Rock Lobster Enhancement & Aquaculture Subprogram: The Nutrition of Juvenile and Adult Lobsters to Optimise Survival, Growth and Condition

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Australian Government
Fisheries Research and Development Corporation

ROCK LOBSTER ENHANCEMENT & AQUACULTURE SUBPROGRAM

CSIRO MARINE RESEARCH

Tasmanian Aquaculture & Fisheries Institute
University of Tasmania

THE UNIVERSITY OF ADELAIDE AUSTRALIA
SUB CRUCE LUMEN

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Rock Lobster Enhancement and Aquaculture Subprogram: The Nutrition of Juvenile and Adult Lobsters to Optimize Survival, Growth and Condition

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1. NON-TECHNICAL SUMMARY

2000/212 Rock Lobster Enhancement and Aquaculture Subprogram: The Nutrition of Juvenile and Adult Lobsters to Optimise Survival, Growth and Condition

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OBJECTIVES

1. Develop manufactured feeds for juvenile rock lobsters that optimize survival and growth by:
    a. Defining the chemicophysical cues that stimulate food consumption in juvenile rock lobsters;
    b. Developing pelleted feeds that remain attractive to lobsters for periods in excess of four hours after immersion; and
    c. Determining the optimum dietary specifications of selected nutrients required by juvenile rock lobsters for growth and development.
2. Develop manufactured feeds for adult lobsters for body maintenance and moult manipulation by:

a. Determining the optimum pellet size and feeding frequency for maintaining the condition of adult rock lobsters; and
b. Providing continued advice to Project 98/305 on feeds development for adult lobster holding.

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE

1. Enhanced knowledge on the chemicophysical factors that affect the lobster's acceptability of a feed and how the attractiveness of the feed can be retained following immersion
2. Expanded database on the apparent digestibility of feed ingredients for southern lobsters *Jasus edwardsii*
3. Increased knowledge of the nutrients required by juvenile southern, *J. edwardsii*, and tropical, *Panulirus ornatus*, lobsters for high rates of growth and survival
4. Awareness that blue, *Mytilus edulis*, or green-lip, *Perna canaliculus*, mussels are not suitable as a sole food source for juvenile tropical lobsters
5. Improved feeding strategies and management of adult *J. edwardsii*

Research in FRDC RLEAS 98/303 established that tropical (*Panulirus ornatus)*, southern (*Jasus edwardsii*) and western (*Panulirus cygnus*) rock lobsters readily consumed formulated pelleted dry feeds and exhibited a dose dependent growth response to dietary protein concentration. The derived optimum dietary crude protein dry matter specification was 49, 37 and 57% for tropical, southern and western lobsters, respectively. However, growth rates of lobsters fed diets of either fresh mussel or expensive (AUD$7,500/tonne) extruded kuruma shrimp (*Penaeus japonicus*) feed pellets were from one third to five-times better than for the laboratory-pelleted diets. The sub-optimal performance of the laboratory-pelleted diets was attributed to a loss of attractiveness of the food after 1–2 hours immersion in the water and/or a sub-optimal supply of nutrients critical to growth and development of the lobsters. Based on these results, the focus of the research in this project (FRDC RLEAS 2000/212) was to enhance the lobster's acceptability of pelleted dry feeds and to advance knowledge on the animal's requirements for critical nutrients and the nutritive value of alternative protein ingredients. Additionally, research was carried out with adult southern rock lobsters to improve feeding and husbandry management practices.

Highlights of the research were:

- For juvenile southern rock lobsters, the attractiveness of pelleted dry feed and pieces of fresh blue mussel decreased with increasing immersion time in the water but pre-soaking an extruded kuruma shrimp diet for up to 8 h did not adversely affect the growth and survival of juvenile southern rock lobsters. The high nutrient specification and high water stability of the kuruma diet are possible reasons why lobster performance was not affected by pre-soaking. In studies examining the chemical cues eliciting feeding behaviour responses of lobsters, the free amino acid glycine was shown to stimulate greater feeding behaviour than either betaine or taurine. Including glycine in the diet formulation may improve the lobster's attractiveness to the feed.

- The chemical composition and time course of nutrients leaching from pelleted feeds containing natural fresh foods (such as mussel, bloodworm, squid and prawn) were

characterized and compared with the preference of juvenile tropical lobsters fed these diets. The study showed a strong correlation between the lobster's feeding preference and the chemical signature of the leachate. Highest correlations were for soluble protein and the free amino acids glycine and taurine while the rate of loss of dry matter, total nitrogen and other free amino acids was only weakly correlated. In contrast to the finding with juvenile southern lobsters, the attractability to juvenile tropical lobsters of kuruma and laboratory-made pelleted feeds and pieces of green-lip mussel decreased with increasing water immersion time. These results suggested that feeding more frequently than twice daily and incorporating protein hydrolysates into the dietary formulation as a rich source of free amino acids and soluble protein constituents might improve productivity of tropical lobsters fed pelleted feeds.

- A pelleted dry diet formulated with krill hydrolysate and krill as rich sources of free amino acids and soluble protein and fed four times daily produced excellent growth of juvenile tropical lobsters. Using this base formulation, the crude protein requirement of juvenile tropical lobsters was reassessed to be not less than 60% dry matter (57% air dry) while a dietary astaxanthin (carotenoid) specification of not less than 50 mg/kg was advocated for maintenance of good lobster colouration and as an aid in reducing the lobster's susceptibility to stress during culture. In these requirement studies, lobsters were additionally fed benchmark diets of green-lip and/or blue mussels. Lobsters grew well on the mussel diets for about 4 weeks but thereafter growth and survival decreased markedly, indicating the unsuitability of mussels as a sole food source for juvenile tropical lobsters. A similar effect of feeding fresh mussels has not been observed with either southern or western rock lobsters.

- The nutritional condition of southern rock lobsters was shown to be highly responsive to dietary carbohydrate to lipid ratio. Optimal condition of lobsters occurred on a diet containing 27% carbohydrate and 13.5% lipid (2:1 ratio) with growth rate and nutritional condition of the lobsters falling as the dietary carbohydrate content increased.

- The apparent digestibility of five marine and five terrestrial protein feed ingredients was determined for juvenile southern rock lobsters. Protein digestibility was highest for lupin flour (100%), mussel meat (98%) and wheat gluten (90%); intermediate (61-77 %) for prawn meal, fishmeal and soybean meal; and poor for defatted fish meal (53%), pea meal (52%), canola meal (38%) and squid meal (7%).

- Consumption of pelleted dry feed was not significantly affected by holding adult *J. edwardsii* at water temperatures of 15 or 23ºC and neither was it affected by pre-soaking the feed for 6 h. Feed intake of adult *J. edwardsii* lobsters, expressed as g dry feed consumed per g wet lobster weight, declined exponentially as a function of body weight over the examined range of 550 to 1,300 g.

2. ACKNOWLEDGEMENTS

This project involved research collaboration between CSIRO Marine Research, Cleveland, Tasmanian Aquaculture & Fisheries Institute, Taroona and the University of Adelaide, Adelaide. Each of these research agencies contributed staff, funds and facilities without which this work would not have been possible. Also gratefully acknowledged is the support of the South Australian Research and Development Institute, Port Lincoln, who agreed to the secondment of Dr Richard Musgrove to take responsibility for the adult lobster feeds management research work that had been planned to be done by Dr Simon Bryars, University of Adelaide, but who resigned at the commencement of the project. On behalf of all of the collaborating research agencies, I thank all staff engaged in the project for their enthusiasm, commitment and contributions without which little would have been achieved. This project is part of a FRDC-funded national research effort on Rock Lobster Enhancement and Aquaculture Subprogram (RLEAS). The support, guidance and technical contribution of the RLEAS coordinator, Dr Robert van Barneveld in this undertaking is acknowledged and I thank him for his critique of this and other Reports emanating from this work.

Kevin C Williams
15 January 2004

3. BACKGROUND

The rock lobster fishery is one of Australia's most important fisheries, worth about $400M pa. or 20% of Australia's total fishery catch; almost all of the catch is exported as live or chilled product to markets mainly in SE Asia and USA. Australia's fisheries are carefully managed to ensure a maximum sustainable yield. However, many other rock lobster fisheries in other parts of the world are heavily exploited while some have collapsed. Suffice to say that there is little prospect that the catch from the wild lobsters fisheries can be increased to meet the ever growing demand for marine lobsters. In Australia, rock lobster aquaculture could develop into a significant industry, equal in value to that of the wild catch, but this requires research to develop appropriate and profitable culture technologies. In the short term, aquaculture could assist in value-adding of the wild fishery by:

- the 'feed-lotting' of legal size lobsters for weight gain and niche marketing; and
- the on-growing of early juveniles collected from wild stocks and reared in either land-based or sea-based holdings.

Collection of juveniles from the wild would have to be based on a policy of local and regional biological neutrality to ensure preservation of the wild stocks. In the long-term, successful larval culture of rock lobster would enable complete domestication of the species and the supply of juveniles for on-growing essentially independent of the wild fishery.

Recognizing that a rock lobster aquaculture industry would not be economically feasible in the absence of a cost-effective pelleted feed for on-growing of adult and juvenile rock lobsters, FRDC supported an 18 month nationally-coordinated Project (FRDC 98/303) as part of the Rock Lobster Enhancement & Aquaculture Subprogram (RLEAS) to develop a formulated feed for the three key Australian rock lobster species – western (*P. cygnus*), southern (*Jasus edwardsii*) and tropical (*P. ornatus*). The Project terminated in December 1999. It showed that the developed dry pelleted diets were readily eaten by each of the lobster species investigated and that the lobsters moulted, grew reasonably well and had high survival rates. However, growth rates were not as good as when the lobsters were fed fresh mussel. The developed dry diet proved equal to fresh mussel for maintaining condition and improving the colour of adult *J. edwardsii* held in sea cages (Project 98/305). This project builds on the findings from the earlier research to develop improved and more cost-effective pelleted diets for rock lobsters.

4. NEED

Because Australia's rock lobster fisheries are carefully managed to ensure a maximum sustainable yield, the value of the industry can be increased only through the development of lobster aquaculture. In the immediate term, this could be achieved by on-growing of juveniles taken from the wild and the holding of adults for weight gain or niche marketing opportunities. In the longer term, domestication of the specie/s and hatchery propagation of the juveniles will enable a sustainable rock lobster aquaculture industry to develop. In Australia, these opportunities are seriously constrained by the lack of a cost-effective and efficacious rock lobster feed. This contrasts with the developing industry in New Zealand where waste from the large mussel industry is an available and inexpensive source of feed. If feed comprises from 40 to 50% of rock lobster production cost, as is the case for prawn and finfish grow-out operations, the availability of a suitable formulated pelleted feed is a necessity for commercial production.

Preliminary feed's development work (FRDC 98/303) for juvenile and adult lobsters was initiated in an 18-month project that terminated in December 1999. Although all lobster species consumed the developed dry feed pellets, the productivity of juvenile animals was inferior to the feeding of fresh mussels. However, with adult *J. edwardsii* held in sea cages, the pelleted diets were equal to fresh mussel in maintaining the condition (moult frequency, weight increase and survival) and colour of the lobsters. The sub-optimal performance of the pelleted diets for the juvenile lobsters was thought to have been due to the reduced attractiveness and/or sub-optimal nutrient specifications of the diet.

Improving the lobster's acceptance of the feed was identified as a key objective to be investigated with juveniles of both *P. ornatus* and *J. edwardsii*. A secondary objective was to extend earlier research to define the animal's requirements for key nutrients and to more closely examine the interaction between different nutrient classes. Strategies to improve feeding management and condition of adult *J. edwardsii* lobsters was also identified as a priority to support existing live-holding practices in South Australia. Collaborators in the research were CSIRO Marine Research (research on *P. ornatus*), Tasmanian Aquaculture and Fisheries Institute (research on juvenile *J. edwardsii*) and the University of Adelaide (research on adult *J. edwardsii*).

5. OBJECTIVES

1. Develop manufactured feeds for juvenile rock lobsters that optimize survival and growth by:
    a. Defining the chemicophysical cues that stimulate food consumption in juvenile rock lobsters;
    b. Developing pelleted feeds that remain attractive to lobsters for periods in excess of four hours after immersion; and
    c. Determining the optimum dietary specifications of selected nutrients required by juvenile rock lobsters for growth and development.
2. Develop manufactured feeds for adult lobsters for body maintenance and moult manipulation by:
    a. Determining the optimum pellet size and feeding frequency for maintaining the condition of adult rock lobsters; and
    b. Providing continued advice to Project 98/305 on feeds development for adult lobster holding.

6. TECHNICAL REPORT

6.1 Effect of water immersion time on the preference of juvenile tropical spiny lobster *Panulirus ornatus* for feeds containing alternative natural foods

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6.1.1 Abstract

The leaching rate and nature of leachates from fresh green-lip mussel *Perna canaliculus* (GLM) and pelleted dry feeds were quantified for immersion periods of 0-1, 1-2.5, 2.5-5 and 5-7.5 h and the preference of juvenile *P. ornatus* for these feeds was assessed relative to kuruma prawn feed (KPF). Four fishmeal-based, gluten-bound pelleted feeds were prepared, with fresh homogenates of GLM (MF), bloodworm *Marphysa sanguinea* (BF), prawn *Metapenaeus bennettae* (PF) and squid *Sepioteuthis spp* (SF) included at 50 g kg^{-1}dry matter (DM). Each of the pelleted feeds and KPF lost 7 to 10% DM in the 0-1 h period and a further 3 to 8% over the next 6.5 h with total loss greatest for KPF (17%) and least for PF and SF (11%). DM loss of GLM was 27% for 0-1 h and a further 7% in the next 6.5 h. In the 0-1 h period, loss of Kjeldahl N, trichloroacetic acid-precipitated protein (TCA-P) and total free amino acids from GLM was from 2.5 to 6-fold greater than for KPF while losses from pelleted feeds were generally slightly lower than for KPF. Pelleted feeds and GLM were pre-soaked for 0, 2.5 or 5 h and individually preference-tested (n = 6) against KPF, either following the same pre-soaking period (E1) or un-soaked (E2). In E1, the lobster's preference for test feeds was greatest for GLM (60.7%) and least for SF (44.6%) and these effects were independent of water immersion time. In E2, increasing the immersion time reduced the lobster's preference of feeds tested but preference for GLM always exceeded that of un-soaked KPF. Regression of the proportional intake of test feeds and the leach rate of the test feed relative to that of KPF identified TCA-P, glycine and taurine as the principal leachate components with the highest positive correlations with the lobster's feeding preference.

Keywords: rock lobster; leachate; soaking; mussel; attractant; homogenates, amino acid

6.1.2 Introduction

The on-growing of tropical spiny lobsters *Panulirus ornatus* from wild seed is a flourishing aquaculture industry in many parts of SE Asia and notably in Vietnam where current annual production is estimated to be about 2,000 tonnes, with a farm-gate value of US$70 to 75 million (Tuan *et al*. 2000; L. H. Tuan, pers. comm.). Culture is based entirely on the feeding of fresh fishery product with swimming crab, rice paddy snail, oysters and fish bycatch being the predominant food types making up the 'trash' fish that is fed to the lobsters. Subject to availability and cost, shellfish is preferred over that of finfish and especially for small juvenile lobsters. The dwindling supply of trash fish and the downstream environmental impacts of its use with intensive cage aquaculture are of increasing concern to aquaculturists and environmentalists alike (Chamberlain & Rosenthal 1995; New 1996; Barg et al. 1997; Harache 2002).

The development of a palatable and efficacious pelleted feed that enable tropical spiny lobsters to grow rapidly and economically to a marketable size is a high priority. We have previously shown that juvenile *P. ornatus* lobsters readily eat dry feed pellets and that lobster growth exhibits a clear dose dependency response to dietary protein concentration (Smith *et al*. 2003b). However, the observed growth rate of the lobsters, even on the best performing laboratory-made feeds, was less than that seen in the wild (Dennis *et al*. 1997) or when lobsters were cultured on trash fish (Tuan *et al*. 2000; L.H. Tuan, pers. comm.). We also observed that the lobsters were less inclined to eat after dry feed pellets had been immersed in the water for 1 to 2 h, whereas the attractiveness of shucked mussel remained for 10 or more hours after immersion. In contrast, immersion of a commercially-extruded kuruma prawn feed pellet in water for up to 8 h prior to feeding to temperate spiny lobster *Jasus edwardsii* had no demonstrable effect on lobster growth during a 16-week experiment (Tolomei et al. 2003). However, the lobster's attractiveness to the soaked feed was much lower than for the non-soaked prawn pellet or mussel flesh.

The objectives of the present work were threefold. Firstly, we sought to characterize the chemical nature and rate that compounds leach from fresh mussel and pelleted feeds during immersion periods of up to 7.5 h. We incorporated various fresh natural foods into laboratory-made pelleted feeds to test how their inclusion influenced the chemical profile of the resultant leachate and compared it to the profile from fresh-shucked mussel or a commercial, extruded, kuruma prawn feed pellet. Secondly, we determined the lobster's feeding preference for the same feeds that had, or had not, been soaked for the same time before feeding. Thirdly, we sought to see if any relationship existed between the chemical profile of the feed leachates and the observed lobster's feeding preference for the feeds. This paper reports the results of this work and discusses how selecting ingredients to optimise the release of chemical attractants and feeding stimulants could aid the development of more attractive pelleted feeds for the culture of tropical spiny lobsters.

6.1.3 Methods

6.1.3.1 Feed leachate study

The nature of the nitrogenous compounds leaching from feeds was investigated by analysing a series of leachates from four laboratory-made pelleted feeds (Table 1), an extruded, kuruma prawn (*Penaeus japonicus*) feed (KPF) (Ebi Star, Higashimaru Co. Ltd., Kagoshima, Japan), and pieces of fresh green-lip mussel (*Perna canaliculus*) (GLM). Weighed feed samples (feed pellets: 2 replicates of ~1 g air-dry; GLM: 5 replicates of ~5 g wet) were immersed in jars containing 70 mL of deionised water and placed at 28°C in a shaking water bath (oscillating at 40 rpm) for periods of 0 to 1, 1 to 2.5, 2.5 to 5 and 5 to 7.5 h. At the end of each immersion period, the samples were removed from the solution onto a 1 mm mesh screen, and re-immersed in another 70 mL of deionised water for the next immersion period. The leachate solution was further filtered through a membrane filter (0.45um Durapore HVLP, Millipore Pty Ltd, Bedford, MA, USA) and stored at -20°C until chemical analysis.

Crude protein (CP) content of the leachate was calculated as nitrogen content (N) x 6.25, with N content determined by a Kjeldahl digestion of a 50 ml sample, followed by distillation of the liberated ammonia into 2% boric acid, and titration of the boric acid with hydrochloric acid to an end point at pH 5.0. Protein content of the leachate was determined by the bicinchoninic method

(BCA Protein Assay, Pierce Biotechnology Pty. Ltd., Rockford, IL, USA) after prior protein precipitation using trichloroacetic acid (TCA), following the protocol recommended by Pierce Biotechnology Technical Notes. Total free amino acids were determined from a sample of the supernatant following centrifugation of the TCA precipitate, using the ninhydrin method described in Blackburn (1968) as modified using dimethyl sulphoxide as the solvent (Moore, 1968). Glutamic acid was used as the standard. Individual free amino acid profiles in the leachates were determined for the 0 to 1 h and 2.5 to 5 h samples by high performance liquid chromatography (HPLC) using the AccQ-Tag method (Waters Pty. Ltd., Milford, MA, USA). Leachate solutions were prepared for analysis by filtration through Ultrafree-MC 5000 NMWL centrifugal filter units (Amicon Bioseparations Pty. Ltd., Bedford, MA, USA). The DM loss (water stability) from the feeds was determined for matching periods of water immersion using standardised procedures (Smith et al., 2002; 2003a).

Table 1. Formulation (g kg^{-1}) of the laboratory-made feeds used to examine the chemical composition of leachates and lobster feeding preference in Experiments 1 and 2

Ingredient	Feed designation			
	MF	BF	PF	SF
Mussel[1]	50	0	0	0
Bloodworm[1]	0	50	0	0
Prawn[1]	0	0	50	0
Squid[1]	0	0	0	50
Fishmeal (68% CP)	600	600	600	600
Wheat flour	200	200	200	200
Squid oil	30	30	30	30
Vitamin premix[2]	2.5	2.5	2.5	2.5
Other ingredients[3]	117.5	117.5	117.5	117.5

[1] DM equivalent of incorporated fresh constituent based on analysed DM content of 194, 210, 207 and 203 g kg^{-1} for mussel, bloodworm, prawn and squid, respectively.

[2] Provided in final feed (mg kg^{-1}): Retinol (A), 1.8; ascorbic acid (C), as ascorbyl-2-polyphosphate, 100; cholecalciferol (D_3), 0.03; menadione (K_3), 10.0; d/l α-tocopherol (E), 200; choline, 500; inositol, 100; thiamine (B_1), 15; riboflavin (B_2), 20; pyridoxine (B_6), 15; pantothenic acid, 50; nicotinic acid, 75; biotin, 0.5; cyanocobalamin (B_{12}), 0.05; folic acid, 5; and ethoxyquin, 150.

[3] Comprised (g kg^{-1} of feed): Wheat gluten, 50; krill meal, 50; cholesterol, 2; lecithin, 12; carophyll pink (8% astaxanthin), 0.5; ascorbyl-2-polyphosphate (Stay-C), 1; and ethoxyquin (Banox E), 2.

6.1.3.2 Lobster preference feeding experiments

Two preference feeding experiments were carried out to test the lobster's response to the feeds examined in the leachate study. In Experiment 1, the lobster's preference for either of five feeds that had been soaked in water for periods of 0, 2.5 or 5 h and presented as a paired choice with KPF was determined; the KPF pellets being immersed in water for the same period of time as the test feed to which it was being choice-compared. The five feeds comprised shucked GLM and four laboratory-made dry pelleted feeds (Table 1) that contained either GLM flesh (MF), freshly caught bloodworm *Marphysa sanguinea* (BF), freshly caught prawn *Metapenaeus bennettae* (PF), or freshly caught whole squid *Sepioteuthis* spp (SF) included as the fresh constituent at a rate equivalent to 50 g kg^{-1} dry matter (DM). The five test feeds were compared simultaneously

using triplicate tanks (15 tanks). The three water immersion treatments were examined sequentially and in random order. Upon completion of this testing, the same lobsters were randomly re-assigned to tanks and the aforementioned testing of the feeds and immersion periods was repeated a second time. Since the experiment was carried out under closely controlled water temperature and photoperiod conditions, the confounding of the sequential testing of the water immersion times was considered to have a negligible effect on the outcome results. Hence, the experiment was analysed as a 3 (immersion time) x 5 (feed type) factorial experiment with six replicates (Venables & Ripley 1994). Preference was measured as the amount of test feed consumed by the lobsters, on a DM basis, expressed as a percentage of the total DM feed eaten.

The second lobster preference feeding study (Experiment 2) was similar to the first except that the feeds were limited to three – the laboratory-made MF, and GLM and KPF but the same water immersion times were applied. The lobster's preference for the test feeds was assessed relative to the consumption of un-soaked KPF. All three immersion-times were compared simultaneously with six replicate tanks (18 in total) applied to each treatment; the different feed types were assessed in sequential but random order. The data were analysed as a 3 (immersion time) x 3 (feed type) factorial design with six replicates.

6.1.3.3 Feed preparation

Each of the fresh natural food constituents was homogenized using a high-speed laboratory blender and incorporated at a rate equivalent to a DM contribution of 50 g kg^{-1} (Table 1). All other major feed ingredients were purchased from commercial sources. The vitamin premix was a custom mix supplied by Rhone-Poulenc, Queensland; ascorbyl-2-polyphosphate sodium (Stay-C, Roche Vitamins Australia) was provided by Ridley Agriproducts, Narangba Queensland; and astaxanthin (carophyll pink, 8% astaxanthin) was donated by Roche Vitamins Australia, Sydney. Other feed additives were purchased from Sigma Chemicals. Ingredients that were too coarse to pass through a 740 µm screen were finely ground (Mikro-Pulverizer, NJ, USA) prior to mixing.

Diets were prepared by thoroughly mixing together all dry ingredients using a planetary dough mixer (Hobart A200, Hobart Corporation, Troy, Ohio, USA). The wet constituents, including the squid oil, were added along with sufficient de-ionised water (approximately 400 ml kg^{-1} DM) to form a soft dough. After mixing to a uniform consistency, the dough was twice cold pressed through a meat mincer (Hobart Corporation, Troy, Ohio, USA), which had a die plate with 2.5 mm holes. After the second extrusion, the spaghetti-like strands were steamed for 5 min. and dried overnight in a forced-draught oven at 40ºC. After drying, the strands were reduced to a pellet length of 3 to 4 mm to enable lobsters of this size to ingest them with minimal fragmentation and wastage. Pellets were stored frozen (-20°C) until immediately before being fed to the lobsters.

6.1.3.4 Experimental animals and management

Approximately 400 juvenile *P. ornatus* of ~ 0.5 to 2 g were collected from Trinity Inlet, Cairns, North Queensland, (16°55' S, 145°45' E) by divers and shipped to the CSIRO Cleveland laboratory. The lobsters were placed in a 2000 L tank and supplied with aerated, flow-through filtered (20 µm) seawater at 28°C and salinity of 33 to 35 g L^{-1}. Lobsters were fed a mixture of

GLM and KPF twice daily to satiety. After 6 weeks, lobsters were weight sorted into two groups (blocks) of 50 lobsters and one group of 35 lobsters for Experiment 1. Within each of these groups, lobsters were randomly and equally distributed to 15 tanks such that stocking density was 10 lobsters per tank for two of the blocks and 7 of the larger group of lobsters per tank for one of the blocks. The mean (± SD) initial weight of the 135 lobsters used in Experiment 1 was 5 ± 3.2 g. For Experiment 2, 138 lobsters of initial weight 83 ± 26.4 g were weight sorted into two blocks each of 48 animals and one block of 42 animals. Within these groups, lobsters were randomly and equally distributed to 18 tanks (eight lobsters per tank for two of the blocks and seven of the larger group of per tank for one of the blocks). Rectangular tanks (1500 x 600 x 500 mm deep, 350 L capacity) constructed of fiberglass with a black gel coat on the inside and the bottom treated to provide a non-skid surface were used in both experiments. Sufficient clay bricks with holes were placed in each tank to provide shelters for the lobsters and tanks were fitted with twin-walled polycarbonate sheeting covers. The tanks were supplied with aerated flowing seawater (1L min^{-1}) at 28°C and salinity of 33 to 35 g L^{-1}. The water intake was placed at the bottom and centre of the tank and the water exited through a standpipe situated in the centre at the water surface, thus minimizing any longitudinal flow. Tanks were housed in an enclosed seawater laboratory where photoperiod was controlled to a cyclical schedule of 12 h light and 12 h dark.

The daily management of the lobsters was similar for both preference feeding experiments. A weighed amount of KPF and the test feed to which it was being compared was separately spread on a circular feed tray (300 mm diameter) placed randomly at opposite ends of the tank. The feed was dispensed twice daily at 0930 and 1300 h and uneaten feed was quantitatively collected 2.5 h after feeding. Collected feed was dried and actual feed consumption reconciled after taking into account expected DM leaching losses over the feeding period. Each experimental sub-set testing period comprised six days acclimatization followed by six days of preference testing. At the conclusion of each testing period, the immersion time treatments (in Experiment 1) or feed treatments (in Experiment 2) were randomly re-assigned to the tanks and the placement of feed on the trays within tanks also randomly re-assigned. The amount of each feed allocated was adjusted daily to ensure an excess of each feed type was present during the 2.5 h feeding period. Tanks were siphoned cleaned on a daily basis. Experiment 1 took 13 weeks to complete all testing periods during which the lobsters increased in size to an average final weight of 25 ± 10.3 g. Experiment 2 was of 5 weeks duration with the lobsters increasing to an average final weight of 106 ± 39 g.

6.1.3.5 Nutrient analyses

Finely ground samples were analysed in duplicate by standard laboratory methods essentially in accordance with AOAC (1999) recommendations, at either the CSIRO Marine Laboratory, Cleveland or at the Queensland Department of Primary Industries' Animal Research Institute, Yerrongpilly. DM was determined by oven drying at 105°C to constant weight; ash by ignition at 600°C for 2 h; total nitrogen (total N) by a macro-Kjeldahl technique on a Kjel Foss automatic analyser using mercury in the digestion; crude protein (CP) was calculated by multiplying total N by 6.25 irrespective of the nature of the N. Total lipid was determined gravimetrically after a Bligh & Dyer (1959) extraction as modified by Christie (1982). The composition of the GLM and pelleted feeds examined in this study is detailed in Table 2.

Table 2. Chemical composition of the feeds used to examine the chemical composition of leachates and lobster feeding preference experiments

Analysis	Feed designation[1]					
	GLM	KPF	MF	BF	PF	SF
	Air dry basis (g kg^{-1})					
Dry matter	193	916	942	944	946	940
	Dry matter basis (g kg^{-1})					
Ash	194	178	116	113	117	115
Crude protein	583	617	604	605	585	593
Total lipid	108	134	154	153	154	150

[1] GLM, Shucked fresh green lip mussel; KPF, commercial extruded kuruma prawn feed pellets; MF, BF, PF and SF, laboratory-made pelleted feeds containing fresh mussel, bloodworm, prawn and squid mantle, respectively.

6.1.3.6 Statistical analyses

In accordance with the factorial design of each Experiment, data were analysed as a two-way ANOVA using statistical packages (Queensland Department of Primary Industries) for balanced data (BALF), taking into consideration the blocked design of the experiment (Venables & Ripley 1994). Percentage feed consumption (preference) data were analysed as the natural and arcsine-transformed values, but as the transformation did not materially alter the significance of the *F*-statistic, only the natural values are reported. Differences between treatment effects were examined *a posteriorly* using Fischer's protected *t*-test (Snedecor & Cochran 1989) wherein differences between means were examined only where the F-test of the ANOVA was significant ($P < 0.05$).

6.1.4 Results

6.1.4.1 Chemical composition of leachates

All four laboratory-made dry pelleted feeds and the extruded KPF had a similar pattern of DM loss of between 7 and 10% in the first h and a further 3 to 8% over the next 6.5 h (Table 3). Overall, DM loss was greatest for KPF (17%) and least for PF and SF (11%). Well-drained GLM lost 27% of its DM in the first h and a further 7% in the following 6.5 h. The total amount of free amino acid protein equivalents leaching from the laboratory-made feeds in the first h was between 15.0 and 18.0 mg g DM^{-1} h^{-1} compared with 21.3 mg g DM^{-1} h^{-1} for KPF and 92.4 mg g DM^{-1} h^{-1} for GLM. Over the next 6.5 h, the hourly rate of total free amino acid loss decreased rapidly for all feeds. For each water immersion time, the rate of total free amino acid loss from GLM generally exceeded that of the pelleted feeds but this difference decreased with increasing water immersion time (Table 3). The rate of leaching of soluble protein assayed as TCA-precipitated protein was more than five times as fast for GLM than for the pelleted feeds for the 0 to 1 h immersion period (16.3 vs 2.5 to 3.2 mg g DM^{-1} h^{-1}, respectively). The rate of soluble protein loss declined rapidly with increasing water immersion time for all feeds but more noticeably with GLM (Table 3). Loss of total CP (N x 6.25) in the 0 to 1 h water immersion period was greatest for GLM (101.4 mg g DM^{-1} h^{-1}) and about 3-fold higher than for the laboratory-made feeds or KPF, which were similar (27.5 to 41.2 mg g DM^{-1} h^{-1}). Thereafter, the rate of total protein loss decreased markedly with increasing water immersion time, particularly

for the GLM, such that the rate of loss after 1 h immersion was similar for all feeds, reducing to between 0.3 and 0.6 mg g DM^{-1} h^{-1} in the 5 to 7.5 h period (Table 3).

