Condition and its assessment in the southern rock lobster *Jasus edwardsii*.
II. Field application of techniques for condition assessment and moult staging developed in the laboratory

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OBJECTIVE

Obtain an understanding of variations in growth and recruitment through the establishment of the potential of selected indices to characterise temporal and spatial changes in condition of lobsters within the fishery.

The above was addressed by subdivision of the study as per the following additional objectives:

1a) Investigation of the use of haemolymph pigmentation as a means of subdividing the moult cycle for use in condition assessment.

1b) Exploration of the relationship between serum protein and percentage weight of dry tissue and examination of the nature and degree of change in these parameters with haemolymph pigmentation during the moult cycle.

1c) Determination of the capacity of those parameters to distinguish between high and low growth sites in the fishery.

1d) Testing of the hypothesis that premoult haemolymph lipid concentration could be used as a predictor of moult increment.

NON-TECHNICAL SUMMARY

OUTCOMES ACHIEVED

A haemolymph colour index or "pigment stage", was developed in an attempt to improve the resolution of serum protein data in the characterisation of temporal and spatial changes in the condition and growth of a wild population of *J. edwardsii*. The results suggested that the combination of moult stage, haemolymph pigment stage and serum protein index, or its correlate, the haemolymph refractive index, could be used as an indicator of nutritional condition if combined with conventional moult staging techniques. The index allowed differentiation of lobsters at the beginning and those at the end of intermoult and, in this study, provided a means to distinguish between high and low growth sites in the fishery.

Haemolymph lipid concentration was also tested, in the laboratory, for its ability to predict moult increment. The study was undertaken to develop a non-lethal means of moult increment prediction, which could then be used in the field. Both phospholipid and triglyceride were significantly correlated with percent moult increment. Phospholipid showed the highest coefficient at $r^2=0.66$. The data suggest that haemolymph phospholipid level has the potential to predict moult increment. However, the haemolymph lipid/moult increment data were gathered over a short time period and within a relatively controlled environment.

> Further field studies are necessary to better understand the relationship between haemolymph lipid level and moult increment in wild populations of this species.

At present, recruitment to the fishery and population growth are assessed using catch per unit effort (CPUE) data, puerulus settlement rates and capture-recapture methods. This work would benefit from the addition of a means of condition assessment, allowing a more accurate assessment of inter-area and inter-annual growth of lobsters in the fishery. Recruitment to the fishery is determined by year-class strength and moult increment. The latter is dependent on lobster size and condition. In South Africa, circumstantial evidence suggests that a reduction in growth caused by poor condition lead to a 50% downturn in catches in the early 1990's. Assessment of biochemical condition of lobsters prior to a moult may allow estimation of future growth and, with data on year class strength, assist in estimation of recruitment to the fishery.

A condition index or group of indices also offers a means of comparison between populations and within populations between years and provides an independent means of growth assessment, for comparison with data gathered by capture-recapture methods.

Each objective was addressed as follows:

1a) Investigation of the use of haemolymph pigmentation as a means of subdividing the moult cycle for use in condition assessment.

This objective was achieved by correlating the colour of the haemolymph of 150 lobsters with the moult stage. Nine colour stages were identified and colour was found to increase in intensity through the moult cycle, providing a useful means of subdividing the cycle, especially during intermoult. The same colour changes were seen in the laboratory and were found to relate to the level of astaxanthin in the haemolymph.

1b) Exploration of the relationship between serum protein and percentage weight of dry tissue and examination of the nature and degree of change in these parameters with haemolymph pigmentation during the moult cycle.

This objective was achieved by taking haemolymph samples from 50 lobsters from each of four sites each month over the 1997/1998 fishing season. Pigment stage, refractive index, serum protein and muscle and hepatopancreas dry weight were measured for each lobster.

There were significant correlations between percent dry weight (abdomen and hepatopancreas) and serum protein. There were also significant increases in serum protein and tissue percent dry weight with pigment stage during the moult cycle.

1c) Determination of the capacity of those parameters to distinguish between high and low growth sites in the fishery.

Examination of data recorded at high and low growth sites showed that the combination of serum protein, pigment stage and moult stage could be used to distinguish between high and low growth sites. The highest mean pigment stage–specific serum protein values were recorded in areas of high growth in both seasons. Abdominal and hepatopancreatic tissue percent dry weight showed similar patterns within pigment stages.

1d) Testing of the hypothesis that premoult haemolymph lipid concentration could be used as a predictor of moult increment.

This objective was achieved by examining the relationship between moult increment and premoult haemolymph lipid level in lobsters housed in the laboratory and the relationship between hepatopancreatic lipid and haemolymph lipid levels from lobsters collected from the field.

Haemolymph lipid, in particular phospholipid, may be useful in the prediction of % moult increment. This outcome is potentially useful, as haemolymph lipid measurement does not require the killing of the lobster, however caution should be exercised in application of these results as haemolymph lipid/moult increment data were gathered over a short time period and within a relatively controlled environment. Further field studies are needed to better understand the relationship between haemolymph lipid level and moult increment in wild populations of this species. For example, questions remain as to whether higher growth sites, showing higher serum protein content, would also show higher moult increments.

In conclusion it is suggested that a condition index that combines measurement of pigment stage and serum protein with conventional moult staging techniques would allow the characterisation of temporal and spatial changes in condition of lobsters within the fishery. Grouping lobsters by pigment stage increases the resolution of serum protein data to allow closer examination of muscle growth and reserve accumulation. The index has the added advantage of simplicity of both concept and methodology.

Furthermore, it may also be possible to estimate percent moult increment using serum lipid levels although work should be done to confirm the relationship between lipid accumulation and moult increment as these data were gathered within a relatively controlled environment. Additional laboratory and field studies are necessary to better understand the relationship between serum lipid and moult increment in wild populations of this species, particularly with regard to the question of whether faster growth necessarily implies a greater moult increment.

ACKNOWLEDGMENTS

CHAPTER 1 GENERAL INTRODUCTION

1.1   Background

The Southern Rock Lobster (*Jasus edwardsii*) supports one of Australia's most valuable fisheries. In 1998/99 the catch was 4615 tonnes of which 2622 tonnes were caught in South Australia, 1485 tonnes in Tasmania and 508 tonnes in Victoria. The catch in each state was valued at $78.3, $46.2 and $16.4 million respectively. In South Australia the industry accounts for about 33% of the value (as landed value only) generated by the State's commercial fisheries.

Over the last 10 years there has been an increasing level of concern about potential over-exploitation in the face of increasing fishing effort and fishing power. In 1993 this resulted in the initiation of a program to study the population dynamics of the Southern Rock Lobster. The program involved the South Australian Research and Development Institute (SARDI) and the South Australian Rock Lobster Research Association (SARLRA) and included data collection and analysis at the recruit level as well as modelling of populations and catch forecasting. Requirements for such analysis included spatial and temporal data on growth, recruitment and fecundity.

The present study comprises the second part of a larger work (refer FRDC 95/017) aimed at enhancing the resolution of such data by establishing a field condition index for the Southern Rock Lobster. It was intended that the index be used as an independent means of support for area-specific growth data gathered by capture-recapture methods and to assist in estimation of future growth and recruitment to the fishery.

The study of condition involves an investigation of an organism's growth response to the biotic and abiotic features of its habitat. Such a study may examine change in body fluid or tissue components or the relationships between body dimensions such as length and weight (Suthers, 1991). As lobsters do not retain hard parts against which changes in weight may be measured, assessment must be made of some fraction of the body's composition.

Linkage between condition and the moult cycle further complicates the picture. The moult cycle in crustaceans includes the moult (ecdysis) itself, the development of a cuticle to harden

the new shell, gonadal and somatic growth and the development of the next shell under the old in preparation for the subsequent cycle (Passano, 1960). Four general stages or phases have been superimposed on this continuous process for ease of description. These are metecdysis or postmoult (stages A and B, C_1–C_3), anecdysis or intermoult (stage C_4), proecdysis or premoult (stage D) and ecdysis (Stage E) (Drach, 1939; Aiken, 1973). Stages A to D may be identified by reference to the state of the cephalothorax integument and development of setae on the appendages using light microscopy (Aiken, 1973; Musgrove, 2000).

In the periodic assessment of condition in lobsters, one is really obtaining a measure of progression through the moult cycle with its attendant changes in somatic or reproductive tissue mass. Just prior to ecdysis the animal begins to take up water, diluting the haemolymph. This is accompanied by some tissue catabolism in preparation for the moult and to supply metabolic needs (Depledge and Bjerregaard, 1989; Mykles and Skinner, 1990; Musgrove and Geddes, 1995) associated with the non-feeding period during late premoult and ecdysis itself. Most haemolymph components reach a minimum concentration during postmoult. Levels then increase slowly to peak in early premoult (Mercaldo-Allen, 1991). Such changes are more the result of variation in haemolymph space than in haemolymph components *per se* (Depledge and Bjerregaard, 1989). For example, rising serum protein and haemocyanin concentrations are concomitant with decreasing blood volume (Smith and Dall, 1982) and a corresponding increase in tissue mass (Stewart and Li, 1969).

Previously, condition assessment in rock lobsters has been problematic because of the long intermoult stage (C_4) (Musgrove, 2000). Although serum protein concentration may be useful as an index because of its correlation with tissue mass (Stewart, *et al* 1967), there has been no way of distinguishing between those at the beginning, middle or end of intermoult (Dall, 1974). This means that moult cycle and starvation effects have been very difficult to separate.

1.2   *Need*

At present, recruitment to the fishery and population growth are assessed using catch per unit effort (CPUE) data, puerulus settlement rates and capture-recapture methods. This work would benefit from the addition of a means of condition assessment, allowing a more accurate assessment of inter-area and inter-annual growth of lobsters in the fishery. Recruitment to the

fishery is determined by year-class strength and moult increment. The latter is dependent on lobster size and condition. In South Africa, circumstantial evidence suggests that a reduction in growth caused by poor condition lead to a 50% downturn in catches in the early 1990's. Assessment of biochemical condition of lobsters prior to a moult may allow estimation of future growth and, with data on year class strength, assist in estimation of recruitment to the fishery.

A condition index or group of indices may also offer a means of comparison between populations and within populations between years. It would provide an independent means of growth assessment, for comparison with data gathered by capture-recapture methods. Finally, such analysis would improve the resolution of planned population growth models. It is anticipated that such an index would be used by scientists rather than by industry *per se*.

1.3   Objectives

Obtain an understanding of variations in growth and recruitment through the establishment of the potential of selected indices to characterise temporal and spatial changes in condition of lobsters within the fishery.

The above was addressed by subdivision of the study as per the following additional objectives:

1a. Investigation of the use of haemolymph pigmentation as a means of subdividing the moult cycle for use in condition assessment.

1b. Exploration of the relationship between serum protein and percentage weight of dry tissue and examination of the nature and degree of change in these parameters with haemolymph pigmentation during the moult cycle.

1c. Determination of the capacity of those parameters to distinguish between high and low growth sites in the fishery.

1d. Testing of the hypothesis that premoult haemolymph lipid concentration could be used as a predictor of moult increment.

CHAPTER 2 INTERACTIONS BETWEEN HAEMOLYMPH CHEMISTRY AND CONDITION

Objectives:

1a   Investigation of the use of haemolymph pigmentation as a means of subdividing the moult cycle for use in condition assessment.

This objective was achieved by correlating the colour of the haemolymph of 150 lobsters with the moult stage. Nine colour stages were identified and colour was found to increase in intensity through the moult cycle, providing a useful means of subdividing the cycle, especially during intermoult. The same colour changes were seen in the laboratory and were found to relate to the level of astaxanthin in the haemolymph.

1b   Exploration of the relationship between serum protein and percentage weight of dry tissue and examination of the nature and degree of change in these parameters with haemolymph pigmentation during the moult cycle.

This objective was achieved by taking haemolymph samples from 50 lobsters from each of four sites each month over the 1997/1998 fishing season. Pigment stage, refractive index, serum protein and muscle and hepatopancreas dry weight were measured for each lobster.

