Condition and Its Assessment in the Southern Rock Lobster *Jasus edwardsii.*

i. Assessment of condition indices and moult staging techniques

R.J.B. Musgrove

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Project 95/017

TABLE OF CONTENTS

List of Tables

List of Figures

NON TECHNICAL SUMMARY

Project 95/017 Condition and Its Assessment in the Southern Rock Lobster *Jasus edwardsii*. i. Assessment of condition indices and moult staging techniques

PRINCIPAL INVESTIGATOR: Dr. Richard Musgrove
ADDRESS South Australian Research and Development Institute (Aquatic Sciences)
P.O. Box 120,
Henley Beach,
SA 5022

OBJECTIVES

1. Moult Staging Techniques. To determine the correlation between moult stage, shell rigidity and pleopod epidermal and setal development for this species. To determine the length of individual moult stages.

2. Development of a condition index or indices .To determine the correlation between selected biochemical components, growth and temperature in the laboratory.

NON TECHNICAL SUMMARY

This work forms the initial part of a study, undertaken for the following reasons. The condition of lobsters has an important effect on growth, catchability, and market value. For example, recruitment to the fishery is determined by year-class strength and moult increment (Project 93/087, Population Dynamics of the Southern Rock Lobster in South Australian Waters). The latter is dependent on lobster size and condition. In South Africa, research has suggested that poor condition has lead to a 50% downturn in catches in recent seasons. Assessment of biochemical condition of lobsters before a moult can provide estimation of moult increment and, with data on year class strength, assist in the estimation of recruitment to the fishery.

A condition index or group of indices is also necessary as a means of comparison of the growth status between populations and within populations between years. Such information provides a means of validation of growth data derived from tag-recapture studies. Analyses of condition also provide clues as to sources of variation in the growth of lobsters.

The moult staging research described here may provide both the cheapest way of establishing moult periodicity, essential for the population model, and a necessary check on tagging data. If a lobster is captured, tagged, measured, moult staged and appears to have grown when recaptured, comparison of moult stages for the two occasions would provide a means of validating growth. Knowing where a lobster is in the moult cycle may also reduce the variability within length:weight relationships derived for the fishery as weight increases from postmoult through to premoult.

Moult-staging techniques also have utility in the processing of rock lobsters, particularly in the reduction of mortality of lobsters in high-density storage. Much of the mortality under such conditions occurs as a result of stress or cannibalism at, or immediately after, moulting. If premoult animals were identified and excluded from long-term storage or export such losses would be reduced. The 'rejected' lobsters could be processed for local markets immediately.

Objective 1 was achieved. A moult staging technique is described and estimates presented for moult stage and moult cycle length in juvenile lobsters. Note that the techniques developed in this study have already been used in the study of rock lobster dynamics (Project 93\087) and field condition (96/160).

The moult cycle can be divided into a total of 12 stages or substages, of which 10 are relatively easy to distinguish using a combination of cephalothorax rigidity, pleopod epidermis retraction and setal formation. The stages are similar to those to those described for other lobsters and crustaceans with interspecific differences including the length of D_0 (apolysis) and the timing of barbule appearance. Postmoult accounted for 6 to 8% of the cycle, intermoult for 64 to 67% and premoult for 26 to 27%. A flow chart is presented to assist with moult stage assessment and the application of the technique to field studies is discussed.

Objective 2 was achieved as far as possible. It is suggested that the establishment of a better correlation between juvenile growth rate and the RNA:DNA ratio awaits the development of a more complete diet. The correlation between the biochemical components and biomass accumulation was established. The components were lipid, protein and the nucleic acids, RNA and DNA and these were compared with variation in dry weight and water content. The digestive gland was an important storage organ with the energy reserves of it and the abdomen progressively utilised as the quantity of food decreased. Protein was the principal catabolic substrate with lipid used mainly for the energetic demands of moulting. DNA was conserved during growth suggesting that cell enlargement not cell proliferation was occurring during the intermoult phase of the moult cycle. RNA concentration was correlated with protein concentration in well-fed animals.

The results of the study suggested that the digestive gland:abdomen weight ratio and organ moisture content would be useful predictors of condition. The RNA: DNA also shows some promise in this regard. Further work is being carried out at present (project 96/160) to determine the usefulness of these indicators, and other indices, in field populations of lobsters. Particular emphasis is being placed on the development of a non-lethal index or indices.

Keywords

Southern Rock Lobster, *Jasus edwardsii*, condition index, RNA:DNA ratio, lipid, protein, food availability.

1. BACKGROUND

In July 1993, a program was initiated by the South Australian Research and Development Institute (SARDI) and the South Australian Rock Lobster Research Association (SARLRA) to study the population dynamics of the Southern Rock Lobster. The study fitted with, and became part of, the tri-state strategic research direction for Southern Rock Lobster. The program is intended to provide a scientific basis for management of the fishery and reflected concerns of potential over-exploitation in the face of increasing fishing effort and fishing power. The program includes data collection and analysis at the recruit level as well as modelling of populations and catch forecasting. Requirements for such analyses include spatial and temporal data on growth, recruitment, and fecundity.

This report details the first part of a study aimed at enhancing the resolution of such data by establishing a field condition index for the Southern Rock Lobster. The work also included the establishment of a system for moult staging in this species. The condition assessment methods tested here include tissue RNA DNA ratios, lipid and protein content.

The study of condition involves an investigation of an organism's growth response to the biotic (i.e. presence or absence of food or conspecifics) and abiotic (i.e. temperature, day length) features of its habitat. Growth may be negative or positive, affect either somatic or gonadal tissues and vary with size and age. 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). The index or indices chosen are limited by the characteristics of the organism under study. For example, during growth lobsters do not retain hard parts against which changes in weight may be measured so assessment must be based on the analysis of body composition.

The above indices were selected for this project because they appear to be the most appropriate for the measurement of condition in this species. They also occur frequently in the literature and their measurement is relatively uncomplicated. Lipid and protein are among the most common parameters measured in biochemical composition and condition studies. Both are found in the digestive gland (DG) and the abdominal (AB) tissue of crustaceans (i.e. Barclay *et al.*. 1983, Trendall and Prescott, 1989) where lipid provides metabolic energy and protein may have both structural and metabolic functions (Barclay *et al...* 1983, Chang and O'Connor, 1983, Musgrove, 1994) depending on species. The indices have also been shown to vary with the nutritional state of lobsters (i.e. Dall, 1974*;* Anger *et. al.,* 1985; Trendall and Prescott, 1989), prawns (Barclay *et al...* 1983), crabs (Vinagre and Silva, 1992) and amphipods (Lehtonen, 1994). Lipid and protein levels also vary with moult stage (Nicol *et al..*, 1992) and with degree of sexual maturation (Mourente and Rodriguez, 1991). Nucleic acids have also been used to assess condition of a wide variety of organisms including fish (i.e. Bulow, 1970, 1987; Buckley, 1979, Wright and Martin, 1985, Suthers, 1991, Malloy and Targett, 1994), crabs (Wang and Stickle, 1988), cuttlefish (Clarke, *et al..*, 1989), post-larval lobsters (Cobb *et al..* 1991, Juinio *et al..*, 1992), oysters (Wright and Hetzel, 1985), prawns (Moss, 1994) and scallops (Robbins *et al..*, 1990). The use of the

relationship between RNA (ribonucleic acid) and DNA (Deoxyribonucleic acid) is based on the assumption that the DNA content of a cell is relatively constant whereas that of RNA will vary with the degree of protein synthesis. Thus the RNA:DNA ratio provides an index of recent (Bulow, 1987) or instantaneous (Clarke *et al..*, 1989) growth. It is assumed that most of the increase in protein synthesis with growth is the result of an increase in ribosomal RNA as opposed to transfer RNA or messenger RNA (Clarke *et al..*, 1989, Miglavs and Jobling, 1989). It is also considered important to measure both total RNA and DNA as well as the ratio as the latter gives no idea of which nucleic acid is varying (Suthers, 1991).

2. NEED

At present, recruitment to the fishery and population growth are assessed using catch per unit effort (CPUE) data, puerulus settlement rates and tag-recapture methods. Assessment of the condition of lobsters can complement this information by providing information on sources of variation in growth and recruitment. For example, 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 has lead to a 50% downturn in catches in recent seasons. Assessment of biochemical condition of lobsters before a moult may allow estimation of moult increment and, with data on year class strength, assist in estimation of recruitment to the fishery.

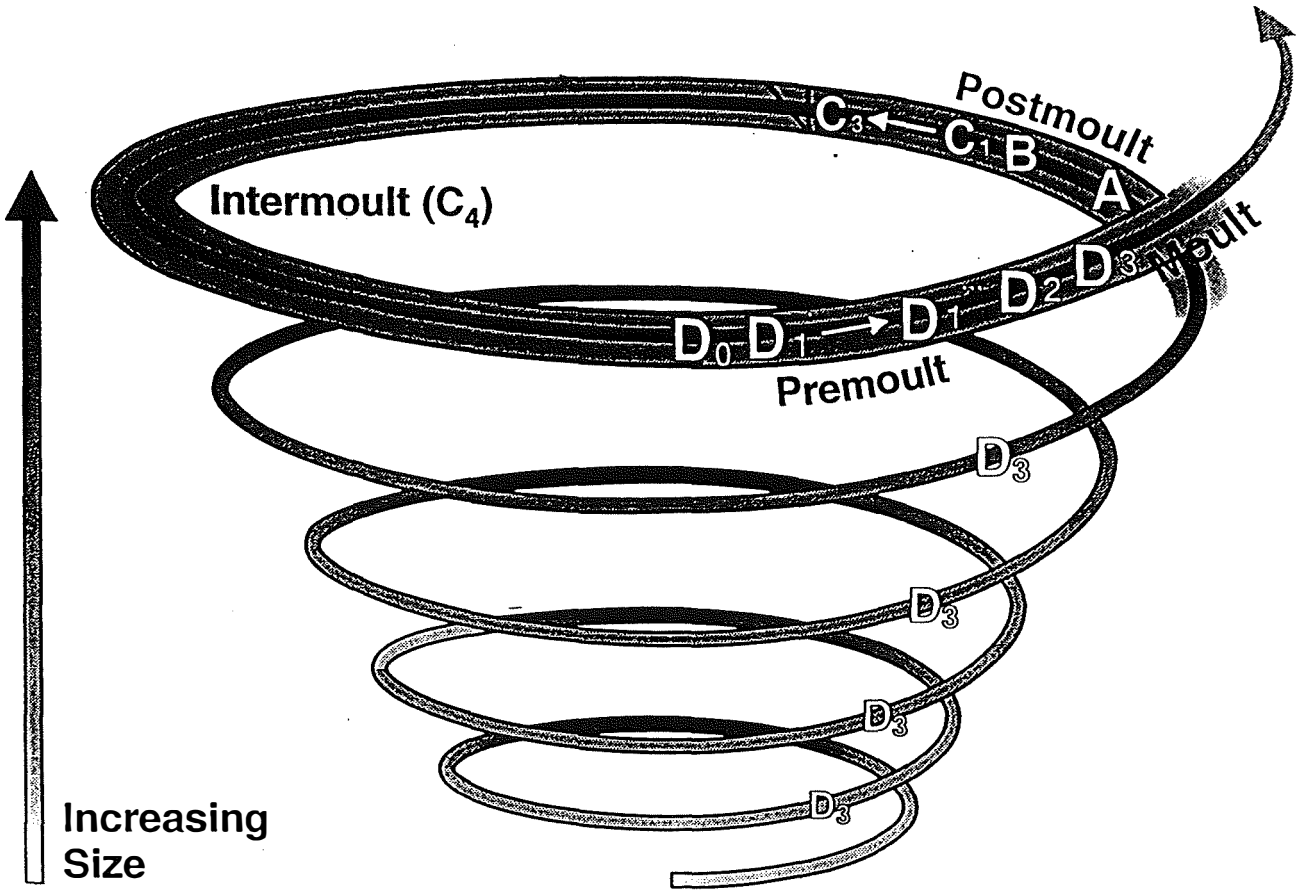
A condition index or group of indices is also necessary as a means of comparison between populations and within populations between years. Such information would provide an alternative to, and a means of validation of, growth data gathered by tag-recapture methods. Finally, such analyses would improve the resolution of population growth models.

The study of moult staging in rock lobsters is an essential adjunct to studies of growth. The moult cycle in crustaceans is broadly separated into 5 stages (Fig.1) : postmoult, intermoult, premoult, late premoult and ecdysis (Aiken, 1973, Aiken, 1980, Aiken and Waddy, 1987). Note that the diameter of each loop of the spiral in Fig 1 increases with size, representing an increase in the length of each successive intermoult period as the lobster grows. The spacing of the moult stages around each loop approximates their length (refer Section 5.2).

Specific stages are devoted to tissue growth, reserve accumulation, ovarian development and the manufacture of the new exoskeleton. This means that the occurrence of an event of interest during the cycle will define the sampling period. For example, most growth, ovarian tissue accumulation, and egg extrusion in lobsters occurs during intermoult/early premoult (Aiken, 1980, Juinio *et al..*, 1992) so it is important to be able to distinguish this stage from others when sampling for condition.

The moult staging work also provides both the cheapest way of establishing moult periodicity, essential for the population model, and a necessary check on tagging data.

Fig 1
The Moult Cycle of the Southern Rock Lobster



If a lobster is captured, tagged, measured, moult staged, and appears to have grown when recaptured, comparison of moult stages for the two occasions would provide a means of validation. The inclusion of moult-staging as a standard method would also reduce the variability within length:weight relationships derived for the fishery, as weight changes significantly within the moult cycle (Aiken, 1980, Anger, 1991, Musgrove, 1994). Finally, the introduction of moult-staging techniques to the processing industry would reduce mortality of lobsters in high density storage or when exported. Much of the mortality under such conditions occurs as a result of stress or cannibalism at, or immediately after, moulting. If premoult animals were identified and excluded from long-term storage or export such losses would be reduced. The 'rejected' lobsters could be processed for local markets immediately.

3. OBJECTIVES

1 Moult Staging Techniques. To determine the correlation between moult stage, shell rigidity and pleopod epidermal and setal development for this species. To determine the length of individual moult stages.

2 Development of a condition index or indices .To determine the correlation between selected biochemical components, growth and temperature in the laboratory.

4. METHODS

Small juvenile rock lobsters (15-17 mm Carapace Length (CL)) were collected from shallow water off the south-east coast of South Australia and the east coast of Tasmania during the summer of 1995. Adult lobsters (80 - 95 mm CL) were also collected during this period from South Australian waters using standard potting techniques.

4.1. Moult Staging Techniques

Forty-five juvenile lobsters were placed in individual flow-through plastic containers within 500 L tanks. Each container was aerated. The water temperature was maintained at 18^{o}C and salinity at 36 g L^{-1}. Lobsters were fed daily on mussel tissue (*Mytilus* sp.) and a pellet feed made from prawn heads (*Penaeus merguiensis*). The adult lobsters were placed in an aerated communal tank under the same conditions of temperature, salinity and food.

At intervals over a five month period the carapace rigidity of juveniles and adults was noted and pleopods removed for observation under 400 and 1000 times (oil immersion) with LEICA system compound and phase contrast microscopes. The degree of cuticle development and pleopod epidermal retraction and setal formation was noted. Pleopod removal intervals were determined by development rate and lobster size. Intervals varied from 1 to 2 days to a week during postmoult and just before and during premoult to between a week and a month during intermoult. Pleopod removal sequence was standardised for each animal.

4.2. Moult Stage duration.

Once a moult staging technique had been established, moult stage timing was examined. 20 Lobsters averaging 26.1 mm CL (SE = 0.47, range 16.9 - 33.8 mm) were placed in individual flow-through plastic containers within each of four 500l flow-through (1000 l/day) tanks. Two tanks were kept at 18^{o}C and two at ambient temperature[1]. Each container was aerated and provided with shelters (rocks and PVC pipes) and there was no mixing of water between the individual containers or between each tank and its containers. Lobsters were fed *ad libitum* on mussels and prawn-head pellets and acclimated to conditions for two weeks before the experiment. The experiment was run for four months to allow each animal to moult at least once. Light periodicity was at 14:10 L:D

Assessment of moult stage length was undertaken using the techniques developed above. Pleopod removal intervals were determined by development rate and lobster size. Intervals varied from 1 to 2 days during postmoult and just before and during premoult to between a week and two weeks during intermoult. Pleopod removal sequence was standardised for each animal. Data from each animal contributed only once to the assessment of the length of a given moult stage.

Changes in the length of each moult stage with size were examined with regression analysis. Tank effects on moult cycle length were tested using ANOVA. Note that size will not affect what defines a given moult stage although it will probably affect the length of each moult stage[2].

4.3 Development of a condition index or indices.

The following questions relate to the establishment of the connection between biochemical condition and growth rate. It was assumed that the intermoult stage of the moult cycle is the best time to sample the animals. Intermoult is the period of tissue growth and there is little chance of interference from the processes associated with the formation of the new exoskeleton which occur during premoult. Small juveniles (18-23mm CL) were used for the following growth experiments. Lobsters of 60-80 mm CL will be analysed for condition in project FRDC 96/160,

The questions were:

i. Do food availability or temperature have an effect on RNA, DNA, total lipid, total protein or the ratios RNA:DNA or Lipid:Protein during growth in juveniles?

ii. Is there a correlation between biochemical composition and moult increment and moult frequency?

---

[1] It was originally planned to use three constant temperature treatments but system failure prevented this.

[2] The 60-80 mm CL group were not tested as initially proposed. Further work has to be carried out on artificial diets and tank systems for larger lobsters before meaningful data can be collected on this question.

4.3.1 Experimental design

As there had been technical difficulties with the temperature control system it was decided not to include temperature as a treatment. Eight wooden tanks were constructed from 16 mm marine plywood and coated with food grade epoxy resin (Fig 2). Two hundred and eighty-eight first-moult juvenile lobsters (13.4 $\pm$ 1.2 mm CL) were randomly assigned to individual 1.2 l black plastic pots within an aerated flow-through seawater system (36 g/l). The system comprised eight tanks, each of which contained 36 pots in rows of six. Each pot was aerated and provided with shelter (rocks) and there was no mixing of water between the individual containers or between each tank and its containers. Within each tank, one of three treatments was then randomly assigned to each pair of rows such that there were 12 lobsters per treatment per tank or 96 lobsters per treatment in the experiment. The treatments comprised three levels of food availability (*ad libitum* daily (approximately 10% body weight) abbreviated to 1/1, 10 % body weight every 5th day (1/5) or 10 % body weight every 10th day (1/10). Light periodicity was 14:10 L:D, flow/pot 10l/hr and temperature 17^{o}C. A further twenty-four lobsters were kept under the same conditions in a ninth tank and used in the initial sample (Day 0 - see below) in the subsequent experiment.

