The Bioenergetics of the juvenile Yabbie (*Cherax destructor* Clark)

Richard Musgrove M.Sc. Hons.

Zoology Department

University of Adelaide

South Australia

Thesis submitted for the degree of

Doctor of Philosophy

March, 1994

Awarded 1994

To my Parents Olwyn and John, to my Sister, Felicity

and to my Grandfather, Fred

Belief, inspiration and the shift and swell of the sea

Contents

*(Part of this Chapter has been published in Freshwater Crayfish 9)

Abstract

The bioenergetics and growth of an organism can be defined through the construction of an energy budget, where the energetic fate of food consumed is quantified in terms of caloric equivalents. In crustacea the process of growth is complicated by the need to produce a new exoskeleton at intervals to house the expanding tissue. This means that in addition to the energetic costs of feeding and growth, as described for vertebrates, an energy budget must also include costs associated with the growth of a new exoskeleton and the shedding of the old. This study was prompted by an interest in the process of energy use in crustacea in general and freshwater crayfish in particular. The work uses the juvenile yabbie in laboratory and field experiments to examine tissue accumulation and energy intake and use and presents a model for juvenile growth in this species.

Chapter 2 investigates a coarse diet to determine its usefulness for further experiments. The influence of size on energy requirements is also examined. Initial experiments tested the effect of three forms of the diet on growth. They were isocaloric (3700 kcal/kg) and contained 15%, 25% and 35% protein respectively. Survival was high on all the diets and the 35% protein form gave the best growth. The marked differences in growth observed probably reflect changing intermoult period as differences in % moult increment were small, although still significant. Growth rate slowed with time in all treatments and there was a significant negative relationship between initial weight and growth rate. This experiment was followed by investigation of consumption, assimilation and respiration rates using the 15% and 35% protein forms of the diet and yabbies ranging in size from 1g to 15g. Consumption varied from 2 to 12 % dry weight of food to wet body weight per day and was inversely proportional to wet weight. The assimilation efficiency of energy varied between 75 and 95% and was independent of size. Protein digestibility was also high (90-97%) and independent of size. Protein was assimilated more efficiently from the 35% protein diet and there were no significant differences between the diets

in terms of weight-specific consumption and assimilation. Calculated Protein:Energy ratios were 43 and 99 mg protein/kcal for the 15% and 35% protein diets respectively. Pooled data are presented which show that energetic requirements declined exponentially with increasing size. Yabbies of 1g assimilated 2.8 to 3 times and respired 3 times more energy than 10g animals. Results are discussed with respect to size-specific energy requirements for growth

Chapter 3 investigates field growth under semi-intensive aquaculture conditions where the 35% protein diet was added to enclosures within an above-ground pool. Uneaten food and waste along with micro organisms provided a detritus system and filamentous algae (*Cladophora*) was also available as a food source. Growth was measured over a fifty-three day period using fifteen groups of yabbies within enclosures in the pool. Third stage juveniles were used in the experiment (Initial size 3.0-3.3 mm : 12-14 mg wet weight). Five groups were harvested at 15, at 30 and at 53 days. Yabbies grew to 19.5 mm OCL (3.6g). The mean instantaneous growth rate over the 53 day period was 0.084 mg/mg ash free dry weight/day although this varied enormously during the experiment partly as a result of temperature fluctuations. Mean growth for the first 15 days was 0.086 mg/mg afdw/day, for days 15 to 30 it was 0.11 mg/mg afdw/day and for days 30 to 50 it was 0.06 mg/mg afdw/day. Tissue accumulation, energy storage and use were examined by determination of moult stage and size-specific body composition. Moult stage determination was based on the literature for this species. Thus the moult cycle comprised four stages and 11 substages. These were postmoult (A and B), intermoult (C_1 and C_2), premoult ($D_{1.1}$ to $D_{1.4}$) and late premoult (D_2 to D_4). Moult stage duration was predicted from numbers of animals caught at each moult stage.

Dry weight and ash free dry weight (organic content) peaked twice over the moult cycle. The first peak occurred in the middle ($D_{1.2}/D_{1.3}$) and the second in the later stages of premoult (D_3). Both peaks were of similar magnitude at about 2 to 2.3 times the postmoult (stage A) weight. Wet weight also showed similar but smaller peaks at

these stages. In this case both were about 1.3 times the postmoult weight. Losses in organic content over the moult as a percentage of tissue accumulation during the previous cycle declined from 51% at 3.1mm OCL to 36% at 17mm OCL.

Percent water content varied between 75 and 85% of wet weight depending on size and moult stage and organic content varied between 74 and 82% of body dry weight. The exuvia contained 50% organic matter.

Water is taken up during D_4 and at the moult itself with uptake during A, B and D_3 as part of that continuum. Tissue accumulation occurred primarily between B and $D_{1.3}$, with further weight gain largely the result of fluid uptake.

Percentage ash varied from 18% at C_2 to 26% at $D_{1.4}$ and was dependent on size. In absolute terms ash increased immediately postmoult and between C_1 and $D_{1.3}/D_{1.4}$ with a major peak occurring during C_1/C_2.

Individuals within two size groups (5 - 7 mm OCL and 12 - 17 mm OCL) were assayed for carbon and nitrogen content using a carbon analyser and Kjeldahl methods respectively. Total carbon varied between 27 and 29% of the body and about 30% of the exuvia. Mean body organic carbon varied between 33 and 35.5% of the body and 49% of the exuvia. Neither form was affected by moult stage. Percent nitrogen varied between 9.4 and 11.3% of body organic content and about 7.3% of the exuvia. It was not affected by moult stage but declined with OCL in the larger of the two size groups. Chitin was assayed to allow estimation of protein and non-protein nitrogen. It varied between 9 and 17% of body organic content and made up about 50.5% of the exuvia. Calculated protein content varied between 47 and 62% of the body and about 25% of the exuvia. It was not affected by moult stage but declined with OCL in the larger size group. Mean energy content varied between 19 and 22 J/mg ash free dry weight depending on size and moult stage and the overall average was 20.73 J/mg ash free dry weight. Caloric content increased with OCL during premoult (A and B) and

early intermoult (C_1). There was a decline with size from C_2 to $D_{1.2}$ inclusive but no size effect from $D_{1.3}$ to D_4.

The relationship between protein, chitin and remaining carbon (organic carbon minus chitin) was then examined after conversion to joules using caloric equivalents. There was a decline in 'remaining carbon' during postmoult accompanied by an increase in chitin. A low level of tissue accumulation during intermoult was followed by an increase in all three tissue types between $D_{1.1}$ and $D_{1.3}$. The exuvia accounted for most of the carbon (chitin and remaining carbon) lost during ecdysis but relatively little of the total protein losses. It is suggested that protein and some carbon is catabolised during the moulting process, possibly to fuel metabolism. Models are presented showing changes in proximate composition over the moult cycle for two sizes of yabbie and tissue and energy accumulation and loss over a series of moult cycles and sizes from 3.1 mm to 17 mm OCL. .

Chapter 4 investigates the effect of temperature on growth and the components of growth in the laboratory. These experiments allowed more accurate estimation of size-specific energetic requirements and expenditure in the laboratory and assisted in the explanation of observed patterns of field growth (Chapter 3). Yabbies were grown at 15, 20, 25, 27.5 and 30°C using the 35% protein diet and the same species of algae found in the pond (*Cladophora*). The first three temperatures covered the range experienced in the field and the last two increased the range of the data set to allow regression analysis. The temperatures spanned the range from zero growth to beyond the optimum reported in the literature. Individuals within two initial size groups (5-6 mm OCL and 10-11 mm OCL) were used in the experiments which were continued until the first group had reached 10-11 mm OCL and the second 19-20 mm OCL. The latter was approximately the size achieved in the field. Instantaneous growth rates (IG) varied from 0.0103 to 0.049 mg/mg ash free dry weight/day and were directly proportional to temperature for the first three treatments. IG peaked for the first group at 25°C and for the second between 25 and 27.5°C. Growth was also inversely

proportional to initial size except at 15°C where rates were approximately equal. By extrapolation, zero growth would have occurred about 13°C. Percent moult increment was independent of size but increased slightly with temperature. Intermoult period was proportional to size and decreased with temperature. Regressions were calculated for the relationship between log intermoult period and premoult OCL (mm). Instantaneous growth rate curves (IG vs weight) were derived for 20, 25 and 27.5°C. A multiple regression equation was derived describing growth as a function of size and temperature for temperatures between 20 and 27.5°C.

Chapter 5 presents work on the effect of temperature on the respiration rate of the individuals in the growth experiment (Chapter 4). Respiration rate was measured at 15, 20 and 25°C. Oxygen consumption in ug/h was positively correlated with wet weight. Coefficients of determination varied from 0.83 to 0.97 depending on temperature. Mass exponents were 0.67, 0.71 and 0.72 at 15, 20 and 25°C respectively. There was a significant increase in respiration rate with temperature, largely as a result of an increase in intercept as the slopes of the lines were not significantly different. Q_{10} was about 2.4 for the 15 to 20°C and 1.4 for 20 to 25°C. A multiple regression equation was derived describing respiration as a function of size and temperature. The usefulness of this approach is compared with that of the Q_{10}.

Chapter 6 presents net growth efficiencies and models of daily energy use for the laboratory growth experiments at each of three temperatures (15, 20 and 25°C, Chapter 4) and for the field growth experiments (Chapter 3). Daily growth and metabolic cost was estimated for the laboratory budget using the multiple regression equations from Chapters 4 and 5 respectively. Metabolic cost (Routine Metabolic Rate, RMR) for the field budget was estimated using the laboratory-derived metabolic rates but with a component added for increased field activity. Exuvial and metabolic losses at the moult were calculated using relationships developed in Chapter 3.

In the laboratory, estimated daily energy assimilation increased with temperature from 15 to 25°C, as yabbies grew from 5 to 15 mm OCL. Energy assimilation for successive size classes varied between 6 and 70 joules per day at 15°C, between 18 and 111 joules per day at 20°C and between 27 and 156 joules per day at 25°C. Net growth efficiency (K_2) increased with size from 21% to 51% at 15°C, from 50 to 64% at 20°C and from 54 to 70% at 25°C.

In the field, estimated daily caloric assimilation ranged from 7 Joules/day on day 1 to 348 Joules/day on day 53. Increasing RMR by 50% elevated these figures to 8 and 361 Joules/day respectively. Mean net growth efficiency varied from 36 to 75%, depending on whether or not exuviae were included in growth and on whether an activity component was added to RMR. Mean K_2 also increased with successive harvests. If exuvia were included and RMR increased by 50%, mean K_2 was 48% for days 1 to 15, 62% for days 16 to 30 and 68% for days 31 to 53.

After 53 days the caloric increment (i.e. growth) for an average sized individual was estimated as 3242.8 joules and the joules respired were 538.8 when RMR values were used and 808.2 when RMR was increased by 50%. Over the same period there were nine moults and exuvial losses totalled 677.5 joules while metabolic losses at the moult totalled 988.9 joules. Thus assimilation was 5448 joules if laboratory RMR was used or 5717 joules if RMR was increased by 50%. Total RMR was 10% of assimilation and 17% of growth. Addition of the activity component to RMR increased these figures to 14 and 25% respectively. Addition of metabolic losses at the moult to RMR increased the contribution of respiration to between 28% and 31% of assimilation and between 47.11 and 55.42% of growth.

Growth and metabolism are discussed with reference to their effect on net growth efficiency. The effect of the metabolic and exuvial losses at the moult are also discussed with reference to the construction of energy budgets.

Chapter 7 presents a summary of the main points and conclusions.

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Signed: Date: 30.3.94.

Acknowledgements

I would like to thank my Supervisor, Dr. Michael Geddes, for encouragement, advice and patience. Many thanks also to Dr. Keith Walker and Dr Andrew Boulton for advice and invaluable assistance with statistical matters. Many thanks to Mr. Clive Chesson for stirling advice on chemical matters and for the use of his freezer and other facilities. Thanks to Mr. Phil Kempster and Mr Terry McKenzie for writing the yabbie measuring program and for setting up the measurement system.

Thanks also to Mr. Steve Clarke of DPI Fisheries and DPI Fisheries for the use of facilities at West Beach Laboratories.

Finally, I would like to thank Ms Fiona Baker for endless support, for putting up with too many late nights at the computer and for invaluable help with production of the figures and diagrams in this thesis.

Chapter 1.

Introduction

Freshwater crayfish are the largest mobile macroinvertebrates in temperate freshwater ecosystems (Holdich and Lowery, 1988). Species are grouped within the superfamilies Astacoidea and Parastacoidea and occur in Europe, North and South America, Eurasia and Australasia. Astacoidea is native to the Northern Hemisphere and contains the families Astacidae and Cambaridae. Superfamily Parastacoidea occurs naturally in the Southern hemisphere and contains the family Parastacidae (Hobbs, 1988). In Australia there may be up to 100 species of parastacid although the taxonomy is 'unsettled' and the status of many species remains unclear (Sokal, 1988). *Cherax destructor* is found in the eastern and south-eastern parts of South Australia and the Northern Territory respectively and in Victoria, New South Wales and southern Queensland (Sokal, 1988). It occurs in farm dams, billabongs, swamps, creeks, rivers and lakes including the Murray River flood-plain. It also occurs in temporary water bodies and may burrow when the water evaporates (Sokal, 1988). Populations are characterised by high resilience (capacity to respond to the stress of exploitation) and low stability of population numbers (Momot, 1984). The species is characterised by high fecundity and fast growth and may live 3 to 8 years (Woodland, 1967, Frost, 1975).

Freshwater crayfish are usually reported as opportunistic polytrophs or omnivores with detritus forming the nutritional mainstay (e.g. *Astacus astacus*, Abrahamsson, 1966; *Cherax destructor*, Woodland, 1967, Lake and Sokal, 1986; *Orconectes spp*. Momot *et al*, 1978; *Paranephrops zealandicus*, Musgrove, 1988a; *Parastacoides tasmanicus*, Growns and Richardson, 1988) However, recent work (McClain *et al* 1992a and b, Momot, in press) suggests that the gut analysis used to determine diet in the above studies produces a significant bias which may misrepresent the contribution of some components. For example, it is argued that the relative abundance of detritus is not indicative of its role as a primary food source. Momot (in press) suggests that crayfish are primarily carnivores, ingesting living plant and detrital material while searching for associated animal prey or in the latter's absence or decline. Animal tissue is destroyed or made unrecognisable by the crayfish's gastric mill

more easily than detrital or fresh plant material, so its real contribution to diet is underestimated by conventional gut analyses. McClain *et al* (1992b) made a similar suggestion based on growth experiments using prepared feed, fresh plant material and various components of the detrital system. Both Jones and Momot (1983) and McClain *et al* (1992a and b) have shown that fresh plant tissue and detritus (without associated fauna) is of little value in promoting growth. Other workers have reported low digestibility and consumption rates with fresh plant material (Moshiri and Goldman, 1969, Kossakowski, 1974, Wiernicki, 1984, Musgrove, 1988b, Brown *et al*, 1990,). For example, Brown *et al* (1990) tested ten aquatic macrophytes on *Orconectes virilis,* six of which showed dry weight digestibilities between 0 and 54% - the remaining four were up to 90% digestible. Consumption for all plants tested was low, the majority of plants were consumed at less than 0.2% (0.04 - 0.2%) of body weight per day with the maximum at 0.87%. Thus it appears that the importance of fresh and detrital plant tissue has been over-emphasised, suggesting that crayfish must ingest large amounts of animal protein (Momot, in press) and/or microbial tissue (McClain *et al* 1992a) to account for observed growth. McClain *et al* (1992a) suggested that growth of juvenile *Procambarus clarkii* in a flow-through culture system was determined by the abundance of detrital microflora and fauna, not by the detritus itself. Plant material may be a source of calories, as suggested by Wolcott and O'Connor (1992) for marine and terrestrial crabs, but its role as a protein-N source (i.e. for growth) is doubtful. That plant products have a role as an energy source is also suggested by work on *Orconectes virilis* and *O. rusticus* (Cambaridae) (Tierney and Atema, 1987) which showed that the sugars cellobiose and sucrose stimulate feeding movements. Cellobiose is a breakdown product of cellulose. Other sugars, compounds and single amino acids gave similar but lower magnitude responses. Furthermore, Musgrove (1988b) reported that the enzyme reservoir of *Paranephrops zealandicus* (Parastacidae) includes enzymes that degrade cellulose derivatives, as well as tissues and storage sugars of fungi and live plants, suggesting that both detrital and fresh macrophyte tissue may provide some nutritional benefit. Although plant material may provide an important source of energy, energy without protein will not produce growth.

The extent to which various components of the diet of crustaceans are used in respiration and growth or are defecated or excreted may provide an index of the efficiency of energy

utilisation. These responses may be further modified by environmental variables such as temperature, water quality and food quality and availability (Capuzzo, 1983). Their quantification within the framework of an energy budget is critical to understanding energy transformations within a population (i.e. Woodland, 1967, Jones and Momot, 1983) and the impact that a population may have on the system it occupies. Knowledge of energy use by a species is also an important consideration when determining nutritional requirements for culture.

1.1 Energy Budgets

The simplest form of an energy budget is Production = Assimilation - Metabolism - Excretion. A more extensive form of the budget that is particularly related to the energetics of freshwater crayfish will be used in this study. The budget takes account of the different metabolic energy demands and incorporates a term related to energy involved in moulting. The energy budget (Brett and Groves, 1979, Capuzzo, 1983, Jones and Momot, 1983, Parry, 1983) for crayfish may be written as

$$Q_c - (Q_u - Q_v) = Q_p + Q_g + Q_s + Q_d + Q_a + Q_m \quad \textbf{Equation 1.1}$$

Thus ingested energy (Q_c) may be lost from the individual in the form of faeces (Q_u) or assimilated but subsequently catabolised and lost as nitrogenous waste products (Q_v). Assimilated energy in the form of carbohydrates, lipids and protein may be converted to tissue (Q_p) or used to fuel the conversion process (Q_g). Q_g is the 'cost of growth' or the 'cost of synthesis of new protoplasm' (Brett and Groves, 1979, Parry, 1983, Wieser and Medgyesy, 1990). In small poikilotherms this has been equated with the increase in metabolic rate immediately after feeding (Weiser and Medgyesy, 1990) which is also known as the specific dynamic effect (SDE, Brett and Groves, 1979) and often referred to as Q_d (Clifford and Brick, 1979, Jones and Momot, 1983). However tissue synthesis may occur during starvation (Parry, 1983) suggesting that Q_d and Q_g are better maintained as separate terms in the energy budget. Other costs include Q_s which refers to the energetic cost of maintenance. This arises because existing tissue (i.e. muscle) is continually turned over and replaced (Calow, 1977). The energy loss may be attributed to the work of synthesising replacement polymers and to the energy lost during breakdown and excretion of replaced molecules (Calow, 1978). Q_a refers

to the mechanical costs involved in activity and includes the cost of the mechanical work of feeding, walking, swimming etc. The last term, Q_m, introduces the caloric losses at the moult and includes energy lost with the shed exuvia plus losses of energy reserves metabolised during the moult (Capuzzo, 1983). For the purposes of this study Q_m is further subdivided into E_v and E_r which refer to exuvial and metabolic losses respectively. For crustaceans the exuvia may be considered as part of production (Kurmaly *et al*, 1989) as although shed it has been part of the body of the organism.

The terms in this budget may be grouped as assimilation (Q_c - Q_u), production (Q_p - including E_v), respiration (Q_g, Q_s, Q_d, Q_a and E_r) and excretion (Q_v) and so related to the simple form of the energy budget:

Production (P) = Assimilation (A) - Respiration (M) - Excretion (E)

The parameters production, consumption and assimilation may be used to calculate growth or conversion efficiencies (Capuzzo, 1983) which are usually expressed as gross or net conversion efficiencies, K_1 and K_2, and derived as :

$$K_1 = \frac{\text{Production}}{\text{Consumption}}$$

$$K_2 = \frac{\text{Production}}{\text{Assimilation}} \qquad \textbf{Equation 1.2}$$

For the purposes of this study, K_2 alone will be considered, as consumption was not measured in the field or laboratory growth experiments used to generate the energy budgets (Chapters 3 and 5). As suggested by Equation 1.2, A = P + M + E so K_2 also equals P/(P + M + E) where, in this case, $P = Q_p + E_v$.

