completion of the oxygen consumption trial 20ml water samples were taken from the respirometers and stored at -18°C until total ammonia nitrogen (TAN = $NH_3 + NH_4^+$) analyses were conducted. Water from the control respirometers was used to determine initial ammonia concentration. TAN was measured using the phenol-hypochlorite method using procedures outlined by Parsons *et al.* (1984). Ammonia excretion (TAN - mg TAN g⁻¹ h⁻¹) was determined from the following equation:

$$TAN = \frac{(TAN_{f} - TAN_{i}) * V}{W * T}$$

where TAN_f is the ammonia as nitrogen in the sample at the end of the measuring period in mg l⁻¹, TAN_i is the ammonia as nitrogen in the sample at the beginning of the sampling period in mg l⁻¹, V is the volume of water in the container in litres, W is the weight of the lobster in grams and T is the Time of the measuring period in minutes.

 Q_{10} values for oxygen consumption and ammonia excretion were determined using the following equation:

$$Q_{10} = \left(\frac{M_2}{M_1}\right)^{10/T_2 - T_1}$$

where M_1 and M_2 = oxygen consumption and TAN at temperatures T_1 and T_2 respectively.

All data were tested by ANOVA with comparisons of means following ANOVA using the Scheffe post-hoc test. Survival data were arcsine-transformed prior to analysis (Sokal and Rohlf, 1995). The growth and survival data were plotted and curvilinear regressions were fitted to obtain the temperature response curves. Least-squares regression was used to assess relationships between body weight and oxygen consumption or ammonia excretion. Regressions were tested for significance by analysis of variance. The optimal response to temperature was predicted as the upper or lower asymptote of the best-fitting quadratic response curve (Lellis and Russell, 1990).

8.2.1.3 Results

The final mean weight and SGR at 22°C were significantly higher (P<0.05) than at 24°C (Table 67). No significant differences in growth were determined between 18, 20 or 22°C. The quadratic regression of temperature on SGR (SGR = $-0.031T^2 + 1.261T - 10.884$, r² = 0.77) (Figure 24A) predicted the optimum temperature for growth to be 21°C.

The intermoult period decreased as temperature increased, with lobsters at 22°C having a significantly lower (P<0.05) intermoult period to those at 18°C (Table 67). At 24°C, the intermoult period increased again and was significantly higher (P<0.05) than at 22°C. The relationship between temperature and intermoult period was described by a quadratic regression (Intermoult period = $1.134T^2 - 48.056T + 532.99$, $r^2 = 0.61$) (Figure 24B). No significant difference was detected in percentage moult increment at the four temperatures (Table 67). The relationship between percentage moult increment and temperature was described by a quadratic regression (Moult increment = $0.625T^2 - 30.55T + 433.3$, $r^2 = 0.99$) showing that as temperature increased there was a reduction in the moult increment (Figure 24C).

Table	67.	Effect	of temp	perature	on	survival,	growth,	feed	consumption	ı, food	conversion	ratio
(FCR)	and	moult in	ncremei	nt of post	t-pue	erulus roc	k lobster	s Jast	us edwardsii.	Values	expressed as	mean
(±SE).	Valu	es in the	e same ro	ow with s	ame	superscrip	ot are not	signif	icantly differe	ent (P>0).05)	

	Temperature			
	18°C	20°C	22°C	24°C
Survival, %	90 <u>+</u> 7 ^a	69 <u>+</u> 8 ^{ab}	82 <u>+</u> 3 ^{ab}	51 <u>+</u> 7 ^b
Initial mean wt (g)	1.02 ± 0.02	0.99 <u>+</u> 0.01	0.96 <u>+</u> 0.04	0.99 <u>+</u> 0.03
Final mean wt (g)	5.97 <u>+</u> 0.23 ^{ab}	6.09 ± 0.38 ab	6.77 <u>+</u> 0.12 ^a	4.79 <u>+</u> 0.43 ^b
Weight gain (%)	486 <u>+</u> 14 ^{ab}	518 <u>+</u> 41 ^{ab}	610 <u>+</u> 19 ^a	382 <u>+</u> 35 ^b
SGR (% BW/d)	1.92 <u>+</u> 0.03 ^{ab}	1.98 <u>+</u> 0.07 ^{ab}	2.13 <u>+</u> 0.03 ^a	1.70 <u>+</u> 0.78 ^b
Total moults	30.33 <u>+</u> 1.86 ^a	29.67 <u>+</u> 1.45 ^a	46.33 <u>+</u> 4.10 ^b	23.67 <u>+</u> 2.03 ^a
Moult increment (%)	86.50 <u>+</u> 8.50	72.00 <u>+</u> 1.00	63.67 <u>+</u> 3.68	60.00 <u>+</u> 3.00
Intermoult period (days)	33.85 ± 4.85^{a}	30.60 <u>+</u> 1.60 ^{ab}	19.80 <u>+</u> 0.76 ^b	34.69 <u>+</u> 1.55 ^a
Feed Consumption (% BW/d)	2.08 ± 0.07	2.10 ± 0.08	2.41 <u>+</u> 0.11	2.21 <u>+</u> 0.05
FCR	1.07 <u>+</u> 0.04	1.07 <u>+</u> 0.03	1.13 <u>+</u> 0.05	1.31 <u>+</u> 0.08

The effects of temperature on the FCR and feed consumption are shown in Table 67 and Figure 25. No significant differences (P>0.05) in FCR or feed consumption were detected. The response of FCR to temperature was described by a quadratic regression (FCR = $0.011T^2 - 0.434T + 5.231$, $r^2 = 0.995$) which predicted that the optimum temperature for feed conversion was 19.3°C (Figure 25A). The response of feed consumption to temperature was described by a quadratic regression (Feed consumption = $-0.0137T^2 + 0.6125T - 4.53$, $r^2 = 0.53$) (Figure 25B).

Temperature affected survival of lobsters, with survival at 24°C being significantly lower (P<0.05) than at 18°C (Table 67). There were no significant differences (P>0.05) in survival at 18, 20 or 22°C. Most mortalities at 24°C occurred as lobsters approached the moult, whilst most mortalities at the other temperatures occurred due to cannibalism immediately after the moult.



Figure 24. Growth response of *J. edwardsii* subjected to elevated temperatures (mean \pm SE). A. Specific growth rate (%BW/day) B. Intermoult period (days) C. Moult increment (%)

As expected, body weight significantly influenced (P<0.01) oxygen consumption rate (Mo₂) of lobsters at all temperatures (Table 68). The exponent *b* value of the Log₁₀-transformed linear regressions relating oxygen consumption (μ g min⁻¹) to body weight (g) varied with the culture temperature. At 18 and 24°C, *b* was greater than 1, meaning the weight-specific Mo₂ increased with increasing body weight. However, at 20 and 22°C, *b* was less than 1 and weight-specific rate decreased with increasing body weight. To take into account the oxygen consumption variation due to body weight, the oxygen consumption data were weight standardised at each temperature (Table 69). The standardised oxygen consumption data were then plotted against temperature (Figure 25C). There was a significant affect of temperature on oxygen consumption (F=5727, P=0.009), which was described by a quadratic regression (Mo₂ = -0.044T² + 1.91T - 18.553, r² = 1.0). Oxygen consumption increased rapidly with temperature up to 22°C, with a decline at 24°C. The Q₁₀ values for oxygen consumption decreased with increasing temperatures (Table 69), ranging from 3.7 (Q₁₀₍₁₈₋₂₀₎) to 0.6 (Q₁₀₍₂₂₋₂₄)).

Table 68. Linear regressions describing the relationship between total oxygen consumption $(M_{02}:\mu g \text{ min}^{-1})$, total ammonia excretion (TAN: $\mu g \text{ min}^{-1}$) and body weight (W:g) of *Jasus edwardsii* at four temperatures

Temperature (°C)	n	Regression model	r ²	F	Р
18	19	$Log M_{O2} = 1.515 \ Log_{10} \ W - 3.138$	0.74	48.7	< 0.0001
20	18	$Log \ M_{O2} = 0.860 \ Log_{10} \ W - 2.597$	0.80	63.9	< 0.001
22	16	$Log M_{O2} = 0.692 Log_{10} W - 2.431$	0.50	12.1	< 0.004
24	23	$Log \ M_{O2} = 1.091 \ Log_{10} \ W - 2.741$	0.72	54.8	< 0.001
18	10	$Log TAN = 0.794 \ Log_{10} \ W + 0.229$	0.75	18.3	< 0.005
20	11	$Log \ TAN = 0.865 \ Log_{10} \ W + 0.185$	0.74	19.9	< 0.003
22	10	$Log TAN = 0.187 \ log_{10} \ W + 0.772$	0.28	3.07	0.118
24	10	$Log TAN = 0.196 Log_{10} W + 0.850$	0.36	2.29	0.205

The Log₁₀-transformed linear regressions relating TAN excretion (μ g min⁻¹) to body weight (g) at each temperature are shown in Table 68. The ammonia excretion rate was influenced by body weight at 18 and 20°C (P<0.01), but not at 22 or 24°C. The ammonia excretion data was standardised as for the oxygen consumption data (Table 69) and then plotted against temperature (Figure 25D). Ammonia excretion increased significantly (F=19.14, P<0.05) with temperature and was described by a linear equation (TAN = 0.127T - 1.174, r² = 0.91). The Q₁₀ value for ammonia excretion was low (1.1) as the temperature increased from 18 to 20°C, but was higher at the upper temperature ranges (Table 69).



Figure 25. The feeding response, oxygen consumption rate and ammonia excretion rate of *J. edwardsii* subjected to elevated temperatures (mean \pm SE). A. Feed conversion ratio B. Feed consumption (%BW/day) C. Oxygen consumption rates (µg/g/min) D. Total ammonia nitrogen (TAN) excretion rates (µg/g/min)

Table 69. The standardised weight-specific oxygen consumption and ammonia excretion rates of *J. edwardsii* at each temperature. The rate data were standardised using the regression equations describing the relationship between body weight and oxygen consumption/ammonia excretion (Table 68). The Q_{10} values for each temperature range are also shown

Temperature (°C)	M _{O2} (µg/g/min)	Q ₁₀ (M _{O2})	TAN (μg/g/min)	Q ₁₀ (TAN)
18	1.66		1.216	
20	2.16	3.7	1.232	1.1
22	2.3	1.4	1.598	3.7
24	2.1	0.6	1.942	2.7

8.2.1.4 Discussion

Previous studies have suggested the optimum temperature range for the culture of *J. edwardsii* is between 18 and 20°C (Hollings, 1988; Manuel, 1991; Booth and Kittaka, 1994). Overall, the calculated optimum temperature for growth (20.6°C) and feed efficiency (19.3°C) from this study support this range. The results of this study also indicate that lobsters collected from the east coast of Tasmania can be cultured at elevated temperatures up to at least 22°C (after a period of acclimation) without affecting growth or survival. The reduced growth, survival and declining oxygen consumption at 24°C are indications that *J. edwardsii* is approaching its upper thermal limits.

Low growth and survival had previously been reported at 22°C for lobsters collected from New Zealand (Hollings, 1988; Manuel, 1991). It has been shown that crustaceans of the same species but collected from geographically different locations exhibit differences in response to the same environmental conditions (Sastry and Vargo, 1977). It is possible that animals collected from the east coast of Tasmania respond differently to elevated temperatures than animals collected in the south of New Zealand. However, the poor performance at 22°C recorded by Hollings (1988) may also be related to the reduced water quality and temperature fluctuations noted in the trial. Lellis and Russell (1990) suggested that the thermal preferences for growth of *Panulirus argus* may differ with development stage. The effects of temperature on *J. edwardsii* at other development stages also need to be examined.

Growth rate is a function of moulting frequency and the weight increment with each moult. Typically, there is a reduction in the intermoult period and the moult increment as temperature increases (Hartnoll, 1982). Both those components of growth were strongly influenced by temperature in this trial and were reflected in the growth rates observed at 18 and 22°C. Although the intermoult period was reduced at 22°C the growth rate did not increase because of the decreased moult increment. A reduced intermoult period and moult increment was also observed in *Panulirus longipes* as the temperature increased from 20 to 26°C (Chittleborough, 1975), although the reduction in moult increment was not great enough to prevent increased growth at 26°C. Temperature did not affect the moult increments of *P. interruptus* (22 and 28°C) or *P. argus* (27 and 30°C) and the observed accelerated growth at the higher temperatures was driven by reduced intermoult periods (Serfling and Ford, 1975; Lellis and Russell, 1990).

As the temperature approached that the upper thermal limit of *J. edwardsii*, the effect of temperature on the components of growth varied. Growth was reduced at 24°C because the intermoult period increased, while the moult increment stayed the same as at 22°C. A similar result was observed with *P. longipes* as the temperature increased from 26 to 29°C (Chittleborough, 1975). Conversely, Manuel (1991) found that the reduced growth of juvenile *J. edwardsii* at higher temperatures was due to a smaller moult increment, with the intermoult period not being affected. Lellis and Russell (1990) found that moult increment decreased and intermoult period increased at higher temperatures. The affect of temperature on the components of growth appear to vary with lobster species, as noted for many crustaceans (Hartnoll, 1982).

Food consumption generally increases with temperature in response to increased metabolic activity (Wyban *et al.*, 1995), although it was not apparent in this study. The growth rates

achieved in this study exceed those reported in other studies of *J. edwardsii* (Hollings, 1988; Rayns, 1991; Bunter and Westaway, 1993; Hooker *et al.*, 1997). Specific growth rates of around 2% BW day⁻¹ and FCRs between 1.1 and 1.3:1 indicates the nutritional suitability of a mixed diet containing fresh mussels and prawn dry growout pellets for post-puerulus *J. edwardsii* (Crear *et al.*, 1999).

The oxygen consumption rate of *J. edwardsii* is similar to that for other crustaceans of comparable size (Weins and Armitage, 1961; McLeese, 1964; Chen and Kou, 1996). Oxygen consumption rates increased with increasing temperature with a decrease occurring at 24°C. Similar respiratory responses for crustaceans at elevated temperatures have been reported (Rutledge and Pritchard, 1981; Zoutendyk, 1989; Varo *et al.*, 1991). Vernberg (1983) described this decline in respiration at elevated temperatures as a strategy for metabolic regulation in energy conservation.