Lobsters were acclimated to laboratory conditions for 2 weeks during which time they were all fed *ad libitum* daily on an artificial diet (Table 1).

Table 1 Ingredients for diet used in condition study

Ingredients	**Inclusion Level %**	**Partial Protein Content %**
prawn head meal	40.0	20.0
fish meal	40.0	26.0
semolina	16.5	1.8
B-carotene	1.0	
sodium alginate	2.0	
calcium sulphate	0.5	
Totals	100.0	47.8

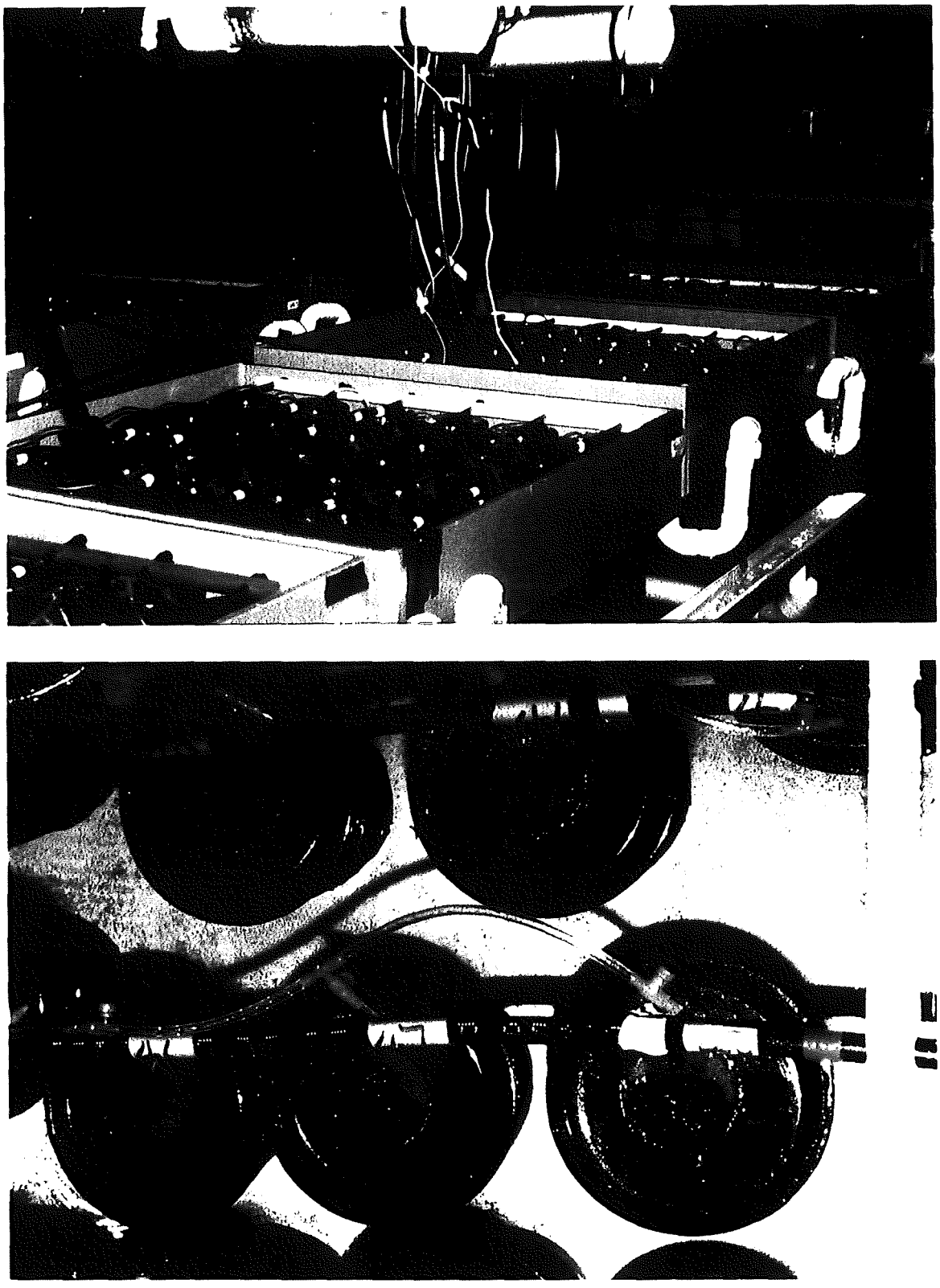


Fig 2 Tank system (top) The large white PVC pipes attached to the tanks (lower right) maintain the water level and are part of the drainage system. The PVC containers at the top of the picture are water mixers for temperature control via a building automation system (BAS). The black polythene pies descending from the mixers carry the water and the wires associated with the pipes connect the temperature gauges in each pair of tanks to the BAS. A close-up (bottom) shows several containers within a tank. Each has water (black polythene pipe) and air (clear tubing) piped in. Water flows out of each container via small holes cut in the side. These holes also control the water level.

They were then measured (to 0.1 mm CL) and the experiment commenced and run for 78 days with 24 lobsters randomly harvested from each treatment on days 0 (Day 1), 23, 43, 63 and 78. At each harvest, small groups of lobsters were collected, moult staged and those which were at intermoult processed further. Those at post- or premoult were returned to their pots. This was continued until the required 72 lobsters had been harvested. These were then weighed (0.1 mg wet weight), measured and randomly assigned to either nucleic acid or lipid and protein analysis. If to be used for nucleic acid analysis, abdominal (AB) tissue was removed, weighed and snap-frozen in liquid nitrogen. Otherwise both AB and digestive gland (DG) tissue was weighed and snap-frozen. All tissue was freeze-dried and stored in desiccators at - 30°C until analysed. Note that hindguts were carefully removed from the AB tissue before freezing.

Other Data collection:
Growth rates were calculated as Dry Weight AB tissue (mg) /day. The relationships between CL and wet and dry tissue weights were also derived for comparison between treatments.

4.3.2. Biochemical Analysis:

4.3.2.1 Nucleic Acids and RNA:DNA

Nucleic acids were assayed with a modification of the two-dye fluorometric method used by Lemmens (1995). RNA and DNA were assayed using the fluophors Ethidium Bromide and Bizbenzimidazole (Calbiochem) as follows. Note that all homogenates and extracts were kept on ice during the process unless otherwise stated.

Between 30 and 50 mg of freeze-dried abdominal tissue was homogenised (motor driven Polytron) for 5 minutes in 2 ml of ice cold Tris buffer (0.05 M Trizma base, 0.1 M NaCl, 0.01 M EDTA, pH 9, 0.2 mg/ml proteinase-K) on ice.

↓

An 800 µl subsample was transfered to an Eppendorf tube. 100 µl proteinase-K solution (0.2 mg proteinase-K ml^{-1} Tris buffer) and 100 µl of SDS solution (20% in Tris buffer) was added. The mixture was then incubated for 15min at room temperature while stirring on a Vortex mixer.

↓

The mixture was centrifuged for 15 min in an Eppendorf centrifuge at 12000 rpm

↓

Duplicate 300 µl subsamples were transfered to Eppendorf tubes

↓

A1: 300 µl of 80% Phenol and 300 µl chloroform/isoamylalcohol (24:1, v/v) was added and the mixture stirred for 10 min on a Vortex mixer at room temperature.

↓

A2: The mixture was centrifuged for 10 min in an Eppendorf centrifuge at 12000 rpm

↓

A3: The phenol-chloroform/isoamylalcohol phase (lower phase) was removed with a micropipette

↓

Steps **A1** to **A3** were repeated with the supernatant, containing the nucleic acids

↓

B1: 300 µl chloroform/isoamylalcohol was added to the supernatant and the mixture stirred for 5 min on a Vortex mixer at room temperature.

↓

B2: The mixture was centrifuged for 5min at at 12000 rpm

↓

B3: The chloroform/isoamylalcohol phase (lower phase) was removed.

↓

Steps **B1** to **B3** were repeated with the supernatant, containing the nucleic acids

↓

The supernatant was transfered to a new Eppendorf tube. This was used for the fluorometric determinations and was kept below 4°C for short term storage (< 1 hr) or at -80oC or below for longer periods.

↓

Fluorometry was performed on the extract. 100 µl of homogenate was added to 2 ml of both fluorophors (bisbenzimidazole (BBZ) and Ethidium Bromide (EB)). Working dye concentrations were 0.002 mg/ml (Stock: 2 mg/ml) and 0.01 mg/ml (Stock: 10 mg/ml) for BBZ and EB respectively. Excitation and emission wavelengths were 320nm and 590nm respectively for EB and 352nm and 448nm respectively for BBZ.

Note that Clemmesen, 1989 and Lemmens, 1995 used 365nm for the EB excitation wavelength. As this was found to be close to the excitation minimum (Fig 3), the peak, at 320nm, was used instead. A similar correction was made by Clarke *et al.*. 1989 who found that the excitation peak was 305nm at pH 7.

Calculation of nucleic acid quantities and ratios followed Clarke *et al.*. (1989) as follows. Three calibration curves were constructed: EB fluorescence against DNA concentration, EB florescence against RNA concentration and BBZ fluorescence against DNA concentration (Fig 4). The BBZ curve was used to calculate the DNA concentration of a given sample. The EB fluorescence due to this DNA was calculated from the EB-DNA curve, subtracted from the total Day EB fluorescence measured and the difference assumed to be due to RNA alone. The amount of RNA in the sample was then calculated from the RNA-EB curve.

4.3.3.2 Lipid and Protein

Freeze-dried tissue was analysed for total lipid and total protein by sulphophosphovanillin (Barnes and Blackstock, 1973) and Biuret methods (Gornall, 1949) respectively. All glass and plastic ware was acid washed.

AB and DG tissue samples were finely ground using a mortar and pestle and weighed into clean plastic test tubes. They were then extracted overnight (16 hr) at 4°C in 2:1 (v/v) chloroform:methanol at a tissue:solvent ratio of 60:1 w/v for DG tissue and 30:1 w/v for AB tissue. The following day each homogenate was filtered (Whatman GF/C) and the residue washed three times with solvent. The extract and washings were combined with 0.2 vol NaCl and mixed with a Vortex mixer then the solvent removed with a rotary evaporator. The lipid was redissolved in 1 ml of solvent and 0.5 ml of that was reduced to dryness *in vacuo* in clean tubes. 0.5 ml concentrated sulphuric acid was then added to the residue, mixed and the tubes plugged with non-absorbant cotton wool. After heating for 10 min in a boiling water bath followed by rapid cooling in an ice bath, duplicate 0.1 ml aliquots of the acid digest were added to clean tubes and 2.5 ml of vanillin reagent added and mixed thoroughly. The

Fig 3 Ethidium Bromide:
Excitation and Emission spectra at
pH = 9

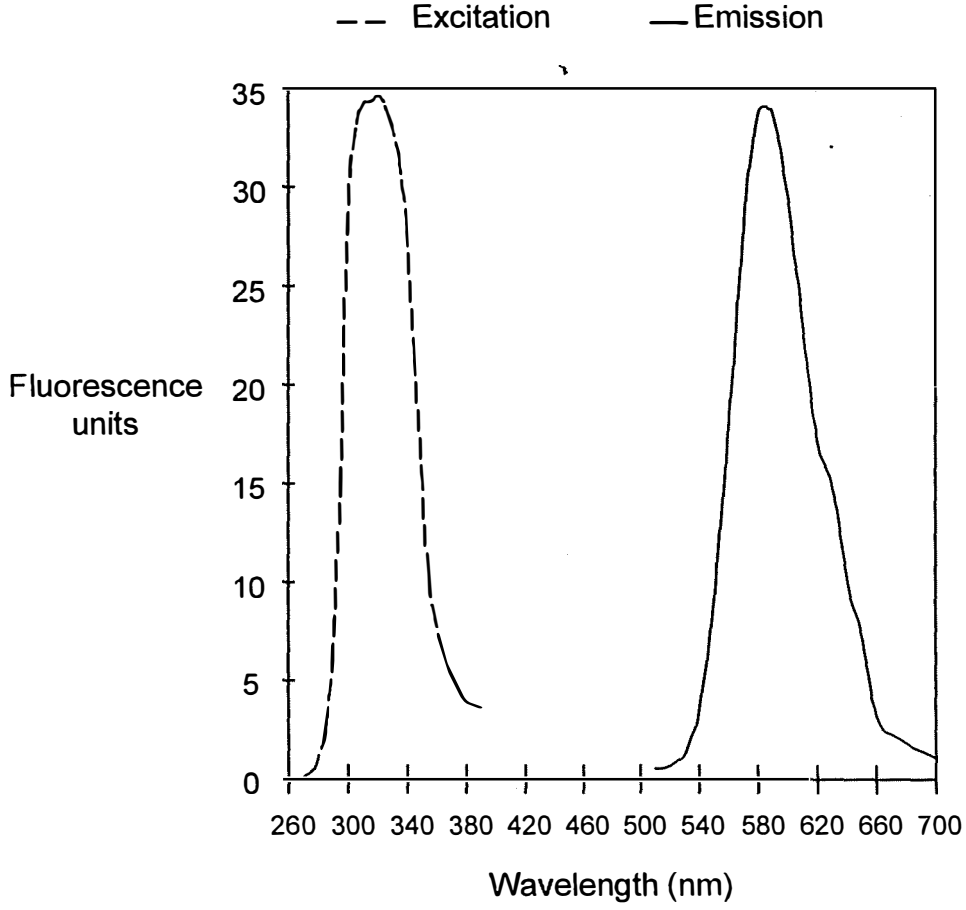
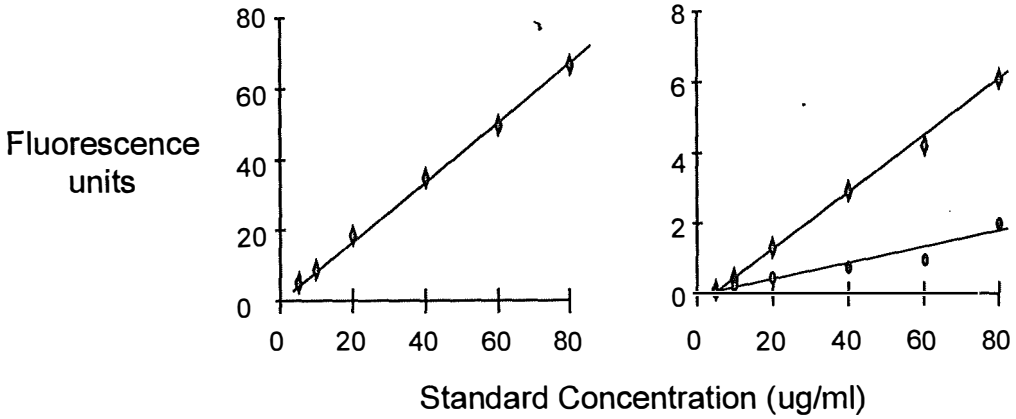


Figure 4 Typical Calibration Curves for Standards DNA (◊) and RNA (•) in Ethidium Bromide (EB) and Bisbenzimidazole (BBZ). Fluorescence vs Standard Concentration (ug/ml)



samples were then allowed to stand for 30 to 60 minutes at room temperature before optical density was measured at 520nm. Cholesterol was used as a standard. The filters containing the remaining tissue were dried (16 hr, 60^{o}C) and cooled in a vacuum desiccator then analysed for biuret protein as follows. Filters plus tissue were placed in plastic centrifuge tubes to which was added 1 ml 10% NaOH (w/v) per 15mg dry weight of tissue. The tubes were then stoppered with non-absorbent cotton wool and samples hydrolysed for 15 minutes at 80^{o}C in a water bath. Samples were then made to volume with a final concentration of 6% NaOH and centrifuged at 5,000 rpm for 15 minutes. Duplicate 1ml aliquots of the supernatant were then added to clean tubes and 4 ml of Biuret reagent added. After mixing thoroughly with a Vortex mixer the samples were left to stand at room temperature (20 to 25oC) for at least 40 minutes before optical density was read at 540 nm. Bovine serum albumin (fraction 5) (Sigma) was used as a standard.

4.3.4 Data Manipulation and Analysis:

Moult increment and moult frequency were measured and growth rates calculated. ANOVA and ANCOVA were used where appropriate. The effect of size on biochemical composition was also considered.

5. RESULTS AND DISCUSSION

5.1. Moult Staging Techniques. To determine the correlation between moult stage, shell rigidity and pleopod epidermal and setal development for this species. To determine the length of individual moult stages.

Moult staging techniques were developed as proposed and the length of individual moult stages was assessed for small juvenile lobsters.

The moult cycle in *Jasus edwardsii* can be divided into a total of 12 stages or substages, of which 10 are relatively easy to distinguish using the techniques described above. The form of each stage does not vary with size so the following is based on observations from all lobsters collected.

Postmoult.
Stage A (Fig 5) was distinguishable by the extreme flaccidity of the exoskeleton and the corresponding absence of cuticular (c) thickening in the pleopods (p) and the setae (s). The setal lumen was wide and contains a granular matrix. The setal bases were poorly developed, showing little separation from the granular interior of the pleopod itself. By Stage B (Fig 6) the exoskeleton had become parchment-like, the setal walls had become thicker and the base of the setae more distinct. Although it has been reported that Stage B ends when the pre-exuvial layers are fully formed (Aiken and Waddy, 1987) this was not readily discernible using light microscopy so stages B and C_1 cannot readily be separated using the pleopod method.

Intermoult

Stages B/C_1 and C_2 may be separated using Aiken's (1980, Fig. 4) method which defined these moult stages in *Homarus americanus* on the basis of the hardness of the carapace exoskeleton. He suggested that if the anteriodorsal region of the carapace could be depressed by light finger pressure then the lobster was in B or C_1. The lobster was in stage C_2 if that area was rigid but the dorsolateral part of the posterior carapace soft, in stage C_3 if only the ventrolateral part of the carapace could be depressed and Stage C_4 or D if all of the areas were hard. In terms of the pleopods, Stage C, and especially C_4 (Figs 7a and b) was visible as increased cuticular thickening. This continues to the extent that the setal lumen of larger individuals may be almost completely occluded.

Premoult

In *Jasus edwardsii* and *Homarus americanus* (Aiken, 1980), a change in colour of the ventral surface of the abdomen may be observed during premoult (D) and in some individuals appears to signal its onset. In *J. edwardsii* this was seen as an orange-pink colouration which darkens, often to a brown-purple, as the lobster gets closer to the moult. However, although the process is associated with the resorption of pigment from the exoskeleton is was found that absence of the initial colour did not necessarily indicate that premoult had not started.