Table 3. Effect of consecutive water immersion periods (CWIP) on loss of dry matter (DM), total free amino acid protein equivalents, TCA-precipitated protein, total protein (N x 6.25) and un-recovered protein equivalents from feeds used in lobster preference feeding Experiments 1 and 2

			Feed designation[1]			
CWIP (h)	GLM	KPF	MF	BF	PF	SF
			DM loss (mg g DM^{-1} h^{-1})			
1	271	93	101	100	71	70
2.5	16	20	08	25	12	10
5	1	13	6	0	4	7
7.5	10	0	0	2	0	0
			Total DM loss (mg g DM^{-1})			
0 to 7.5	340	172	129	157	107	112
			TCA-precipitated protein (mg g DM^{-1} h^{-1})			
1	16.3	2.9	2.6	3.2	2.8	2.5
1 to 2.5	4.7	2.7	1.8	1.8	1.7	1.4
2.5 to 5	2.8	1.7	0.9	1.1	1.1	1.0
5 to 7.5	1.8	0.8	0.5	0.8	0.6	0.5
			Total free amino acid protein equivalents (mg g DM^{-1} h^{-1})			
1	92.4	21.3	16.4	18.0	16.7	15.0
1 to 2.5	11.2	5.5	3.7	5.2	4.6	4.6
2.5 to 5	2.0	1.4	0.8	1.3	1.2	1.3
5 to 7.5	0.6	0.4	0.3	0.6	0.3	0.4
			Total crude protein (N x 6.25) (mg g DM^{-1} h^{-1})			
1	101.4	41.2	27.5	36.9	31.2	29.7
1 to 2.5	14.3	15.3	9.2	15.4	11.2	10.8
2.5 to 5	4.5	6.4	2.7	5.8	3.3	4.3
5 to 7.5	1.7	2.2	1.4	3.3	1.7	1.7
			Un-recovered protein equivalents (mg g DM^{-1} h^{-1})[2]			
1	-8.7	17.0	8.5	15.7	11.7	12.2
1 to 2.5	-1.6	7.1	3.7	8.4	4.9	4.8
2.5 to 5	-0.3	3.3	1.0	3.4	1.0	2.0
5 to 7.5	-0.7	1.0	0.6	1.9	0.8	0.8

[1] See Table 2 for full description of feeds.

[2] Difference between total crude protein and the sum of total free amino acid protein equivalents and TCA-precipitable protein.

The free amino acids present at the greatest concentrations in the 0 to 1 h leachate were glycine, taurine and alanine for all feeds: concentrations of proline, histidine and arginine were also comparatively high in the leachates from the pelleted feeds. Glycine and taurine were present at the highest concentrations in the 2.5 to 5 h leachate of all feeds (Table 4). The rate of total free amino acid loss from GLM was 6 to 10-fold higher than for all pelleted feeds in the 0 to 1 h immersion period and from 5 to 12-fold higher for the 2.5 to 5 h immersion period (Table 4).

Table 4. Effect of water immersion time on the loss of individual free amino acids (mmol g DM^{-1} h^{-1}) from feeds used in lobster preference feeding Experiments 1 and 2

	Feed designation[1]					
Amino acid	GLM	KPF	MF	BF	PF	SF
	Hourly rate of loss when immersed for 1 h					
Ala	29.0	19.4	14.8	12.1	12.4	12.1
Arg	23.4	7.0	5.7	2.9	3.1	3.3
Asp	26.3	1.6	1.4	1.3	1.3	1.6
Glu	25.1	5.2	5.9	3.4	4.3	5.4
Gly	417.9	32.7	16.3	6.4	19.7	32.5
His	0	7.6	8.1	5.8	6.8	7.1
Ile	3.8	2.4	2.0	1.9	1.7	1.9
Leu	4.7	4.3	4.0	3.9	3.2	3.7
Lys	10.1	3.7	3.3	2.3	2.5	3.0
Met	0	0	0.4	0.6	0	0.2
Phe	1.9	1.4	1.5	1.5	1.1	1.2
Pro	6.0	9.2	9.6	7.8	6.7	7.3
Ser	14.5	2.7	1.9	1.2	1.6	1.7
Tau	290.4	38.6	15.5	26.2	31.6	24.0
Thr	5.3	1.9	0	0.6	0	4.3
Tyr	2.0	0.8	0.8	0.9	0.5	0.5
Val	4.8	3.5	3.3	2.8	2.6	3.1
Total	865.2	142.0	94.7	81.9	99.2	113.2
	Hourly rate of loss when immersed for 2.5 to 5 h					
Ala	2.7	1.3	1.3	1.3	0.5	0.5
Arg	2.7	0.8	1.1	0.5	0.3	0.9
Asp	1.8	0.2	0.3	0.3	0.2	0.2
Glu	2.3	0.4	0.9	0.6	0.3	0.3
Gly	20.8	1.7	1.4	0.5	0.6	0.9
His	1.1	0.9	1.2	0.9	0.6	0.7
Ile	0.4	0.3	0.3	0.3	0.1	0.2
Leu	0.5	0.4	0.5	0.5	0.2	0.4
Lys	1.0	0.5	0.6	0.4	0.3	0.7
Met	0.2	0.0	0.0	0.1	0.0	0.0
Phe	0.3	0.2	0.3	0.3	0.1	0.2
Pro	1.3	1.1	1.3	1.2	0.7	0.8
Ser	1.2	0.2	0.3	0.2	0.1	0.1
Tau	17.9	2.0	1.4	2.7	0.7	0.7
Thr	0.6	0.2	0.1	0.1	0.0	0.0
Tyr	0.3	0.1	0.2	0.2	0.0	0.1
Val	0.6	0.3	0.4	0.4	0.1	0.2
Total	55.7	10.6	11.4	10.4	4.7	6.6

[1] See Table 2 for full description of feeds.

6.1.4.2 Lobster Experiment 1

Feed type and immersion time both significantly affected ($P < 0.05$) the lobster's preference for KPF but the interaction between these main effects was not significant ($P > 0.05$) (Table 5). The proportional intake of the test diet was greatest with GLM for all immersion periods, and lowest

with SF, but intermediate and similar to that of KPF with the other laboratory-made feeds. Immersion of the feed for 2.5 h resulted in lower proportions of test feed being consumed than non-immersed feeds and feeds that had been immersed for 5 h, which were similar.

Table 5. Effect of water immersion time and feed type on the proportion of test feed eaten by juvenile *P. ornatus* lobsters in Experiment 1, expressed as % of all DM eaten[1]

	Immersion time			
Feed type[2]	0 h	2.5 h	5 h	Feed mean
GLM	65.5^{A}	57.0^{BC}	59.6^{B}	60.7^{P}
MF	52.6^{DEF}	45.2^{G}	50.8^{EF}	49.5^{R}
BF	55.7^{CD}	46.8^{G}	52.9^{DEF}	51.8^{Q}
PF	55.4^{CDE}	46.5^{G}	53.7^{CDEF}	51.9^{Q}
SF	43.7^{G}	38.1^{H}	52.1^{EF}	44.6^{S}
Immersion mean	54.6^{X}	46.7^{Y}	53.8^{X}	$(\pm 1.25)^{3}$

[1] Comparison was between test feeds soaked for various times against KPF that had been soaked for the same time. The DM quantity of test feed consumed by the lobsters is expressed as a percentage of the total DM eaten by the lobsters. Since the analysis of the natural and arcsine-transformed data showed identical statistical significance for all effects, only the natural percentage data are shown.

[2] Refer to Table 2 for full description of the feeds.

[3] Standard error of the mean for the interaction term (Soak time x Feed type).

[A, B, C, D, E, F, G & H; P, Q, R & S; X & Y] Within respective interaction (Soak time x Feed type) and main effects (Soak time and Feed type) respectively, means without a common superscript letter differ ($P < 0.05$).

6.1.4.3 Lobster Experiment 2

The interaction between the main treatment effects was not significant ($P > 0.05$) but the lobster's preference was significantly affected by both feed type and immersion time (Table 6). The proportional intake of test diet was greatest ($P < 0.05$) with GLM, but for MF and KPF was similar to that for the un-immersed KPF. Preference for the test diets progressively decreased ($P < 0.05$) with increasing water immersion time.

Table 6. Effect of water immersion time and feed type on the proportion of test feeds eaten by juvenile *P. ornatus* lobsters in Experiment 2, expressed as % of all DM eaten[1]

	Immersion time			
Feed type[2]	0 h	2.5 h	5 h	Feed mean
GLM	64.2	62.4	60.2	62.3^{A}
MF	39.6	36.2	32.2	36.0^{B}
KPF	48.0	32.2	27.4	35.8^{B}
Immersion mean	50.6^{X}	43.6^{Y}	39.9^{Y}	$(\pm 3.43)^{3}$

[1] Comparison was between feeds immersed in water for various times and non-immersed KPF. The DM quantity of test feed consumed by the lobsters is expressed as a percentage of the total DM eaten by the lobsters. Since the analysis of the natural and arcsine-transformed data showed identical statistical significance for all effects, only the natural percentage data are shown.

[2] Refer to Table 2 for full description of the feeds.

[3] Standard error of the mean for the interaction term (Soak time x Diet type).

[A, B; X, Y] Within respective main effects, means without a common superscript letter differ ($P < 0.05$).

6.1.5 Discussion

6.1.5.1 Composition of the feed leachate

Immersion of the feed in water for 7.5 h resulted initially in a rapid loss of DM and soluble and particulate nitrogenous compounds with all feeds. Nitrogenous compounds appeared to be the major chemical compounds leaching from the feeds, accounting for 70 to 80% of the DM lost in the first hour, except for MF and BF pellets where the proportions were 46 and 58%, respectively. Why these two last mentioned feeds should have more of the DM lost as non-nitrogenous compounds is unknown but possibly relates more to subtle differences in diet manufacture than to the test ingredient included *per se*. However, it cannot be satisfactorily explained as being due to inadequate feed binding since the water stability of these feeds (measured as total DM loss during immersion) was no worse than for other pelleted feeds. In the first hour following immersion, loss of nitrogenous compounds was 2.5-fold or greater for GLM compared with all pelleted feeds. Differences between the pelleted feeds in their losses of individual nitrogenous compounds were small, with the commercially extruded KPF having a seemingly higher loss rate of total free amino acids, which was due predominantly to the leaching of free taurine, glycine and alanine (Table 4). Differences in leaching losses between the laboratory-made pellets in the first hour of immersion were comparatively minor: DM loss appeared to be greater for the PF pellets while total CP loss was greatest for the BF pellets (Table 3). Losses of individual free amino acids from the laboratory-made pellets, reflecting the nature of the constituent fresh foods included in the feed, differed between feeds with the loss of glycine being highest for SF, and similar to the commercially extruded KPF (32.5 vs 32.7 mmol g DM^{-1} h^{-1}, respectively), and lowest for BF (6.4 mmol g DM^{-1} h^{-1}). The rate of taurine loss was low for the MF pellets and only about half that of the other laboratory-made pellets.

The rate of loss of DM and nitrogenous compounds from the feeds declined rapidly as the immersion time extended beyond 1 h and as a result, differences in the leaching rate between the various feeds also diminished. However, GLM consistently had a higher rate of loss of TCA-precipitated protein for the whole 7.5 h immersion period and a higher loss of total free amino acids for the 2.5 to 5 h immersion period compared to other feeds (Tables 3 and 4). The higher loss rate of free amino acids for GLM was predominantly due to the leaching of glycine and taurine, which together accounted for about 70% of the loss of total free amino acids.

There are very few studies where the temporal leaching loss of nutrients from pelleted feeds have been studied. Loss of amino compounds from natural herring and polyurethane-bound artificial lobster baits was shown by Daniel and Bayer (1987, 1989) to be most rapid immediately upon immersion in water and for the loss rate then to decline exponentially. A similar effect was observed by Lokkeborg (1990) who compared the rate at which total dissolved amino acids were released from pieces of fresh mackerel or a carrageenan-bound aqueous methanol shrimp extract upon immersion in water. Amino acids were leached most rapidly within the first 1.5 h of immersion and for the release rate to continually decrease over the next 2 to 24 h.

The quantitative and temporal changes in the chemical composition of the leachate observed for the various diets should not be regarded as being indicative of the ingredient composition of the pelleted diet *per se*. In each of the pelleted diets, the test ingredient made up only a small proportion (less than 10%) of the total protein content of the diet. While these constituents would

have made some contribution to the chemical composition of the resultant leachate, the contribution from the other dietary ingredients would have been equally, if not more important. Moreover, the leach rate and subsequent composition of the leachate could also have been significantly affected by subtle differences in the way each diet was manufactured. In the context of the purpose of the study, it was not important to understand what ingredient or manufacturing effect resulted in the observed differences in leachate composition or leaching rate loss. Rather, the diets, including that of the fresh GLM, provided an array of leachates, varying quantitatively and temporally in chemical composition, by which differences in the lobster's feeding preference might be compared and possible cause and effect relationships determined.

6.1.5.2 *Lobster's feed preference*

In designing the preference feeding experiments, the KPF was chosen as the feed to which all other feeds would be compared because of its previously demonstrated superiority over laboratory-made lobster pellets (Smith *et al*. 2003b). Selection of the fresh natural foods for incorporation into the laboratory-made pelleted feeds was based on the observation that invertebrates, particularly molluscs, crustaceans and polychaetes, were the dominant natural foods eaten by wild *P. ornatus* sub-adults (Joll & Phillips 1986). This food selection is similar to that observed for other spiny lobsters (Mayfield et al. 2000a, b; Goni et al. 2001). Although squid appears not to be an important item in the diet of spiny lobsters, it was included in this study because previous work had demonstrated its inclusion in formulated pelleted dry feeds was equally efficacious as fresh mussel in increasing the lobster's attractiveness for the feed (Williams 2001).

In the preference testing of the feeds, it came as no surprise that fresh mussel was found to be more attractive than KPF, being consumed in a significantly greater amount. Perhaps what was surprising was that the attractiveness of the mussel persisted after it had been immersed in water for 5 h (Tables 5 and 6). Even when compared with non-immersed KPF, the lobsters consumed more of the 5 h-immersed mussel than KPF (Table 6). However, there was a progressive loss of attractiveness with increasing period of immersion for each of the three feeds – GLM, MF and KPF – when compared to non-immersed KPF in Experiment 2 (Table 6). The loss of attractiveness of pelleted feed following immersion in water is undoubtedly due to the slowing down, or exhaustion, of leached soluble compounds that act as attractants to stimulate foraging and feeding. Much is known about the role of chemoreception in the feeding behaviour of crustaceans (see particularly Mackie *et al*. 1972; Carr 1988; Lee & Meyers 1997). Compounds that evoke the greatest feed attractant stimulus are characterized as being generally less than 1000 dalton molecular weight, soluble in water, non-lipid and amphoteric or basic in charge. Thus, they are typically free amino acids, nucleotides, nucleosides, organic acids or tertiary amine compounds and rarely lipids. Chemicals that elicit the greatest feed attractant stimulus are the free amino acids taurine and glycine, the nucleotide adenosine 5-triphosphate, the quartenary ammonium compound betaine and combinations of the former. In contrast, ammonia is inhibotory of feeding behaviour. However, as clearly demonstrated for both spiny (Lynn et al. 1994; Derby 2000) and clawed (Mackie 1973; Daniel & Derby 1991) lobsters, mixtures evoke a greater feeding behavioural response than what would be expected additively for the individual constituents. However, there are very few studies with crustaceans where behavioural responses to attractants have also been extended to examine their effectiveness in increasing the animal's

feed consumption or growth rate. Studies that have been done have given highly equivocal results: a positive enhancement was reported by Deshimaru & Yone (1978), Murai et al. (1985) and Pittet et al. (1996) with penaeid shrimp and by Costa-Pierce & Laws (1985) with the fresh water prawn whereas no effect was observed by Hartati & Briggs (1993) with penaeids.

We sought to see if the observed feeding preference of lobsters in the present study could be correlated with the nature and rate at which chemicals leached from the feeds. We regressed the proportional consumption of the test feed consumed by the lobsters against the rate that each of the main chemical groups and free amino acids leached from the feed. The chemical leach rate was expressed as a ratio of the leach rate of the respective chemical for KPF. Only the leach rate data for the first 2.5 h water immersion time were used in the analysis since this was the time period over which feed intake preference of the lobsters was measured. The relationships for the main chemical classes – DM, TCA-precipitated protein, total CP and total free amino acids – are shown in Figure 1 while Figure 2 shows the relationships for the four most abundant free amino acids – glycine, taurine, alanine and arginine.

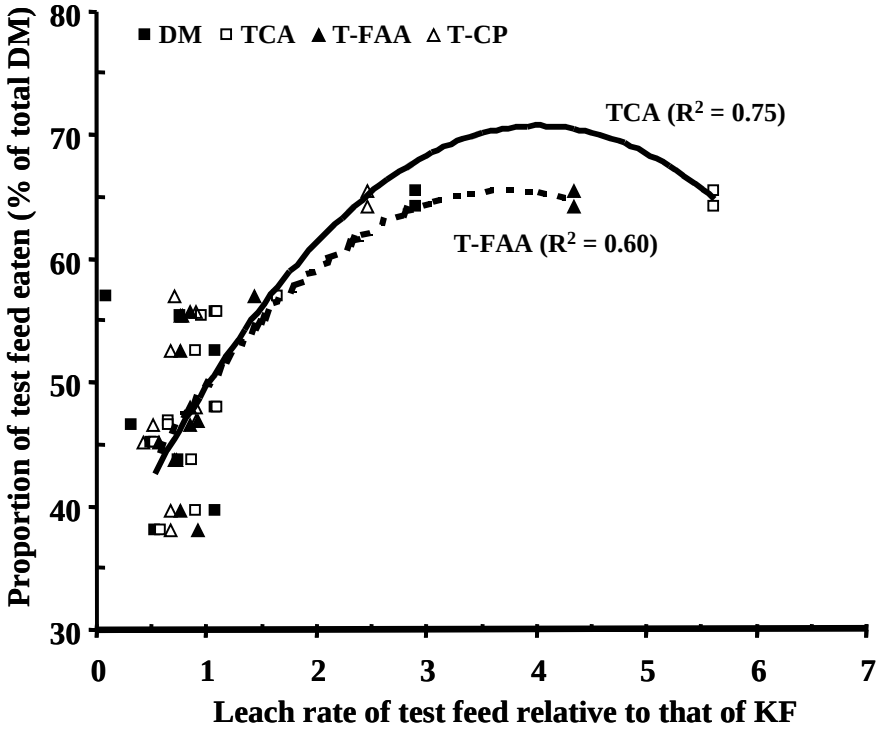

Figure 1. Relationship between the proportion of test feed eaten by juvenile *P. ornatus* lobsters in Experiments 1 and 2 (Y; % of total dry matter (DM)) and the rate at which test feeds leach DM, TCA-precipitable protein (TCA), total free amino acid (T-FAA) or total protein (T-CP) when expressed as a ratio of the respective leach rate for the reference kuruma feed. All data are shown but only the most significant ($P < 0.05$) relationships are drawn for reasons of clarity.

For the major chemical groups, there were significant ($P < 0.05$) correlations between the lobster's feed preference and the relative leaching rate of TCA-precipitated protein and total free amino acids. The rate of DM and total-CP loss from the feeds was only weakly correlated with

feeding preference. For the free amino acids, only glycine and taurine showed strong and significant correlations between leach rate and the lobster's feeding preference. These correlations suggest that the feeding preference of the lobsters was most responsive to the rate at which free amino acids and soluble protein (TCA-precipitable protein) compounds leach from the feeds. Glycine and taurine were the most abundant of the free amino acids in the feed's leachate and at particularly high concentrations in the leachate of GLM (Table 4) which possibly accounts for the strong correlations found between the lobster's feeding preference and the chemical signature of the feed's leachate. The strong correlation for TCA-precipitable protein but the not so strong correlation for total kjeldahl N (total-CP) suggests that the smaller nitrogen compounds such as small peptides may have been the key substances eliciting the lobster's preference for the different feeds. However, in the absence of more dicriminating chemical analyses of the constituents of the TCA-precipitable protein fraction of the feed's leachate, this supposition must be treated with caution.

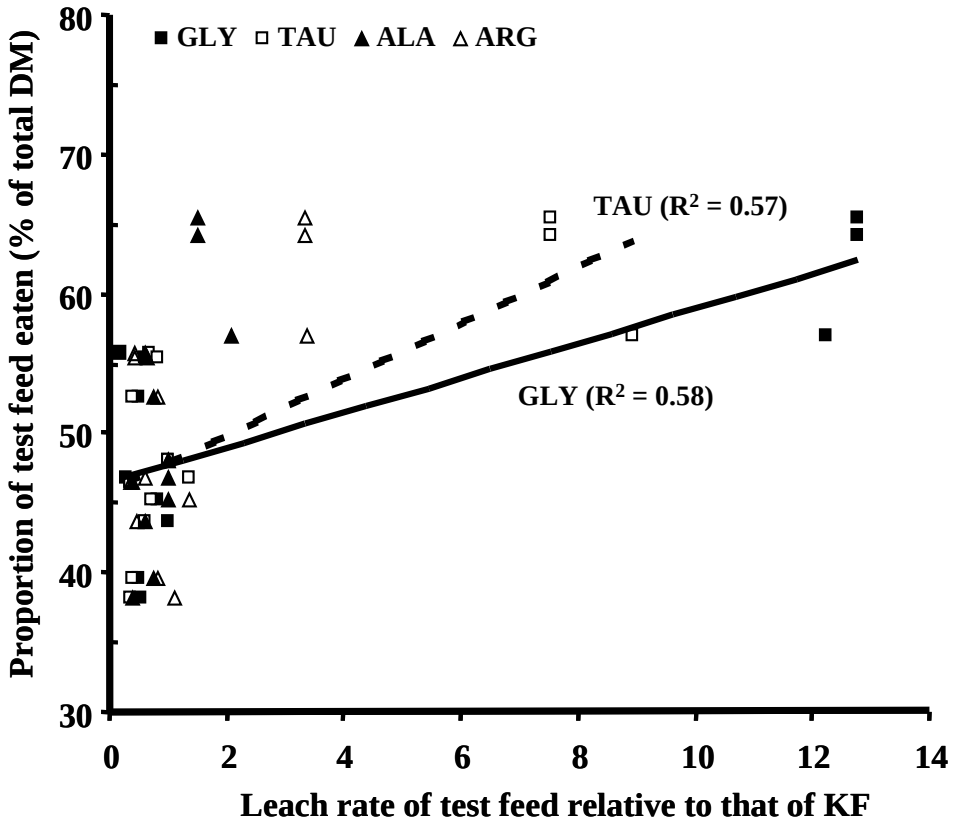

Figure 2. Relationship between the proportion of test feed eaten by juvenile *P. ornatus* lobsters in Experiments 1 and 2 (Y; % of total dry matter (DM)) and the rate at which test feeds leach the free amino acids glycine (GLY), taurine (TAU), alanine (ALA) or arginine (ARG) when expressed as a ratio of the respective leach rate for the reference kuruma feed. All data are shown but only the most significant ($P < 0.05$) relationships are drawn for reasons of clarity.

In conclusion, the study has shown that the lobster's attractiveness to GLM and pelleted feeds containing small inclusions of a variety of fresh natural prey items decreases with increasing water immersion time. However, pieces of GLM remained more attractive to juvenile *P. ornatus* lobsters after 5 h water immersion compared to previously non-immersed kuruma and laboratory-made pelleted feeds. This finding differs to that for juvenile *J. edwardsii* lobsters where immersion of kuruma shrimp pelletes for up to 8 h had no effect on the lobster's subsequent growth, survival, food conversion or carapace colour (Tolomei et al. 2003). Interestingly in the study of Tolomei et al. (2003), the excitatory capacity of non-soaked kuruma pellet, soaked kuruma pellet and fresh mussel was similar while the attractability of the non-soaked kuruma pellet was greater than for either soaked kuruma pellet or mussel flesh. These different responses to water immersion suggest that there are marked differences between lobster species in their feeding stimuli. The findings from the present study suggest that increasing the frequency that pelleted diets are fed and including protein hydrolysates in the formulation as a rich source of feeding stimulatory free amino acids and small peptides may be practical ways of improving the productivity of juvenile *P. ornatus* fed on pelleted dry diets.

6.1.6 Acknowledgements

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6.2 Diet immersion time: effects on growth, survival and feeding behaviour of juvenile southern rock lobster, *Jasus edwardsii*

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6.2.1 Abstract

The effect of water immersion time of a formulated shrimp diet on growth, survival, food conversion and carapace colour of juvenile southern rock lobster, *Jasus edwardsii*, was determined during a 16-week growth trial. Lobsters were fed one of five diets: pre-soaked shrimp diet (0.5, 2, 4, and 8 h) or non-soaked shrimp diet (0 h). Despite a significant loss in dry matter, protein, ash and astaxanthin from the diet as a result of pre-soaking, there was no effect on the growth, survival, food conversion or carapace colour of lobsters.

Feeding behaviour of juvenile *J. edwardsii* was assessed using glycine, taurine and betaine, previously identified as strong chemoattractants to other crustaceans. Quantification of the antennular grooming frequency (AGF) was identified as the best method of assessing chemoattractant detection in *J. edwardsii*. The highest rate of AGF was observed in response to glycine at concentrations of 10^{-4} to 10^{-6} mol L^{-1}. AGF was subsequently used to assess the excitatory capacity of shrimp diet extract and fresh mussel (*Mytilus edulis*) extract.

Two separate experiments were performed to determine the influence of water immersion on the excitatory capacity (ability to detect the source - AGF) and the attractability (capacity to attract to the source – foraging preference) of the shrimp diet in comparison to that of fresh mussel flesh. The excitatory capacity of the non-soaked shrimp diet, soaked shrimp diet and fresh mussel extracts were similar. However, the attractability of the non-soaked shrimp diet was greater than soaked shrimp diet and mussel flesh. Lobsters are capable of detecting the shrimp diet regardless of prolonged exposure to water, however they will selectively move towards a “fresh” rather than a “stale” diet.

The results of this study show that the loss of chemoattractants and nutrients from a shrimp diet, caused by leaching, had little impact on the culture performance of *J. edwardsii*. Thus, even though some lobsters have a slow feeding response and may not feed for several hours after being fed, the dispensation of feed only once per day will not impact on culture performance. However, the influence of leaching on the performance of diets specifically developed for *J. edwardsii* will need to be re-assessed.

Keywords*:* chemoattractants; *Jasus edwardsii*; lobster; nutrient leaching; shrimp diet

6.2.2 Introduction

Increasing global demand, a high market value, and concern for the sustainability of wild stock has created a significant incentive for the development of rock lobster aquaculture (Jeffs and Hooker, 2000). The success of intensive *Jasus edwardsii* culture will invariably depend on providing a cost-effective, nutritionally balanced, water-stable formulated diet. One major problem identified with regard to culture of crustaceans is their slow, intermittent feeding response (Marchetti et al., 1999). In the culture of *J. edwardsii* juveniles, they do not always feed immediately after a diet is introduced; the diet sometimes remaining untouched for several hours (pers. obs.). Upon exposure of a diet to water, loss of nutritional and attractive properties occurs (D'Abramo and Sheen, 1994).

A nutritionally-balanced diet exhibiting poor water stability will quickly become nutritionally impoverished as a result of nutrient loss through leaching. Cuzon et al. (1982) for example demonstrated a loss of 19% dry matter, 11% protein, and 8% carbohydrate from a shrimp diet after 1 h immersion in sea water. Water-soluble vitamins were the most vulnerable to leaching with an 89% loss of vitamin C within the first hour and 99% after three hours. Thus, a diet needs to contain sufficient levels of chemoattractants to encourage rapid detection, location and ingestion of the diet.

At this stage juvenile *J. edwardsii* grown on mussels (*Mytilus edulis* and *M. galloprovincialis*), a natural component of their diet, elicit superior growth and survival compared to those grown on formulated diets (Kington, 1999; Ward et al., in press; Crear et al., 2000); a result consistent with other spiny lobsters (Ryther et al., 1988). This fact is likely to be attributed to the superior nutritional value of mussels, however, a comparison of the attractability of mussels to formulated feeds is necessary before this can be confirmed.

Identification and assessment of chemoattractants for crustaceans has resulted in the creation of a general feeding model (Lee and Meyers, 1996). This model describes and classifies chemical stimuli based on their ability to induce a full feeding response, as defined by a graded sequence of behavioural events. The model illustrates that animals follow a general pathway in response to food-related stimuli from detection through to cessation of feeding, with each behavioural step dependent on the presence of specific chemical stimuli. Simply stated, detection does not necessarily lead to attraction or ingestion (Chamberlain, 1995; Lee and Meyers, 1996). This has provided a foundation for chemoreception research and has enabled researchers to make specific conclusions about the stimulatory capacity of a single chemical or group of chemicals. Among the plethora of chemostimulatory substances identified, glycine, taurine and betaine account for the majority of intense, positive responses when averaged over the range of individual chemicals and crustacean species tested (Carr, 1978; Zimmer-Faust, 1991; Coman et al., 1996).

The principal aim of this study was to quantify nutrient leaching from a formulated diet and examine the effect of diet immersion time on growth, survival, food conversion and colouration of *J. edwardsii*. An additional aim was to assess the effect of immersion time on the excitatory capacity and attractability of a formulated diet, using mussel extract or flesh as a reference point. Finally, we aimed to assess if *J. edwardsii* elicits similar stimulatory responses to chemoattractants as other crustaceans using known chemostimulatory substances and further use

this behavioural response in the assessment of diet detection (excitatory capacity). The results of this study will be used to recommend a feeding regime suitable for the culture of juvenile *J. edwardsii*.

6.2.3 Materials and Methods

6.2.3.1 Experimental animals

Juvenile *J. edwardsii* used in all experiments were obtained from stock maintained at the Tasmanian Aquaculture and Fisheries Institute (TAFI) Marine Research Laboratories in Hobart, Tasmania. They were held in 1000 L Rathburn tanks at ambient temperature (13-18°C) under a 10L:14D h photoregime and fed a mixed diet of fresh blue mussels (*M. edulis*) ($2 \times week^{-1}$) and commercial *Penaeus japonicus* (Higashi Maru #12) pellets ($3 \times week^{-1}$). Lobsters were caught as puerulus off the east coast of Tasmania and were approximately three months old at the start of the experiments.

6.2.3.2 Effect of diet immersion on growth, survival and food conversion: Growth Trial

The recirculation system used for the growth trial consisted of twenty circular, 350 L plastic tanks each with a central standpipe. The system was held under a 10L:14D h photoregime with light intensity at the water surface ranging from 0.5 - 1.52 μmol $s^{-1}m^{-2}$ (Gossen-Prosifix). Temperature (19.3 ± 0.3°C) (mean ± SD), dissolved oxygen (7.12 ± 0.18 mg L^{-1}), total ammonia nitrogen (< 0.26 mg L^{-1}), pH (8.4 ± 0.1) and salinity (35.2 ± 0.5 ppt) were measured weekly. Three concrete building blocks were placed in each tank to provide refuge.

Quadruplicate tanks each containing ten pre-acclimated lobsters (initial weight 13.1 g ± 0.2 (mean ± SE)) were randomly allocated one of five treatments: fresh diet (0 h – non-soaked) or diet which had been pre-soaked at 19.3°C for different periods in flowing seawater (0.5, 2, 4, 8 h). The commercial shrimp (*P. japonicus*) pellet was used as the diet in the growth trial. Mussels were not used in the growth trial as sufficient data for baseline comparisons were already available. All treatments were fed at 1730 h (30 min after lights out) in an attempt to limit the amount of time between feeding and ingestion; *J. edwardsii* exhibits peak foraging activity during nocturnal periods (Fielder, 1965). Feed was evenly distributed in the front half of the tank to minimize agonistic interactions associated with food clumping (Barki et al., 1997).

Uneaten food was siphoned onto a 150 μm screen each morning at 0900 h, rinsed with fresh water and stored at -18°C ready for drying and calculation of feed consumption. Feed rate was adjusted so that approximately >90 % of the food was consumed each day. All animals were individually identified by gluing (Loctite 454®) a numbered, polymer tag to their carapace. Lobsters that moulted overnight were re-tagged the following morning.

All lobsters were weighed on days 31, 62, 91 and at the conclusion of the experiment (day 120). Lobster performance was assessed with respect to growth (SGR), survival, food conversion ratio (FCR), moult increment, intermoult period and carapace colour. At the end of the trial, intermoult lobsters from each treatment between 10 and 30 d post-moult were colour-graded (Crear et al., 2002). Feed intake, FCR and protein efficiency ratio (PER) were calculated, taking into account dry matter loss during both the initial immersion period and the feeding period to

collection. Dry matter was calculated as the ratio of leached pellet dry matter to the original pellet dry matter multiplied by 100.

Water stability of the diet was assessed by immersing quadruplicate samples (≈5 g) of the diet in flowing water (≈ 40 L h^{-1}, 19.3°C) for 0.5, 2, 4 and 8 h. After the required immersion time, the samples were rinsed with distilled water to remove residual salt, freeze-dried for 24 h and re-weighed. The samples were then ground with a mortar and pestle and their proximate composition determined.