The positive correlation between increasing respiration rate and weight in this study has been well documented for other crustaceans (Wolvekamp and Waterman, 1960; Cockcroft and Wooldridge, 1985). Weymouth *et al.* (1944) gave the average value of *b* for 54 crustaceans species of 0.85. Bridges and Brand (1980) reviewed *b* for crustaceans and found they typically ranged between 0.286-0.877 at temperatures 8.5-17.8°C. The *b* values from this study 0.86 (20°C) and 0.69 (22°C) are within the generally accepted range. However, *b* values at 18°C and 24°C are high and are probably a reflection of the limited weight range in the study, cf. Rao (1957) who found *b* of 1.05 for *Metapenaeus monoceros*.

 Q_{10} typically vary between 2 and 3 near optimal temperatures (Wolvekamp and Waterman, 1960) and the Q_{10} recorded for this study for 18 to 22°C fits within this range. A similar Q_{10} of 2.3 was recorded for larger (186-2180 g) *J. edwardsii* between 17 and 21°C (Crear, 1998). The increase in metabolic rate could not be sustained at 24°C, resulting in a Q_{10} value of less than 1 for the 22-24°C temperature range. Q_{10} generally decreases as temperature moves towards the thermal maximum (Varo *et al.*, 1991). Zainal *et al.* (1992) also reported Q_{10} values less than 1 for squat lobsters, *Munida sarsi* and *Munida rugosa*, at the upper temperature maximum.

Mortalities at 24°C appeared to be stress-related, mostly occurring during the pre-moult period. Penkoff and Thurberg (1982) have shown that the American lobster, *Homarus americanus*, has an increased oxygen requirement during the pre-moult period. Additionally, the availability of oxygen decreases as water temperature increases. This suggests that the mortalities at 24°C in this study may have been due to respiratory failure caused by the inability of lobsters to meet the increased oxygen requirements of moulting. Respiratory stress leading to mortality at the moult has been reported for the western rock lobster, *P. cygnus*, and the South African rock lobster, *J. lalandii*, when lobsters were cultured at low oxygen levels (Chittleborough, 1975; Beyers *et al.*, 1994).

Under intensive culture conditions, stocking levels will be governed by the system's capacity to provide oxygen for metabolic activity. The implications for systems design are that animals maintained at 22°C will consume approximately 40% more oxygen than those at 18°C. However, water holds 6.5% less oxygen at 22°C (35 ppt and 100% saturation). The equations relating temperature, body weight and oxygen consumption can assist in determining the appropriate

stocking levels and flow rates at a particular temperature based on the oxygen requirements The effects of activity and feeding on oxygen consumption will require further investigation.

Ammonia excretion rates increased in response to increasing temperature, a response that has been documented for crustaceans (Regnault, 1987; Villarreal and Rivera, 1993; Chen and Kou, 1996; Crear, 1998). Ammonia excretion was influenced by body weight particularly at the lower temperatures. The exponent *b* at 18°C (0.794) and 20°C (0.865) were consistent with *b* values of 0.88 for *Xiphopenaeus kroyeri* (Carvalho and Phan, 1997) and 0.75 for *Penaeus japonicus* (Marangos *et al.*, 1990). However, *b* values were low at 22°C and 24°C, probably due to the limited size range of animals. Ammonia excretion accounts for approximately 80% of the nitrogenous excretion in *J. edwardsii* (Crear, 1998). Ammonia has been shown to decrease growth and cause mortality in aquatic organisms, and recommended levels of total ammonia for aquatic organisms is generally below 0.5 mg l⁻¹ (Forteath, 1990). Therefore, the rate of ammonia production in intensive recirculating systems is of primary concern for lobster culturists. These preliminary results will assist in calculating the ammonia excretion rates of small *J. edwardsii* and the biofiltration requirements for ammonia removal. Feeding has been shown to increase TAN excretion rates for large *J. edwardsii* (Crear, 1998). The effect of feeding on ammonia excretion levels for small juveniles will require further investigation.

In conclusion, this study shows that a temperature range of between 19.3 and 21°C is optimal for post-puerulus *Jasus edwardsii* in terms of survival, growth and FCR. At higher temperatures, the measured performance criteria were reduced and the upper thermal limits appeared to be 24°C. Culturists would need to consider the economic advantages of lobsters reaching market size in the shortest possible time against the increased costs associated with heating water. In addition, lobsters at higher temperatures have greater respiratory requirements and excrete greater amounts of ammonia. If lobsters are to be cultured at elevated temperatures in intensive recirculating aquaculture systems, then there will be even greater reliance on water treatment, to ensure water quality is not limiting growth. Finally, if lobsters were to be grown in flow-through systems, or in cages in the sea (Hollings, 1988) these results indicate that it will be necessary to select sites where summer water temperatures do not rise above 22°C.

8.3 <u>Objective 3.2</u> Conduct a literature review of southern rock lobster growout

8.3.1 Ongrowing of the spiny lobster, *Jasus edwardsii*: a review

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8.3.2 Abstract

There is worldwide interest in the development of aquaculture for spiny lobsters (family Palinuridae). However, there is relatively little published information about the requirements for the aquaculture of these species. The aim of this article was to review the available research about ongrowing of the southern rock lobster, Jasus edwardsii, which is probably the best known of all the spiny lobsters in terms of aquaculture. More than 50 relevant research articles have been reviewed and a synthesis of their findings presented. The results indicate that while a great deal is known about basic husbandry and containment of this species, their nutritional and environmental requirements are poorly defined and require further research for aquaculture With the advance of intensive aquaculture an increasing number of health development. problems have been encountered. These will also require further identification, with corresponding development of methods of prevention and treatment. Overall, the performance of this species in ongrowing experiments indicates good prospects for further commercial aquaculture development. Given the similarities in the biology of spiny lobsters it is likely that these good prospects will also apply to the development of aquaculture for other spiny lobster species, particularly species from tropical waters that generally have faster growth.

8.3.3 Introduction

World production of spiny lobsters from fisheries remains static at around 75,000t, while there is increasing demand for this valuable seafood (Lipcius and Eggleston, 2000). Consequently, there is strong international interest in the development of aquaculture techniques for spiny lobsters. The species for which aquaculture techniques are most advanced is the southern rock lobster,

Jasus edwardsii, which is found in southern Australia and New Zealand. This species has been the subject of extensive aquaculture research for more than 20 years and commercial farming commenced recently in New Zealand and Australia. Much of this research has been communicated only in local publications, unpublished reports, or bound in student research theses. The purpose of this article was to draw together the information contained in this extensive "grey" literature and provide a synthesis of the current state of knowledge for the aquaculture of this species. It is intended that this synthesis will provide direction for future research into areas where there is a current lack of knowledge for the development of efficient ongrowing of this lobster. It is also anticipated that the findings of this review will provide direction to the development of techniques for the aquaculture of other species of spiny lobsters around the world.

8.3.4 Seed lobsters for aquaculture

There are two potential sources of seed for J. edwardsii aquaculture, larval culture and collection of early settled juveniles from the wild. The larval culture of early juvenile lobsters is not commercially feasible due to the extended larval period (up to 24 months) and a lack of knowledge about rearing techniques (Kittaka, 2000). Large collections of early settled lobsters for commercial ongrowing have taken place in New Zealand for around five years, and are about to commence in Tasmania. The catching of these early stages largely relies on setting collectors in shallow waters and which imitate the natural crevices into which the swimming puerulus stage prefer to settle. Some development work has been undertaken on collector design in both Tasmania and New Zealand to improve catching performance. Commercial ongrowing operations in New Zealand have experienced enormous inter-annual variations in catches of early settled lobsters which have made it difficult to obtain sufficient seed in years of poor settlement. Relatively little is known about the settling behaviour of the puerulus of this, or any other species of spiny lobster, making it difficult to optimise collection techniques (Lipcius and Eggleston, 2000). This is a priority area for further research for wild collection of seed lobsters for aquaculture.

8.3.5 Biology of Jasus edwardsii

8.3.5.1 Behaviour

One of the primary advantages of culturing spiny lobsters, such as, *J. edwardsii* compared to Homarid lobsters is that they are generally gregarious and can be successfully reared in communal tanks. However, we need to understand the social interactions that occur within a group of lobsters in order to maximize growth and survival of cultured individuals. Death of low-ranking individuals can occur as a result of feeding dominance (Thomas, unpub. data).

The different ecological and habitat requirements of different life stages of an animal can lead to ontogentic behavioural changes in a species. Studies have shown that post-settlement *J. edwardsii* are typically solitary in nature but juvenile lobsters (>30mm carapace length - CL) are highly gregarious (MacDiarmid, 1994; Edmunds, 1995; Butler *et al.*, 1999), and in the wild they have been found in aggregations of up to105 individuals (MacDiarmid, 1994). Cohabitation is greatest in lobsters between 30-90mm CL, with larger lobsters becoming more solitary, especially mature males (>140mm) during the mating season (MacDiarmid, 1994). Laboratory experiments on *J. edwardsii* show that in 83% of the trials late juvenile lobsters (40-80mm CL)

are attracted to a water-borne chemical produced by conspecifics, but early juveniles (<40mm CL) were not (MacDiarmid, 1994). The authors suggest that aggregation of late juveniles decreases the risk of predation due to communal defence whereas the aggregation of defenceless early juveniles would only increase the detection risk. This hypothesis is supported by results from a field study where early and late juveniles were tethered to their shelters, either alone or in a group of three. The authors found that there was no difference in the predation rate between the early juveniles, on their own or in groups, however, groups of late juveniles survived three times longer than solitary late juveniles (MacDiarmid, 1994; MacDiarmid *et al.*, 1998).

Several authors have recorded the occurrence of growth depensation, mortality and cannibalism in communal cultures of *J. edwardsii*, especially when food is limited and densities are high (Manuel, 1991; Rayns, 1991; James and Tong, 1997; Westbury, 1999; Crear *et al.*, 2000). Larger lobsters are more aggressive than smaller individuals and tend to dominate the feeding territories, although no strict dominance hierarchy exists (Manuel, 1991). Growth depensation can be reduced if lobsters are fed at multiple sites as this allows smaller lobsters to feed simultaneously with the dominant individuals (Westbury, 1999). Cannibalism in *J. edwardsii* primarily occurs in the post-moult period when lobsters are soft and vulnerable (Crear *et al.*, 2000), although it is not a problem if lobsters have sufficient food and adequate shelters are provided (Crear *et al.*, 2000). Adult lobsters often show synchronised moulting, which further decreases the incidence of cannibalism (Rayns, 1991).

8.3.5.2 Growth rates

Tagging studies on wild J. edwardsii have shown that wild lobsters from central North Island, New Zealand, populations can reach marketable size (75mm CL) 3 years after settlement (McKoy and Esterman, 1981; Booth and Kittaka, 2000), while Stewart Island lobsters grow more slowly to reach a carapace length of 62-73mm after 3 years (Annala and Bycroft, 1985). Growth rates of cultured lobsters have exceeded predicted wild growth rates¹ when conditions are near optimal (Booth 1988; Booth and Kittaka 1994), however, growth rates of cultured lobsters vary greatly. Hooker et al. (1997) found that in the first year after settlement the growth of cultivated lobsters² matched growth estimates from wild populations of lobsters from Gisborne (McKoy and Esterman, 1981), but subsequently grew slower and were estimated to reach approximately 150g after 3 years while the wild lobsters reached 215g after 3 years. Estimates by Hollings (1988) of 2-3 years to culture a lobster from settlement to marketable size may be over optimistic (Hooker et al., 1997; Tong and James, 1997). Hooker et al. (1997) estimated that it would take 3.3 years for cultured lobsters to reach 180g. However, lobsters captured as puerulus and held at 18°C, reached 50 g after 1 year, 190 g after 2 years and an average of 337g after 3 years in captivity (Crear, unpub. data). Growth of larger juveniles, which have been held since puerulus is greater than that previously reported for wild caught larger juveniles (Hooker et al., 1997), suggesting there is no cumulative negative effects of captivity.

¹ McKoy and Esterman, (1981) estimated growth rates of wild *J. edwardsii* by fitting mark and recapture data and to the Von Bertalanffy model. Annala and Bycroft (1985) used cohort analysis of size-frequency data.

² Lobsters were grown in tanks at ambient temperature (13.4-23.3°C) at a density of 100 indivuals m⁻². They were fed mussels (*Perna canaliculus*) at least 3 times per week.

The growth of captive *J. edwardsii* is influenced primarily by temperature (Hollings, 1988; Manuel, 1991; McClary, 1991; Rayns, 1991; Thomas *et al.*, 2000), photoperiod (Brett, 1989; Berry, 1997), stocking density (Rayns, 1991; Tong, 1994) and diet (Kington, 1999, James and Tong, 1997). Most studies have show that *J. edwardsii* shows optimal growth when cultured at 18-20°C with a L:D regime of 18:6 and fed mussels (*Perna canaliculus* and *Mytilus galloprovincialis* in New Zealand and *M. edulis planulatus* in Australia) (see relevant sections for more detail).

Female *J. edwardsii* grow slower than males once they reach sexual maturity (72-121mm CL, Annala *et al.*, 1980), both in culture and in wild populations (McKoy and Esterman, 1981; Annala and Bycroft, 1985; Hooker *et al.*, 1997; McGarvey *et al.*, 1999), with females having lower moult frequencies and smaller growth increments than males (McKoy and Esterman, 1981). For smaller animals, there was minimal difference in growth of males and females up to around 100 g (58mm CL) in weight, but after that, growth of males was approximately 13% greater than females (Crear, unpub. data). The faster growth of male *J. edwardsii* may mean that it is more economic to culture male lobsters, however the difference in growth rates between the two sexes is small, and there is very high natural variability in growth rates between individuals (McKoy and Esterman 1981; Kington, 1999; McGarvey *et al.*, 1999).

Rayns (1991) showed that small lobsters held downstream of larger and similar sized individuals showed reduced growth due to smaller moult increments and moult inhibition. He suggests that results could be evidence of a type of 'long-range hormonal control'. These results may have significance to the design of culture systems, however, the difference in growth between upstream and downstream animals was very small (2.2mm CL.yr⁻¹). The effect did not increase when the number of upstream animals was doubled, indicating that any inhibition of growth is likely to be limited.