Excretion, Q_v, was not considered in this study; therefore the nett conversion efficiency can be written $K_2 = P/(P + M)$. Ammonia is the major nitrogenous waste product in *C. destructor* (Fellows and Hird, 1979) and in many other crustaceans (Klein Breteler, 1975, Nelson *et al*, 1977, Anger, 1991) but contributes relatively little to the overall energy budget. Klein Breteler (1975) reported a value of less than 1% for juvenile shore crabs (Carcinus maenas) fed mussels, Nelson *et al* (1977) reported values of between 1.8 and 2.4% for juvenile prawns (*Macrobrachium rosenbergii*) fed either an algae (*Cladophora*), a mixed source commercial diet or tubificid worms and Anger suggested values of between 2 and 5% for larvae of the spider crab (*Hyas araneus*). It is suggested that the contribution of Q_v to the overall yabbie energy budget would be of the same order.

Each of the parameters within the energy budget vary with animal size and maturity, food type, and environmental conditions. For example, within the metabolism component, growth accounts for most of the heat production in juveniles so the cost of growth is relatively high but as biomass increases growth slows with a larger fraction of metabolic expenditure progressively diverted to maintenance (Parry, 1983). The progressive increase in the energetic cost of maintenance also results in a decline in growth efficiency with size (Calow, 1977).

It is difficult to measure the components of respiration, Q_d, Q_g, Q_s and Q_a, particularly in juveniles. In order to measure maintenance (Q_s) it is necessary to stop growth (Parry, 1983). As this is practically impossible in juveniles, the usual measurements of metabolism involve more than one of the components; the most commonly taken measurements (Lampert, 1984) are standard metabolic rate (SMR), routine metabolic rate (RMR) and total metabolic rate (TMR). Standard metabolic rate (SMR) is defined as that measured on a fasting animal at rest in a constant temperature regime (Dejours, 1975, Lampert, 1984); when added to the costs of activity (incl. feeding) and digestion, absorption and transportation of absorbed food the combined total represents total metabolic rate (TMR). Routine Metabolic Rate (RMR) (Lampert, 1984) is determined from animals that have been fed up to the time of measurement and which are allowed some freedom of movement. Both SMR and RMR will include Q_s and Q_g but SMR will not include Q_d. RMR will also include some of the metabolism associated

with activity, Q_a. However the extent of activity will depend on the conditions under which respiration is measured and the characteristics of the respirometer.

If the data is to be used to estimate energy budgets then routine metabolic rate is the appropriate measurement and conditions of food availability, light, temperature and water quality must be as close as possible to those of the growth conditions (Lampert, 1984). A difference of 10% such as that caused by application of respiration rates of starving animals (i.e. SMR) to a field energy budget could result in up to a 50% error in calculated production (Lampert, 1984) especially if metabolism was a large part of the overall energy budget. In this study routine metabolic rate measured on animals growing and feeding in laboratory conditions has been used.

Estimated metabolism based on the RMR requires two further elements to take account of total metabolic costs in the energy budget of the animal. The moult is a time of high metabolic rate in some crustaceans (i.e. *Geocarcinus lateralis*, Skinner, 1962; *Hyas araneus*, Anger, 1991). Skinnner (1962) found that integumentary tissue metabolism increased up to 85% relative to earlier stages during late premoult /early postmoult and Anger (1991) reported a 30% increase in metabolic rate of larvae over the same period. Thus the energy used during the moulting process, from D_4 of one moult cycle to A of the next moult cycle, should also be calculated as part (E_r) of the metabolic component of the energy budget.

Secondly, the contribution of activity (Q_a) must be added to RMR. Q_a varies with species and the nature and degree of movement. For example, swimming may increase standard metabolic rate by 130% in the prawn *Penaeus esculentus* (Dall *et al* 1990), 250% in the amphipod, *Gammarus oceanicus* (Halcrow and Boyd, 1967) or 350% in the grass shrimp *Palaemonetes vulgaris* (McFarland and Pickens, 1965). The combination of feeding and walking has less effect, increasing SMR by about 45% in *P. esculentus* (Dall *et al* 1990) and 46% in the tanner crab *Chionoectes bairdi*. (Paul and Fuji, 1989).

1.2 Growth

Energy budgets are constructed to better understand the energy transformations that contribute to the growth process, therefore growth itself must be understood and defined.

1.2.1 Growth in Length

Growth in crustacea is usually described in terms of % moult increment and moult frequency (Hartnoll, 1982, Botsford, 1985). It is seen as a stepwise process where growth in length occurs only at and immediately after the moult. Once the new exoskeleton has hardened further growth in length is limited by the extension or unfolding of the arthrodial membranes (Botsford, 1985). Thus there are rapid increases in size at moult followed by relatively long intermoult periods during which size remains relatively unchanged (Botsford, 1985, Somerton, 1980).

There are two distinct strategies of growth in crustacea (Hartnoll, 1983). Animals such as *Daphnia* (Branchiopoda), *Balanus* (Cirripedia), the lobster *Homarus,* and the freshwater crayfish *Austropotomobius pallipes*, *Cherax* spp., *Paranephrops* spp. and *Pacifastacus lenuisculus* display indeterminate growth (Woodland, 1967, Flint, 1975, Devcich, 1979, Pratten, 1980, Hartnoll, 1983, Momot,1984, Musgrove, pers. obs. 1987) having no terminal anecdysis, but rather moulting occurring until death. Some ostracods and copepods as well as decapods such as *Carcinus* and *Portunus* (Hartnoll, 1983) display determinate growth, having a terminal anecdysis with maturity occurring at or before the final moult. In both cases growth rate is influenced by size. The determinate growth strategy is usually characterised by an increasing intermoult period and an increasing or constant % moult increment, leading to a declining growth rate with size. Those crustacea with indeterminate growth patterns also show a declining growth rate with size, visible as a progressively declining % moult increment and increasing intermoult period (Hartnoll, 1983). In both cases absolute moult increment is generally found to increase with increasing size but may be independent of temperature (Pratten, 1980). The intermoult period appears to be more plastic than moult increment as it is usually inversely proportional to both size and temperature (Hartnoll, 1982).

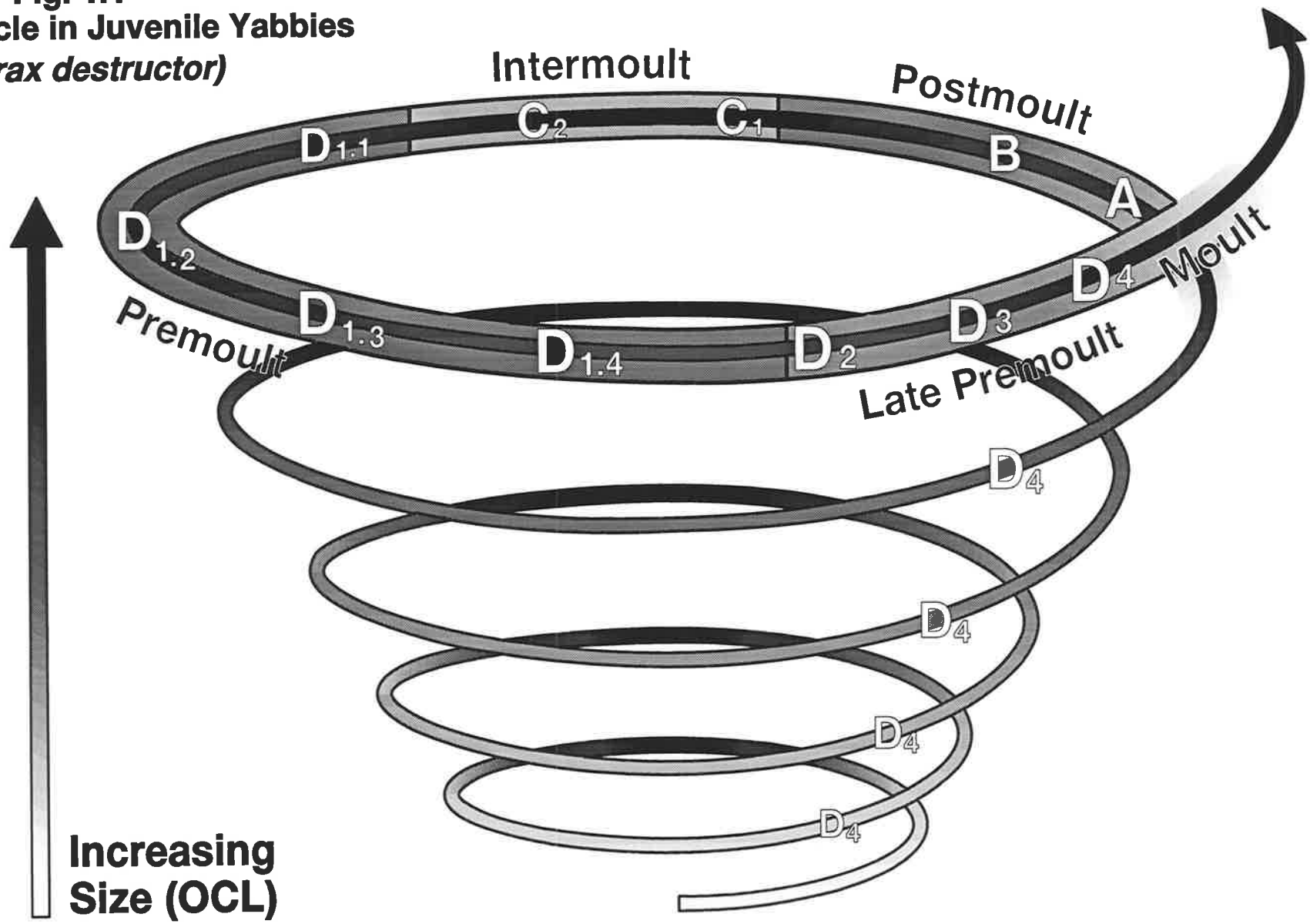
1.2.2 Tissue accumulation and the Moult cycle

The intermoult period and the moult itself comprise the 'moult cycle' (Drach, 1939, Knowles and Carlisle, 1956 - in Aiken and Waddy, 1987). The changes in body length and volume at the moult are out of phase with the real growth processes of cell proliferation and protein synthesis which occur primarily during the intermoult period (Aiken and Waddy,

1987). In this context, growth includes muscle and exoskeleton anabolism and the associated energy and calcium storage and use as animals proceed through the stages of the moult cycle.

Although the moult cycle is a continuum it may be subdivided into substages by reference to morphological changes in the integument which accompany physiological events. Such changes have been catalogued in decapods in general (Drach, 1939), in stomatopods (Reaka, 1975), lobsters (Aiken 1973) and several species of freshwater crayfish (Vranckx and Durliat, 1978, Burton and Mitchell, 1987). Although there is a great deal of species-specific variation in the nature of the changes and author-specific variation as to what exactly constitutes a substage, four general categories have been recognised. In freshwater crayfish these are usually labelled A to D (Aiken and Waddy, 1987) and may be identified by reference to integument state (i.e. soft, firm, hard) and appendage setal development using light microscopy. For the purposes of this study Stage E (ecdysis) (Aiken and Waddy, 1987) is referred to as an event, not a stage. In yabbies the four stages have been divided into 11 substages (Burton and Mitchell, 1987) as shown in **Fig 1.1**. The classification is based on the development of uropod setae and on the integument state. Stages A and B comprise what is usually referred to as postmoult, substages C_1 and C_2 are intermoult, $D_{1.1}$ - $D_{1.4}$ are premoult and D_2 - D_4 are late premoult. Stage A (**Fig 1.1**) begins as soon as the animal is free of the exuvia (Aiken and Waddy, 1987) and progression through this and the other postmoult stage (B) is characterised by formation of the endocuticle, changes in the epicuticle and the progressive hardening of the exoskeleton through calcium deposition (Drach, 1939, Aiken and Waddy, 1987). Stage C begins once these changes are completed and the exoskeleton is at maximum rigidity (Drach, 1939, Burton and Mitchell, 1987). This stage is a time of energy storage, especially for ovarian maturation in adult female lobsters (Aiken, 1980) and freshwater crayfish (i.e. *Orconectes nais,* Armitage *et al*, 1972). The onset of stage D (**Fig 1.1**) marks the beginning of preparation for the next moult which includes gastrolith formation, development of new setae in the appendages and energy storage (Aiken, 1980, Aiken and Waddy, 1987). The begining of this stage is also marked by the retraction of the epidermis from the old cuticle (apolysis). The absolute timing of events such as gastrolith formation varies within and between species. In yabbies, gastroliths have been observed during late intermoult stage C_2 (pers obs) and their formation is acknowledged to be too

Fig. 1.1
The Moult Cycle in Juvenile Yabbies
(Cherax destructor)



variable to be useful as an indicator of the begining of stage D in other crayfish (Rao *et al*, 1977; Stevenson *et al*, 1968). Setal development is completed by stage $D_{1.4}$ (Aiken and Waddy, 1987, Burton and Mitchell, 1987) and the beginning of late premoult (D_2) is characterised by the secretion of the pre-exuvial layers which include the epicuticle and the exocuticle (Aiken and Waddy, 1987). During D_3 the old cuticle is resorbed (Aiken and Waddy, 1987) and in D_4 ecdysal sutures rupture at the bases of the legs and the thoraco-abdominal junction opens (Aiken, 1980, Burton and Mitchell, 1987 , pers obs).

As indicated by the spacing of stages in **Figure 1.1,** moult stages/substages are not all the same length. Early postmoult and late premoult are generally the shortest and stage D the longest in crayfish (Huner and Avault, 1976, Rao *et al*, 1977) and other decapods (Reaka, 1975) although the length of all stages vary with species, size, experimental conditions (Passano, 1960) and probably food sources and availability. The spacing in the figure was based on the results of this study and will be further discussed in Chapter 3.

There is a great deal of interspecific variation in the moult stage-specific body composition (Read and Caulton, 1980, Barclay *et al* 1983, Anger, 1988, 1991, Anger *et al* 1989 and Nicol *et al*, 1992). However there do appear to be some common trends which relate to growth. In juvenile prawns (Read and Caulton, 1980) and marine decapod larvae (Anger 1991) protein deposition continues until late premoult. This suggests that tissue growth is determined more by moult stage than the physical constraints of the exoskeleton. Biomass accumulation is also accompanied by cyclic changes in nitrogen and carbon content as well as micronutrients such as phosphate (Aiken and Waddy, 1987) and elements such as calcium, copper, magnesium, potassium, and sodium (Nicol *et al* 1992). Lipid (Armitage *et al*, 1972) or protein (Barclay *et al*, 1983) or both (Anger, 1991) fuel the growth process depending on species and lipid provides precursors for structural materials such as chitin (Aiken and Waddy, 1987).

1.3 Growth and Nutrition

When studying energy relationships and growth, the nature and formulation of the test diet is important. For example, crustaceans feed to satisfy energy requirements for metabolic processes concerned with maintenance, growth (D'Abramo and Robinson 1989) and reproduction. Whereas feeding rate may be determined by energy intake, growth itself may be determined by protein and energy sources (D'Abramo and Robinson 1989, Reigh *et al* 1990 , Brown *et al* 1990) as well as the Protein : Energy (P : E) ratio (Sedgewick, 1979). Sedgewick (1979) found that prawns (*Penaeus merguiensis* De Man) were feeding to satisfy immediate energy requirements, irrespective of dietary protein content. Food consumption was depressed at high energy levels and growth restricted because energy needs were satisfied before sufficient protein could be ingested. He concluded that the protein : energy ratio was an important consideration in the formulation of artificial diets. Hubbard *et al* (1986) found that optimal growth in the Red Swamp Crayfish (*Procambarus clarkii* Gerard) occurred at an energy : protein ratio of 120 mg protein/kcal (30% protein and 2.5 kcal/g). They suggested that decreased growth in diets with ratios below 80 (more energy per g protein) resulted from depressed consumption of these high energy diets. Similarly, Ackefors *et al* (1992) found that juvenile *Astacus astacus* grew the best on diets with P : E ratios between 114 and 153 mg/kcal.

1.4 Definitions and Conventions in the areas of Nutrition and Energy relations

The following details the diets used here and defines units adopted for respiration and growth studies.

1.4.1 Diets

Coarse diets were used in the following experiments in the absence of precise knowledge of the nutritional requirements of the yabbie. Crayfish growth trials using refined diets have been highly variable. For example, *Procambarus clarkii* (Huner and Meyers, 1979) grew at a

rate close to that under 'austere' field conditions when fed a group of refined diets and *Cherax tenuimanus* grew at less than 35% of the observed field rate (Morrissy, 1989) when fed two widely tested refined diets (BML 81 S and HFX CRD 84). Castell *et al* (1989) found that *C. tenuimanus* was one of two species out of ten crustaceans investigated that would not feed on either of these diets. Growth of marron was also limited in Villareal's (1989) study of the aquaculture potential of the species, although he rigorously followed diets recommended in the literature. Thus, it is apparent that the requirements for refined diets for freshwater crayfish are not well understood. It was assumed that a diet containing a wide variety of nutrient sources would produce better growth than a refined diet.

The diets were based on a locally available 'yabbie' pellet (Laucke Mills, Greenock, SA) modified to approximate the specifications recommended by D'Abramo and Robinson (1989). Three isocaloric (3692 kcal/ kg afdw, 15445 kJ/kg afdw) forms of the diet were made, each with a different protein level (15%, 25% or 35% protein). The digestible energy content and digestible protein : energy ratios were determined in Chapter 2. The diets were made from crop meals (wheat, barley, oats, lucerne), meat meal and fish meal (**Table 1.1a**). The amino acid composition of the 35% protein diet was similar to that found in the yabbies tail muscle (**Table 1.1b**, **Fig 1.2**). **Fig 1.2** uses the approach suggested by Cowey and Tacon (1983) to determine the coincidence between amino acid profiles of the intended diet and requirements of the target species. The diagonal line represents agreement between diet and muscle tissue. Cowey and Tacon suggested this method as a guide to the essential amino acid profile of the diet, especially when feeding young, growing animals where the greatest proportion of body weight gain is in the form of muscle. Out of the ten essential amino acids recommended for crayfish by D`Abramo and Robinson (1989) only tryptophan was undetectable in the tail muscle and the diet. The latter was also undetectable in *Procambarus clarkii* tissue (D'Abramo and Robinson, 1989). The coincidence between the relative percentages in diet and muscle presented in **Fig 1.2** suggests that the 35% diet had an adequate amino acid profile.

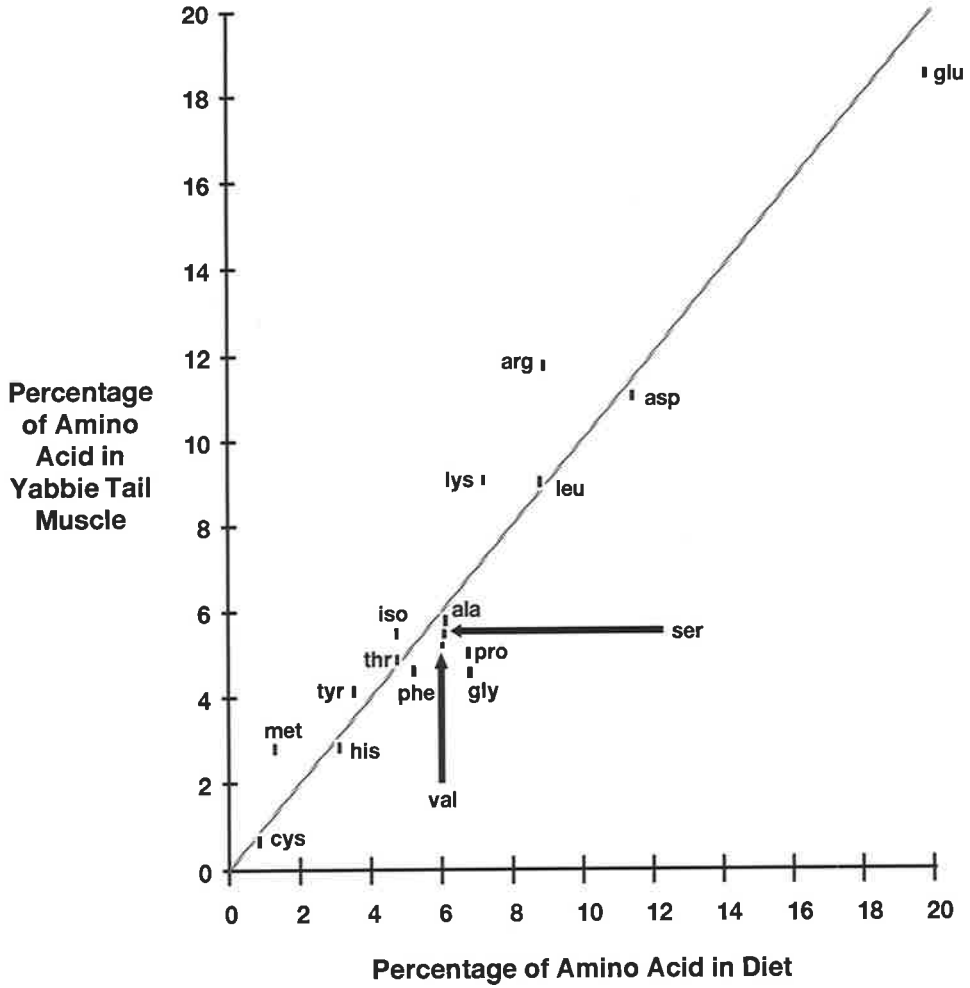
Table 1.1a Diet Composition (refer text). Carboxymethyl Cellulose was used as a binder. P:E = Protein : Digestible Energy Ratio.