Lipid was analysed using chloroform methanol extraction (Bligh and Dyer, 1959); nitrogen (Kjeldahl with selenium catalyst), allowing calculation of crude protein (N × 6.25). Energy was quantified using bomb calorimetry (Gallenkamp autobomb™ calibrated with benzoic acid) and ash was estimated after combustion at 450°C for 16 hours (Carbolite™ furnace). Astaxanthin content was obtained by acetone extraction and compared to an astaxanthin (3,3′-Dihydroxy-β,β-carotene-4,4′-dione - Sigma®) standard using spectrophotometry (472 nm).

6.2.3.3 Assessment of J. edwardsii feeding response to chemoattractants: System and protocol used for the assessment of excitatory capacity

Five glass aquaria (15 cm^3) supported by a glass-topped stand were used to observe and record the excitatory behaviour of *J. edwardsii* in response to potential excitants. Two litres of filtered (5 μm) seawater was added to each aquarium and maintained at 18°C. Water movement and aeration was provided to each aquarium via regulated airlines. Intermoult lobsters were acclimated to the aquaria (one lobster/aquaria) for 10 to 15 min prior to behavioural observations. They were not used if they did not appear settled after this period (Sarac and Smith, 1997). All equipment was thoroughly washed with fresh water after each trial to remove residual chemoattractants and conspecific scent.

Red (0.02 μmol s^{-1} m^{-2} at the water surface) and infrared lighting enabled the observer to operate the experimental system without affecting lobster behaviour (Kennedy and Bruno, 1961). A black and white camera and high-resolution monitor allowed observation of the lobster's physical movements from underneath. Black plastic optically isolated each aquarium from the observer and con-specifics.

6.2.3.4 Excitatory capacity of glycine, taurine and betaine

The chemostimulatory response of *J. edwardsii* was assessed using two amino acids; glycine (aminoacetic acid - MERCK Pty Ltd.) and taurine (2-aminoethanesulfonic acid - ICN Biomedicals Inc.) and a quaternary ammonium compound; betaine HCL (ICN Biomedicals Inc.) on a dose-response basis. Each chemical was weighed (± 0.00001 g) (Metler Delta Range™), dissolved in filtered sea water (FSW) in a 100 mL volumetric flask (stock solution), and serially diluted in FSW to the required concentration (2, 2 × 10^{-2}, 2 × 10^{-4}, 2 × 10^{-6} mol L^{-1}). The pH of each solution was measured and adjusted to 8.34. Ten mL volumes of each concentration were added via syringe in a Z pattern to the surface of each aquarium (2 L), resulting in final chemoattractant concentrations of 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8} mol L^{-1}. Lobsters were always in the corner of the aquarium when chemoattractants were introduced. It should be noted that

subsequent results are based the on the final concentrations of excitants following full dilution in the experimental aquaria, while in reality the concentrations initially detected by antennule chemoreceptors may have been slightly higher.

Following acclimation, each lobster was observed for 3 min (pre-attractant phase), during which time the antennular grooming frequency (AGF) was recorded. Ten mL of the chemoattractant solution was added to each aquarium and allowed to disperse for 10 s (previously determined to be sufficient time for dispersal by addition of dye). Following dispersal, AGF was recorded during the 3 min post-attractant phase. The mean difference in AGF during the pre- and post-attractant phases were used to plot dose response curves. A control group (n = 19) of lobsters were subjected to 10 mL of FSW containing no chemoattractant.

6.2.3.5 Excitatory capacity of shrimp pellet and mussel extracts

Extract solutions were prepared in 100 mL of FSW using the following diets; non-soaked shrimp diet (0.26 g); soaked (8 h) shrimp diet (0.26 g); mussel flesh (0.8 g). This amount of diet was calculated to approximate the diet to water volume ratio employed in the growth trial, this giving an indication of the excitatory environment within the growth trial (comparing non-soaked and soaked shrimp diet). Following a standing period of 10 min, the solutions were decanted, centrifuged at 4500 rpm for 10 min and the pH adjusted to 8.34. The excitatory capacity (quantified as AGF) of the three extracts was assessed using the same methods described above.

6.2.3.6 Attractability of shrimp pellet and mussel flesh

Three juvenile lobsters (10-20 g) were placed in a 50 L tank together with two standard house bricks to provide refuge (outlet end of tank). Lobsters were allowed to acclimate to the tank for a minimum of 48 h prior to the introduction of the diet tubes. Three experiments were performed: A) non-soaked vs. soaked, B) non-soaked vs. mussel, and C) soaked vs. mussel. Two perforated (500μm) tubes, into which the diets were placed, were suspended at the water inlet end of the tank, a minimum of 5 cm from the tank walls, with approximately 10 cm separating the tubes. Gentle aeration and a flow rate of 40 L h^{-1} were supplied to each tank. To quantify the attractability of the diets, the number of grasps onto each tube by the lobsters was recorded over a 30-minute period from when the tubes were first introduced into the tank. A control tube containing no diet was randomly placed in 10 of the experimental tanks.

6.2.3.7 Statistical analyses

Nested analysis of variance was used to determine if either tank or diet had any effect on growth (SGR), where tank was nested within diet. One-way ANOVA was used assess the effect of immersion time on the composition of the diets as well as FCR and PER. Regression analysis of variance was used to assess the relationship between moult increment and weight, as moult increment generally decreases as weight increases. The effect of immersion time on carapace colour was assessed using Chi-square analysis. It should be noted that 25 % of the expected values in this analysis were not greater than 5. The excitatory capacity of betaine, glycine and taurine was assessed using two-way ANOVA where chemical and concentration were fixed factors and AGF was the dependent variable. Once a significant interaction was determined, one-

way ANOVA was use to compare pooled data. One-way ANOVA was used to compare the excitability of the three diet extracts. The attractability of the diets in terms of grasps diet^{-1} was assessed using paired t-tests. Homogeneity of variance was examined using residual box plots, and where necessary, an appropriate transformation was performed. The Tukey-Kramer HSD test was used for post-hoc comparisons following ANOVA tests. Statistics were executed using SPSS version 8.0; P = 0.05.

6.2.4 Results

6.2.4.1 Effect of diet immersion on growth, survival and food conversion: Growth trial

Diet immersion time had no significant impact on SGR ($F = 0.33_{(4, 15)}$, P = 0.85); no influence of tanks on SGR was found ($F = 0.90_{(15, 180)}$, P = 0.56). There was no effect of weight on moult increment, therefore all moult increment data for each treatment were pooled. Diet had no significant effect on moult increment ($F = 1.92_{(4, 68)}$, P = 0.12) or inter-moult period ($F = 0.8_{(9, 30)}$, P = 0.62). Food conversion ratio was not influenced by diet immersion ($F = 2.69_{(4, 15)}$, P = 0.07), nor was PER ($F = 2.42_{(4, 15)}$, P = 0.09) or colour index ($X^2 = 11.2_{(8)}$ P = 0.19). Survival in all treatments was 100% (Table 1).

Table 1. Growth performance (mean ± SE) of juvenile *J. edwardsii* fed non-soaked (0 h) and soaked (0.5, 2, 4, 8 h) *P. japonicus* diet. Means bearing the same superscript letter are not significantly different (P < 0.05)

Attribute	Pellet immersion time (h)				
	0	0.5	2	4	8
Initial wt (g)	13.1 ± 0.23[a]	13.2 ± 0.21[a]	13.1 ± 0.23[a]	13.1 ± 0.26[a]	13.2 ± 0.31[a]
Final wt (g)	35.3 ± 0.74	34.3 ± 0.78	34.1 ± 0.83	34.3 ± 0.77[a]	34.2 ± 0.82[a]
SGR (% day^{-1})	0.82 ± 0.08[a]	0.79 ± 0.08[a]	0.79 ± 0.09[a]	0.80 ± 0.07[a]	0.80 ± 0.07[a]
Survival (%)	100 ± 0.0[a]	100 ± 0.0[a]	100 ± 0.0[a]	100 ± 0.0[a]	100 ± 0.0[a]
FCR	1.29 ± 0.02[a]	1.28 ± 0.05[a]	1.37 ± 0.01[a]	1.38 ± 0.05[a]	1.42 ± 0.03[a]
PER (%)	147.5 ± 2.1[a]	154.7 ± 7.0[a]	150.6 ± 1.1[a]	161.4 ± 5.8[a]	163.8 ± 3.4[a]
Moult increment (%)	52.2 ± 2.11[a]	48.5 ± 3.7[a]	53.2 ± 2.0[a]	51.0 ± 3.0[a]	48.8 ± 4.2[a]
Intermoult period (d)	49.3 ± 1.6[a]	50.2 ± 2.1[a]	49.4 ± 1.1[a]	52.6 ± 2.8[a]	51.2 ± 2.6[a]
Mean colour index	2.8 ± 0.1[a]	2.4 ± 0.2[a]	2.6 ± 0.2[a]	2.4 ± 0.2[a]	2.0 ± 0.3[a]

SGR = (ln final weight (g) – ln initial weight (g)) × 100/number of days
FCR = dry weight feed intake (g) : lobster wet weight gain (g)
PER = [(weight gain (g)/feed intake (g) × (protein content of diet × 0.8))] × 100
Moult increment = ((weight gain at moult (g)/premoult weight (g)) × 100)
Intermoult period = number of days between moults
Colour index = scale 1-5, where 1 = lightest (pink/light brown) and 5 = darkest (dark red/maroon)

6.2.4.2 Water stability and proximate composition

The pellets maintained their physical stability during immersion, although swelling and softening of the pellets were noted, along with loss of colouration. Dry matter content of the pellets decreased significantly ($F = 6738.3_{(4, 15)}$, $P < 0.001$) at each immersion time, with the majority of the loss occurring during the first 2 hours (33% by 0.5 h and 61% by 2 h) (Table 2). Crude protein decreased with immersion time, resulting in significantly lower ($F = 11.8_{(4, 33)}$, $P < 0.001$) nitrogen levels to that in the non-soaked diet after 4 h. Crude protein made up a large proportion of dry matter loss from the diet, being 66% of the total after 8 hours of soaking.

Table 2. Dry matter (DM), crude protein (CP), ash, total lipid, gross energy (GE) and astaxanthin (Astax) concentration (mean ± SE) of the *P. japonicus* diet subjected to water immersion over 8 hours

Analyte	Pellet immersion time (h)				
	0	0.5	2	4	8
DM (% initial DM)	100 ± 0.0^{a}	94.0 ± 0.1^{b}	89.0 ± 0.1^{c}	85.1 ± 0.1^{d}	82.0 ± 0.1^{e}
CP (% DM)	65.6 ± 1.3^{a}	63.2 ± 1.9^{a}	60.4 ± 2.0^{ab}	56.3 ± 0.9^{bc}	53.8 ± 0.4^{c}
% DM lost as CP	N/A	27	47	62	66
Total lipid (% DM)	12.0 ± 0.05^{a}	12.3 ± 0.05^{b}	12.9 ± 0.05^{c}	12.9 ± 0.03^{c}	12.7 ± 0.08^{c}
GE (MJ kg^{-1} DM)	18.2 ± 0.05^{a}	18.7 ± 0.06^{b}	18.9 ± 0.07^{b}	18.7 ± 0.03^{b}	18.6 ± 0.05^{b}
CP:GE (g $.MJ^{-1}$)	36.1	33.8	31.9	30.2	28.9
Ash (% DM)	18.8 ± 0.18^{a}	17.7 ± 0.20^{b}	17.1 ± 0.15^{b}	17.1 ± 0.23^{b}	17.5 ± 0.25^{b}
Astax ($mg.kg^{-1}$)	256 ± 5.7^{a}	132 ± 4.9^{b}	127 ± 7.4^{b}	134 ± 15.0^{b}	105 ± 4.8^{b}

^{a,b,c,d,e} Means bearing the same superscript letter are not significantly different ($P > 0.05$).

Immersion also resulted in small, but significant ($F = 18.36_{(4, 10)}$, $P < 0.001$) changes in the gross energy content of the diets. At all immersion times the energy content was greater than the non-soaked diet, although gross energy peaked and remained level after 2 h of immersion. The large decrease in the dietary crude protein content resulted in parallel decreases in the crude protein : gross energy ratio of the diet. There were significant decreases in the ash ($F = 13.58_{(4, 15)}$, $P < 0.001$) and astaxanthin ($F = 50.12_{(4, 25)}$, $P < 0.001$) contents of the diet. For both components the decrease occurred during the first 0.5 h of immersion, with no subsequent decreases. Although the decrease in ash content was relatively small, the astaxanthin content decreased by 51% during the first 0.5 h immersion period. Dietary lipid levels increased as a proportion of the diet over the first 30 min of immersion, however remained in similar proportions thereafter ($F = 44.72_{(4, 5)}$, $P < 0.001$).

6.2.4.3 Assessment of J. edwardsii feeding response to chemoattractants: Excitatory capacity of glycine, taurine and betaine

Preliminary trials found that the observed detection responses of *J. edwardsii* to chemoattractants were: increased frequency in antennular grooming using the third paired maxillipeds, increased second maxillae movement (gill bailing), and a general increase in pereiopod activity (sweeping, probing). Antennular grooming was easily observed and quantified and was accepted as the best

method for assessing chemical detection. In all experiments conducted using AGF, no significant difference was found in the initial AGF count (before the addition of excitants) when pooled ($F = 1.18_{(15, 217)}$, $P = 0.28$), so all following analyses compared final AGF counts only. A significant interaction between chemical and concentration was found in the two-way ANOVA ($F = 21.56_{(9, 238)}$, $P < 0.001$).

The dose response functions show that AGF was not affected by any chemical at the lowest concentration tested ($F = 1.43_{(3, 57)}$, $P = 0.24$) (Fig 1.). Increased AGF was observed when lobsters were exposed to glycine and taurine at 10^{-6} mol L^{-1}, with the response to glycine clearly the strongest ($F = 64.44_{(3, 61)}$, $P < 0.001$). A further increase in excitant concentration to 10^{-4} mol L^{-1} induced a significant increase in AGF for all three chemicals tested compared to the control; glycine again inducing the strongest response ($F = 72.24_{(3, 61)}$, $P < 0.001$). At the highest concentration tested, the response to glycine was significantly lower than what was observed for glycine at 10^{-4} mol L^{-1} ($F = 54.71_{(3, 60)}$, $P < 0.001$), however lobsters exposed to taurine at this concentration exhibited the highest response of all three excitants compared to the control ($F = 70.47_{(3, 59)}$, $P < 0.001$). Betaine had no significant effect on AGF at any of the concentrations tested except at 10^{-4} mol L^{-1} when compared to the control ($F=5.62_{(7, 124)}$, $P < 0.001$). Addition of FSW did not affect AGF ($F=0.005_{(1, 36)}$, $P = 0.94$).

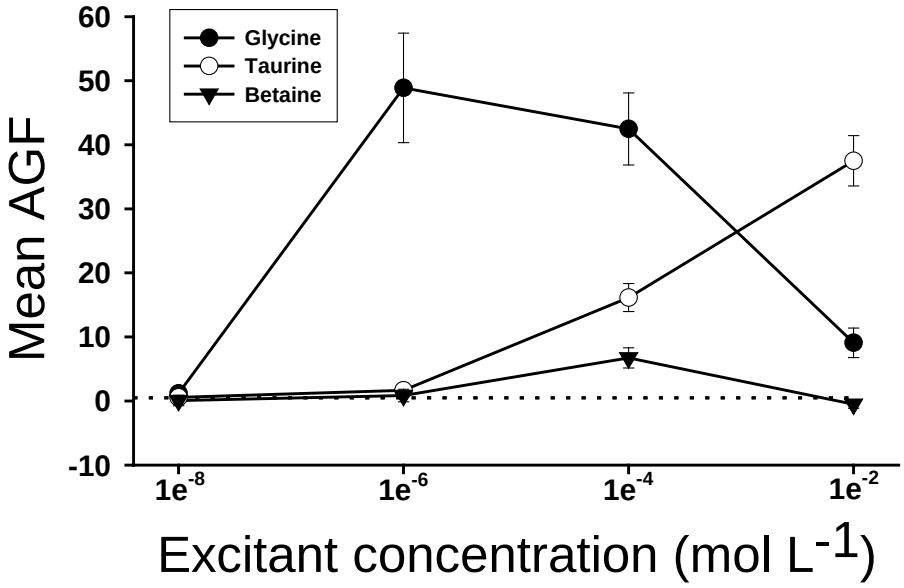

Figure 1. Excitatory response (mean ± SE antennular grooming frequency - AGF) of juvenile *J. edwardsii* to increasing concentrations of glycine, taurine and betaine. AGF (± SE) = (number of antennule grooms during the three min post-attractant phase) - (number of antennule grooms during the three min pre-attractant phase). Between 13 and 17 individual lobsters were trailed at each concentration for each chemical tested. Dotted line represents control AGF level (0.05 ± 0.01) (n = 19).

6.2.4.4 Feeding response to shrimp pellet and mussel

All three diet extracts induced a significant excitatory response compared to the control ($F=62.2_{(3, 51)}$, $P < 0.001$) although there was no significant difference in the mean AGF between diets ($F=1.37_{(2, 33)}$, $P = 0.27$) (Fig. 2).

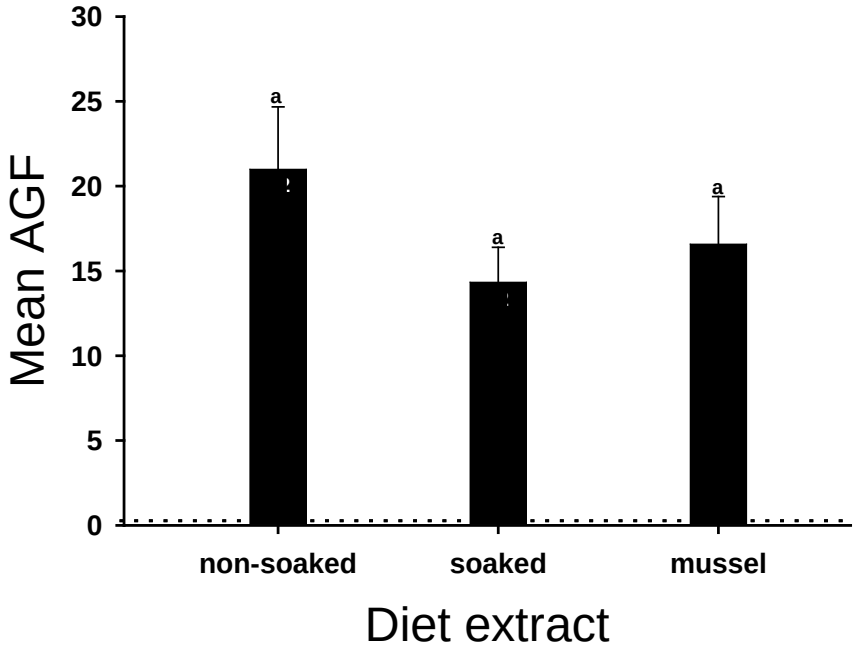

Figure 2. Excitatory response (mean ± SE antennular grooming frequency - AGF) of juvenile *J. edwardsii* to shrimp diet extract (non-soaked and soaked (8 h)) and mussel tissue extract (*M. edulis*). AGF (± SE) = (number of antennule grooms during the three min post-attractant phase) - (number of antennule grooms during the three min pre-attractant phase). Dotted line represents control AGF level (0.05 ± 0.01) (n = 19). Columns bearing the same superscript are not significantly different (P = 0.05), n is indicated.

Immersion for 8 h significantly ($t = 8.63_{(19)}$, $P < 0.001$) reduced the attractability of the shrimp diet (Fig. 3A). The non-soaked shrimp diet was also significantly ($t = -3.61_{(21)}$, $P = 0.002$) more attractive than fresh mussel flesh (Fig. 3B). Mussel flesh did however elicit a significantly ($t = -8.33_{(15)}$, $P < 0.001$) stronger attractability than the soaked shrimp diet (Fig. 3C). None of the lobsters were attracted to the empty control tubes (n = 10).

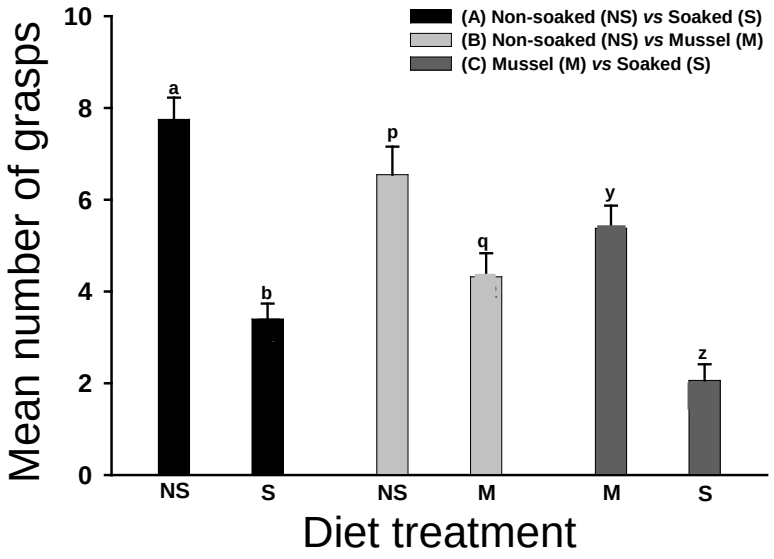

Figure 3. The attractability of diets in paired comparisons (A): non-soaked shrimp diet vs soaked shrimp diet (immersed in flowing sea water for 8 h); (B): non-soaked shrimp diet *vs* fresh mussel flesh (*M. edulis*), and (C): fresh mussel flesh *vs* soaked (8 h) shrimp diet. Mean number of grasps (± SE) = mean number of times a mesh tube containing a specified diet was grasped by a juvenile lobster over a 30 min period. Columns bearing the same superscript are not significantly different (P = 0.05), n is indicated.

6.2.5 Discussion

This study has shown that after an extended period of immersion, the changes in dietary composition from the shrimp diet were not sufficient to affect growth, survival, food conversion or carapace colour of juvenile *J. edwardsii*. The diet still contained sufficient levels of feeding excitants, attractants and incitants to stimulate detection of, attraction to and consumption of the diet, respectively. This is despite the fact that a significant proportion of the nutritional components leached from the diet, particularly within the first 30 minutes of exposure.

The formulated shrimp diet used in the present study was over fortified with many nutrients compared to known optimum levels for juvenile *J. edwardsii* (protein, protein/energy ratio – Ward et al., 2003). Immersion did not reduce the levels of those nutrients to sub-optimal levels, and in the case of both protein and protein/energy ratio, they moved towards optimum levels. The crude protein to gross energy ratio of the shrimp diet decreased from 36 to 28 g CP.MJ GE^{-1} over the 8 h immersion period. The optimal digestible crude protein (DCP)/digestible energy (DE) ratio for *J. edwardsii* is approximately 29 g DCP.MJ DE^{-1} (Ward et al., 2003). Assuming protein and energy are equally digestible, as found by Ward et al. (2003), then the protein/energy ratio in the diet after 8 hours immersion is very close to the optimum. A formulated diet must provide an optimal protein/energy ratio to optimise protein efficiency and growth (D'Abramo and Sheen, 1994). A diet formulated to meet the specific requirements of *J. edwardsii* may however be significantly affected by nutrient loss, whereby any amount of leaching would result

in a sub-optimal nutritional profile. A possible solution may involve the provision of a diet containing water-soluble nutrients at slightly higher levels than determined to be optimal to compensate for nutrient losses associated with leaching.

Crustaceans, like all animals, are incapable of *de novo* synthesis of carotenoids and thus require an exogenous source to meet their specific requirements (Latscha, 1991). Although immersion resulted in high rates of astaxanthin leaching from the diet, which is consistent with loss of other water-soluble nutrients (Cuzon et al., 1982), it did not adversely affect lobster colour (darker red is preferred) as the level remained above the minimum requirement for this species (115 mg kg^{-1}) (Crear et al., in press).

The soaked, non-soaked shrimp diet and fresh mussel extracts were found to induce similar detection responses. The fact that diet immersion had no effect on the excitatory capacity of the formulated diet is interesting considering that amino acids (potential excitants) from a variety of "baits" are rapidly lost within the first 1-2 h of water exposure (Mackie et al., 1980; Daniel and Bayer, 1987; Løkkeborg, 1990). This suggests that lobsters fed on pre-soaked diet in the growth trial, which ate the same amount as lobsters fed on non-soaked diet, may develop the ability to initiate a detection response when lower concentrations of excitants are present. Associative learning in this manner has been demonstrated in other spiny lobster species whereby animals can be trained to discriminate between the quality and intensity of chemical stimuli (Fine-Levy and Derby, 1991). Another possible explanation considers the minimum threshold concentration of excitants required to initiate a detection response (Zimmer-Faust, 1991). It appears the shrimp diet contained sufficient levels of excitants above the minimum threshold level even after eight hours of immersion to initiate a detection response similar to lobsters exposed to non-soaked diet extract.

Water immersion significantly reduced the attractability of the shrimp diet. These results appear to conflict with those reflected in the growth trial, whereby similar feed intake was observed across all treatments. One assumes that diet attractability would be reflected in feed consumption, however, chemicals associated with attractability may not necessarily be associated with chemicals required to incite feeding (Lee and Meyers, 1996). This study clearly demonstrates feeding behaviour of *J. edwardsii* is dependent on chemicals specific to detection, attraction and ingestion. A broad range of chemoattractants must be provided in the diet to ensure feeding efficiency occurs from detection through to cessation of feeding.

Interestingly, the non-soaked shrimp diet was more attractive than mussel flesh. The superior attractability of the *P. japonicus* diet possibly originates from the dietary ingredients used in its formulation. Ingredients such as fish, shrimp and squid meals are extensively incorporated into dietary formulations to impart chemoattractive properties and enhance the nutritional value of the diet (Tacon, 1990). Shrimp meal for example contains a variety of amino acids and low molecular weight organic compounds, which are highly stimulatory to a range of crustaceans (Meyers, 1987).

Glycine, taurine and betaine are chemicals commonly found in high concentrations in prey tissue and have been shown to stimulate specific chemoreceptor neurons in crustaceans (Fuzessery et al., 1978; Carr and Derby, 1986; Ache et al., 1988; Carr, 1988; Zimmer-Faust, 1991). The

detection behaviour (AGF) in response to the three chemicals was variable, which is consistent with other reports (Zimmer-Faust et al., 1984a; Barbato and Daniel, 1997; Daniel et al., 2001). The presence or absence of these chemicals in natural foods of *J. edwardsii* may reflect the apparent differences in each chemical's excitatory capacity found in the present study. For example, Sheppard (2001) found glycine to significantly increase feed intake of *J. edwardsii* when incorporated into a formulated diet compared to taurine and betaine.

Conversely, betaine, glycine and taurine were found to be ineffective excitants to *Panulirus argus* at 5×10^{-4} mol L^{-1} (Barbato and Daniel, 1997). L-glutamate was the only chemical found to increase antennular grooming. The authors proposed the response to L-glutamate was non-olfactory mediated whereby the electrostatic attraction of L-glutamate to aesthetasc receptors induced grooming behaviour. It is believed the primary role of antennular grooming has evolved from the necessity of decapod crustaceans to remove biofouling from olfactory appendages, necessary if the organism is to successfully locate food, mates or avoid predators in dynamic aquatic environments (Bauer, 1989). However, the author demonstrated neither contamination of antennules with sediment or carmine particles caused any apparent increase in AGF in several decapods (not named) except *Pandalus danae* (shrimp). Antennular grooming in *Panulirus interruptus* was shown to significantly increase following the introduction of natural food (abalone) to the experimental tank and was hence used as an indicator of appetitive feeding (Zimmer-Faust et al., 1984b).

The present study verifies the results of Fielder (1965); *J. edwardsii* forages selectively when presented with a variety of food types. The strong feeding response to the formulated diet (both soaked and non-soaked) suggests that the superior growth performance of *J. edwardsii* juveniles fed with mussels, as shown in several previous studies (Ward et al., in press; Crear et al., 2000), was not due to the superior attractiveness of mussels, but was probably due to their better nutritional value or digestibility. In both those previous studies, feed intake was the same for both mussel-fed and formulated-diet-fed lobsters.

This study has shown that feeding incitants appear to be maintained in the soaked diet even after 8 h of immersion. Although some attractive and nutritional components decreased with leaching, these were not sufficient to affect growth, survival, food conversion, or carapace colouration. Thus, it appears that *J. edwardsii* juveniles need only be fed once daily, thus simplifying husbandry requirements. In the future, when a diet is formulated specifically for *J. edwardsii*, there may need to be a re-assessment of that protocol.

6.2.6 References

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6.3 Apparent digestibility of potential ingredients as protein sources in formulated feeds for the southern rock lobster *Jasus edwardsii*

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6.3.1 Abstract

Recent advances in defining the protein and protein energy requirements of southern rock lobster, *Jasus edwardsii* using fish meal based diets have provided baseline information to produce formulated diets for commercial culture of the species. Protein sources supplied in a diet should provide an adequate balance of amino acids, and be highly digestible and contain few anti-nutritional factors. Selecting ingredients with the highest nutrient availability to provide adequate nutrients and energy for rapid growth is crucial to formulating effective diets. A range of commercially available protein sources available to agriculture and aquaculture feed producers was selected and apparent digestibility tested using 70:30 reference diet to test ingredient method. Changes in digestibility of three ingredients over time were assessed over three consecutive week-long sampling periods. The lack of significant changes in digestibility over time prompted the further testing of 15 test ingredients over one week-long period. The ingredients were ranked according to their apparent protein digestibility. The current standard protein source, fish meal, fell mid range (62.5%) among the ingredients tested, and it appears substantial improvements to the provision of protein from current diets may be possible through the use of more digestible protein sources.

6.3.2 Introduction

The proposed aquaculture of the southern rock lobster, *Jasus edwardsii* will require the production of a formulated diet to guarantee optimal animal growth throughout the growout period. Research into protein requirements is underway and diets are being refined compositionally to provide the best nutrient balance for growth of *J. edwardsii* (Ward et al., 2003). However to date, the maximum growth possible on experimental diets or using commercial prawn pellets is still lower than the growth of lobsters fed fresh mussels (Crear et al., 2002; Ward et al., 2003). Diets tested to date have used fishmeal as the major protein source. However, the possibility that fishmeal may be restricting growth and the need to address fish meal replacement in future aquaculture feeds stimulates the need for alternate protein sources that may meet or increase the present growth levels using fish meal based diets.

Producing the most efficiently utilized diet involves selecting highly digestible nutrient sources to promote growth. Measuring the apparent digestibility of crude protein (AD_{CP}) of potential dietary ingredients is essential to ensure the most available protein sources are being provided to the lobster. Although true digestibility more comprehensively accounts for the endogenous losses of nutrients from sources like cell sloughing and periotrophic membranes, these nutrient losses are small and apparent digestibility is the most practical measure of digestibility(Jobling, 1983).

Measuring the capacity of lobster to digest various ingredients then allows an effective selection of suitable ingredients to be included in formulated feeds.

The apparent digestibility of a diet may be affected by many factors e.g. anti nutritional factors like phytases, processing level and temperature, antagonistic interactions between diet ingredients, amino acid profile and the digestive enzyme complement of the target species available to digest the nutrients. The increasing cost of fish meal and need to replace the use of marine meals in commercial diets has seen the incorporation of novel protein sources in many marine species (Carter and Hauler, 2000; Farhangi and Carter, 2001; Fleming et al., 1998). Plant meals such as soybean meal, lupin meal, canola meal, wheat gluten and pea meal have been identified as potential protein sources due to their low cost and relatively high protein levels, although the maximum inclusions levels may be restricted by anti-nutritional factors such as oligosaccharides, high starch levels, high NSP and lectins (Farhangi and Carter, 2001). Alternate marine protein meals from seafood by-products and processing waste such as squid, prawn and mussel meals also offer potential fish meal replacements.