Further long-term studies need to be undertaken to monitor the cumulative effects of captivity on growth rates from settlement to market size. Most studies have extrapolated early juvenile growth rates to estimate later stage growth rates and time to reach market size, which may be inaccurate. For example, Manuel (1991) found that lobsters reared at 18°C had a specific growth rate (SGR) of 1.03% body weight (bw) day⁻¹ after the first 168 days of culture but after a further 168 days the SGR had dropped to 0.67% bw.day⁻¹. Where late juvenile growth has been examined it has generally been carried out on wild caught individuals, rather than lobsters cultured from settlement (Hollings, 1988; Rayns, 1991; Hooker *et al.*, 1997), therefore excluding the cumulative effects of captivity.

8.3.5.3 Endocrinology (eyestalk abalation)

There has been a lot of interest in the possibility of manipulating hormonal levels to improve growth in spiny lobsters. Moulting in crustaceans is regulated by the interaction of two hormones, the steroid moulting hormone 20-hydroxyecdysone (20-HE) and the moult inhibiting hormone (MIH) (Chang *et al.*, 1993) which is produced in the eyestalk (Khoo, 1996). Studies have tested the effects of ablating the eyestalks of lobster to accelerate growth by eliminating the site where the MIH is produced. In juvenile *J. edwardsii* both bilateral and unilateral (except Bunter and Westaway, 1993) eyestalk ablation have been found to accelerate growth (Rayns, 1991; Berry, 1997). These two ablation treatments worked differently to increase growth;

unilateral ablation increased moult frequency, while bilaterally ablated animals had larger moult increments (Rayns, 1991). Highest growth rates have been attained in bilaterally ablated lobster, but these animals also had high (70%) mortality after 500 days (Rayns, 1991). Berry (1997) also found bilaterally ablated lobsters grew faster than unilaterally or intact lobsters³, but results were less robust due to limited replication and the relatively short duration of the experiment. In contrast to Rayns' (1991) results he found very low mortality rates could be achieved after ablation over a 30 day period (Berry, 1997), although ablated lobsters often became lethargic and moribund, and were very prone to lose limbs. It is possible that post-ablation mortality may be more related to the higher moult rate than the actual ablation procedure, and thus, increased mortality effects may only be apparent over a longer period of time. Due to the unsuitability of ablated lobster for the live lobster market (ablation decreases the value of lobsters) further research into this issue may be of a lower priority.

8.3.6 Nutrition

8.3.6.1 Digestive system

The digestive system of spiny lobsters appears morphologically and functionally similar to that described for other decapod crustaceans (Mikami and Takashima, 1994). Nishida *et al.* (1990) studied and described the morphology of the mouthparts and foregut of first-moult juvenile *J. edwardsii.* They concluded that the mandible with its calcified molar and incisor is functionally specialised for grinding large, calcified material, whereas the gastric mill with its well developed gastric teeth and setae is specialised for further masticating these and softer food materials into smaller pieces and mixing them with digestive fluids. Based on these observations and the analyses of gut contents by Edmunds (1995) it would appear that juvenile *J. edwardsii* is well equipped to ingest and mechanically process a broad range of food items.

Little is known of the digestive capability or the types and concentrations of digestive enzymes produced by *J. edwardsii*. Juvenile *J. edwardsii* appear to have similar capability to other crustaceans to digest protein and energy (80% digestibility of crude proteins, 78% digestibility of energy - Ward, 1999) suggesting that a wide range of enzymes (e.g. proteases and lipases) is produced. Ontogenetic shifts in diet (Edmunds, 1995) are likely to be accompanied by changes in the enzyme complement. Characterisation of the digestive capabilities of *J. edwardsii*, through an assessment of the digestive enzymes produced in pueruli, juveniles and adults is currently the focus of a study by Johnston (Australian Research Council grant, 2000). Although much can be presumed about the digestive system of *J. edwardsii* based on studies of other decapod crustaceans, the development of species-specific diets and feeding practices will require further studies.

8.3.6.2 Natural diet of wild lobsters

The natural diet of *J. edwardsii* comprises a diverse spectrum of benthic prey, which is dominated by molluscs, echinoderms and crustaceans (Fielder, 1965; Edmunds, 1995). *J. edwardsii* appears to be a discriminate feeder (Edmunds, 1995) rather than a random scavenger,

³ Ablation produced a non-significant increase in growth rate for 2 out of 3 trials due to very high variance in growth in ablated lobsters (Berry, 1997).

as proposed by Fielder (1965). Lobsters prefer food of marine origin compared to terrestrial, and prefer the feed to be fresh rather than decomposing (Fielder, 1965). An ontogenetic shift in diet occurs at about 30mm in carapace length, which could be a function of differences in feeding behaviour and abilities, foraging habitats or because of differences in the calorific and nutritive values of the various prey types (Edmunds, 1995).

8.3.6.3 Feeding in culture

An economic analysis of *J. edwardsii* aquaculture found that when mussels are used as the feed they comprise one of the largest cost components of producing a marketable lobster (Jeffs and Hooker, 2000). The authors suggested that the development of a low cost formulated diet has the potential to greatly increase growth rates and reduce labour input for farming spiny lobsters. At present there is no specifically formulated feed for *J. edwardsii* and diets for culture have mainly consisted of natural seafood (Jeffs and Hooker, 2000). Mussels (*Perna canaliculus* and *Mytilus galloprovincialis* in New Zealand, and *M. edulis* in Australia) have been shown to be excellent feeds, supporting good growth rates and high survival (Hooker *et al.*, 1997; James, 1998; Kington, 1999; Crear *et al.*, 2000).

8.3.6.3.1 Natural foods

Mussels can constitute a reasonably large component of the diet of wild *J. edwardsii* (McKoy and Wilson, 1980), and may be used as a viable food supply for farmed rock lobsters (Tong and James, 1997). As a result of the large mussel industry, commercial lobster farmers in New Zealand have access to large quantities of mussels. Two types of mussel product are available; farmed mussels (usually *P. canaliculus*) and waste mussels (usually *M. galloprovincialis*). Both species have proven to be excellent food for *J. edwardsii* (James and Tong, 1997). It is estimated that the farming and processing of mussels in New Zealand produces at least 500 t/yr of waste mussel meat (Jeffs and Hooker, 2000). However, problems with collection, seasonal variation in quality, storage and handling (Rayns, 1991; Booth and Kittaka, 1994) may make the use of mussels unfeasible and/or uneconomical. Due to a restricted access to mussels in Australia it is thought that the availability of a suitable formulated diet is critical to successful commercial production (Crear *et al.*, 2000).

There are two options for the feeding of mussels to lobsters: mussels can either be fed live or opened. The method used may have important implications for the economic viability of lobster culture. Given that the growth of lobsters fed live mussels is the same as those fed opened mussels (James, 1998) there are significant advantages to be obtained through feeding live mussels. James and Tong (1998*a*) showed that lobsters can open suitably sized mussels readily, and once opened all of the flesh is consumed. Thus, the use of live unopened mussels minimises fouling of the water and would eliminate the need to provide fresh food each day (James and Tong, 1998*a*). There is also the possibility that a live mussel feeding regime would decrease labour costs, as there would be a decreased requirement for feeding and tank cleaning (James, 1998). The maximum or critical size of mussel that *J. edwardsii* can open is strongly correlated with carapace length (James and Tong, 1998*a*), although very small juveniles require mussels to be opened (Tong and James, 1997). The optimum (or preferred) mussel size was approximately half the critical size, which means for a lobster of 60-70 mm CL (100-120 g) the preferred size of cultured mussel is about 30-35 mm shell length (James and Tong, 1998*a*). However, it should be noted that

Jeffs and James (2001) found that *J. edwardsii* (40-65mm CL) held in sea cages were unable to fed on live unopened mussels due to the mussels binding tightly together with byssal threads. The use of live mussels to feed lobsters shows promise, however its application to commercial scale culture needs to be investigated.

Feeding opened mussels presents problems in that if they are not eaten immediately, they may disintegrate, reducing water quality and increasing the need to clean the holding tanks (James and Tong, 1998*a*). When lobsters have been fed opened mussels, feeding every third day as compared to daily has resulted in decreased growth (James and Tong, 1997). Although the difference in growth was not large the authors suggested that over a growout period the time taken by lobsters fed mussels every three days to reach marketable size, would be much longer than that taken by those fed daily. However, the differences in growth may not justify the extra labour costs of daily feeding (Jeffs and Hooker, 2000). Feeding strategy is likely to be controlled by environmental variables. For example, Hooker *et al.* (1997) found that the lowest survival and growth of lobsters coincided with peak water temperatures during summer. Decomposing food may have been an indirect cause of mortality and slower growth, rather than the higher water temperatures. Therefore, daily feeding and cleaning may be necessary during periods of high water temperature. During periods of low water temperature, where the rate of decomposition of uneaten food is low, feeding every second or third day may be feasible.

To overcome some of the problems of the seasonality of supply of mussels, lobsters could be fed frozen mussels. Although growth of lobsters fed frozen mussels, compared to fresh mussels, is reduced (James and Tong, 1997), the authors suggested that the short-term use of frozen mussels when supplies of fresh mussels are scarce should not greatly disadvantage farmers.

J. edwardsii appears to utilise mussel flesh well, with most studies achieving feed conversion ratios (FCR) of around 2:1 (Table 1). James and Tong (1998*a*) found that conversion rates appeared to be strongly influenced by lobster size, with larger lobsters having poorer conversion rates. However, the conversion rates in James and Tong's (1998*a*) study were high, and subsequent studies indicate that there may be little difference in the ability of lobsters of different sizes to utilise food (Kington, 1999; Crear, unpub. data). Other factors, such as temperature, may influence the FCR, however, Thomas *et al.* (2000) found that FCR was not affected over the temperature range of 18-22°C. The methodology used to determine consumption and conversion rates has varied significantly and if meaningful comparisons between studies are to be made then methods need to be standardised.

In captivity, *J. edwardsii* will feed on a wide range of natural food items not normally available to them in the wild (Fielder, 1965; Rayns, 1991). Thus, there is the possibility to reduce the cost of production through the use of less expensive natural feed sources. Kington (1999) found that the waste products from fish processing plants (finfish, abalone, paua, kina) could constitute 50% of a diet without affecting growth or survival. However, such products may suffer from many of the same problems (outlined above) that are likely to limit the suitability of mussels as a feed for the commercial ongrowing of lobsters. From a practical perspective, similar growth can be obtained from a mixed diet, consisting primarily of a relatively inexpensive formulated shrimp feed supplemented with mussels once per week. Such a scenario offers real possibilities for significantly reducing the cost of production (Crear, unpub. data)

Lobster size (g)	FCR ¹	Mussel species	Reference
12-40	4.2:1	P. canaliculus	James and Tong, $1998a^2$
58-105	6.6:1		
1-31	1.5:1	P. canaliculus	Hooker (unpub. data) in Jeffs
60-120	2.1:1		and Hooker, 2000 ²
120-180	3.0:1		
1-15	1.4:1	P. canaliculus	Kington, 1999 ²
20-40	1.3:1		-
2-15	2.5:1	M. edulis	Ward, 1999
5-30	1.6:1	M. edulis	Crear et al., 2000; Williams,
50-150	1.7:1		2001

Table 1. The feed conversion ratios (FCR) of Jasus edwardsii fed mussels.

¹ FCR (Feed conversion ratio) = dry weight feed fed:wet weight lobster gain

² Mussel flesh assumed to be 70% moisture

8.3.6.3.2 Formulated diets

Little research has been devoted to the development of formulated diets for spiny lobsters, even though their availability is seen as an important component for the development of spiny lobster culture (Booth and Kittaka, 1994). *J. edwardsii* is attracted to formulated diets, and consumes them readily, however to date growth and survival of lobsters fed mussels have been better than those fed formulated feeds (Kington, 1999; Crear *et al.*, 2000).

8.3.6.3.3 Form of diet

Based on the gut contents of lobsters in the wild (Edmunds, 1995), even small J. edwardsii appears physically capable of handling and ingesting a range of food sources. Nishida et al. (1990) examined the gross morphology of the mouthparts and foregut of post-puerulus J. edwardsii and suggested that it was capable of masticating large, hard or soft, materials. Both moist and dry diets have been employed in dietary studies and lobsters readily accept and consume both (Crear et al., 2000). Like many crustaceans, detection and location of food by J. edwardsii appears to be mainly via chemical cues (Fielder, 1965); therefore, they may be slow to locate and consume food. Thus, characteristics of diets developed for prawns, such as high attractiveness, palatability and water stability, should suit the feeding behaviour of J. edwardsii. Commercial prawn grow-out diets have proven to be highly attractive to J. edwardsii (Crear et al., 2000; Tolomei, 2000). To successfully develop formulated diets for J. edwardsii there is a need to understand its feeding and ingestive mechanisms as these will help determine the optimal form (shape, size and texture) of diets suitable for particular developmental stages. Fielder (1965) concluded that the mechanism of feeding of J. edwardsii is not efficient and much potential food is lost due to the external mastication of food items. Sheppard (2001) found that pellet size was a significant factor in reducing food losses through disintegration of moist pellets during lobster feeding. In addition he found that there were marked changes in the abilities of different sized juvenile lobsters to handle different sized pellets and that this in turn affected food consumption and wastage. Rapid ingestion of long cylindrical pellets by a sucking action where no wastage occurs has been observed and may also have some potential for J. edwardsii, but is yet to be tested (David Smith, personal communication). Such diet formats may result in a significant reduction in the loss of nutrients through disintegration. From a practical perspective a dry diet, in the form of a pellet, would be easiest to store and handle for commercial scale lobster ongrowing.

8.3.6.3.4 Feed intake

Feed intake of spiny lobsters is controlled by a number of factors, with water temperature, lobster size and the moult cycle probably having the greatest influences (Zoutendyk, 1987; Booth and Kittaka, 1994). Feed intake increases with temperature, for example feed intake was found to be 8-17 % higher at 18°C than at 16°C (Crear *et al.*, 2000). The higher feed intake can lead to faster growth, as feed efficiency is not affected at the higher temperature (Crear *et al.*, 2000). As temperature exceeds the optimum (>22°C) feed intake declines (Thomas *et al.*, 2000). Absolute feed intake increases with lobster weight (James and Tong, 1998*a*), although on a weight specific basis feed intake decreases with weight (Figure 1).