	Proximate Composition		
Components	**15% Protein**	**25% Protein**	**35% Protein**
Wheat	18.6		
Barley	30	10	10
Oats	20		
Peas		10	10.4
Lupins		10	20.1
Bran		10	
Malt Low Combs		10	12.6
Lucerne Meal	15	18.5	15
50% Meat Meal	4.7	5	7.7
Samtein Fish Meal		2.5	8
Chilean Fish Meal	5.0	7.5	13.7
Full Fat Soya		10	
Feed Oil	4.2	2.5	
Salt	1	1	1
Limestone	0.4	1.5	
Cellulose	1.5	1.5	1.5
Protein	**15**	**25**	**35**
Energy (kcal/kg)	**3700**	**3700**	**3700**
P:E (see text)	**43.37**	**-**	**110.5**

Table 1.1b Amino Acid Analysis of 35% Protein Diet and Yabbie Tail Muscle.
It was assumed that the other diets would have a similar composition. Units are g/kg.
Those marked with an asterisk are considered essential (D'Abramo and Robinson, 1989).

Amino Acid	Diet (g/kg)	% of total AA in Diet	Tail (g/kg)
Aspartic acid	21.3	10.27	74.9
Threonine*	8.8	4.24	33.0
Serine	11.2	5.40	35.8
Glutamic acid	36.9	17.78	125.2
Proline	12.7	6.12	31.1
Glycine	12.6	6.07	34.3
Alanine	11.4	5.49	39.3
Cystine	1.6	0.77	4.6
Valine*	11.3	5.45	37.3
Methionine*	2.4	1.16	19
Isoleucine*	8.8	4.24	37.3
Leucine*	16.4	7.90	61.3
Tyrosine	6.6	3.18	28
Phenylalanine*	9.7	4.67	31.4
Lysine*	13.4	6.46	61.5
Histidine*	5.8	2.80	19.3
Arginine*	16.6	8.00	79.7
Tryptophan*	not detectable		-

Fig 1.2
Relationship between Patterns of Amino Acids in 35% Protein Diet and Yabbie Tail Muscle



Key

ala	Alanine
arg	Arginine *
asp	Aspartic acid
cys	Cystine
glu	Glutamic acid
gly	Glycine
his	Histidine*
iso	Isoleucine*
leu	Leucine*
lys	Lysine*
met	Methionine*
phe	Phenylalanine*
pro	Proline
ser	Serine
thr	Threonine*
tyr	Tyrosine
val	Valine *

Note: those marked with an asterisk are considered essential (see text)

1.4.2 Respiration

The measurements of 'metabolic rate' made in this study represent 'Routine Metabolic Rate' (RMR) (Anger, 1991) as it was measured on animals in constant temperature conditions which had access to food up until three hours before testing. SMR was not measured for two reasons. Firstly, it is considered inadequate in ecologically based studies because the costs associated with tissue production are not measured (Lampert, 1984) and secondly, it is rarely 'standard' in the sense of comparable between species as it is seldom clear if, or to what extent, growth has been stopped (Parry, 1983). TMR was not measurable in any meaningful way as it includes the cost of activity which is hard to standardise, although RMR will approach TMR if growth and respiration are measured in similar conditions in the laboratory. For the purposes of this study RMR is referred to as MO_2 (mg/h or ug/h) or weight-specific MO_2 (mg or ug/unit weight/unit time).

1.4.3 Growth Measurement

Growth in crustaceans may be measured in terms of either length (i.e Total Carapace Length (TCL), Orbital Carapace Length (OCL)) or weight (i.e. wet, dry or ash free). Although the data collected depends on the aims of the experiment, length measurements alone are not adequate when describing growth in crustaceans as tissue growth and cell proliferation occur during the intermoult period and are thus out of phase with growth in length (Aiken and Waddy, 1987). Thus a postmoult or intermoult animal (i.e. moult stages B or C_1) would weigh less than an animal of the same length but at late premoult stage D_2. However, if the growth rate was based on length and expressed as mm OCL/day both would be considered to have grown at the same rate.

Growth in weight may be measured using wet weight, dry weight or ash free dry weight (afdw). Wet weight changes little in yabbies during the moult cycle as water is replaced by tissue (pers obs) and so it is not a good reflection of tissue growth (Anger, 1991). Dry weight includes ash, especially in the form of the calcium carbonate of the exoskeleton. As an energy

budget is primarily concerned with the accumulation and loss of organic matter, ash free dry weight is the most suitable parameter.

Growth may be described in absolute terms (mg afdw/day) or in weight-specific terms (mg/mg afdw/day). The first is limited in usefulness for long-term experiments as it is dependant on initial weight. Weight-specific or 'instantaneous' descriptions of growth are also not truly independent from weight as they have been shown to decline with size in field studies of crayfish (*Austropotamobius pallipes*; Pratten, 1980, Brewis and Bowler, 1982), amphipods (*Gammarus pulex*; Sutcliffe *et al*, 1981) and in laboratory studies of larval marine decapods (Anger, 1991). However, instantaneous growth rates (IG) provide reasonable average growth estimates in short experiments with juveniles of similar initial weight. The calculation of IG assumes exponential growth in weight over the period growth is measured and this has been reported for some juvenile crustaceans (Pratten, 1980, Sutcliffe *et al* 1981, Brewis and Bowler, 1982).

Therefore, growth in this study is expressed in terms of ash free dry weight and rates calculated assuming a exponential model by estimation of instantaneous growth rates.

1.5 Aims and Experiments

This study was prompted by an interest in the process of energy use in crustacea in general and freshwater crayfish in particular. The work uses the juvenile yabbie in laboratory and field experiments to examine changing patterns of tissue accumulation and energy intake and use with size and during the moult cycle. Models of moult stage-specific tissue accumulation, size and temperature-specific growth and metabolism and size-specific energy use are presented for this species. This latter model can provide a guide for intensive culture of juvenile yabbies.

The study was initiated by selecting and testing a diet (Chapter 2) to determine its usefulness in further experiments. Energy and protein digestibility and the effect of energy and protein content on food intake were determined. Experiments were also carried out on the relationship between energetic requirements and size, and therefore the appropriate feeding

rate given a certain diet. Finally, the experiment was combined with measurement of the size : metabolic rate relationship to determine the correlation between energy intake and energy expenditure on metabolism. Chapter 3 investigates field growth under semi-intensive aquaculture conditions where juvenile yabbies were grown within enclosures in an above-ground pool. Tissue accumulation, energy storage and use were examined by determination of moult stage and size-specific body composition. Chapter 4 investigates the effect of temperature and size on growth and the components of growth in the laboratory. Chapter 5 presents work on the effect of temperature and size on the respiration rate of the individuals in the growth experiment (Chapter 4). Chapter 6 presents an energy budget and growth model in terms of tissue accumulation and energy use over the moult cycle during the juvenile phase and Chapter 7 summarises the main points and presents the conclusions.

Chapter 2.

The Effect of different Protein levels on Growth, Feeding, Assimilation and Energy use in the Laboratory.

2.1 Introduction

Crustaceans feed to satisfy energy requirements for metabolic processes concerned with maintenance, growth (D'Abramo and Robinson 1989) and reproduction. Whereas feeding rate may be determined by energy intake, growth itself may be determined by protein and energy sources (D'Abramo and Robinson 1989, Reigh *et al* 1990 , Brown *et al* 1990) as well as the Protein : Energy (P : E) ratio (Sedgewick, 1979). Thus the nature and formulation of the diet is important when studying energy relationships and growth.

The coarse pelleted diets discussed in Chapter 1 were evaluated to determine their potential for growth promotion and thus their suitability for future experiments. The use of three diets of differing protein levels also allowed investigation of the relationship between 'protein nutrition' and bioenergetics for this species. Varying the protein content at a given energy level also provides information on the necessary protein level for good growth.

The first experiment in this chapter considers the three diets in terms of the effect of dietary protein levels on growth. The second experiment uses two of the diets (15% and 35% protein) to investigate the relationship between energy intake and energy use in the laboratory. Energy and protein digestibility as well as the effect of energy content on food intake were investigated and 'effective' P : E ratios calculated. The experiment used different sized individuals and so provided information on the relationship between energetic requirements and size, and therefore the appropriate feeding rate given a certain diet. Finally, the experiment was combined with measurement of the size : metabolic rate relationship to determine the correlation between energy intake and energy expenditure on metabolism.

Growth in this chapter is expressed in terms of instantaneous growth (mg/mg afdw/day). Metabolism was measured as routine metabolic rate (RMR, Lampert, 1984, Anger, 1991).

As discussed in Chapter 1, this is measured on animals in constant temperature conditions which have had access to food prior to testing. For the purposes of this study RMR is referred to as MO_2 (mg/h or ug/h) or weight-specific MO_2 (mg or ug/unit weight/unit time). RMR was used following Lampert's suggestion that metabolism should be measured on fed animals under as natural conditions as possible. It was also considered appropriate because a fed animal is presumably growing and growth accounts for most of the expenditure on metabolism in juveniles (Parry, 1983).

2.2. Methods

Diets were received from the manufacturer in pellet form and were kept in a -10°C freezer until needed. Prior to their use in feeding experiments, portions of the diets were dried overnight at 60°C then stored in a dessicator over silica gel. On the basis of the results of Experiment 1, all diets were ground to one mm and repelleted for use in Experiment 2 and subsequent work. This involved mixing the ground diet to a stiff dough with distilled water (125ml/500g diet), rolling it out into a sheet of approximately 5 mm thickness and cutting it into 5-10 mm squares. After drying at 60°C overnight the diet was stored in a dessicator as above. There was no significant loss in Kjeldahl nitrogen during this process (Students *t*, P >0.05). Pellet integrity also appeared to have been improved by this process.

Data were analysed with parametric and nonparametric methods using the package $SYSTAT_{(TM)}$ on a microcomputer. Analyses were carried out after testing for normality. If the data were normal or could be normalised methods including Student's *t* , ANOVA and ANCOVA were applied. In one case data could not be normalised and a Mann-Whitney U test was used.

2.2.1 Growth of juvenile *Cherax destructor* on diets with different protein levels in the laboratory under clean conditions

In order to test growth caused by the three diets without physical interference from other yabbies and with minimal bacterial contribution, a `clean` battery system was designed in which the animals were held individually.

a) The Animals

Two size classes of animals were chosen for the growth experiment. Eighty juvenile yabbies (0.685 ± 0.012 g) were randomly selected from a group collected from a local pond. One hundred stage three (14 mg) juveniles were randomly selected from a brood provided by a berried female collected from the same pond.

b) The System

A clean, flow through, filtered battery system was designed **(Fig 2.1).** Yabbies were held individually in 1.25 L plastic 'Petalite' (Coke) bottles which had been modified and integrated into the flow-through system **(Fig 2.1)**. The bottles were placed horizontally within large plastic trays (670 x 410 x 70mm). Water flowed in a small hole cut in one side (i.e. the 'top') and out through the mouth of each bottle. The larger juveniles were in about 750 ml of water while bottles were cut down to about 550 ml capacity for stage three juveniles. Each bottle was provided with a piece of plastic mesh `substrate' and a tube shelter and each tray covered with onion mesh to minimise disturbance. Five large bottles or ten small bottles were held in each tray.

Dechlorinated tapwater was pumped from a reservoir tank to a header tank via a charcoal filter and a 0.45um bacterial filter. The reservoir and header tank were connected by 35 mm plastic tubing and there was a 35mm 'overflow' return to the reservoir. Passive flow was directed down from the header tank via a plastic manifold to individual 13 mm tubes servicing each tray. Each tube was controlled by a tap. Within each tray the tube was positioned in an arc which covered all bottles in the area of the inlet. The end of the tube was stoppered and flow directed into each bottle through hypodermic needles attached to threaded `Nylex' hose fittings which had been screwed into the tubing. One end of each tray was elevated slightly and the water flowed through the tray to a small hole and then via a tube to a biological filter before trickling into the reservoir.

Water flowed through the system five times a day. The reservoir was vigorously aerated. The system was flushed and cleaned and the water replaced once a week.

c) The Experiment

The water temperature was maintained at 24°C and the light at 12:12 LD. The experiment comprised two growth trials, the first using 14 mg stage three juveniles and the second juveniles that were 0.685 ± 0.012 g. In each trial twenty to thirty intermoult animals from each size class were randomly assigned to each of four treatments. These were: unfed, 15, 25 and 35% protein. Animals were randomly assigned to bottles within trays irrespective of diet. Yabbies were fed at about 10% body weight per day every third day. Exuviae were left to be eaten. OCL was measured on all yabbies at the beginning and end of each trial and during the experiment it was measured once a week for the stage three juveniles and once a fortnight for the 0.69 g animals. This allowed estimation of moult increment in millimetres. Wet weight was also measured (to the nearest 0.1mg) and moult stage determined (Burton and Mitchell, 1987) at the beginning and end of the each trial. The first trial lasted 120 days and the second 102 days.

As the stage three juveniles were too small to measure with vernier callipers, measurements were made using a microscope/video camera and a digitiser (**Fig 2.2**). A binocular dissecting microscope with a drawing head was attached to a video camera which was connected to a TV monitor. A digitiser was positioned under the arm of the drawing head. A mouse with an optical fibre light fixed to its top was used on the digitiser and the light was reflected up through the drawing head. The light, appearing as a sharply defined dot, was thus also reflected back up into the body of the microscope into the video camera and onto the TV screen. As the specimen was also visible on the screen, measurements could be taken by moving the mouse on the digitiser so that the light moved from one end of the carapace to the other. The device was calibrated using a standard microscope calibration scale with divisions at 0.1mm. Calibration was based on the mean of ten measurements. The mean and the resulting conversion factor were calculated by a computer program which used the factor to convert incoming coordinates from the digitiser into millimetres OCL.

The OCL measurements were used to calculate moult increment in length. The wet weight data were converted to ash free dry weight by back-calculating from wet weight using a mean conversion based on animals of similar size and moult stage analysed in Chapter 5. Ash free

Fig 2.1
Flow-Through Battery System

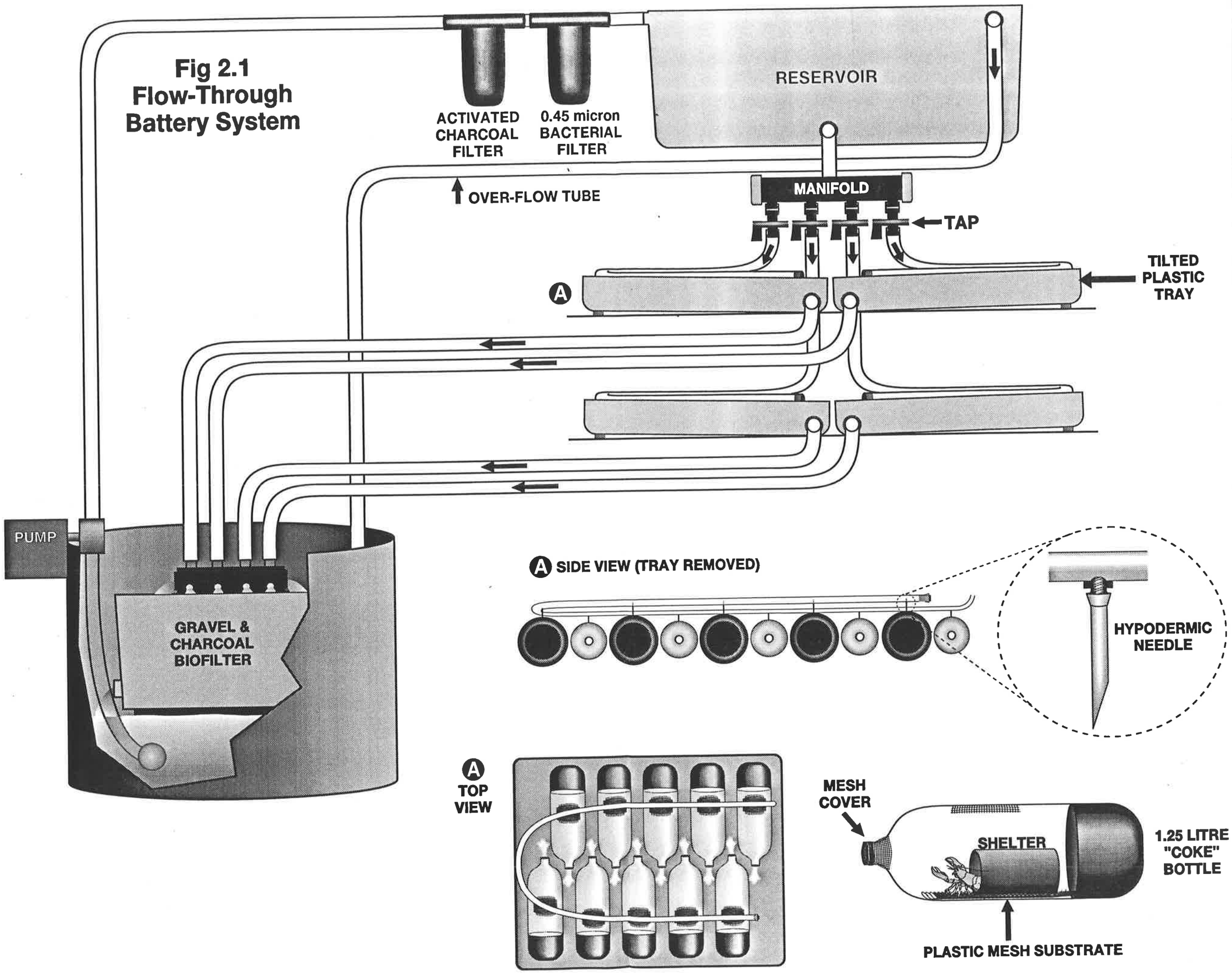
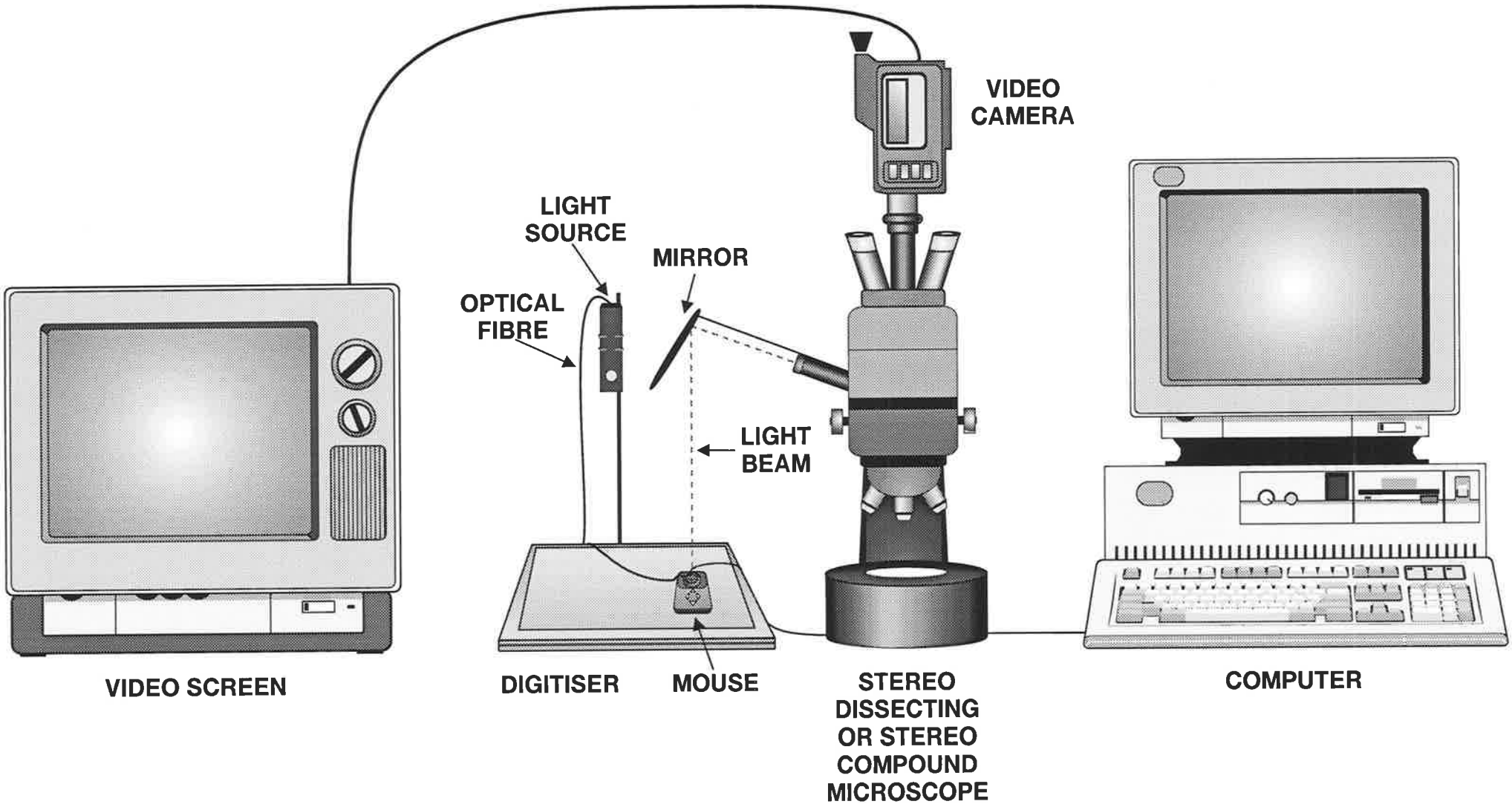