With respect to change under the exoskeleton, premoult was initiated by the apolysis or retraction of the epidermis from the old cuticle, leaving a clear space between the two tissues. As retraction occurred, new setae began to form, signalling D_1' (Fig 8), so D_o is very short in this species, particularly in small juveniles. During D_1' the tips of new setae (ns) were visible as erect cones of tissue projecting from the epidermis (ep) into the space between the epidermis and the cuticle. Invagination papillae (ip) were also visible around the 'bases' of the developing setae. D_1'' (Figs 9a and b) commenced with invagination (i) of the setae and continued until D_1''' (Fig 10) when barbules (bb) appeared along the setal axes. At this stage the proximal ends of the setae were not well defined. Stage D_2 (Fig 11) was indicated by the deposition of epicuticle (ep) and bifurcation (bi) of proximal ends of the setae. D_3 (Fig 12) is the final stage, beginning when the new exoskeleton develops folds (f) in preparation for expansion after the moult. The carapace also softens at the beginning of this stage and will give way slightly ("crunch") if light pressure is applied. No further changes were visible in the pleopods until the moult occurs. Just before the moult the ecdysial sutures at the bases of the legs open in preparation for the shedding of the exoskeleton.

Fig 5 Stage A. Juvenile. The cuticle (c) of the pleopod (p) and setae (s) is very thin and the setal bases poorly developed. Scale bar = 150 um

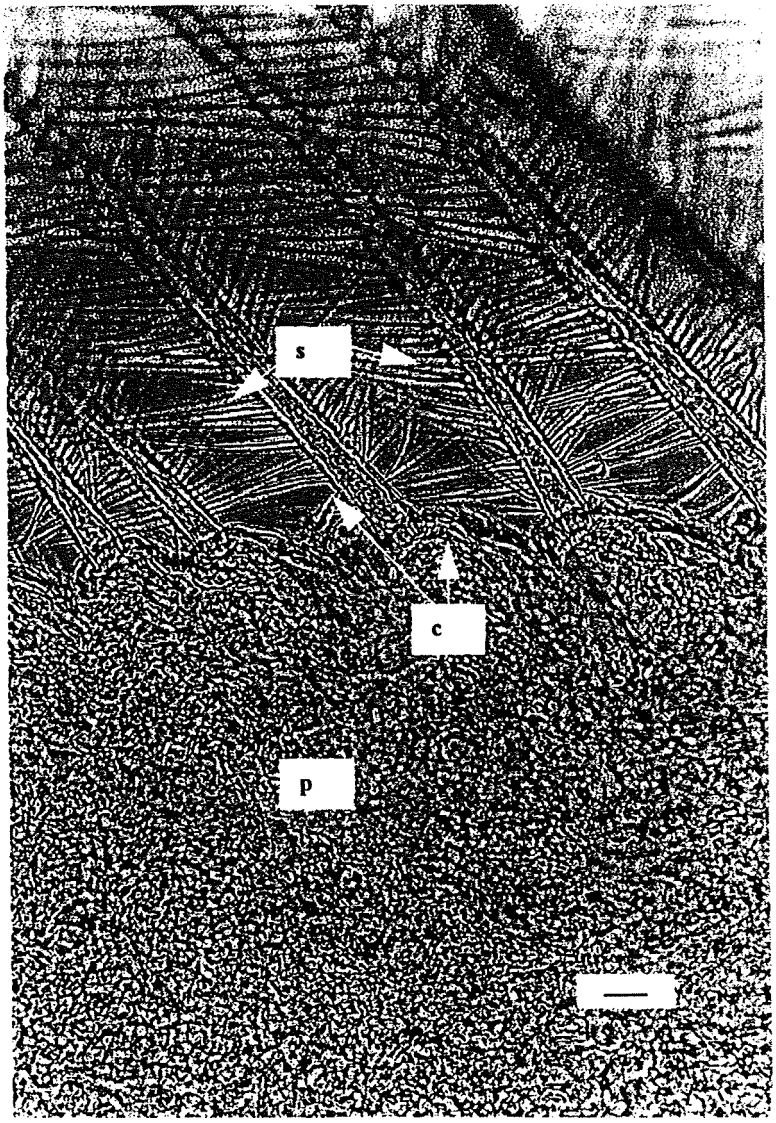


Fig 6 Stage B. Juvenile. Thickening has progressed in the pleopod and setae but the cuticle is still parchment-like. Scale bar = 150 um

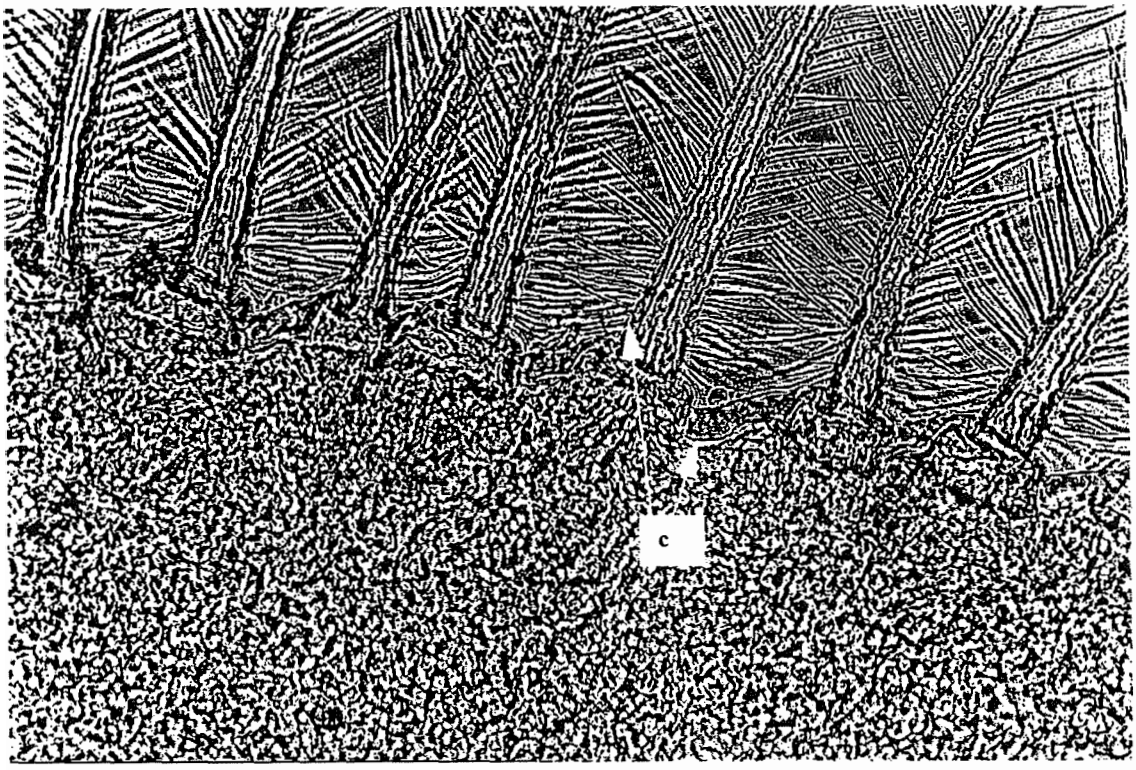


Fig 7a Stage C4. Juvenile (scale bar = 150 um). The cuticle is rigid.

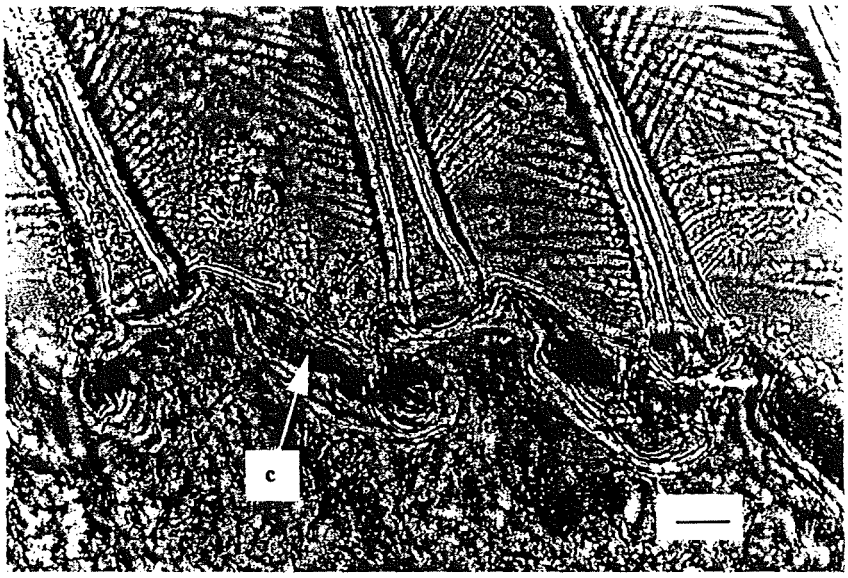


Fig 7b Stage C4. Adult (scale bar = 50 um). The cuticle is rigid.

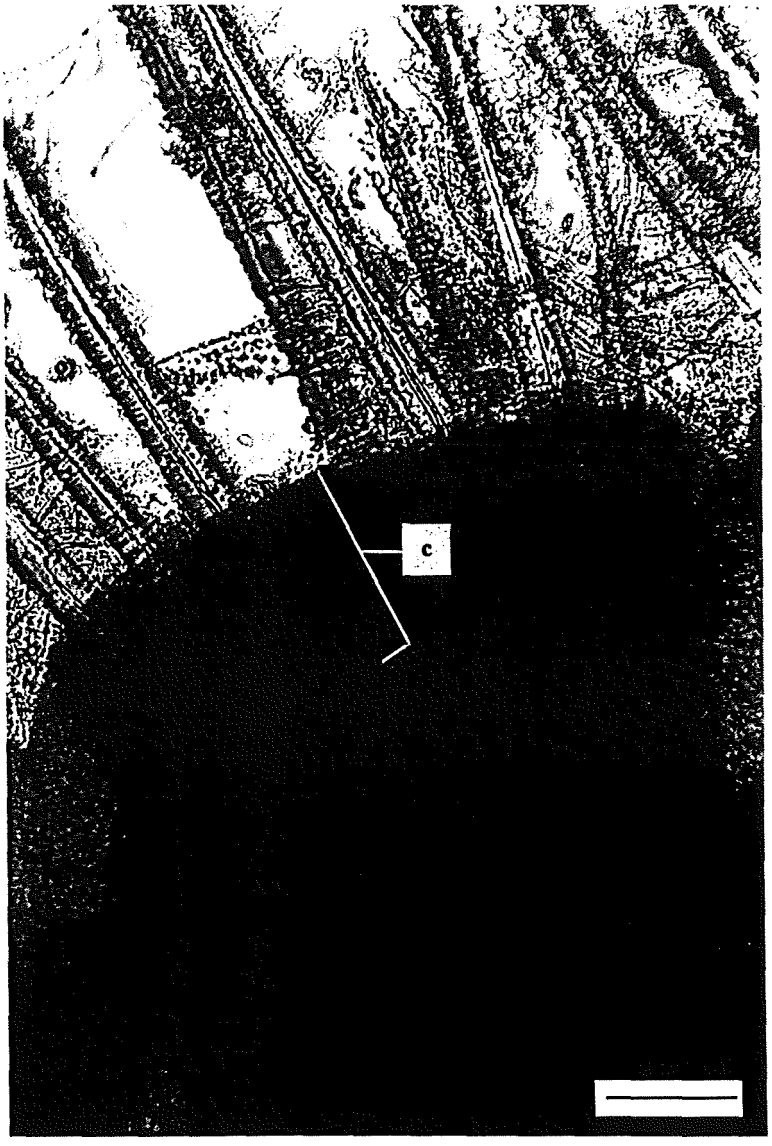


Fig 8 Stage D1'. Juvenile. Apolysis has occurred as shown by the retracted epidermis (ep). The new setae (ns) have started to develop under the old. Invagination papillae (ip) are visible around the developing setae. Scale bar = 150 um

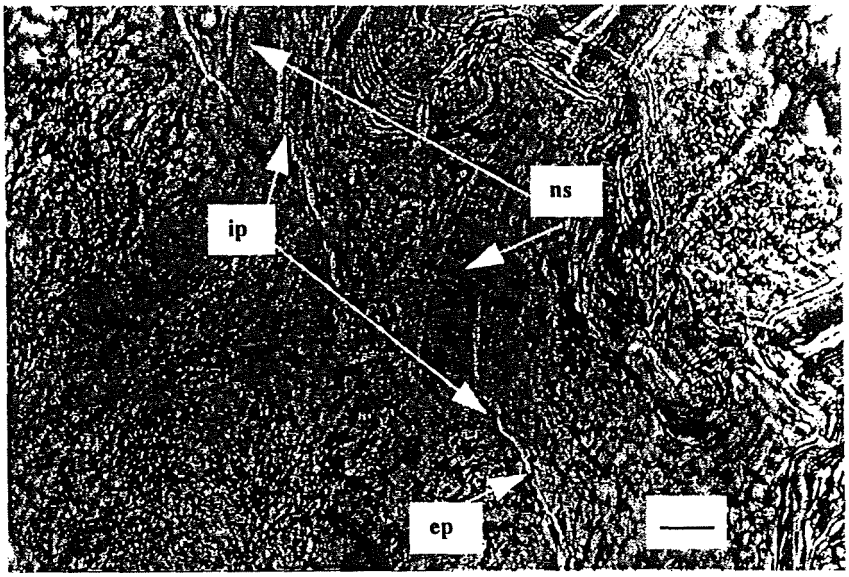


Fig 9a Stage D1'' Juvenile. (scale bar = 150 um)
Invagination (i) has begun.

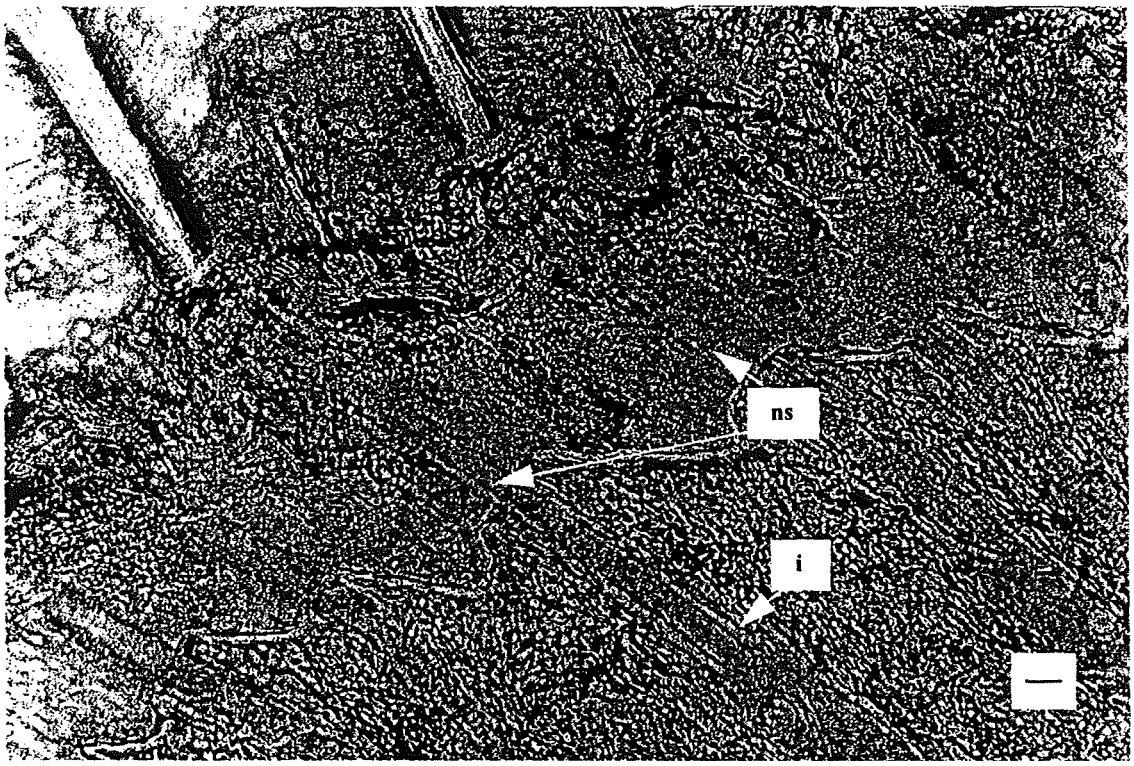


Fig 9b Stage D1'' Adult (scale bar = 50 um). Invagination (i) has begun.