The effect of moult cycle on feed intake of *J. edwardsii* needs to be clearly understood. Cyclical patterns of feeding activity that appear to be related to moult cycles have been observed in communally held lobsters (Crear, pers. ob). Lowest feeding rates usually occurred just prior to some of the lobsters within a tank moulting, with feeding rates increasing after they had moulted. The feed intake of communally held lobsters varied markedly on a daily basis (James and Tong, 1998*b*; Sheppard, 2001). Moulting of lobsters in communally held tanks tend not to be synchronised, especially where lobsters are held in indoor recirculating systems. The large variations in feed intake (both daily and over time) observed in tanks where all other factors appear to be constant need to be accounted for when feeding. The amount of feed to be fed on a daily basis needs to be adjusted based on observations of leftover feed.



Figure 1. The dry matter food consumption (% lobster weight/day) of various weight *Jasus edwardsii* fed mussels (*Mytilus edulis*) at 18°C (from Ward, 1999 and Crear, unpub. data)

For commercial farmers, it is vital that information such as recommended feeding rates is available, as it will enable optimisation of feed management practices and minimisation of feed wastage. More data on feed intake are required across the full spectrum of temperature and weight ranges.

8.3.6.3.5 Feeding method

In captivity, it is recognised that feeding time should coincide with the active feeding period for a particular species (Cuzon *et al.*, 1982) so that the food may be quickly ingested, thus decreasing problems associated with the leaching of nutrients, especially from formulated diets. Also, it commonly coincides with peaks in digestive enzyme activity (Cuzon *et al.*, 1982). During normal daily cycles activity of *J. edwardsii* takes place throughout the night (Williams and Dean, 1989; Berry, 1997; Westbury, 1999). Fielder (1965) and Berry (1997) found that the feeding activity of *J. edwardsii* also takes place throughout the night with the majority occurring at or just after dusk, although captive juvenile lobsters tend to lose their diurnal patterns of activity in culture systems (Kington, 1999, James *et al.*, 2001). There is a need to more clearly quantify feeding patterns throughout the night so that an optimum-feeding regime can be determined. The optimum feeding frequency may change with size. Smaller juveniles feeding at higher weight specific rates may require more frequent daily feeding. Feeding trials which examine the effect of feeding frequency, coupled with information on gastric evacuation rate, time for the return of appetite and enzyme excretion patterns are required.

8.3.6.4 Nutritional requirements

Information on the nutritional requirements of *J. edwardsii* is critical to the development of effective formulated diets for its' culture (Williams and Ritar, 1997; Ward, 1999; Crear *et al.*, 2000). Smith (1998) developed a "best guess" set of nutrient specifications for formulated diets for lobsters drawn from published information about the nutritional requirements of other decapod crustaceans. The formulation of experimental diets for *J. edwardsii* nutritional research has largely been based on those specifications (Ward, 1999; Crear *et al.*, 2000). However, nutritional research with *J. edwardsii* is at the point where diets designed specifically for their needs can begin to be formulated.

There are likely to be significant differences in the nutritional requirements of *J. edwardsii* as it grows from puerulus to market size. For example, the requirements for post-puerulus juveniles are likely to be specific considering the puerulus uses large quantities of stored energy reserves (lipid) during its shoreward migration (Jeffs *et al.*, 1999) and those lipid reserves are further decreased during the moult to the post-puerulus stage (Pearce, 1997). The ontogenetic shift in diet observed in the wild (Edmunds, 1995), may indicate a phase where changes in nutritional requirements occur.

The aim of this section is not to discuss the need to include specific nutrients in formulated diets, but to concentrate on outlining the nutritional information known for *J. edwardsii*. Several nutritional reviews are available and provide excellent background reading. Kanazawa (1994) and Smith (1998) reviewed the nutrition of spiny lobsters. On a broader scale, Fox *et al.* (1994)

and D'Abramo *et al.* (1997) reviewed the nutrition of crustaceans. This review will also outline nutritional research priorities for *J. edwardsii*.

8.3.6.4.1 Digestibility

To be able to make meaningful comparisons of ingredients, and between studies, the biological availability of feed ingredients needs to be known. Limited data on the digestibility of feed ingredients is known for small juvenile *J. edwardsii*. Ward (1999) determined the crude protein, energy and dry matter digestibility of fishmeal-based diets using the chromic oxide method. The apparent digestibility coefficients were 80%, 78% and 59%, respectively, which is similar to other crustaceans fed fishmeal-based diets (Brunson *et al.*, 1997). Research needs to focus on providing digestibility data for selected feed ingredients in order to ensure that diets are formulated on a digestible nutrient basis. Data for larger juveniles are very important at this stage of the development of formulated feeds.

8.3.6.4.2 Protein and amino acids

The overall optimum protein requirements of small (3-10g) juvenile *J. edwardsii* have been determined, with the optimal level being dependent on lipid content of the diet (Ward, 1999). Growth was optimal at 29% digestible crude protein (DCP) for diets containing 5% lipid, and at 31% DCP with diets containing 9% lipid. Large (60-110 g) juvenile *J. edwardsii* appear to require a minimum dietary level of 45-50 % crude protein (Williams, 2001). Assuming a similar protein digestibility to small juvenile *J. edwardsii* (\approx 80%) (Ward, 1999) then the minimum digestible crude protein requirements would be 36-40%. Although protein requirements tend to decrease with age and size (Guillaume, 1997), the higher level measured for larger *J. edwardsii* may be a reflection of the ontogenetic shift in diet observed in the wild (Edmunds, 1995). Determination of the optimal dietary levels of other ingredients (e.g. lipid, carbohydrate, and energy) may lead to a re-evaluation of the optimal dietary protein level.

The quality of a protein as a feed source is determined by the closeness of its essential amino acid profile to that of the animals' whole body protein. It is assumed that *J. edwardsii* would require the same 10 essential amino acids as determined for other crustaceans (Guillaume, 1997). The amino acid profile of *J. edwardsii* needs to be determined as a first step in establishing the quality of protein sources for inclusion in diets for *J. edwardsii*. Although fishmeal is likely to be the protein source during the developmental phase of diets for this species, there needs to be some investigation of the use of alternative protein sources (e.g. plant-based material).

8.3.6.4.3 Protein:energy ratio

The correct dietary protein:energy balance is essential for efficient feed formulation (Fox *et al.*, 1994). The optimal protein:energy ratio determined for small (3-10 g) juvenile *J. edwardsii* for maximum weight gain ranges between 27.3 -31.7 g DCP.MJ DE⁻¹, with maximum growth occurring at 29 g DCP.MJ DE⁻¹ (Ward, 1999). There is a need to determine the optimal ratio for larger lobsters.

8.3.6.4.4 Non-protein energy

Juvenile *J. edwardsii* are capable of utilising both lipid and carbohydrate as an energy substrate (Calvert, 2000) although they appear to use lipid preferentially to carbohydrate (starch) for metabolic energy. A non-protein energy ratio of 2:1 carbohydrate:lipid (27% of dry matter:13.5% dry matter) resulted in the best growth of juveniles.

8.3.6.4.5 Lipids - fatty acids, phospholipids, cholesterol

Calvert (2000) found that juvenile *J. edwardsii* were capable of utilising relatively high lipid levels, with a level of 13.5% (range studied 3-19%) lipid producing best growth. However, best growth in Tasmania has been best achieved when mussels (*M. edulis*), which contain total lipid levels of between 5 and 10%, have been used as the feed (Ward, 1999; Crear *et al.*, 2000). There was no effect on growth of *J. edwardsii* when fed diets containing either 5 or 9% lipid (Ward, 1999). Good growth has been achieved with a *Penaeus japonicus* diet that contains almost 13% lipid (Crear, unpub. data). Diets containing lipid levels of 5, 10 and 18% did not cause significant differences in growth (Gerring, 1992), although the overall poor growth and survival in that study made it hard to draw conclusions about lipid inclusion levels. The inclusion of lipids in the diets is based upon the need to satisfy the requirements for specific nutrients such as fatty acids, sterols, phospholipids and energy. The above broad range of results with diets containing different dietary lipid levels probably indicates that little is known of the requirements for those specific nutrients. Smith (1998) recommended that the total lipid content for a rock lobster diet can be safely set between 6 and 10% of the dry matter, although there would appear to be some scope for including higher levels in diets for *J. edwardsii*.

Gerring (1992) found the growth rates of lobsters fed diets containing cod liver oil appeared to be better then those fed diets containing coconut oil or corn oil, most probably due to the better fatty acid profile of cod liver oil (high levels of highly unsaturated fatty acids). Cod liver oil has generally been used in experimental diets for *J. edwardsii* research (Ward, 1999; Crear *et al.*, 2000; Calvert, 2000). The fatty acid profile of juveniles (Pearce, 1997) should form the basis for determining the optimum type of lipid to include in diets.

Phospholipids are normally included at low levels in crustacean diets although the need to include specific sources of phospholipids may depend on inclusion of other dietary ingredients (Kean *et al.*, 1985). The survival of *J. edwardsii* fed casein-based diets was low, with most deaths occurring at the moult (Gerring, 1992). The symptoms of the deaths were consistent with moult death syndrome (MDS) and phospholipid deficiency (Bowser and Rosemark, 1981). Commercial soy lecithin containing about 40% phosphatidylcholine led to a marked reduction in the occurrence of moult death when added to the diets at 10% of the dry weight (Gerring, 1992). There was no effect when it was added at 5% dry weight. There has been no observance of MDS in other studies with formulated diets where lecithin has been included at 1.2% (Ward, 1999; Williams, 2001). An optimum level of phospholipid can lead to increased growth and survival (Piedad-Pascual, 1986) thus there is a need to determine the level and composition required by *J. edwardsii*.

Crustaceans are unable to synthesise sterols *de novo*, and require dietary sources of sterols for growth, development and/or survival. No research in this area has been undertaken with *J. edwardsii*. Smith (1998) suggests that cholesterol be included in spiny lobster diets at 0.2%. As there

are likely to be species-specific requirements, the optimum levels for J. edwardsii need to be determined.

No lipid levels can be recommended for a species without considering the total energy content of the diet, the lipid source, essential fatty acid composition, as well as the protein level of the diet. Further research aimed at optimising the levels and types of lipid in formulated diets is essential.

8.3.6.4.6 Minerals and vitamins

No research on the mineral or vitamin requirements of *J. edwardsii* could be found in the literature. It is likely that the requirements would not differ significantly from other marine crustaceans, which in general have not been clearly defined (Conklin, 1997; Davis and Lawrence, 1997). Inclusion of minerals and vitamins at the levels recommended by Smith (1998) would appear to be a safe option at this stage and further research in this area is not a priority given the lack of knowledge about more significant components of formulated feeds.

8.3.6.4.7 Feed attractants and stimulants

Crustaceans have a well developed chemosensory ability that plays an important role in the detection of food and its subsequent ingestion. The effective use of chemical stimuli in diets can reduce the time a feed is subjected to leaching and increase the amount of feed ingested by the animal (Fox et al., 1994). Fielder (1965) described the response of J. edwardsii to a chemical stimulant; the antennules appear to play a large part in the detection of and orientation to a food source. To aid in determining the response of J. edwardsii to chemical stimuli a behavioural model, as outlined by Lee and Meyers (1997) needs to be established. J. edwardsii shows a behavioural response (antennule wipe) to glycine, taurine and betaine Tolomei, 2000), compounds that have proven to be strong stimulants to other crustaceans (Zimmer-Faust, 1991). Fishmeal-based formulated diets have shown a good ability to attract and encourage J. edwardsii to feed on them. For example, a formulated prawn diet induced a strong feeding response in juvenile J. edwardsii (Tolomei, 2000): and the attractiveness and palatability of a formulated prawn diet remained high, even after it had been soaked for 8 hours (Tolomei, 2000). Similar levels of feed intake by J. edwardsii have been measured with formulated diets and mussels (Ward, 1999; Crear, unpub. data; Crear et al., 2000). In the future, there is likely to be a need to replace fishmeal with terrestrial protein sources that may not contain the same chemo-attractive properties. Gerring (1992) found that casein based diets were not attractive to J. edwardsii. Hence, the inclusion of feed attractants and stimulants into diets may become necessary, and further research should be conducted on identifying possible compounds. Sheppard (2001) tested the efficacy of 13 potential chemical attractants in increasing consumption of a bland moist pelleted diet. He found that mussel (P. canaliculus) liquor induced the greatest consumption of feed along with L-lysine, glycine and DL-methionine. These recent results would suggest that chemical attractants could be used to greatly increase formulated diet consumption. However, it is important that growth trials are conducted to validate the behavioural assays undertaken in laboratory systems (Tolomei, 2000).

8.3.6.4.8 Carotenoids

The production of cultured *J. edwardsii* that have red exoskeletons may have economic advantages, as there is a consumer preference for red lobsters in some countries. Although other

factors, such as background colour, have been found to affect the colouration of cultured *J.* edwardsii (Manuel, 1991), diet has been found to be the major modifier (Rayns, 1991; James and Tong, 1997; Crear, unpub. data). Dietary carotenoid is responsible for inducing colouration (Meyers and Latscha, 1997) and levels of around 115 mg/kg are required to achieve a colour equivalent to that of wild caught juveniles (Crear, unpub. data). Astaxanthin has been identified as the major colour pigment in the carapace of *J. edwardsii* (Liyanage, 1999). As an expensive ingredient it would be useful to establish if carotenoid can be included in diets at low levels during most of the grow-out period and then included at higher levels to obtain a "market colour" lobster just prior to harvest. Results from Sheppard (2001) indicate that this is certainly possible within the moult in early juveniles, but Rayns (1991) concluded that the colour could be changed over three moult cycles in larger juveniles. Carotenoids have other biological functions besides colouration (Meyers and Latscha, 1997) and dietary levels that meet those requirements need to be determined. For example, higher levels of dietary carotenoids led to higher survival in early juvenile lobsters (Sheppard, 2001).

8.3.6.4.9 Feed conversion / nutrient retention

Feed conversion ratios (FCR) with formulated diets have varied between 1.3 and 6, although values of between 1.3 and 2.5 have generally been achieved with commercial prawn diets (Ward, 1999; Crear, unpub. data; Tolomei, 2000). These compare favourably to other crustaceans (range of FCRs 1-7 - Capuzzo, 1983) and to mussel-fed *J. edwardsii* (Table 1). FCR is affected by the protein and protein:energy levels, increasing as the levels move away from the optimum (Ward, 1999). However, FCR does not appear to be altered by size, with 7-20 g lobsters having similar ratios to 60-110 g lobsters fed the same diets (Crear, unpub. data; Williams, 2001). The comparatively high FCRs in the study of Ward (1999) may have been partially driven by the relatively low water stability of the diets. Research into further methods of minimising the FCR, such as through better diet manufacture or through better feeding regimes should be undertaken.