Fig 2.2
Measuring System for Stage Three Juveniles (refer text)



dry weight for animals from stage C_1 to D_3 averaged 20.416 % (± 0.2637 %; n=149) of wet weight. In the present experiment animals were at these moult stages when initial and final weights were taken.

Instantaneous growth rates (IG, Pratten, 1980) were calculated in terms of ash free dry weight according to the formula:

$$IG = Ln(W_t/W_o)/T \qquad \text{Equation 2.1}$$

where W_o = initial weight

W_t = final weight

T = Time

Growth data were analysed using ANOVA and ANCOVA.

2.2.2 Feeding, Assimilation and Energy use in the laboratory on 15 % and 35% protein diets.

The low (15%) and high (35%) protein diets from the growth experiment were used in feeding, assimilation and respiration experiments. Yabbies were kept under the same conditions (12:12 LD, 24°C) as before. An apparatus was designed in which both feeding and respiration experiments would be run (**Fig 2.3**). The feeding chamber was a 1.25 L plastic 'Petalite' bottle with a hole cut in its 'upper' surface to allow contact with the surrounding water. The thread of the bottle allowed attachment to the respirometer. All apparatus was submerged in bins lined with black plastic and filled with aerated water during trials. The bins were covered with onion bags to reduce disturbance.

Respirometry was conducted using a semi-closed system. Five respirometers were made (**Fig 2.3**) based on a design by Titulaer (1991). Each unit was a sealable perspex tube with a volume of 300 ml. Water was circulated by a propeller attached to a 12 volt motor housed in a sealed chamber. All motors were attached to a common variable-speed controller. The motor chamber formed one end of the respirometer and the other end was closed using a perspex

Fig. 2.3 Respirometer-Feeding Chamber

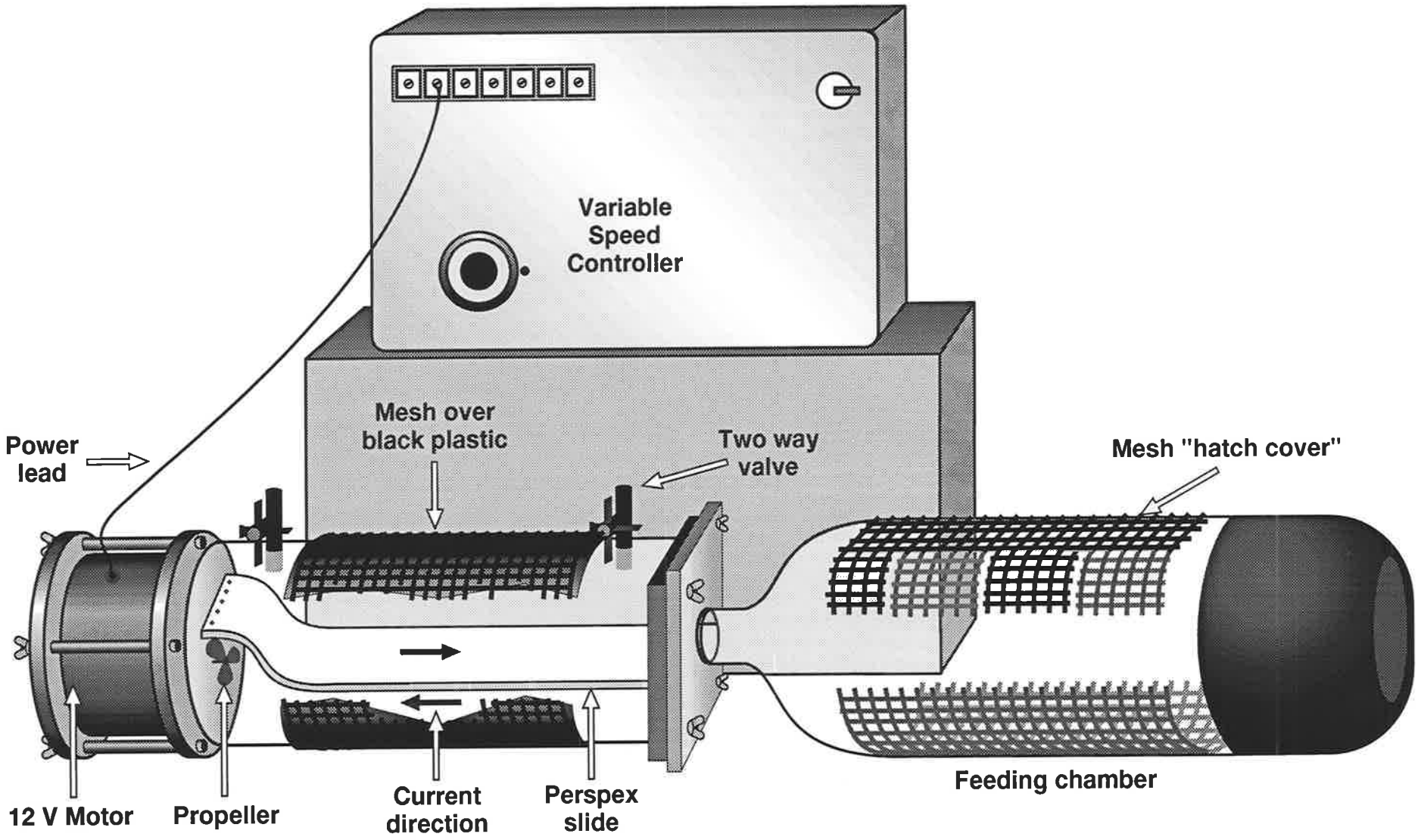


plate. Flat 'food quality' rubber washers were inserted between the plate/motor and the respirometer to form water-tight seals. Water samples were taken through two-way valves glued to small holes drilled through the dorsal surface of each perspex tube. These sample ports were located at either end of the tube (i.e. at the motor end (proximal) and at the plate end (distal)).

The body of each respirometer was wrapped in black plastic and a moulded perspex slide provided a platform for the yabbie. Water was directed through the slide via tapered holes drilled in either end (**Fig 2.3**) An alternative endplate was designed to receive the threaded end of the feeding chamber (**Fig.2.3**) during acclimation. Each yabbie was acclimated to the conditions for two days prior to the experiment in a unit comprising a respirometer and a feeding chamber. All animals were observed to readily `adopt` their respirometer as a shelter. This behaviour made it possible to reduce handling as the animals would move into their shelters when the covers were removed from the bins.

All yabbies were acclimated to diets and conditions for two weeks prior to experiments. They were weighed (to the nearest mg) and moult staged before and after each experiment. Intermoult to early premoult animals (Stages C_1 to $D_{1.1}$ - Burton and Mitchell, 1987) were used throughout.

a) Feeding and Assimilation

In the feeding and assimilation experiments, total faecal collection was used in preference to inert marker methods (e.g. chromic oxide); this is consistent with the recommendations of Brown *et al* (1986) and Reigh *et al* (1990).

Feeding and assimilation experiments were done on crayfish in three size groups (1-3g, 5-8g and 10-15g) with twenty-four animals used for each diet. Animals were randomly assigned (within size classes) to the 15% or the 35% protein diet and acclimated for two weeks during which time they were fed *ad libitum* daily. This period allowed estimation of the appropriate ration of food to be provided in the experiments. In the feeding trials animals were fed in excess with preweighed dried food and allowed to feed for two hours; this process was carried out twice a day. The aeration in the bins was turned off during this period to prevent loss of

food. Oxygen levels did not fall below 70% saturation in any chamber. At the end of the period excess food was siphoned from the chamber into a small plastic container with a Pasteur pippette attached to a length of plastic hose. The faeces were removed in the same way before and after each feeding period. Food was then collected onto preweighed glass fiber filters (Whatman No. 1), freeze-dried and weighed. Faecal material was not filtered as it proved impossible to remove from filters for further analysis. Individual faecal samples were siphoned from each feeding chamber into small plastic containers, frozen at - 10°C and freeze-dried. As individual daily faecal production was generally low, faeces produced by each crayfish during the experiment were pooled before weighing.

Feeding in each animal was measured on five days, evenly spaced over approximately a two week period. Daily consumption, as dry weight of food consumed, was calculated as food provided minus uneaten food collected . Daily faecal production was calculated as total faeces divided by the five day feeding period for each animal. The animals were fed using the same routine on days between experiments.

The food and faeces were analysed for nitrogen and energy content using Kjeldahl Total N analysis (Dowgiallo, 1975) and a Phillipson microbomb calorimeter (Gentry Instruments) respectively. Benzoic acid (26.454 kJ/g, Brafield, 1985) was used as a standard for the calorimetry. Daily assimilation of energy was calculated as daily consumption minus daily faecal production for each individual. Protein digestibility was calculated as total consumption minus total faecal production for each individual.

Both consumption and assimilation were corrected for leaching as follows. Dry samples (120 g) of each diet were weighed into 2 L plastic containers. Five containers were used per diet for each of six time intervals (0,15, 30, 45, 60 and 120 mins). Containers within diets were randomly assigned to time intervals. At the appropriate time (i.e. at 0,15, 30 mins etc.) five containers were harvested per diet. The contents were collected onto preweighed glass fiber filters (Whatman No. 1), freeze dried and weighed. Loss was measured in terms of organic content (Ashing, 500°C, 24 hrs) and nitrogen (Kjeldahl). It was assumed that energy content was proportional to organic content. Nitrogen was converted to protein by comparing

the manufacturer's assayed protein content with the measured Kjeldahl nitrogen content. Regressions were then calculated between organic content, protein content and time.

b) Respirometry

Respiration rates (RMR) were measured on 43 yabbies. Thirteen randomly selected crayfish ranging in size from 1.5 - 9 g were fed the 15% protein diet and a further 13 animals within the same size range fed the 35% protein diet. The remaining 17 crayfish, ranging in size from 0.25 to 1.5g were fed the 35% protein diet. Four animals were tested simultaneously, with the fifth respirometer used as a control. In each case RMR was measured for a time appropriate to the size of the animal (25 mins -1 hour) such that oxygen levels in the respirometers did not fall below 70% saturation. At the end of each test period duplicate 5 ml samples were taken from the distal port of experimental and control respirometers while at the same time introducing an equal volume of bin water from the proximal port. Tests using rhodamine dye suggested that there was no contamination of the samples with incoming water. Water samples were analysed using a blood-gas analyser (PHM 71 mk2 Acid Base Analyser). Samples of bin water were also taken and measured just before each respirometer was closed.

The respirometers were then opened for an hour so that they could be flushed with well oxygenated water. This time was more than adequate as replacement of water within a respirometer took about 3 mins (determined using rhodamine dye). The open end was covered with plastic mesh (2 ml) during this period to contain the yabbie.

Each animal was tested over three consecutive days and the respiration rate measured three times a day. The animals were allowed to feed for two hours before and after each day's run. Diurnal variation in respiration rate was not considered as previous workers (Woodland, 1967; Fradd, 1974) have been unable to find any such rhythm.

2.3 Results

2.3.1 Growth of juvenile *Cherax destructor* on diets with different protein levels in the laboratory under clean conditions

Survival was good in all except the unfed treatment in both trials. (**Table 2.1**). All the small yabbies in the unfed treatment died within two weeks of the beginning of the experiment and 25% of the large unfed yabbies survived the entire period although 75% were still alive after 80 days. No unfed animals moulted during the experiment. All animals showed some loss of pigmentation during the experiment.

Maximum growth was achieved in the thirty five percent diet in both trials. In that treatment the small individuals grew from 14 mg to 268mg and the large yabbies grew from an average of 680mg to 4020mg (**Table 2.1**). All individuals in the fed treatments showed positive growth in terms of wet weight throughout the experiment, although there was considerable variability (**Fig 2.4a to f**). Instantaneous growth rates (IG) were calculated for the whole period (Average IG) and for 14 to 25 day periods within each trial. Calculation was based on ash free dry weight and carried out for each individual in all treatments. In the first trial (14 mg initial weight) there was a significant relationship between Average IG and protein level (Students *t*; P = 0.05,0.001, **Table 2.2**); all animals were very similar-sized stage 3 juveniles in this trial. Mean growth rates ranged from 0.0154 (± 0.0006) mg/mg afdw/day for crayfish fed the 15% diet to 0.0266 (± 0.0004) mg/mg afdw/day for animals fed the 35% diet. In the second trial, mean IG was 0.017 (± 0.0009) mg/mg afdw/day for the 15% diet, 0.0186 (± 0.001) mg/mg afdw/day for the 25% diet and 0.0236 (± 0.0012) mg/mg afdw/day for the 35% diet.

Table 2.2 The Relationship between Average Instantaneous Growth Rate (Average IG) and Dietary Protein Level for Trial 1

Treatment	Mean	SE	Compared diets	t_{calc} (DF)	P
15	0.0154	0.0006	15,25	1.728 (41)	0.05
25	0.0168	0.0005	25,35	14.304 (46)	0.001
35	0.0266	0.0004			

In this trial IG was significantly affected by initial size (**Fig 2.5**).($b < 0$, $P<0.001$) in all treatments as shown by the regression statistics in **Table 2.3.** Comparisons between line intercepts show that growth increased significantly with dietary protein level (ANCOVA, **Table 2.4**). The slope of the line was also significantly different between 25 and 35% Protein

Table 2.1 Survival and Growth in the lab when fed one of three isocaloric diets containing either 15, 25 or 35% protein.

a) Trial 1 Length of Experiment = 120 days

Treatment (% Protein)	Number of Yabbies		Mean Initial Wet Weight (mg) (± SE)	Mean Final Wet Weight (mg) (± SE)	Mean Initial AFDW (mg) (± SE)	Mean Final AFDW (mg) (± SE)
	Initial	Final				
Unfed	25	0	14.30 (0.15)	NA	2.910 (0.03)	NA
15%	25	19	14.04 (0.15)	91.33 (4.93)	2.866 (0.032)	18.647 (1.007)
25%	25	24	14.33 (0.18)	106.22 (4.09)	2.926 (0.036)	21.685 (0.834)
35%	25	24	14.60 (0.14)	268.40 (12.82)	2.981 (0.028)	54.800 (2.616)

b) Trial 2: Length of Experiment = 102 days

Treatment (% Protein)	Number of Yabbies		Mean Initial Wet Weight (mg) (± SE)	Mean Final Wet Weight (mg) (± SE)	Mean Initial AFDW (mg) (± SE)	Mean Final AFDW (mg) (± SE)
	Initial	Final				
Unfed	20	5	678.5 (21.09)	759.8 (61.66)	140.250 (5.08)	NA
15%	20	17	689.41 (25.08)	2222.00 (139.56)	140.750 (5.120)	453.644 (28.492)
25%	20	19	693.89 (26.75)	2482.50 (168.28)	141.664 (5.461)	506.827 (34.357)
35%	20	18	685.00 (25.49)	4017.17 (356.89)	139.850(5.205)	820.145(72.864)

Table 2.3 The relationship between Initial AFDW (mg) and IG for animals fed three different diets in Trial 2.
*** P<0.001. The equation is IG = a+b (Initial AFDW).