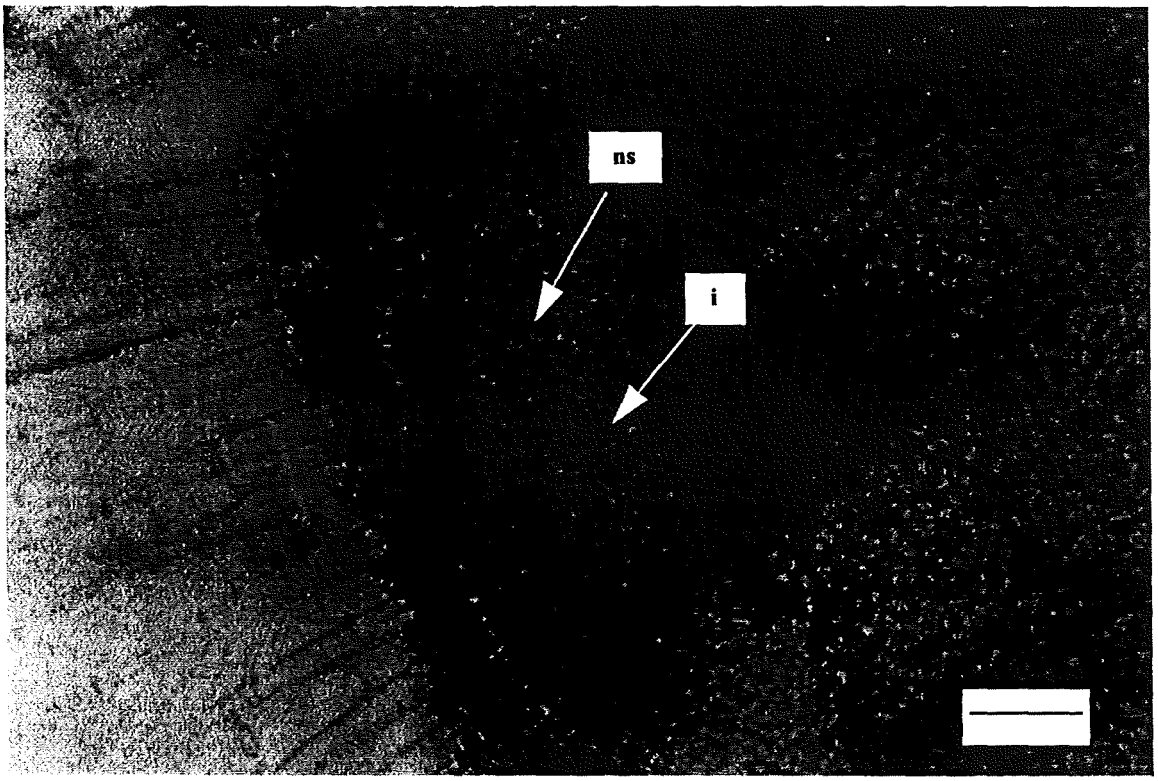


Fig 10 Stage D1''' Juvenile. Barbules (bb) have appeared along the setal axes. Scale bar = 150 um

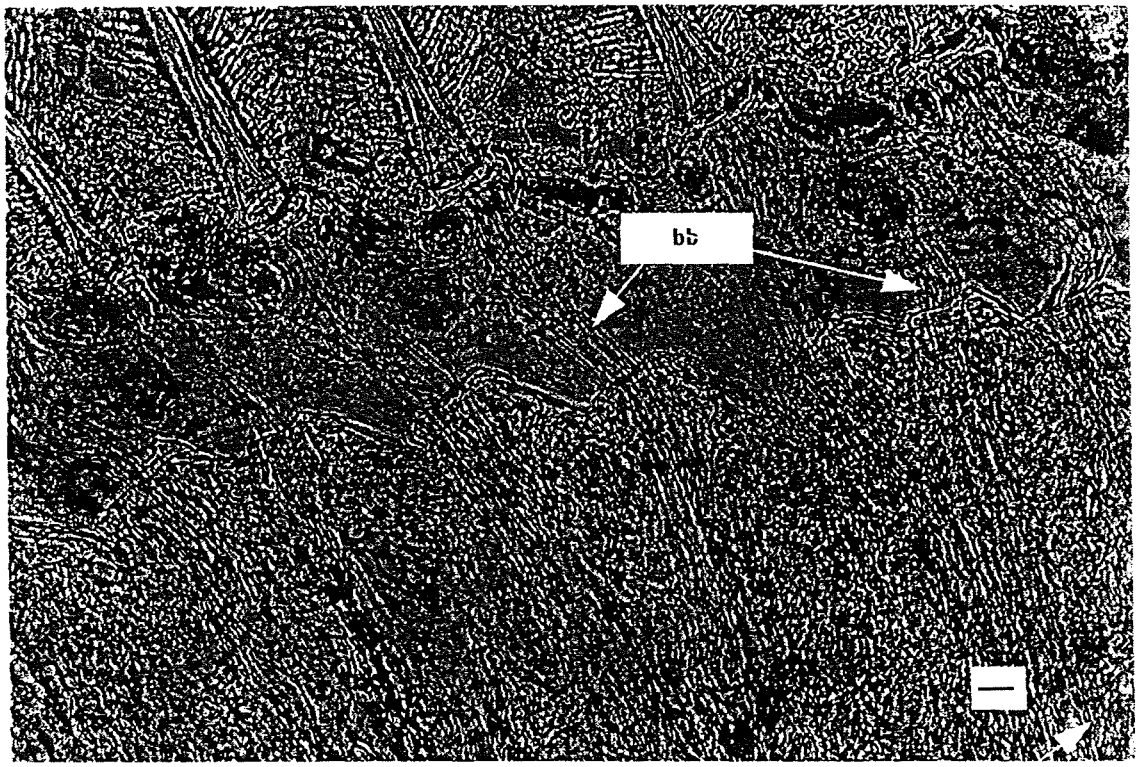


Fig 11 Stage D2. Adult. The epicuticle (ep) has begun to develop and bifurcation (bi) is visible in the proximal ends of the setae. Scale Bar = 50 um.

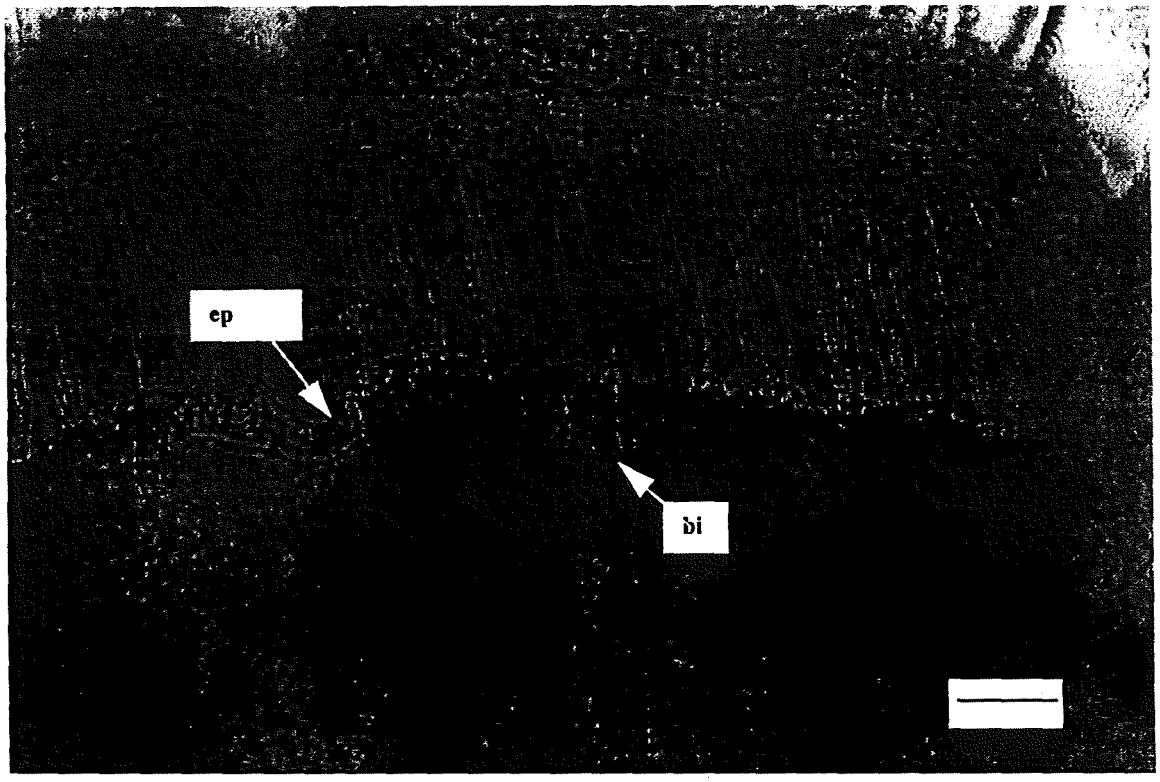
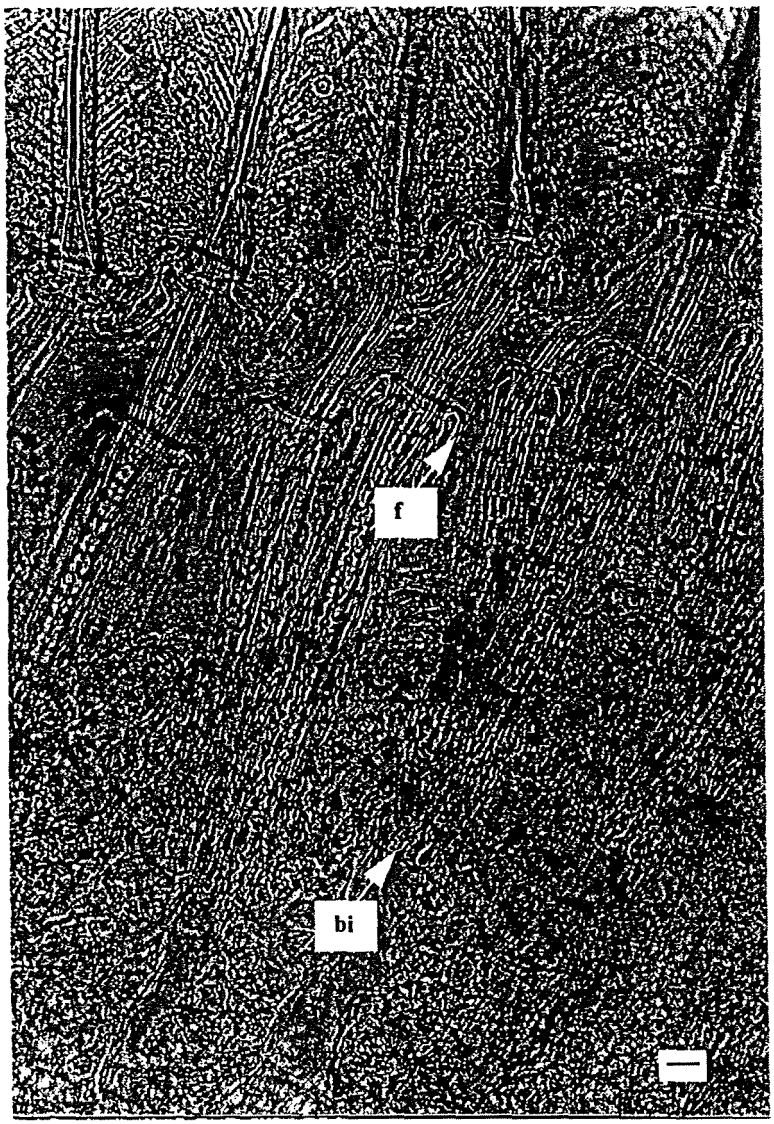


Fig 12 Stage D3. Juvenile. Folding (f) of the new exoskeleton has begun in preparation for ecdysis. Bifurcation (bi) is much clearer at this size. Scale bar = 150 um.



5.2 To determine the length of individual moult stages.

During the experiment, temperature in the ambient treatment rose from 12 to 18ºC. (mean = 16.05 $\pm$ 0.06, n = 489). The intermoult period (as distinct from the Intermoult Stage - C_4) was extremely variable. The mean intermoult period varied from 64 to 70 days (Table 2) and was independent of treatment ($P > 0.05$) and CL ($P > 0.05$) within the size range used (16.9–33.8 mm CL). The proportional contribution of each moult stage to the moult cycle was also not affected by temperature ($P>0.05$) or CL ($P>0.05$). As a result of the variability, these figures did not change significantly when data were added from lobsters which moulted only once (Table 2). In the latter case the beginning of C_4 was not precisely known.

Table 2. Moult Stage Length in Days.
Mean cycle length calculated from lobsters which had moulted twice during the experiment. Summed cycle length calculated as the sum of all observations, each individual used no more than once for a given moult stage

Temperature (ºC)	Mean Cycle Length			Summed Cycle Length	
	Mean	SE	n (animals)	Total	n (observations)
Ambient	69.9	3.6	5	71	67
18	64.2	6.4	9	68	41

Intermoult (C_4) was the longest stage, lasting between 44 and 48 days and accounting for more than sixty percent of the cycle (Table 3) This was followed by premoult at 27 to 29%, and postmoult at 6 to 8%.

Table 3. Length of each Moult Stage as % of Moult Cycle. Calculated using all observations. The length of D_o was estimated.

	Temperature (°C)	
	Ambient	**18**
Stage	**% of moult cycle**	**% of moult cycle**
A	1.4	1.5
B/C$_1$	1.4	2.2
C$_2$	2.1	2.4
C$_3$	1.6	1.5
C$_4$	67.2	64.9
(D$_O$)	$\leq$ 1.4	$\leq$ 1.5
D$_{1.1}$	9.6	9.8
D$_{1.2}$	6.6	4.0
D$_{1.3}$	5.0	9.3
D$_2$	2.3	1.5
D$_3$	1.4	1.5
	100.0	100.0
postmoult	6.5	7.6
intermoult	67.2	64.9
premoult	26.3	27.5

Moult Stage Techniques and Duration Discussion

The stages shown for *Jasus edwardsii* were similar to those described for *Homarus americanus* (Aiken, 1973) and *Panulirus ornatus* (Turnbull, 1989). However, the relative lengths of the stages differ among species. Aiken (1973) described D_o as a "broad transition zone" and Rao *et al.*. (1977) found that it occupied 50 to 70% of the moult cycle of the freshwater crayfish *Faxonella clypeata*. *Panulirus ornatus* (Turnbull, 1989) of similar size (22 to 33mm CL compared with 17- 35 mm CL) also has a relatively long D_o, its length measured in days. In contrast, this stage was extremely short for juvenile *J. edwardsii*, to the extent that it was difficult to detect. Setal development begins very shortly after apolysis and continues as the epidermis retracts. In absolute terms, this period probably increases with size as suggested by a related study (Prescott *et al.*., 1997) where, out of 6108 pleopods removed from legal-sized adult *J. edwardsii*, (>98.5 mm CL), 5877 were at C_4, 14 at D_o and 183 at other premoult stages up to D_1''' (Table 4). Stage A and D_2 or D_3 lobsters were not caught, presumably because they are non-feeding stages. The study was carried out over six months from October 1995 to March 1996, which includes the peak summer moult.

Table 4 Moult Stages of a sample of lobsters caught in commercial pots between October 1995 to March 1996. The moult staging method was as described above. Data: Prescott *et al.*, 1997.

Moult Stage	Number
C_3	34
C_4	5877
D_o	14
D_1'	164
D_1''	17
D_1'''	2

In common with the laboratory work this data suggests that D_o is a very short stage and C_4 the longest. The field data also suggests that premoult is proportionally longer than postmoult, in common with the laboratory study Fielder (1964) reported a similar result with captive *J. lalandei* (now *J. edwardsii*), lobsters ranging in size from 50 to 80 mm CL. He found that intermoult lasted from 73 to 287 days (between 78 and 91% of the cycle), generally increasing with size. In approximate percentage terms, postmoult and premoult decreased with size from 6% to 1.9% and 15% to 4.5% respectively.

Setal development is early in contrast to that described in *P. ornatus* as Turnbull (1989) reported it begining once the epidermis was fully retracted, a feature the species shares with H. americanus (Aiken, 1973). *J. edwardsii*'s early setal development is, however, similar to that found in other decapods (Stevenson., 1968, Mills and Lake, 1975 and Smith and Dall, 1985). Finally, barbules appear on the new setae of *J. edwardsii* before deposition of the epicuticle - in stage D_1'''. This feature is also common to *Panulirus ornatus* (Turnbull, 1989) and various stomatopods (Reaka, 1975) but not to *Homarus americanus* (Aiken, 1973), *P. marginatus* (Lyle and MacDonald, 1983) and *Chionoectes opilio* (O'Halloran and O'Dor. 1988) where barbules are not present until D_2.

From the point of view of determination of moulting periodicity in the management of the fishery, premoult and postmoult are generally the events of interest. Superimposed upon this is catchability with respect to moulting as the range of lobsters caught in pots would not usually include the non-feeding stages A and D_3. In the field, postmoult was fairly obvious but premoult was more difficult to distinguish because pigmentation of the haemolymph, visible through the ventral surface of the abdomen, may not be synchronised with the beginning of premoult. However, the onset of premoult may be determined in the field by looking for the apolysis in the tail fan or the pleopods, usually visible with the aid of a good hand lens. In the event that this is not visible, the lobster is likely to be in late intermoult. Once the stage is established, the distal half of the appendage may be clipped, put on ice and taken back to the laboratory for more precise definition. The flow chart in Appendix 1 may be of some use in moult staging work.

5.3 Development of a condition index or indices .To determine the correlation between selected biochemical components, growth and temperature in the laboratory.

Data were tested for normality and transformed where necessary. If data could not be normalised, non-parametric methods were used. Analysis was carried out using GLM, MANOVA or Kruskal-Wallis (KW) protocols within Systat$_{(TM)}$. Examination was made of total carapace length (CL), treatment and interaction effects between treatments within Days and within treatments between Days. Significance was accepted at $\alpha < 0.05$. For clearer graphical presentation data were standardised to the mean CL (14.4 mm $\pm$ SE) for the whole experiment with standard errors calculated from the appropriate regression statistics (Zar, 1984). Regression statistics and means and their standard errors are given in Appendix 1.