Digestibility of ingredients for southern rock lobster has not been investigated and digestibility studies in other lobster species have been receive little research attention. This is partly due to the difficulty involved with performing digestibility studies in crustaceans. The slow and messy feeding characteristics of these animals make high dietary water stability essential to prevent the loss of nutrients prior to feed consumption. Inert markers traditionally used to calculate the nutrient density gradient from feed to faces have been associated with decreased feed intake and partitioning in the digestive tract. In particular, the most commonly used digestibility marker, chromic oxide, has been shown to decrease feed intake at inclusion levels above 1%, and shows inconsistent movement through the digestive tract (Leavitt, 1985). Partitioning of chromic oxide has been observed in freshwater crayfishes *Procambarus clarkii*, (Brown et al., 1986), *Cherax tenuimanus*, *C. destructor* (Jones and De Silva, 1997) and also in clawed lobster *Homarus americanus* (Leavitt, 1985). More recent nutrient digestibility studies with fish and crustaceans are now using yttrium oxide and ytterbium acetate and cholestane as inert markers at much lower inclusion levels, with more sensitive detection methods to avoid these partitioning problems (Carter et al., 2003; Deering et al., 1996; Ishikawa et al., 1996; Smith et al., 1985). This study aims to measure apparent digestibility of potential protein sources in the southern rock lobster.

6.3.3 Materials and Methods

6.3.3.1 Experimental animals

Juvenile southern rock lobster *J. edwardsii* were obtained from the Marine Research Laboratories (TAFI) where they had been maintained under ambient conditions (12:12 photoperiod, 18°C) in a flow-through seawater system. They were fed mussels 3 days per week and commercial penaeid prawn diet (Higashi Maroo) four days per week. Prior to experimentation at the School of Aquaculture, University of Tasmania, they were held under identical conditions, and fed Higashi Maroo 7 days per week in a recirculating seawater system with a flow rate of 1.00 ±0.2 $L.min^{-1}$ giving 100% turnover every 1.5 h. Water quality was measured twice per week and parameters remained within recommended ranges for lobsters (Crear and Allen, 2002).

6.3.3.2 Diet manufacture

Test protein ingredients were selected from commercially available marine meals and plant sources. Squid meal and prawn meal were donated by Ridley Aquafeeds, QLD. Powdered blue mussel meal was obtained from NIWA, NZ. Fish meal was defatted by double hexane:ethanol (2:1) extraction (Ward et al., 2003). Canola meal, soybean meal and pea meal were from Pivot Agrifeeds, Tasmania. Lupin flour (*Lupinus albus*) was donated by M.C. Croker Pty. Ltd, NSW. All ingredients used in both test and reference diets, were passed through a 1 mm screen.

The reference diet used South American fish meal (Skretting Australia, TAS) as the protein source and fish oil (Skretting Australia, TAS) as the lipid source (Table 1). Wheat gluten and pre-gelatinised maize starch (Sigma Chemicals) were used to balance the protein and energy levels. Sodium alginate (Kelco International, Sydney) and trisodium phosphate (Ajax Chemicals, Sydney) were mixed with the dry ingredients to avoid premature calcium binding until the final pellet form was achieved.

Table 1. Ingredient composition of the reference diet g.kg^{-1}

Ingredient	Inclusion
Fishmeal (Pivot)	639.67
Pre-gelatinised maize starch	143.54
Wheat gluten	120.00
Fish oil	30.00
Manucol	46.77
Trisodiumbisphosphate	20.00
Yttrium oxide	1
Water	As required[1]

[1] Water added at about 40% to form a soft dough for extrusion

Dry components of the reference diet (Table 1) were mixed and stored at –20ºC. The test protein ingredient was added at 30% to 69.9% reference diet with 0.1% yttrium oxide as an inert digestibility marker (Table 2), which was mixed in a Hobart mixer. Fish oil was added and when thoroughly mixed, water was added to form a stiff dough. The dough was passed through a 3 mm die, and the dough strands placed into a 10% CaCl setting bath for 3 mins, after which the strands were dried at 30ºC in an oven. When below 10% moisture, strands were transferred to a -20ºC freezer until feeding.

Table 2. Ingredient composition of the digestibility test diets g.kg^{-1}

Ingredient	FMT	DFM	PRM	SQM	MM	CM	SBM	LMA	PEA	WG	REF1	REF2	REF3
Reference diet	699	699	699	699	699	699	699	699	699	699	999	999	999
Yttrium	1	1	1	1	1	1	1	1	1	1	1	1	1
Ytterbium	1	1	1	1	1	1	1	1	1	1	1	1	1
Fish meal (Triabunna)	300												
Defatted fish meal		300											
Prawn meal			300										
Squid meal				300									
Mussel meal					300								
Canola meal						300							
Soy bean meal							300						
Lupin meal								300					
Pea meal									300				
Wheat gluten										300			
Chemical composition g.kg^{-1}													
Nitrogen test ingredient	105.0	110.8	50.9	110.6	85.9	63.0	76.0	59.7	35.7	116.1	81.7	81.7	81.9
Nitrogen diet	88.7	90.5	72.4	90.3	83.1	76.1	79.9	75.1	67.8	92.1	81.7	81.7	81.9

6.3.3.3 Digestibility experiment

Three lobsters were randomly allocated to each of nine conical 40-L experimentation containers, and the tanks were assigned one of three test diets. Lobsters were fed equal rations to satiation twice daily (0900 h and 1600 h) for a one hour feeding period, after which uneaten feed was removed, and tanks flushed clean.

After one week of acclimation to the allocated diets and tanks, lobsters were blotted dry using paper towel, weighed and returned to their assigned tank. Lobsters were allowed to feed over a 1 h feeding period. After feeding, the tanks were flushed of all uneaten feed and faecal collectors were fitted to each tank and immersed in an ice slurry. Faecal collection continued until just before the next feed, when collectors were removed. The faeces were gently tipped onto a 150µm screen and rinsed into a sample container using chilled distilled water to remove salts (Brunson et al., 1997). All faecal samples were frozen immediately. During experiment 1, three sampling periods were tested, each of 5 consecutive day duration. During each pooling period, the evening (pm) and morning (am) feeds were pooled separately. In Experiment 2, both am and pm faeces over each of the 5 days were pooled into one sample, which was freeze dried to constant weight and stored at –20°C for chemical analysis. As feeding was generally immediate and of short duration, protein loss from pellets prior to ingestion was assumed to be minimal. If exudiae were consumed, or cannibalism occurred, collection ceased for 48 h but normal feeding retained to evacuate digestive tract of non-pellet materials.

6.3.3.4 Chemical analyses

Nitrogen and carbon were determined by elemental analysis (Leco CHNS-932 using cysteine standard, and BCR Haricotts Bean standard, in Sn crucibles). Crude protein was calculated (N X 6.25). Yttrium was measured by digesting feed and faecal samples in 3 ml HNO_3:H_2SO_4 (2:1) at 90˚C until the residue was clear. A 1:5 dilution in distilled water was analysed for yttrium by flame atomic absorption spectrometry (Varian SprectrAA 300), using a yttrium lamp (Varian, Australia) and N_2O/C_2H_2 flame.

6.3.3.5 Calculations

Apparent digestibility (%) of the reference diet was calculated:

$$AD\% = 100 - [100(\%I_{diet} / \%I_{faeces}) \text{ X } (\%N_{faeces} / \%N_{diet})]$$

(Maynard and Loosli, 1969) where I is the inert marker (chromic oxide) and N the nutrient. Apparent digestibility (%) of the test ingredient was calculated according to (Sugiura et al., 1998) based on the 70:30 ratio of reference diet to test ingredient in each test diet

$$AD_{Ing}\% = (Nutr_{TD} \times AD_{TD} - 0.7 \times Nutr_{BD} \times AD_{BD})/(0.3 \times Nutr_{Ing})$$

Where AD_{Ing} = apparent digestibility (or availability) of nutrients in test ingredient; $Nutr_{TD}$ = nutrient concentration in test diet; AD_{TD} = apparent digestibility of test nutrients in the test diet; $Nutr_{BD}$ = nutrient concentration in the basal diet; AD_{BD} apparent digestibility of the basal diet; $Nutr_{Ing}$ = nutrient concentration in the test ingredient.

6.3.3.6 Statistical analysis

Differences in apparent digestibility coefficients between the diets were tested for using one-way ANOVA unless otherwise stated. Significant differences were identified using Tukey-Kramer HSD.

6.3.4 Results

6.3.4.1 Experiment 1

There were no significant effects on apparent digestibility of crude protein (AD_{CP}) due to an interaction of sampling time (am or pm) with diet ($F_{2,5}$=0.918, P=0.408) (Table 3.). There was no significant interaction between diet and the sampling week ($F_{2,6}$=0.997, P=0.421), and overall differences in AD_{CP} between the three sampling blocks were not significant ($F_{2,16}$=2.609, P=0.085). Differences between the AD_{CP} of individual ingredients over the three sampling weeks were not significantly different in the pea meal and lupin diets (respectively $F_{2,10}$=0.63, P=0.557; $F_{2,17}$=0.321, P=0.73), however there was a significant decrease in the AD_{CP} of the reference 1 diet from between first two weeks and the third week of sampling ($F_{2,16}$=9.91, P=0.002).

Table 3. Percentage apparent protein digestibility coefficients (AD_{CP}) for various ingredients fed to juvenile *Jasus edwardsii* over a three one-week sampling periods (after 1 week of dietary acclimation)

Diet	Sampling week	AD_{CP}	Standard error
Lupin meal	1	104.84	6.9
	2	97.79	5.9
	3	97.73	6.3
Pea meal	1	69.01	26.0
	2	54.40	31.0
	3	32.54	8.3
Reference diet	1	96.94 [a]	5.5
	2	80.84 [a]	9.7
	3	54.51 [b]	4.2

Significant differences are indicated by different superscripts

6.3.4.2 Experiment 2

Because there were no differences in AD_{CP} between am and pm in Experiment 1, daily collections in Experiment 2 from am and pm were pooled over the sampling week and stored for analyses. There were significantly different AD_{CP} between the different test ingredients ($F_{12,37}$=6.026, P<0.001) (Table 4.). Lupin flour, wheat gluten and mussel meal were the best digested of the diets and had significantly higher AD_{CP} than the squid meal, canola meal and fish meal (Pivot). The squid meal was the poorest digested test ingredient with a significantly lower AD_{CP} than the reference diets and prawn meal. The processing level of the test ingredients had a significant effect on the digestibility of that ingredient. Flours and powdered ingredients had significantly higher AD_{CP} than hammer milled grains and coarse meals ($F_{2,37}$=11.968, P=0.001).

Table 4. Percentage apparent protein digestibility (AD_{CP}) of various marine protein and plant protein sources for juvenile southern rock lobster *J. edwardsii* over a week sampling period

Diet	AD_{CP}	Standard error
Marine protein sources		
Mussel meal	97.64	0.1
Prawn meal	77.21	19.1
Pivot fish meal	62.52	1.4
Defatted fish meal	53.08	5.7
Squid meal	7.26	2.3
Plant protein sources		
Lupin flour	100.12	6.0
Wheat gluten	90.11	9.7
Soybean meal	60.53	19.0
Pea meal	51.98	8.7
Canola meal	38.34	13.7
reference 1	77.43	9.02
reference 2	70.31	4.61
reference 3	75.00	10.74

6.3.5 Discussion

Digestibility values for dietary ingredients for use in rock lobster diets have not been previously investigated. Some data exist for ingredient digestibility for commercially cultured crustacean species (prawns, homarid lobsters and some species of crayfish). Where comparisons to *Jasus sp.* is not possible, other crustacean species provide the closest biological reference. Differences in the AD_{CP} between the species and between different forms of ingredient are prevalent as the AD_{CP} is influenced not only by form of ingredient, but also the age of test animal, experimental conditions, acclimation period to diet, faecal collection method, inert marker used etc. Comparisons made are to draw a best guide as to the suitability of a given ingredient and to explain the results observed.

Digestibility trials on crustaceans have rarely measured the digestibility of individual ingredients, more often assessing the entire diet digestibility, and often of complex formulations with many combinations of protein sources. The present study measures individual ingredient digestibility, to allow comparison to select superior ingredients for future formulation. Therefore the relative digestibility of these ingredients have been ranked in order to make recommendations for further development of ingredients. Comparisons, where possible, have been made to studies in the literature, but caution is recommended when comparing ingredient to diet digestibility as the effects of ingredient additivity and possible interactions have not been quantified in lobster to date. The values cited aid comparison of the present results to current literature, but recommendations are based on within study comparisons of apparent digestibility.

Marine meals are the primary protein source in most aquaculture diets, and also included as attractants and as sources of lipids, minerals and pigments. Of the marine meals tested, mussel meal was the most efficiently digested of the ingredients (97.5%). This corresponds

well to reported literature where fresh mussels and mussel meal based diets have supported superior growth when compared to fish meal based diets; the standard used for formulated commercial and experimental diets (Crear et al., 2002; Ward et al., 2003).

Direct comparisons for the same prawn meal used in this experiment were not possible, however the AD_{CP} calculated (77%) suggests a high digestibility, although with the high variability (CV=0.42), considerable caution in interpretation is recommended. Crustacean meals are regularly used in diets as attractants and as protein sources. High diet digestibility values were reported for *Penaeus monodon* fed protein combinations of shrimp and fish wastes (92.0%), scallop and shrimp wastes (92.5%) and commercial fish meal (source not specified) and shrimp meal (92.8%) and a sardine and lobster processing waste (85.4%) (Sudaryono et al., 1996). A crab protein based purified diet of similar composition to the current reference diet (46.8% protein, 15.9% lipid) for juvenile homarid lobsters had AD_{CP} of 92.6% , where crab protein comprised (Bordner et al., 1983).

Fish meal is the main protein source used in aquaculture diets, and experimental rock lobster diets to date. There is no data for fish meal digestibility as an ingredient in *J. edwardsii*. The fish meal digestibility measured in this study (62.5%) was slightly lower than AD_{CP} observed in an ingredient digestibility study in *P. monodon* 77.5% (Smith and Tabrett, 2002) and in *P. setiferus* where a diet containing menhaden fish meal had AD_{CP} of 75.9% (Brunson et al., 1997). The fish meal digestibility is lower than previously measured protein digestibility for *J. edwardsii* using a more complex fish meal based diet (80.3%) (Ward et al., 2003), which may partly be due to the higher digestibility of other diet ingredients. The hexane:methanol fat extraction of fishmeal slightly reduced the digestibility of the resultant de-fatted fishmeal (53.0%).

The apparent digestibility of squid meal was surprisingly low (7.3%), which was confirmed by minimal growth in a subsequent growth trial using the same meal (Ward, unpublished). Total volatile nitrogen analysis of this meal suggested levels above industry standard were present and probable poor batch quality is the likely cause of the low digestibility. Squid meal in other crustacean studies has relative high digestibility.

Plant materials are increasingly being used to replace fish meal in aquaculture diets as they are readily available, inexpensive and with protein levels suitable for formulating diets. The pulses, field peas and lupins and oil seeds such as soybean and canola are available in various forms and stages of processing and as protein concentrates and isolates for inclusion in aquaculture feeds. Meals in this study were commercial grains processed to a uniform level, rather than high protein concentrates as used in many studies. Of the plant meals in the present study, the lupin flour had the highest apparent digestibility with a value of 100.1%. This compares well to a study by (Smith, 1998) in *P. monodon* where a dehulled lupin meal protein was 95% digestible.

Cruz-Suarez et al., (2001) reported 89.0% AD_{CP} for a whole raw pea meal in *Litopenaeus stylirostris* and Bautista-Teruek et al., (2003) similarly reported 84.4% AD_{CP} in *P. monodon*. The high processing levels of the reported meals may have increased the amount of protein digested when compared to the present study, using a twice hammer milled pea meal, which had a protein digestibility of 52.0%. Soybean meal in the present study was also hammer milled twice, and had a digestibility value of 60.5%. Although variable, the AD_{CP} appears far lower than the reported value of 92.0% for *P. monodon* (Smith, 1998). Canola meal was not

well digested in rock lobster (38.3%), which contrasts to reported studies in *P. monodon* which had 78.0% AD_{CP} (Smith, 1998).

Wheat gluten was also very well digested (90.1%) by rock lobster. *P. monodon* have high been reported to digest wheat gluten well also with 102% digestibility (Smith, 1998). High grade wheat flours have been used in diets for *P. monodon* with protein digestibility of around 80% (Shiau et al., 1991).

The levels of ingredient processing especially for plant proteins should also be examined further to assess what level of processing is necessary to enable sufficient digestibility. The filtering and rejection of large particles from ingested homogenates is well documented, and to avoid the selective rejection of dietary components ensuring that the particles suit the size of the animal to be fed is essential. Further trials to examine the ingredient particle size effect of digestibility are underway for this species.

From this study, it appears that the marine meals; mussel meal and prawn meal are more digestible than the current protein source fish meal being used. The potential to increase the growth observed in growth trials to date is suggested, and further trials to quantify the nutrient value of these ingredients over long term growth trials is warranted. Considerable potential to replace fish meal with plant proteins was evident especially with the lupin, wheat gluten and soybean meals, however these must also tested for longer terms growth and health effects in this species. Further examination of the processing level of these plant ingredients may enhance the digestibility values reported and provide potential partial marine meal replacements in future diets.

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6.4 Optimising dietary protein content for the tropical rock lobster *Panulirus ornatus*

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6.4.1 Abstract

In an 8-week growth assay, juvenile *P. ornatus* lobsters have been shown to grow best on a diet containing at least 610 g kg^{-1} crude protein (CP) on a dry matter basis (DM) and a digestible protein to energy (P/E) ratio of 29.8 mg kJ^{-1}. Wild-caught *P. ornatus* of mean (± SD) initial weight of 2.5 ± 0.19 g were randomly allocated within weight stratification groups to 400 L tanks (1500 x 600 x 300 (h) mm) in accordance with a six treatment x four replicate randomized block design. The lobsters were fed either of five isolipidic diets (130 g kg^{-1} DM total lipid) in which the CP was serially incremented between 330and 610 g kg^{-1} DM, or New Zealand green-lip mussels, which were included as a reference diet. Each of the 18 tanks within three of the blocks contained 10 lobsters (1.9 ± 0.68 g), while each of the six tanks in the fourth block contained seven lobsters (4.3 ± 0.73 g). Tanks were provided with individual air-stones and supplied with heated (29 ± 0.5 ºC) and filtered (20 µm) flow-through (60 L h^{-1}) seawater (33 - 35 ‰) and sufficient brick shelters for all lobsters. Formulated dry-pelleted feeds were fed four times daily to satiety, while the mussels were fed twice daily to satiety. Digestibility of the pelleted feeds was high and similar across treatments (mean apparent crude protein digestibility 91%, mean apparent digestible energy 91%). Lobsters fed the pelleted diets responded to increasing dietary CP content with progressively higher growth rates, which increased from 0.36 g/wk with 330 g kg^{-1} CP to 0.91 g/wk with 610 g kg^{-1} CP (SE ± 0.07 g/wk). Survival was high (79 ± 4.5%) with the pelleted feeds. Both growth rate and survival were low with the mussel diet (0.42 g/wk and 41 ± 4.5%, respectively). These results demonstrate that tropical rock lobsters do grow well with high-protein, pelleted diets with a P/E ratio of 29 to 30 mg CP kJ^{-1} DE. However, the use of ingredients and feeding strategies that stimulate feed intake appear to be particularly important in maintaining high growth rates in this species.

6.4.2 Introduction

An earlier study at this laboratory examined the productivity responses of juvenile tropical rock lobster *Panulirus ornatus* fed pelleted diets that varied in crude protein (CP) content from 320 to 600 g kg^{-1} dry matter (DM) in factorial combination with lipid concentrations of either 60 or 100 g kg^{-1} DM (Smith et al., 2003). At both dietary lipid concentrations, increasing the CP content of the diet resulted in a curvilinear improvement in lobster growth rate with asymptotic responses occurring at 474 g kg^{-1} for the lower lipid series and at 533 g kg^{-1} for the higher lipid series. While that study clearly demonstrated lobster growth rate was responsive to dietary protein and lipid concentration, the absolute rate of growth of the lobsters observed in the 12-week study was low, with a maximum of around 0.2 g $week^{-1}$, as compared to calculated rates of 0.7-0.8 g $week^{-1}$ for tagged and re-captured lobsters of a similar size in the wild (Dennis et al., 1997). The comparatively poor growth of lobsters in the earlier study (Smith et al., 2003) prevented a firm conclusion being drawn as to the optimal dietary protein specification for juvenile *P. ornatus*.

Subsequently, the nature and rate of nitrogenous compounds leaching from thawed fresh-frozen mussels and pelleted feeds were determined and related to the feeding preference of juvenile *P. ornatus* for these same feeds (Williams et al. in prep). That work identified

soluble protein compounds and the free amino acids, glycine and taurine, as the principal leachate components affecting the lobster's preference for the feeds. Accordingly, the inclusion of protein hydrolysates in the diet as a rich source of soluble feeding stimulants was proposed as a mechanism for improving the lobster's acceptance of the feeds. This hypothesis was tested in the present study by re-examining the dietary protein requirement of juvenile *P. ornatus* when krill hydrolysate was included as a constant inclusion in pelleted dry diets that provided an incremental increase in dietary CP. This paper reports the results of this work and compares the efficacy of the developed pelleted dry diets against that of fresh-frozen green-lip mussel *Perna canaliculus* as feeds for juvenile *P. ornatus*.

6.4.3 Materials and Methods

6.4.3.1 Diet formulation and manufacture

A growth assay experiment was carried out to determine the optimal dietary protein specification for juvenile *P. ornatus*. An additional treatment of fresh-frozen mussels (*P. canaliculus*) was included in the treatment array for comparative purposes. Diets were formulated to provide an incremental increase in dietary CP over an intended range of 330 to 610 g kg^{-1} DM while maintaining the lipid content constant at 130 g kg^{-1} DM. The dietary protein gradient was obtained by varying the inclusion of Peruvian fishmeal at the expense of corn starch and diatomaceous earth (to balance ash), while cod liver oil was concomitantly reduced to balance lipid and energy (Table 1). All formulations contained a constant inclusion of krill hydrolysate, homogenized freshly caught bloodworm *Marphysa sanguinea* and Antarctic krill meal to enhance the attractiveness of the diet.

Table 1. Ingredients and formulation of the pelleted dry diets used in the growth assay (g kg^{-1} as used unless otherwise stated)

Ingredient	Treatment label				
	330CP	400CP	470CP	540CP	610CP
Fishmeal[1]	0	90	180	270	360
Starch[2]	271	204	136	68	0
Fish oil[3]	44	33	22	11	0
Diatomaceous earth	56	44	33	22	11
Wheat flour	140	140	140	140	140
Krill meal[4]	300	300	300	300	300
Krill hydrolysate[5]	80	80	80	80	80
Gluten[6]	55	55	55	55	55
Lecithin[7]	12.5	12.5	12.5	12.5	12.5
Other dry ingredients[8]	41.5	41.5	41.5	41.5	41.5

[1] Peruvian fish meal, 68% CP, supplied by Ridley Aquafeeds Pty Ltd, Narangba, Queensland.
[2] Corn starch, Janbak Industries Pty Ltd, Brisbane, Queensland, Australia
[3] Cod liver oil, Melrose Laboratories, Box Hill, Victoria, Australia
[4] Antarctic krill, Inual, Santiago, Chile
[5] Wheat gluten, 76% CP, Janbak Industries Pty Ltd, Brisbane, Queensland
[6] Krill hydrolysate, Speciality Marine Products Ltd, Vancouver, B.C., Canada
[7] Soybean lecithin, 70% lipid, Janbak Industries Pty Ltd, Brisbane, Queensland
[8] Other ingredients (g kg^{-1}): bloodworm, 120 (equivalent to 21 g kg^{-1} of 95% dry product); cholesterol (80 g kg^{-1}), 3; choline chloride (700 g choline kg^{-1}), 0.25; astaxanthin (Carophyll pink, 80 g kg^{-1}, donated by Roche Vitamins Australia), 1; ethoxyquin (Banox E), 0.25; vitamin premix (Williams et al., 2004), 11, manufactured by Rabar Pty Ltd, Beaudesert, Queensland; and trace mineral premix (Williams et al., 2004), 5.

All dietary dry ingredients were finely ground to pass through a 710 μm screen and the bloodworm was homogenized using a high-speed kitchen blender. Dry ingredients were mixed in a planetary dough mixer (Hobart A 200, Hobart Corporation, Troy, OH, USA) before adding the oil, homogenized bloodworm and sufficient water to form a dough of approximately 40 to 50% moisture. The dough was twice extruded through a 2.5 mm diameter die plate of the mincer attachment for the dough mixer and the resultant feed strands transferred to a steam oven for 5 min. After steaming, the feed strands were dried overnight at 40ºC in a forced draught oven, broken into pellets of 3 to 4 mm length and stored at -20°C until just before use.

Fresh-frozen, food grade, green-lip mussel *P. canaliculus* (New Zealand Greenshell™ mussel) were used for the fresh mussel dietary treatment. When feeding mussels to the lobsters, the meat was removed from the frozen half-shells, chopped into approximately 2 cm cubes, thawed and immediately placed in the tanks.

6.4.3.2 Experimental design, animals and management

The growth assay consisted of a randomised block experiment with each of the six treatments (five pelleted diets and fresh mussel) randomly assigned to one of 6 tanks in each of four blocks of tanks. Approximately 250 recently settled *P. ornatus* of approximately 2 to 5 g were collected from Trinity Inlet, Cairns, North Queensland, (16°55' S, 145°45' E) by divers and shipped to CSIRO Cleveland. The lobsters were held for one month to acclimatize to the holding tanks, during which time they were fed a mixture of green-lip mussel and extruded shrimp feed (Lucky Star, Taiwan Hung Kuo Industrial Co, Taiwan), formulated for the kuruma shrimp, *Penaeus japonicus*. Prior to the start of the experiment, lobsters were sorted by weight into one group of 180 lobsters of mean (± SD) weight of 1.9 ± 0.68 g and another group of 42 lobsters of 4.3 ± 0.73 g. Within each block, lobsters were equally distributed to tanks, with three blocks assigned from the smaller lobster group (10 lobsters per tank) and one block from the larger lobster group (7 lobsters per tank). The experimental tanks (1500 x 600 x 500 mm deep, 400 L water capacity) were constructed of fiberglass with a black gel coat on the inside and the bottom treated to provide a non-skid surface; sufficient clay bricks were placed in each tank to provide shelters for the lobsters, and the tanks were fitted with twin-walled polycarbonate sheeting covers. The tanks were supplied with heated (29 ± 0.5 ºC) and filtered (20 μm) flow-through (60 L h^{-1}) seawater (33 - 35 ‰) and were provided with individual air-stones. Tanks were housed within an enclosed seawater laboratory and lighting provided on a 12:12 dark-light cycle. Automatic feeders were used to issue the pelleted diets to the lobsters four times daily, nominally at 0930, 1630, 2200 and 0400 h. Feeding was to slight excess of apparent satiety with approximately two thirds of the daily allocation being provided at the nocturnal feeding times. Lobsters on the fresh mussel treatment were hand-fed the mussels to apparent satiety twice daily and nominally at 0930 and 1630 h. All uneaten feed was removed daily by siphon cleaning and tanks brushed to maintain good system hygiene. Lobsters were individually weighed at the start of the experiment and re-weighed at 4-weekly intervals until the experiment ended at 8 weeks. A daily record was kept of mortalities and moults in each tank.

6.4.3.3 Apparent digestibility

Attempts to collect faeces from the lobsters used in the growth assay were unsuccessful. The feces did not form a cohesive mass and dispersed almost immediately after defecation. Hence, larger lobsters (*P. ornatus*) of 600 to 800 g were purchased from a commercial fishing company (MG Kalis, Cairns, Queensland, Australia), and shipped to CSIRO, Cleveland. The lobsters were held individually in 100 l tanks (600 mm diameter, 400 mm depth, red

polyethylene) supplied with heated (29 ± 0.5 ºC) and filtered (20 µm) flow-through (60 L h^{-1}) seawater (33 - 35 ‰) and provided with individual aeration, until they moulted or commenced feeding on pelleted diets. As the faeces from these lobsters were similar those of the smaller lobsters, in that they did not form cohesive pellets and dispersed almost immediately after defecation, an alternative method of collection was developed (Irvin and Tabrett, 2004). A plastic flange (10 mm diameter) was attached to the exoskeleton, surrounding the anus of the lobsters. A small, rubber balloon was placed over the flange, enabling the collection of faeces that were mostly unexposed to the tank water. The balloons was placed on the lobsters prior to feeding in the mornings and removed 6 h later. The faeces were removed from the balloon or inside the flange and transferred to a vial for storage at -4ºC. Faeces from individual lobsters were collected and pooled for about 3 weeks until sufficient material had been collected for analysis, or until the first appearance of necrosis of the exoskeleton inside the flange. Four replicate samples were collected from lobsters fed either one of three of the diets used in the growth assay (330CP, 47CP and 610CP).

6.4.3.4 Growth rate calculations

Growth rate was calculated and expressed as linear (AWG), specific (SGR) or growth coefficient (DGC) according to the following equations:

$$AWG\,(g\,week^{-1}) = \left(\frac{W_e - W_s}{wk}\right)$$

$$SGR\,(\%\,d^{-1}) = \left(100\,\frac{\ln W_e - \ln W_s}{d}\right)$$

$$DGC\,(\%\,d^{-1}) = \left(100\,\frac{W_e^{1/3} - W_s^{1/3}}{d}\right)$$

Where W_e and W_s are the weights of the lobsters at the end and start of the growth period, respectively, ln is the natural logarithm, and d and wk are the number of days and weeks, respectively in the growth period. Growth rates were calculated for periods 0 to 4 weeks, 5 to 8 weeks and 0 to 8 weeks.

6.4.3.5 Chemical and statistical analyses

Samples of finely ground raw ingredients or diets were analyzed in duplicate by standard laboratory methods essentially in accordance with AOAC International (1999) recommendations, at either the CSIRO Marine Laboratory, Cleveland or at the Animal Research Institute, Yerrongpilly. Dry matter (DM) was determined by oven drying at 105°C to constant weight; ash by ignition at 600°C for 2 h; total nitrogen (total N) by a macro-Kjeldahl technique on a Kjel Foss automatic analyzer using mercury in the digestion; crude protein (CP) was calculated by multiplying total N by 6.25 irrespective of the nature of the N. Total lipid was determined gravimetrically after a Bligh and Dyer (1959) extraction as modified by Christie (1982). Gross energy (GE) was determined by isothermal bomb calorimeter using a microprocessor-controlled Leco AC 200 automatic bomb calorimeter. The fatty acid composition of the lipid extracts was determined as methyl ester derivatives (FAME). Lipids were esterified by the method of Morrison and Smith (1964) and analyzed by gas chromatography (GC) using a Hewlett Packard 5890 capillary GC with direct on-column injection and flame-ionization detector. The FAME were separated on a 50 m polar column (BP20, 0.33 mm i.d., 0.5 µm film thickness, Scientific Glass Engineering) with a

hydrogen carrier gas flowing at 2.7 ml min^{-1}. Amino acid composition was determined by ion-exchange chromatography using a Waters HPLC following hydrolysis of samples with 6 **M** HCl at 110°C under an atmosphere of N_2 for 18 h. Cystine was measured as cysteic acid and methionine as methionine sulfone after performic acid oxidation. Tryptophan was determined by the colorimetric method of Allred and MacDonald (1988) with 4.2 **M** NaOH at 110°C under an atmosphere of N_2 for 20 h. Carotenoids were estimated by extraction with 90% acetone followed by phase separation into hexane and measurement of absorbance at 470nm using E (1%) = 2100 (Britton 1995). Feed and feces samples, that were analysed for chromic oxide and ytterbium content, were digested in a multi-acid solution of nitric, sulphuric and perchloric acids. Chromium in the digestion solution was analyzed by GF-AAS and ytterbium analyzed by ICP-MS. The determined chemical composition of the pelleted diets and fresh mussel is detailed in Table 2.

Table 2. Analyzed chemical composition of the pelleted dry diets and green-lip mussels used in the growth assay (g kg^{-1} dry matter unless otherwise stated)

Composition	Treatment label					
	330CP	400CP	470CP	540CP	610CP	Mussel
Dry matter (g kg^{-1})	924	943	945	948	942	197
Crude protein	338	404	466	536	612	574
Digestible protein [1]	310	368 [2]	419	489 [2]	563	n.d.
Ash	125	131	134	134	138	116
Total lipid	130	137	138	128	128	115
Gross energy (kJ g^{-1})	19.6	19.6	20.2	20.5	20.8	19.6
Digest.energy (kJ g^{-1}) [1]	17.9	18.2 [2]	18.4	18.7 [2]	19.0	n.d.
Carotenoid (mg kg^{-1}) [3]	103	nd	106	nd	112	67
Fatty acids (mg kg^{-1})						
18:2n-6	78	76	74	67	66	10
18:3n-3	12	11	11	10	10	7
20:4n-6	4	5	5	5	6	10
20:5n-3	78	80	82	80	84	104
22:6n-3	67	71	74	74	79	73
Amino acids						
Arg	19.6	nd	nd	nd	30.9	44.3
Met + Cys	10.3	nd	nd	nd	16.0	13.8
Lys	17.6	nd	nd	nd	34.2	38.0
Thr	11.3	nd	nd	nd	22.9	23.5
Try	3.6	nd	nd	nd	6.1	6.2

[1] Digestibility determined with 600 g rock lobsters.
[2] Values derived using mean apparent digestibility data.
[3] In pelleted diets, values are from astaxanthin; in the mussel, values are from a combination of astaxanthin and other pigments, most likely chlorophyll, which co-eluted with astaxanthin.
n.d. not determined.