There were significant correlations between percent dry weight (abdomen and hepatopancreas) and serum protein. There were also significant increases in serum protein and tissue percent dry weight with pigment stage during the moult cycle.

1c   Determination of the capacity of those parameters to distinguish between high and low growth sites in the fishery.

Examination of data recorded at high and low growth sites showed that the combination of serum protein, pigment stage and moult stage could be used to distinguish between high and low growth sites. The highest mean pigment stage–specific serum protein values were recorded in areas of high growth in both seasons. Abdominal and hepatopancreatic tissue percent dry weight showed similar patterns within pigment stages.

2.1   Introduction

The study of condition involves investigation of an organism's growth response to the biotic and abiotic features of its habitat. Such a study may examine change in body fluid or tissue components or the relationships between body dimensions such as length and weight (Suthers, 1991). As lobsters do not retain hard parts against which changes in weight may be measured, assessment must be made of some fraction of the body's composition. Linkage between condition and the moult cycle further complicates the picture. The moult cycle in crustaceans includes the moult (ecdysis) itself, the development of a cuticle to harden the new shell, gonadal and somatic growth and the development of the next shell under the old in preparation for the subsequent cycle (Passano 1960). Five general stages or phases have been superimposed on this continuous process for ease of description.   These are postmoult (subdivided into stages A, B, and C_1 to C_3), intermoult (C_4), premoult (D) and ecdysis (E) (Drach, 1939; Aiken, 1973). Stages A to D may be identified by reference to the state of the cephalothorax integument and development of setae on the appendages using light microscopy (Aiken, 1973; Musgrove, 2000).

In the periodic assessment of condition in lobsters, one is really obtaining a measure of progression through the moult cycle with its attendant changes in somatic or reproductive tissue mass.   Just prior to ecdysis the animal begins to take up water, diluting the haemolymph. This is accompanied by some tissue catabolism in preparation for the moult and to supply metabolic needs associated with the period of inanition during late premoult and ecdysis itself (Depledge and Bjerregaard, 1989; Mykles and Skinner, 1990; Musgrove and Geddes, 1995). Some haemolymph components reach a minimum concentration during postmoult (e.g. protein, haemocyanin, Ca^{++}, Mg^{++}). Levels then increase slowly to peak in early premoult (Mercaldo-Allen, 1991). Such changes are more the result of variation in haemolymph space than in haemolymph components *per se* (Depledge and Bjerregaard, 1989). For example, rising serum protein and haemocyanin concentrations are concomitant with decreasing blood volume (Smith and Dall, 1982) and a corresponding increase in tissue mass (Stewart and Li, 1969).

Condition assessment in rock lobsters is problematic because of the long intermoult stage (Musgrove, 2000). Although serum protein concentration may be useful as an index because

of its correlation with tissue weight (Stewart, et al., 1967), there has been no way of distinguishing between lobsters at the beginning, middle or end of intermoult (Dall, 1974).

This chapter has three objectives. Firstly, to investigate the use of haemolymph pigmentation as a means of subdividing the moult cycle for use in condition assessment. Secondly, to explore the relationship between serum protein and percentage weight of dry tissue in the abdomen and hepatopancreas and to examine the nature and degree of change in these factors with change in the colour of the haemolymph during the moult cycle. Thirdly, to look at the capacity of those factors to distinguish between high and low growth sites in the fishery as a means of testing the index. This chapter was published in 2001 in Marine Biology, Vol 139, pages 891-899.

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2.2   Methods

2.2.1   Field collection of lobsters for haemolymph and tissue analysis.

Approximately 50 lobsters of both sexes and 80-101.9 mm carapace length (CL) were collected from fishers from each of two low growth (Robe and Cape Jaffa, Fig. 2.1) and two high growth sites (Marion Bay and Kangaroo Island) each month from November 1997 to March 1998. Estimates of growth rates were obtained from Prescott *et al*. (1997). During the last three months (January to March) of the following season (1998/99) haemolymph samples were also taken from similar size lobsters at Marion Bay and Cape Jaffa.

Initial processing of the lobsters and data collection took place adjacent to the study site. After noting latitude, longitude and water depth as supplied by the fishers, each animal was sexed, weighed (nearest 0.1g) and measured (CL , to the nearest 0.1mm). The state of the lobster's exoskeleton was also assessed in terms of hardness and fouling to assist with moult staging. Any damage to shell or appendages was also noted. Pleopod samples were taken during the 1997/98 season for moult stage determination by examination of setal development (Musgrove, 2000). Pleopod collection was not possible during the 1998/99 season.

Fig. 2.1 Sites for monthly rock lobster collection and sampling of lobster haemolymph during 1997/98 fishing season.
Marion Bay (mb), Kangaroo Island (ki), Cape Jaffa (cj) and Robe (ro). During the 1998/99 fishing season only Marion Bay and Cape Jaffa were used for monthly surveys of lobster haemolymph.
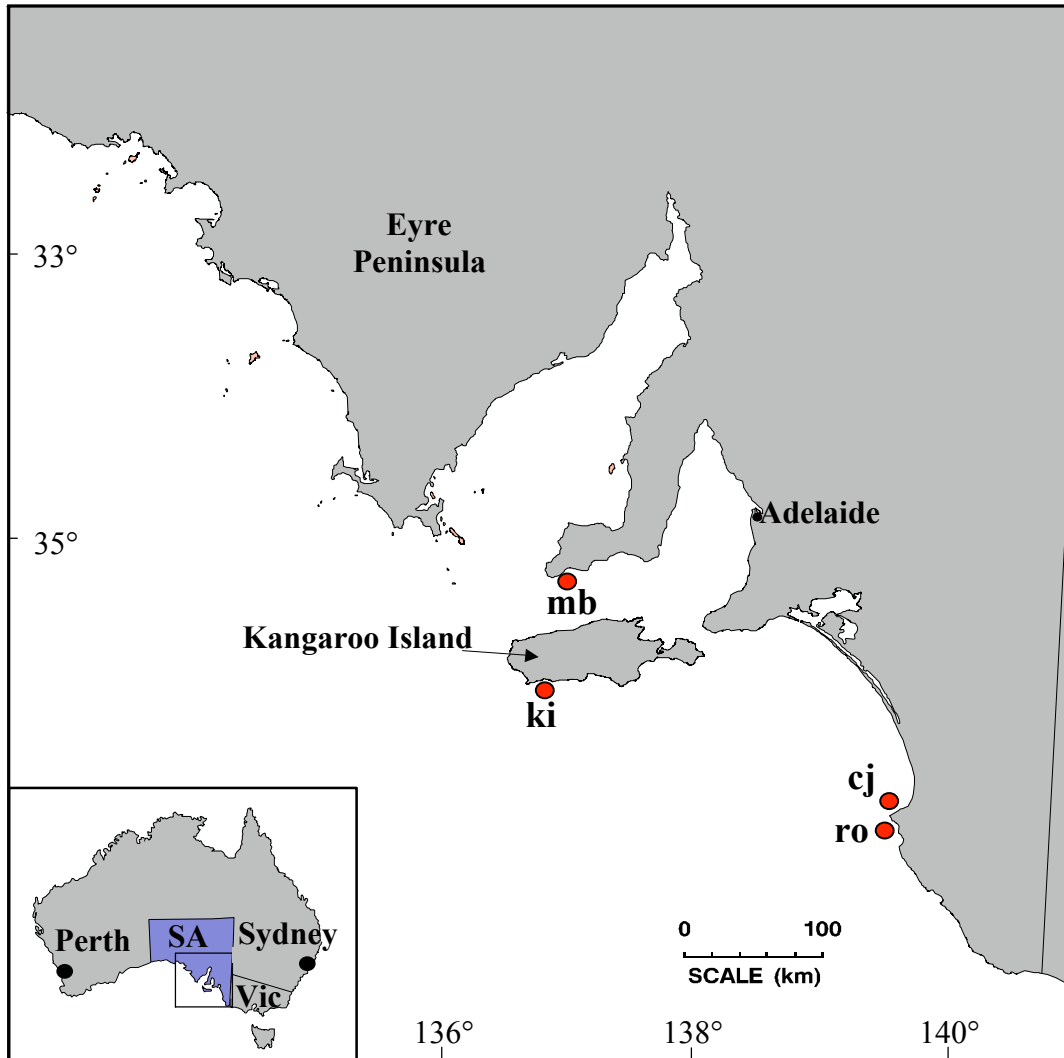
2.2.2 *Haemolymph pigmentation of lobsters in the field*

A haemolymph sample (1 ml syringe, 22 gauge needle) was taken by pericardial puncture. Colour assessment was standardised as follows. During the first month, photographs were taken of about 150 haemolymph samples to be sure of covering the range of haemolymph colours. The photographs were taken of syringes full of freshly extracted, uncoagulated haemolymph placed on a small light box. All photographs were compared and nine "pigment" stages (PS 0.5 to PS 4.5) identified, representing visually separate points along the continuum of increasing colour intensity (Fig. 2.2) from light blue, through beige to deep orange. The photographs were then used to standardise interpretation of the pigment stage for subsequent haemolymph samples using the same light box.

Fig 2.2 Haemolymph pigment stages for the southern rock lobster *Jasus edwardsii.*
Stage PS0.5 occurs during postmoult but is not included because it is highly variable and it is more practical to assess carapace rigidity at this stage.
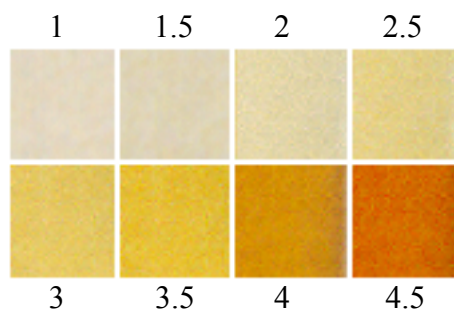
2.2.3 *Haemolymph protein analysis*

An aliquot of the sample was also placed in a hand-held refractometer (Model UR-2, Industrial and Scientific Supply Co.) to measure the refractive index (RI) (Leavitt and Bayer, 1977). The remainder of the sample was snap-frozen at $-196^{\circ}C$ for later serum protein analysis.

At the laboratory, a range of whole haemolymph samples was randomly selected from the field collection for serum protein analysis. The clotted haemolymph was broken up gently with a glass stirring rod and the sample centrifuged (15 minutes at 17,280 g) to extract the serum. One aliquot of serum was then taken for measurement of RI and another analysed for serum protein using the Biuret method (Sigma Aldrich test kit 542) on a Cobis Mira Autoanalyser with Bovine Serum Protein as the standard. Accuracy was maintained at $\pm1g/l$

using commercially available quality controls (Nycomed Farmer). The resulting linear regressions were used to convert field whole haemolymph RI to serum RI and the resulting data converted to serum protein (g/l).

2.2.4 Tissue percent dry weight

After each haemolymph sample was taken, lobsters were tagged then frozen at -20°C to -30°C in a blast freezer, transported back to Adelaide in cool boxes containing dry ice and stored at -30°C. Within two weeks of collection, lobsters were thawed and the abdominal tissue and hepatopancreas removed, weighed and then dried to constant weight (60°C, 72 h). The tissue was then allowed to come to room temperature in a desiccator over silica gel and reweighed to the nearest 0.1 mg.

2.2.5 Change in haemolymph pigmentation during the moult cycle in the laboratory

Haemolymph from captive lobsters was analysed for astaxanthin level in order to standardise pigment stage. Forty lobsters (mean CL: 89.88$\pm$ 0.6 mm, mean weight 364.6 $\pm$ 6.54g) were individually housed in 30 l plastic tanks in a flow through system (0.4 l/min/tank). Each tank was independently supplied with air and water (mean temperature 17.7^{o}C, average summer temperature in the area of capture). The temperature of the latter was controlled through a sensor attached to a Building Automation System. Day length was set at 14:10 LD and the lights (Daylight, 36w) covered with red cellophane to minimise disturbance. Lobsters were fed *ad libitum* daily on a mixed diet of artificial pellets, cockles and fish in a rotation. Excess food was removed and tanks cleaned each day, taking care to minimise disturbance to the lobsters. The experiment was run for 185 days.