Treatment : % Protein	**n**	**Mean IG (±SE)**	**Slope (b)**	**SE(b)**	**Intercept (a)**	**SE (a)**	**r^2**
15	16	0.017 (0.0009)	-0.00013	0.00002	0.02779	0.00167	0.7450**
25	17	0.0186 (0.001)	-0.00009	0.00003	0.0265	0.002	0.4450**
35	17	0.0236 (0.0012)	-0.00021	0.00004	0.0383	0.0031	0.6230**

Fig 2.4a
Laboratory Growth on the 15% Protein Diet :
Initial weight 14 mg

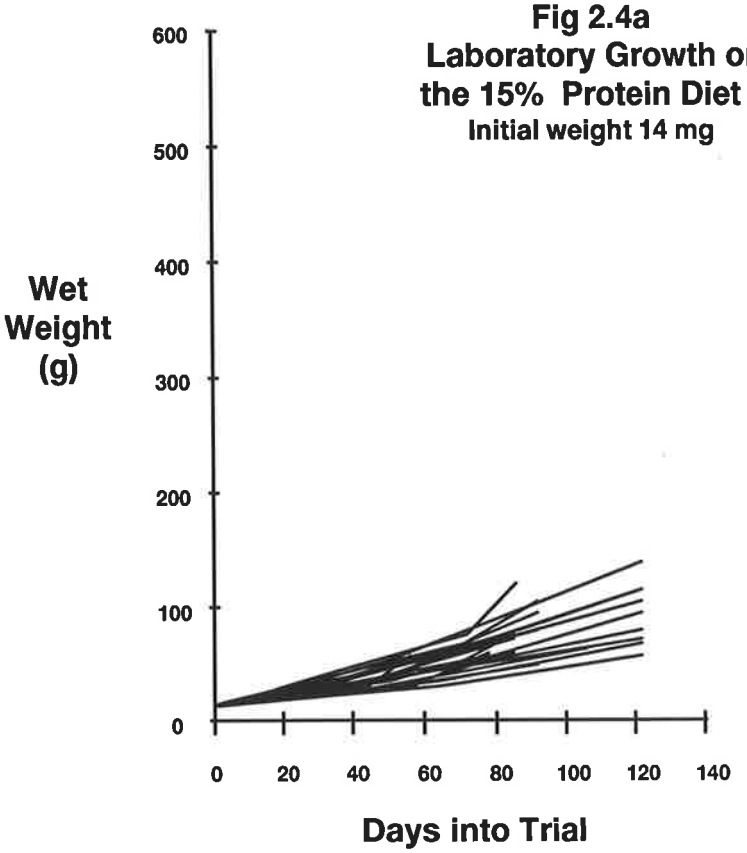


Fig 2.4b
Laboratory Growth on the 25% Protein Diet :
Initial Weight 14 mg

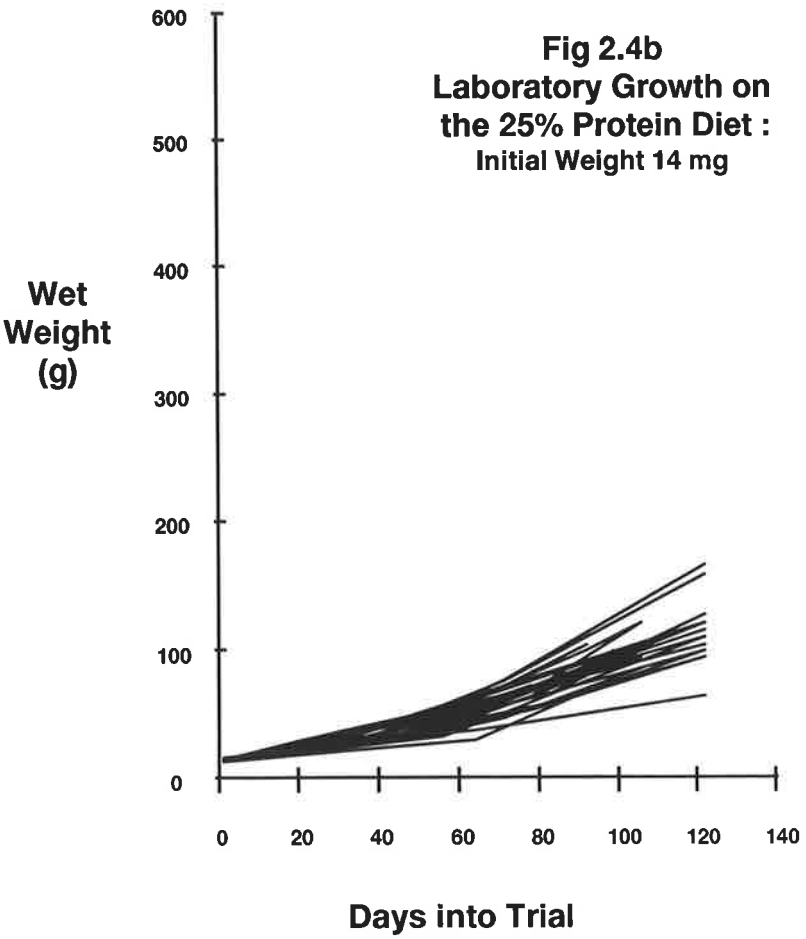


Fig 2.4c
Laboratory Growth on the 35% Protein Diet :
Initial Weight 14 mg

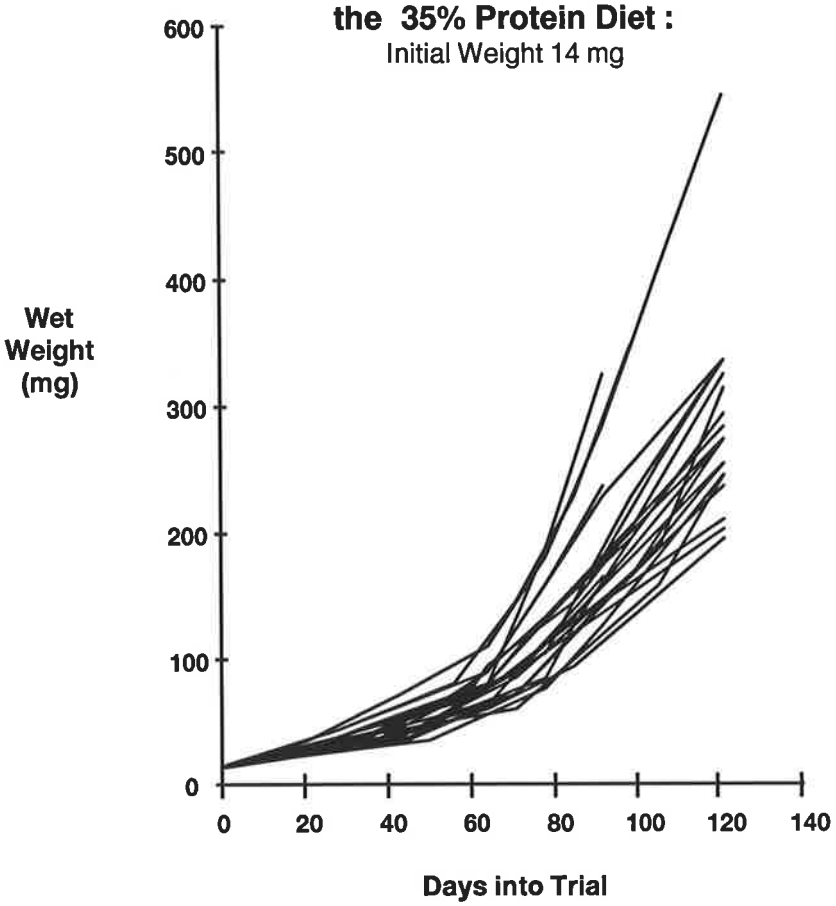


Fig 2.4d
Laboratory Growth on the 15% Protein Diet:
Initial Weight 680 mg

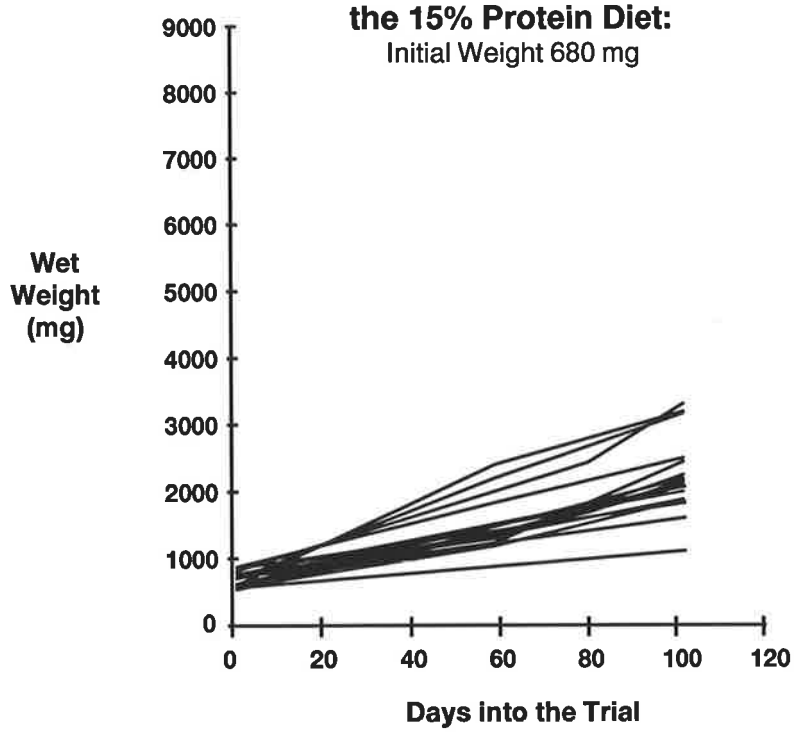


Fig 2.4e
Laboratory Growth on the 25% Protein Diet:
Initial Weight 680 mg

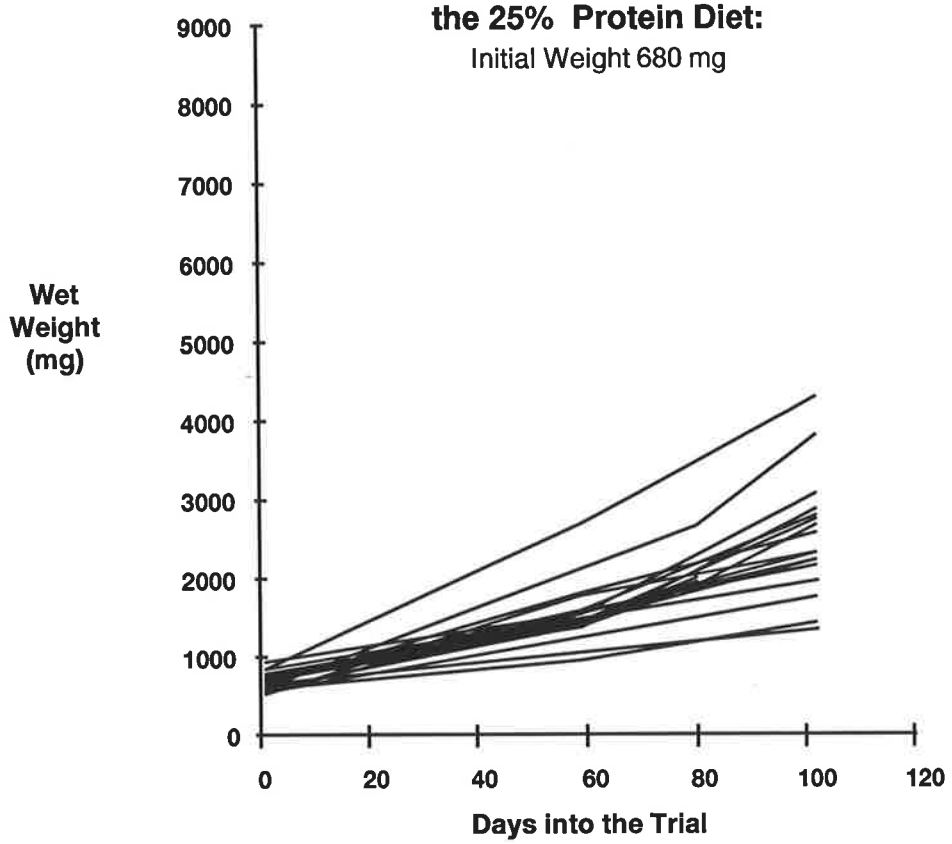


Fig 2.4f
Laboratory Growth on the 35% Protein Diet :
Initial Weight 680 mg

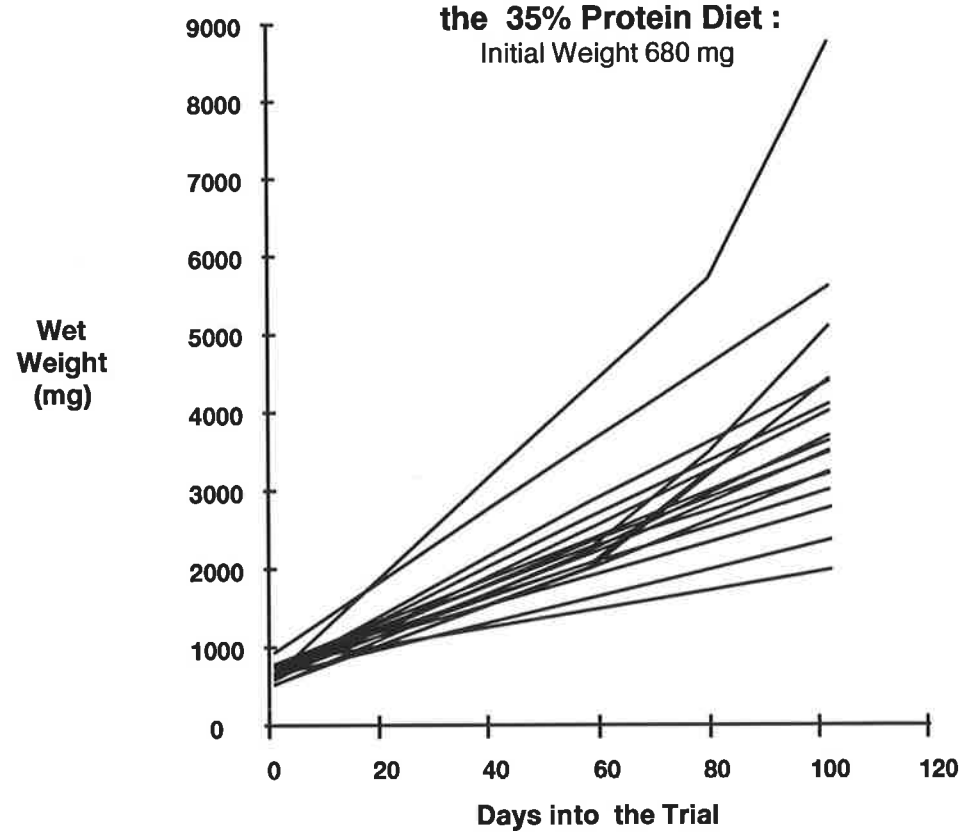
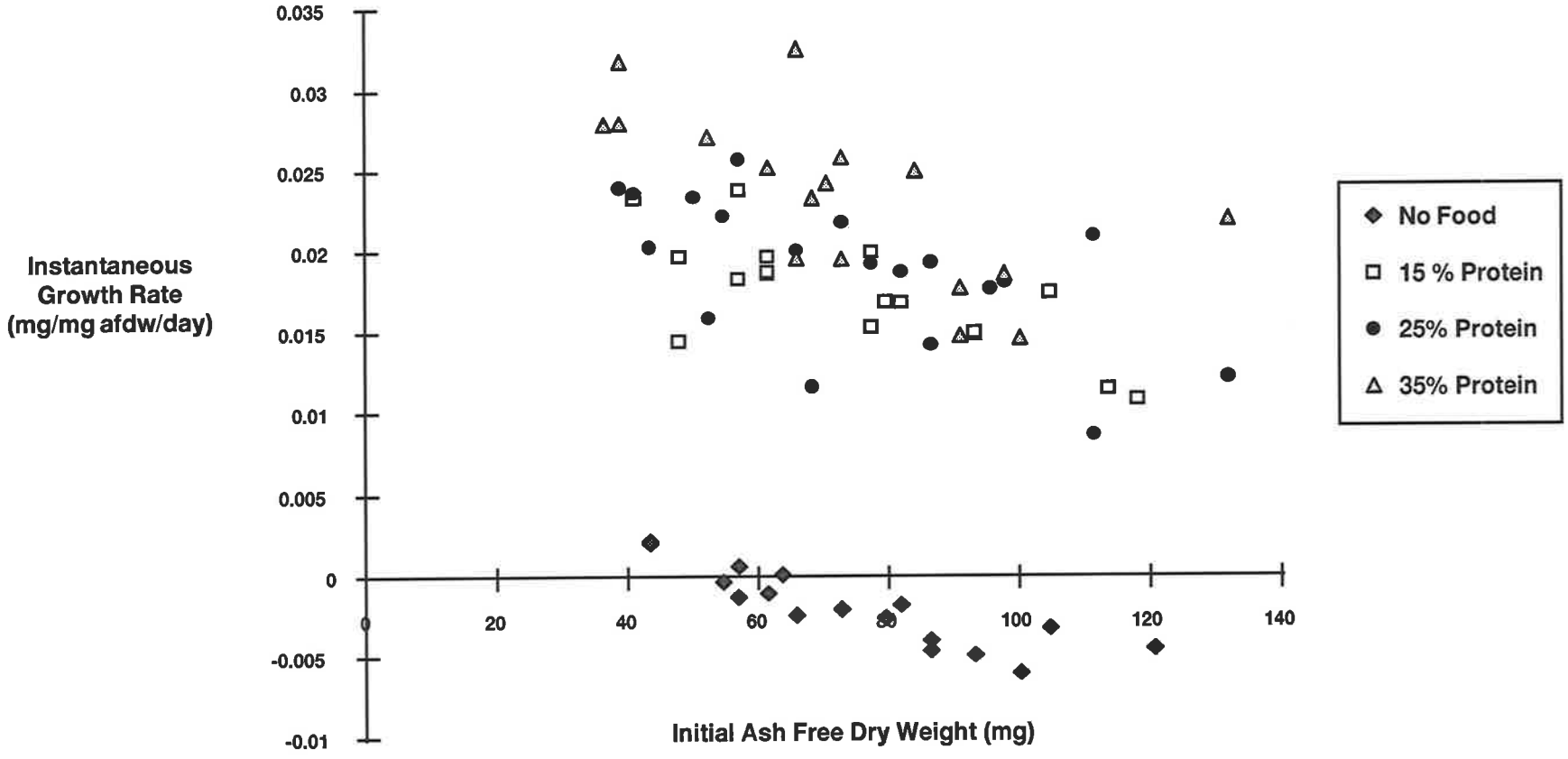


Fig 2.5
Instantaneous Growth Rate (IG: mg/mg afdw/day) vs Initial AFDW (mg): Initial Size 0.68g



(P<0.001) but there was no consistent trend as 'b' for 35% was no different from that for 15% protein (P>0.05).

Table 2.4 Comparisons between Diet-specific IG vs Initial AFDW relationships in Trial 2 using ANCOVA. * P<0.05, ** P<0.025, *** P<0.001.

Compared Treatments (% Protein)	f_{calc} (slope)	df	f_{calc} (intercept)	df
15,25	1.5898	1,29	5.3631*	1,32
25,35	6.3903**	1,30	7.2214**	1,33
15,35	2.911	1,29	21.5312***	1,32

IG values calculated for shorter periods within each individual's growth trajectory are shown in **Fig 2.6**. Each IG value is an average for the preceding growth period and was calculated to include at least one moult per animal. **Fig 2.6 a to c** suggest that there was no size effect on the growth rate of yabbies in Trial 1 whereas **Fig 2.6 d to f** show a decline in IG with size for the larger yabbies in Trial 2.

Moult increment as a proportion of premoult length ranged between 11 and 25 %. The means (±SE) for each moult within each treatment are presented in **Table 2.5**. Animals in the 15% Protein treatment had a lower number of moults per trial than animals on higher protein diets with an average of only 4 moults in Trial 1 and 3 moults in Trial 2.

Therefore, so that similar growth stages were being compared between treatments, the first four moults for each animal in the Trial 1 and the first three moults for each animal in the Trial 2 were used for analysis of moult increment within trials between diets. Variance in % moult increments was homogeneous (fmax, P>0.05) between moult numbers within treatments and subsequent *t* tests (Students *t*, Sokal and Rohlf, 1981) showed no significant differences between mean moult increments at each successive moult (P>0.05). Therefore this data for each treatment was pooled and tested within trials between treatments using ANOVA. Data were normalised using an arcsin transformation (Sokal and Rohlf, 1981).