The protein efficiency ratios (PER) and the energy efficiency ratios (EER) of juvenile *J. edwardsii* range from 50 to 152 % and 170 to 292 %, respectively (Ward, 1999; Williams, 2001). These values were similar to those found in other crustacean studies (Baillet *et al.*, 1997) although they are at the low end of the scale.

The protein productivity value (PPV) ranged between 7.4 and 14.6 % and was affected by the protein level in the diet (Ward, 1999). There was a noticeable decrease in PPV at high dietary protein levels (high protein:energy ratio). The PPV is higher at the optimal protein:energy levels, suggesting that protein is being efficiently used for growth when energy is not limiting. The energy productivity value (EPV) was related to the protein level in the diet, indicating inefficient use of dietary energy outside the optimal protein range (Ward, 1999).

8.3.6.4.10 Experimental methods

In the development of formulated diets, it is important that results from different studies and institutions are comparable (D'Abramo and Castell, 1997). To achieve this with *J. edwardsii* methodologies need to be standardised wherever possible. For example, mussels have traditionally been used as the reference treatment for nutrition experiments, however, variability in the nutritional condition of mussels, both spatially and temporally, makes comparisons

between studies difficult. In studies undertaken at the Tasmanian Aquaculture and Fisheries Institute, a commercial *P. japonicus* growout diet has also been used as a control. The results indicate that it may be an excellent reference diet for use in nutritional studies of *J. edwardsii*. Ideally, a formulated diet which supports good growth needs to be developed as a reference diet for *J. edwardsii* nutritional studies.

Nutritional information on larger animals is very important, as they ingest a larger part of the total feed required for production (Guillaume, 1997). However, one of the major problems with undertaking nutritional studies with large, relatively slow growing animals, such as *J. edwardsii*, is the extended time-period required to achieve a result. Thus, there is a need to develop methods, other than weight gain and survival, to aid in the determination of optimal requirements. Methods such as physiological responses (oxygen consumption and ammonia excretion), nucleic acid analyses and histopathological responses may prove useful. Calvert (2000) found that histological examination of the digestive gland supported the results of a trial, which examined the effect of dietary lipid and carbohydrate on growth and condition of juvenile *J. edwardsii*.

Although there have been relatively few studies on the nutritional requirements of *J. edwardsii* a significant amount of information has emerged. As yet no formulated diet has provided growth equivalent to that of mussels, thus decreasing the strength of the results of studies which have aimed to define nutritional requirements. Even so, there is a reasonable understanding of the nutritional requirements of *J. edwardsii* and this can be used to optimise the formulation of future experimental diets. It is suggested that future research should focus on several aspects to optimise the development of formulated diets for the southern rock lobster. Of key importance are data on:-

- the availability and digestibility of key nutrients
- the requirements for different types of lipids and proteins
- the optimal protein:energy requirements
- changes in nutritional needs with size

8.3.7 Aquaculture systems

8.3.7.1 Systems (tanks, cages)

There has been virtually no research on the optimal tank or cage design for culturing *J. edwardsii.* Most studies to date have reared lobsters in land-based tanks with either a recirculating or flow-through water system (e.g. Manuel, 1991; Rayns, 1991; Hooker *et al.*, 1997; Crear *et al.*, 2000). Lobsters held in cascade tanks show differential growth, with lobsters in the first or top tank growing faster than lobsters held in subsequent tanks (Hollings, 1988; Rayns, 1991). Rayns (1991) suggests that *J. edwardsii* produce a water-borne chemical that inhibits the growth of small conspecifics.

Tanks used for land-based aquaculture of spiny lobsters have usually consisted of readily available plastic tanks, but painted wood, fibreglass, glass and stone tanks have all been used to ongrow lobsters. There is no evidence that any particular type of tank has advantages over and above other designs. Dark plastic tanks with lids have been used in a number of laboratory studies on the basis that providing constant low light may encourage continual feeding and thereby improve growth. However, there is little evidence in support of this contention (Brett, 1989; Manuel, 1991; Berry, 1997; Hooker et al., 1997). It is unclear if background colour influences the intensity of the colour of J. edwardsii in culture as it commonly does in other crustaceans. Stuart et al. (1996) found that tank colouration did not affect lobster carapace colouration, however, Manuel (1991) found lobsters grown on a black background had a darker red coloration than those raised on a white background. Rectangular tanks have frequently been used as they a readily available and can generally be conveniently positioned within buildings, including through stacking to make use of available space (Jeffs and Hooker 2000). Water levels are often kept to a minimum, just sufficient to cover the body of the animal and not necessarily the antennae. Self draining tanks are a feature of a number of commercial lobster ongrowing operations as the juvenile lobsters survive better out of water in the event of an interruption to water flow. Water flow rates for juvenile lobster culture have been reported at 0.5 to 1 l/min/kg of biomass, but the basis for this information is unclear (Jeffs and Hooker, 2000) and requires further investigation.

There have been two studies on the possibility of rearing *J. edwardsii* in sea cages. Jeffs and James (2001) reared post-settlement lobsters in cages⁴ in three locations; the Hauraki Gulf and Waitemata and Wellington harbours. Growth in the first year (mean weight \leq 36.9g) was as good as, if not better than land-based cultures (31g, Hooker *et al*, 1997), and lobsters were raised at much higher densities (425 m⁻² cf. 100 m⁻²). However, mortality in the sea cages (78% in Hauraki and 51% in Waitemata) was greater than for land-based cultures at similar temperatures (25%), although mortality was only 14% in Wellington.

In southern Australia, some fishers are holding commercially fished *J. edwardsii* in sea cages to maximise on the market price fluctuations. A sixteen week pilot study was conducted to investigate the effects of holding fed and unfed lobsters (≥ 102 mm CL) in sea cages⁵ (Lorkin *et al.*, 1999). The results showed that while survival was high (77% unfed, 96% fed), both fed and unfed lobsters lost weight, and 93% suffered unsightly tail damage. The ambient water temperature during the study (21-24°C) was higher than recommended for *J. edwardsii* (Hooker *et al.*, 1997; Thomas *et al.*, 2000) and this may have contributed to the negative growth and tail damage. However, despite the small decrease in weight, survival was high and if lobsters are stock-piled for a short time when market prices are low, a small decrease in weight and quality would be more than compensated for by higher market prices (Lorkin *et al.*, 1999).

⁴ Post-settlement lobsters were reared at a density of 425 ind.m⁻² in 20*l* polyethylene cylindrical pails (30cm diameter, 40cm high) with 6mm holes drilled throughout the pails to assist water flow. Lobsters were fed opened mussels twice weekly. After 9 months, lobsters were transferred to 380mm x 570mm x 310mm polyethylene ventilated crates at a density of 55 ind.m⁻².

⁵ Lobsters were held in a 4-tiered cage with each compartment being 1m wide, 1.7m long and 0.3m high and covered with 12mm x 12mm polyethylene oyster mesh. Densities were less than 10 m⁻².

Further research is needed on optimal tank or cage design and the relative costs of land and seabased cultures. The results from Jeffs and James (2001) show that equivalent growth rates can be achieved in land and sea cultures, however, culture sites must be selected carefully as they strongly influence growth and mortality rates. It may be better to situate sea cages in regions of lower water temperatures (e.g. Wellington), where growth rates will be slower but survival will be higher, as obtaining optimal growth rates is likely to be less important in the less expensive sea cultures.

8.3.7.2 Habitat (hides)

In their natural habitat, *J. edwardsii* are most often associated with physical shelters, especially as juveniles (MacDiarmid, 1994) and consequently there has been a tendency to provide artificial shelter in culture systems for lobsters. It has generally been assumed that shelters provide necessary protection for moulting lobsters against cannibalism, and reduce captive stress. However, lobster shelters can restrict water flow, create areas of low oxygen concentration and provide greater surface area for the build-up of fouling organisms that need to be cleaned and may be a source of disease. Lobsters may also need to be disturbed more during cleaning, and the provision of shelters may be costly.

An appropriate shelter for an aquaculture system should theoretically mimic the biological requirements of the lobster during its development. Observations of shelter use of *J. edwardsii* in the wild provide clues to these requirements. For example, several studies have described the matching changes in shelter size to body size, and found a marked shift in sheltering behaviour and gregariousness at around 35mm carapace length (MacDiarmid, 1994; Edmunds, 1995; Butler *et al.*, 1999). Edmunds (1995) suggested that smaller lobsters (<35mm carapace length) may have a greater requirement for shelter because they are more solitary and prefer shelters that closely fit their body size and are enclosed on three sides. An artificial shelter for aquaculture must allow for easy observation of occupants, and must be easy to clean and have minimal impact on water flow and oxygen dispersion (Crear *et al.*, 1998). Artificial shelters that have been used include small plastic "tables" and pipes made of clay, plastic or mesh (Kington, 1999; Crear *et al.*, 2000; James *et al.*, 2001; Jeffs and James, 2000).

Experimental results for tank reared small juveniles (mean carapace length 12.5mm) indicate that shelter did not affect growth but improved survival by around 6% (James and Tong, 1998*b*; James *et al.*, 2001). These authors also observed that juveniles reared from pueruli tended to stop using artificial shelters after 3-6 months in captivity. Similarly, Crear *et al.* (2000) found that the provision of shelters increased the survival of post-settlement lobsters (2.3-8.9 g) by 23-38% but did not affect growth rate. Ninety percent of the observed mortalities were due to cannibalism, and these could be almost completely eliminated by the provision of shelters (98% survival). Kington (1999) also found that shelters did not affect the growth of larger juveniles (mean carapace length 44mm). However, he did not detect any significant difference in survival either, although this could have been due to the high variability in the results, or differences in shelter designs⁶. Booth and Kittaka (1994) suggested that the lack of a rough surface results in mortality

⁶ Kington (1999) found that there was higher mortality in tanks without shelters however his results were not significant due to high variability. He used half terracotta pipes for shelters in his study, while Crear *et al.* (2000) used cylindrical hides of 3mm oyster mesh and James and Tong (1998b) used fibrolite tables.

in moulting lobsters due to the inability of lobsters to get sufficient grip to pull themselves out of their shells. Crear *et al.* (2000), however, found no difference in survival between lobsters grown in smooth tanks and in tanks with a mesh floor. Overall, it appears that shelters are beneficial to the survival of early juvenile lobsters, but may not be for larger animals, and problems of cost, difficulty of cleaning and extra disturbance of animals may outweigh the benefits of shelters for larger animals. A significant improvement in the growth and survival of larger juvenile lobsters in culture through the provision of shelters could provide significant savings to a commercial operation and warrants further research.

8.3.7.3 Stocking densities

Over-crowding can significantly reduce growth rates and survival of captive juvenile *J. edwardsii* (Tong, 1994; Tong *et al.*, 1998; Rayns 1991). Rayns (1991) stated that there was greater size variability and moult asynchrony in lobsters held at high densities, which lead to depressed growth and higher mortality of smaller individuals due to larger lobsters dominating food and shelters. High stocking densities also increased the incidence of disease and transmission rates (Tong *et al.*, 1998).

Optimal stocking densities have been poorly defined for the culture of *J. edwardsii* and are likely to vary greatly with different tank or cage arrangements, artificial shelters and feeding. Results of research to date suggest that optimal stocking densities alter dramatically with increasing size of juvenile lobsters (Jeffs and Hooker, 2000). Rayns (1991) investigated the growth and survival of two size classes (35 and 75mm CL) of J. edwardsii held at a range of densities between 7 and 183 ind.m⁻². The best growth and survival were attained at densities of 37 ind.m⁻² for 35mm CL animals and 15-30 ind.m⁻² for 75mm CL animals. However, these low densities may not be commercially viable (Jeffs and Hooker, 2000). James et al. (2001) reared⁷ post-pueruli J. edwardsii (mean weight 1.3g) at densities of 50, 100, 150 and 200 ind.m⁻². They found that the growth rate decreased with increasing density, and after 118 days lobsters cultured at 50 ind.m⁻² were 36% heavier than lobsters held at 200 ind.m⁻². Mortality was not significantly different between the treatments. Similarly, Tong et al. (1998) found that growth was inversely related to density, and post-pueruli J. edwardsii (mean weight 3.3g) cultivated at 200 ind.m⁻² grew 40% slower than those at 50 ind.m⁻². However, mortality was much higher in the high density tanks, although a fungal infection throughout the tanks may have magnified the effect in the high density tank.

To be economically feasible, the optimal stocking density needs to be a balance between optimal growth, minimum mortality and maximum yield. Further research is needed on optimal stocking densities, especially in larger animals. Results show that it is possible to have low mortality rates at high culture densities, although lobsters should be fed daily to avoid cannibalism, and careful maintenance of the tank environment is needed. Lobsters cultivated at high densities should also be regularly size graded to avoid large lobsters dominating the food. Tong *et al.* (1998) and James *et al.* (2001) suggested that early juvenile *J. edwardsii* could be successfully reared in densities up to 100 ind.m⁻² of tank floor area.

⁷ Lobters were cultivated in 36*l* flow-through tanks held at 18°C. The lobsters were fed to excess daily with *Mytilus galloprovincialis*. Shelter was provided.
8.3.8 Environmental factors

8.3.8.1 Temperature

Knowledge of the temperature requirements for optimal growth and survival of J. edwardsii are important as they will greatly influence the culture method, selection of sites, and the profitability of any ongrowing facility. Chittleborough (1975) showed that lobster growth will increase with temperature to a threshold above which both growth and survival decline as lobsters reach the upper limit of their thermal range. As the temperature rises, the moult rate increases linearly and the moult increment tends to decrease, although the relationship between increment and temperature is less clear (Rayns, 1991). Optimum temperatures for growth and survival of J. edwardsii are from 18°C to 20°C (Hollings, 1988; Manuel, 1991; Bunter and Westaway, 1993; Booth and Kittaka, 1994; Thomas et al., 2000). However, Thomas et al. (2000) showed that J. edwardsii could be successfully cultured in temperatures up to 22°C. Growth was fastest at 20.6°C (up to 2% body weight/day⁻¹) and optimum food conversion occurred at 19.3°C (Thomas et al., 2000). Culturing lobsters at higher temperatures could reduce costs through shortening the time necessary to ongrow lobsters to a marketable size (Hooker et al., 1997), however, any advantages have to be offset against the increased cost of heating water. Lobsters held at higher temperatures have lower food conversion efficiencies (Thomas et al., 2000), elevated activity, greater incidence of disease, lower survival, and usually higher food consumption (Manuel, 1991), although not all of these effects were apparent in Thomas et al.'s (2000) study. At higher temperatures, there is also a greater risk of problems with water quality, which are compounded by the increased ammonia excretion and oxygen demand of lobsters. Therefore, water treatment and exchange systems need to be more efficient to ensure that poor water quality does not limit growth at higher growing temperatures.