Haemolymph samples were taken fortnightly from each lobster by pericardial puncture. 200 μl of each whole haemolymph sample was mixed with an equal volume of 10% Na Citrate anti-coagulent in 3% NaCl then snap-frozen in a –80°C freezer until analysed for astaxanthin. The method used was a modification of that described by Dall, Smith and Moore (1995). All extractions were carried out in dim light to minimise pigment breakdown. After thawing, 200 μl of 90% acetone was added to each sample and mixed well using a Vortex mixer. 800 μl of heptane was then added and the sample centrifuged for 15 mins at 17,280 g. The sample was then washed twice with Reverse Osmosis water and 400 μl of heptane (containing the

pigment) removed to a clean glass tube. The heptane was then block evaporated at 60$^{\text{o}}$C under nitrogen. Once cooled to room temperature, the samples were redissolved in 200 µl hexane:dichloromethane:isopropanol (92:1:7 v/v/v) containing 5mg% butylated hydroxytoluene (BHT) antioxidant and analysed using a Waters 996 Photodiode Array Detector attached to a Waters 510 HPLC Pump and a Waters WISP-710B Water Sampler. Millenium 32 Chromatography software was used. A calibration curve was constructed using astaxanthin (purity 97%, assayed by Roche Vitamins) in heptane. Standards were extracted and measured in the same way. The exact concentration of the standard solution was estimated using a spectrophotometer and an extinction coefficient of 2100 µl%/1cm (Schierle and Hardi, 1994).

If the lobster was about to moult, haemolymph samples were taken before and after ecdysis. Pigment stage was also noted whenever haemolymph was taken. A pleopod was also taken to determine moult stage as described above.

2.2.6   Environmental Data

Bottom temperature was continuously monitored during both seasons using submersible data loggers (Hastings Data Loggers). In the 1997/98 season loggers were tied into fishers' pots. This was also done during 1998/99 at Marion Bay but at Cape Jaffa the data were obtained from a fixed logging station attached to a buoy.

2.2.7   Data Analysis

The analysis of the field data was completed using either the General Linear Models or the Nonparametric modules of the Statistics Package for the Social Sciences. The data were subjected to tests for normality and homogeneity of error variances and transformed if necessary. If data could be normalised they were analysed by multivariate ANOVA with Tukey's HSD and S-N-K tests used for *post hoc* comparisons. ANCOVA was also used where appropriate. If data could not be normalised, the Kruskal-Wallis Non Parametric ANOVA or the Wilcoxan Rank Sign were used. In all cases significance was accepted at $P<0.05$.

## 2.3	Results

### 2.3.1	Change in haemolymph pigmentation during the moult cycle

Haemolymph colour changes from light blue-grey, through beige to deep orange during the
moult cycle as shown by field samples (Fig 2.2). The same colour changes were seen in the
laboratory and may be related to increasing astaxanthin levels (Fig. 2.3). As the moult cycle
progressed, differences between pigment stage-specific astaxanthin levels increased.
Significant differences were identified using Wilcoxon Rank Sign Test (Sokal and Rohlf,
1981).

Fig. 2.3 Mean astaxanthin ($\pm$SE$_{mean}$) (mg/l) by pigment stage for whole haemolymph.
Dissimilar superscripts denote significant differences.
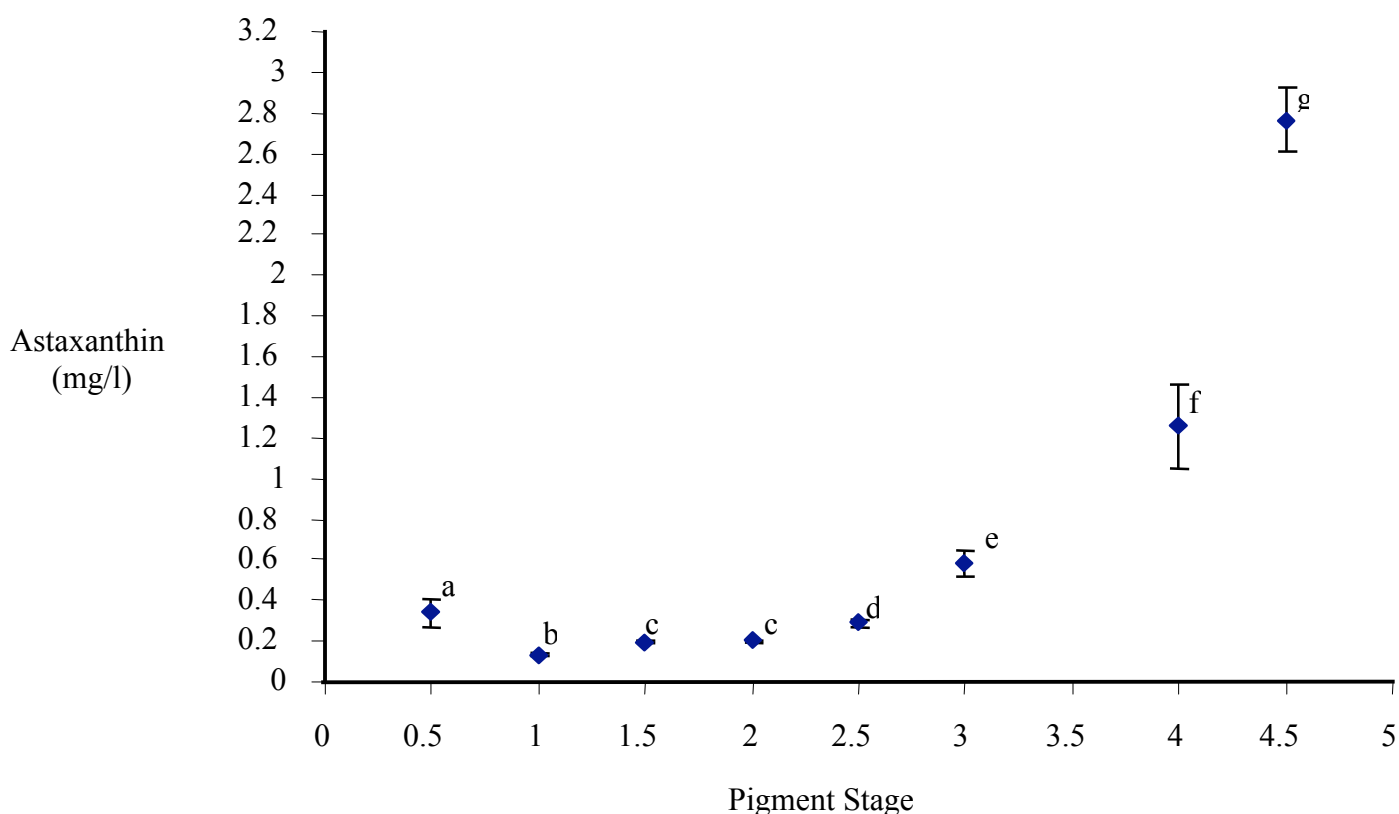
The contribution of each moult stage to each pigment stage (eg. how many C_4 lobsters were
found to be at PS2) was tested between sites to validate inter-site comparisons. It was found
that the only significant difference occurred at PS 4.5 (Kruskal-Wallis, X^2=10.632, P =

0.014), otherwise $P>0.123$. The significant difference was partly due to a very small number of lobsters at this pigment stage (Table 2.1).

Table 2.1 Percentage of lobsters of each moult stage within pigment stage 4.5 for each site within the field sample 1997/1998. n=25

Site	Moult Stage						Total %	Number of lobsters
	C_4	D_1'	D_1''	D_1'''	D_2	D_3		
Cape Jaffa	25.0		12.5		62.5		100	8
Robe	60		20	20			100	5
Marion Bay					83.3	16.7	100	6
Kangaroo Island	33.3	16.7		16.7	33.3		100	6

Furthermore, the significance is somewhat irrelevant with respect to the analysis of condition differences between sites (see below), as there were no site-specific differences beyond PS 3. Data on the remaining pigment stages were pooled between sites for comparison (Fig 2.4).

Fig. 2.4 Field sample 1997/1998: percentage contribution of lobsters of each moult stage to each pigment stage. C_4 is intermoult, D_1' to D_2 are premoult stages. The data for PS 4.5 are shown in Table 2.1 because of the site-specific differences in the distribution of moult stages within the pigment stage. n=886.
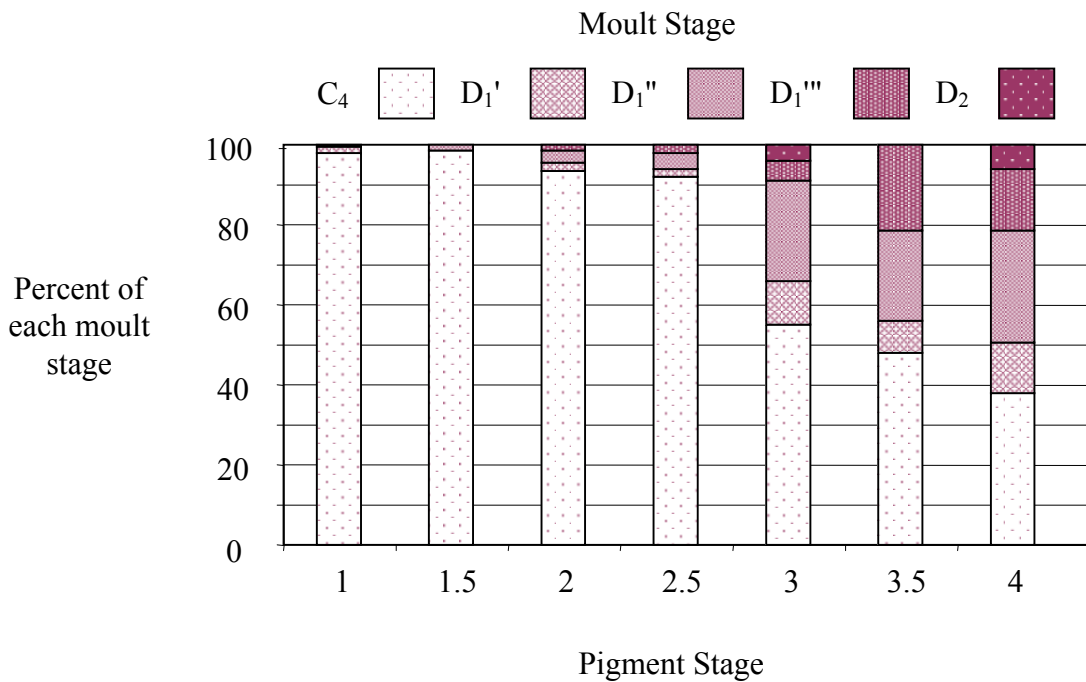
In the field, PS1 to PS2.5 occurred almost exclusively during C_4 (intermoult) (Fig. 2.4), PS3 occurs during late intermoult/ early premoult and the remaining stages follow as ecdysis approaches. PS0.5 did not appear in the field data because no postmoult lobsters were caught.

The length of the stages may be estimated using both laboratory and field data. In the field, within the bounds imposed by catchability, numbers of lobsters of a given moult/pigment stage, in a large sample such as this, may reflect relative moult/pigment stage length (Table 2.2).

Table 2.2 Number and percentage of each moult stage and each pigment stage within the field sample 1997/1998 (mean length of lobsters=97.6$\pm$ 0.19mm CL)
For comparison the number and percentage of each moult stage in the sample taken by Prescott *et al* (1997, FRDC 93/087) are also included (*).