Lobster response data were analyzed as a one-way ANOVA and the effect of varying dietary protein on productivity responses was examined using regression analysis (Snedecor and Cochran 1989) with statistical packages (Queensland Department of Primary Industries) for balanced data (BALF) and regression (REGN). Percentage survival data were analyzed as

the natural and arcsine-transformed values, but as the transformation did not materially alter the significance of the *F*-statistic, only the natural values are reported.

6.4.4 Results

The apparent dry matter digestibility (ADMD), apparent crude protein digestibility (ACPD) and apparent digestible energy (ADE) of the two diets at the extreme ends of the range used (330CP and 610CP) and an intermediate diet (470CP) were all similar and not significantly different. The mean (± s.e.) of ADMD across diets was 86.5 ± 0.63%; for ACPD 91.2 ± 0.66% and for ADE 90.8 ± 0.57%. Increasing the CP and digestible CP content of the pelleted diets fed to the lobsters resulted in a marked improvement in growth rate but survival rate was not significantly ($P > 0.05$) affected (Table 3). Lobsters fed the fresh green-lip mussel grew and survived well during the first four weeks of the experiment but these traits declined abruptly during the 5 to 8 week period. For the whole eight weeks of experimentation, lobsters fed the mussel diet had the lowest survival rate and the growth rate was among the worst of all treatments.

Table 3. Productivity responses of lobsters to dietary treatment in the growth assay for periods 0 to 4, 5 to 8 and 0 to 8 weeks

Trait[1]	Treatment label						
	330CP	400CP	470CP	540CP	610CP	Mussel	± sem
				Period 0-4 weeks			
AWG (g wk^{-1})	0.24^{d}	0.28^{cd}	0.29^{cd}	0.42^{ab}	0.45^{a}	0.34^{bc}	0.033
SGR (% d^{-1})	1.17^{c}	1.46^{bc}	1.49^{bc}	1.93^{a}	1.95^{a}	1.67^{ab}	0.124
DGC (% d^{-1})	0.54^{c}	0.66^{bc}	0.69^{bc}	0.91^{a}	0.95^{a}	0.78^{ab}	0.061
Survival (%)	80.3^{a}	87.5^{a}	90.0^{a}	80.4^{a}	88.9^{a}	78.9^{a}	5.50
				Period 5-8 weeks			
AWG (g wk^{-1})	0.49^{c}	0.49^{c}	0.78^{bc}	0.95^{b}	1.37^{a}	0.49^{c}	0.124
SGR (% d^{-1})	1.69^{d}	1.79^{cd}	2.29^{bc}	2.53^{ab}	2.92^{a}	1.46^{d}	0.160
DGC (% d^{-1})	0.90^{cd}	0.96^{cd}	1.27^{bc}	1.44^{ab}	1.80^{a}	0.81^{d}	0.130
Survival (%)	91.0^{a}	91.4^{a}	93.3^{a}	96.9^{a}	88.8^{a}	57.8^{b}	5.71
				Period 0-8 weeks			
AWG (g wk^{-1})	0.37^{c}	0.41^{c}	0.53^{bc}	0.68^{b}	0.91^{a}	0.42^{c}	0.071
SGR (% d^{-1})	1.43^{d}	1.63^{cd}	1.89^{bc}	2.23^{ab}	2.44^{a}	1.56^{cd}	0.093
DGC (% d^{-1})	0.72^{d}	0.81^{cd}	0.98^{bc}	1.18^{ab}	1.38^{a}	0.80^{cd}	0.076
Survival (%)	72.8^{a}	79.0^{a}	84.0^{a}	77.8^{a}	79.0^{a}	40.8^{b}	4.51

[1] AWG = average weekly gain; SGR = specific growth rate; DGC = daily growth coefficient.
[a,b,c,d] Means in the same row with a common letter do not differ ($P > 0.05$).

Each of the different methods of calculating growth rate displayed similar productivity response trends but DGC showed the greatest treatment differentiation, especially when the whole 8-week experimentation period was examined. Regression analysis of the data for the 0-8 week period, showed growth rate of lobsters improved linearly with increasing dietary digestible CP content with the best fit ($R^2 = 0.84$) being found for DGC (Figure 1). There was no indication of a break-point in the growth rate response to increasing dietary digestible CP over the range examined in the experiment.

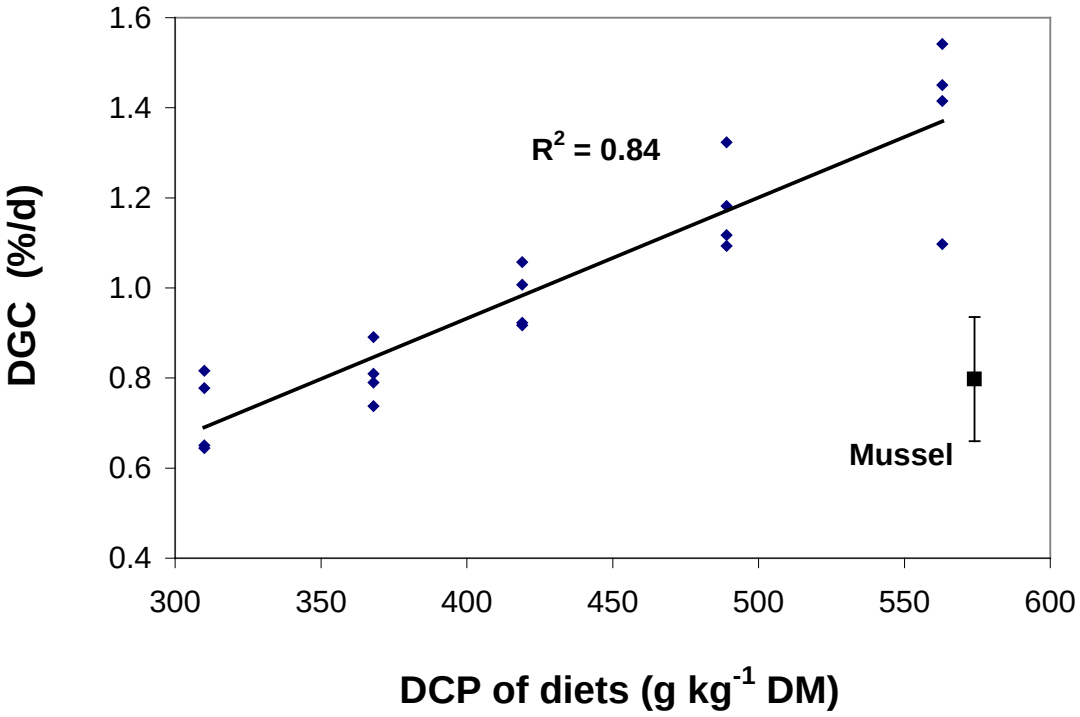

Figure 1. Relationship between the digestible crude protein (DCP) content of the diet (on a dry matter basis) (X) and daily growth coefficient (DGC) of lobsters fed pelleted diets for the 0-8 week period of the growth assay (Y). The DGC of lobsters fed thawed fresh green-lip mussel is shown at its corresponding crude protein content (dry matter basis). Error bars are ± sem

6.4.5 Discussion

Lobster growth exhibited a linear dose response to estimated dietary digestible protein concentration (Table 3, Figure 1) with no indication that the response was diminishing even at the highest level of dietary CP examined, viz 612 g kg^{-1} DM (equivalent to 576 g kg^{-1} air-dry basis). Moreover, the rate of growth exhibited by the lobsters fed the highest CP diet was excellent, surpassing that of fresh-frozen green-lip mussel and was more than four-fold greater than that observed in the previous study with *P. ornatus* of a similar size (Smith et al., 2003). However, the observed DGC on the highest CP diet (1.38 % d^{-1}) was less than that of 1.9 % d^{-1} extrapolated for similar size *P. ornatus* in the wild (Dennis et al. 1997).

The high growth rates of lobsters fed the pelleted feeds observed in this study are jointly attributed to the inclusion of krill hydrolysate in the formulation and to the adoption of four times daily feeding, as apposed to twice daily feeding in the previous study (Smith et al., 2003). Apart from the inclusion of krill hydrolysate, the test diets differed from those of the earlier study in that they contained higher lipid content (130 cf. 106 g kg^{-1} DM). The lipid, and hence energy content, of the diets were increased to 130 g kg^{-1} DM in response to both the better performance of lobsters fed a commercial shrimp feed, and the increased growth response with increasing lipid content in the diets used to assess response to dietary protein (Smith et al., 2003). This level of dietary lipid might be considered as being higher than optimal as in most previous studies with crustaceans the best survival and growth responses

have been achieved with between 50 and 80 g kg^{-1} of lipid (D'Abramo, 1997). However, the commercial shrimp feed, formulated for *Penaeus japonicus*, which resulted in the highest growth rate observed in the study by Smith et al. (2003), contained 122 g kg^{-1} total lipid on DM basis, with 640 g kg^{-1} CP and a P/E ratio of 31.7 mg CP kJ^{-1} GE.
In this study, the diet that gave the highest growth rate (610CP) had the highest protein content (563 g kg^{-1} of digestible CP) and contained 18.9 kJ g^{-1} of digestible energy, which when combined equates to a digestible P/E ratio of 29.8 mg protein kJ^{-1}. As growth response did not show a peak and a decline with the series of diets in this study, the optimal P/E ratio has not been demonstrated. However, optimal performance in the two series of protein/lipid diets (Smith et al. 2003) was obtained with P/E ratios of 29.1 and 29.6 mg CP kJ^{-1} GE. These values are similar to those reported as being optimal with the southern rock lobster, *Jasus edwardsii*, (Ward et al., 2003) and close to optimal for the black tiger shrimp, *Penaeus* monodon (Hajra, 1988; Shiau and Peng, 1992). It is possible that similar performance would be obtained with a lower dietary protein content, provided that the P/E ratio was maintained at between 29 to 30 mg kJ^{-1}, and the diets were sufficiently attractive to the lobsters to incite high feed intake.

The krill hydrolysate product used in this study was prepared from Antarctic krill (*Euphausia superba*), which was subjected to a proprietary enzymatic process (Marine Speciality Products Pty Ltd, Canada) to produce a dry hydrolysate composed predominantly of short chain peptides. As observed in this laboratory with *P. ornatus* (Williams et al. in prep) and for other crustaceans (Mackie 1973; Derby 1984; Carr and Derby 1986), small soluble peptides and free amino acids are potent feed attractants that induce strong feeding behaviour in crustaceans. The inclusion of krill hydrolysate at 82 g kg^{-1} in an otherwise non-marine protein, soybean-based diet was shown by Floreto et al. (2001) to be as effective as fresh blue mussel *M. edulis* or fishmeal-based formulated feeds for rearing juvenile American clawed lobster *Homarus americanus*. However, increasing the amount of krill hydrolysate in the diet to levels of 492 g kg^{-1} and above resulted in significantly better body weight gains being achieved by the lobsters. Providing the feed pellets as four discrete meals per day in the present study would have assisted in maintaining a strong presence of feeding stimulatory cues in the water for the lobsters. Further research is needed to determine the productivity benefits of increased feeding frequency and to design feeding strategies that take into account the increased cost and potentially greater feed wastage of such practices.

The comparatively poor growth and high mortality of lobsters fed the thawed fresh-frozen mussel was an unexpected observation. Mussels have typically been used as a reference or benchmark food source for both the larval (Kittaka and Kimura, 1989; Kittaka et al., 1997; Matsuda and Yamakawa, 2000) and grow-out (James and Tong, 1997; Crear et al., 2000; Glencross et al. 2001; Ward et al. 2003) rearing of spiny lobsters and alternative live or formulated feeds have invariably resulted in substantially poorer rates of productivity. The mussels used in this study were food grade, frozen half shell green-lip mussel *P. canaliculus* (New Zealand Greenshell™ mussel) that was kept frozen until fed to the lobsters. A New Zealand study (James and Tong 1997) compared the effect of feeding juvenile *J. edwardsii* on either of two species of mussels, blue *Mytilus galloprovincialis* or greenlip *P. canaliculus* when provided as either fresh, thawed fresh-frozen, or aged (fresh mussel fed every third day) product. Lobsters grew better on the blue mussel, though not significantly so when fed the fresh product, while feeding thawed fresh-frozen mussel resulted in a 15 to 30 % slower lobster growth rate than those fed fresh mussel; growth of lobsters on the aged mussel was intermediate between that of fresh and thawed fresh-frozen. James and Tong (1997) offered no explanation for the observed differences in the lobster growth efficacy between the

alternate mussel products. Nutritional differences unrelated to the way the mussel was handled may have contributed to the observed result since the fresh mussels were held alive on mussel lines or in cages throughout the course of the 3-month experiment whereas mussels for the thawed fresh-frozen product treatment were held frozen in a domestic freezer at -20ºC from the outset and until required to be fed. However, Juinio-Menez and Ruinata (1996) also found thawed fresh-frozen green mussel *Perna viridis* to be an inadequate sole diet for bilaterally ablated juvenile *P. ornatus* in a 4-month experiment and suggested that the low survival rate (6%) of these lobsters was due to the nutritional inadequacy of the thawed fresh-frozen mussel. Deaths of lobsters fed thawed fresh-frozen mussel in our experiment coincided with molting. This observation was similar to that reported by Juinio-Menez and Ruinata (1996), implying some form of nutritional inadequacy of the mussel, and akin to the well recognized 'molt death syndrome' of juvenile clawed lobsters (Conklin et al. 1980; Floreto et al. 2000).

While freezing may have deleteriously altered the nutritional quality of the mussel, it is also possible that the nutritional requirements of *P. ornatus* are sufficiently different to that of other spiny lobsters as to make mussels an inappropriate sole diet for this tropical species. The natural diet of *P. ornatus* appears remarkably similar to that of other spiny lobsters with invertebrates such as polychaete worms, gastropods, bivalves and crustaceans being heavily preyed upon (Joll and Phillips 1986; Pitcher 1993). However, *P. ornatus* is unusual in that it does not readily enter baited traps or pots and is caught commercially only by diving. Whether or not this is because conventional dead baits are unattractive to *P. ornatus* or a predilection of this species to not enter pots is unknown. A further possible reason for the poor performance of lobsters fed the thawed fresh green-lip mussel is that the particular batch of mussel used in the study had deteriorated prior to freezing or contained dinoflagellates or other toxic contaminants (Matsuyama et al. 1997; Sallenave et al. 1999; Amzil et al. 2000). These seem unlikely to be the case since juvenile *P. ornatus* fed at this laboratory on a different consignment of green-lip mussel or on blue mussels showed a similar marked retardation of growth with both types of mussels after a feeding period of 4 weeks (Barclay et al., in prep). It must be concluded that thawed fresh-frozen and probably fresh mussels as well, are not suitable as a sole diet for juvenile *P. ornatus*.

In conclusion, the study has demonstrated that a pelleted dry diet comprising fishmeal, krill meal and krill hydrolysate as the protein sources and fed four-times daily enable juvenile *P. ornatus* to grow at rates equal to tag and re-capture lobsters in the wild. However, we were not able to determine an optimal dietary protein concentration since lobster growth increased linearly with increasing protein up to the examined maximum of 603 g kg^{-1} DM, which had a digestible P/E ratio of 29 mg CP kJ^{-1} DE.. This protein concentration is considerably higher than the earlier reported optima of 474-533 g kg^{-1} for slowly growing juvenile *P. ornatus* (Smith et al. 2003) and higher than optima of 500-550 and 360-380 g kg^{-1} DM respectively for *Panulirus cygnus* (Glencross et al. 2001) and *J. edwardsii* (Ward et al. 2003), but when combine with dietary energy provides a similar P/E ratio. An aspect for further study is whether this increase in growth performance is a function of increased dietary digestible protein or an increase in metabolisable energy. A diet solely of green-lip mussel appears to be nutritionally inadequate for *P. ornatus*.

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6.5 Dietary carbohydrate:lipid ratios and nutritional condition in juvenile southern rock lobster, *Jasus edwardsii*

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6.5.1 Abstract

The availability of formulated diets is considered economically imperative if rock lobster aquaculture, based on the collection and ongrowing of puerulus, is to progress. Central to the development of diets is an understanding of the nutritional requirements of the species. This study determined the effect of different dietary carbohydrate:lipid ratios (17:1, 5:1, 2:1, 0.8:1) on the growth and nutritional condition of juvenile southern rock lobsters, *Jasus edwardsii*, by measuring standard growth parameters, proximate composition of the whole body and digestive gland, and from a histological investigation of the digestive gland. Four replicate groups of 8 lobsters (initial weight 5.08 ± 0.98g (mean ±SD)) per diet treatment were held in 50 l tanks, in a recirculating system at 18^{o}C for 84 days. Maximum growth and the highest levels of lipid and dry matter in digestive glands and whole bodies was found in lobsters fed a diet containing 27% carbohydrate and 13.5% lipid (2:1 ratio) suggesting that of the four experimental diets, this diet provided the best balance of lipid and carbohydrate. Digestive gland histology supported this conclusion and lobsters fed low carbohydrate, high lipid diets were in the best nutritional condition, with high lipid accumulation, and structurally sound epithelial cells. Digestive gland epithelial cells of lobsters fed the high carbohydrate, low lipid diets were compressed, of inconsistent shape and size, with low lipid accumulation. Histology is therefore seen as a feasible method, in addition to growth and proximate composition data, to further examine the effect of diets in nutritional studies of crustaceans.

Keywords: lobster, nutrition, formulated diet, aquaculture, lipid, carbohydrate, histology

6.5.2 Introduction

High market value and a shortage of wild stocks (Lipcius and Cobb, 1994) have generated considerable interest in the development of rock lobster aquaculture (Kittaka and Booth, 1994). The ability to capture pueruli in specialised collectors has encouraged researchers and commercial farmers to undertake the 'ongrowing' of puerulus, postpuerulus and juveniles to marketable size. Such an industry, based on *Jasus edwardsii* (Hutton, 1975), has already commenced in New Zealand (Jeffs and Hooker, 2000). The availability of cost-effective artificial feeds is likely to improve the economic outlook of culturing *J. edwardsii* (Jeffs and Hooker, 2000), however their development has been slow, largely due to a lack of understanding of dietary requirements. This study aims to aid the development of a formulated diet by determining the appropriate dietary carbohydrate:lipid ratio for culture of the southern rock lobster, *J. edwardsii*.

Protein, essential for tissue growth and maintenance, is an expensive component of formulated diets. When insufficient energy is available in a diet from non-protein sources, protein may be catabolised to meet the energy requirements at the cost of nutrient supply somatic growth (Capuzzo and Lancaster, 1979; Sedgwick, 1979). The most efficient diets contain sufficient non-protein energy sources (lipid and carbohydrate) that are metabolised

preferentially to protein to meet general energy requirements, leaving an organism to direct the maximum level of available dietary protein into growth (Sedgwick, 1979; Bautista, 1986). The majority of studies on the use of dietary lipid and carbohydrate have focused on penaeid prawns due to their extensive worldwide culture. These studies have shown that lipid has a limited role in optimising protein retention (Ackefors et al., 1992; Kanazawa and Koshio, 1994; D'Abramo, 1997), with greater reliance on carbohydrates, such as starch, as a non-protein energy source (Shiau and Peng, 1992; Cruz-Suarez et al., 1994; Ahamad Ali, 1996). A limited number of studies have examined carbohydrate:lipid ratios: and suggested ratios of 4:1 for the freshwater prawn *Macrobrachium rosenbergii* (D'Abramo, 1998) and 3:1 for juvenile *Macrobrachium malcolmsonii* (Das et al., 1995). However, it is not known whether these findings are broadly applicable to other species of crustaceans generally and *J. edwardsii* specifically.

Traditionally, growth, survival, feed conversion ratio, digestibility values, digestive gland index, and the condition factor have been used to test the biological value of a diet. However, one of the primary roles of the digestive gland in decapod crustaceans is energy storage in the form of lipid and glycogen (Gibson and Barker, 1979; Vogt, et al., 1985; Icely and Nott, 1992). These reserves, which are stored as granules, fat bodies or lipid droplets in R-cells, and are used for energy during the moult cycle (Al-Mohanna and Nott 1987; Takahashi et al., 1994; Nishida, et al., 1995), have been shown to be an indicator of nutritional condition (Storch, 1984; Vogt et al., 1985, 1986; Vogt, 1987). Studies on the effect of dietary regime on the nutritional condition of prawns have shown that R-cell structure and lipid content changes markedly with diet (Papathanassiou and King, 1984) and that these cells could therefore be used to monitor the nutritional value of diets (Vogt et al., 1985).

The specific aims of this study were to determine the effect of different dietary carbohydrate:lipid ratios on growth, body composition and nutritional condition of juvenile southern rock lobsters, by measuring standard growth parameters and proximate composition, and by undertaking a histological investigation of the digestive gland. Diets were formulated to have lower than optimum protein contents in order to promote maximum use of non-protein energy sources.

6.5.3 Materials and methods

6.5.3.1 Experimental diets

Four experimental diets were formulated to contain about 32% crude protein using South American fish meal (68% crude protein, Skretting, Australia), de-fatted using a 2:1 hexane:ethanol extraction (Ward et al., in press). Lipid (cod liver oil, Melrose Laboratories Pty Ltd., Box Hill, VIC) content in each diet was manipulated to between 3 - 18%. Carbohydrate (gelatinized maize starch, BO11C, Earlee Products, Qld) content was added to maintain a calorific value (gross energy) of around 15 MJ kg^{-1} DM. Carbohydrate levels ranged from 15 to 50% and the final carbohydrate:lipid ratios of the four diets were 17.4:1, 4.8:1, 2:1 and 0.8:1 (Table 1).

Table 1. Ingredient composition and proximate analysis of four experimental diets containing different ratios of carbohydrate:lipid

	Diet			
	17.4:1	4.8:1	2:1	0.8:1
	Ingredient composition (g.kg^{-1})			
Fish meal (de-fatted)	250	250	250	250
Fish meal	50	50	50	50
Wheat gluten	100	100	100	100
Pre-gelatinized starch	400	288	175	60
Cod liver oil	0	50	100	150
Cholesterol	2	2	2	2
Lecithin (soya)	12	12	12	12
Mussel	50	50	50	50
Manucol	60	60	60	60
Banox E	0.2	0.2	0.2	0.2
Carophyll Pink	1.5	1.5	1.5	1.5
Choline chloride	0.2	0.2	0.2	0.2
Diatomaceous earth	50	110	180	240
Vitamin C (Stay C)	1	1	1	1
Vitamin premix	2	2	2	2
TSP phosphate	20	20	20	20
	Chemical composition (% dry matter)			
Dry matter	96.70	97.70	98.50	99.00
Crude protein	32.50	31.88	31.88	31.25
Total lipid	2.90	8.10	13.50	18.70
Carbohydrate	50.50	38.90	27.00	15.70
Ash	14.10	20.50	27.70	34.20
Gross energy (MJ. kg^{-1}DM)	15.61	13.95	14.80	14.21
CP:GE[2]	20.82	22.85	21.54	21.99

[1] Carbohydrate = Dry matter – (%crude protein + %total lipid + %ash).
[2] Crude protein:gross energy (gCP.MJ GE^{-1}).

Binders (sodium alginate, Manucol DM, Germantown International and wheat gluten, Goodman Fielder, Summer Hill, NSW), vitamin C (Roche Chemicals), lecithin (Lucas Meyer), Banox E (Rhone-Poulenc, Qld), Carophyll Pink (Roche Chemicals), cholesterol, choline chloride, TSP phosphate and diatomaceous earth (Sigma Chemicals, Castle Hill, NSW) were added in constant proportions (Ward et al., in press). Fresh blue mussel (*Mytilus edulis*) was added to enhance the attractiveness of the diet to the lobsters.

The ingredients were mixed thoroughly and extruded into 25 mm x 3 mm pellets. Pellets were placed into a bath of 10% aqueous calcium chloride for 3-5 min to activate the binding properties of the sodium alginate. The pellets were dried at 50°C for 48 h, and stored at –20°C. The proximate composition (Table 1) and water stability of each diet was determined.

6.5.3.2 Growth experiment

J. edwardsii puerulus collected from the east coast of Tasmania during Dec-Feb 1999/2000 were on grown to juveniles in a 1000 l Rathbun tank flow-through system at 15-18°C on a diet of blue mussels and commercial prawn diet (*Penaeus japonicus*) (Higashi Maru #12).

One hundred and twenty eight juveniles (initial mean weight 5.08g ± 0.98g (SD)) were randomly allocated to 16 x 50 l black polyethylene tanks (i.e., 8 lobsters per tank), which were part of a recirculating system. Temperature was 17.9 ± 0.7ºC, salinity was between 33-36 ‰ and photoperiod was 12L:12D. Hides and an airstone were provided in each tank for refuge and maintenance of oxygen levels, respectively.

Each treatment was replicated four times and randomly allocated to the tanks. Lobsters were acclimated to the tanks and respective test diet for one week. During this period, mortalities were replaced with similar sized animals from the stock population. Following acclimation, lobsters were individually blotted dry with tissues, weighed and tagged with small circular tabs of waterproof paper glued to their carapace (Locktite 454). The lobsters were fed at a rate of 4% $BW.d^{-1}$ (3.82% $BW.day^{-1}$ on a dry weight basis) for the duration of the experiment. The diet was fed at two times during the day: 30% at 0830 h and 70% at 1730 h at the start of the dark photoperiod when the majority of feeding activity occurs. Uneaten pellets were collected at 1700 and 0800 h, siphoned onto a 1 mm mesh screen, rinsed with freshwater to remove residual salt and stored at -20°C. Uneaten feed was dried at 100°C for 16-20 hours.

The growth trial was conducted for 84 days. Animals were weighed every 21 d and daily rations altered based on the biomass in each tank. Mortalities were excluded from the analysis. Moults were recorded and those lobsters re-tagged. After 84 days the lobsters were starved for 24 h to clear the gastrointestinal tract of ingested food (Davis and Robinson, 1986) and then placed in a chill coma. Ten intermoult lobsters, stages C or D1, were sampled from each diet treatment, the digestive glands removed and weighed, and both body parts stored frozen in liquid nitrogen (-86°C) for later proximate analysis. Digestive glands were dissected from an additional three lobsters from each diet treatment, immediately fixed and used for histological examination of the digestive gland. Sixteen lobsters from the initial population (controls) were sampled for proximate analyses, while five were dissected for histology.

6.5.3.3 Proximate analysis

Digestive glands and whole bodies were freeze-dried (at -45°C and 200 Pa vacuum in a Dynavac Freezedrier) to constant weight. Dry matter was calculated as the difference between wet weighed samples and their weight after freeze drying. Samples were then ground using a mortar and pestle. Total lipid was determined by chloroform methanol extraction (Bligh and Dyer, 1959), protein by Kjeldahl digestion (crude protein = % nitrogen x 6.25) and energy by bomb calorimetry (Gallenkamp autobomb, calibrated with benzoic acid). Ash was determined by burning samples in a muffle furnace at 550°C for 16 h. Carbohydrate was determined by difference (Carbohydrate = Dry matter – (%crude protein + %total lipid + %ash). Insufficient material prevented direct energy estimates for digestive gland tissue. Instead, energy was calculated using standard gross energy values for protein (23.6 kJ g^{-1}), lipid (38.6 kJ g^{-1}) and carbohydrate (17.2 kJ g^{-1}) (Jobling, 1994).

Whole body (WB) proximate composition (% dry matter) values were calculated using a ratio of the two analysed body parts in relation to the whole body weight. Dry weight compositions were then adjusted using mean moisture contents for each treatment, to give composition on a wet weight basis. Efficiency of nutrient retention was calculated for protein and energy using the formula:

Nutrient retention (%) = 100 x (total nutrient gain (g) / total nutrient intake (g))

To ensure that food consumed by lobsters that died during the trial did not distort the calculation of feed intake data, the number of lobster feeding days was calculated (based on mortality data at each weighing) and used to calculate daily weight gain and feed intake for each lobster.

6.5.3.4 Histology

Digestive gland tissue was fixed in Bouin's for 48 h, dehydrated, infiltrated and embedded in paraffin wax (Tissuetek® II, RX-11B), sectioned at 5μm on a microtome (Microm, Heidelberg) and stained with haemotoxylin and eosin. Sections were examined on a Leitz Wild MPS 52 microscope.

To assess the amount of lipid stored in the R-cells of the digestive gland, tissue was fixed in 2.5% gluteraldehyde in 0.1M cacodylate buffer (pH 7.1) for 2 h at room temperature (20°C). Following 3 x 10 min washes in 0.1M cacodylate buffer (pH 7.1), tissue was post-fixed in 1% osmium tetroxide in 0.1M cacodylate buffer for 1 h at room temperature. Following further washes in the 0.1M cacodylate buffer, the tissue was dehydrated and embedded in JB4 resin. Resin blocks were sectioned at 2μm on a microtome (Microm, Heidelberg) using glass knives, and stained with polychrome blue. Sections were examined on a Leitz Wild MPS 52 microscope.

6.5.3.5 Statistical analysis

Growth performance of lobsters fed the experimental diets is presented as the mean and standard error of replicate tanks. Proximate composition whole body and digestive gland values were for 10 pooled animals per diet treatment. Analyses were conducted in duplicate and the mean of duplicate values used for data points. The effect of diet on growth performance, proximate composition of body and digestive gland, and nutrient retention were determined by one-way ANOVA (SPSS version 8) and post-hoc comparison by Tukey's HSD. The probability level for rejection of the hypotheses was 0.05. Data that did not show homogeneity of variance using Levene's statistic and visual interpretation of residual plots, was transformed using log10 or square root.

6.5.4 Results

6.5.4.1 Growth performance

Dietary treatment had no significant influence on survival (mean of 82 ±4.8%, Table 2). However, diet significantly affected growth with weight gain significantly greater for lobsters fed the 2:1 carbohydrate:lipid ratio diet (4.49 ± 0.59 g) and 0.8:1 diet (4.18 ± 0.35 g) than lobsters fed the 17.4:1 diet (2.36 ± 0.23 g; P=0.004). Lobsters fed the 17.4:1 carbohydrate:lipid ratio diet also had the lowest mean weight at the conclusion of the trial (8.08 ± 0.21 g) and lobsters fed the 2:1 carbohydrate:lipid ratio diet had the highest (9.91 ± 0.84 g), although the difference between the two was not significant. There were no significant differences in dry matter feed intake (FI), food conversion ratio (FCR), intermoult period or moult increment between the dietary treatments (Table 2).

Table 2. Growth performance (mean ±SE, n=4) of juvenile *J. edwardsii* fed four diets containing different carbohydrate:lipid ratios over a 84 day growth experiment

Attribute	Diet				Statistical analyses		
	17.4:1	4.8:1	2:1	0.8:1	df	*F*-test	P
Initial wt[1]	5.7±0.35[a]	5.0±0.30[a]	5.4±0.51[a]	5.3±0.35[a]	3,36	0.64	0.592
Final wt[2]	8.1±0.21[a]	8.5±0.50[a]	9.9±0.8[a]	9.5±0.42[a]	3,36	2.45	0.079
Wt gain	2.4±0.23[a]	3.5±0.39[ab]	4.5±0.59[b]	4.2±0.35[b]	3,36	5.22	0.004
Survive (%)	71.8±7.87[a]	87.5±5.11[a]	81.3±6.25[a]	87.5±0.00[a]	3,12	1.72	0.216
Feed intake[3]	0.15±0.01[a]	0.14±0.01[a]	0.16±0.01[a]	0.15±0.00[a]	3,12	1.28	0.325
FCR[4]	5.8±0.46[a]	4.2±0.19[a]	3.9±0.14[a]	4.0±0.09[a]	3,12	2.79	0.086
IP[5]	54.8±6.54[a]	58.9±1.86[a]	50.6±3.30[a]	51.0±2.77[a]	3,10	1.47	0.280
MI[6]	23.8±4.53[a]	16.8±1.00[a]	26.2±3.39[a]	27.7±4.14[a]	3,10	2.37	0.132

[1] Initial mean weight (g) of lobsters per diet treatment.