Growth rate:

Growth rate was calculated as mg dry weight (dwt) AB tissue per day ($\pm$ SE) and varied between 0.055 and 0.654 mg dwt/ day depending on treatment (Fig 13). It was also independent of CL ($P_{regression} > 0.05$) within the size range tested. Growth declined significantly in all treatments during the experiment and was significantly greater in the *ad libitum* treatment than in the 1 day in 10 (1/10) treatment by Day 43 (P=0.011). By Day 78, all treatments were significantly different from each other. As mean moult increment (Table 5a) was similar in all treatments, growth rate differences were due to changes in intermoult period. This was especially apparent after Day 23 as significantly more lobsters had moulted for a second time in the *ad libitum* treatment (1/1) than in the other two treatments (X^2, $P < 0.001$) (Table 5b)

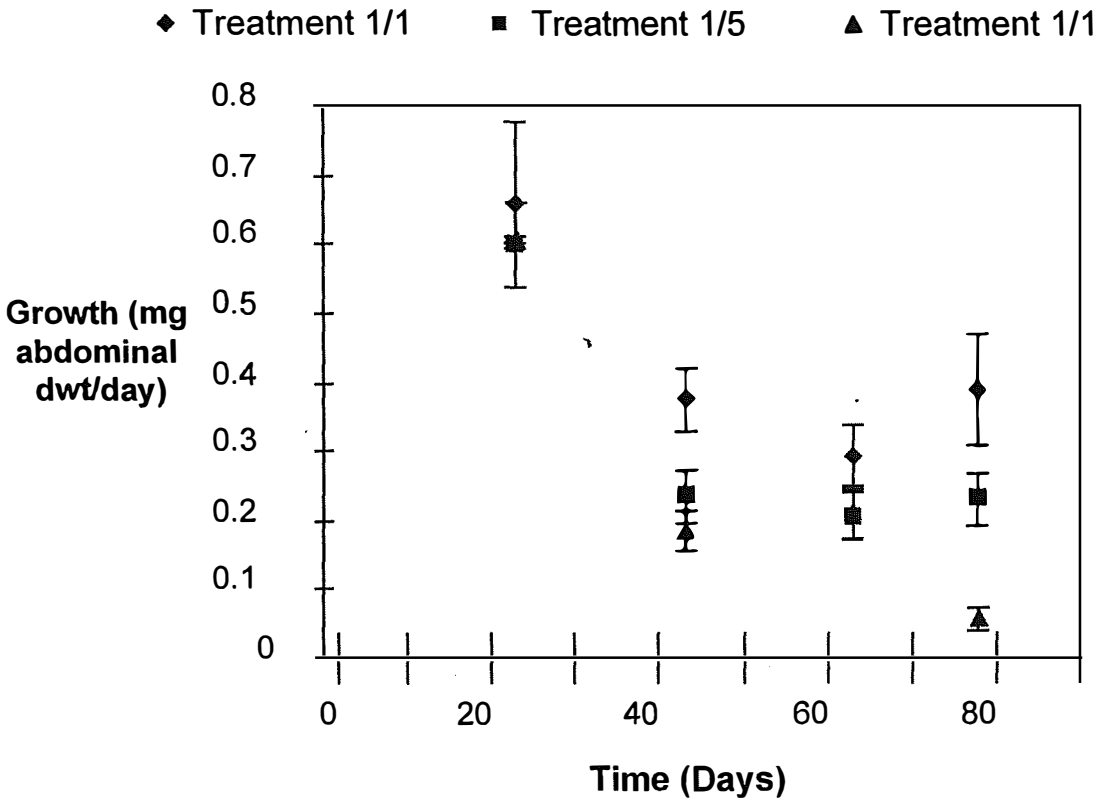
Table 5a Mean moult increment ($\pm$ SE) and number of lobsters moulting in each of two moults

Treatment	Mean Moult Increment (SE)	number 1st molt	number 2nd molt
1/1	1.3 (0.10)	71	21
1/5	1.1 (0.05)	61	7
1/10	1.1 (0.06)	64	1

Table 5b Number of lobsters moulting between sampling days within each treatment

		1st moult			2nd moult			Number moulting in given period		
		Treatment			Treatment			Treatment		
		1/1	1/5	1/10	1/1	1/5	1/10	1/1	1/5	1/10
Day	<0	14	17	18	0	0	0	14	17	18
	0 to 23	43	34	42	0	0	0	43	34	42
	23 to 43	10	8	3	17	2	0	27	10	3
	43 to 63	3	2		2	2	1	5	4	1
	63 to 78	1		1	1	3	0	2	3	1
	Total	*71*	*61*	*64*	*20*	*7*	*1*	*91*	*68*	*65*

Fig 13 Growth Rate (mg abdominal dry weight/day) (±SE) vs Time (Days). Treatment 1/1 = fed *ad libitum* daily, 1/5 = fed every fifth day and 1/10 = fed every tenth day.



Dry weight:

AB and DG dry weight changed significantly during the experiment (Fig 14a and b). Significant differences are indicated by dissimilar superscripts. Those within Days are indicated by letters and those within Treatments between Days, by numbers. Animals in Treatment 1/1 were generally heavier than those in the other treatments and the greatest effects were shown by Treatment 1/10. There were also interaction effects at days 23 and 78, indicating a difference in slope of the compared CL:weight regressions. In both cases this was due to a reduction in b within treatment 1/10. Maximum AB weight was achieved by Day 23 in all treatments.

DG weight fluctuations were also treatment-dependent and showed a reduction during the experiment (Fig 14b). Treatments 1/1 and 1/5 were generally similar, with 1/10 again showing the greatest effect. Combining the tissue weight data (Fig 15) clearly shows the relative drop in DG weight by Day 23, although the significant fall in H:A for Treatment 1/1 was due to a rise in AB weight, not a fall in DG weight as in the other two treatments.

Water:

In absolute terms fluctuations in water content with treatment and Day were the same as those already reported for dry weight (above). However, during the experiment percent tissue water content rose, the rate of increase depending upon treatment and tissue (Fig 16). Also % water in both tissues in Treatment 1/10 remained significantly higher than that of the other treatments throughout most of the experiment (Abdomen: $P \leq 0.013$, DG: $P \leq 0.034$) although there was no further change in Treatment 1/10 after Day 23.

Protein:

AB protein content (Fig 17a) increased with time in Treatments 1/1 and 1/5, peaking at Day 43. At this point both were significantly higher than that of Treatment 1/10 ($P<0.001$) which did not change significantly during the experiment. There was a significant initial drop in % Protein in all treatments ($P<0.001$) and Treatments 1/1 and 1/5 were significantly different by Day 43 ($P=0.005$) (Fig 17b). In the DG both absolute and % protein showed a general decline ($P \leq 0.001$, Day 0 vs Day 78) (Figs 18a and b) as the experiment progressed although % protein increased to Day 43 ($p=0.044$) then fell ($P=0.002$). There were no Treatment effects in % Protein in the DG so the data were pooled.

Lipid:

There were no differences between AB lipid levels in any treatments so the data were pooled within Days and differences between Days examined. After an initial fall (Fig 19a and b), AB % lipid rose between Days 43 and 63 ($P=0.001$) as did total lipid. In the DG there was no change in the % lipid although there were two effects of note in total lipid (Fig 20a and b). Firstly, the pooled data for Day 23 showed a significant decline from Day 0. Secondly, a treatment effect was apparent by Day (78) where Treatment 1/10 was reduced in comparison to 1/1.

Fig 14a Mean Abdominal Dry Weight (mg) (±SE) for each Treatment vs Time (Days). Different letter and number superscripts refer to significant (P≥0.05) differences within and between sampling days, respectively (refer text).

◆ Treatment 1/1 ■ Treatment 1/5 ▲ Treatment 1/10

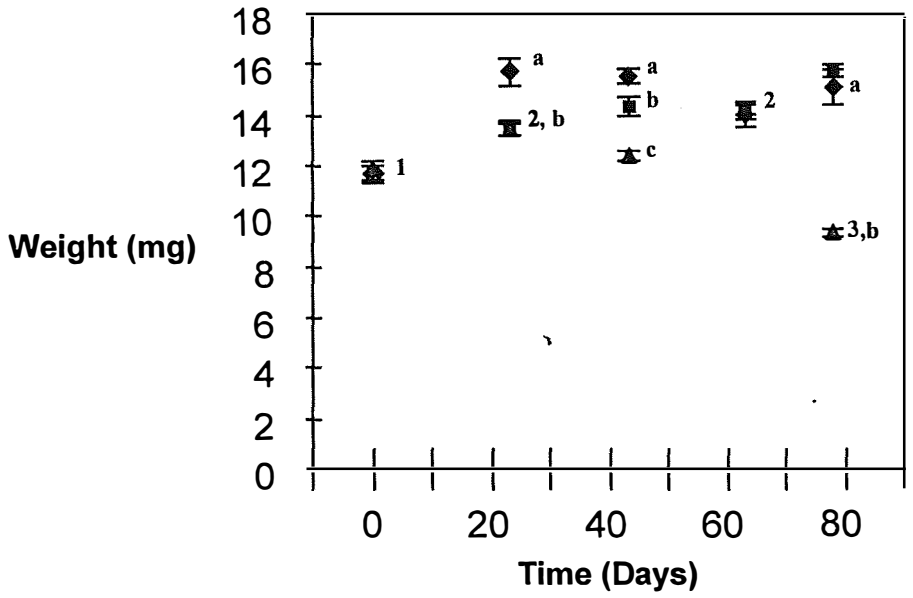


Fig 14b Mean Digestive Gland Dry Weight (mg) (±SE) for each Treatment vs Time (Days). Different letter and number superscripts refer to significant (P≥0.05) differences within and between sampling days, respectively (refer text).

◆ Treatment 1/1 ■ Treatment 1/5 ▲ Treatment 1/10

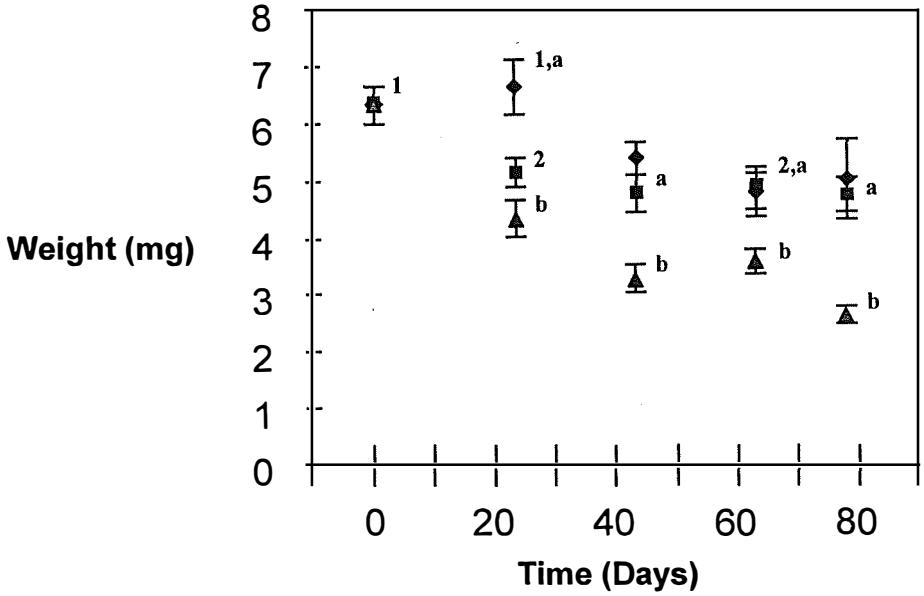


Fig 15 Mean Digestive Gland Dry Weight as a % of Abdominal Tissue dry weight (±SE) by Treatment vs Time (Days). Different number superscripts refer to significant (P≥0.05) differences between sampling days (refer text).

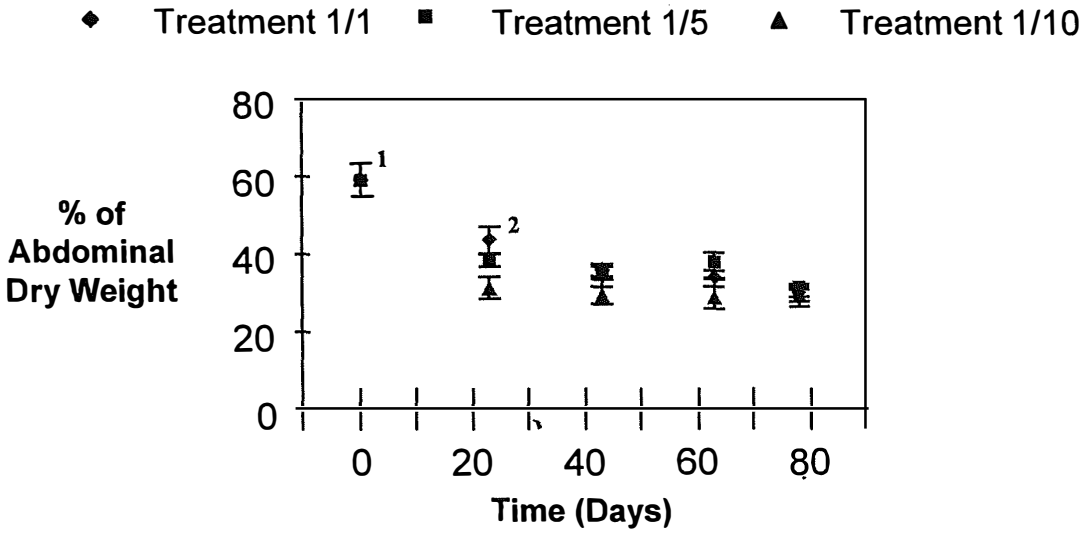


Fig 16a Mean Water Content (% wet weight) (±SE) of Abdominal Tissue by Treatment vs Time (Days). Different letter and number superscripts refer to significant (P≥0.05) differences within and between sampling days, respectively (refer text).

◆ Treatment 1/1 ■ Treatment 1/5 ▲ Treatment 1/10

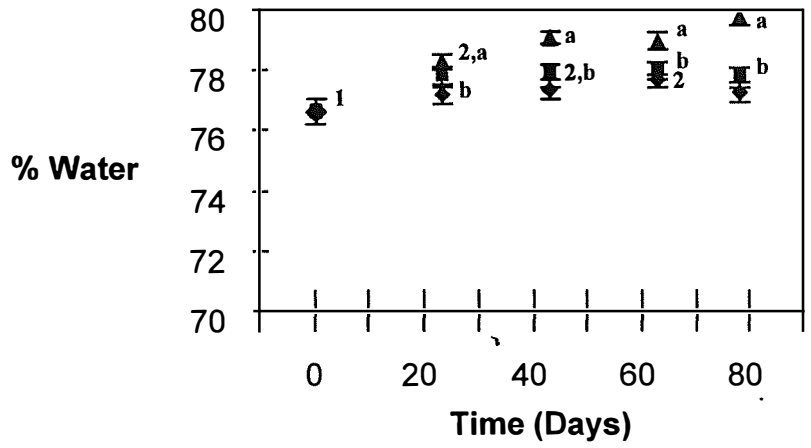


Fig 16b Mean Water Content (% wet weight) (±SE) of Digestive Gland by Treatment vs Time (Days). Different letter and number superscripts refer to significant (P≥0.05) differences within and between sampling days, respectively (refer text).

◆ Treatment 1/1 ■ Treatment 1/5 ▲ Treatment 1/10

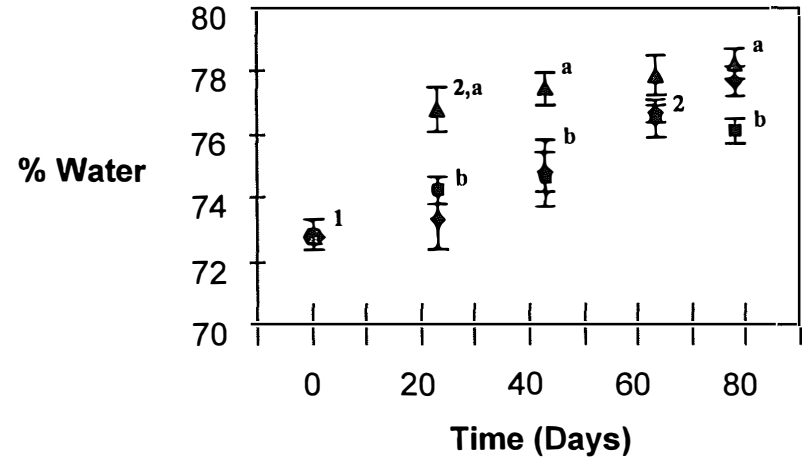


Fig 17a Mean Protein content (mg dry weight) (±SE)of Abdominal Tissue by Treatment vs Time (Days). Different letter and number superscripts refer to significant (P≥0.05) differences within and between sampling days, respectively (refer text).

◆ Treatment 1/1 ■ Treatment 1/5 ▲ Treatment 1/10

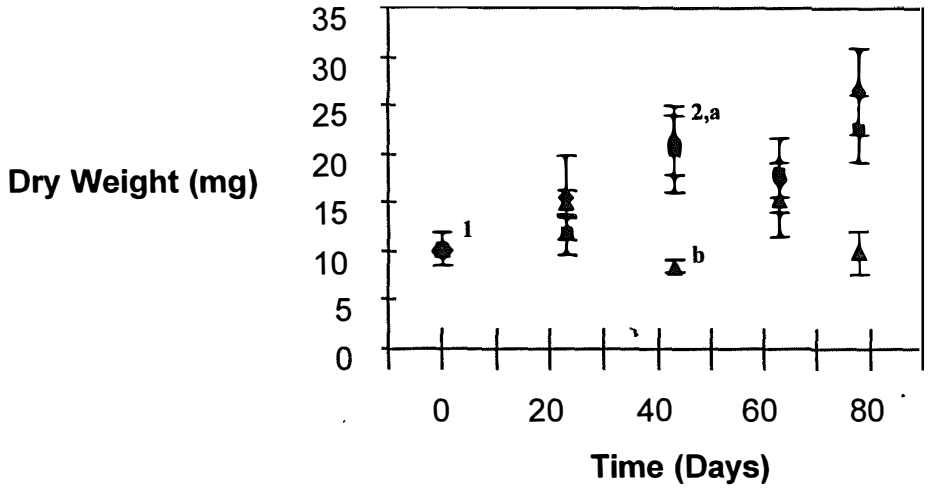


Fig 17b Mean Protein content as a % of Abdominal Tissue dry weight (±SE) by Treatment vs Time (Days). Different letter and number superscripts refer to significant (P≥0.05) differences within and between sampling days, respectively (refer text).

◆ Treatment 1/1 ■ Treatment 1/5 ▲ Treatment 1/10

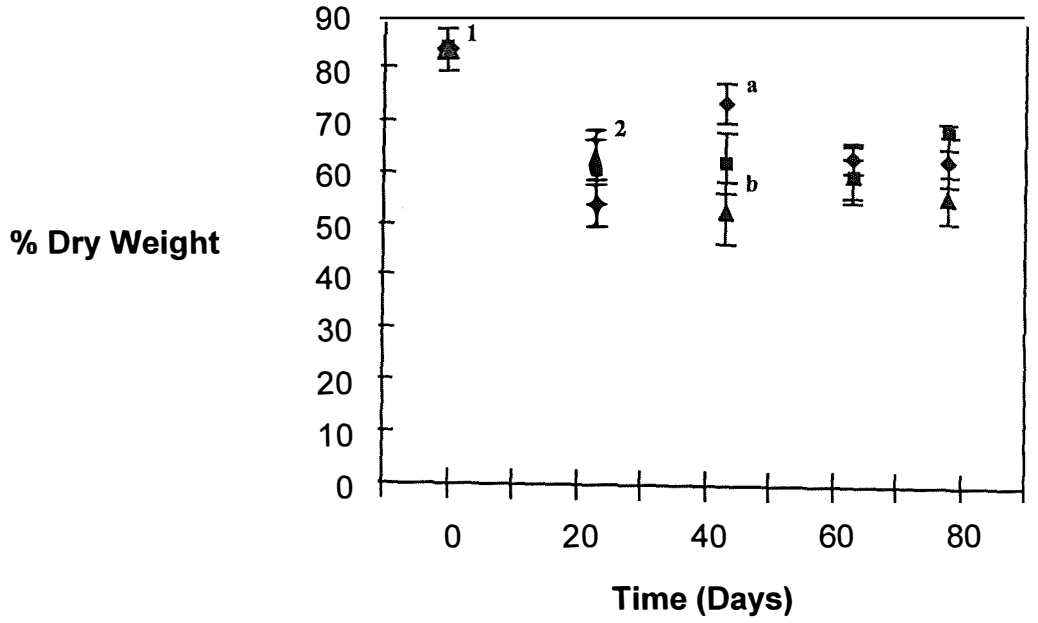


Fig 18a Mean Protein content (mg dry weight) (±SE) of Digestive Gland vs Time (Days). Treatments pooled.