		Moult Stage		
MS	Number	Percent	Number*	Percent*
C_3			34	0.56
C_4	815	89.46	5877	96.22
D_o			14	0.23
D_1'	22	2.41	164	2.69
D_1''	39	4.28	17	0.28
D_1'''	18	1.98	2	0.03
D_2	15	1.65		
D_3	1	0.11		
Total	911		6108	

	Pigment Stage	
PS	Number	Percent
1	301	33.04
1.5	111	12.18
2	245	26.89
2.5	78	8.56
3	85	9.33
3.5	40	4.39
4	26	2.85
4.5	25	2.74
Total	911	

Within intermoult, PS1 is the longest followed by PS2 then PS1.5. There is a progressive decline in PS length until ecdysis. In the laboratory, slightly smaller lobsters (89.8 $\pm$ 0.60 mm CL compared to the field average of 97.52 + 0.20 mm CL) took approximately 112 days to go through a moult cycle (Table 2.3). From the moult to PS2 took approximately 80 days. At the onset of PS2, ecdysis was about a month away and once the lobsters had reached PS4 to 4.5, they moulted within 2 weeks.

Table 2.3 Mean Length (days ± SE) of pigment stages in laboratory experiment.
Mean length of lobsters= 89.6 ± .60 mm CL. (n=40) Total length of moult
cycle approximately 112.33 days (± 10.84, n=4)

| | Pigment Stage | | | | |
	1	1.5 to 4/4.5	2 to 4/4.5	3-4/4.5	4/4.5 - 1
Days	60.50	36	27.25	20.33	13.80
SE	3.86	1.87	2.11	2.52	1.68
n	4	3	8	6	5

2.3.2   Haemolymph protein analysis

Whole haemolymph RI, measured in the field, was directly correlated with Serum RI according to the equation: Serum RI = 0.9121 x Whole Haemolymph RI + 0.1172 (r^2 = 0.99, P<0.001). Serum RI was also correlated with Serum Protein according to the equation: Serum Protein = 4936 x Serum RI - 6609.3 (r^2=0.99, P<0.001). These relationships were used to convert whole haemolymph RI to serum RI then to serum protein (g/l).

2.3.3   Tissue percent dry weight

Tissue percent dry weight was used in the following analysis as it proved to be independent of CL and so unaffected by any CL x site interactions. In detail, regression analysis showed % abdominal tissue to be independent of CL   (r^2= 0.001, F=1.944, P=0.164, n=911) and, although % hepatopancreatic tissue appeared to be significantly affected by CL (F= 19.15, P<0.001, n=911) the very low correlation coefficient (r^2= 0.020) suggested that it was effectively independent. Total dry abdominal tissue was significantly affected by CL (r^2 =0.526, F= 1015.701 P<0.001), as was total dry hepatopancreatic tissue (r^2 = 0.24, F= 285.652, P<0.001), although the correlation was again weak.

2.3.4   Correlations between serum protein and tissue percent dry weight

There were significant correlations between percent dry weight (abdomen and hepatopancreas) and serum protein as follows. Serum protein = 0.00005 x % dry abdomen$^{4.2943}$ (r^2 = 0.78, n=871, P<0.001) and Serum protein = 0.4666 x % dry hepatopancreas$^{1.3930}$ (r^2 = 0.64, n=864, P< 0.001)). Sex had no effect on the relationships (P>0.05).

2.3.5 Change in serum protein and tissue percent dry weight with pigment stage during the moult cycle.

Serum protein varied between 13.3 and 105 mg/ml (mean = 57.06 $\pm$ 0.747, n=895) and was not significantly affected by carapace length ($P>0.05$) or sex ($P>0.05$) although it did increase significantly with pigment stage ($P< 0.001$) (Fig. 2.5), as did tissue percent dry weight ($P< 0.001$, Fig. 2.6) for both hepatopancreas and abdomen. In all cases the pattern was similar, with an increase to PS3-3.5, then a levelling off towards ecdysis. Site-specific differences in tissue percent dry weight are examined below.

Fig. 2.5 Mean serum protein (mg/ml) ($\pm$SE$_{mean}$) by pigment stage for each site.
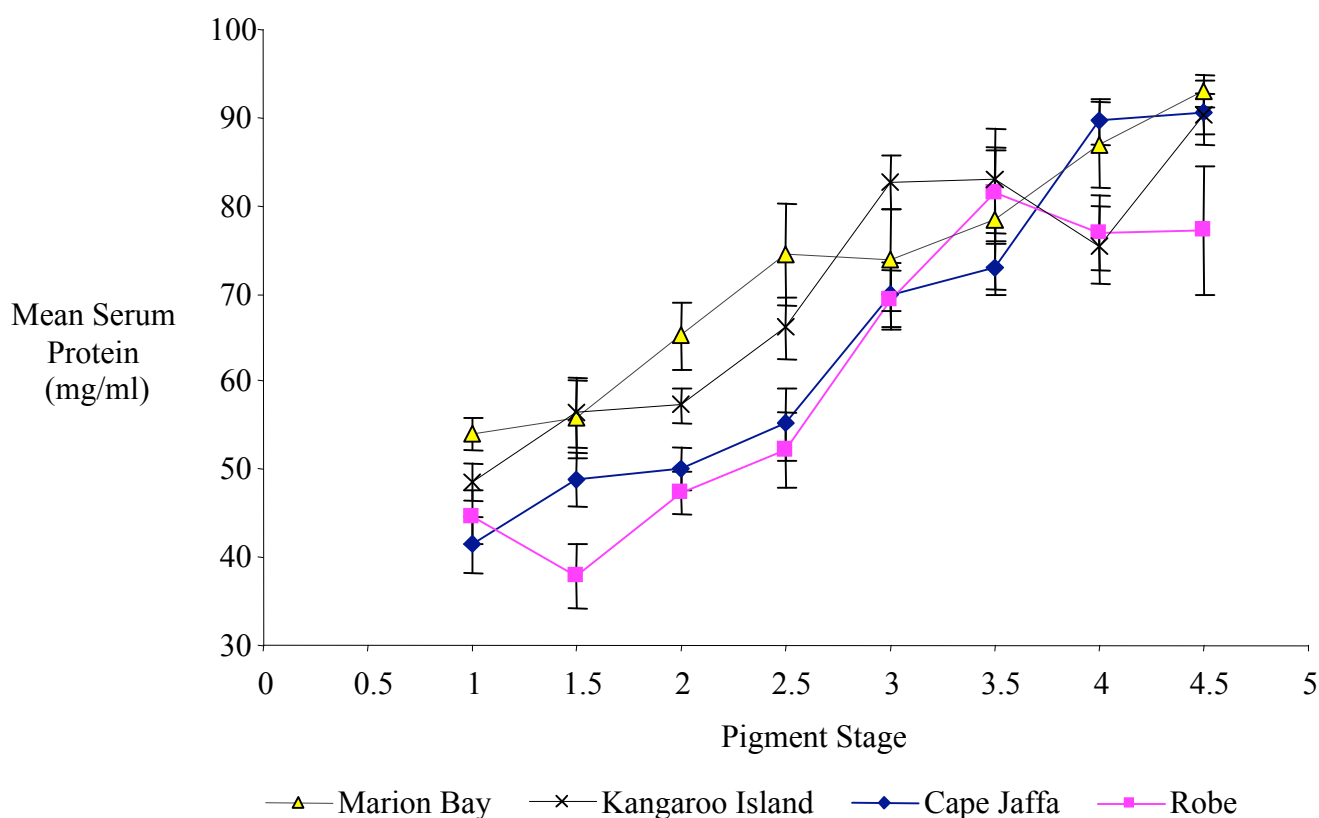
2.3.6 Distinguishing between high and low growth sites using pigment-stage specific serum protein and tissue percent dry weight

Mean serum protein varied with site (Fig. 2.5, Table 2.4). The variation amongst sites was correlated with the estimated specific growth rate derived for adjacent marine fishing areas using the population dynamics model developed for the fishery (Prescott et al., 1997) (Table 2.4). There were no significant sex effects on site-specific serum protein ($P>0.05$, Kruskal Wallis), so the data were pooled and pigment stages individually analysed for site effects (Kruskal Wallis). Month was not considered because of the low numbers of lobsters at some pigment stages in some months. Also, as there were no significant PS effects in stages above PS3, data were pooled. There were significant differences between sites from PS1 to PS3 inclusive, with the highest mean values recorded in areas of high growth *viz* Marion Bay and Kangaroo Island. Furthermore, significant differences between site-specific mean serum protein levels occurred in both seasons (Table 2.4). Pigment stages were not recorded for the 1998/99 season.

Abdominal and hepatopancreatic tissue percent dry weight data were analysed in the same way as serum protein, and showed similar patterns within pigment stages, that is, it was generally higher at the sites with high growth rates (Fig. 2.6). There were no significant sex or CL effects ($P>0.05$). Within pigment stages, significant differences ($P<0.05$) are shown by dissimilar superscripts (Kruskal Wallis non-parametric ANOVA).

Table 2.4 Growth rate (GROTAG* parameters in mm/yr) for a 100 mm CL lobster for the period 1993 to 1998 by sex and mean serum protein (mg/ml) and the pigment stage for the fishing seasons 1997/98 and 1998/99. GROTAG is the southern rock lobster population dynamics model developed by Prescott *et al* (1997) and was extracted with permission. Different superscripts within each column point to significantly different serum protein levels (Kruskel Wallis non-parametric ANOVA, $P \leq 0.05$).

| Site	Growth Rate (mm/yr) Males	Females	Mean Serum Protein mg/ml)	1997/98 Pigment Stage 1	1.5	2	2.5	3	>3	1998/99 Pigment Stage not recorded
Marion Bay	20.61	14.48	Mean	54.12[a]	55.79[a]	65.2[a]	74.48[a]	73.97[b]	86.09	57.92[a]
			SE	1.87	4.53	3.8	5.7	5.79	3.32	1.88
			n	127	21	40	9	13	18	156
Kangaroo Is.	17.10	10.05	Mean	48.56[b]	56.37[a]	57.25[b]	66.12[a,b]	82.63[a]	83.30	
			SE	2.21	3.8	2.05	3.42	3.02	2.85	
			n	95	21	86	19	19	17	
Cape Jaffa	16.96	9.10	Mean	41.47[c]	48.96[a]	50.1[c]	55.12[b]	69.79[b]	80.03	43.59[b]
			SE	3.13	3.1	2.54	4.14	3.64	2.38	2.13
			n	38	45	59	25	27	36	88
Robe	16.96	9.10	Mean	44.57[b,c]	37.93[b]	47.4[c]	52.26[b]	69.25[b]	78.27	
			SE	3.09	3.6	2.41	4.3	3.48	3.01	
			n	41	20	56	21	24	17	

Fig. 2.6 Percent tissue dry weight (±SEmean) for each pigment stage and site.
Dissimilar superscripts denote significant differences at a given pigment stage.