[2] Final mean weight (g) of surviving lobsters at end of experiment per diet treatment.

[3] Feed intake (g lobster^{-1} day^{-1}) = g dry feed consumed-dry matter loss from pellet.

[4] Food conversion ratio FCR = g dry feed consumed/g wet weight gain.

[5] IP = Intermoult period: number of days between first and second moult.

[6] MI = Moult increment (%): (weight gain after moult/weight before moult) X 100.

[a,b] Values which share the same superscript are not significantly different ($P > 0.05$).

6.5.4.2 Body composition

6.5.4.2.1 Whole body

Dry matter in lobsters fed the 17.4:1 carbohydrate:lipid ratio diet (28.77 ± 0.07%) was significantly (P<0.001) higher than in those fed the 0.8:1diet (25.56 ± 0.07%) (Table 3). There was a general decline in crude protein level in lobsters fed diets with higher levels of dietary lipid (12.10 ± 0.03% lobsters fed the 17.4:1 diet to 10.35 ± 0.03% lobsters fed the 0.8:1 diet). Lobsters fed the diet with the lowest lipid (17.4:1) had the highest level of whole body lipid (0.92 ± 0.01%), while lobsters fed the high lipid diet (0.8:1), had the lowest level of whole body lipid (0.77 ± 0.01%) (P<0.001).

Lobsters fed the 17.4:1 diet had significantly (P< 0.001) higher gross energy levels (mean calorific value 3.26 ± 0.01 MJ.kg^{-1}) than lobsters fed the 0.8:1 diet (2.66 ± 0.01 MJ.kg^{-1}). Increasing dietary carbohydrate increased whole body carbohydrate levels. Lobsters fed the 17.4:1 carbohydrate:lipid ratio diet had the highest carbohydrate content (7.21%), whereas lobsters fed the 0.8:1 diet had the lowest (5.95%). While there were significant differences in ash content between treatments, there were no particular trends with diets (Table 3). Although differences were not statistically significant lobsters fed the 2:1 carbohydrate:lipid ratio diet showed a tendency to have greater energy and protein retention (Fig. 1).

Table 3. Chemical composition (% wet weight) and gross energy (GE, MJ kg^{-1}) of control (initial) lobsters (mean, ±SE, n=8 for whole body and mean, ±SE, n=3 or n=10 for digestive gland) and lobsters fed diets with four differing carbohydrate (CHO):lipid ratios (mean, ±SE, n=10), over an 84 day growth experiment

		Diet				Statistical analyses		
	Control	17.4:1	4.8:1	2:1	0.8:1	df	*F*-value	P
Whole Body[1]								
DM	$24.8^{\pm 0.76}$	$28.8^{\pm 0.07d}$	$26.1^{\pm 0.05b}$	$26.8^{\pm 0.07c}$	$25.6^{\pm 0.07a}$	3,36	432	0.00
CP	$10.7^{\pm 0.02}$	$12.1^{\pm 0.03d}$	$10.5^{\pm 0.02b}$	$10.9^{\pm 0.03c}$	$10.4^{\pm 0.03a}$	3,36	850	0.00
Lipid	$1.56^{\pm 0.01}$	$0.92^{\pm 0.01c}$	$0.81^{\pm 0.01 b}$	$0.80^{\pm 0.01b}$	$0.77^{\pm 0.01a}$	3,36	1041	0.00
GE	$2.94^{\pm 0.01}$	$3.26^{\pm 0.01d}$	$2.72^{\pm 0.01b}$	$2.91^{\pm 0.01c}$	$2.66^{\pm 0.01a}$	3,36	1379	0.00
CHO	5.70	7.21	7.13	6.35	5.95			
Ash	$6.86^{\pm 0.02}$	$8.54^{\pm 0.02b}$	$7.74^{\pm 0.02a}$	$8.67^{\pm 0.02c}$	$8.49^{\pm 0.02b}$	3,36	393	0.00
DGI	$6.52^{\pm 0.19}$	$5.31^{\pm 0.22b}$	$4.44^{\pm 0.31ab}$	$4.46^{\pm 0.23ab}$	$3.90^{\pm 0.25a}$	3	5.20	0.00
Digestive gland								
DM	$30.4^{\pm 1.20}$	$21.3^{\pm 0.73ab}$	$18.6^{\pm 0.43a}$	$22.2^{\pm 1.32b}$	$20.7^{\pm 0.85ab}$	3	2.96	0.04
CP	$15.6^{\pm 1.01}$	$10.4^{\pm 0.03c}$	$10.0^{\pm 0.01ab}$	$10.1^{\pm 0.13bc}$	$9.6^{\pm 0.01a}$	3	23.74	0.01
Lipid	$11.63^{\pm 0.13}$	$4.39^{\pm 0.06b}$	$3.32^{\pm 0.09a}$	$8.46^{\pm 0.06d}$	$7.79^{\pm 0.07c}$	3	1247	0.00
GE[2]	8.43	4.96	4.24	5.99	5.58			
CHO	1.56	4.72	3.48	1.91	1.73			
Ash	1.61 ± 0.04	$1.76^{\pm 0.01b}$	$1.77^{\pm 0.05b}$	$1.68^{\pm 0.01b}$	$1.50^{\pm 0.02a}$	3	20.12	0.01

[1] Values are calculated as a ratio of the two body part compositions.
Digestive gland index DGI = (digestive gland wet weight g / whole body wet weight g) x 100
Carbohydrate = Dry matter – (%crude protein + %total lipid + %ash)

[2] Due to low digestive gland mass, energy was calculated using values of 23.6 kJg^{-1} for protein, 38.6 kJ^{-1} for lipid and 17.2 kJ^{-1} for carbohydrate (Jobling, 1994).

DM = Dry matter; CP = Crude protein; CHO = Carbohydrate.

[a,b,c,d] Values which share the same superscript are not significantly different ($P > 0.05$).

6.5.4.2.2 Digestive gland

The digestive gland index (DGI) decreased significantly from lobsters fed the 17.4:1 diet (5.31 ± 0.22%) to those fed the 0.8:1 diet (3.90 ± 0.25%). Crude protein decreased significantly as dietary lipid increased from 10.41 ± 0.03% in lobsters fed the 17.4:1 diet to

9.63 ± 0.01% in lobsters fed the 0.8:1 diet (P=0.005) (Table 3). Digestive gland lipid content was significantly higher (P<0.001) in lobsters fed high lipid (2:1 and 0.84:1) diets (Table 3), than those fed low lipid (17.4:1 and 4.8:1) diets, with the 2:1 diet highest (8.46 ± 0.06%) and the 4.8:1 diet lowest (3.32 ± 0.09%). Lobsters fed the 2:1 diet also had the highest gross energy values 5.99MJ.kg^{-1}, but in this case animals fed the 4.8:1 diet had the lowest at 4.24MJ.kg^{-1}. Apparent carbohydrate levels in the digestive gland rose incrementally, with increases in carbohydrate content in the diets (Table 3). Lobsters fed the 0.8:1diet had the lowest digestive gland ash content (1.50 ± 0.02%), which was significantly different from the other dietary treatments (P=0.007).

6.5.4.3 Digestive gland histology
6.5.4.3.1 Control lobsters (initial population)

Epithelial cells were reasonably uniform in shape and size in control lobsters with B-, R- and F-cells all evident in the digestive gland tubules. Vacuolation was clearly observed in the cytoplasm of the R-cells, which were columnar in shape. There were a large number of lipid droplets in the cells, with little variation in droplet size.

6.5.4.3.2 Diet 17.4:1

There was no apparent difference in B- and R-cell numbers in the digestive gland tubules between lobsters fed the 17.4:1 diet and the control lobsters. However, the R-cells were swollen, with very little vacuolation and a large amount of granular material compared to control lobsters (Fig. 2A). In general epithelial cells were not consistent in shape or size. The R-cells in particular were compressed and appeared cuboidal in 17.4:1 fed lobsters, while they were more uniform, and columnar in shape in the control lobsters. There was also a large amount of haemocyte accumulation around the tubules and a small number of lipid droplets in the R-cells of 17.4:1 diet fed lobsters (Fig. 2B).

6.5.4.3.3 Diet 4.8:1

The epithelial cells of animals fed the 4.8:1 carbohydrate:lipid ratio diet were more uniform in shape and not as compressed as in the lobsters fed the 17.4:1 diet. Granular material was also present in the R-cells of 4.8:1 fed lobsters, giving them a swollen appearance (Fig. 2C). A low amount of vacuolation in the R-cells of lobsters fed 4.8:1 diets corresponded with a low amount of lipid droplet accumulation, compared to the control lobsters. There was, however, a noticeable increase in lipid droplet number in 4.8:1 fed lobsters compared to those fed the 17.4:1 diet. The tubules in 4.8:1 fed lobsters were either devoid of lipid droplets, contained a small amount of small droplets, or a few large lipid droplets (Fig. 2D).

6.5.4.3.4 Diet 2:1

There were several distinct differences in the condition of the digestive gland of lobsters fed the 2:1 diet compared with those fed the 17.4:1 and 4.8:1diets. There was greater vacuolation in the R-cells of lobsters fed the 2:1 diet and the epithelial cells were more columnar in shape giving the tubules a similar appearance to the control lobsters. Granulation within the R-cell cytoplasm was reduced (Fig. 3A) in 2:1 fed lobsters. Vacuolation in the R-cells corresponded with a high density of lipid droplets, at densities similar to the control lobsters. The droplets were dispersed evenly throughout the tubules, and were a reasonably uniform size (Fig. 3B).

6.5.4.4.5 Diet 0.8:1

Similar to lobsters fed the 2:1 diet, lobsters fed 0.8:1 diets displayed a prolific amount of vacuolation within the R-cells (Fig. 3C). Lobsters fed these two diets were similar, in that the tubules were structurally similar to the control lobsters, on account of the uniformity in size and shape of the epithelial cells. The digestive gland tubules in 0.84:1 fed lobsters were also dominated by the presence of lipid droplets (Fig. 3D).

6.5.5 Discussion
6.5.5.1

Over the 84 days of the trial southern rock lobster grew on all diets but there were few statistical differences between treatments. Growth performance was suggestive of the 2:1 and 0.8:1 carbohydrate: lipid diets being superior to the higher carbohydrate diets. It is proposed that the 2:1 diet was closest to the optimum ratio because it produced the numerically highest growth and significantly higher lipid levels in the digestive glands. This indicated the 2:1 diet promoted the greatest protein retention as well as storage of the largest energy reserves in the digestive glands, which are crucial for moulting (Chang, 1995)**.** The 17.4:1 diet was clearly the poorest diet with significantly lower weight gain compared with the other treatments. The 2:1 dietary carbohydrate:lipid ratio performance is similar to that found for other crustaceans of both of marine and freshwater origin. A 3:1 ratio maximised protein sparing and growth in *M. malcolmsonii* (Das et al. 1995) and *M. rosenbergii* (Clifford and Brick, 1979). Growth of the marine shrimp *Penaeus monodon* and *Litopenaeus stylirostis* was compromised with dietary carbohydrate:lipid ratios greater than 3:1 (ratios determined from data in studies by Catacutan (1991) and Rosas et al. (2000), respectively).

The protein content of the experimental diets (32% and 21-22 g CP.MJ GE^{-1}) was lower than recently determined requirements of 29 g CP.MJ GE^{-1} for *J. edwardsii* (Ward et al., in press) and 27.7 g CP .MJ GE^{-1} for the best performing diet fed to western rock lobster (Glencross et al., 2001). This meant that the amount of protein incorporated into our test diets was too low to meet this protein requirement. However, the over-supply of potentially available non-protein energy tested how efficiently these sources were used for energy when the needs for reducing the use of protein as an energy source and for maximising protein retention were increased. The potential for relative differences in protein retention was maximised under this design providing the non-protein energy sources were available. The 2:1 diet promoted numerically higher protein retention (11.95%) but there was no significant difference between the diets. In addition, protein retention can be higher than 12% in juvenile *J. edwardsii*, it was 19% when mussels (*M. edulis*) were used as the diet (Crear et al., 2000). The low energy retention (<8%) and similarity across diets indicated low energy availability from both dietary carbohydrate and lipid sources. A consequence of decreasing the carbohydrate: lipid ratio is decreasing the amount of dietary carbohydrate and increasing the dietary lipid. Decreasing digestibility of carbohydrate and or lipid with increasing inclusion level would have been reflected by differences in digestible energy. Although, differences in digestible non-protein energy between the diets may have explained the results more clearly the purpose of the present experiment was to determine the best carbohydrate: lipid ratio for use at a given protein inclusion in current rock lobster diets. In addition, digestibility values are currently unavailable, difficult to determine, arguably less accurate than growth data and were beyond the scope of this study.

Previous studies have found that high dietary levels of lipids are generally associated with significant reductions in growth rate of crustaceans (D'Abramo, 1997), with a maximum of around 10% being tolerated. In contrast, dietary lipid levels of at least 13.5% and up to 18.7% did not cause a decrease in growth rate of *J. edwardsii* juveniles in the present study. *J. edwardsii* juveniles grew well on commercial shrimp feeds that contained relatively high (12-13%) levels of lipids (Crear et al., 2002). Gerring (1992) also found that a diet containing approximately 18% total lipid produced similar growth rates in *J. edwardsii* to diets containing 5 and 10% lipid, although the low growth rate and survival make it difficult to predict the optimal level. However, the relatively high levels of dietary lipid tolerated by *J. edwardsii* appear to provide more scope for its inclusion as a non-protein energy source than with other species of cultured crustaceans.

The preferential utilisation of carbohydrate over lipid has been demonstrated in many species of lobster (Capuzzo and Lancaster, 1979; Ackefors et al., 1992) and shrimp (Cruz-Suarez, et al., 1994; Ahamad Ali, 1996; Boonyaratpalin, 1998). Maximum dietary carbohydrate levels of 20-25% are tolerated by some crustaceans (Catacutan, 1991; Rosas et al., 2000), which may also be the case for *J. edwardsii*. In the present study the 17.4:1 and 4.8:1 diets contained 40% and 29% pre-gelatinized starch, respectively. The growth performance and digestive gland data suggest that 17.5% pre-gelatinized starch was the highest useable level out of the current diets and the maximum available-carbohydrate inclusion is, therefore, between 17.5 and 29%. Since rock lobsters are lower order carnivores (King and Shepherd, 1982) and feed predominantly on molluscs, echinoderms and crustaceans and only a small amount of carbohydrate rich plant material (Edmunds, 1995), it is not surprising that they use protein and lipid, rather than carbohydrate, to meet the majority of their energy requirements.

The overall size of the digestive gland, measured as digestive gland index, was highest in lobsters fed the high carbohydrate, low lipid diets. This finding is contrary to expectations as most studies have shown that a high digestive gland index corresponds with a high level of nutritional condition and growth (McClain, 1995; Jussila, 1997; Jones and Orbst, 2000). Nevertheless, this trend was also observed in juvenile *J. edwardsii* in a previous study (Ward et al., in press). It is possible that the total amount of lipid provided by the digestive gland in these lobsters is more important than gland size. There was more lipid (absolute amount per lobster) from smaller digestive glands on account of their lipid concentrations being higher, whereas lobsters on poorer (low lipid, high carbohydrate) diets had more carbohydrate in the digestive gland, both in terms of percentage composition and on an absolute basis.

6.5.5.2 Histology

Digestive gland histology supported the trends in lipid deposition and growth, with lobsters fed the high lipid diets (i.e., the 2:1 and 0.8:1 ratio diets) in the best nutritional condition. These lobsters had structurally sound epithelial cells and substantial lipid accumulation compared with lobsters fed high carbohydrate, low lipid diets (i.e., 17.4:1, 4.8:1) which had digestive gland epithelial cells that were compressed, of inconsistent shape and lacking in lipid deposits. Lipid accumulation and cell structure of digestive gland epithelia (R-cells in particular) is known to be indicative of nutritional condition in crustaceans (Storch, 1984; Vogt et al., 1985, 1986; Vogt, 1987). This study validates histological examination of digestive glands as a feasible method for determining the nutritional condition of lobsters, and in combination with proximate nutritional analyses, can be used as a tool for ascertaining a relatively simple, qualitative diagnosis of the nutritional value of lobster diets.

While the histological changes observed here were not as severe as in studies where crustaceans were purposefully starved (Papathanassiou and King, 1984; Vogt, 1987; Niles et al., 1993), there is evidence to suggest that the nutritional condition of lobsters fed low lipid diets (17.4:1; 4.8:1) was poorer than that of the high lipid diets (2:1; 0.8:1). Epithelial cell structure in lobsters fed low lipid diets showed signs of deterioration, the R-cells in particular were more compressed than in the control lobsters, or those fed high lipid diets (2:1, 0.8:1). This compression is believed to be due to decreased accumulation of lipid droplets, a condition previously observed in *J. edwardsii* post-puerulus following moulting (Nishida, et al., 1995) and in juvenile *H. americanus* fed a sub-optimal diet (Rosemark, et al., 1980). A high haemocyte infiltration was observed in lobsters fed 17.4:1 diets and to a lesser extent 4.8:1 diets. Rosemark et al. (1980) also found that progressive haemocyte infiltration occurred in conjunction with decreased vacuolation of the R-cells, and atrophy of the tubule, when juvenile *H. americanus* were fed a sub-optimal diet. This was believed to be a function of nutritional stress.

In conclusion, this study indicates that high lipid - low carbohydrate diets provide more available energy to juvenile *J. edwardsii* than high carbohydrate – low lipid diets. However, further research on the availability of non-protein energy sources is still required. For example the digestibility of specific non-protein ingredients such as cod liver oil and pre-gelatinised starch is required to further quantify the findings of this study. The effectiveness of other sources of lipid and carbohydrate also needs to be tested to ensure the best ingredients are used as non-protein energy sources for *J. edwardsii*. An important aspect of the present research was that the digestive gland histology provided a sensitive indicator of the nutritional status of lobsters that complemented the growth data alone. Lobsters fed the high lipid - low carbohydrate diets were in the best nutritional condition, with high lipid accumulation, and structurally sound epithelial cells. In comparison the digestive gland epithelial cells of lobsters fed the high carbohydrate - low lipid diets were compressed, of inconsistent shape and size, with low lipid accumulation. Histology is therefore seen as a feasible method, in addition to growth and proximate composition data, to further examine the effect of diets in nutritional studies of crustaceans.

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6.6 Dietary astaxanthin requirement of juvenile tropical spiny lobster *Panulirus ornatus*

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6.6.1 Abstract

Carotenoids are an expensive but critical component of crustacean feeds but little is known of the requirements of spiny lobsters. We conducted a 12-week growth assay, assessing growth, survival and tissue carotenoid levels with juvenile *P. ornatus.* Lobsters were fed either pelleted feeds supplemented with astaxanthin and containing 36, 62, 84 or 106 mg total carotenoid kg^{-1} (0, 26, 52 and 81 mg free astaxanthin kg^{-1}) or one of two fresh mussel reference feeds; green-lip *Perna canaliculus* (GLM) and blue *Mytilus edulis* (BM). Eighty-four lobsters (mean ± SD, 18 ±10.0 g) were randomly and equally allocated to weight stratified groups in a randomized block design (6 treatment x 4 replicate) and subsequent weights measured every 4-weeks. Growth rate (g wk^{-1}) showed no clear dose response to increasing dietary astaxanthin content (3.2. 2.4, 3.0 and 3.3, respectively) and was intermediate for GLM (2.5) and inferior for BM (1.7). Twelve-week survival ranged from 79 to 92% and was unaffected by treatment but survival of mussel-fed lobsters was significantly lower than pelleted diet-fed lobsters for the 9 - 12 week period. Whole body lobster carotenoid content increased with increasing dietary astaxanthin (4.7, 16.7, 27.8 and 32.8 mg kg^{-1}); pre-treatment value was 22.2 mg kg^{-1}. Total carotenoid content of the mussel-fed lobsters was unexpectedly high due to interference by other pigments. HPLC-analysis of free astaxanthin levels varied from a pre-treatment value of 7.3 mg kg^{-1} to 2.0, 7.6, 12.5 and 23.6 mg kg^{-1} with increasing dietary astaxanthin, and 3.5 (GLM) and 5.9 (BM) mg kg^{-1} for the mussel-fed lobsters. Although dietary astaxanthin, over the investigated range, did not affect growth rate or survival, there was a dose-response increase in tissue carotenoid content and darkening of the exoskeleton pigmentation, which may have important implications for immunocompetence and marketing. A dietary astaxanthin specification of not less than 50 mg kg^{-1} is advocated for juvenile tropical lobsters.

6.6.2 Introduction

In a previous study at this laboratory examining the optimum dietary protein specification of juvenile *P. ornatus*, a benchmark diet of thawed fresh green-lip mussel *Perna canaliculus* was found to be markedly inferior to pelleted dry diets in supporting lobster productivity (Smith et al. in prep). Growth rate of lobsters fed the mussel was only half that of the highest protein formulated diet (0.42 *vs* 0.91 g $week^{-1}$, respectively) and survival was about half that of those fed formulated diets (40 *vs* 73 to 84%, respectively). Mortality of lobsters fed the green-lip mussel generally occurred at times of moult and those animals that did survive the moulting were a pale pink colour compared to those fed the formulated diets, which remained a dark brown-black colour. These observations suggest that the astaxanthin content of green-lip mussel was insufficient to meet the requirements of the lobsters. This finding differs from most other laboratories engaged in diet development for spiny lobsters (Crear et al. 2000; Glencross et al. 2001; Ward et al. 2003) where mussel has been used as a benchmark diet and without exception has produced the best growth rate and highest survival of the lobsters. However, the mussel used in those studies has typically been the blue mussel, *Mytilus edulis*. New Zealand researchers (James and Tong 1997; A. Jeffs, pers. comm.) have found that the southern lobster *Jasus edwardsii* prefers blue to green-lip mussels as juveniles but that this

preference is less noticeable with the adult. We could not find any other published reports comparing the growth and survivorship efficacy of green-lip and blue mussel as sole feeds for *P. ornatus*. The natural diet of *P. ornatus* appears remarkably similar to that of other spiny lobsters with invertebrates such as polychaete worms, gastropods, bivalves and crustaceans being heavily preyed upon (Joll and Phillips 1986; Pitcher 1993).

It is uncertain whether the poor performance of lobsters fed the green-lip mussel in the earlier work of Smith et al. (in prep) was due to the species of lobster being studied or to the species of mussel used. With juvenile *J. edwardsii*, Crear et al. (2002) found that a dietary carotenoid concentration of 115 mg kg^{-1} dry matter (DM) was required to produce animals of a similar colour to wild-caught juveniles. In comparison, the carotenoid concentration of the blue mussels used as a reference diet in their studies was 31 mg kg^{-1}DM. Although the object of our research was not to discover how to improve the nutritional value of mussel as a food for lobsters, a better understanding of the lobster's requirements for astaxanthin is critical for diet development since it is one of the most expensive dietary supplements. For example, the ingredient cost of supplementing a diet with 100 mg kg^{-1} of astaxanthin is about AUD$440 per tonne. For the above reasons we sought to establish the dietary astaxanthin requirement of juvenile *P. ornatus* lobsters and to directly compare the efficacy of blue and green-lip mussels as sole feeds for these animals. This paper reports the findings of this work.

6.6.3 Methods

6.6.3.1 Animals and Management

Small juvenile *Panulirus ornatus* were collected by divers from Trinity Inlet, Cairns, North Queensland, and shipped to CSIRO Cleveland. The lobsters were placed in 2000L tanks provided with aerated, flow through filtered seawater (temperature, 28°C and salinity 33 - 35 g L^{-1}) and were fed twice daily to excess a high-protein, high-carotenoid kuruma shrimp *Penaeus japonicus* feed. Four weeks prior to the experimental period the lobster feeding was switched to a low carotenoid commercial, black tiger prawn feed. One-week prior to the experiment start the lobsters were acclimated to their respective allocated diets and tanks.

Lobsters were kept in an array of 24 fibreglass tanks (1500 x 600 x 500mm; 400L) and supplied with filtered (20 μm), heated (28°C) seawater at a flow rate exceeding 1 L min^{-1}; aeration was continuously provided via two air stones per tank. Sufficient shelters were provided for the size and number of animals. Tanks were housed within an enclosed seawater laboratory and lighting regulated to a 12:12 photoperiod. Water temperature was monitored using a TPS thermometer. Lobsters were fed the formulated (pelleted) diets four times daily at 0930, 1630, 2000 and 0400 h. The daily food allocation was apportioned between the four feeds at a ratio of 0.1:0.3:0.1:0.3, respectively and distributed using automatic feeders. The lobsters fed the fresh mussel diets were fed twice daily at 0930 and 1630 at a ratio of 0.5:0.5. Food allocation was recorded and adjusted daily to minimize food wastage but always ensuring excess food was provided. Mortalities and exuviae were removed and recorded. Tanks were siphoned-cleaned of uneaten food and faecal matter on a daily basis.

6.6.3.2 Experimental Design

The experiment compared six dietary treatments – four pelleted diets providing an incremental supplementation of astaxanthin, and two reference diets, one of blue mussel *Mytilus edulis* and the other of green-lip mussel *Perna canaliculus*. Each treatment had four tank replicates. Lobsters were sorted by weight into four size classes (blocks) to reduce size

variation between tanks and within each experimental block. The four blocks were assigned to each of the six dietary treatments. Each tank within a block contained four lobsters (except for two blocks which contained three lobsters per tank). The animals were individually weighed at the start of the experiment and then every four weeks until termination at 12 weeks. The lobsters were individually tagged and the number of moults recorded. Digital photographs of lobsters were taken at the start and end of the experiment.

6.6.3.3 Diets

Four diets were formulated on a dry matter (DM) basis to provide free astaxanthin concentrations that varied at 25 g kg^{-1} increments between 0 and 75 mg kg^{-1}. Stabilised astaxanthin was added to the diet in the form of Carophyll Pink (Roche). The basal (non-astaxanthin supplemented) diet contained a background carotenoid concentration of 36 mg kg^{-1}. Dietary concentrations of lipid and crude protein (CP) were kept constant at 125 g kg^{-1} and 640 g kg^{-1} respectively (Table 1).

Table 1. Formulation of the pelleted diets (g kg^{-1} air dry basis) and determined chemical composition of diets and blue *M. edulis* (BM) and green-lip *P. canaliculus* (GLM) mussels fed to juvenile *P. ornatus* in the 12-week growth assay

Attribute	Treatment label					
	0A	25A	50A	75A	BM	GLM
	Formulation of pelleted diets					
Wheat flour	140	140	140	140		
Fishmeal	360	360	360	360		
Krill meal	300	300	300	300		
Krill hydrolysate	80	80	80	80		
Bloodworm[1]	21	21	21	21		
Starch	4	3.6	3.2	2.8		
Wheat gluten	55	55	55	55		
Carophyll-pink	0	0.4	0.8	1.2		
Diatom. earth	7	7	7	7		
Supplements[2]	33	33	33	33		
	Composition (g kg^{-1} dry matter unless otherwise stated)					
Dry matter (g kg^{-1})	944	938	930	935	183	178
Crude protein	619	620	623	624	654	680
Ash	132	133	131	131	138	125
Total lipid	125	127	122	128	121	127
Gross energy (kJ g^{-1})	20.68	20.75	20.78	20.80	19.37	19.83
Free astaxanthin (mg kg^{-1})	0	25.7	51.5	81.3	1.2	0.3
Total carotenoid (mg kg^{-1})	36.1	62.5	84.3	105.6	70.7	47.7
Cholesterol	5.9	6.2	6.4	6.3	2.9	2.4
Total sterol	6.8	7.1	7.3	7.2	8.4	9.2
Fatty acids						
18:2n-6	6.7	6.7	6.8	6.7	0.6	0.5
18:3n-3	0.9	0.9	1.0	1.0	0.5	0.4
20:4n-6	0.6	0.6	0.6	0.6	1.5	0.6
20:5n-3	7.8	8.0	8.2	8.0	4.7	3.5
22:6n-3	7.4	7.6	7.7	7.6	6.1	6.0

[1] Homogenated fresh bloodworm *Marphysa sanguinea* equivalent to 21 g kg^{-1} of 95% dry product.

[2] provided to each diet (g kg^{-1}): cholesterol (80 g kg^{-1}), 3; lecithin (700 g kg^{-1}), 12.5; choline chloride (700 g choline kg^{-1}), 0.25; ethoxyquin (Banox E), 0.25; vitamin premix (Williams et al., 2004), 11; and trace mineral premix (Williams et al., 2004), 5.

Two reference diets of blue mussel *Mytilus edulis* and green-lip mussel *Perna canaliculus* were obtained as frozen food grade mussels from Capalaba Seafoods, Brisbane. The day prior to feeding the mussels were shucked while still frozen and cut roughly in to 20 mm square pieces, some defrosting did occur during this period and any resulting liquid present was drained away before weighing. The weighed tank aliquot was replaced in the freezer until feeding the next day.

The formulated diets were prepared as follows. Dry feed ingredients were finely ground (all particles passing through a 710 μm screen) and dry-mixed in a Hobart planetary dough mixer (Hobart Corporation Pty Ltd, Utah, USA) before the oil and sufficient water were added to form a dough of approximately 40 to 50% moisture. The dough was twice extruded through a 2.5 mm diameter die plate and the resultant feed strands transferred to a steam oven for 5 min. After steaming, the feed strands were dried overnight at 40ºC in a forced draught oven, broken into pellets of 3 to 4 mm length and stored at -20°C until just before use.

6.6.3.4 Chemical Analysis

Diet and tissue samples were analysed essentially by AOAC (1999) procedures: DM from weight change following heating in a thermogravimetric analyzer (Leco TGA-601) at 105°C to a constant weight; ash from weight change following burning in a thermogravimetric analyzer (Leco TGA-601) at 600°C to constant weight; CP (N x 6.25) by the Dumas combustion method, calibrated using aspartic acid (Elementor Rapion Analyzer, AOAC 1999); Total lipids were extracted from samples with chloroform/methanol using the method of Bligh and Dyer (1959). The DM loss (water stability) from the diets was determined as described by Smith et al. (2002).

Fatty acids were estimated by analysis of the total lipid extracts. These were derivatised to their methyl esters (FAME) by the method of Van Wijngaarden (1967) and separated by capillary gas chromatography on a HP6890 gas chromatograph (Agilent Technologies), using a split injection, a flame ionization detector. Cholesterol and sterols were estimated by extraction with hexane after direct saponification in 0.1N KOH:ethanol (2:1) as described by Kovacs et al. (1979). The sterol content of the extracts was determined by capillary gas chromatography using an HP1 non-polar column and quantified using cholestane as an internal standard.

For the analysis of lobster whole body (WB) and digestive gland total carotenoid content, a sample of three lobsters was taken at the start of the experiment and all lobsters in each tank at the end of the experiment were dried and weighed, frozen, then sagitally sectioned, dissected and freeze-dried. The combined dried lobster tissue from each tank was homogenised in a water-cooled Knifetec grinder (Tecator, Sweden) and stored at -24 C° until analysis. Total carotenoid was extracted with 90% acetone followed by phase separation into hexane. Absorbance was measured at 470 nm and concentration calculated using $E^{1\%} = 2100$. Astaxanthin and its esters were estimated by analysis of the total carotenoid extract by high performance liquid chromatography (HPLC) on a silica column using the method of Dall et al. (1995). The chemical composition of the pelleted diets and the two mussel feeds is detailed in Table 1.

6.6.3.5 Growth rate calculations

Growth rate was calculated and expressed as linear weekly growth rate (AWG), specific growth rate (SGR) or daily growth coefficient (DGC) according to the following equations:

$$AWG\,(g\,week^{-1}) = \left(\frac{W_e - W_s}{wk} \right)$$

$$SGR\,(\%\,d^{-1}) = \left(100\,\frac{\ln W_e - \ln W_s}{d} \right)$$

$$DGC\,(\%\,d^{-1}) = \left(100\,\frac{W_e^{1/3} - W_s^{1/3}}{d} \right)$$

Where W_e and W_s are the weights of the lobsters at the end and start of the growth period, respectively, ln is the natural logarithm, and d and wk are the number of days and weeks, respectively in the growth period. Growth rates were calculated for periods 0 to 4, 5 to 8, 9 to 12 and 0 to 12 weeks.