Lobsters cultured at ambient sea water temperatures in north eastern New Zealand grew faster than those in southern localities (Hooker, 1996; Hooker *et al.*, 1997; Kington, 1999) with growth rates of up to 0.17g day⁻¹ reported (Kington, 1999). It is possible to transport pueruli from areas of high settlement to areas with warmer ambient seawater for ongrowing and hence avoiding water heating costs, however, peak summer water temperatures must be kept within the tolerance levels of *J. edwardsii* (Hooker *et al.*, 1997; Ruru, 2000). Alternatively, potential sources of inexpensive water heating could include warm-water discharges from power stations, solar heat or geothermal heat (Booth, 1988).

When lobsters are reared in water temperatures near their thermal tolerance their growth is retarded (Manuel, 1991; Hollings, 1988; Thomas *et al.*, 2000). Thomas *et al.* (2000) found that when *J. edwardsii* were reared at 24°C the moult increment remained constant but the intermoult period increased, thus, growth was slower overall. The apparent upper thermal limit of *J. edwardsii* has not been clearly identified (see Table 2). Different holding systems or water quality between studies, or different sources of experimental lobsters could be the cause of the apparent disparity in these results. Temperature tolerances could also vary with age and more mature lobsters may be able to withstand higher temperatures (Booth, unpubl.). Hooker *et al.* (1997) found that the highest mortality occurred in small juveniles (< 6months post-settlement) during periods of high temperatures, while there was much lower mortality in older juveniles (>18

months post-settlement) over the same period and held in similar conditions. Several authors have suggested that fluctuations in temperature may be as important as the temperature itself (Ayres and Wood, 1977; Hollings, 1988), although Hooker (1996) did not find a correlation between temperature fluctuations of between 1.5-2.3°C and mortality events. If larger juveniles are more tolerant to elevated temperatures they could be ongrown at higher temperatures after the first year, potentially reducing the time needed to reach marketable size without reducing survival. Further study may also be needed into the effect of age or geographic origin on temperature tolerance, and the interactive effect of other stressors and elevated water temperatures.

Mean weight	Lobster origin	Survival	(%)	Reference				
(g)		10°C	14°C	18°C	20°C	22°C	24°C	-
0.99	Tasmania	-	-	90	69	82	51	Thomas et al., 2000
2.5-3.8	Gisbourne, NZ	90	88	78		68	-	Manuel, 1991
2-4	Gisbourne, NZ	-	76	-		50	-	McClary, 1991
na ^A	Gisbourne, NZ			45		12.5		Hollings, 1988
0.7	Tasmania		75 ^в	20.5				Bunter and Westaway,
								1993

Table 2. Effect of temperature on the survival of captive *Jasus edwardsii*. Mean weight refers to the initial mean weight. na=not available.

^AMean carapace length=54mm. Hollings (1988) notes that system failures caused several fatalities throughout his experiments.

^BLosters were held at ambient temperature (10-16°C).

8.3.8.2 Photoperiod/light intensity

Lobsters are more active at night, but only 16% of this activity is feeding (Berry, 1997). Therefore, increased photophases may promote growth by reducing unnecessary energy expenditure associated with nocturnal activity. Long day length may also provide a physiological cue to initiate moulting (Brett, 1989). However, in trials with a single light:dark (LD) cycle every 24 hours, increasing the photophase has not resulted in significantly increased growth to that attained under a LD12:12 cycle. (Brett, 19989; Berry, 1997; Crear *et al.*, this report Section 8.4). Some dark period appears to be necessary to optimise foraging and promote moulting because constant light has consistently produced the slowest growth (Brett, 1989; Crear *et al.*, this report Section 8.4). No difference in growth rates between juveniles reared in constant darkness and those reared under a LD12:12 regime has been found (Manuel, 1991; Crear *et al.*, this report Section 8.4). The use of multiple daily photoperiods to increase growth shows some promise, as fastest growth was attained with three light cycles every 24 hours (Brett, 1989). This type of photoregime is thought to raise moult increment through increasing feeding opportunity. It needs further investigation, however, as replication in Bretts' study was low due to high mortality.

Photoperiod may only have an effect on physiological processes like growth, moult or reproduction if a lobster is outside its optimum temperature range (Conan, 1985). If sea

temperatures are unusually cold, an increasing photoperiod may promote growth and reproduction for summer. In the studies by Brett (1989) and Manuel (1991), water temperatures were close to optimal so some of the effects of differing photoperiod on growth may have been masked.

There appears to be no effect of different light conditions on lobster colouration (Brown, 1961). Likewise, no colour difference has been attained with *J. edwardsii* under different light conditions (Manuel, 1991; Crear *et al.*, this report Section 8.4). Light intensity can affect the survival of lobsters (Eagles *et al.*, 1986); the effect of light intensity on growth and survival of *J. edwardsii* has not yet been adequately addressed. However, it is likely that the light intensity should be kept to a minimum during the dark periods as only low levels of illumination are necessary to suppress activity (Williams and Dean, 1989).

One overriding outcome of the various studies on photoperiod is that there has been no treatment that has resulted in significantly better growth or survival than that under the standard LD12:12 cycle. Thus, further research on photoperiod is probably not warranted, although the effects of multiple photoperiods and light intensity need to be clarified. All studies concerning photoperiod to date have tested early juveniles in their first year after settlement and there is no information available on the response of older *J. edwardsii* to photoperiod. Further information is needed on activity patterns of captive lobsters under different light regimes.

8.3.8.3 Water quality (oxygen, salinity, ammonia, pH, nitrite, nitrate)

The southern rock lobster *J. edwardsii* is sensitive to water quality (Kensler, 1967). In an ongrowing situation there is more potential for water quality to deteriorate, due to the high temperatures and continual feeding regimes required to produce optimum growth.

8.3.8.3.1 Oxygen

Lobsters are oxygen regulators, they can vary the flow rate of water which passes over their gills to compensate for low ambient oxygen levels (Waldron, 1991), and can thus maintain oxygen consumption rates down to low saturation levels (35-50% - Waldron ,1991; Crear and Forteath, 1998). Below this level, consumption decreases and becomes dependant on the ambient oxygen level (Crear and Forteath, 1998) and the lobster will build up an oxygen debt through the use of anaerobic metabolism. However, studies in other spiny lobster species show growth and survival can be inhibited at oxygen levels much higher than the critical oxygen level (67% saturation - Beyers *et al.*, 1994; 55% saturation - Chittleborough, 1974). The minimum oxygen levels recommended for holding spiny lobsters range from 40 to 80% saturation (Anon., 1980; Beard and McGregor, 1991; Forteath *et al.*, 1993). However, it is not apparent how these estimates were obtained and they may be based on trial and error rather than quantitative research or long-term aquaculture studies, as opposed to short term holding of wild caught stock. As oxygen saturations of 100% promote rapid recovery from stress (Crear and Forteath, 1998), and the best growth (Beyers *et al.*, 1994) it would seem that this level should be aimed for in culture systems and oxygen should be kept above 80% saturation at all times.

Culture systems must anticipate large fluctuations in demand for oxygen as there are a number of factors, which affect oxygen consumption in *J. edwardsii*, including temperature and body

weight. However, activity has the most marked effect, increasing oxygen consumption by as much as three fold from resting (Crear and Forteath, 2000). Consequently, due to high levels of nocturnal activity oxygen consumption has been found to be 42% higher at night (Crear and Forteath, 2000). The resting rate of oxygen consumption increased 1.5-1.72 times with feeding, peaking 1-10 h after feeding (weight dependent) and remaining elevated for 48 h (Crear and Forteath, 2000; Lefever, 2000). Lobsters have a large capacity for anaerobic respiration with relatively rapid recovery when oxygen becomes available again. It is this capacity that is utilised in the aerial transport of live J. edwardsii to markets around the world. Oxygen consumption following re-immersion in water is more than 2.5 times the resting rate and can remain elevated for up to 8 hours (Waldron, 1991; Crear and Forteath, 2000). However, lobsters held in deoxygenated water expire quickly due to rapid loss of their available oxygen. For this reason most lobster holding and aquaculture facilities are designed to be self-draining in the event of a systems failure that would prevent the circulation of oxygenated water. Oxygen concentration has been found to influence the growth of other spiny lobster species (Chittleborough, 1975; Beyers et al., 1994) but it has not yet been tested on J. edwardsii. There is already a significant amount of information available on oxygen consumption rates (Waldron, 1991; Crear and Forteath, 2000; Lefever, 2000; Thomas et al., 2000) which may be used to ensure that sufficient oxygen is supplied to the lobsters, either via aeration or water flow. As oxygen saturation is relatively easy to maintain in culture systems, it is doubtful that further research effort directed in this area would produce worthwhile benefits to aquaculturalists.

8.3.8.3.2 Nitrogenous waste

Few studies have investigated the nitrogenous output of *J. edwardsii*. Lobsters excrete nitrogen through urine and faecal matter, but most is lost by simple diffusion through permeable membranes like the gills (Binns and Peterson, 1969; Zoutendyk, 1987; Crear and Forteath, 1998). Ammonia is the principal excretory product of lobsters constituting 72% of the total nitrogen output of *J. edwardsii* (Binns and Peterson, 1969). Ammonia is known to be toxic to crustaceans if allowed to accumulate in their holding water (Tomasso, 1994) and can inhibit growth even at low levels (Chen and Lin, 1992). The negative effects of ammonia in holding water are further compounded with low oxygen concentrations, salinity and pH (Wajsbrot *et al.*, 1989; Chen and Lin, 1992). In flow-through sea water systems, a high flow rate and adequate water mixing will prevent ammonia build-up, whereas in a recirculating system a well designed and managed biofilter is critical. Knowledge of the ammonia excretion rate of *J. edwardsii* is therefore essential. Crear and Forteath (1998) examined the effect of temperature, body weight, daily rhythm, feeding, handling and emersion on ammonia excretion in adult *J. edwardsii*.

Temperature and body weight had large influences on the rate of ammonia excretion and lobsters excreted more ammonia at night. The resting rate of ammonia excretion increased more than six times with feeding, peaking 7 hours after feeding and remaining elevated for 26 h (Crear and Forteath, 1998). By contrast, activity had a minimal effect on ammonia excretion rates, and after a 30 min emersion period, rates returned to normal within an hour (Crear and Forteath, 1998). Other nitrogen waste products commonly found in sea water systems, nitrates and nitrites, are also believed to be toxic to lobsters. Nitrite has a maximum recommended concentration in seawater for lobsters of about 1 mg L⁻¹ (Harvie, 1993). However, there is very little known about the effect of nitrite on lobster physiology although it is thought that nitrite may affect the oxygen-carrying capacity of the blood (H.H. Taylor and W.A. Titulaer, unpubl.; cited in Taylor *et al.*,

1997). Nitrate is less toxic than ammonia or nitrite and the maximum recommended level is 100-140 mg L^{-1} (Harvie, 1993).

The maximum recommended concentration of ammonia in water used for culturing lobsters should be below 0.5 mg L⁻¹ (Bunter, 1992; Harvie, 1993). Biological filters need to be designed to cope with especially high loading due to the high rate of nitrogenous waste excreted by feeding animals. Additionally, the high temperatures (18-20°C) which produce optimum growth also increase the excretion of nitrogenous waste. Crear and Forteath (1998) examined these two factors separately, but further research could investigate the combined effect of temperature and feeding on the excretion rates of *J. edwardsii*. This is especially important for smaller lobsters as the ammonia excretion rate of small juveniles (5-10 g) is 30-50 times greater than that of adult lobsters at a similar temperature (Crear and Forteath, 1998; Thomas *et al.*, 2000).

8.3.8.3.3 pH

The pH of seawater normally varies between 7.8 and 8.4 and lobsters appear to be able to survive in water over a wider range of pH. Extended periods of exposure to lower pH has been associated with carapace erosion in other lobster species, however, the effect of lowered pH on *J*. *edwardsii* is unclear. Low pH of seawater can occur in recirculating systems. It appears to stress lobsters, which then become more active and aggressive (Harvie, 1993).

8.3.8.3.4 Salinity

Adult *J. edwardsii* can readily acclimatise to a salinity change of less than 5‰ but mortality increases after a 7‰ drop (Stead, 1975). However, lobsters can become acclimatised to larger changes in salinity if they are allowed to adjust gradually in successive changes, each no more than 3-5‰ (Stead, 1975). Moss *et al.* (2000) investigated the growth and survival of juvenile *J. edwardsii* at ambient (33‰), moderately low (28‰), low (25‰) and fluctuating salinities (33-25‰). Growth at moderately low salinity was comparable to that at ambient salinity but reduced growth was detected in the low and fluctuating salinity treatments. Although survival was high, most deaths occurred at the lowest salinity. Therefore, for culturing lobsters the salinity should be kept as close as possible to normal seawater levels of 33-35‰.