— △ — Marion Bay   — ✕ — Kangaroo Island   — ◆ — Cape Jaffa   — ■ — Robe

a) abdominal tissue
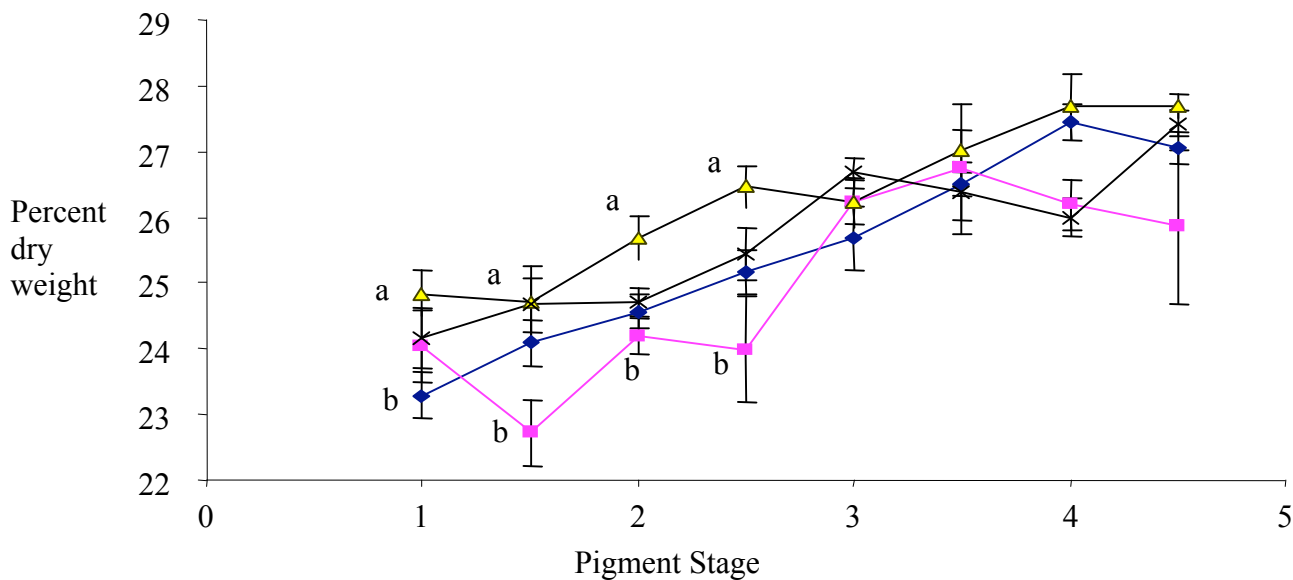
b) hepatopancreatic tissue
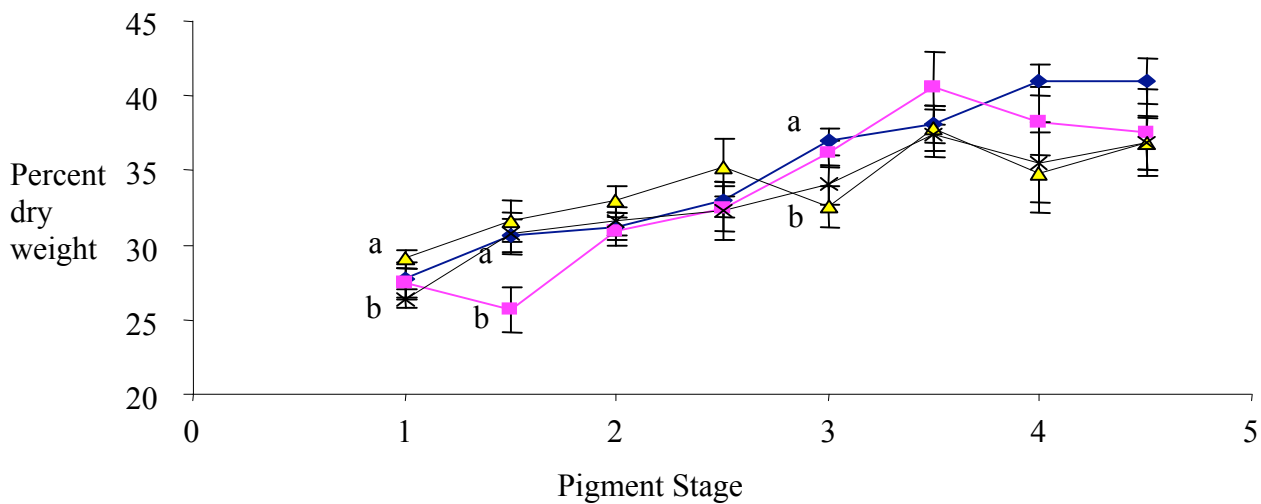
2.3.7 *Environmental Data*

In 1997/98 temperature was highest and depth shallowest at the sites with the highest growth (Table 2.5). The Cape Jaffa and Robe data were combined in the table because the areas are adjacent and there are unlikely to be any differences between sites for water temperatures at a given depth. The shallower and deeper temperature logs were provided by fishers working out of Cape Jaffa and Robe respectively. In 1998/99 the temperature data from Cape Jaffa were obtained from a fixed depth, but the pattern is similar to that of the previous season.

Table 2.5 Mean temperature and depth fished for the fishing seasons 1997/98 and 1998/99.
In the 1998/99 season fishing was carried out at Marion Bay and Cape Jaffa. *The depth for Cape Jaffa in this season has no variance because it originates from a logging station attached to a buoy.

	Marion Bay	Kangaroo Is.	Cape Jaffa/Robe	
	Mean (SE,n)	Mean (SE,n)	Mean (SE,n)	Mean (SE,n)
1997/98				
Temperature (^{o}C)	17.63 (0.02,2363)	16.51 (0.03,2266)	15.02 (0.03,2714)	12.72 (0.03, 2493)
Depth fished (m)	31.37 (1.09, 100)	35.51 (2.03, 105)	31.32 (1.63,106)	65.52 (2.63, 86)
1998/99				
Temperature (^{o}C)	16.95 (0.02,2492)		12.51 (0.021, 1776)	
Depth fished (m)	26.84 (0.90,13)		52.00*	

2.4   Discussion

This study shows that the combination of serum protein and pigment stage may be used as an indicator of nutritional condition if used with conventional moult staging techniques. Condition differed significantly between areas, probably influenced in part by temperature-mediated effects on growth rate. The addition of pigment stage to the serum protein index allowed the differentiation of lobsters at the beginning and at the end of intermoult, thus solving a problem raised by Dall (1974) when discussing the work of Stewart et al. (1967) and Stewart and Li (1969).

The serum protein/pigment stage index also allowed closer examination of the lobster tissue growth than obtained by conventional moult staging techniques alone. The combination of pigment stage and serum protein, and their relationship with percent dry weight, shows that the intermoult resource accumulation phase is important in determining differences between areas of different growth. Given that the length of intermoult has not been determined for the study sites and that it is likely to be shorter in fast growth rather than slow growth areas, the real differences in condition are likely to be greater. The data also indicate that there is a range of serum protein values to be expected within a given pigment stage, with high values pointing to relatively better condition. Monitoring of proportions of the various pigment stages within a given population might allow assessment of moulting activity although one would still need to clip pleopods and analyse setal development. Thus both spatial effects and temporal effects within sites could be more thoroughly monitored. The combination of serum protein and pigment stage also has some use within aquaculture, in grading lobsters prior to grow-out to select those which are likely to moult earliest and in monitoring tissue accumulation (i.e. weight vs PS) during grow-out.

Current moult staging methods are based on appendage setal development and carapace rigidity (Kurup, 1964; Aiken, 1973; Musgrove, 2000) with noticeable changes occurring during postmoult and premoult. However, as the methods do not allow further subdivision of intermoult, a problem arises when intermoult is long, as it is in lobsters. It has often been assumed that animals at intermoult were all physiologically the same or at the same point in their development. The present study shows (Table 2.4, Fig. 2.6) that significant tissue accumulation does occur during intermoult, potentially adding a confounding effect to such work.

Tissue growth should be considered as a variable if the crustacean under study has a long intermoult period. This study has offered a way of monitoring such growth.

CHAPTER 3 THE RELATIONSHIP BETWEEN HAEMOLYMPH CHEMISTRY AND MOULT INCREMENT

> **Objective 1d. Testing of the hypothesis that premoult haemolymph lipid concentration could be used as a predictor of moult increment**.
>
> This objective was achieved by examining the relationship between moult increment and premoult haemolymph lipid level in lobsters housed in the laboratory and the relationship between hepatopancreatic lipid and haemolymph lipid levels from lobsters collected from the field, as described in the previous chapter.
>
> Haemolymph lipid, in particular phospholipid, may be useful in the prediction of % moult increment. This outcome is potentially useful, as haemolymph lipid measurement does not require the killing of the lobster, however caution should be exercised in application of these results as haemolymph lipid/moult increment data were gathered over a short time period and within a relatively controlled environment. Further field studies are needed to better understand the relationship between haemolymph lipid level and moult increment in wild populations of this species. For example, questions remain as to whether higher growth sites, showing higher serum protein content, would also show higher moult increments.

3.1   Introduction

Growth data are essential to rock lobster fisheries stock assessment. At present, predictions of growth for a given year are based on data from previous years with the accuracy of estimates being unknown until measures of actual growth are obtained for the year in question.

The shedding of all hard parts at ecdysis complicates measurements of growth in rock lobsters and other crustaceans. No structures are retained (*sensu* fish otoliths) from which age at size, and therefore growth information, may be gathered. During work in South Africa, Cockcroft (1997) suggested that growth may be estimated from hepatopancreatic lipid level having found a significant relationship between moult increment and percent hepatopancreatic lipid during premoult in *Jasus lalandii*. The finding of this relationship was a significant advance, although it was still necessary to kill the animal to gather the data, a step that might be avoided by isolation of an equally useful haemolymph component. In Chapter 2, haemolymph protein, in combination with haemolymph pigment level and moult stage, was useful in distinguishing between lobsters at

high and low growth sites within the South Australian fishery. Grouping serum protein data by pigment stage with reference to the major pigment, astaxanthin, allowed the differentiation of lobsters at the beginning and those at the end of intermoult. Given the correlation between serum protein and % dry weight, differences in lobster condition between high and low growth sites could be examined more thoroughly using this method. Haemolymph protein has been used successfully in other studies as a measure of condition (Leavitt and Bayer, 1977; Musgrove, 2001) but has not been shown to be useful in predicting moult increment. Given Cockcroft's work, premoult haemolymph lipid appeared to be the most likely to show a predictive relationship with moult increment. If haemolymph lipid could be used in place of total hepatopancreas lipid to predict moult increment, the necessity to kill the lobster would be avoided and multiple samples may be taken over time from the same individual.

Phospholipids are the major circulating lipid and triglycerides the major storage lipid in crustaceans. Both are found in the haemolymph and hepatopancreas (Chang and O'Connor, 1983). The hepatopancreatic lipid component of *Jasus lalandii* is largely triglycerides (neutral lipids) with phospholipids (polar) of less importance (<14%) Cockcroft (1997). In the hepatopancreas, ingested neutral lipids are cleaved to mono or diglycerides, which are then converted to phospholipids. These are expelled into the haemolymph and transported to various tissues, either for use as membrane components or conversion to triglycerides and storage (Chang and O'Connor, 1983).

Given that the hepatopancreas of rock lobsters increases in size and lipid content through the moult cycle, reaching a maximum just before ecdysis (Present study; Cockcroft, 1997), it may also be expected that other chemical compounds would show similar patterns. Thus, as the hepatopancreas reached maximum storage during late premoult (Chapter 2 present study; Mercaldo-Allen 1991), so haemolymph lipid would reach maximum concentration. Furthermore, as Cockcroft (1997) found that hepatopancreas lipid was an indicator of moult increment in the field, so may haemolymph lipid be, as increasing amounts of phospholipid would be used for both the cell membranes of the expanding hepatopancreas and, after conversion to triglyceride, as the main lipid store.

This chapter tests the hypothesis that premoult haemolymph lipid concentration may be used as a predictor of moult increment, in the laboratory, and examines the relationship between haemolymph and hepatopancreatic lipid content and tissue weight. The study was undertaken

to develop a non-lethal means of moult increment prediction, which could then be used in the field. This chapter was published in 2003 in the Journal of Shellfish Research, Vol 22 (1), pgs 235-240.

3.2 Methods

3.2.1 Laboratory Experiment 1: The relationship between moult increment and premoult haemolymph lipid level

Forty lobsters (mean CL : 89.88$\pm$ 0.60 mm, mean weight 364.6 $\pm$ 6.54g) were individually housed in 30L plastic tanks in a flow through system (0.4 L/hour/tank) for 185 days. Each tank was independently supplied with air and water of a constant temperature (18°C, which was similar to the average summer temperature in the area of capture). Day length was set at 12 hours and the lights covered with red cellophane to minimise disturbance. Lobsters were fed *ad libitum* daily on a mixed diet of artificial pellets (4 pellets/feed, Geddes et al 2000) and cockles (4 cockles/feed, *Donax deltoides*) in a rotation. Daily consumption was assessed by eye from day 52 and categorised as zero, <25%, 25-50% and >50%. Excess food was removed and tanks cleaned each morning, taking care to minimise disturbance to the lobsters.