6.6.3.6 Statistical Analysis

Productivity responses of the lobsters were determined for each 4-week period of the growth assay as well as for the whole 12-week period of experimentation. Data were analysed by 1-way ANOVA and the variation due to dietary treatment was partitioned into the following three orthogonal *a priori* one-degree of freedom comparisons (Sokal and Rohlf 1981):

(i) All pelleted diets versus all mussel reference feeds;
(ii) Blue mussel feed versus green-lip mussel feed; and
(iii) Linear (and quadratic) contrasts of the effect of dietary astaxanthin concentration of the pelleted diets.

Survival percentage data were analysed as the natural and arcsine transformed values (Snedecor and Cochran 1989). However as the transformation did not materially alter the *F*-statistic of the ANOVA, only the natural values have been reported. Differences between treatment effects were also examined *a-posteriorly* using Fischer's protected 't' test (Snedecor and Cochran, 1989) wherein differences between means were examined only where the 'F' test of the ANOVA was significant ($P < 0.05$).

Table 2 Statistics for ANOVA and *a priori* comparisons for response traits of daily growth coefficient (DGC), specific growth rate (SGR), average weekly gain (AWG) and survival of juvenile and post-treatment whole body (WB) carotenoid content of *P. ornatus* lobsters fed pelleted diets varying incrementally in astaxanthin content or thawed fresh blue *M. edulis* or green-lip *P. canaliculus* mussel

Response trait	ANOVA		*A priori*		*A priori*		*A priori*	
	Treat MS	Error MS	Pelleted diets vs mussel		Blue vs green-lip mussel		Effect of diet astaxanthin	
	df=5	df=14	*F*-test	*P*	*F*-test	*P*	*F*-test	*P*
DGC (% d^{-1})								
0-4 wk	0.19554	0.09458	3.20	ns	6.71	< 0.05	< 1	ns
5-8 wk	0.31743	0.14709	9.12	< 0.01	< 1	ns	1.38	ns
9-12 wk	0.25794	0.19634	4.27	0.061	< 1	ns	1.92	ns
0-12 wk	0.11990	0.05446	4.73	< 0.05	3.33	ns	2.94	ns
SGR (% d^{-1})								
0-4 wk	0.18073	0.09044	4.01	0.069	5.65	< 0.05	< 1	ns
5-8 wk	0.25051	0.10860	9.48	< 0.01	< 1	ns	1.66	ns
9-12 wk	0.15156	0.11989	4.52	0.053	< 1	ns	1.58	ns
0-12 wk	0.07877	0.03761	3.90	ns	3.47	ns	3.11	ns
AWG (g wk^{-1})								
0-4 wk	1.13859	0.57568	1.56	ns	6.97	< 0.05	1.35	ns
5-8 wk	2.28052	1.33498	7.52	< 0.05	< 1	ns	< 1	ns
9-12 wk	3.19548	2.29687	4.01	0.069	< 1	ns	2.35	ns
0-12 wk	1.22077	0.59751	5.13	< 0.05	2.32	ns	2.76	ns
Survival (%)								
0-4 wk	104.167	62.5	< 1	ns	1.25	ns	6.88[1]	< 0.05
5-8 wk	41.6667	55.5555	< 1	ns	1.41	ns	2.11	ns
9-12 wk	104.167	76.3889	4.26	0.061	1.02	ns	1.53	ns
0-12 wk	191.667	416.667	< 1	ns	< 1	ns	2.05	ns
WB (mg kg^{-1} DM)								
Total carotenoid	1185.23	19.7438	125	< 0.01	100	< 0.01	74.9	< 0.01
Free Astaxanthin	215.367	5.9922	41.9	< 0.01	136	< 0.01	1.93	ns

1 Although a significant amount of variation in the survival rate of lobsters fed the pelleted diets in the 0-4 week period could be attributed to supplementary astaxanthin, neither linear nor quadratic relationships to dietary astaxanthin supplementation were significant ($P < 0.05$).

6.6.4 Results

6.6.4.1 Health

The health of the lobsters appeared to be generally good throughout the experiment. Animals fed the zero astaxanthin-supplemented diet and those fed mussels became progressively paler in colour. This was most noticeable for the tail fan (Plate 1) since the holding of the lobsters in internally black tanks caused all lobsters to adopt a dark colouration of the exoskeleton. Lobsters fed the green-lip mussel were noticeably paler than those fed blue mussels. One tank of lobsters on treatment diet 75A was removed from the experiment in the ninth week when dislodgement of the drainage standpipe resulted in all water being lost from the tank overnight.

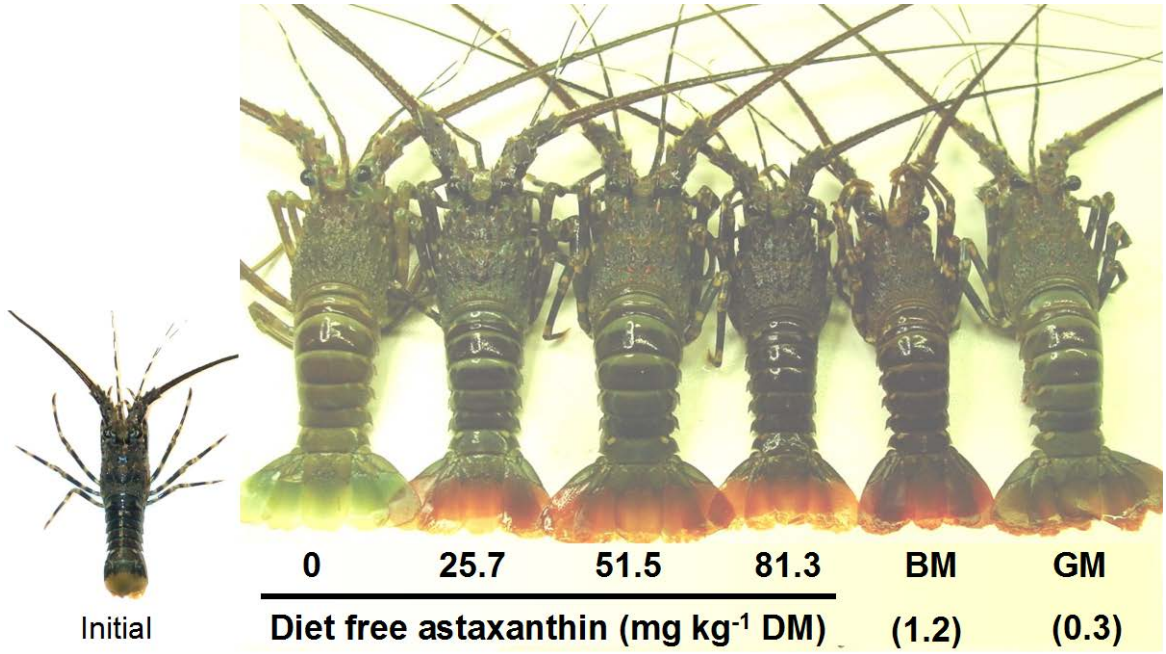

Plate 1 The initial and post-treatment appearance of lobsters fed either pelleted diets supplemented with astaxanthin or two types of thawed fresh mussel: blue mussel *Mytilus edulis* (BM) or green-lip mussel *Perna canaliculus* (GLM). Analysed free astaxanthin concentrations of the feeds (mg kg^{-1} DM) are indicated.

6.6.4.2 Lobster productivity responses and tissue astaxanthin

A summary of the statistics for the *a priori* one-degree of freedom treatment comparisons for all productivity responses of the lobsters and the post-treatment carotenoid and free astaxanthin concentrations in the lobsters is detailed in Table 2 and *a posteriori* testing of the treatment means is shown in Table 3. The WB total carotenoid and free astaxanthin content of the sacrificed pre-experiment lobsters was 21.6 and 7.3 mg kg^{-1} DM, respectively.

There was no significance difference in growth rate or survival of lobsters fed either mussels or pelleted feeds in the 0-4 week growth period. For the subsequent growth periods and for the overall 0-12 week growth period, lobsters fed mussels consistently grew more slowly and survival tended to be lower (P = 0.061) than for those fed pelleted diets during the 9-12 week period. The post-treatment WB carotenoid and free astaxanthin concentrations of the lobsters

were significantly higher and lower, respectively for lobsters fed mussels compared to those fed pelleted diets. Significant productivity differences between the two mussel diets were confined to the 0-4 week growth period where feeding blue mussel resulted in slower growth rates and lower survival of lobsters compared to green-lip mussel. WB carotenoid and free astaxanthin concentrations were significantly higher for lobsters fed blue mussel than green-lip mussel.

Table 3 Productivity responses[1] and whole body carotenoid content[2] of juvenile *P. ornatus* fed either pelleted diets supplemented with astaxanthin or thawed fresh blue *M. edulis* (BM) or green-lip *P. canaliculus* (GLM) mussel for growth assay periods of 0 to 4, 5 to 8, 9 to 12 and 0 to 12 weeks

Trait	Diet label						
	0A	25A	50A	75A	BM	GLM	± sem
DGC (% d^{-1})							
0-4 wk	1.27	1.18	1.18	1.07	1.13	1.70	0.159
5-8 wk	1.45	1.12	1.35	1.45	0.77	0.91	0.198
9-12 wk	1.42	0.97	1.17	1.26	0.71	0.90	0.229
0-12 wk	1.38	1.09	1.23	1.26	0.87	1.17	0.121
SGR (% d^{-1})							
0-4 wk	1.34	1.23	1.21	1.19	1.25	1.76	0.155
5-8 wk	1.37	1.06	1.27	1.38	0.76	0.90	0.170
9-12 wk	1.20	0.88	1.00	1.07	0.66	0.77	0.179
0-12 wk	1.30	1.06	1.16	1.21	0.89	1.14	0.100
AWG (g wk^{-1})							
0-4 wk	2.38	2.22	2.39	1.71	1.88	3.30	0.392
5-8 wk	3.37	2.54	3.23	3.21	1.59	1.84	0.597
9-12 wk	4.09	2.42	3.48	3.50	1.64	2.47	0.783
0-12 wk	3.28	2.39	3.03	2.81	1.70	2.54	0.399
Survival (%)							
0-4 wk	100	94	88	100	94	100	4.0
5-8 wk	100	100	94	100	100	94	3.7
9-12 wk	94	100	100	100	88	94	4.4
0-12 wk	94	94	81	78	81	88	10.2
WB (mg kg^{-1} DM)							
Carotenoid	4.8^{d}	17.2^{c}	28.7^{b}	33.9^{b}	56.2^{a}	29.0^{b}	2.29
Free Astax	2.0^{e}	7.6^{c}	12.5^{b}	24.3^{a}	5.9^{cd}	3.5^{de}	1.26

[1] DGC = daily growth coefficient; SGR = specific growth rate; AWG = average weekly gain.
[2] WB = whole body; DM = dry matter..
[a, b, c, d, e] Means in the same row without a common letter differ ($P < 0.05$).

Growth rate and survival of lobsters fed the pelleted diets showed no significant relationship to dietary astaxanthin content but WB carotenoid and free astaxanthin concentration of the lobsters increased linearly and curvilinearly respectively with increasing dietary astaxanthin supplementation (Figure 1).

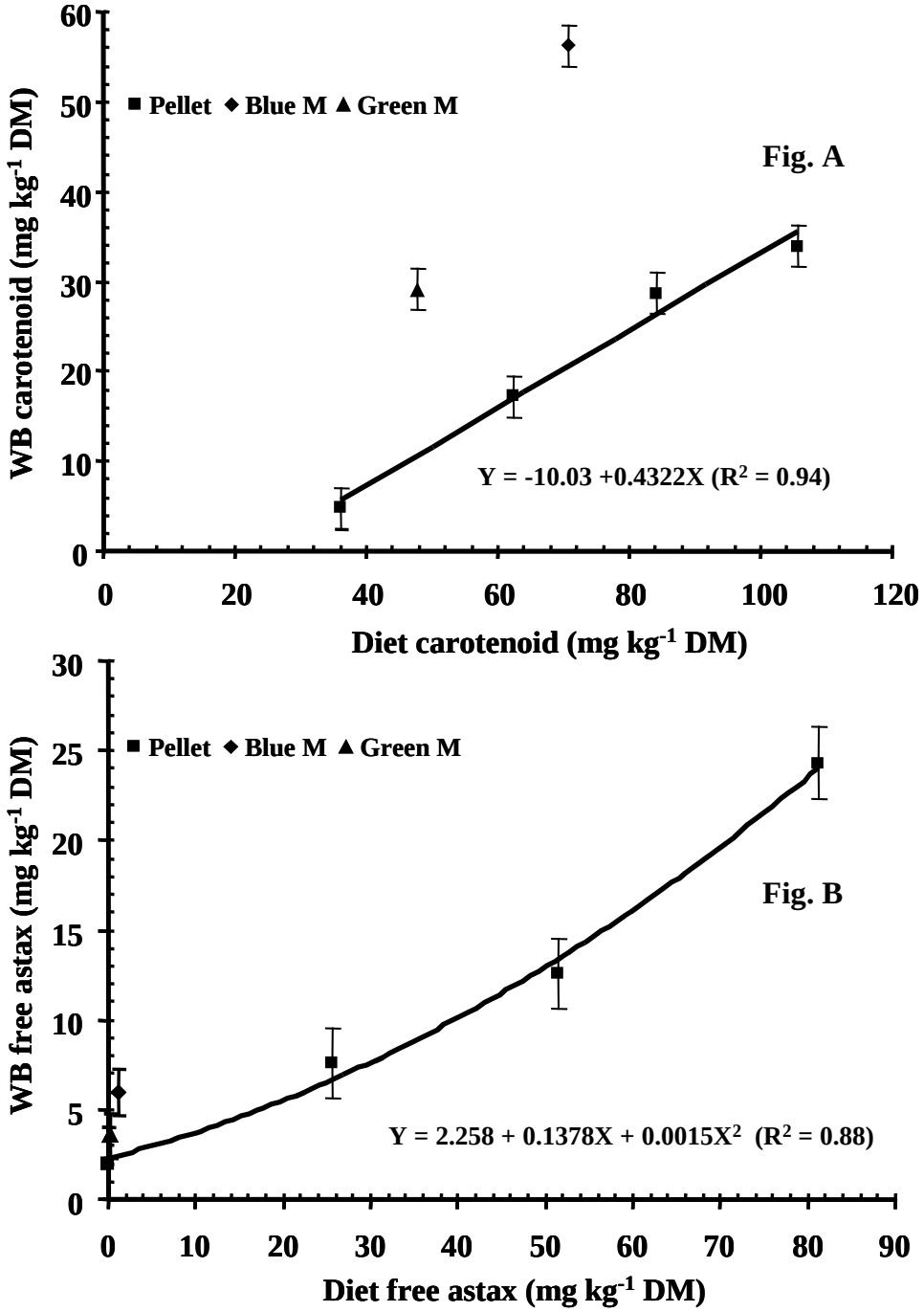

Figure 1 Relationships between dietary dry matter (DM) carotenoid concentration and whole body (WB) DM carotenoid concentration (Fig. A) and between dietary free astaxanthin (astax) DM concentration and WB DM free astax concentration (Fig. B) for lobsters fed pelleted diets (■) in the 12 week growth assay. Corresponding data for lobsters fed blue mussel (♦) or green-lip mussel (▲) are also shown. Error bars are ± sem (n = 4).

A posteriori testing of treatment means (Table 3) revealed significant differences only in respect of WB carotenoid and free astaxanthin concentration. This analysis revealed no additional information to that already noted from the *a priori* analysis.

6.6.5 Discussion

Increasing the dietary astaxanthin concentration above the total carotenoid content of the zero-supplemented diet (36 mg kg^{-1} DM) failed to elicit a significant improvement in either growth rate or survival of the lobsters. However, astaxanthin supplementation did result in a marked linear and curvilinear increase in lobster WB total carotenoid and free astaxanthin concentration, respectively (Figure 1). The colour of the exoskeleton of the lobster also darkened with increasing dietary astaxanthin supplementation (Plate 1). Most interesting however, were the differences seen between the two types of mussels in their carotenoid content and the effect this had on lobster WB carotenoid concentration and exoskeleton colouration. Total carotenoid concentration was higher for blue compared to green-lip mussel (70.7 vs 47.7 mg kg^{-1} DM, respectively) and this resulted in lobsters fed the blue mussel having higher WB total carotenoid values compared to those fed green-lip mussel (56.2 vs 29.0 mg kg^{-1} DM, respectively). The colour of the exoskeleton of lobsters fed the green-lip mussel also was much lighter than those fed the blue mussel, which induced a distinctly pinkish tinge to the lobsters (Plate 1). By comparison, lobsters fed pelleted diets supplemented with 25 mg kg^{-1} DM of free astaxanthin and which contained a total carotenoid concentration of 62.5 mg kg^{-1} DM, had much darker exoskeleton pigmentation but a lower WB total carotenoid value (17.2 mg kg^{-1} DM). This apparent anomaly is due to the very low concentration of astaxanthin in the mussels, 1.2 and 0.3 mg kg^{-1} DM of free astaxanthin and 3.9 and 2.1 mg kg-1 DM of total astaxanthin (free plus esters) for the blue and green-lip mussel, respectively. Although we made no attempt to characterize the nature of the carotenoid compounds present in the mussels, Matsuno (1989) report the principal carotenoids in molluscs to be β-carotene, lutein A, zeaxanthin, alloxanthin and astaxanthin along with several other isoprenoid compounds. Additionally, chlorophyll and other xanthophyll pigments originating from the microalgae consumed by the mussels, will contribute to the spectral absorbance making up the analysed total carotenoid value. Animals are unable to synthetise carotenoids *de novo* and thus are dependent on an exogenous dietary supply to meet their requirements (Meyers & Latscha 1997). In crustacea, astaxanthin is the main carotenoid and among its many functions, is responsible for the colour of the animal's exoskeleton. Since astaxanthin is only poorly bio-converted in crustacea from other ingested carotenoids, with β-carotene being the best with a bio-availability of about 50% (Meyers & Latscha 1997), it is not surprising that lobsters fed mussels in the present study should have poor exoskeleton pigmentation. Of significance was the low concentration of free astaxanthin in the WB of lobsters fed on mussels (5.9 and 3.5 mg kg^{-1} DM for blue and green-lip mussels, respectively) as compared to values of 7.6 to 24.3 mg kg^{-1} DM for lobsters fed pelleted diets supplemented with free astaxanthin at inclusion rates of 25 to 75 mg kg^{-1} DM, respectively.

Carotenoids have many biological functions in animals in addition to those involved with pigmentation. Other generally accepted physiological functions of carotenoids include being a source of provitamin A, stimulating the animal's immune system, enhancing reproductive performance and increasing an animal's tolerance to stress (Bendich 1989; Meyers & Latscha 1997; Linan-Cabello et al. 2002). In the present study, survival of lobsters fed mussels was significantly poorer than those fed pelleted diets during the 9-12 weeks phase of the experiment with deaths occurring mostly at moulting. This was similar to a previous finding with juvenile *P. ornatus* fed green-lip mussel (Smith et al. in prep) and was one of the main reasons why the present study was undertaken. However, it seems unlikely that the poor survival of lobsters fed mussels in the present experiment can be attributed to the low amount of astaxanthin they contained. Lobsters fed the non-astaxanthin supplemented pelleted diet (Diet 0A) showed the least amount of exoskeleton pigmentation (Plate 1) and the lowest WB

concentration of free astaxanthin compared to all other dietary treatments but survival rate was not adversely affected (Table 3). This suggests that factors other than dietary astaxanthin were responsible in the present study for the poor growth rate and low survival of lobsters fed mussel diets. In the absence of dietary astaxanthin being solely implicated as the cause of the higher death rate of lobsters fed mussels, the next most probable cause is a dietary inadequacy of cholesterol.

Cholesterol is an essential nutrient for crustaceans where it is an integral constituent of cell membranes and, as a metabolic precursor of steroid hormones, has a crucial role in the moulting process (Teshima 1997). A dietary cholesterol deficiency is most commonly manifested as a reduced growth rate and occasionally as increased mortality of rapidly moulting juveniles with death occurring at time of moulting (see review of Teshima 1997). The cholesterol content of the blue and green-lip mussels in the present study was 2.9 and 2.4 g kg^{-1}, respectively and about half those of the pelleted diets, which ranged between 5.9 and 6.4 g kg^{-1}. These cholesterol values for mussels are somewhat higher than those reported by Murphy et al. (2002), which ranged between 0.8 and 1.0 g kg^{-1} for blue mussel and between 1.4 and 1.9 g kg^{-1} for green-lip mussel. We have not seen any published reports of the dietary cholesterol requirement of *P. ornatus*. For juvenile *Homarus americanus* lobsters, cholesterol requirement estimates have ranged from 2 to 5 g kg^{-1} (Castell et al. 1975; Bordner et al. 1986) while we determined the requirement for sub-adult giant tiger shrimp *Penaeus monodon* to be 1.7 g kg^{-1} (Smith et al. 2001). It is not inconceivable that the low cholesterol content of mussels in combination with the low amount of astaxanthin they contained was sufficient to cause the observed growth rate depression and lower survival of lobsters in the present study.

Similar to that previously observed for this formulation (Smith et al. in prep.), the pelleted diets fed in the present study supported excellent growth and survival of the lobsters. While diets varying in free astaxanthin concentration between 0 and 75 mg kg^{-1} were indistinguishable from each other in terms of lobster growth and survival, exoskeleton colouration of the lobsters darkened with increasing astaxanthin supplementation (Plate 1). This creates a dilemma in deciding what is the most appropriate dietary astaxanthin specification to advocate for juvenile *P. ornatus*. The colour of the lobster has a great bearing on the market price of the product with pale animals being heavily penalised. However, free astaxanthin is a very expensive additive, costing about AUD$440 to achieve a dietary supplement of 100 mg kg^{-1}. Crear et al. (2002) advocated a dietary carotenoid specification of 115 mg kg^{-1} to achieve good colouration of southern rock lobster, *J. edwardsii*. Although the free astaxanthin concentrations of the diets were not determined in the study of Crear et al. (2002), it is likely that the 115 mg kg^{-1} carotenoid diet would contain a free astaxanthin concentration of about 70 mg kg^{-1}. For juvenile *P. ornatus*, we would advise a dietary specification of not less than 50 mg kg^{-1} free astaxanthin to ensure good lobster colouration and as insurance for maintaining good health and vitality of the cultured animal.

6.6.6 Acknowledgements

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6.7 Studies with adult *J. edwardsii* on improved feeding management

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6.7.1 Introduction

The effect of soakage time on pelleted diet consumption has been identified as an issue in live-holding of southern rock lobsters with respect to future feeding strategies.

The aims of this research were two-fold. Firstly, to characterize the effect soaking time has on the subsequent consumption of dry food pellets by adult *J. edwardsii*. Secondly, to examine the effect feeding frequency and stocking density has on the productivity and incidence of tail fan necrosis (TFN) of adult *J. edwardsii*. Two experiments were carried out with adult *J. edwardsii* lobsters to address these research objectives.

6.7.2 Methods

6.7.2.1 Experiment 1 – Effect of soak time on consumption of pelleted diets

An experiment was run to determine the effect of soak time on diet consumption over 14 d. The diet used was experimental rock lobster diet RL35D (Table 1), a formulation manufactured at the SARDI Pig and Poultry Production Institute.

After capture by local fishermen at a depth of about 60m, 56 male lobsters, ranging in size from 530 g to 1.3 kg, were purchased from a processor at Robe (37º10” S 139º46”). The lobsters were acclimated for 2-weeks after capture in 500 L communal tanks held at 18ºC. Lobsters were randomly assigned to individual 30 L experimental tanks with false mesh floors, aeration and flow-through water (Figure 1) where they acclimated for a further week. Equal numbers of tanks were maintained at either 15 or 23ºC using a Building Automation System. The upper temperature was chosen to represent the maximum summer temperature likely to be experienced by lobsters (Geddes et al 2000). Lobsters were also assigned to one of two pellet treatments (soak times of 0 or 6 h) and either of two pellet types (new or old) within each temperature treatment. During the acclimatization period, lobsters were fed on alternating days with Goolwa cockles (*Donax deltoides*) at 3 per lobster/day or their assigned diet at 2% body weight/day.

Two batches of the same diet were used, one made 2 weeks and the other 6 months before the experiment commenced. Both were dried to constant weight (55ºC, 16h), sealed in plastic bags with silica gel and frozen (-30ºC) until needed. A further two treatments, soaked versions of the aforementioned diets, were also included in the experiment. Diets were soaked for 6 h in fine mesh (30 μm) bags within tanks identical to those used to house the lobsters. Separate tanks were used for soaking each diet. After soaking, the bagged diets were briefly immersed in fresh water to remove salt, dried in an oven for 16 h at 55ºC and stored frozen (-30ºC) until needed.

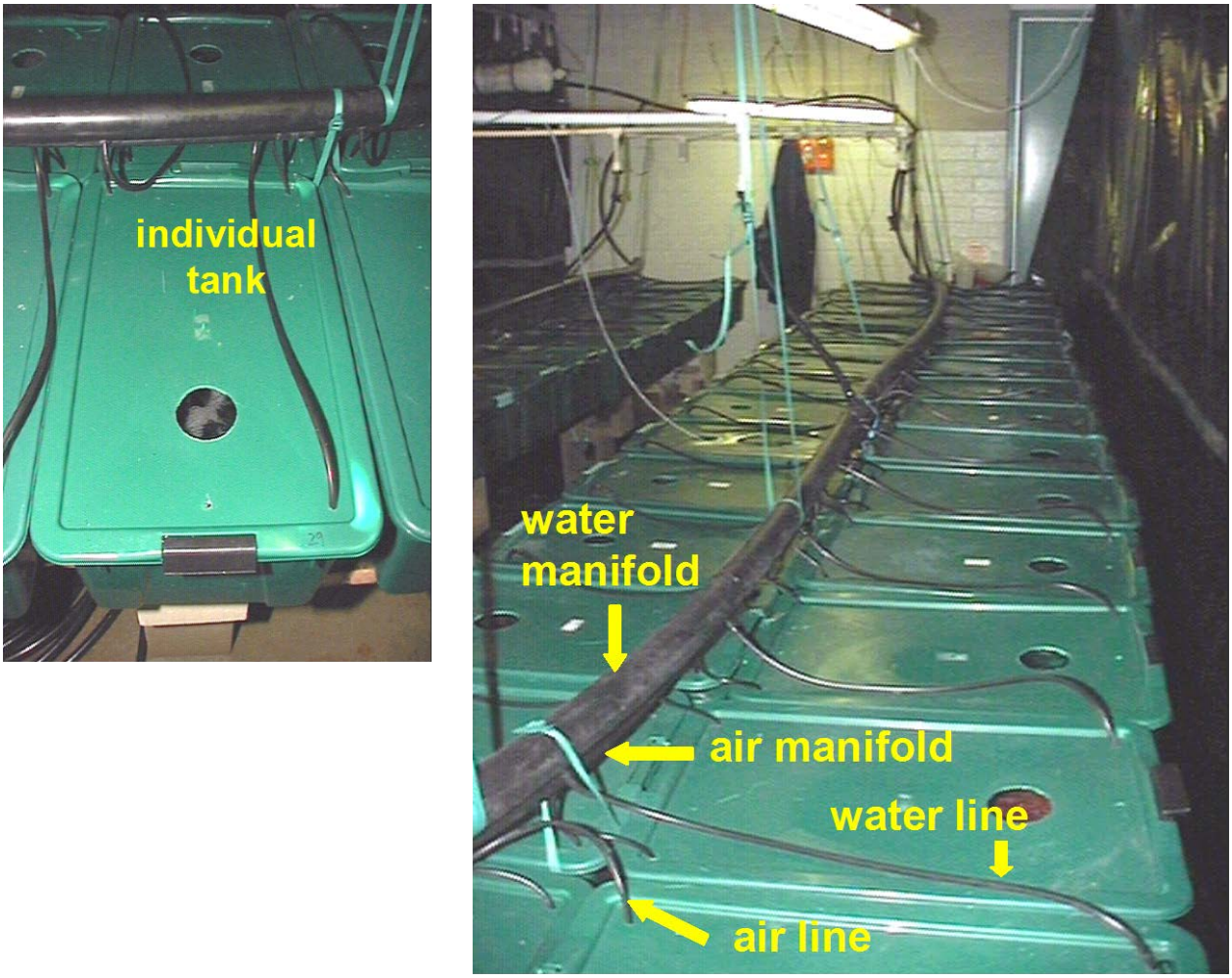

Figure 1 Tanks used for diet consumption experiment

Table 1 Formulation (RL35D) of the diet fed in Experiment 1

Ingredient	Inclusion rate (%)
Fish meal	45.7
Wheat gluten	6.0
Wheat flour	22.9
Crustacean meal	20.0
Fish oil	0.6
Aquabind	3.0
Premix[1]	1.8

[1] Provided in final diet (%): Vitamin C, 0.1; Vitamin mixture, 0.2; carophyll pink (8% astaxanthin), 0.07; cholesterol, 0.2; lecithin, 1.2 and ethoxyquin (Banox E), 0.1

Lobsters were fed their assigned treatment diet at 2% body weight per day, as per the routine shown in Table 2, on alternate (i.e. non-experimental) days. On the other day, lobsters were fed cockles. On day of feeding, sufficient of each treatment diet was allowed to come to room temperature and weighed into alotments corresponding to the daily feed allocation of 2% body weight per day. The water supply was temporarily turned off for 14 h at feeding

time in order to retain all uneaten food particles; aeration (two 25 mm air-stones per tank) was continued during this time. Feeding took place in the late afternoon and uneaten pellets were collected the following morning, dried overnight (55ºC, 16 h), allowed to cool to room temperature over silica gel and weighed to 0.1 mg. Feed consumption data were converted to g consumed per g wet weight of lobster.

Table 2 Feeding routines for lobster consumption experiment (asterisks indicate the days that lobsters at each temperature were fed soaked or un-soaked versions of each of two different diets)

Day	Temperature (°C)	
	15	**23**
1	*	cockles
2	cockles	*
3	*	cockles
4	cockles	*
5	*	cockles
6	cockles	*
7	*	cockles
8	cockles	*
10	*	cockles
11	cockles	*
12	*	cockles
13	cockles	*
14	*	cockles
15	cockles	*

Leaching controls were run for all diets using a variant of the standard CSIRO method: 12-14 g samples of each diet (soaked and un-soaked) were weighed (to 0.1 mg) into tared screw-top 70 ml plastic jars, the ends of which were covered with 20 µm nylon mesh to allow water movement but retention of all but the finest diet particles (Figure 2).

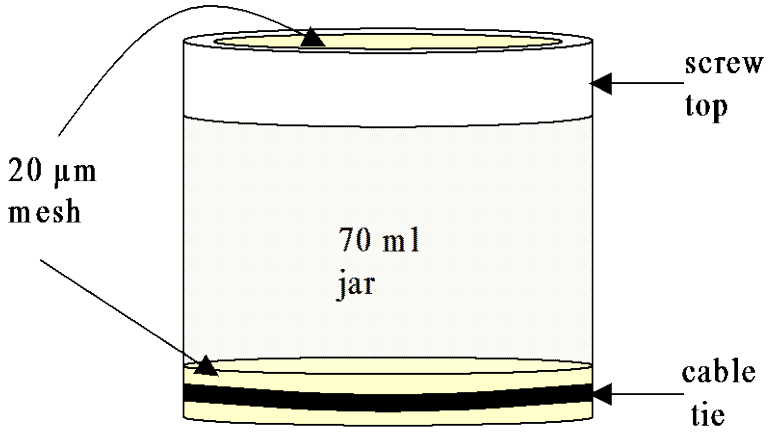

Figure 2 Container used for determining the water stability (leach-rate loss) of the diets

The jars were placed into tanks identical to those housing the lobsters, including aeration and water supply, and soaked for 1, 2, 4, 6 or 20 h. After the designated immersion time, the

residual feed was removed intact, dried as described above, and weighed. The difference in dry weight of the original and residual feed sample was recorded as the leach loss and expressed as a percentage of the original sample weight.

Data were subject to Type III Repeated Measures ANCOVA, with weight as the covariate, using Genstat$_{TM}$. Significant differences were accepted at P <0.05.