8.3.9 Disease

There is a general lack of knowledge about parasites and diseases in spiny lobsters either in culture or in the wild (Evans and Brock, 1994), therefore it is not surprising that there is little known about the diseases of *J. edwardsii*. This species appears to have a low incidence of parasites and disease when kept in captivity (Van Olst *et al.*, 1980; Booth and Kittaka, 1994). However, a number of infections have been reported and most of these have affected juveniles and have been related to poor water quality (Booth, 1988; Rayns, 1991; Crear *et al.*, 1998; Diggles, in press; Diggles, 1999), or poor nutrition (Hollings, 1988; Rayns, 1991; Diggles, 1999). Fungal and bacterial infections, including vibriosis, shell disease (Rayns, 1991) as well as infections by parasitic nematodes and ciliates have been reported (Diggles, in press). Low levels of mortality of captive adults have also been attributed to a disease of unknown aetiology termed 'Turgid Lobster Syndrome' (Diggles, in press). Most of the pathogens involved in these incidents appear to be opportunistic, only causing problems when lobsters are damaged or are

stressed, from handling, or are held under sub-optimal conditions (Evans and Brock, 1994). Handling damage that results in the loss of appendages also can allow a point of entry for infection and increases lobster susceptibility to disease (Booth and Kittaka, 1994). For example, adult lobsters held in sea cages developed "tail fan rot", which was thought to be associated with infection entering damaged posterior margins of the telson and uropods (Lorkin *et al.*, 1999). Treatment methods have been developed for some pathogens identified in *J. edwardsii* (Diggles, in press), however, it would seem that poor health of captive lobsters can best be prevented through good husbandry, particularly by maintaining water quality, tank cleanliness and good nutrition. For example, the non-pathogenic disturbances, 'Moult Death Syndrome', and the problem of shell erosion appear to be related to poor nutrition and water quality (Diggles, 1999). With further commercial farming of this species it can be expected that new health problems and their causative agents will be identified. A routine lobster health monitoring programme has recently been initiated by the Tasmanian Aquaculture and Fisheries Institute, and knowledge gained from this should be sufficient to keep abreast of any emerging disease problems (Crear *et al.*, 1998).

8.3.10 Conclusions

This review has drawn together research that has been conducted on *J. edwardsii* biology and culture. In some cases, the results of studies are difficult to interpret, as there has been poor experimental design and procedures. To increase the robustness of the results, it is important that future studies are undertaken using appropriate experimental designs. It is obvious that there are many areas of research that can be pursued. However, much of that research may have little impact on the profitability of a commercial aquaculture venture. Thus, research priorities for lobster ongrowing need to be established. There have been several studies which have investigated the economic aspect of *J. edwardsii* culture (Hollings, 1988; Jeffs and Hooker, 2000; Ruru, 2000). These studies have identified processing and transport, labour and feed as the highest cost components of a growout operation. Research is not likely to result in a reduction of processing and transport costs except if it resulted in the development of premium markets closer to growout facilities. Labour costs are likely to decline as appropriate systems and feeds are developed. Thus, to reduce the cost of ongrowing operations, future research should therefore concentrate on four critical areas:

- Appropriate growout system design. Sea cage culture may result in decreased grow out costs, however, further research is required on cage design and feeding methods. Other possible farming systems worth investigating in an effort to reduce costs include developing operations in conjunction with existing land-based operations, such as abalone farms
- Once suitable growout systems are developed, then research needs to be conducted on maximising production (growth, stocking density and survival) within the system. These factors have a lower impact on profitability, but will become more important as the costs of other growout factors are reduced.

- The development of formulated diets and feeding protocols which promote good growth and survival while reducing costs, both directly through lower feed costs and indirectly through lower labour requirements.
- There is a need for an ongoing health monitoring programme to ensure the disease risk is minimised

8.3.11 Acknowledgements

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8.4 <u>Objective 3.3</u> Determine growth rate and survival in relation to photoperiod

8.4.1 The effect of photoperiod on growth and survival of the southern rock lobster, *Jasus edwardsii*.

8.4.1.1 Introduction

Photoperiod has been shown to affect growth of crustaceans although the results, both within and between species, have generally been inconsistent (Chittleborough, 1975; Aiken and Waddy, 1976; Mason, 1978; Bordner and Conklin, 1981). Several studies have investigated the effect of photoperiod on growth and survival of *J. edwardsii*; results have been mixed and inconclusive (Brett, 1989; Manuel, 1991; Berry, 1997b). In those studies a broad size range of lobsters subjected to varying conditions (i.e. temperature, system design, diet, density) and feeding regimes have been used, making comparisons between studies difficult. Additionally, none of the studies subjected lobsters to the full range of photoperiods (complete light to complete dark).

Photoperiod manipulation may affect growth through a number of processes, including alterations in behaviour, physiology, feed intake and/or activity. However, there has been little attention focused on determining how it acts on *J. edwardsii*. Brett (1989) and Berry (1997b) examined activity of lobsters under different photoperiods and determined that activity was significantly determined by photoperiod: most activity occurring during the dark period. Thus, it is suggested that long photophases may support better growth, as activity and therefore energy use is decreased, and more energy can be directed towards growth (Berry, 1997b). This study examined if, and how, photoperiod affected the growth and survival of small juvenile *J. edwardsii*.

8.4.1.2 Materials and Methods

The experiment was conducted in a flow-through system consisting of 20 black culture tanks (diameter 430 mm, depth 300 mm) containing a total of 33-1 of water. A flow-through system was used to prevent the interaction of animals that may occur in a recirculation system through water-borne chemical messages (Rayns, 1991). The reservoir tank was heated using 1.2-2.4 kW titanium heaters. Vemco minilog data loggers (TR data loggers) monitored temperatures in the tanks at 10-min intervals. Temperatures were also manually checked each day. Mean temperature was 16.9°C and ranged from 15°C at the start of the experiment during winter to 19.3°C at the completion of the experiment during summer. The flow to each tank enabled a complete water exchange every 50-60 minutes and aeration was supplied via airstones. Dissolved oxygen concentration (>90% saturation), total ammonia nitrogen (<0.25 mg/l), pH (8.1-8.3) and salinity (33-35‰) were measured fortnightly. During light periods there was an average light intensity of 41.6 µmol s⁻¹ m⁻² (range 37-47 µmol s⁻¹ m⁻²) at the water surface. No light could be detected at the water surface during dark periods, except during cleaning and feeding times (approx. 20 min day⁻¹) when a light intensity of 0.1 μ mol s⁻¹ m⁻² (range 0.03-0.24 μ mol s⁻¹ m⁻²) was detected. Hides, in the form of a 10-hole house brick and pieces of fish trawl mesh, were provided to each tank.

Five photoperiod treatments (0L:24D; 6L:18D; 12L:12D; 18L:6D; 24L:0D) were investigated, with 4 replicate tanks assigned randomly to each treatment. Fifteen juvenile *J. edwardsii* (initial mean weight \pm SE = 1.28 \pm 0.01 g) were stocked into each 33-l tank and were acclimatised over 7 days prior to the first weight measurement. There was no significant difference between the weights in each of the treatments (F = 0.45, P = 0.77). During the first week after the initial weight measurement, mortalities were replaced with animals of similar weight. These mortalities were assumed to be due to stresses associated with handling and not included in the final mortality data. The experiment ran for 112 days. Mortalities and moults were recorded daily.

Lobsters were fed twice daily with freshly opened cultured mussels (*Mytilus edulis planulatus*) in the evening (1700 h), and dry growout pellets for penaeids (*Penaeus japonicus*) in the morning (0900 h). Food was provided slightly in excess and was based on the previous days' consumption.

Feed consumption was quantified during three five-day periods over the course of the experiment (1st, 5th and 9th week), where only mussels were fed to the lobsters and all uneaten mussel flesh was recovered and dried at 50°C. A ratio of mussel wet weight (including shell) to dry mussel flesh was calculated for each period, and used to calculate dry weight of food fed. The ratio was the same for mussels opened and soaked in a tank overnight (no lobsters), therefore, there was no need to apply a correction factor for leaching losses. Food consumption was calculated as dry matter consumed per wet weight lobster biomass and expressed as a percentage of wet lobster body weight per day.

Lobsters were weighed to the nearest 0.01 g on days 0, 28, 56, 84 and 112. Animals were dried on absorbent paper for 30 s to remove excess water prior to weight measurement. Growth was evaluated in terms of specific growth rate (SGR) and percentage weight gain (%WG). These parameters were defined as: SGR = (ln Final weight -ln Initial weight) *100 / number of days; % WG = (final weight - initial weight) * 100 / initial weight. At the end of the experiment all lobsters were colour graded using a colour scale developed at the Tasmanian Aquaculture and Fisheries Institute. This colour scale has five levels with one the lightest colour (pink/light brown) and five the darkest (dark red/maroon).

The 24-h activity of lobsters within each treatment was quantified by video recording. For each treatment, two tanks were filmed from above for two days (total of 4 days of recordings). The video camera was placed above the tank. A grid system placed just above the water surface dissected the tank into 6 equal pie-shaped sectors. Activity was quantified on an hourly basis by counting the number of times lobsters moved from one sector to another over a 5-minute period (a lobster had to move entirely into the next sector to be counted as a movement). An infra-red light source allowed lobsters to be observed during the dark periods. The infra-red light was left on permanently for each 24-hour recording period; previous research had shown that it did not interfere with the lobsters' normal behaviour (Westbury, 1999).

All data were analysed using one-way analysis of variance and Fisher's LSD test for significant differences at the 0.05 confidence level (JMP Version 3.1.6.2). Percentage data were arc sine $\sqrt{}$ transformed prior to analysis.

8.4.1.3 Results

Lobsters grown under the 6L:18D and 24L:0D photoperiods had significantly lower (P<0.05) weight gain and specific growth rate than lobsters in any of the other regimes (Table 70). Photoperiod did not affect survival or colour but it had an effect on the mean number of moults per tank. Although growth was high in the 0L:24D regime, the number of moults was significantly lower than in the other treatments, apart from the 6L:18D regime.

Table	70.	Effect	of photo	perio	od on	gro	wth,	sur	viva	al, (colour	and	mou	ılting	of	juvenile .	Iasus
edward	sii.	Values	expressed	as r	nean	± SE	E. Va	alues	in	the	same	row	with	differe	ent	superscript	s are
signific	antly	y differe	ent (P<0.05	5)													

	Photoperiod				
	0L:24D	6L:18D	12L:12D	18L:6D	24L:0D
Initial mean wt (g)	1.27 ± 0.01	1.28 ± 0.01 1.29 ± 0.01		1.29 ± 0.01	1.29 ± 0.01
Final mean wt (g)	$7.84\pm0.16^{\rm a}$	$7.06\pm0.06^{\rm b}$	$7.90\pm0.31^{\rm a}$	$7.95\pm0.25^{\rm a}$	$7.15\pm0.18^{\rm b}$
Weight gain (%)	$517\pm8^{\rm a}$	$451\pm8^{\rm b}$	$513\pm26^{\mathrm{a}}$	$517\pm14^{\mathrm{a}}$	$455\pm12^{\rm b}$
SGR (% BW d ⁻¹)	$1.62\pm0.01^{\rm a}$	$1.52\pm0.01^{\rm b}$	$1.62\pm0.04^{\rm a}$	$1.62\pm0.02^{\rm a}$	$1.53\pm0.02^{\rm b}$
Survival (%)	88 ± 3	93 ± 3	88 ± 5	88 ± 2	78 ± 9
Colour	4.0 ± 0.4	4.0 ± 0.6	4.1 ± 0.5	3.8 ± 0.5	4.4 ± 0.3
Total moults per	$41.3\pm2.8^{\rm a}$	46.3 ± 2.8^{ab}	$47.5\pm1.3^{\rm b}$	$51.0\pm1.6^{\rm b}$	$51.3\pm0.9^{\rm b}$
tank					

Food consumption in the first five-day period was higher (P<0.05) in the 12L:12D and 18L:6D regimes than in the other treatments (Table 71). There was no significant difference between treatments in the other five-day periods.

Table 71. Effect of photoperiod on feed consumption (% BW/d) of juvenile Jasus edwardsii. Values expressed as mean \pm SE. Values in the same row with different superscripts are significantly different (P<0.05)

	Photoperiod									
	0L:24D	6L:18D	12L:12D	18L:6D	24L:0D					
Feed consumption (% BW/d) – 1 st week	$1.49\pm0.10^{\rm b}$	$1.53\pm0.09^{\rm b}$	$2.05\pm0.15^{\rm a}$	$1.96\pm0.07^{\rm a}$	$1.58\pm0.14^{\rm b}$					
Feed consumption (% BW/d) - 5 th week	2.27 ± 0.20	2.15 ± 0.12	2.06 ± 0.03	2.22 ± 0.15	2.39 ± 0.23					
Feed consumption (% BW/d) - 9 th week	2.47 ± 0.11	2.04 ± 0.10	2.45 ± 0.07	2.60 ± 0.35	2.58 ± 0.18					

Activity of the lobsters varied with photoperiod (Figure 26). In the 24L:0D and 0L:24D regimes, activity was at a low level and mostly constant over the whole day. During the dark phases of the remaining treatments peak activity was observed which coincided with the onset of darkness. A secondary peak commencing between 05.00 and 07.00 was also observed in the 6L:18D or 12L:12D regimes. Activity levels during the light periods in the 6L:18D, 12L:12D, 18L:6D regimes were generally low.



Figure 26. Effect of photoperiod on activity (average number of movements over a 5 minute period) of lobsters. The dotted lines indicate when lights were switched on and the solid lines indicate when lights were switched off

8.4.1.4 Discussion

Growth in juvenile *J. edwardsii* was affected by photoperiod, with constant light and an 18-h scotophase (dark phase) reducing the growth. These results are surprising and indicate that there may be more than one factor determining the photoperiod response.

The effect of increasing the photophase above the standard 12-h light was variable. Brett (1989) found similar trends with *J. edwardsii* and concluded that there appears to be an upper limit to the total photophase period that lobsters are able to tolerate. As lobsters normally feed during darkness (Fielder, 1965), a dark period may be necessary for feeding. Berry (1997*a*) also found highest growth with a long photophase and suggested that a dark period of 8 hours per night was sufficient to feed. However, in this study, feed intake was similar under all photoperiods, suggesting decreased food consumption was not the reason for the decreased growth in the 24L:0D regime. Brett (1989) suggested that a photoperiodic cue (i.e a minimum period of darkness) might be necessary to phase physiological rhythms. An investigation into the physiological condition (stress levels) of lobsters in this regime may provide a clue to their poor growth.

The effect of reducing the photophase was also variable. A reduction in growth under a 6L:18D regime was also observed by Brett (1989). Again, there appears to be a limit to the effect of reducing the photophase, as lobsters grown in a 0L:24D regime had similar growth to that in the standard 12L:12D regime. Manuel (1991) also observed similar growth under 12L:12D and 0L:24D regimes. Decreased feed consumption was not the reason for the decreased growth under the 6L:18D regime.