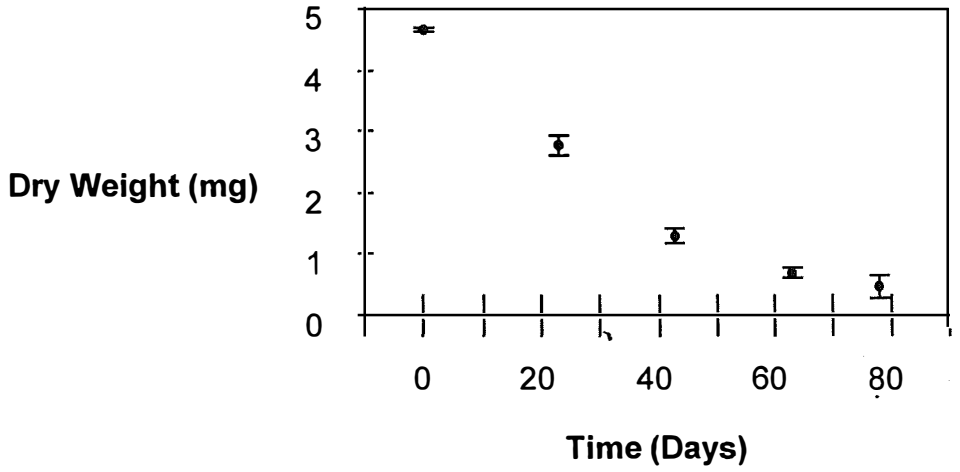


Fig 18b Mean Protein content as a % of Digestive Gland dry weight (±SE) vs Time (Days). Treatments pooled

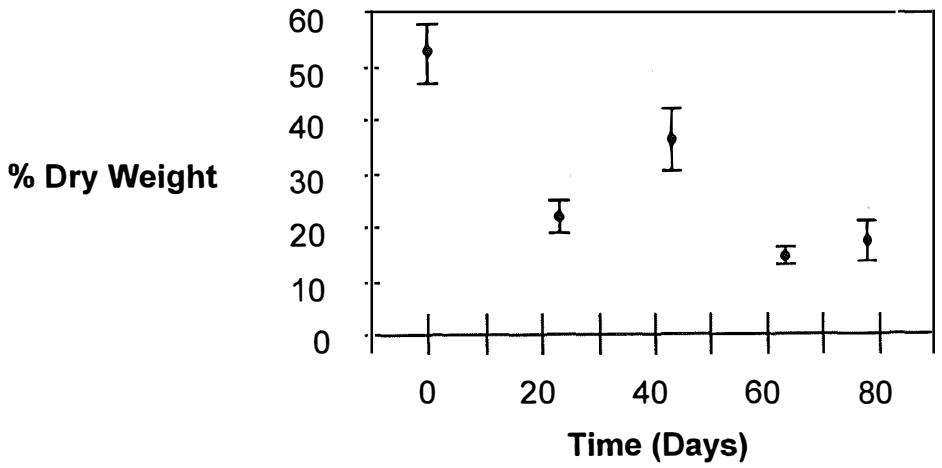


Fig 19a Mean Lipid content (mg dry weight) (±SE) of Abdominal Tissue dry weight (mg) vs Time (days). Treatments pooled.

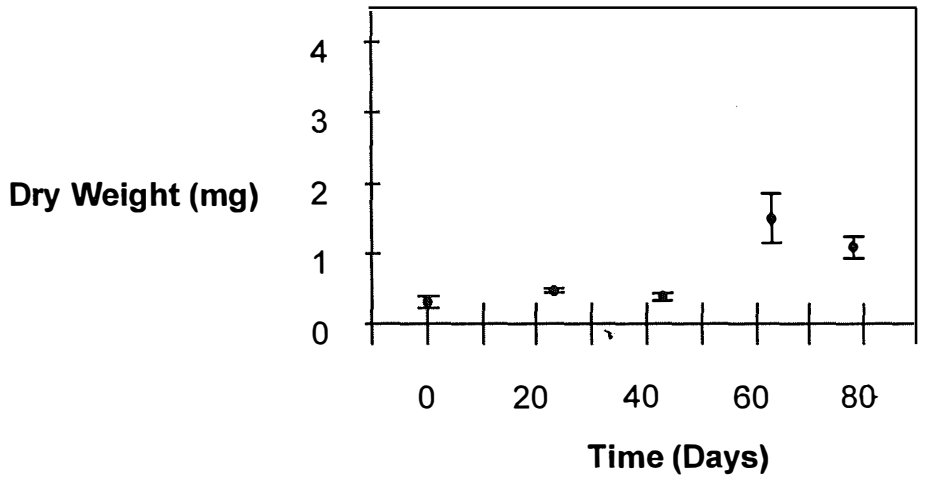


Fig 19b Mean Lipid content as a % of Abdominal Tissue dry weight (±SE) vs Time (days). Treatments pooled.

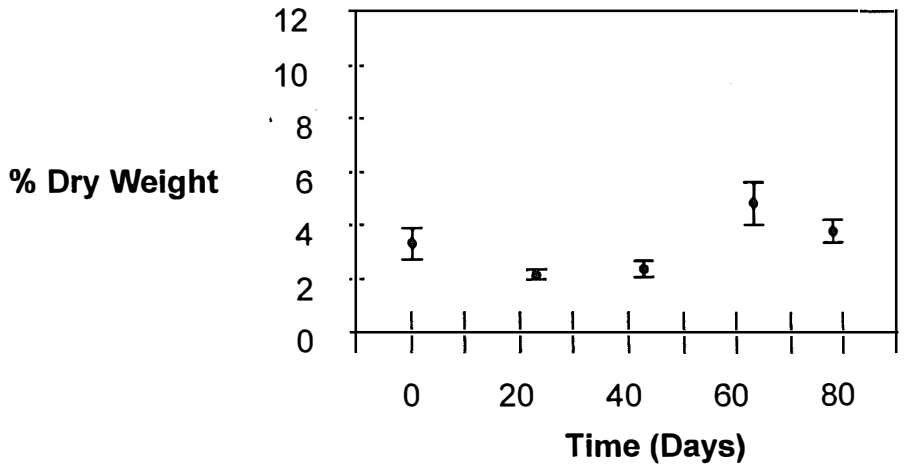


Fig 20a Mean Lipid content (mg dry weight) of Digestive Gland by Treatment (±SE) vs Time (Days).

◆ Treatment 1/1 ■ Treatment 1/5 ▲ Treatment 1/10

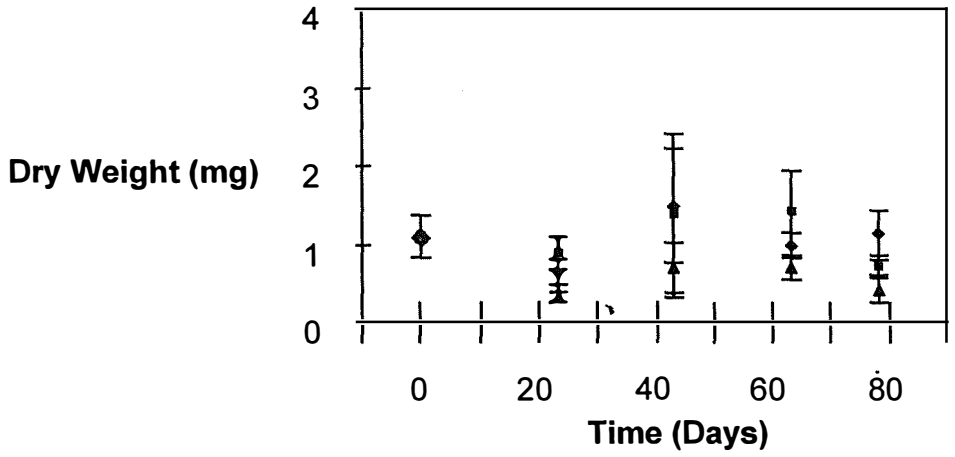
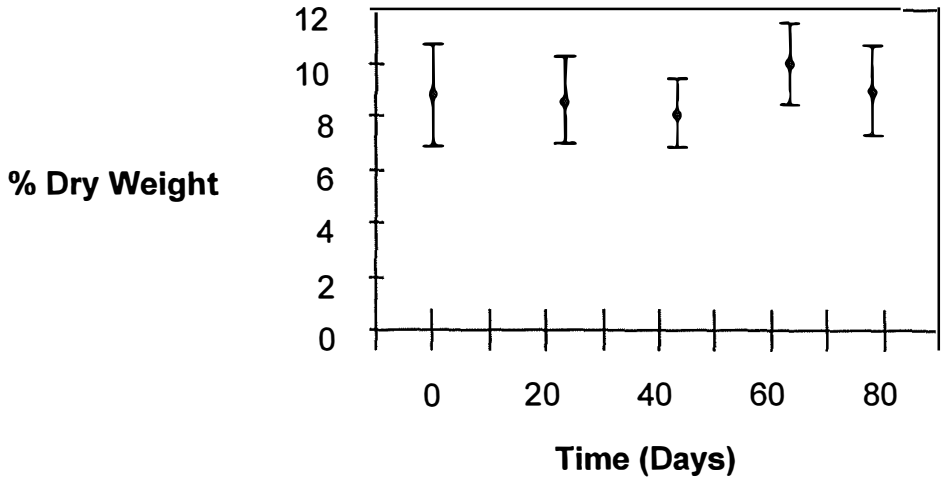


Fig 20b Mean Lipid content as a % of Digestive Gland dry weight (±SE) vs Time (Days). Treatments pooled.



Nucleic acids

There were no treatment effects in weight -specific (ug/mg dwt ABD) or total DNA or RNA ($P \geq 0.05$) so these data were pooled within Days and differences between Days examined. Apart from a significant rise between Days 0 and 23, there were no changes in total DNA during the experiment. Similar trends were observed in DNA concentration (ug/mg dwt ABD) where there was a general decline with increasing CL (Fig 21) and during the first 23 days ($P<0.001$) then no further change. Both total RNA (Fig 22) and RNA concentration (Fig 23) rose markedly from the Day 0 to Day 43 then fell to Day 63. Similar trends were shown in total protein (Fig 22) except for treatment 1/10 on Day 43. In contrast, only the protein concentration in Treatment 1/1 appeared to follow RNA concentration (Fig 23). RNA:DNA varied between 1.6 and 3.3 (Table 6) and, predictably, showed the same trend as TRNA. There was also a difference in RNA:DNA between Treatments at Day 23; the ratio RNA:DNA being higher in treatment 1/5 than in treatment 1/10 ($P=0.04$).

Table 6 Mean RNA:DNA (± SE) for each Treatment within each Day. I = Initial Sample.

Day	Treatment	Mean	SE	n
0	I	1.60	0.308	8
23	1/1	1.66	0.221	7
	1/5	2.53	0.525	7
	1/10	1.63	0.237	5
43	1/1	3.11	0.666	7
	1/5	3.34	0.472	8
	1/10	2.73	0.398	7
63	1/1	2.11	0.115	9
	1/5	2.35	0.331	13
	1/10	1.70	0.361	7

Fig 21 Mean DNA (ug/mgdry weight) vs Carapace Length (mm) by Sample Day (± 95% confidence limits). Days 23, 43 and 63 were not different (P>0.05) and have been pooled

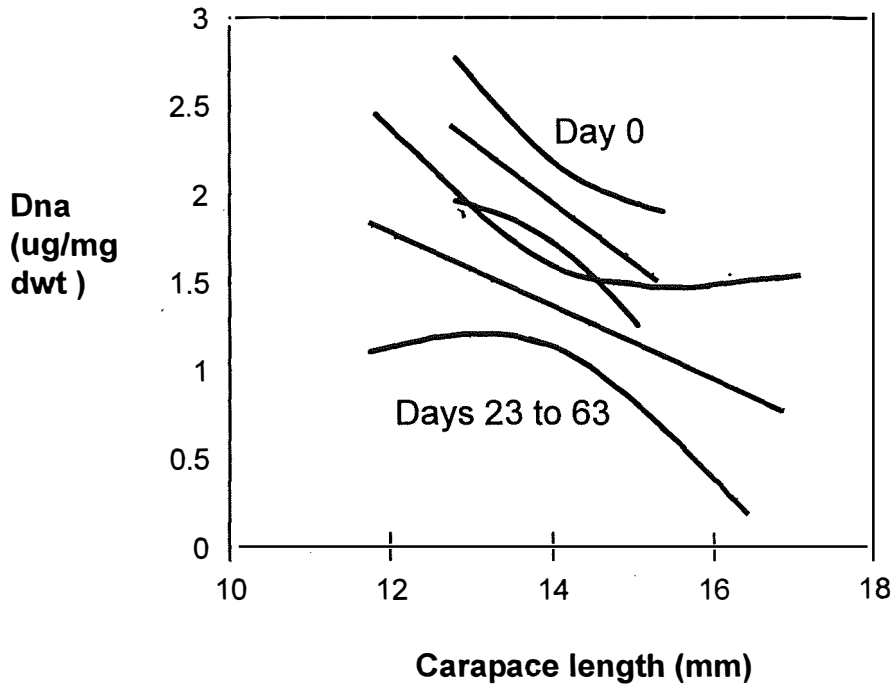


Fig 22 Mean Pooled RNA(ug dry weight) (±SE) and Treatment - specific Mean Protein content (mg dry weight) (±SE) vs Time (Days)

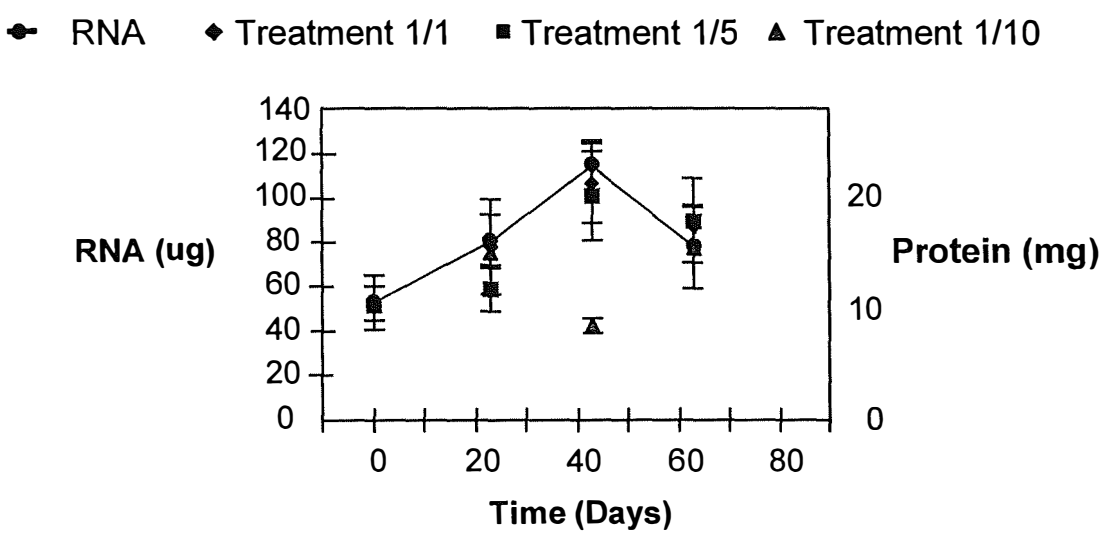
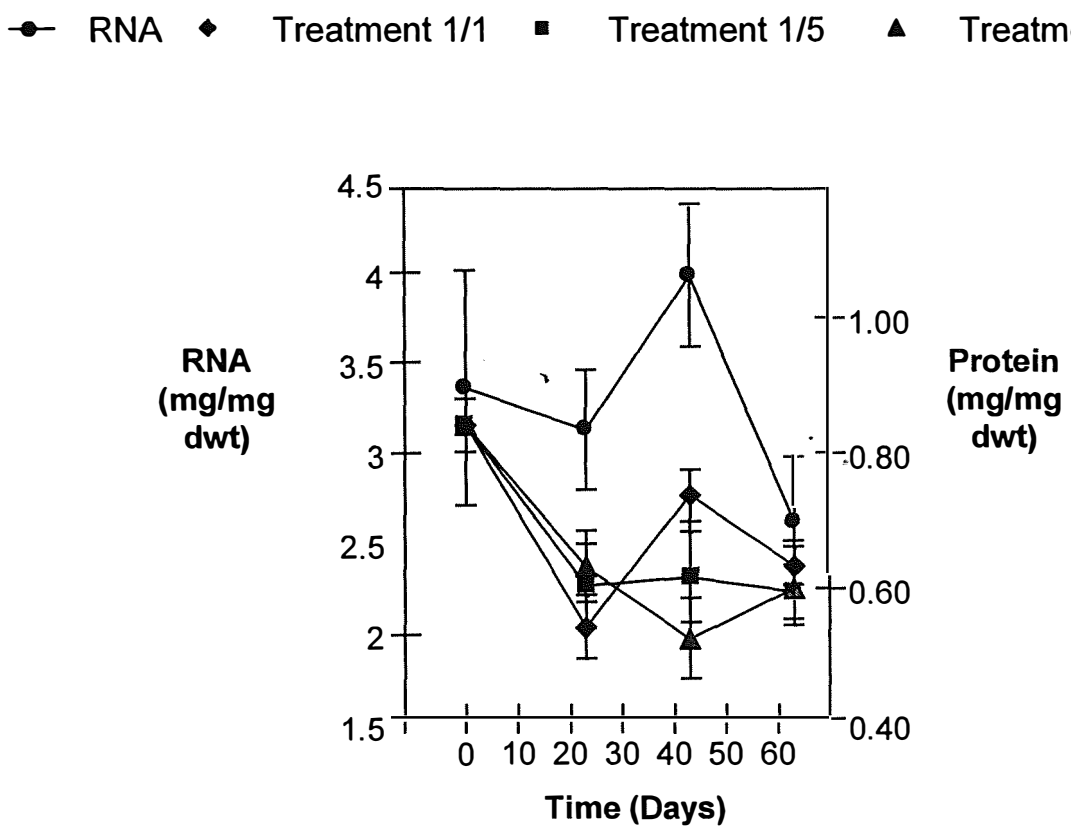


Fig 23. Mean RNA concentration (mg/mg dry weight) and Mean Protein concentration (mg/mg dry weight) vs Time (Days)



Condition Index/Indices Discussion

It is apparent that growth was independent of CL over the size range used in this experiment. Given the expectations of the logistic growth model this appears to be unlikely. However, it occurred here because of the way growth rate was calculated (mg dwt ABD/day) and because each lobster underwent no more than two moults. It is also an indication of the fact that growth occurs under the exoskeleton between moults, independent from any change in length.