Haemolymph samples (0.5 ml) were taken fortnightly from each lobster by pericardial puncture for analysis of haemolymph serum. Once the pigment stage of each haemolymph sample had been noted (Chapter 2, present study) it was snap-frozen (-196°C) for later analysis. Pigment stage refers to the colour of the haemolymph, which changes from light blue, through beige to deep orange during the moult cycle, the beige becoming visible during intermoult (Musgrove, 2001). If the lobster was immediately pre-moult, samples were taken before and after ecdysis. Pre and postmoult carapace length was also measured, the latter delayed until C_4 was reached, and pleopod samples taken periodically to track moult stage by examination of setal development (Musgrove, 2000).

3.2.2 Laboratory Experiment 2: The relationship between moult increment and premoult haemolymph lipid level in a less controlled environment.

Seven premoult lobsters were selected from animals that had been kept in an outside tank for several months with other species (echinoderms, other decapods) and fed two to three times a week on blue mussels (*Mytilus* sp.). The tanks were at ambient temperature (about 16°C) and contained abundant limestone rocks, *Macrocystis* sp., *Ulva* sp. and other aquatic macrophytes. The selected lobsters were measured (range – 65.3 to 103.8 mm CL), moult staged (after Musgrove 2000) and placed in plastic cages within the aquaria. They were fed mussels *ad*

libitum 3 to 4 times a week. Pleopods were taken regularly to keep track of the moult stage and, during late premoult (Stage D_3) a 0.2ml haemolymph sample was taken, pigment staged, then snap frozen. Once each lobster had hardened (i.e. at intermoult) it was re-measured. The data were then compared to those collected from laboratory experiment 1.

For experiments 1 and 2, blood was taken during the afternoon to standardise post-prandial effects on haemolymph lipid (*sensu* Dall, 1981). Lobsters were fed after extraction was completed. In both cases, lobsters were not observed to feed during daylight.

3.2.3 Field Study: The relationship between tissue lipid and haemolymph lipid level.

139 rock lobsters were collected from the wild fishery as described in Chapter 2 and haemolymph samples taken as described above within 3 hours of capture, the pigment stage noted and the sample snap-frozen (-196°C) for later serum lipid analysis. A pleopod was also taken for moult stage determination by examination of setal development (Musgrove, 2000). The lobsters were then frozen (-30°C) and retained for dissection and tissue analysis.

Within two weeks of collection, lobsters were rapidly thawed and the abdominal tissue and hepatopancreas removed, weighed then dried to constant weight (60°C, 72 h). The tissue was then allowed to cool to room temperature in a desiccator over silica gel, reweighed (to nearest 0.1 mg) and dry weight and percent dry weight calculated.

3.2.4 Haemolymph serum analysis

All whole haemolymph samples from the laboratory study and a random selection of samples from the field collection (n=139) were analysed for triglyceride and phospholipid. The clotted haemolymph was thawed then broken up gently with a glass stirring rod and the sample centrifuged (Hettich EBA12 centrifuge, 15 minutes, 17,280 x g) to extract the serum. Serum aliquots were analysed on a Cobas Mira Autoanalyser for triglyceride and phospholipid using commercially produced test kits (Roche). To test for phospholipid the triglyceride kit (Roche, No. 07 3679 1) was modified as follows. 250 units phospholipase C (Sigma No. P4014) were added to a 30ml bottle of triglyceride reagent. The modified reagent was then incubated with the serum sample for 15 min at 37°C (cf 6 min for triglyceride) to convert the serum phospholipids to diglycerides, which were then converted to glycerol by the

lipase in the kit. The incubation time was chosen by incubating a lecithin solution (2 mM) to give a result equivalent to 2mM triglyceride. Accuracy was maintained for all tests using commercially available quality controls (Nycomed Farmer).

3.2.5 Data Analysis:

If data were normally distributed or could be normalised, analyses were performed using ANCOVA or ANOVA with the GLM module (General Linear Models) on SPSS. If data could not be normalised, the Kruskal Wallis Non Parametric ANOVA or the Wilcoxan Rank Sign were used. In all cases significance was accepted at $P<0.05$.

3.3 Results

3.3.1 Laboratory Experiments 1 and 2

Percent moult increment, tank placement and feeding regime

Premoult CL had no effect on % moult increment ($P>0.05$, ANCOVA) and there was considerable overlap between the ranges of % moult increment recorded in the outside tanks (2.4 to 8.0% of premoult CL, n=7) and those inside (0.8 to 5.2%, n=9).

The slopes of the percent moult increment: lipid regressions were the same for inside and outside tanks ($P>0.05$, ANCOVA). For this reason, data from inside and outside tanks were pooled for further analyses.

*Haemolymph serum lipid and moult increment*

Both lipid fractions were significantly correlated with percent moult increment (Table 3.1). Phospholipid showed the highest coefficient at $r^2=0.66$. Both phospholipid and triglyceride showed a progressive increase with pigment stage (Fig 3.1) until PS3.0 to 4.0 then declined to PS4.5.

Table 3.1 Relationship between percent moult increment and haemolymph lipid (mmol/l) for phospholipid, triglyceride and TP (triglyceride + phospholipid).
The regression model is Log % Moult Increment = $\alpha(\log (\text{Lipid}))^\beta$, n=16

Parameter	α	β	r^2	F	P
Phospholipid	0.0715	2.072	0.66	30.062	<0.001
Triglyceride	0.1140	2.248	0.403	11.123	0.005
TP	-0.0786	1.765	0.641	27.781	<0.001

Fig. 3.1 Laboratory: mean haemolymph serum triglyceride (mmol/l) ($\pm$ SE) , phospholipid (mmol/l) ($\pm$ SE) and triglyceride +phospholipid (TP) (mmol/l) ($\pm$ SE) vs Pigment Stage.
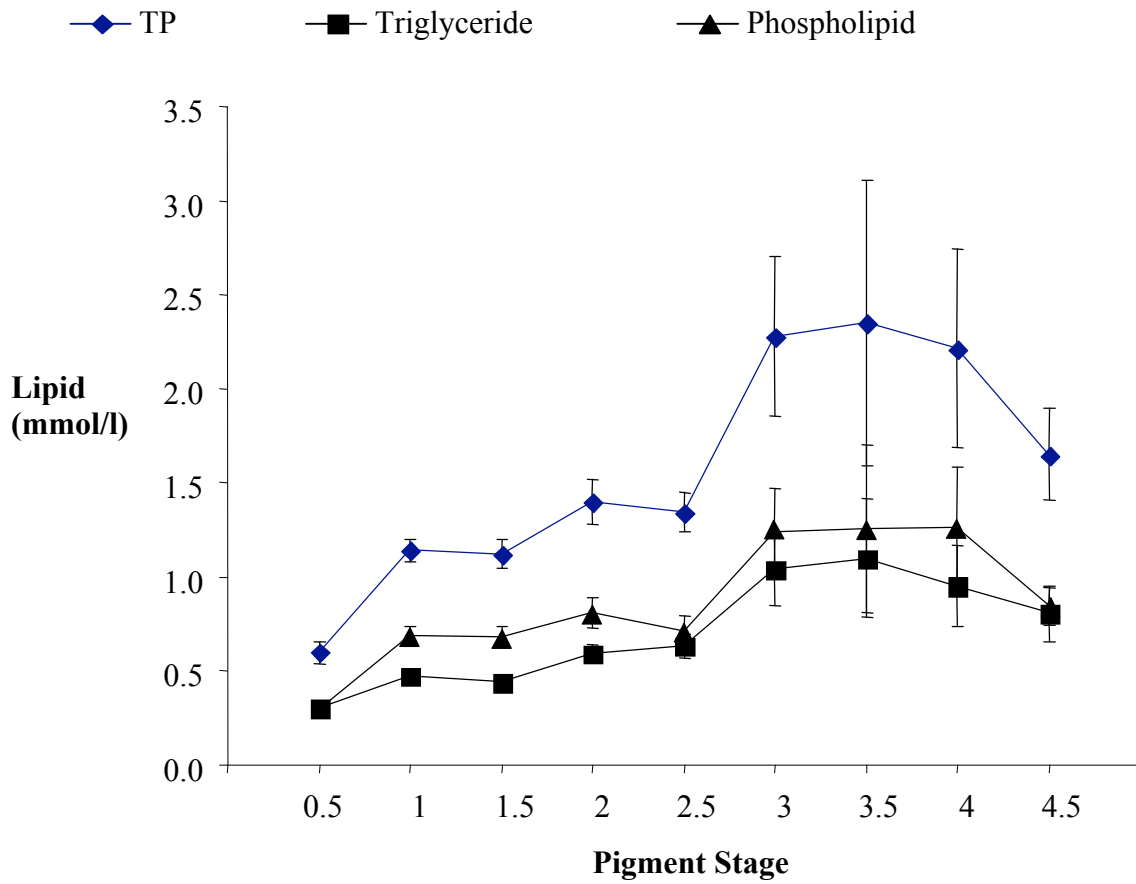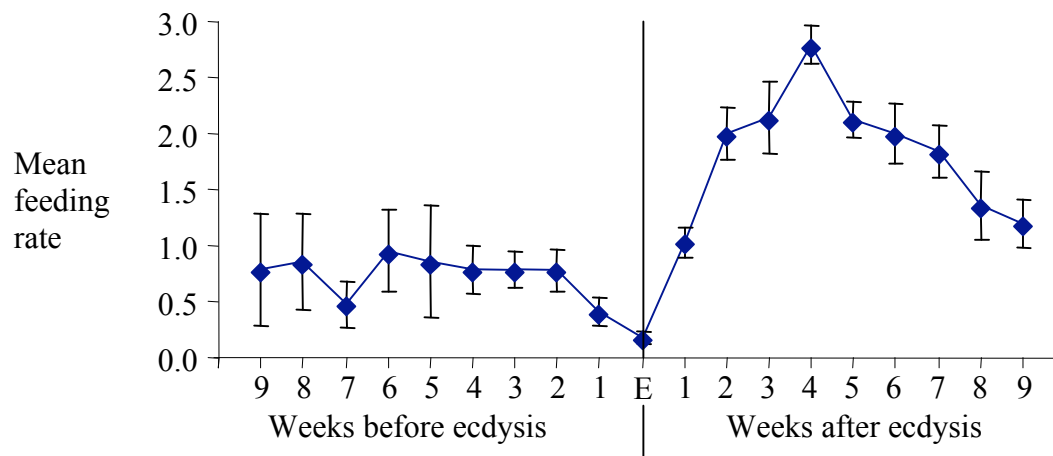
Feeding rates

Feeding rate increased fourfold after the moult in those lobsters for which there were data (Fig 3.2). There were no sexual differences in feeding rate ($P>0.05$).

Fig 3.2 Mean feeding rates ($\pm$ SE) for 9 weeks before and after ecdysis (E).
Four daily consumption categories (0,<25%,25-50% , >50%) were used and assigned numbers from 1 to 4. n=18


3.3.2   *Field Study*

Haemolymph serum lipid

The field serum lipid data showed a progressive increase in lipid content with pigment stage (Fig. 3.3) in a similar fashion to that found in the laboratory, although in this case the peak occurred at PS4.