Fig 2.6a
Laboratory Growth
on the 15% Protein Diet :
Trial 1

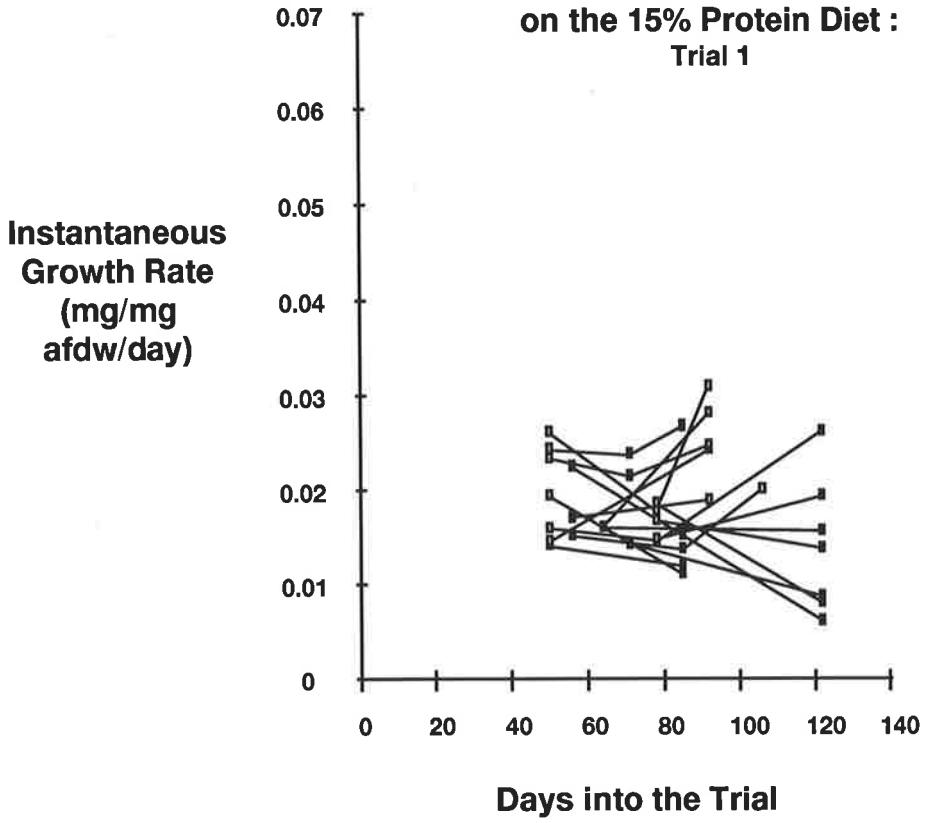


Fig 2.6b
Laboratory Growth
on the 25% Protein Diet:
Trial 1

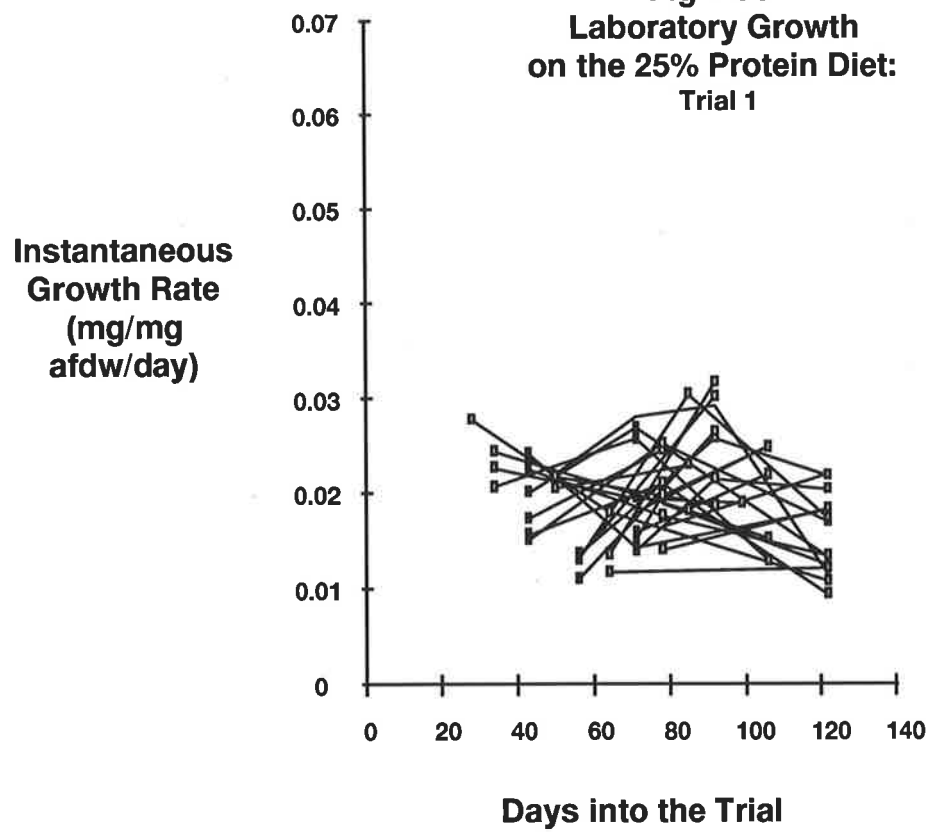


Fig 2.6c
Laboratory Growth on the 35% ProteinDiet:
Trial 1

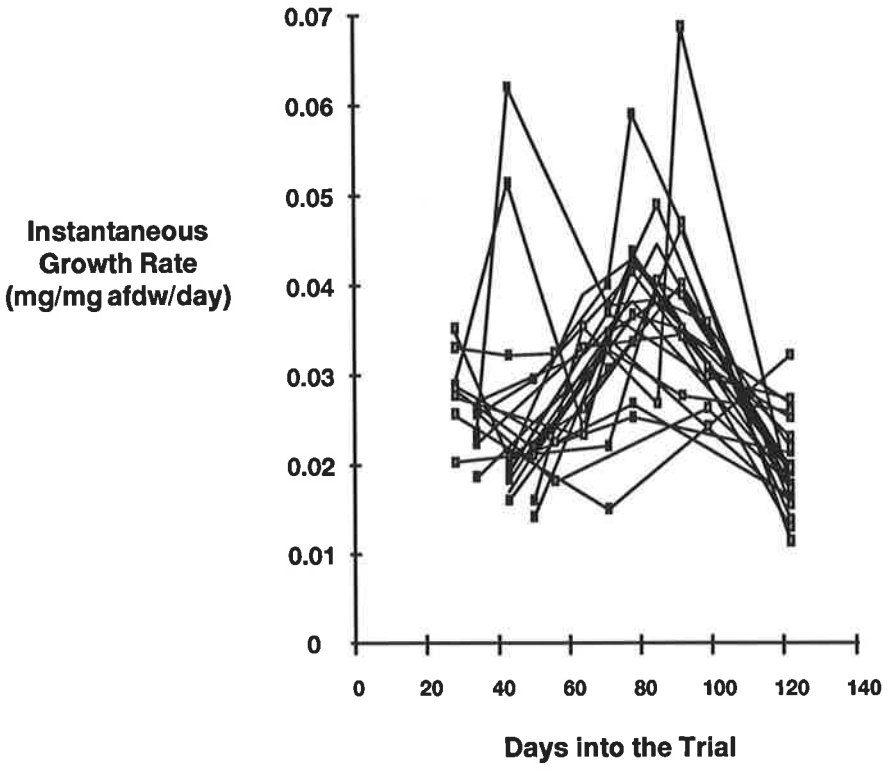


Fig 2.6d
Laboratory Growth on the 15% Protein Diet :
Trial 2

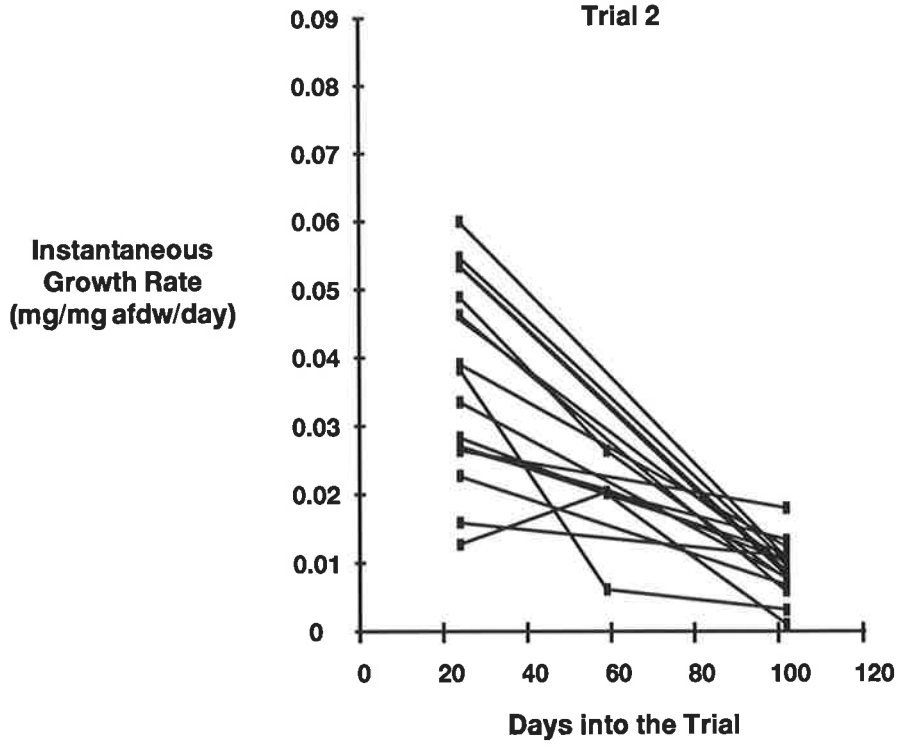


Fig 2.6e
Laboratory Growth
on the 25% Protein Diet :
Trial 2

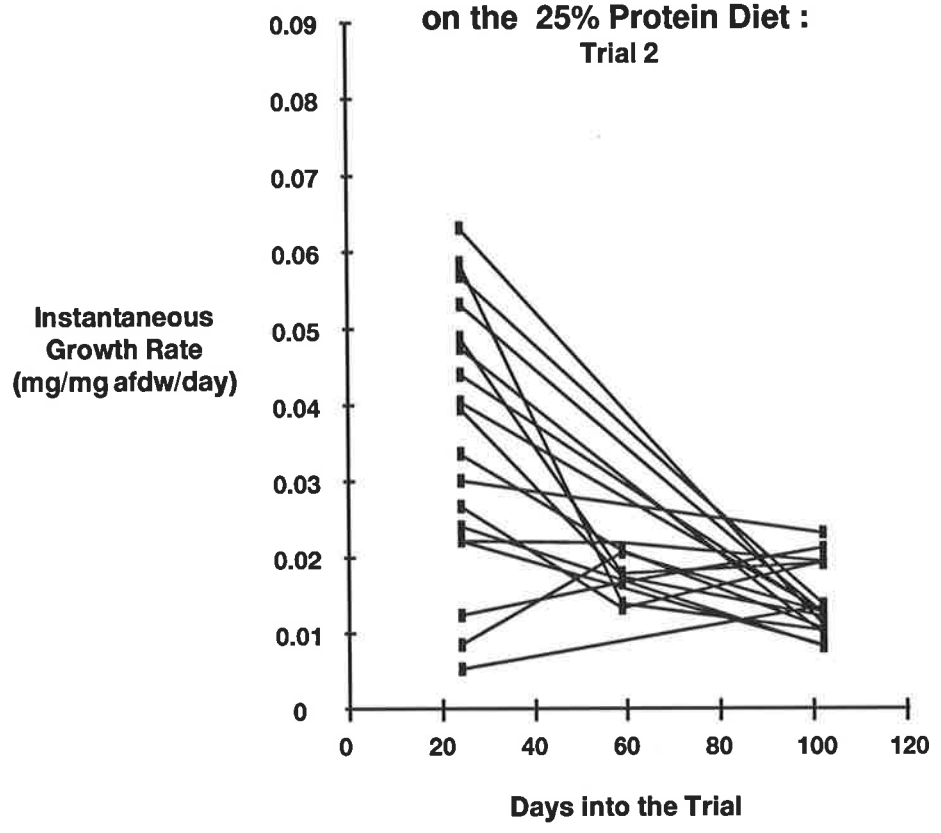


Fig 2.6f
Laboratory Growth
on the 35% Protein Diet :
Trial 2

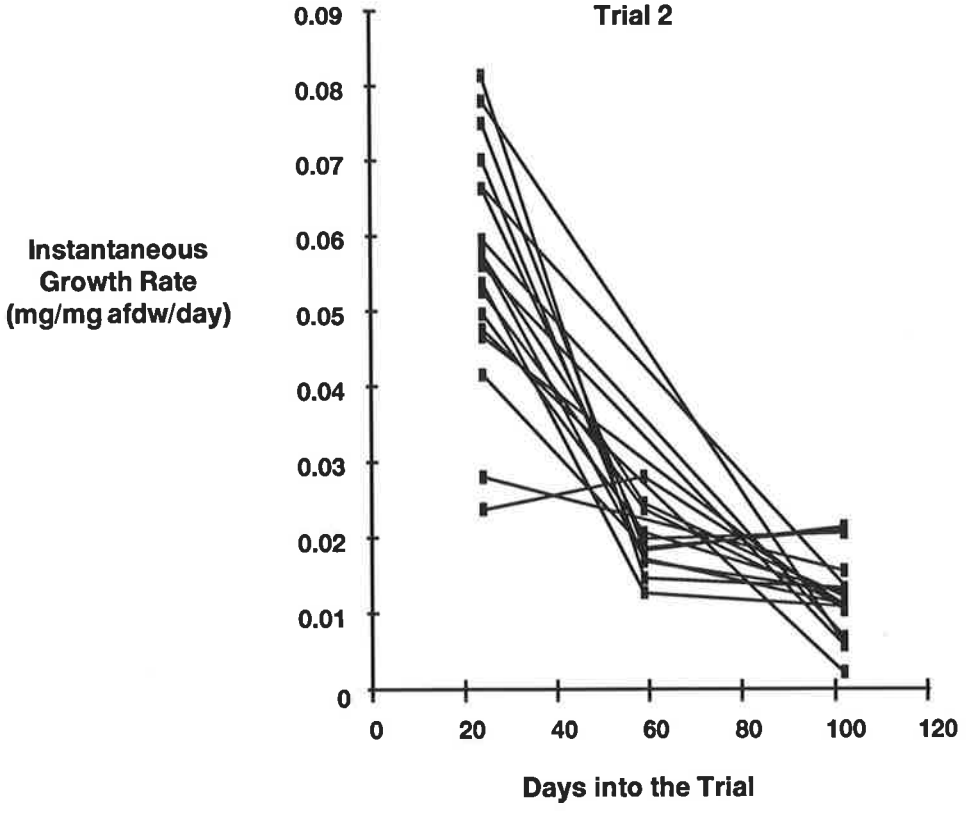


Table 2.5 Mean % Moult Increment (± SE) for each diet treatment within each trial.

Treatment (% Protein)	n	Mean	SE
a) Trial 1			
15	63	13.543	0.285
25	92	13.503	0.333
35	91	15.984	0.354
b) Trial 2			
15	48	13.368	0.409
25	37	13.526	0.340
35	42	15.764	0.521

As shown in **Table 2.6** diet had a significant effect on % moult increment in the second trial.

Table 2.6 Comparisons of % Moult Increment between diets within trials using ANOVA.

	Source	Sums of Squares	Mean Square	F	P
1. Trial 1					
Diet	0.028	2	0.014	3.003	0.051
Error	1.184	255	0.005		
2. Trial 2					
Diet	0.033	2	0.016	9.252	<0.001
Error	0.185	105	0.002		
***Post Hoc* Comparisons : Trial 2 only**	**Compared Diets**	**F_{calc}**	**P**		
	15,35	15.687	<0.001		
	15,25	0.826	0.366		
	25,35	10.508	0.002		

Post hoc tests for this trial showed that % moult increment increased significantly with the 35% protein diet (P= 0.002) but that for the 15% and the 25% diets there was no significant difference (P> 0.05).

2.3.2 Feeding, Assimilation and Energy use in the laboratory on 15% and 35% protein diets.

Data relating consumption and respiration to body weight were analysed using geometric mean regression (Ricker, 1973) and lines compared using ANCOVA (Sokal and Rohlf, 1981). The *t* statistic was used to test the significance of the slopes. (t_S ; Sokal and Rohlf, 1981). Two animals died during the experiments. Data from these individuals were incomplete and were not included in the analyses.

The leaching of organic material was low for both food types. Regression of organic content remaining over time in the 24 hour period showed that leaching was low but significant in both treatments (b<0, P<0.001; **Table 2.7a**). The 15 % diet lost about 2% and the 35% diet about 3% of their organic contents over three hours. Protein leaching was also low but was significant in the 35% treatment (b<0, P<0.001, **Table 2.7b**). These figures probably underestimate leaching and thus lead to a possible overestimate of consumption and assimilation efficiency as samples were not disturbed during the tests as they would have been if yabbies were feeding.

Table 2.7 Leaching of organic material and nitrogen from diets initially containing 15 and 35% protein respectively. ANOVAR on Leachate vs Time. All slopes are significant at P<0.001

a) Organic content (%)

i. 15 % protein	**Slope (b)**	**SE (b)**	**Intercept (a)**	**SE (a)**	**r^2**
n=18	-0.0187	0.0014	94.2622	0.0829	0.918
ii. 35 % protein	**Slope (b)**	**SE (b)**	**Intercept (a)**	**SE (a)**	**r^2**
n=18	-0.0255	0.0038	93.466	0.2257	0.738

b) Nitrogen content (%)

i. 15 % protein	**Slope (b)**	**SE (b)**	**Intercept (a)**	**SE (a)**	r^2
n=20	-0.002	0.001	2.984	0.059	0.189
ii. 35 % protein	**Slope (b)**	**SE (b)**	**Intercept (a)**	**SE (a)**	r^2
n=20	-0.004	0.001	4.619	0.069	0.431

Most feeding occurred in the first half hour of each feeding period and so leaching was estimated over this time using the regressions above (**Table 2.7a and b**). The same maximal feeding period and thus leaching period was used by Reigh *et al* (1990). Consumption and assimilation data were corrected for leaching by subtraction of the half hour estimate from total consumption.

a) Feeding and Assimilation

Mean daily consumption for individual yabbies across both trials and both diets varied from 2 to 12 percent dry weight of food to wet body weight per day and was inversely proportional to wet weight (**Fig.2.7**). The error bars attached to each point refer to 1 standard error calculated from the repeated feeding trials from each animal and suggest that consumption was more variable in smaller yabbies. Size and dietary effects on consumption were analysed after normalisation with log-log transformation. Regression analysis showed that weight-specific consumption was negatively correlated with wet weight (**Table 2.8).** Comparisons between the lines with ANCOVA showed no significant dietary effect on consumption (**Table 2.8**) although there was more variability in the 15% protein treatment - the coefficients of determination (r^2) were significantly different (**Table 2.8**, $P < 0.001$).

Fig 2.7a Consumption
(g dry weight/g wet weight/day) : 15% Protein Diet

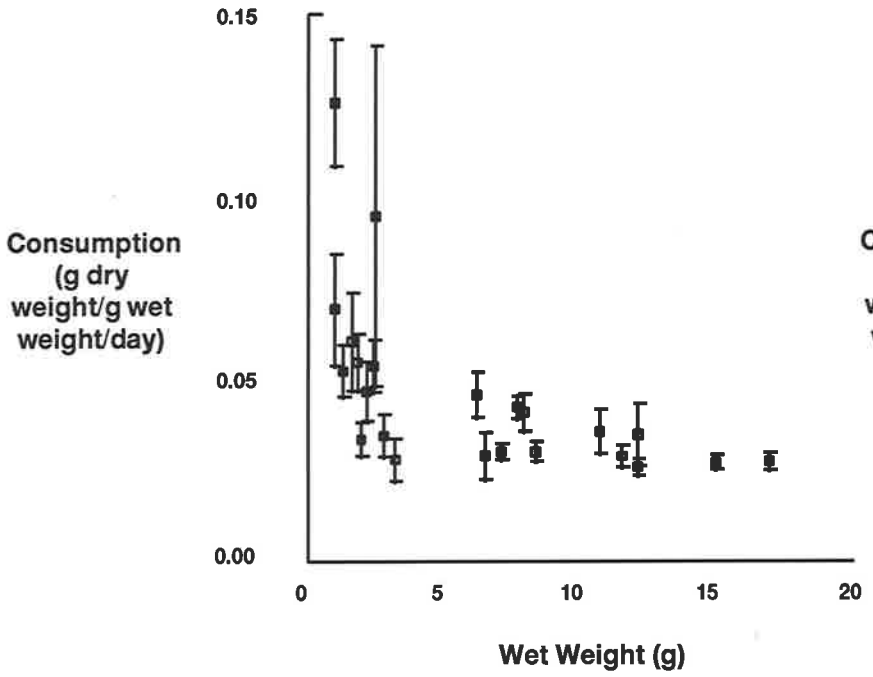


Fig 2.7b Consumption
(g dry weight/g wet weight/day) : 35% Protein Diet

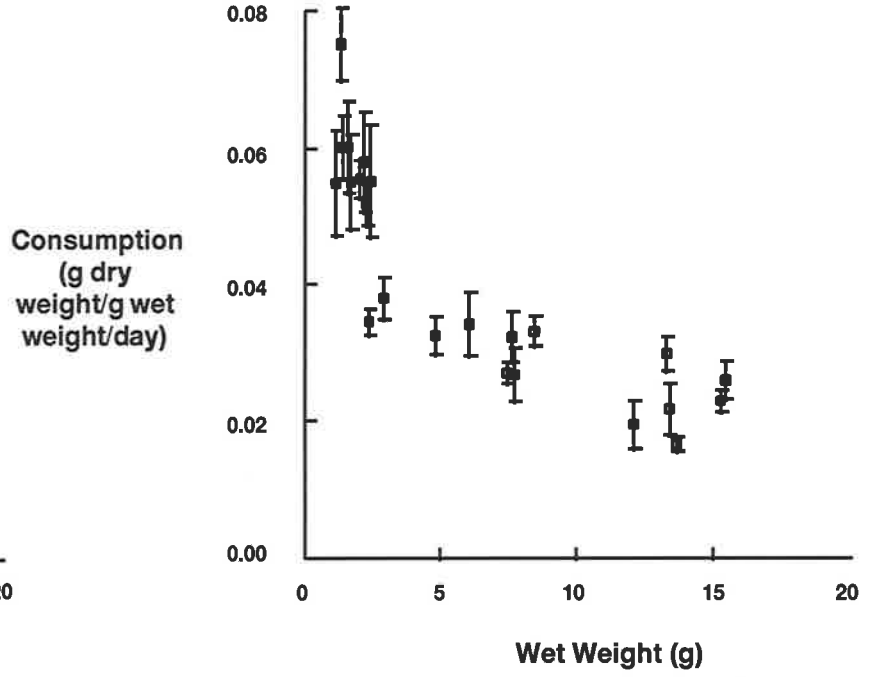


Table 2.8. Weight specific Consumption (joules/g/day) vs Wet Weight (g).
Equation : log (y) = log(a) + blogX . Significance levels on slopes indicate lines are different from zero (see text); * = P<0.05, **= P<0.001. df = degrees of freedom. n=23 per diet.