If both lipid and protein are converted to their caloric equivalents (lipid 36.4 kj g^{-1}, protein 23.9 kj g^{-1}: Brett and Groves, (1979)) it was apparent that protein varied a great deal more than lipid in response to the treatments and to the experimental conditions in general treatment (Fig 24 and 25). In the abdomen there was a reduction in protein accumulation with treatment and in the DG, a reduction in protein level with time. Lipid declined in the DG between Days 0 and 23 and by Day 78 between Treatments 1 and 3. There are two channels for that energy, one towards growth, digestion, and activity i.e. non-moult metabolism, the other toward the anabolic and metabolic requirements of the moult. This second pathway is very important, especially in juveniles. Musgrove and Geddes (1995) found that anywhere from 36 to 50% of the energy accumulated by juvenile yabbies (*Cherax destructor*) during a given moult cycle could be lost over the period of inanition before the next moult and over the moult itself.

Fluctuations in DG protein and AB lipid were independent of treatment (Fig 24 and 25). The same could be said for the DG lipid, apart from the significant effect on Day 78. It is suggested that the marked fluctuations in DG protein and the early reduction in DG lipid were largely due to the moults that occurred, especially in the first 23 days of the experiment. This corresponded to the steepest part of the DG protein curve (Fig 25) and was the period over which occurred 68% percent of the moults noted from days 23 to 78, equivalent to 41% of lobsters in the experiment (Table 7).

Table 7 Percentage of lobsters moulting out of all the moults that took place during the experiment and % moulting out of lobsters remaining at the beginning of each period.

Day	% of total moults	% moulting out of lobsters remaining
0 to 23	68.0	41.3
23 to 43	22.9	18.5
43 to 63	5.7	7.0
63 to 78	3.4	8.8

During the following 20 day period (Days 23 to 43) a further 23% (19% of lobsters) of those moults occurred, accompanied by a continued, but shallower, decline in protein and a rise in total lipid. Presumably this reduction in the substrate loss (to the point of a net gain in lipid) was the result of the substrate accumulation by the 81% of lobsters that did not moult. The continuation of the decline in DG protein into the period where very few (less than 10%) of lobsters were moulting suggests that there was effect on the condition of the remaining animals, probably induced by the experiment conditions. Over the premoult-moulting period, protein and lipid are involved in chitin formation and energy provision (Chang and O'Connor, 1983, Sasaki *et al..*, 1986).

Apart from the moult-stage-specific effects there were two levels at which a change in condition with time may be discussed. The first is in terms of the effects caused by the conditions under which the lobsters were held, stressors such as confinement, degree of disturbance and non-normal diet (i.e. treatment independent holding effects). The second level is in terms of treatment effects. The first has already been discussed with regard to DG protein. That effect probably contributed to the overall decline in protein from the beginning of the experiment. It is also seen in the decline in growth rate and DG weight, the latter particular evident in the DG:AB ratio. Both influence condition, so both are interesting and pertinent to this discussion.

Lipid and protein are considered to be the major energy reserves in decapod Crustacea (Trendall and Prescott, 1989) and the primary storage organ for moult cycle-related processes is the digestive gland (Chang and O'Connor, 1983, Chandumpai *et al..*, 1991). For a decapod to moult under 'normal' conditions, it has been postulated that energy stores must be accumulated to the "point of reserve saturation", also called the "D_o threshold" after the point in the moult cycle where it occurs (Anger and Dawirs, 1981; Gore, 1985). This latter research on larvae may also apply to later stages as suggested by Cockcroft (in press) working on a South African population of the rock lobster *Jasus lalandii*. At D_o, the decapod is effectively independent of exogenous factors with respect to the energy requirements of moulting (Anger and Dawirs, 1981). The DG appears to be the most responsive organ to physiological stress (Trendall and Prescott, 1989) so use of its reserves to cope with a stressor may delay moulting or force catabolism of other tissues. In the face of continued stress the reserves of the digestive gland are rapidly depleted without replenishment and moulting is delayed or the moult cycle arrested as reported by for decapod larvae (Anger, 1984), postlarvae (Sasaki *et al..*, 1986), juveniles under sub-optimal feeding conditions (Juinio *et al.* 1992, Cockcroft, in press) and the juveniles in the present study.

Utilisation of various reserve tissues varies among (Trendall and Prescott , 1989) and within species (Sasaki *et al.* 1986). For *Jasus edwardsii*, there was a clear effect of food supply on the water content of storage tissues (Fig 26). When holding conditions alone are affecting growth the DG supplies most of the energy reserves in the form of protein and lipid unless the lobsters moult, in which case both tissues are involved. In this sense Treatment 1/10 is a control for holding effects. As the food limitation increases, abdominal protein becomes more important. At peak abdomen weight (Day 43, Fig 24), the difference in protein level between Treatments 1/1 and 1/10 was over 300 joules, suggesting that protein is the major energy source during food shortage. It may be that DG lipid is conserved for the energetic and anabolic requirements of the moulting period, the latter a frequent occurrence in lobsters of this size. This was reported by Dall (1981) for the Norwegian Lobster (*Nephrops*

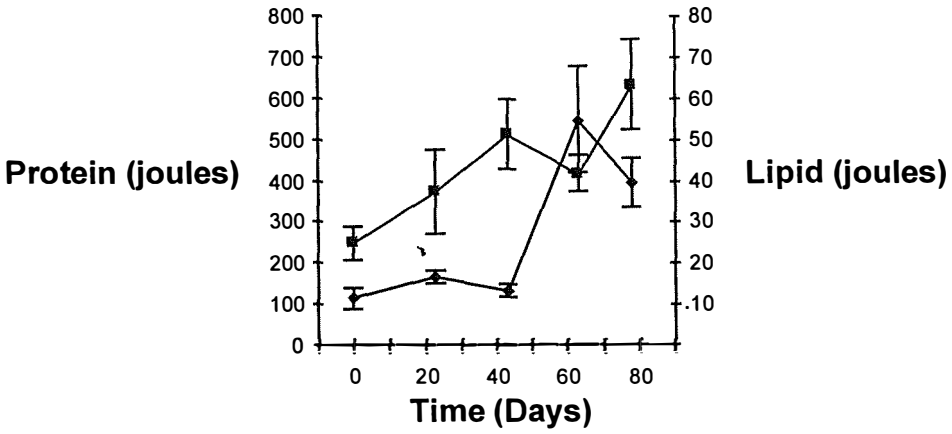
norvegicus). The dominance of protein over lipid catabolism under sub-optimal nutritional conditions was also reported by Chu and Ovsianico-Kowlikowsky (1994) for the shrimp, *Metapenaeus ensis*; by Barclay *et al.*, (1983) for the Tiger Prawn *Penaeus esculentus*; by Dall(1974) for the Western Rock Lobster *Panulirus longipes*; and by Sasaki *et al.*, (1986) for postlarvae of the American Lobster, *Homarus americanus*. Juinio *et al.*, (1991) also suggested that protein catabolism was dominant during starvation in juvenile *H. americanus* although they did not measure lipid.

The sequence of reserve use was also found to vary during starvation associated with emigration in the lobster, *Panulirus ornatus* (Trendall and Prescott, 1989). They reported that digestive gland carbon (primarily lipid) was the most important energy reserve, followed by abdominal protein, then digestive gland protein as starvation progressed. In the present study, differences in the degree of initial catabolism of DG tissue and AB tissue (Fig 27) probably reflected the different composition of lipids in the two tissues. The neutral lipid triacylglycerol makes up the bulk of the lipid fraction in the digestive gland where it functions as the body's primary energy reserve (Chang and O'Connor, 1983, Fraser, 1991, Chandumpai *et al.*, 1991). In contrast, Chandumpai *et al.* (1991) found that the abdomen of the prawn *Penaeus esculentus* contained mostly the structural phospholipids with the major neutral lipid being cholesterol. Phospholipids may function as a secondary energy reserve (Chandumpai *et al.*, 1991) as cell contents then walls are catabolised during starvation, but this was not evident in the present study. In fact, the relative constancy of the total DNA level at intermoult suggests that reduction in abdominal cell size rather than complete cell catabolism occurred. There is an advantage in the maintenance of cell integrity in that it allows faster recovery when food supply improves (Wang and Stickle, 1986). Wang and Stickle (1986) found that DNA concentration in the blue crab (*Callinectes sapidus*) was conserved, whereas the RNA concentration decreased during starvation as did Lemmens (1995) for puerulus of the Western Rock Lobster *Panulirus cygnus* and Juinio *et al.* (1992) for postlarval *H. americanus*. The relative conservation of DNA during starvation or intermoult stage growth in this study suggests that most weight change was the result of abdominal cell size reduction or enlargement (i.e. protein catabolism or synthesis) not cell number reduction or proliferation.

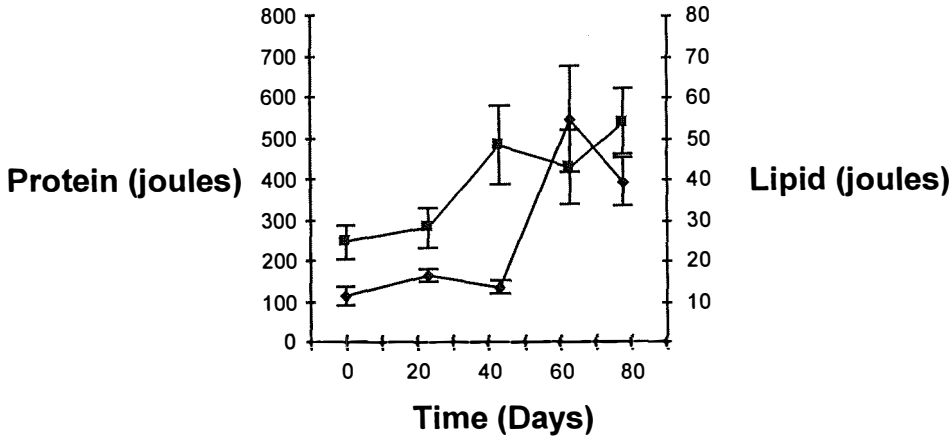
Fig 24 Mean Abdominal Lipid (joules) (±SE) and Mean Protein (joules) (±SE) for three Treatments; a) 1/1, b)1/5 and c)1/10

▪ Protein ◆ Lipid

a) Treatment 1/1



b) Treatment 1/5



c) Treatment 1/10

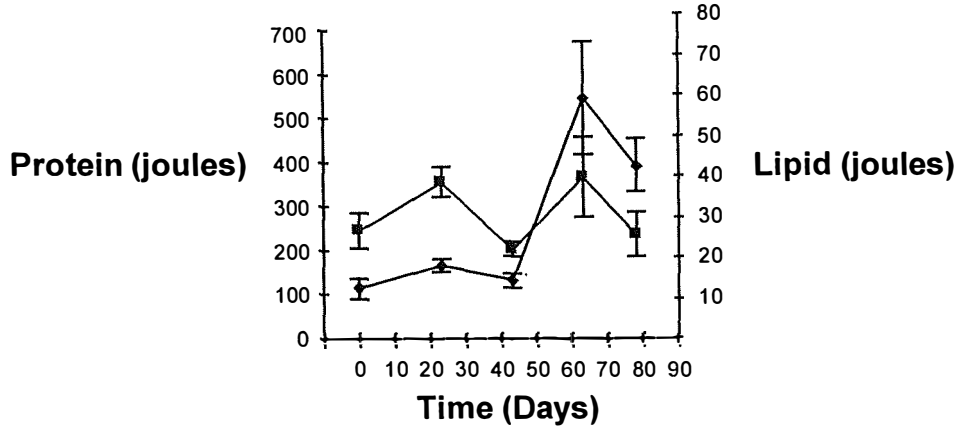
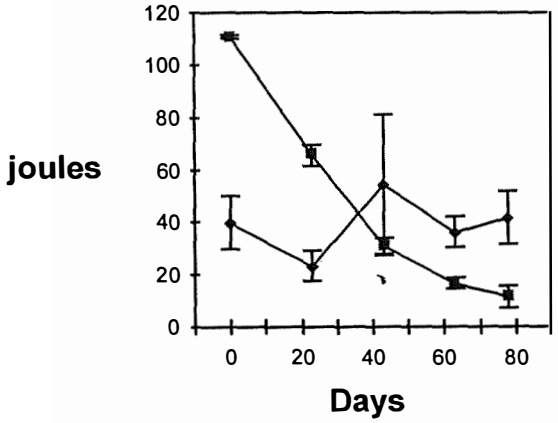


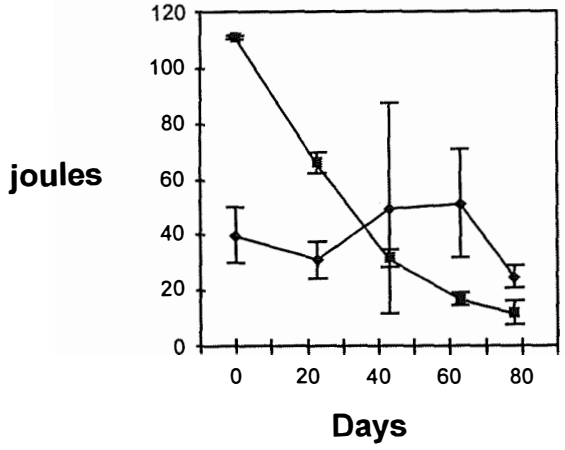
Fig 25 Mean Digestive Gland Lipid (joules) (±SE) and Mean Protein (joules) (±SE) for three Treatments; a) 1/1, b)1/5 and c)1/10

■ Protein ◆ Lipid

a) Treatment 1/1



b) Treatment 1/5



c) Treatment 1/10

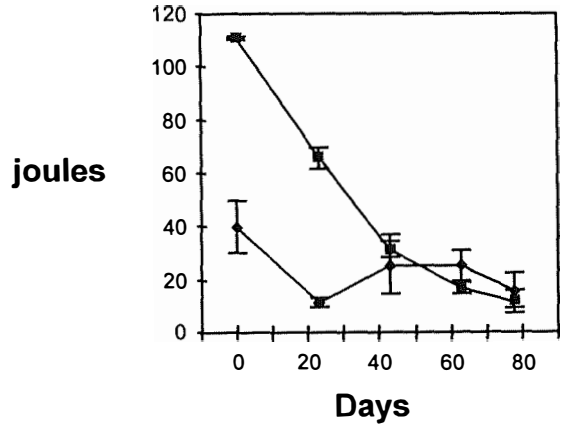


Fig 26 Mean % Water in Abdominal Tissue vs Mean % Water in Digestive Gland. Each point represents a sample day within each treatment. This figure reads from left to right as indicated by (↗)

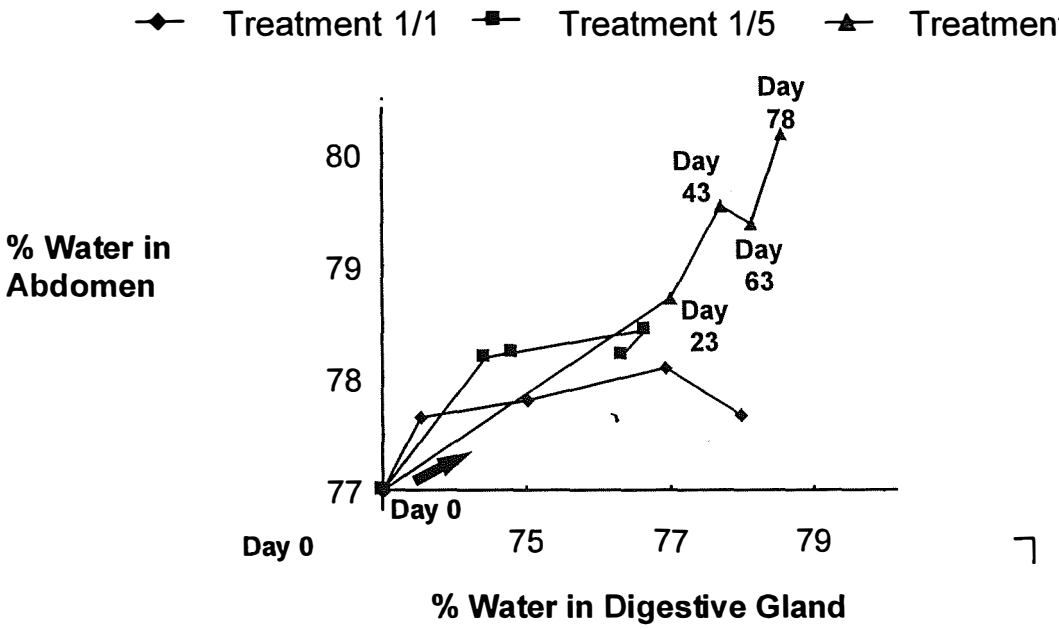
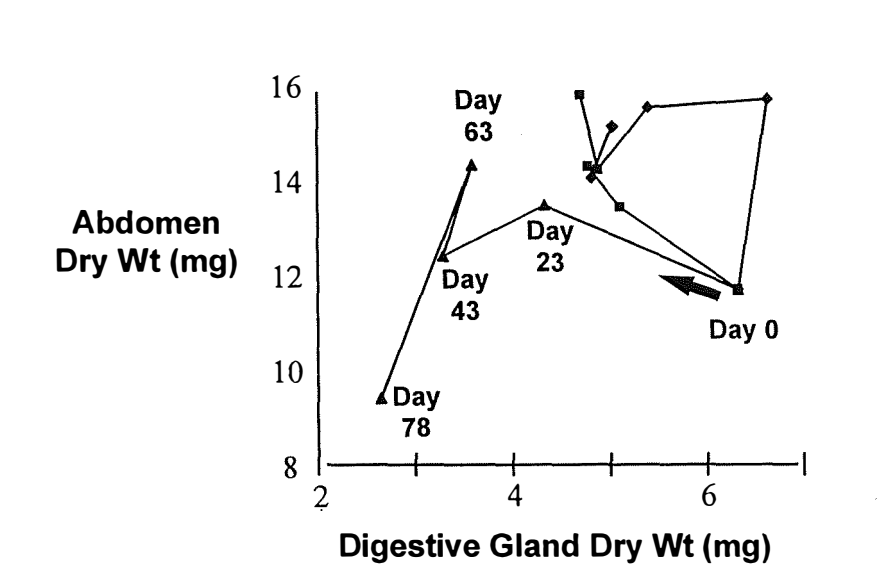


Fig 27 Mean Abdominal Dry Weight (mg) vs Mean Digestive Gland Dry Weight (mg). Each point represents a sample day within each treatment. This figure reads from right to left as indicated by (↖).