6.7.2.2 Experiment 2 – effects of holding density, feeding regime and bagging on the development of TFN in a raceway holding system

A field experiment was set up at Southern Australian Seafoods (SAS) in Port Lincoln to test the effect of feeding frequency and lobster density on growth. The work was carried out in conjunction with the project "Rock Lobster Enhancement and Aquaculture Subprogram: Investigation of tail fan necrosis in live-held adult southern rock lobsters (FRDC 2000/211)".

In November 2000, 450 lobsters were bought from local fishermen and 420 were selected for the experiment. The remainder was used to replace mortalities in the first two weeks of the experiment. The time from capture to release into onshore collection tanks varied between 7 and 14 d depending on the length of time a given fisher remained out fishing.

At the beginning of the experiment all lobsters were weighted (to 0.1 g), measured (to 0.1 mm) and the pleopods clipped for moult staging and identification. Blood colour and refractive index were also noted. Lobsters that moulted during the experiment were noted. At the end of the experiment the surviving lobsters were measured etc, as above.

The experiment commenced on 27.11.00 in conjunction with FRDC 2000/211 and was completed at the end of March 2001. It was set up using seven outside tanks at SAS. The tanks were rectangular, about 4 m long and were continuously aerated and supplied with water (flow-through). Tanks were situated within a shade cloth enclosure. Temperatures were monitored using data loggers. Each tank contained 4 PVC/oyster mesh cages – 28 in total. There were three treatments of feeding frequency (daily feed, weekly fed and starved) and two of density (10 per cage (6.3/m^2) and 20/cage (12.7/m^2) (Figure 3). These treatments were duplicated, except for the starved treatment. Lobsters were assigned randomly to density and feeding treatments then were placed in the cages and the cages secured in the raceways (Figure 4). Water temperature data, recorded 4-hourly, were provided by SAS staff.

d/1	**d/1**
d/2	**d/2**

d/1	**d/1**
d/2	**d/2**

w/1	**w/1**
w/2	**w/2**

w/1	**w/1**
w/2	**w/2**

s/1	**s/1**
s/2	**s/2**

Figure 3 Experimental design for feeding frequency density trial. d=daily feed, w=weekly feed, s=starved.

Figure 4 Cages and tanks used in Experiment 2. Top left, a cage being placed in a tank. Bottom right, cages setup and ready for the introduction of lobsters.

6.7.3 Results

6.7.3.1 Experiment 1 – Effect of soak time on consumption of pelleted diets
Mean feed consumption varied from 0.653 ± 134 to 0.529 ± 0.063 g dry weight pellet per g wet lobster weight (Figure 5) (n = 392). There were no interaction effects. Feed intake was affected by weight ($p<0.001$) but not by temperature, pellet or soak time (Table 3). Consumption declined significantly ($r^2=0.2974$, $F= 22.86$, $P<0.001$) with increasing weight (Figure 6) and increased significantly between days 8 and 12 (Figure 7, Table 4).

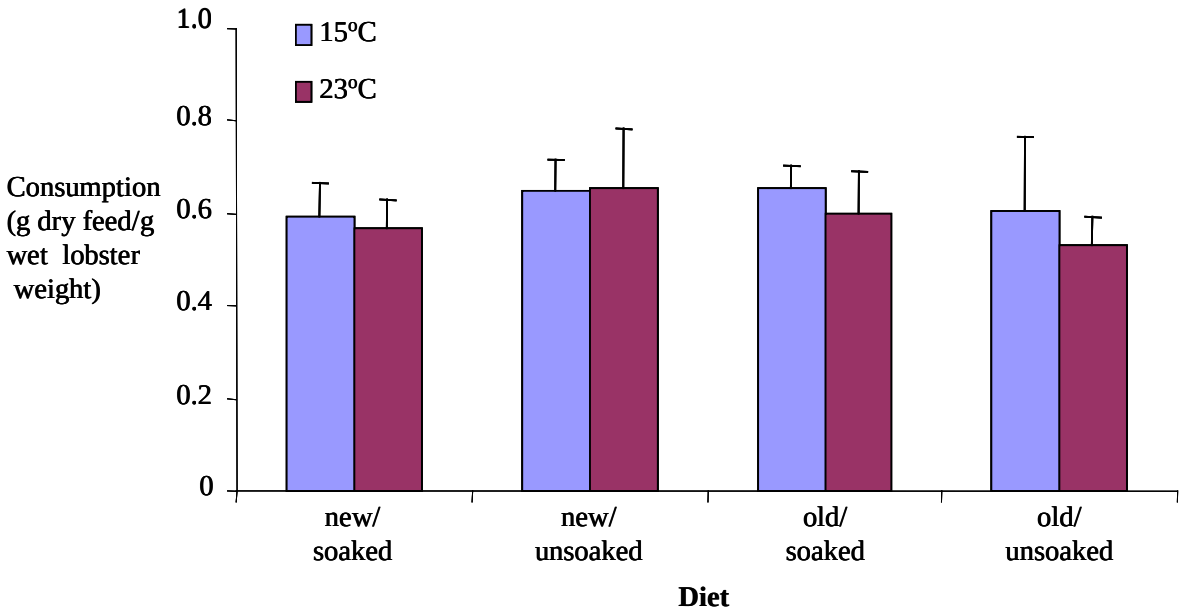

Figure 5 Lobster wet weight (g) vs weight-specific consumption (g dry weight/lobster wet weight) for each of four diet forms and two temperatures (15 and 23ºC).

Table 3 **Type III Repeated Measures ANCOVA on the effect of weight (covariate), temperature (T), pellet type (P) and soaking (S) on consumption by lobsters over a 2-week period. Data were arcsine-transformed.**

Source	df	Sum squares	Mean square	*F*-statistic	Signif
Intercept	1	0.197	0.197	164.2	<0.001
Weight	1	2.584E-02	2.584E-02	21.54	<0.001
T	1	4.217E-03	4.217E-03	3.51	0.067
P	1	1.079E-03	1.079E-03	0.90	>0.05
S	1	3.031E-05	3.031E-05	0.03	>0.05
T x P	1	1.905E-07	1.905E-07	<0.01	>0.05
T x S	1	1.618E-04	1.618E-04	0.14	>0.05
P x S	1	1.794E-03	1.794E-03	1.50	>0.05
T x P x S	1	1.279E-04	1.279E-04	0.11	>0.05
Error	47	5.639E-02	1.200E-03		

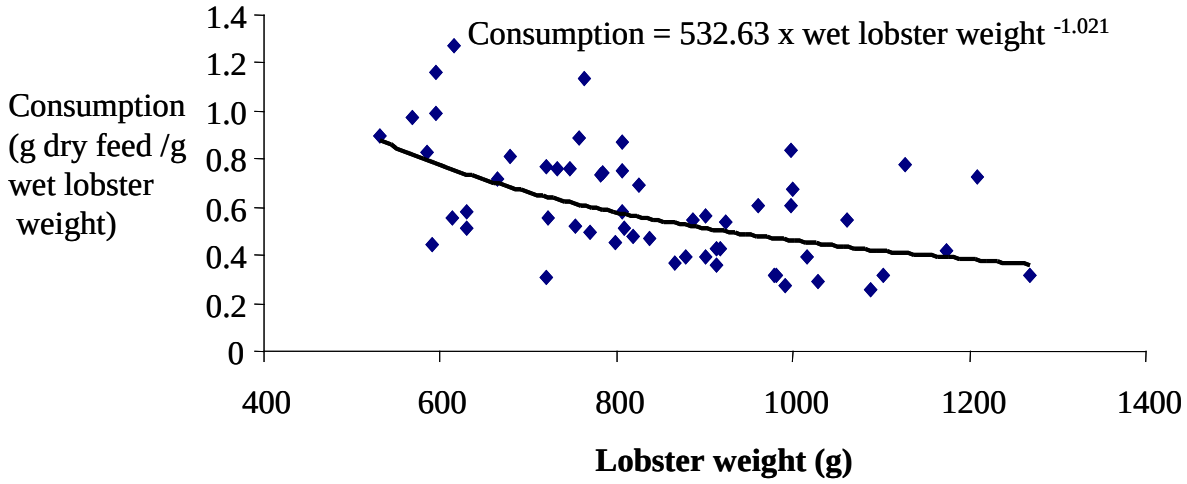

Figure 6 **Lobster wet weight (g) vs pooled weight-specific consumption (g dry weight/g lobster wet weight)**

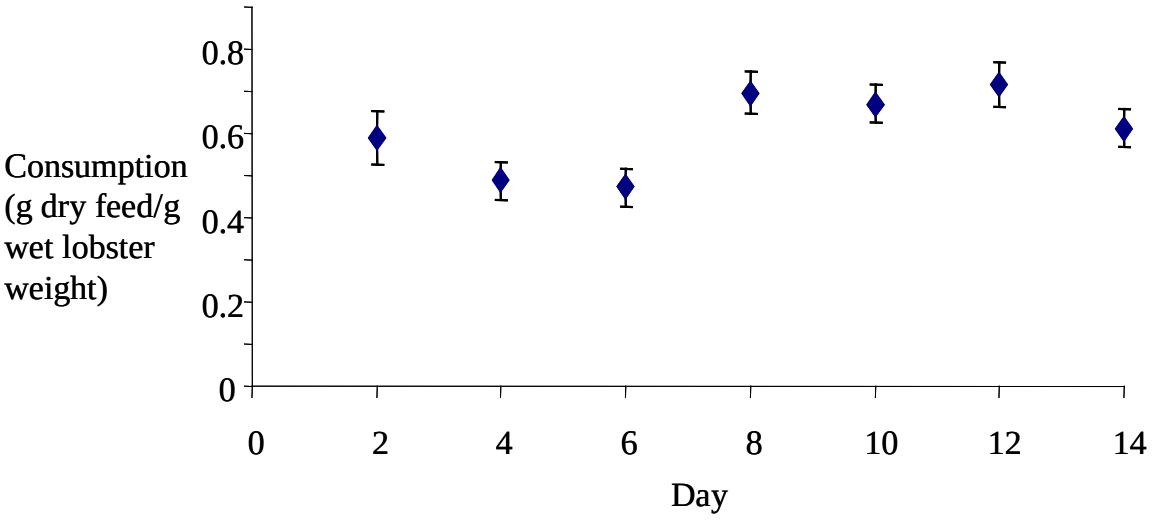

Figure 7 **Weight-specific consumption (g dry weight/lobster wet weight) (pooled data) *vs* day.**

Table 4 Pair-wise comparisons, Type III Repeated Measures ANCOVA on the effect of weight (covariate), temperature, pellet type and soaking on feed consumption by lobsters over a two week period. Data were arcsine-transformed.

Day	2	4	6	8	10	12	14
2					0.035	0.008	
4				0.001	<0.001	<0.001	0.012
6				0.001	<0.001	<0.001	0.012

6.7.3.2 Experiment 2 – effects of holding density, feeding regime and bagging on the development of TFN in a raceway holding system

The experiment was completed on schedule. However, there were serious problems with high water temperatures (Figure 87) and poor water quality. Mean weekly water temperatures reached 23.0ºC (± 0.10, n = 7) in week 9, near the end of January, and were an average of 2.6ºC (± 0.24, n = 18) warmer than the corresponding period in 2001/02. Survival was low at 45, 49 and 65% in the daily, weekly and starved treatments respectively. It appeared that most of the lobsters at pigment stages 3 to 4.5 (a field indicator of impending moult, Musgrove, 2001) died or were cannibalised at ecdysis. Because of this, the results of the experiment could not be used to assess the effects of density and feeding frequency on growth.

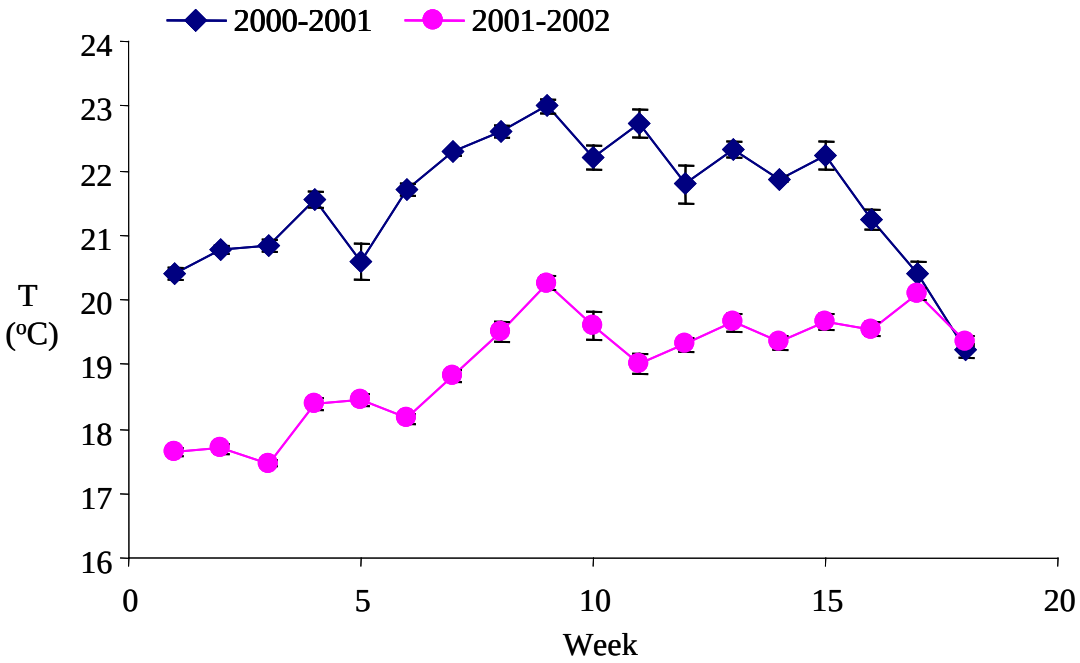

Figure 8 Mean water temperatures (TºC) (± SE) at Southern Australian Seafoods from 27 November to 31 March 2000/2001 and 2002/2003

6.7.4 Discussion

Feed consumption was generally less than 1% body weight per day in Experiment 1 and this was not unexpected for lobsters of this size. Since feed intake did not decline during this laboratory trial, this suggests that the alternate-day feeding design was an appropriate feeding schedule.

However, neither age of the diet (2 weeks vs 6 months since manufacture) nor pre-soaking of the diet for 6 h had any significant effect on the lobster's feed consumption. Since the diets were held at -30ºC immediately after manufacture, it perhaps not surprising that the age of the diet had no effect on its acceptance to lobsters. The lack of an effect of pre-soaking of the diet for 6 h was more of a surprise. Glencross et al. (2001) working with juvenile western rock lobsters *Panulirus cygnus* and Smith et al. (2003b) working with juvenile tropical rock lobsters *Panulirus ornatus* attributed poor growth of the animals fed pelleted dry diets to a loss of its attractiveness due to rapid leaching of chemical attractants. This suggestion has been confirmed in subsequent work with *P. ornatus* (Williams et al. 2004) where pre-soaking was found to reduce the lobster's acceptance of pelleted dry feeds. In contrast, but similar to what was observed in the present study, Tolomei et al. (2003) found that immersing dry shrimp pellets for up to 8 h had no effect on the subsequent growth, survival, food conversion or carapace colour of juvenile *J. edwardsii*. These apparent contradictory results indicate the likely existence of marked differences between lobster species in the chemical cues that stimulate feeding responses.

The lack of an increased feed intake of lobsters held at 23ºC compared with those at 15ºC was quite unexpected. Higher water temperatures usually increase the feed consumption of crustaceans, at least within the animal's thermal tolerance (Wyban et al 1995). However, a similar lack of feed intake response to increasing temperature was observed in another *J. edwardsii* study (Thomas et al, 2000). They fed juvenile *J. edwardsii* on mussels (*Mytilus edulis planulatus*) or dry grow-out pellets for penaeids (*Penaeus japonicus*) but did not detect any differences in food consumption over the temperature range of 18 to 24ºC. In terms of survival, growth and FCR, Thomas et al (2000) considered the optimum water temperature to be between 19 and 21ºC and a temperature of 24ºC being close to the upper thermal limit for this stage. Juveniles live in inshore waters, subject to warmer temperatures and greater temperature fluctuations than adults. As previously noted, the lobsters used in Experiment 1 were caught off Robe, at a depth of about 60m (Musgrove 2001). Musgrove (2001) reported a mean bottom water temperature of 12.7ºC at 65.5 m in this area during the 1996/97 lobster-fishing season and 12.5ºC at 52 m during the 1997/98 season off nearby Cape Jaffa. Although no adult lobsters died during the short laboratory trial in the present study, it is likely that they were well outside their optimum temperature range. Considerable mortality did occur in the field trial (Experiment 2), some undoubtedly as a result of respiratory stress in the immediate post-moult period as reported for juveniles under similar high temperature conditions (Thomas, 2000). Crear and Forteath (1998) reported that *J. edwardsii* attained maximum oxygen consumption at 13ºC. This temperature was a typical environmental water temperature for the lobsters as well as being the temperature too which they were acclimated in the laboratory. This also probably represents the preferred body temperature (Bennet, 1978). Aerobic scope for activity declined from 3.00 at 13ºC to 1.68 at 21ºC. Further work is required to determine what effect water temperature has on feed consumption of adult *J. edwardsii*, particularly in view of the interest in holding adult lobsters in shallow water pontoons or above-ground tanks with limited or non-existent temperature control.

6.7.5 References

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7. STATUS OF DIET DEVELOPMENT FOR JUVENILE ROCK LOBSTERS

Summarized below are the main research findings on the nutritional requirements of juvenile rock lobsters emanating from this and previous FRDC project work. Also detailed are the formulation and nutrient specifications of a diet that is recommended to be used as a reference feed for future nutritional research on juvenile rock lobsters.

7.1 Nutritional information for rock lobster diet development

1. Marked differences were observed between species in their responses to nutritional manipulation with the more rapidly growing tropical *P. ornatus* being more vulnerable to nutritional deficiency than the temperate rock lobster *J. edwardsii*. Table7.1 provides a suggested dietary specifications for juvenile rock lobsters.

Table 7.1 Recommended dietary specifications for juvenile[1] rock lobsters

Nutrient and energy	**Specification (kg^{-1} DM)**	**Comments (J.e=*J. edwardsii*; P.c=*P. cygnus*; P.o=*P. ornatus*)**
Crude protein (g)	400 – 600	Lowest for J.e; intermediate for P.c; highest for P.o
Digestible protein (g)	300 – 460	Comment as for crude protein
GE (MJ)	19 – 20	
Digestible energy (MJ)	14 – 14.5	
CP:GE[2]	22 - 29	Comment as for crude protein
Total Lipid (g)	60 – 100	Lowest for P.o; highest for P.c and J.e
Total saturated fatty acids (g)[3]	20 – 30	
Total monounsat fatty acids (g)[3]	30 – 40	
Eicosapenataenoic (g)[3]	5 – 10	
Docosahexaenoic acid (g)[3]	5 – 10	
Phosphatidylcholine (g)[3]	4 – 10	
Cholesterol (g)[3]	2	
Astaxanthin (mg)	50	Excludes all non-astaxanthin carotenoid
Carbohydrate:lipid	<2:1	Studies done only for J.e.
Vitamins and stabilisers[4]	Premix	
Amino acids (g/16 g N)[3]		
Arginine	5.8	
Lysine	5.3	
Methionine + cystine	3.6	
Threonine	3.6	
Tryptophan	0.8	

[1] There is insufficient data available to make recommendations for adult animals. However, specifications for adult lobsterss are expected to be much lower than those for juveniles.
[2] Protein to energy ratio in units of g crude protein: MJ gross energy.
[3] In the absence of information specific to lobsters, estimates are based on requirements for penaeid shrimp.
[4] Complete vitamin premix and nutrient stabilisers as advocated for penaeid prawns.

2. While blue mussel (*M. edulis*) and green-lip mussel (*P. cannaliculus*) were a suitable reference food for *J. edwardsii* and *P. cygnus*, each was nutritionally inadequate for juvenile *P. ornatus*. Prolonged feeding (from 6 to 10 weeks, depending on initial size) of juvenile *P. ornatus* on a sole diet of mussels resulted in an abrupt reduction in growth rate and increased mortality at time of moulting. The reason why mussels were an inadequate

sole food for *P. ornatus* was not established but a deficiency of a micronutrient, perhaps a vitamin or cholesterol, is postulated.

3. The capacity of lobsters to digest marine and terrestrial feeds appears to be very similar to that of penaeid prawns. The apparent digestibility of lupin and wheat gluten protein was very high (>90%) while that of soybean, pea and canola was low (<60%). The protein of mussel meat was highly digestible (98% ADP), that of fishmeal and prawn meal was intermediate (63-77%) while that of a commercial dried squid meal was exceptionally poor (7%). It is stressed that only limited ingredient digestibility studies have been done with rock lobsters and as such, these data need to be used cautiously.
4. In common with penaeid prawns, rock lobsters have limited capacity to utilize dietary carbohydrate or lipid as an energy source with a natural propensity to oxidize protein for cellular energy functions.
5. Providing a rich source of free amino acids and small peptides in the diet is beneficial for promoting high feed intake and good growth rates in juvenile lobsters. Dried whole krill (*Euphausia* spp) and enzymic hydrolysed krill are good sources of these feed attractants.

7.2 Suggested reference diet for future lobster diet development work

A formulation and nutrient specification of a diet that supports excellent growth of juvenile *P. ornatus* and which could be used as a reference diet for future nutritional studies are detailed in Table 7.2.

Table 7.2 Formulation and nutrient specification of a pelleted dry diet[1] recommended to be used as a reference diet for nutritional studies with juvenile rock lobsters

Ingredient	Formulation ($g\ kg^{-1}$)		Diet attribute	Specification (kg^{-1} DM)
Wheat flour	144		Crude protein (g)	600
Fishmeal (65% CP)	360		Ash (g)	125
Dry whole krill	300		Total lipid (g)	125
Dry krill hydrolysate	80		Phospholipid (g)	12.5
Fresh marine invertebrate[2]	100		EPA (20:5n-3) (g)	8
Wheat gluten	60		DHA (22:6n-3) (g)	7.5
Soy lecithin (70%)	12.5		Astaxanthin (mg)	50
Supplements[3]	23.5		Cholesterol (g)	6
Total (as mixed weight; g)	1080		4 h water stability	>92% DM retention

[1] Moisture content after manufacture of 920-940 $g\ kg^{-1}$.

[2] Homogenised fresh mussel (or other bivalve) or polychaete. Amount is for wet product with an expected DM content of about 20%.

[3] Added to provide in final diet ($g\ kg^{-1}$): cholesterol (8%), 3; choline chloride (70%), 0.25; ethoxyquin (or other antioxidant), 0.25; and shrimp comprehensive vitamin premix, 20.

8. BENEFITS AND ADOPTION

The project has demonstrated that juvenile tropical rock lobsters fed on appropriately formulated pelleted dry feeds will grow at rates that are as good as, if not better than, those achievied by animals of the same size in the wild. The RLEAS Steering Committee has adopted this dietary formulation as the reference diet for future lobster feeds development work in RLEAS.

Although it was not possible in the present project to formally evaluate the reference diet with lobster species other than *P. ornatus*, NIWA (NZ) scientists are currently evaluating its efficacy for the southern rock lobster, *J. edwardsii*. In this evaluation, the reference pelleted diet made by CSIRO Cleveland is being compared against the feeding of blue mussel or a mixture of blue mussel and the reference pelleted feed. After 8 weeks of feeding and midway through the experiment, growth rate and survival of lobsters fed the reference pelleted feed were as good as those fed only mussels while those fed the mussel and pelleted feed mixture appeared to be growing slightly better (Dr Michael Bruce, NIWA, pers. comm.). This is a most encouraging result and indicates that the developed dietary formulation may have general application for rearing both warm- and cold-water species of rock lobsters.

A data set on the apparent protein digestibility of marine and terrestrial feed ingredients for southern rock lobster has been produced at TAFI. This research has shown that the lobster digests terrestrial feed ingredients such as lupin and wheat gluten as effectively as that of mussels. This finding offers much promise that cheaper and more environmentally renewable feeds can be developed for the grow-out culture of rock lobsters.

9. FURTHER DEVELOPMENT

The Project has demonstrated that the developed pelleted dry diet supports excellent growth and survival of lobsters and at rates that are equal to those achieved by lobsters in the wild. However, the formulation of the pelleted diet is quite expensive with an ingredient cost of about \$2.70 kg^{-1}. By comparison, the cost of manufactured pelleted diets for the giant tiger prawn *P. monodon* is about \$1.80 kg^{-1} while extruded pelleted kuruma prawn *P. japonicus* diets cost from \$7 to 10 kg^{-1}. Significantly, the kuruma diet will support reasonable growth and good survival of juvenile lobsters whereas the growth and survival of lobsters on the *P. monodon* diet is poor and unsatisfactory. The high ingredient cost of the developed pelleted diet is due to high inclusion rates of expensive marine ingredients, particularly krill hydrolysate, krill and fresh bloodworm, and high usage rates of expensive additives such as astaxanthin and cholesterol.

No work was done in the present project to optimize the dietary inclusion rate of the marine protein ingredients. Instead, high and most likley excessive usage rates were employed to ensure maximal lobster productivity. Reducing the ingredient cost of the diet by examining whether these expensive marine protein ingredients can be reduced/replaced with cheaper marine or terrestrial protein alternatives is a high priority for continued feed development for grow-out lobster culture. Similarly, a critical examination of the lobster's requirements for cholesterol may enable the ingredient cost of the diet to be further reduced. In the area of defining the nutritional requirements of lobsters, further research is necessary to examine requirements for essential fatty acids and a more thorough investigation of whether more energy-dense diets will enable even higher rates of animal productivity.

In addition to the aforementioned need to optimize the inclusion rate of the expensive ingredients, further research is needed to determine the most cost-effective feeding strategy. The high productivity of tropical rock lobsters in this project is most likely due, at least in part, to feeding four times daily instead of tweice daily as was practised in the previous project (FRDC 1998/303). Frequent feeding would assist in having 'fresh' feed available to the lobsters at closer time intervals and this could be expected to increase the stimulus for the lobsters to seek and consume food. However, increasing the frequency of feeding would add to the labour costs and thus some balance would need to be drawn between optimizing feed intake of the lobsters against the increased labour cost of more frequent feeding. Optimizing feeding frequency for both juvenile and sub-adult lobsters is considered to be a high priority for future research.

Almost all of the nutrient requirement research carried out in this and the previous lobster project (FRDC 98/303) focused on the juvenile lobster, typically over the weight range of ~1 g to less than 100 g. Lobsters are marketed at weights greater than 600 g and premium prices paid for animals above 1 kg. It is likely that dietary specifications will change with increasing body size as with most other animals. There is thus a need to examine the nutrient requirements of lobsters approaching harvest weight with the expectation that this will allow dietary specifications to be reduced without affecting the animal's productivity but increasing profitability.

10. OUTCOMES

1. Enhanced knowledge on the chemicophysical factors that affect the lobster's acceptability of a feed and how the attractiveness of the feed can be retained following immersion

2. Expanded database on the apparent digestibility of feed ingredients for southern lobsters *Jasus edwardsii.*

3. Increased knowledge of the nutrients required by juvenile southern, *J. edwardsii*, and tropical, *Panulirus ornatus*, lobsters for high rates of growth and survival.

4. Awareness that blue, *Mytilus edulis*, or green-lip, *Perna canaliculus*, mussels are not suitable as a sole food source for juvenile tropical lobsters.

5. Improved feeding strategies and management of adult *J. edwardsii*

11. CONCLUSIONS

The project has successfully achieved all planned research objectives otherthan for research to define the optimal pellet size for adult *J. edwardsii*, which was deleted in lieu of examining the effect of feeding frequency in conjunction with stocking density. These findings have been widely disseminated to stake holders and to the scientific community by way of presentations and publications.

Particular highlights of the research are outlined below:

1. For juvenile southern rock lobsters, the attractiveness of pelleted dry feed and pieces of fresh blue mussel decreased with increasing immersion time in the water but pre-soaking an extruded kuruma shrimp diet for up to 8 h did not adversely affect the growth and survival of juvenile southern rock lobsters. The high nutrient specification and high water stability of the kuruma diet are possible reasons why lobster performance was not affected by pre-soaking. In studies examining the chemical cues eliciting feeding behaviour responses of lobsters, the free amino acid glycine was shown to stimulate greater feeding behaviour than either betaine or taurine. Including glycine in the diet formulation may improve the lobster's attractiveness to the feed.

2. The chemical composition and time course of nutrients leaching from pelleted feeds containing natural fresh foods (such as mussel, bloodworm, squid and prawn) were characterized and compared with the preference of juvenile tropical lobsters fed these diets. The study showed a strong correlation between the lobster’s feeding preference and the chemical signature of the leachate. Highest correlations were for soluble protein and the free amino acids glycine and taurine while the rate of loss of dry matter, total nitrogen and other free amino acids was only weakly correlated. In contrast to the finding with juvenile southern lobsters, the attractability to juvenile tropical lobsters of kuruma and laboratory-made pelleted feeds and pieces of green-lip mussel decreased with increasing water immersion time. These results suggested that feeding more frequently than twice daily and incorporating protein hydrolysates into the dietary formulation as a rich source of free amino acids and soluble protein constituents might improve productivity of tropical lobsters fed pelleted feeds.

3. A pelleted dry diet formulated with krill hydrolysate and krill as rich sources of free amino acids and soluble protein and fed four times daily produced excellent growth of juvenile tropical lobsters. Using this base formulation, the crude protein requirement of juvenile tropical lobsters was reassessed to be not less than 60% dry matter (57% air dry) while a dietary astaxanthin (carotenoid) specification of not less than 50 mg/kg was advocated for maintenance of good lobster colouration and as an aid in reducing the lobster's susceptibility to stress during culture. In these requirement studies, lobsters were additionally fed benchmark diets of green-lip and/or blue mussels. Lobsters grew well on the mussel diets for about 4 weeks but thereafter growth and survival decreased markedly, indicating the unsuitability of mussels as a sole food source for juvenile tropical lobsters. A similar effect of feeding fresh mussels has not been observed with either southern or western rock lobsters.

4. The nutritional condition of southern rock lobsters was shown to be highly responsive to dietary carbohydrate to lipid ratio. Optimal condition of lobsters occurred on a diet containing 27% carbohydrate and 13.5% lipid (2:1 ratio) with growth rate and nutritional condition of the lobsters falling as the dietary carbohydrate content increased.

5. The apparent digestibility of five marine and five terrestrial protein feed ingredients was determined for juvenile southern rock lobsters. Protein digestibility was highest for lupin flour (100%), mussel meat (98%) and wheat gluten (90%); intermediate (61-77 %) for prawn meal, fishmeal and soybean meal; and poor for defatted fish meal (53%), pea meal (52%), canola meal (38%) and squid meal (7%).

6. Consumption of pelleted dry feed was not significantly affected by holding adult *J. edwardsii* at water temperatures of 15 or 23ºC and neither was it affected by pre-soaking the feed for 6 h. Feed intake of adult *J. edwardsii* lobsters, expressed as g dry feed consumed per g wet lobster weight, declined exponentially as a function of body weight over the examined range of 550 to 1,300 g.

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APPENDIX 1: Intellectual Property

The focus of the work was to conduct public domain research so that all stakeholders can benefit from the findings.

Research results have/are being published and have/are being presented at national and international forums. It is not anticipated that any patents or commercial intellectual property will arise from the project.

APPENDIX 2: Project Staff

Name and affiliation	Position	FTE on Project (%)
	CSIRO Marine Research, Cleveland	
KC Williams	Principal Scientist	15
DM Smith	Senior Research Scientist	10
SJ Irvin	Senior Experimentalist	40
MC Barclay	Senior Experimentalist	10
SJ Tabrett	Senior Experimentalist	5
	UTAS/TAFI, Taroona	
BJ Crear	Research Scientist	25
S Battaglene	Program Leader	5
CG Carter	Senior Lecturer	5
DJ Johnston	Lecturer	10
A Tolomei	Honors student	50
LR Ward	Honors student	50
KA Calvert	Technician	10
	UA/SARDI, Port Lincoln	
M Geddes	Senior Lecturer	5 (Yr 1 only)
R Musgrove	Research Scientist	50 (Yr 1 only)

APPENDIX 3: Publications and Presentations

Papers published or accepted for publication

Barclay, M.C., Irvin, S., Williams, K., Smith, D., 2004. Dietary astaxanthin requirements of juvenile tropical spiny lobster *Panulirus ornatus.* 7th International Conference on Lobster Biology and Management, Hobart, February 2004. Book of Program and Abstracts, Conference Design P/L, Hobart. p. 112 (abstr.).

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