Diet (%protein)	**a**	**b**	**SE a**	**SE b**	**r^2**
15	3.1081	-0.454*	0.0493	0.0659	0.5574
35	3.0792	-0.4697**	0.0296	0.0392	0.8534

	Comparisons between diets	
f_{calc} intercepts (1,42 df)	**f_{calc} slopes** (1,45 df)	**f_{calc} r^2** (1,45 df)
1.7569	1.5069	98.07**

Consumption of energy in joules was calculated using an initial caloric content of 15,445 j/g afdw (ref Chapter 1) and assuming a leaching period of half an hour. Thus the conversion factor used for the 15% diet was 15,358 j/g and that for the 35% diet was 15,328 j/g. **Figure 2.8a and b** shows the consumption in terms of joules/g/day and its relationship with assimilation and faecal production for both diets. Assimilation (consumption minus faeces) of energy was strongly correlated with wet weight but was not effected by diet (**Table 2.9**) although, as before, there was more variability in the 15% protein treatment as shown by the significantly different coefficients of determination (**Table 2.9** P <0.001).

Fig 2.8a
Consumption, Assimilation and Faeces (Joules/g/day) vs Wet Weight (g) - 15% Protein Diet

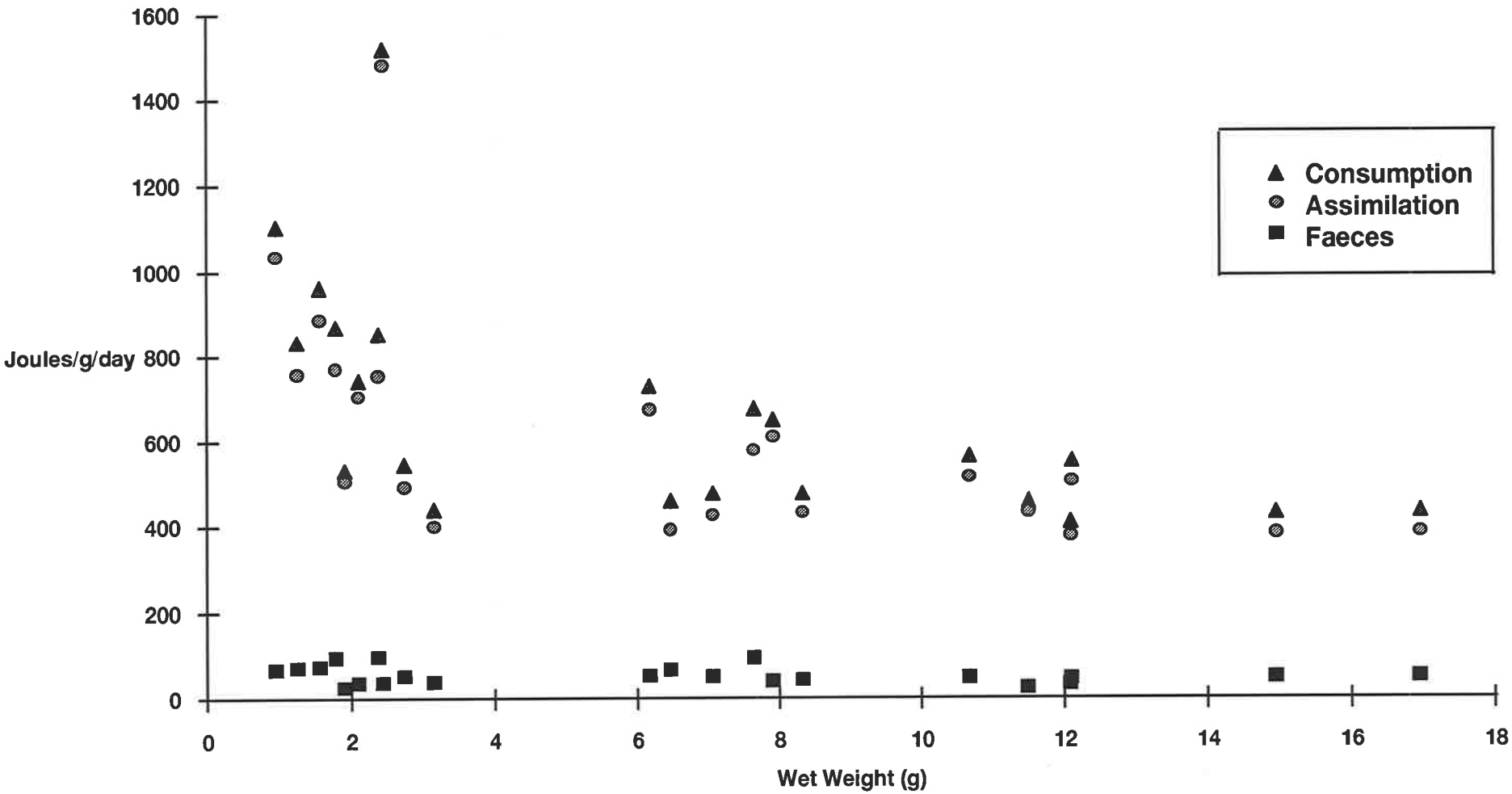


Fig 2.8b)
Consumption, Assimilation and Faeces (Joules/g/day) vs Wet Weight (g) - 35% Protein Diet

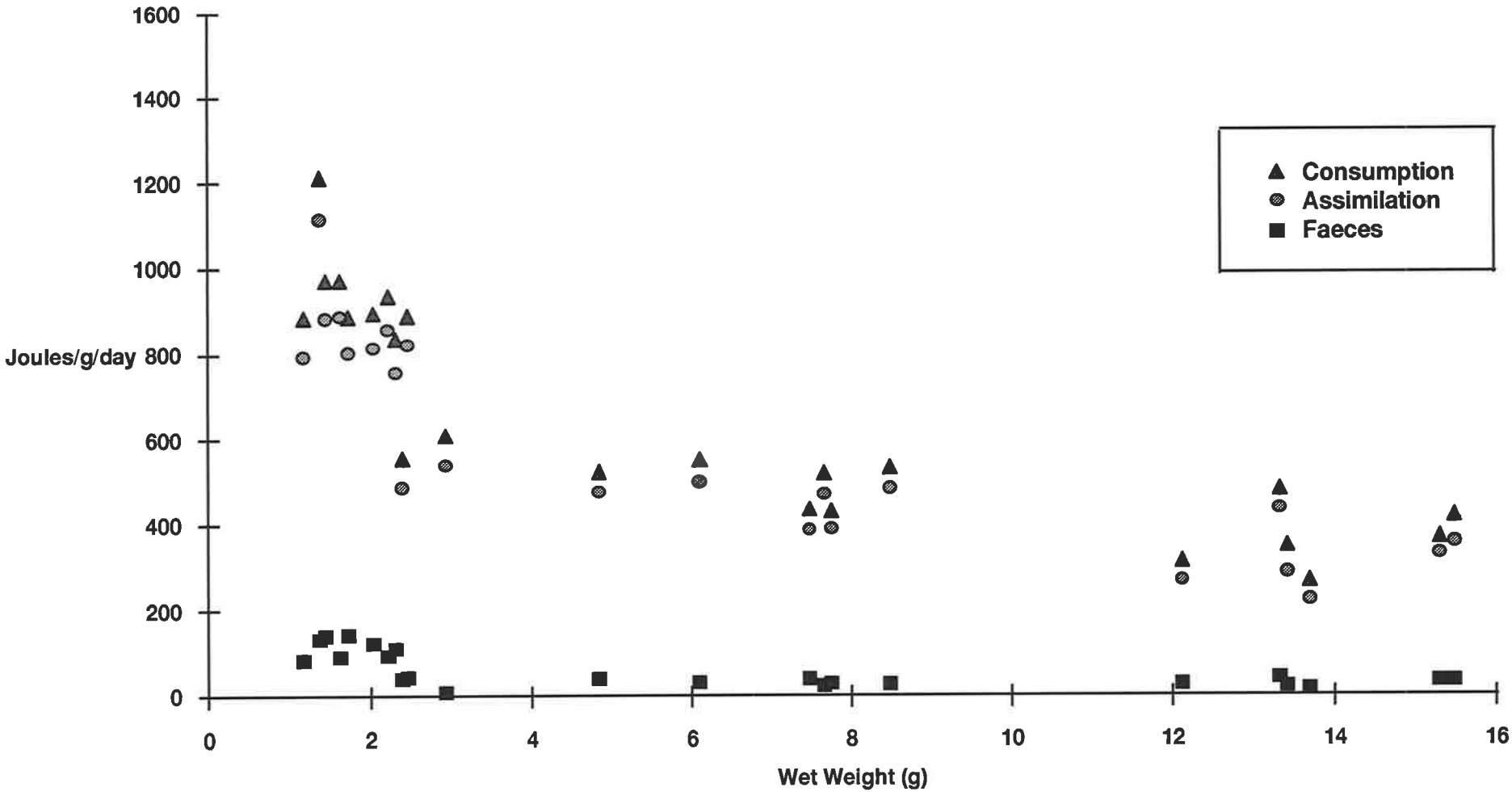


Table 2.9. Weight specific Assimilation (joules/g/day) vs Wet Weight (g).
Equation : log (y) = log(a) + blogX . Significance levels on slopes indicate lines are different from zero (see text); * = P<0.05, **= P<0.001. df = degrees of freedom. n=23 per diet.

Diet (%protein)	a	b	SE a	SE b	r^2
15	3.0806	-0.4724*	0.0514	0.0687	0.5554
35	3.0265	-0.4478**	0.0291	0.0385	0.845

	Comparisons between diets	
f_{calc} **intercepts** (1,42 df)	f_{calc} **slopes** (1,45 df)	f_{calc} r^2 (1,45 df)
1.6548	0.5633	92.976**

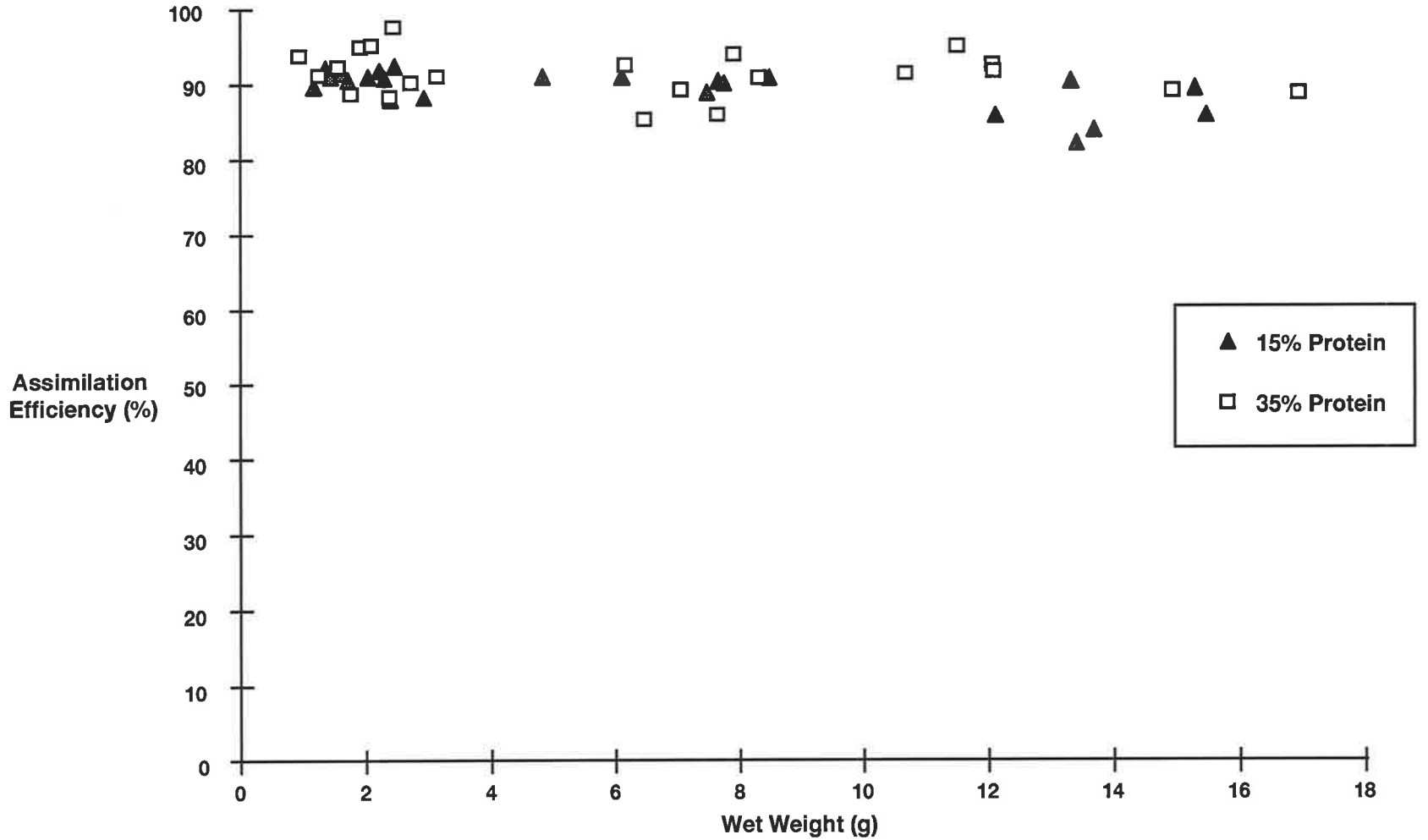
Assimilation efficiency of energy (AE_E) was high and decreased slightly with size (**Fig.2.9, Table 2.10**) but was independent of diet. Mean AE_E on the 15% protein diet was 89.363 ± 0.560 % and that for the 35% protein diet was 91.235 ± 0.654 %.

Table 2.10. Assimilation Efficiency of Energy (%) vs Wet Weight (g).
Equation : log (y) = log(a) + blogX . Significance levels on slopes indicate lines are different from zero (see text); * = P<0.05, **= P<0.001. n=23 per diet.

Diet (% protein)	a	b	SE a	SE b	r^2
15	-0.0156	-0.0371*	0.0057	0.0076	0.1246
35	-0.0438	-0.067**	0.0062	0.0082	0.2568

The 15 % and the 35% diets contained 2.998% (± 0.1179%) and 4.800% (± 0.1462%) nitrogen respectively. This gives % nitrogen to % protein conversions of 5.0409 x and 7.2934 x respectively. These conversions were used to estimate % protein from measured % nitrogen in leached food and faeces which lead to estimation of the assimilation efficiency of

Fig 2.9
Assimilation Efficiency of Energy (%) vs Wet Weight (g)
for yabbies fed 15% and 35% Protein Diets



protein. The latter was high and independent of size (**Fig 2.10**) but the 15% diet (94.83 ± 0.575%) showed a significantly lower value (Mann Whitney U, $P<0.019$) than the 35% diet (96.191 ± 0.284).

The above data on protein and energy content, leaching and assimilation efficiency were used to calculate the protein : energy ratio of the assimilated food (the 'effective' P : E ratio, *sensu* Sedgewick, 1979) as described in **Table 2.11**. The P : E ratios are described in terms of mg protein/kilocalorie not kilojoule as this is the more usual method found in the literature (Sedgewick, 1979, Hubbard *et al*, 1986, Ackefors, 1992). The effective P : E ratios for the 15% and the 35% diets were 43.37 and 99.352 respectively.

b) Respirometry

The data from the respiration experiments were analysed by investigating the relationship between mean routine respiration rate and wet weight of individual animals.

The log form of the respiration rate equation ($RMR=aW^b$) is used:

$$Log(RMR)=Log(a)+bLog(W) \qquad \textbf{Equation 2.2}$$

where RMR = oxygen consumption (mg/h); W = Wet Weight (g); a (mass coefficient) and b (mass exponent) were derived from the GM regression.

Dietary effects on respiration rate were tested by comparing oxygen consumption versus wet weight relationships of animals within a similar size range (1.5-13 g) fed either the 15% or the 35% diet. ANCOVA suggested that the respiration rate did not differ between the diets (**Table 2.12**, Equations A and B; $P>0.05$). This was because of the variability within the data as shown by the coefficients of determination (r^2). Comparisons with the regression for yabbies less than 1.5 g (Eq. D) showed no significant differences ($P>0.05$) therefore the data were pooled (Eq. E) and that line, based on a wide size range, used in further analysis. **Fig. 2.11** shows the pooled data in terms of mean oxygen consumption in mg O_2/h for each individual yabbie, with a bar representing one standard error around the mean.