Fig. 3.3 Field: mean haemolymph serum triglyceride (mmol/l) ($\pm$ SE) , phospholipid (mmol/l) ($\pm$ SE) and triglyceride + phospholipid (TP) (mmol/l) ($\pm$ SE) vs Pigment Stage.
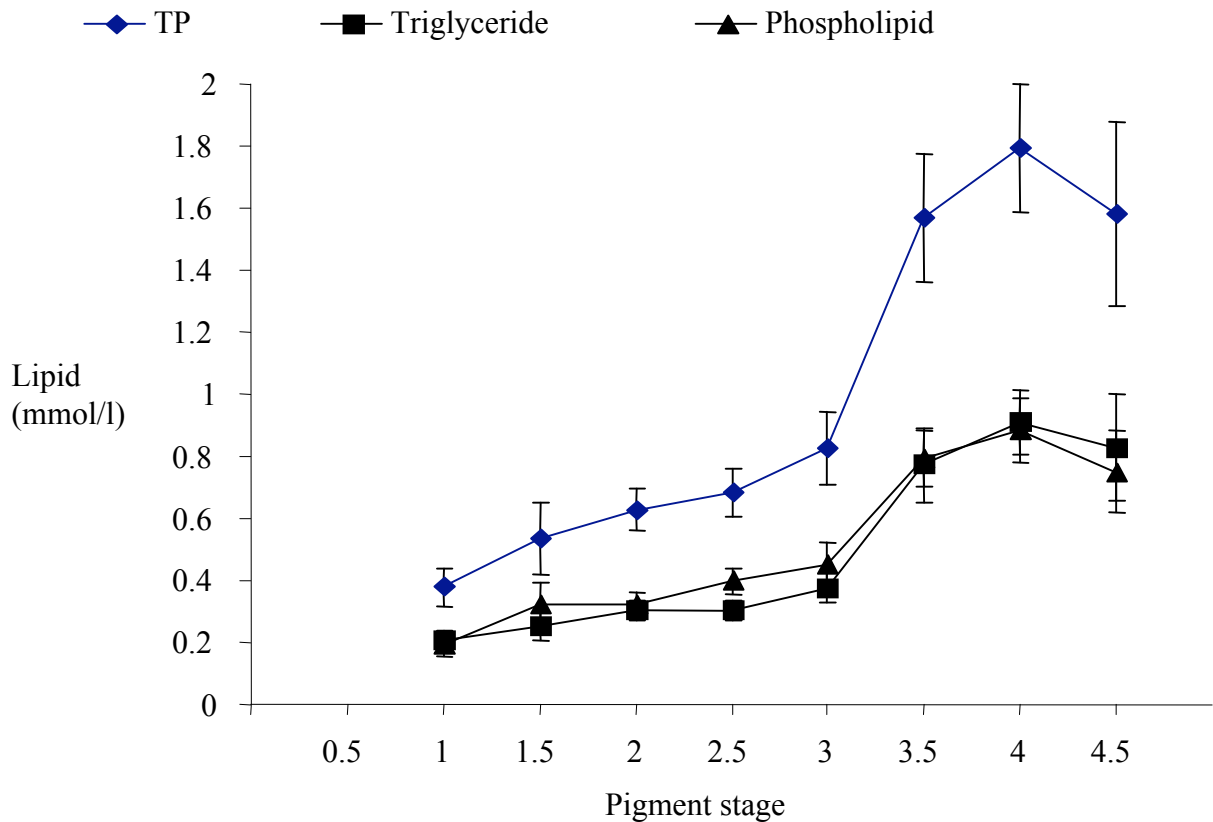
Haemolymph lipid and hepatopancreas weight

Haemolymph lipid increased with hepatopancreas dry weight, on both a total weight and a percentage basis (Fig 3.4a and b) up to PS4. However, while haemolymph lipid was significantly correlated with tissue weight during intermoult (Table 3.2), the relationship declined after PS2-2.5.

Fig. 3.4 Comparison of (a) hepatopancreatic mean dry weight (g) (± SE) , (b) mean percent dry weight (± SE) and Triglyceride +Phospholipid (TP) vs Pigment Stage.
Mean dry weight (g) standardised for Carapace Length using GLM analysis on SPSS. Data are displayed for a 97.9 mm CL lobster. ◆— TP   ■— Dry weight (g)
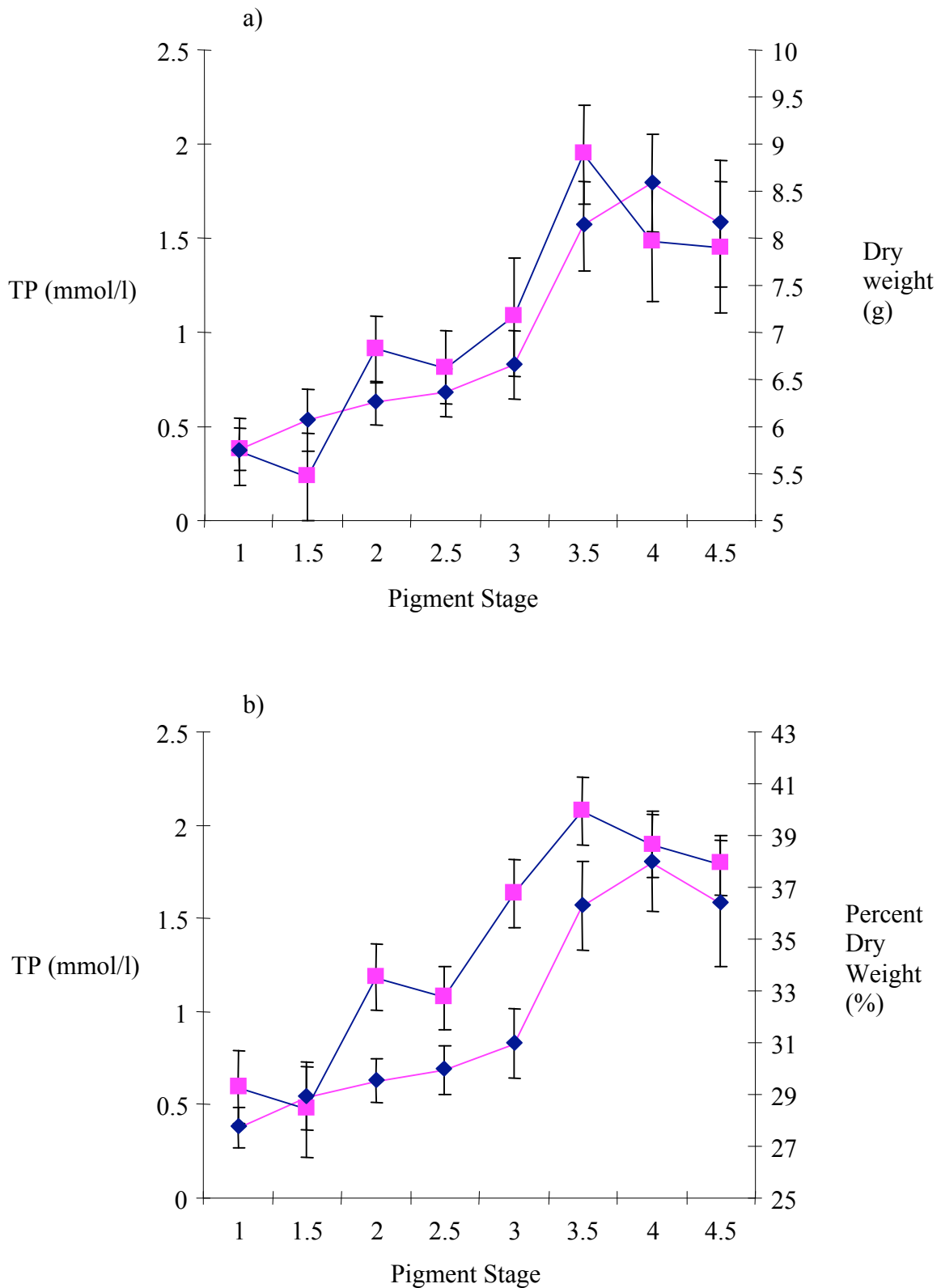
Table 3.2 Field data regression statistics for pigment stage-specific hepatopancreas percent dry weight and total dry weight vs total lipid (T+P) (mmol/l).
Regression model is Lipid $=\alpha$Weight$^{\beta}$ except for percent dry weight at pigment stage 1, where the best fit was given by the cubic model (Lipid$= \alpha + \beta_1$Weight $+ \beta_2$Weight$^2+\beta_3$Weight^3)

| Percent or Total Weight	PS	r^2	F	P	n
Percent	1	0.722	17.33	<0.001	25
	1.5	0.887	118.29	<0.001	17
	2	0.599	29.93	<0.001	24
	2.5	0.685	41.39	<0.001	21
	3	0.448	8.10	0.017	12
	>3	0.067	1.93	0.176	29
Total Weight	1	0.532	24.96	<0.001	25
	1.5	0.767	49.50	<0.001	17
	2	0.389	13.37	0.001	24
	2.5	0.282	7.47	0.013	21
	3	0.000	2.9^{-04}	0.987	12
	>3	0.078	2.29	0.142	29

3.3.3   Laboratory experiments and field study comparisons:

Moult stage and pigment stage

The relationship between moult stage and pigment stage was similar in the laboratory and the field (Fig 3.5). In the following analysis, comparisons are made between pigment stage-specific laboratory and field haemolymph lipids. Before this was done, analysis was undertaken to check that the same pigment stages had similar distributions of moult stages in the laboratory and the field. To facilitate the analysis each moult stage was assigned a number (1-11).

The laboratory distribution of moult stages within each pigment stage was similar to that in the field (Mann-Whitney U, Zar, 1984). The only significant difference was in PS 4 (U = 37.5, P=0.014, mean moult stage$_{lab}$ = 9.46 $\pm$ 0.39, mean moult stage$_{field}$ = 8.15 $\pm$ 0.249), otherwise P$\geq$ 0.212.

Fig. 3.5 Mean moult stage (± SE) within each pigment stage for laboratory experiments (pooled) (n=40) and field study (n=135). ◆ Laboratory ■ Field
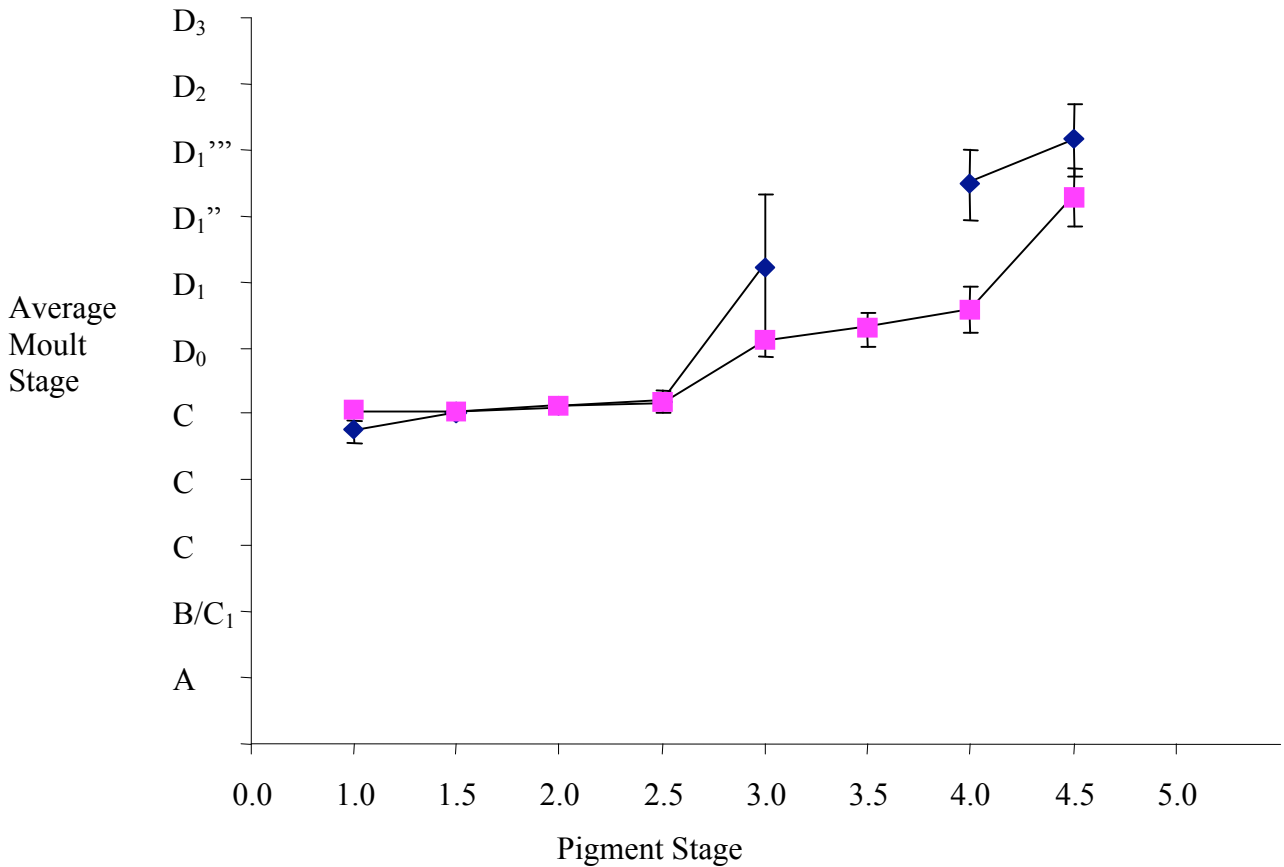
Haemolymph serum lipid

Pigment stage-specific total lipid of laboratory animals was greater than that in the field until PS3.5 (Mann-Whitney U, P< 0.05) (Fig 3.6). The patterns in the relative importance of the two lipid fractions were also different. In the field, the proportion of phospholipid increased until PS 2.5 (Fig 3.7) then fell until PS 4.5, in contrast to the laboratory where the peak was reached during PS1. Both laboratory and field showed the same trends after PS2.5.