As the above suggests, most of the abdominal tissue accumulation and loss was in the form of protein. Apart from Treatment 1/10 on Day 43, fluctuations in total protein level follow those of total RNA. However, protein concentration does not follow RNA concentration as expected. This apparent anomaly may be explained by the overshadowing of protein synthesis by catabolism, the result of inadequate nutrition.

Unlike Juinio *et al.* (1992), the present study did not show that food availability had an effect on the RNA:DNA ratio or that growth rate was correlated with RNA concentration. However, it was apparent that RNA concentration followed protein concentration under *ad libitum* feeding conditions. This suggests that once the nutritional requirement are better understood for this species, it should be possible to establish a correlation between RNA concentration and growth rate as has been done for other crustaceans (Wang and Stickle, 1986; Moss, 1994 and Juinio *et al.*, 1992).

This study has shown that the biochemical response to nutritional stress in juvenile *Jasus edwardsii* involves firstly the digestive gland then the abdominal tissue with the protein component contributing the most energy and abdominal muscle being the largest energy reserve. These same responses may also apply to later stages as they were reported for adult *Panulirus ornatus* and *Jasus lalandii* by Trendall and Prescott (1989) and Cockcroft (in press) respectively. Thus, the digestive gland:abdominal tissue ratio and the digestive gland composition itself are useful indicators of condition.

REFERENCES

Aiken, D.E. (1973) Proecdysis, setal development and molt prediction in the American lobster *Homarus americanus*. Journal of the Fisheries Research Board of Canada 30, 1337-1344.

Aiken, D.E. (1980) Molting and growth. In: The Biology and Management of Lobsters. Vol. 1. (Eds: Cobb,J.S., Phillips,B.F.) Academic Press, New York, 91-163.

Aiken, D.E. and Waddy, S.L. (1987) Molting and growth in crayfish: a review. Canadian Technical Report of Fisheries and Aquatic Sciences 1587, 1-33.

Anger, K. and Dawirs R. R. (1981) Influence of starvation on the larval development of *Hyas araneus* (Decapoda: Majiidae). Helgolander Meeresunters 34, 287 - 311.

Anger, K. (1984) Influence of starvation on moult cycle and morphogenesis of *Hyas araneus* larvae (Decapoda: Majiidae). Helgolander Meeresunters 38, 21-33.

Anger, K. (1991) Developmental changes in the bioenergetics of decapod larvae. Memoirs of the Queensland Museum 31, 289-308.

Anger, K., Storch, V., Anger, V. and Capuzzo, J.M. (1985) Effects of starvation on moult cycle and hepatopancreas of Stage 1 lobster (*Homarus americanus*) larvae. Helgolander Meeresunters 39(2), 107-116.

Barclay, M.C., Dall, W. and Smith, D.M. (1983) Changes in lipid and protein during starvation and the moulting cycle in the tiger prawn *Penaeus esculentus* Haswell. Journal of Experimental Marine Biology and Ecology 68, 229-244.

Barnes, H. and Blackstock, J. (1973) Estimation of lipids in marine animals and tissues: Detailed investigation of the sulphophosphovanillin method for 'total' lipids. Journal of Experimental Marine Biology and Ecology. 12, 103-118.

Brett, J.R. and Groves, T.D.D. (1979) Physiological Energetics. In 'Fish Physiology. Vol. 8. Hoar, W.S., Randall, D.J. and Brett, J.R. (eds.) Academic Press, New York. pp. 279-351

Buckley, L.J. (1979) relationships between RNA-DNA ratio, prey density and growth rate in Atlantic cod (*Gadus morhua*) larvae. Journal of the Fisheries Research Board of Canada 36, 1497-1502

Bulow, F.J. (1970) RNA-DNA ratios as indicators of recent growth rates of a fish. Journal of the Fisheries Research Board of Canada 27, 2343-2349

Bulow, F.J. (1987) RNA:DNA ratios as indicators of fish growth: a review. In: The Age and Growth of Fish. R.C. Sommerfelt and G.E. Hall (eds). Iowa State University Press, Iowa. pp 45-64.

Chandumpai, A., Dall, W., and Smith, D.M. (1991) Lipid-class composition of organs and tissues of the tiger prawn *Penaeus esculentu*s during the moulting cycle and during starvation. Marine Biology 108, 235-245

Chang, E.S. and O'Connor, J.D. (1983) Metabolism and transport of carbohydrates and lipids. In: The Biology of Crustacea Vol. 5. Internal Anatomy and Physiological Regulation. L.H. Mantel (ed). Academic Press Inc. N.Y. pp 263-287.

Chu, K. and Ovsianico Koulikowsky, N.N. (1994) Ontogenetic changes in metabolic activity and biochemical composition in the shrimp, Metapenaeus ensis. . Journal of Experimental Marine Biology and Ecology 183, 11-26.

Clarke, A., Rodhouse, P.G., Holmes, L.J. and Pascoe, P.L. (1989) Growth rate and nucleic acid ratio in cultured cuttlefish *Sepia officinalis* (Mollusca, Cephalopoda). Journal of Experimental Marine Biology and Ecology 133(3), 229-240.

Clemmesen, C. (1989) RNA/DNA ratios of laboratory-reared and wild herring larvae determined with a highly sensitive fluorescence method. Journal of Fish Biology 35, 331-333

Cobb, J.S., Juinio,M.A. and Bengtson, D.A. (1991) Estimation of recent growth of field caught postlarval lobsters from RNA:DNA ratios. Journal of Shellfish Research 10(1), 284.

Cockcroft, A.C. (in press) Biochemical composition as a growth predictor in male West Coast Rock Lobster (*Jasus lalandii*). Marine and Freshwater Research.

Dall, W. (1974) Indices of nutritional state in the western rock lobster *Panulirus longipes* (Milne Edwards). I. Blood and tissue constituents and water content. Journal of Experimental Marine Biology and Ecology 16, 167-180

Dall, W. (1981) Lipid absorption and utilisation in the Norwegian lobster, Nephrops norvegicus (L.). Journal of Experimental Marine Biology and Ecology 50, 33-45.

Fielder, D.R. (1964) The spiny lobster, Jasus lalandei (H. Milne-Edwards), in South Australia. Australian Journal of Marine and Freshwater Research 15, 77-92.

Fraser, A.J. (1989) Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. Canadian Journal of Fisheries and Aquatic Science 46, 1868-1873.

Gore, R.H. (1985) Molting and growth in decapod larvae. In: Crustacean Issues Volume 2, Larval Growth, Wenner, A.M. (ed). A.A. Balkema, Rotterdam. p. 52-65.

Gornall, A.G., Bardawill, David, M.M. (1949) Determination of serum protein by means of the Biuret reaction. Journal of Biological Chemistry 177, 751 - 766.

Juinio, M.A.R., Cobb, J.S., Bengston, D. and Johnson, M. (1992) Changes in nucleic acids over the molt cycle, in relation to food and temperature, in *Homarus americanus* postlarvae. Marine Biology 14, 1-10.

Lehtonen, K.K. (1994) Metabolic effects of short-term starvation on the benthic amphipod *Pontoporeia affinis* Lindstroem from the North Sea. Journal of Experimental Marine Biology and Ecology 176 (2), 269-283.

Lemmens, J.W.T.J. (1995) Nucleic acid levels, cellular activity and growth during the puerulus stage of the western rock lobster (*Panulirus cynus* (George); Decapoda: Palinuridae). Journal of Experimental Marine Biology and Ecology 194, 143-156.

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

Malloy, K.D. and Targett, T.E. (1994) Effects of ration limitation and low temperature on growth, biochemical condition and survival of juvenile summer flounder from two Atlantic coast nurseries. Transactions of the American Fisheries Society 123(2), 182-193

Miglavs, I. and Jobling, M. (1989) Effects of feeding regime on food consumption, growth rates and tissue nucleic acids in juvenile Arctic charr, *Salvelinus alpinus*, with particular respect to compensatory growth. Journal of Fisheries Biology 34, 947-957.

Mills, B. J. and Lake, P.S. (1975) Setal development and moult staging in the crayfish *Parastacoides tasmanicus* (Erichson) (Decapoda:Parastacidae). Australian Journal of Marine and Freshwater Research 26, 47-73.

Moss, S.M. (1994) Use of nucleic acids as indicators of growth in juvenile white Shrimp, *Penaeus vannamei*. Marine Biology 120, 359-367

Mourente, G. and Rodriguez, A. (1991) Variation in the lipid content of wild-caught females of the marine shrimp *Penaeus kerathurus* during sexual maturation. Marine Biology 110, 21-28

Musgrove, R.J. (1994) The bioenergetics of the juvenile Yabbie (*Cherax destructor* Clark). Ph.D. Thesis, University of Adelaide, South Australia

Musgrove, R.J.B. and Geddes, M.C. (1995) Tissue accumulation and the moult cycle in juveniles of the Australian freshwater crayfish *Cherax destructor*. *Freshwater Biology* 34, 541-558.

Nicol, S., Stolp, M. and Nordstrom, O. (1992) Changes in gross biochemistry and mineral content accompanying the moult cycle in the Antarctic krill *Euphausia superba*. Marine Biology 113, 201-209

O'Halloran, M. J. and O'Dor, K. O. (1988) Molt cycle of male snow crabs, *Chionoecetes opilio*, from observations of external features, setal changes, and feeding behaviour. Journal of Crustacean Biology 8(2), 164-176.

Prescott, J.H. (1997) Population dynamics of the Southern Rock Lobster in South Australian waters. FRDC Report, Project 93/087.

Rao, K. R., Mohrherr, D. Reinschmidt, D., and Fingerman, M. (1977) Gastrolith growth during proecdysis in the crayfish *Faxonella clypeata* (Hay1899) (Decapoda:Astacoidea). *Crustaceana* 32, 256-264

Reaka, M. L (1975). Molting in stomapod crustaceans. Journal of Morphology 146, 55-80.

Robbins, I., Lubet, P. and Besnard, J.Y. (1990) Seasonal variation in the nucleic acid content and the RNA:DNA ratio of the gonad of the scallop *Pecten maximus*. Marine Biology 105, 191-195.

Sasaki, G.C., Capuzzo, J.M., and Biesiot, P. (1986) Nutritional and bioenergetic considerations in the development of the American Lobster *Homarus americanus*. Canadian Journal of Fish and Aquatic Science 43, 2311-2319.

Smith, D.M. and Dall, W. (1985) Moult staging in the tiger prawn *Penaeus esculentus* Haswell. Second Australian National Prawn Seminar. P.C. Rothlisberg, B.J. Hill, and D.J. Staples (eds). Cleveland, Queensland. Pp 85-93.

Stevenson, J. R. (1968) Metecdysial moult staging and changes in the cuticle in the crayfish *Orconectes sanbornii* (Faxon). Crustaceana 4, 169-177.

Suthers, I.M. (1991) The use of condition indices in larval fish. In: Larval Biology. Australian Society for Fish Biology Workshop, Proceedings No. 15. D.A. Hancock (ed) Australian. Government. Publishing Service. pp 49-57.

Trendall, J.T. and Prescott, J. (1989) Severe physiological stress associated with the annual breeding emigration of *Panulirus ornatus* in the Torres Strait. Marine Ecology Progress Series 58, 29-39.

Turnbull, C.T. (1989) Pleopod cuticular morphology as an index of moult stage in the Ornate Rock Lobster, *Panulirus ornatus* (Fabricius, 1789). Australian Journal of Marine and Freshwater Research 40, 285 - 293.

Vinagre, A.S. and da Silva, R.S.M. (1992) Effects of starvation on the carbohydrate and lipid metabolism in crabs previously maintained on a high protein or carbohydrate-rich diet. Comparative Biochemistry and Physiology 102A, 579-583.

Wang , S.Y. and Stickle, W.B. (1988) Biochemical composition of the blue crab *Callinectes sapidus* exposed to the water-soluble fraction of crude oil. Marine Biology 98, 23-30

Wright, D.A. and Martin, F.D. (1985) The effect of starvation on RNA:DNA ratios and growth of larval striped bass, *Morone saxatalis*. Journal of Fish Biology 27 (4), 479-485.

Wright, D.A. and Hetzel, E.W. (1985) Use of RNA:DNA ratios as an indicator of nutritional stress in the American oyster *Crassostrea virginica*. Marine Ecology Progress Series 25(2) 199-206.

Zar, J.H. (1984) Biostatistical Analysis, Second Edition. Prentice hall Inc., New Jersey, U.S.A. 718 pp

BENEFITS

This work has benefited project 93/087 (Population Dynamics of the Southern Rock Lobster in South Australian Waters) by developing moult staging techniques, used to gain a better understanding of moulting periodicity in the fishery. This study has also benefited Project 96/160 (Condition and its Assessment in the Southern Rock Lobster *Jasus edwardsii*. ii. Field application of the techniques for condition assessment and moult staging developed in the laboratory) again by developing moult staging techniques and by allowing refinement of condition assessment methods and by identifying the lobster's likely physiological response, in terms of condition, to various stressors.

The two projects (95/017 and 96/160) were intended to function as a unit, the establishment of a continuum of research from the laboratory to the field. Thus, in more general terms, the benefits as stated in the initial proposals still valid. These included improving the accuracy of growth rate estimation (i.e. moult staging) and providing an alternative means of growth assessment (i.e. a condition index). It is still intended that the project will enhance the growth components of the yield per recruit analysis and the population model. 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

INTELLECTUAL PROPERTY

The intellectual property developed during this project included an understanding of relevant morphological changes during the moult cycle, of the biochemical techniques involved in condition assessment and of the likely response of the lobster to stress in terms of the selected indices.

FURTHER DEVELOPMENT

The intellectual property developed in this study will be used in Project 96/160 and formally presented in refereed papers to be submitted.

ACKNOWLEDGMENTS

Thanks to the South Australian Rock Lobster industry for supporting the project and to FRDC for providing the funding. Thanks also to Mr Phillip Cooper, a lobster fisherman of Kingston, for taking us out on his boat to catch the adults for the moult staging work. Finally, thanks to the Department of Primary Industries and Fisheries (Tasmania) and particularly Dr. Stuart Frusher, for supplying the small juveniles.

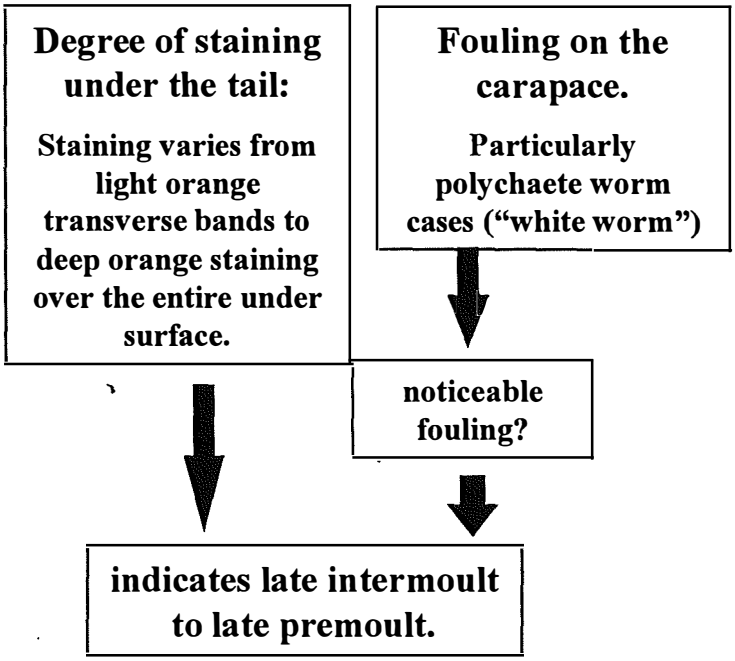
STAFF

(Both externally funded)
Dr. Richard Musgrove
Mr. Luis Corrales

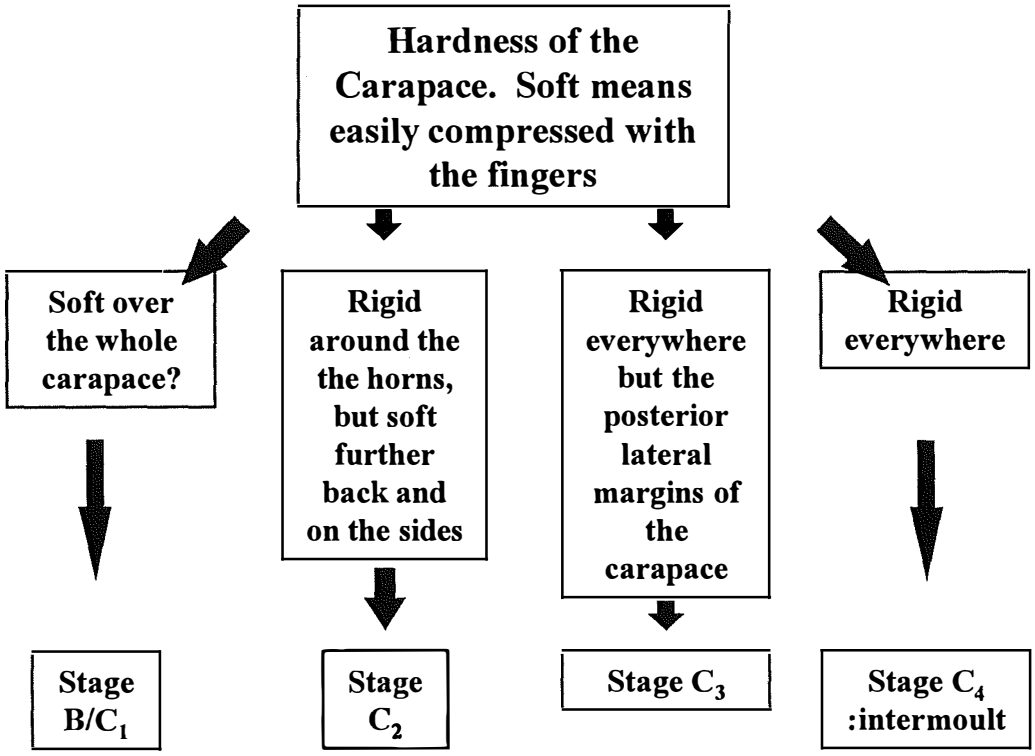
Appendix 1

Three ways of approaching moult staging

1. Shell appearance



2. Shell rigidity



3. Setal Development

As described in text. Apolysis is visible in the telson using a good hand-lens