Activity of *J. edwardsii* is usually restricted to the hours of darkness (Fielder, 1965; Williams and Dean, 1989; Westbury, 1999), with only low levels of illumination (0.03 μ E m⁻² s⁻¹) required to suppress activity during the light phase (Williams and Dean, 1989). In this study, lobsters in the standard 12L:12D regime exhibited a typical nocturnal rhythm; generally, activity was higher at night than during the day but there were also crepuscular increases in activity. Similar responses were observed in the other regimes where there was a dark/light cycle (6L:18D and 18L:6D). The activity level under constant light or dark conditions was generally more regular than under the other regimes, with no clearly identifiable peaks. Activity does not appear to increase with an increase in scotophase, indicating that such conditions would not limit lobster growth through increased energy usage, as suggested by Berry (1997*b*). In comparison, a decrease in the photophase also appeared to reduce activity levels, although there was no apparent increase in lobster growth.

Fielder (1965) found that adult *J. edwardsii* (referred to as *J. lalandei*) had a rapid response to changes in light cycles, changing its activity and feeding pattern to match the new cycle. However, based on feed consumption, it appears that juvenile lobsters in this study took a considerable time to acclimatise to a photoperiod. During the first week of the experiment, feed consumption in some treatments were low, even though they had been acclimated to the system and light regime for one week prior to the experiment commencing, but by the fifth week, feed consumption was similar in all treatments.

Feeding usually takes place during darkness with an apparent feeding stimulation produced by the change from light to dark (Fielder, 1965). If food consumption could be increased through provision of a longer scotophase, allowing more foraging time, then the provision of two daily feeds would ensure that fresh food was available to lobsters at all times. However, in this study, this feeding regime did not result in increased feed intake under increased scotophase conditions. In comparison, food consumption and growth of juvenile *Homarus americanus* was significantly higher under a 1L:23D regime then under a 15L:9D regime (Bordner and Conklin, 1981).

In the treatments where growth was high and not significantly different (0L:24D, 12L:12D and 18L:6D) the mean number of moults increased with the total number of light hours (41.3, 47.5 and 51, respectively). To maintain the same growth, lobsters in the 0L:24D and 12L:12D regimes needed to have greater moult increments at each moult. Light regime also affected the total number of moults of *P. cygnus*, increasing with increases in the light period (Chittleborough, 1975). However, moult increment did not vary with light regime in that study, therefore growth was lower in lobsters maintained in the dark.

The effect of photoperiod is likely to be variable and depends on a number of factors such as age/size of the lobsters and the water temperature (Aiken and Waddy, 1976; Bordner and Conklin, 1981). These may influence feeding processes such as satiation rate, time for the return of hunger and digestive capacity. Thus, it may be necessary to determine the optimum photoperiod across a range of experimental conditions and lobster sizes (Brett, 1989).

One overriding outcome of the various studies on photoperiod and its effect on growth of *J. edwardsii* (Brett, 1989; Manuel, 1991; Berry, 1997*b*; this study) is that there has been no regime that has resulted in significantly greater growth compared to the standard 12L:12D cycle. This suggests that photoperiod has only minimal influence on growth compared to other factors. Furthermore, no studies have found an effect of photoperiod on survival. If lobsters were to be cultured in an indoor system, then husbandry practices can be significantly simplified, as it is possible to subject the lobsters to reasonably long photophases (12-18 hours of light) without affecting growth or survival.

9 <u>Objective 4.1</u> To provide a Code of Practice for sea-based live-holding of rock lobsters

9.1 Recommendations for sea-based live-holding of adult southern rock lobsters.

As there is presently no live-holding industry involving feeding and long-term holding, it is inappropriate to develop a code of practice at this time. However, several recommendations can be made for industry. The following recommendations are based on the results of Component I of the present project for lobsters that are around the legal minimum length (102mm carapace length) and that are held for several months (either November to March, or April to November) at densities of 15-20 per sea-cage compartment (approximately 2-4 per m² of total surface area):

- Handling of lobsters during holding should be minimised as this results in stress and leg loss. Leg loss represents weight loss and legs can only be replaced through a moult.
- Use only healthy lobsters that have been well handled with a known history. Initial handling/holding conditions may have long-term effects on biomass returns.
- Lobsters need to be fed with either a natural or manufactured diet in order to optimise survival, growth at moult, and physiological condition. Suitable diets are live mussels (*Mytilus edulis*), octopus (*Octopus maorum*), and prepared pellets.
- A sea-based cage system using feed shutes and fine (3mm) mesh-lined cage bottoms is adequate for supplying lobsters with natural or manufactured feeds.
- Feeding lobsters at rates of 2 or 3 times per week was adequate in keeping lobsters alive, maintaining the condition of lobsters, and increasing the weight of lobsters.
- Lobsters can be held successfully during both the open fishing season (i.e. "summer") and the closed season (i.e. "winter"). This creates both strategic marketing and product enhancement opportunities.
- Holding lobsters during the closed season (i.e. "winter") rather than during the open fishing season (i.e. "summer") may result in greater survival, moulting activity, growth at moult, and biomass returns.
- Holding males rather than females during winter may result in greater growth at moult and biomass returns. Holding females poses the added risk of spawning during winter.
- Holding compartments in both sea-based and land-based sytems need to be covered from direct sunlight. Direct sunlight causes unsightly algal fouling on lobsters.
- There was no evidence to suggest that speckled/white lobsters cannot be successfully liveheld for extended periods. Indeed, in a winter trial the speckled/white lobsters had equivalent biomass returns to red lobsters. Some improvement in the colour of speckled/white lobsters through a moult can also be expected.

- Tail fan damage is a problem with live held lobsters and needs to be considered with regard to marketing when undertaking live holding operations at the present time.
- Land-based raceway holding systems offer the potential for live holding in conditions of greater control over feeding and husbandry. Densities of 18.5 per m² of bottom surface and flow rates of 60 litres per minute provide a guide for raceway live holding practices. System failures need to be monitored closely in land-based raceway holding systems.

10 <u>Objective 4.2</u> Evaluate existing system design and management regimes for captive growout of tropical rock lobster

10.1 An evaluation of existing system design and management regimes for captive growout of tropical rock lobster.

During the course of running three experiments addressing temperature, salinity and density in the definition of production technologies for *P. ornatus*, considerable experience was gained at a broader level in the development of optimal system design and management protocols for their growout. Both flow-through and recirculation systems were applied, and both are suitable for the growout of *P. ornatus*. Clearly, *P. ornatus* will perform optimally with pristine marine water quality conditions, however, the species robustness allows for moderate resistance to perturbations. Both system types have their relative merits and should be judged on economic grounds. Some of the key features of either system as they might be applied to the culture of *P. ornatus* are listed below.

- Elongated, rectangular raceways would appear to be the most suitable tank specification, to provide efficient access to animals, while maintaining good water quality and maximising self-cleaning of tank.
- Filtration of seawater to at least 40 micron and preferably 1 micron.
- Ideal recirculation system would include in sequential order; mechanical filtration for effective solids removal, bead or other fluidised bed filter, foam fractionator, rotating disk filter and ozone injection.
- Recirculation systems should focus on maximum solids removal, as elevated levels have been associated with high nitrate readings.
- Shelters are critical, but need to further develop optimal specifications.
- Light level should be kept low, and disturbances minimised.
- Reduce feeding if water quality is compromised. *Panulirus ornatus* are tolerant to reduced salinity for brief periods. Feeding activity drops, but otherwise no perceptible adverse effect. Mortalities may result if ammonia levels go above 0.5mg/l.
- Feeding of prawn diet (*Penaeus japonicus*), several times per night or continuously each night (belt feeders), supplemented with prawn or mollusc flesh twice per week.
- Provision of coral rubble is important as a dietary supplement.
- Textured surface to tank, to provide grip.
- Maintain minimal size variation to minimise aggression and cannibalism. For growout from 1g to 1 kg, grading at mean of 250g and 500g is recommended.

11 Extension of results

11.1 Component I

Presentations

- RLEAS Scientific Committee meetings: 7 September 1998, 10 March 1999, 24 September 1999, 23 February 2000.
- RLEAS workshops: 11 March 1999, 24 February 2000.
- World Aquaculture '99, 30 April, 1999, Convention Centre, Sydney.
- 3rd International Lobster Congress, 22 September, 1999, Stamford Grand Hotel, Glenelg, Adelaide.
- Industry meeting, 21 September, 1999, Stamford Grand Hotel, Glenelg, Adelaide.
- Industry meeting, 8 December, 1999, Civic Centre, Port Lincoln.

Written articles

- RLEAS Progress Reports: January 1999, June 1999, December 1999.
- RLEAS 'Lob Release' newsletter, Volume 1, Issue 1, August 1999.
- South Australian Rock Lobster Advisory Council newsletter, Issue 8, September 1999.
- South Australian Rock Lobster Advisory Council website, 1999,

http://www.rocklobster.org.au/rocklobster.nsf/pages/home (see current projects here and Congress Page)

Media releases

- Television interview, Channel 9 News, Adelaide, 24 November, 1999.
- Radio interview, ABC Country Hour, 29 March, 1999.
- Television interview, Regional station, Port Lincoln, 8 January, 1999.

11.2 Component II

Other than RLEAS activities for which contributions were made to all, the only other extension activity has been direct consultation with the live lobster sector of industry in Cairns in response to enquiries. Results are to be presented at the 6th International Lobster Conference in Key West Florida, September 10 to 15, 2000.

11.3 Component III

Presentations

Results of this work have been presented at the Rock Lobster Enhancement and Aquaculture Sub-program annual workshops at Geraldton (March, 1999) and Hobart (Feb., 2000).

Publications

Thomas, C., Crear, B. and Hart, P. (2000) The effect of elevated temperature on growth, survival and metabolic activity of the southern rock lobster, *Jasus edwardsii*. *Aquaculture* **185**: 73-84.

12 Benefits

Results from Component I of the project will enable rock lobster fishers and processors to make better informed decisions about holding systems, holding times, feeding regimes, feed types, and selection of lobsters for long-term live-holding of adult *Jasus edwardsii*.

Results from Component II of the project have delivered the following benefits:

- Temperature, salinity and density optima have been defined for tank culture of *P. ornatus*
- Basic production protocols for the growout of *P. ornatus* have now been defined
- Commercial aquaculture potential of *P. ornatus* has been confirmed

There is presently a rock lobster aquaculture industry in New Zealand based on the ongrowing of wild-caught puerulus. A range of culture systems are being used (see Hooker *et al.* 1997; Jeffs and Hooker, 2000). The development of similar culture operations is envisaged in Tasmania in the near future. Information obtained from Component III of this study will help determine optimum growout sites, system designs and culture methods in both New Zealand and Tasmania.

13 Further Development

13.1 Live-holding of adult Jasus edwardsii

Results from the present project were promising and will enable informed decisions to be made about a number of issues relating to long-term live-holding of adult *Jasus edwardsii*. However, further research is required to refine this decision-making process and optimise live-holding practices. Further research should include an economic analysis of different holding scenarios. Some results from the present project will be written up as a formal paper.

13.2 Grow-out of juvenile Panulirus ornatus

In investigating the specific parameters of temperature, salinity and density for the culture of *P. ornatus*, the priority of other parameters for defining optimal culture conditions were identified. Specification and availability of shelter is very important and will need to be investigated with regard to size specific requirements and importance of refuge for moulting individuals. Feeding strategy is equally important in maximising the efficiency of food delivery and utilisation, and minimising cannibalism mediated through food availability.

In regard to commercial development of technologies established through the research, this is well underway through the establishment of a joint venture in January 2000, between DPI Queensland and MG Kailis Pty Ltd, which aims to commercialise tropical rock lobster aquaculture over the next 5 years.

13.3 Grow-out of juvenile Jasus edwardsii

The literature review (see Section 8.3) outlines future research priorities in the area of system design for ongrowing of juvenile *J. edwardsii*. In the meantime, it is envisaged that the results of this study will be used during the development of *J. edwardsii* culture operations in Australia and the further development of them in New Zealand.

14 Conclusion

All objectives for Component I of the project were completed successfully. A variety of diets were assessed for their effectiveness in promoting growth at moult and in maintaining/improving the condition of red and speckled/white, male and female adult southern rock lobsters (*Jasus edwardsii*). Diets of live mussel, octopus, and manufactured pellets were all successful in promoting growth at moult and in maintaining/improving the condition of lobsters. Speckled/white lobsters fed on octopus and manufactured pellets improved in colour, and long-term live-held lobsters were able to survive a simulated overseas export. The taste of long-term live-held lobsters fed on either live mussels or manufactured pellets was excellent. Whilst results from the study were generally very encouraging, tail fan damage was found to be a major problem with long-term live-held lobsters.

All objectives for Component II of the project were completed successfully. *Panulirus ornatus* was shown to have excellent potential as an aquaculture candidate. Requirements for growout have been sufficiently well defined to be commercialised immediately. Commercial development will be restricted until the life cycle is closed and a reliable and cost-effective supply of juveniles is guaranteed.

All objectives for Component III of the project were completed successfully. Temperature and photoperiod affect growth and survival of juvenile southern rock lobsters (*J. edwardsii*). Temperature has the largest affect, and a temperature range of between 19 and 21°C was optimal in terms of survival, growth, and food conversion ratio. At higher temperatures the measured performance criteria were reduced and the upper thermal limits appeared to be 24°C. Culturists would need to consider the economic advantages of lobsters reaching market size in the shortest possible time against the increased costs associated with heating water. If lobsters were to be grown in flow-through systems, or in cages in the sea these results indicate that it will be necessary to select sites where summer water temperatures do not rise above 22°C. Photoperiod only has minimal influence on growth and survival compared to other factors, such as temperature and diet.

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16 Appendix 1: Intellectual Property

There is no intellectual property from this project that should be protected. All the information will benefit the general community, the lobster industry, and the scientific community best if it is made freely available.

17 Appendix 2: Staff (In alphabetical order)

Bill Addington William Bowman Simon Bryars Steven Clarke **Bradley Crear** Michael Geddes Brett Glencross Piers Hart **Darren Hicks** Druce Horton Andrew Jeffs **Clive Jones** Larnie Linton Melissa Lorkin Stephen Madigan Coby Mathews **Darryl Metters Richard Musgrove Todd Packer Ruth Reuter** Chris Robertson Keith Rowling Carina Sim-Smith Craig Thomas

18 Appendix 3: Colour Plates

- (a) Sea-based cage live-holding facility at Port Lincoln, South Australia
- (b) Sea-based cage system used in experimental trials
- (c) Pellet feeding in sea-based cages
- (d) 'White' lobsters used in the Kangaroo Island trials
- (e) Tail fan damage showing 'raggedness'
- (f) Tail fan damage showing 'erosion'







(b)

(c)







(e)

(f)