Fig. 3.6 Mean triglyceride + phospholipid (TP) (mmol/l) (± SE) : field vs laboratory data by pigment stage.
Significant differences (Mann- Whitney U) between laboratory and field data are indicated by asterisks (** P < 0.001) ─■─ **Laboratory TP** ─◆─ **Field TP**
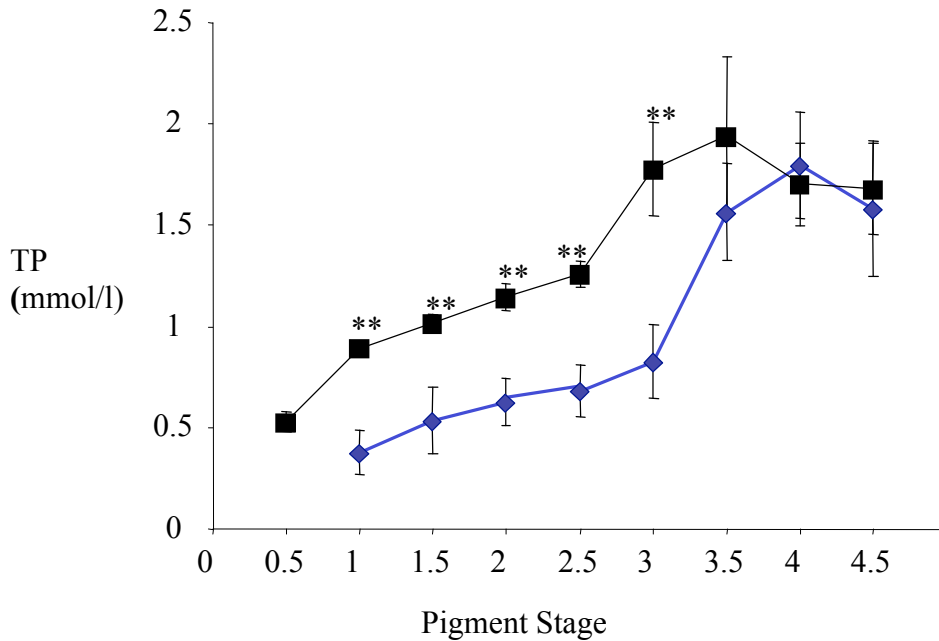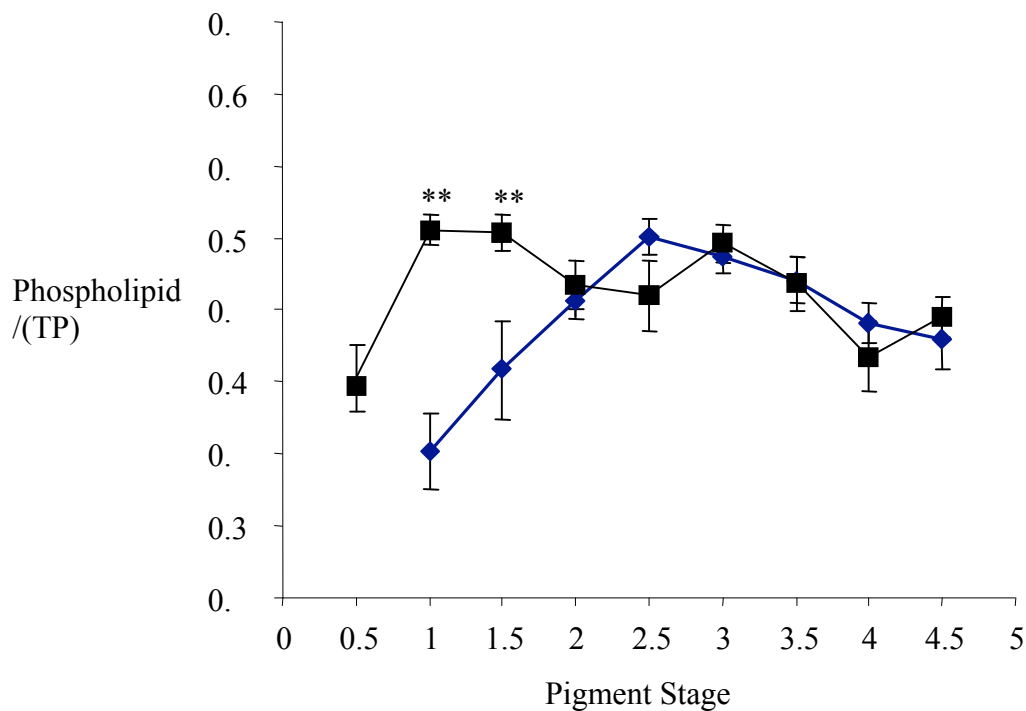

Fig. 3.7 Haemolymph serum phospholipid/(Triglyceride + Phospholipid (mmol/l)) (± SE) : Field vs Laboratory data by Pigment Stage.
Significant differences (Mann- Whitney U) between laboratory and field data are indicated by asterisks (** P < 0.001) ─■─ **Laboratory TP** ─◆─ **Field TP**

3.4 Discussion

The key result to come out of this study is the potential use of haemolymph lipid in the prediction of % moult increment. While further field studies are needed to be sure of the result, this outcome is potentially useful, as haemolymph lipid measurement does not require the killing of the lobster. Questions remain as to whether higher growth sites, showing higher serum protein content would also have higher moult increments. In this regard, significant differences were reported in mean serum protein level between sites by Musgrove (2001). The differences occurred mainly during intermoult, which is the period when haemolymph lipid is significantly correlated with both serum protein and hepatopancreas percentage dry weight. This may suggest a relatively higher degree of lipid accumulation at those sites, pointing to a higher moult increment. Dall (1981) suggested that the principal function of digestive gland lipid in *Nephrops norvegicus* was in the moulting process so one might expect that lipid accumulation in *Jasus edwardsii* would be similarly focussed.

Cockcroft (1997) also found a significant relationship between moult increment and hepatopancreas lipid level, for *Jasus lalandii*. He reported that moult increment was positively related to peak % lipid values occurring during late premoult in the hepatopancreas, similar to the present study, where the significant relationship was between haemolymph lipid (μmol/l) and % moult increment. Furthermore, he suggested a "window" period of reserve accumulation, essential for growth. This occurs from intermoult to early premoult, especially the former, as suggested for *J. edwardsii* by the relative increase in feeding rate after ecdysis. The period of reserve accumulation (PRA) would probably lead up to a "reserve saturation point" as suggested by Anger (1987) for crustacean larvae. Cockcroft found that lobsters starved during PRA, then fed during premoult, showed severely reduced growth rates and even shrinkage. Those starved prior to moulting but fed during PRA moulted with similar growth increments to those of control lobsters, which were fed throughout. So it is this PRA which is critical to future growth, influencing both moult increment and intermoult period (Cockcroft, 1997).

The question is, why should the percent moult increment be correlated with the lipid level in the haemolymph at PS4.5, when it was not related to the hepatopancreas percent dry tissue at that stage? At PS4.5 about 95% of lobsters were beyond D_1'''. The rigorous investigation of this question is outside the framework of this study but it may be that the apparent decoupling

of the relationship between hepatopancreas weight and haemolymph lipid at the later pigment stages is due to a mobilisation of lipid reserves from the hepatopancreas to the haemolymph in preparation for the energetic demands of ecdysis. The correlation may arise because the higher the level of stored lipid in the hepatopancreas, the greater the reserve that may be mobilised in readiness for ecdysis

The importance of phospholipid in the relationship fits in with Chang and O'Connor's (1983) contention that phospholipid is the main circulating lipid in crustaceans. Bligh and Scott (1966) reported that 65% of the total lipid in the haemolymph of the lobster, *Homarus americanus,* was phospholipid, with the remainder almost equally divided between triglycerides and sterols. The latter has a primarily structural role (Fraser, 1989). Free fatty acids comprised only about 2.4% of the total lipid. O'Connor and Gilbert (1969) reported similar results for the land crabs, *Gecarcinus lateralis* and *Cardiosoma guanhumi*.

Finally, the relative levels of haemolymph protein and lipid in the laboratory and the field suggest that the rate of accumulation differs, particularly in the early stages of the moult cycle. One would assume that these differences occur because captive lobsters did not have to hunt for food, more nutrients being directed to muscle accumulation and lipid storage earlier in the moult cycle.

The data suggest that haemolymph phospholipid level has the potential to predict moult increment. However, the haemolymph lipid/moult increment data were gathered over a short time period and within a relatively controlled environment. Further field studies are essential to better understand the relationship between haemolymph lipid level and moult increment in wild populations of this species.

BENEFITS

As stated in the original application, the work will enhance the growth components of the yield per recruit analysis and the population model by providing an alternative means of growth assessment. A more accurate assessment of the population dynamics of the South Australian Southern Rock lobster Fishery will assist in the development of a better management strategy and, in doing so, benefit all those associated with the industry.

FURTHER DEVELOPMENT

Further work should be carried out to better establish the connection between serum lipid level and moult increment, particularly in the field. There is also the question of whether high growth sites would show differences in moult increment as well as serum protein levels. Answers to these questions would enhance our understanding of the dynamics of the fishery.

PLANNED OUTCOMES

The pigment stage- serum protein index will be used in the Southern Zone of the fishery during the 2003/04 season to assess changes in growth resulting from increased abundance. The method was also used in FRDC Project 98/305 (Rock Lobster Enhancement and Aquaculture Subprogram Project 5 : Determination of the optimum environmental and system requirements for juvenile and adult rock lobster holding and growout) and is in use in FRDC 2002/238 (Rock Lobster Post-Harvest Subprogram: Quantification of Shell Hardness In Southern Rock Lobster). There has also been considerable interest in the method from workers in Victoria, New Zealand and the United States.

CONCLUSION

The objective of this study was to obtain an understanding of variations in growth and recruitment through the establishment of the potential of selected indices to characterise temporal and spatial changes in condition of lobsters within the fishery. In Chapter 2 it is suggested that a condition index that combines measurement of pigment stage and serum protein with conventional moult staging techniques would allow the characterisation of such changes. Grouping lobsters by pigment stage increases the resolution of serum protein data to

allow closer examination of muscle growth and reserve accumulation. The index has the added advantage of simplicity of both concept and methodology.

It may also be possible to estimate percent moult increment using serum lipid levels (Chapter 3). Further work should be done to confirm the relationship between lipid accumulation and moult increment as these data were gathered within a relatively controlled environment. Additional laboratory and field studies are necessary to better understand the relationship between serum lipid and moult increment in wild populations of this species, particularly with regard to the question of whether faster growth necessarily implies a greater moult increment.

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APPENDIX 1: INTELLECTUAL PROPERTY

The FRDC's proportion of ownership of the project intellectual property, based on Part C of the application or unless otherwise justified, is 50%.

APPENDIX 2: STAFF

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