Table 2.11 Calculation of Protein : Energy ratios. Diets were isocaloric and contained 3.692 (± 0.0285) kcal/g.

a) Energy Content	**15 % Protein Diet**	**35% Protein Diet**
1. Initial energy content (kcal/g)	3.692	3.692
2. % Loss after half an hour leaching (ref Table 2.7a)	0.561	0.765
3. Remaining kcal/g	3.671	3.664
4. Asimilation Efficiency	0.8936	0.9124
5. Remaining Digestible Energy (kcal/g) (3. x 4.)	3.280	3..3428
b). Protein Content		
1. % Loss after half an hour leaching (ref Table 2.7b)	~0	0.473
2. Remaining Protein (%)	15	34.527
3. Assimilation Efficiency	0.9483	0.9619
4. Digestible Protein (%) (2. x 3.)	14.225	33.212
5. Remaining Digestible Protein (mg/g)	142.245	332.115
6. P:E Ratio (mg Protein /kcal) (b5./a5.)	**43.367**	**99.352**

Table 2.12 Oxygen Consumption (mg/h) **vs Wet Weight** (g). **a)** Eqn: log (respiration) = log(a) + b log(wet weight). Significance levels indicate slopes are different from zero; * = P<0.05, **= P<0.001. **b)** Comparisons between regression slopes and intercepts using ANCOVA. No significant differences found, df = degrees of freedom

a)

Equation (see text)	Lines and Comparisons (see text)	n	a	b	SE_a	SE_b	r^2
A	15 % Protein	13	-0.5907	0.5941*	0.051	0.099	0.6971
B	35 % Protein	13	-0.6388	0.7493*	0.082	0.158	0.5092
C	15 % + 35 % Protein	26	-0.6175	0.678**	0.09	0.046	0.5783
D	Yabbies <1.5g	17	-0.5711	0.6184**	0.092	0.031	0.6694
E	All Data	43	-0.5987	0.6112**	0.04	0.021	0.8241

b)

Comparisons	f_{calc} a	df a	f_{calc} b	df b
A vs B	0.1001	25	0.0427	22
C vs D	0.659	42	0.0049	39

Fig 2.10 Assimilation Efficiency of Protein (%) vs Wet Weight (g)

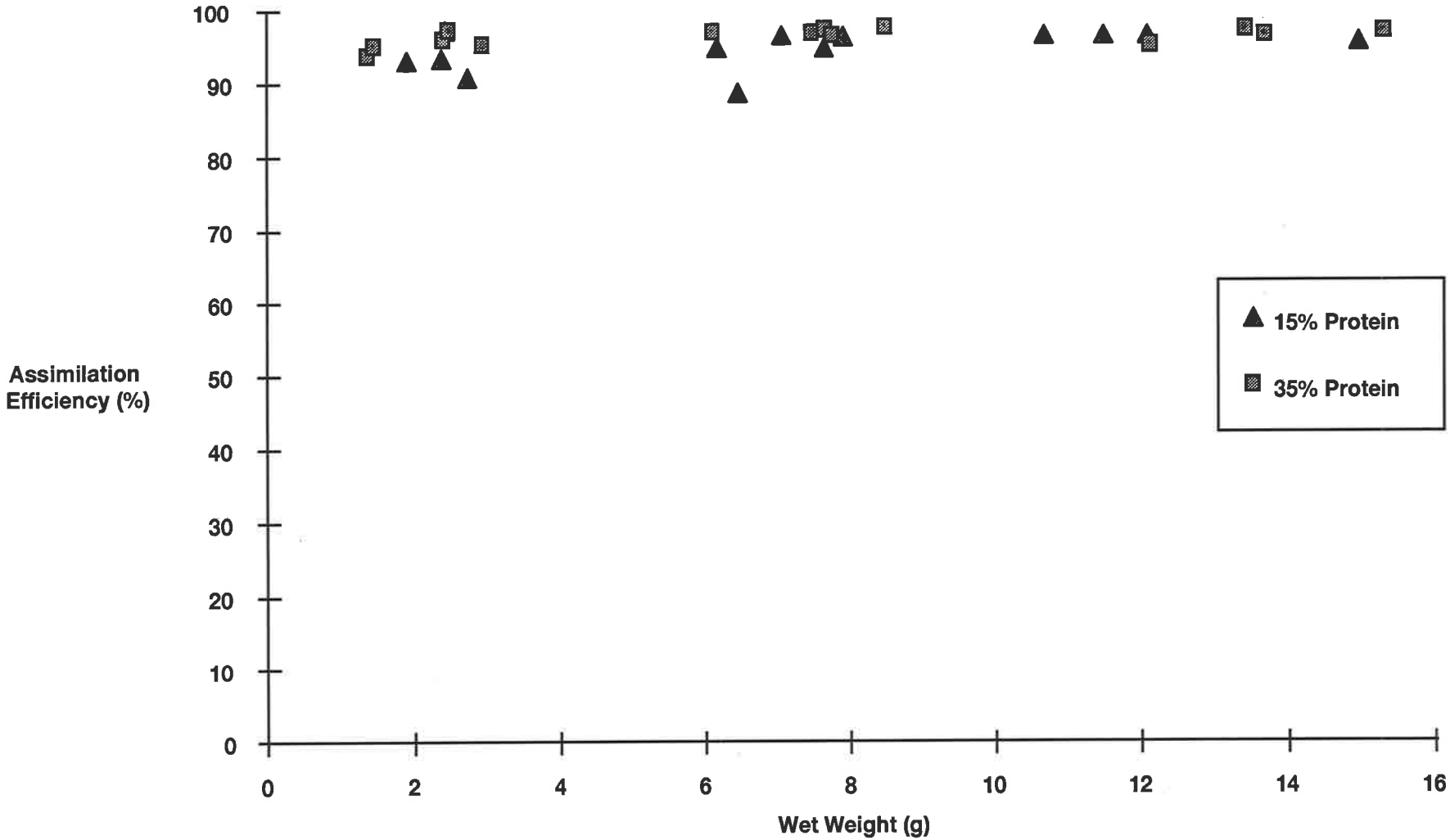
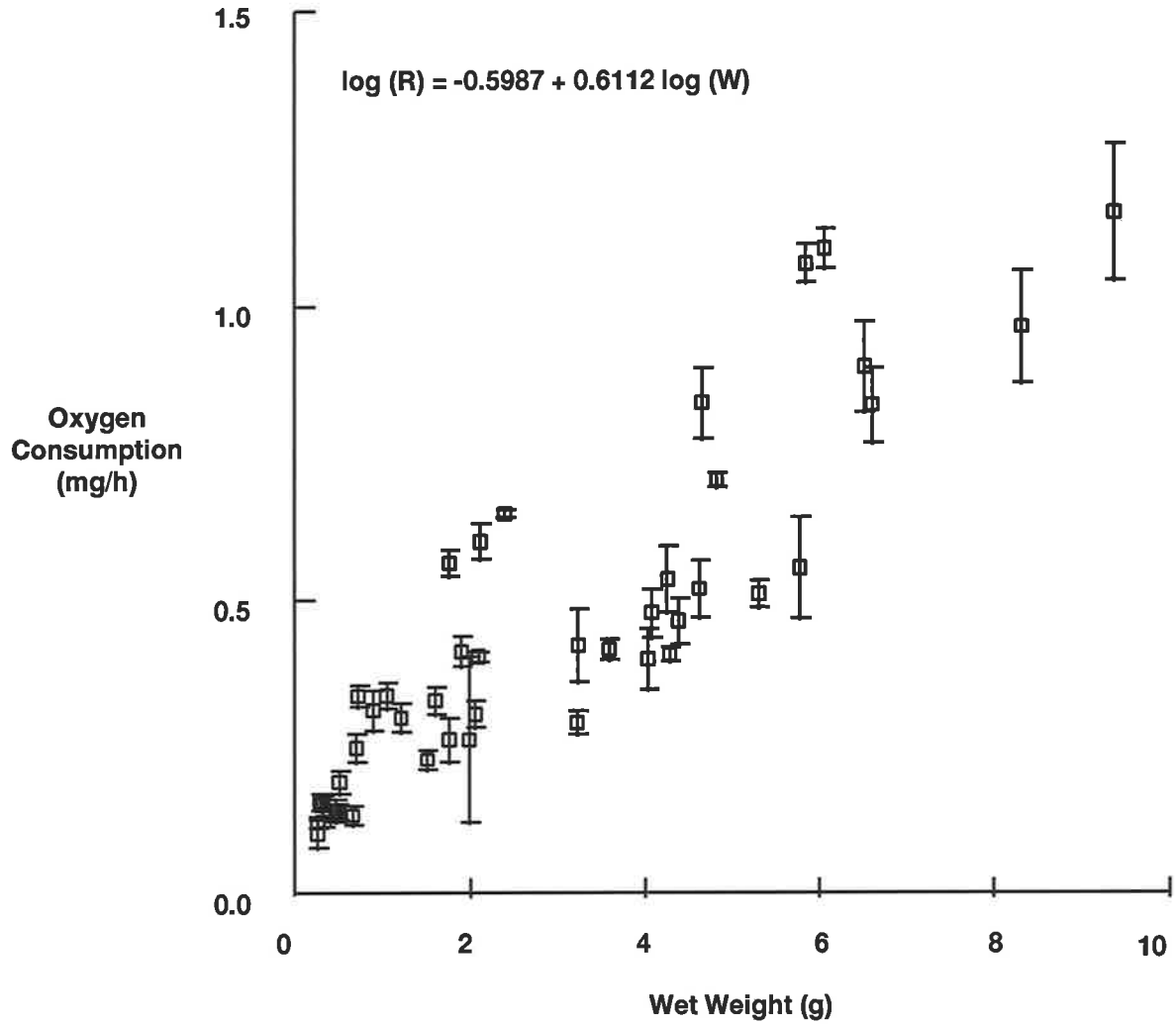


Fig 2.11 Oxygen consumption (mg/h) vs Wet Weight (g)



The routine respiration rates (RMR) in **Fig 2.11** were then converted to metabolic rates in Joules/g/day. The data were divided by the appropriate weights to yield mg O_2/g/h then converted to daily metabolic rates (Joules/g/day) using a general conversion factor of 14.14 Joules/mg O_2 respired (Elliot and Davidson, 1975). The relationship between metabolic rate and size was calculated as:

$$\text{Metabolic rate (J/g/day)} = 92.683 \times W(g)^{-0.514} \qquad \textbf{Equation 2.3}$$

As there was no significant dietary effect on consumption, assimilation or metabolic rate the treatments were pooled and the data combined and back-transformed to exponential functions (i.e. $Y = aW^b$). The relationships between weight and weight-specific consumption, assimilation and metabolic rate and their respective equations are shown in **Fig 2.12a and b**. Energetic requirements declined exponentially with increasing size. Yabbies of 1g assimilated 2.9 times and respired 3.3 times more energy than 10g animals **(Table 2.13)**.

Table 2.13 Proportional Energy use in relation to a 10 g Yabbie (refer text)

		Consumption		Assimilation		Metabolic Rate	
	Diet (% protein)	15	35	15	35	15	35
Wet Weight (g)	1	2.84	2.95	2.98	2.91	3.27	3.27
	2	2.07	2.13	2.15	2.11	2.29	2.29
	5	1.37	1.39	1.39	1.38	1.43	1.43
	10	1		1		1	

Metabolic energy use accounted for less than ten percent of assimilated energy (15% protein diet = 7.6 ± 0.54% ; 35% protein diet = 8.02 ± 0.31 %) and was independent of size (b=0, P>0.05).

2.4 Discussion

2.4.1 Growth of juvenile *Cherax destructor* on diets with different protein levels in the laboratory under clean conditions

Although survival was good, growth rates were low compared to other laboratory studies on yabbies. Geddes *et al* (1988) constructed a model of average growth using individually raised

Fig 2.12a
Consumption, Assimilation and Metabolic Rate
(all Joules/g/day) vs Wet Weight (g)

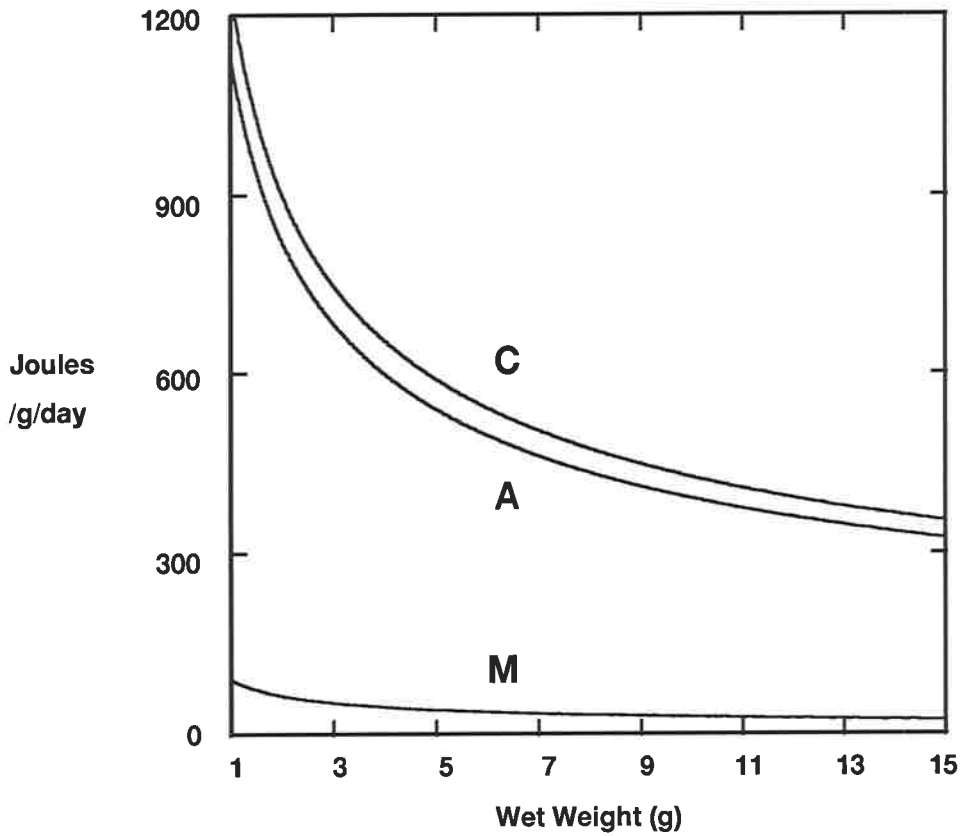
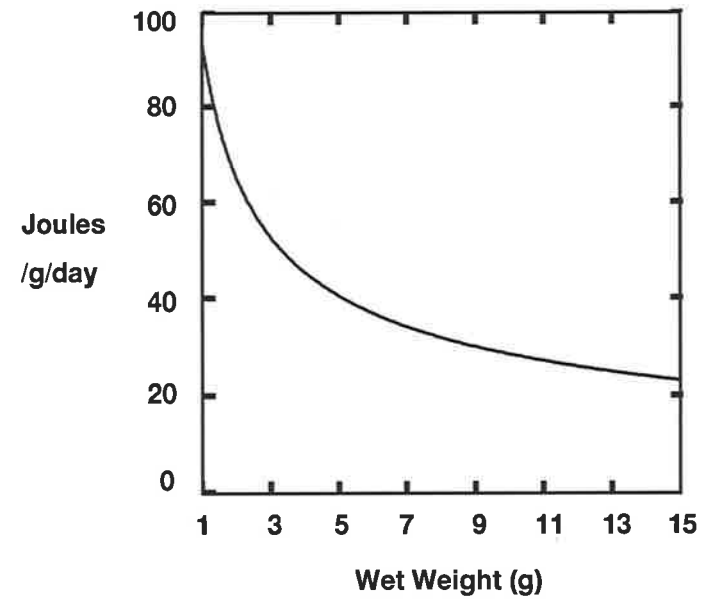


Fig 2.12b
Metabolic Rate
(Joules/g/day) vs Wet Weight (g)



C - Consumption

Eq: $C = 1247.096 \times W^{-0.465}$

A - Assimilation

Eq: $A = 1138.676 \times W^{-0.464}$

M - Metabolic Rate

Eq: $M = 92.683 \times W^{-0.514}$

Where W = Wet Weight (g)

yabbies at 26°C. They used equations of log weight increment on premoult length ($r^2 = 0.918$, $P< 0.001$) and log intermoult period on premoult length ($r^2=0.044$, $P<0.01$). Using their data an instantaneous growth rate of 0.044 mg/mg wet weight/day may be calculated for the interval 1g to 6g. This is higher than the fastest growth (0.033 mg/mg afdw/ day) achieved by the large yabbies on the 35% protein diet. Note that IG estimates derived from ash free dry weight may be up to 20% higher than those derived from the same animals using wet weight (pers obs). Field growth estimates for crayfish vary widely but are generally higher than laboratory growth rates (Morrissy, 1984, Geddes *et al*, in press). Rates calculated from Geddes *et al* (in press) suggest values ranging from 0.02 - 0.14 mg/mg wet weight/day for yabbies raised from 15 mg for 42 days in outdoor nursery ponds. Their best growth averaged 15 mg to 800 mg over the 42 day period (M. Geddes, pers comm) which is equivalent to 0.095 mg/mg wet weight/day. Mean temperature in these trials varied between 20 and 25°C. However, instantaneous growth rates of yabbies calculated from Woodland (1967) suggest 0.023 mg/mg wet weight/day for the weight interval 1.5 to 7.5 g and 0.018 mg/mg wet weight/day for the weight interval 3.5 - 11.9g. These were measured in a farm dam at summer temperatures which ranged from 12.5-30°C and averaged about 18.8°C. (Note that Woodland's reported instantaneous growth rates were not described here as he did not include time period in the calculation).

The depressed growth in the present study relative to Geddes *et al* (1988) and Geddes' *et al* (in press) field study was probably the result of a combination of nutritional inadequacy related to system cleanliness, food availability and lack of variation in diet (Morrissy, 1984, Hartnoll, 1982).

Percent moult increment ranged from 13.5 to 15.8% and changed little between treatments suggesting that differences in growth rates in both trials were largely the result of changes in intermoult period. Mean % moult increment was lower than the values of 17 to 17.8% reported by Geddes *et al* (1988). The depression of one or both growth components by treatment and other environmental effects has been previously suggested for crustaceans. For example, Hartnoll (1982) reported that food quality and quantity affected both components, although his review suggests that intermoult period is the most influenced by environmental conditions whereas moult increment may be more affected by factors such as sex and the

onset of maturity. More recent reports (i.e. Botsford, 1985, Wilber *et al*, 1989) have supported this view. For example, Wilber *et al* (1989) found that holding space and diet affected growth of the West Indian spider crab (*Mithrax spinosissimus L.*) primarily through changes in intermoult period.

In the second trial involving larger juveniles there was a negative relationship between IG and size. It is well accepted that growth slows with increasing size as progressively more nutrients are used in the replacement of existing protoplasm (i.e. maintenance) rather than the manufacture of new tissue (Calow, 1977, Parry, 1983). However, it might have been expected that during the juvenile stage yabbies would grow exponentially, that is with a constant IG. The drop in IG shown in the second trial suggests that growth rate decline begins in the juvenile phase. As suggested above, the nutritional inadequacy of the diets and the cleanliness of the system may have also contributed to the decline in IG as the growth trials progressed. Given that IG declines with size, it would be expected that animals in Trial 1 (initial weight: 14 mg) would grow faster than those in Trial 2. (initial weight: 680 mg+). However, growth rates of third stage juveniles (Trial 1: 0.015 - 0.027 mg/mg afdw/day) were lower or similar to those of larger animals on the same diet (**Fig 2.6**). This may relate to the differing nutritional histories of the two groups. The smaller juveniles were used in the experiment as soon as the whole brood had been released from the female and so had little chance to forage as independent animals. Their previous nutrition would have been derived largely from the yolk reserves. In contrast, those in the second trial would have had reserves derived from a relatively long period of independent foraging. Particle size may have also had an influence as it was noted that some elements of each diet (i.e. seeds) were not consumed in both trials and the smaller animals may have been particularly affected. In response to this observation, diets were reground and repelleted for experiment 2 of this chapter and for subsequent experiments. Crayfish of all sizes appeared to readily accept and consume the reground pellets.

The clear distinctions in growth rates between diets showed that an increase in P : E ratio from 43 to 99 mg/kcal improved growth. Thus the response to changing P : E ratios is similar to that found in other studies (Sedgewick, 1979, Hubbard *et al*, 1986, Ackefors, 1992). The P : E ratio of the 35% protein diet (99 mg/kcal) was lower than that proposed by Hubbard (*et al*

1986) for *P. clarkii* (i.e. 120) but within the range they recommended as optimal (80-120). It was also close to that suggested for *Astacus astacus* (114 to 153) by Ackefors (1992). The amino acid profiles of the 35% diet and the yabbies tail muscle were similar (ref Chapter 1). This suggests that a coarse diet containing 35% protein with an appropriate amino acid profile and a P:E ratio of 99 mg/kcal is adequate for growth studies on this species. The further addition of carotenoids may be necessary to preserve carapace pigmentation. In a less controlled system and with higher feeding rates it is probable that growth would improve. Therefore, it was decided to use the diets in further work.

2.4.2 Feeding, Assimilation and Energy use

a) Consumption and Digestibility

The assimilation efficiency of energy or energy digestibility was within the ranges reported in the literature for artificial diets. Reigh *et al* (1990) used a range of similar coarse artificial diets on *Procambarus clarkii* and found digestibilities of 70 - 100%. Bordner *et al* (1983) tested hybrid lobsters (*Homarus* sp) using refined artificial diets and found that the energy component was 88-91% digestible.

Protein digestibility was also within the ranges reported in the literature. For example, Jones and Momot (1983) reported assimilation efficiency of 95% when *Orconectes virilis* was fed fish. The data also fall within the top of the range (69-100%) reported by Reigh *et al* (1990) for a variety of common protein sources used in diets for *Procambarus clarkii.*

Natural plant diets generally show a lower digestibility (Brown *et al*, 1990, Kossakowski, 1974, Moshiri and Goldman, 1969, Wiernicki, 1984, Musgrove, 1988b). Brown *et al* (1990) tested ten aquatic macrophytes on *Orconectes virilis* (18 g wet weight), six of which showed dry weight digestibilities between 0 and 54% - the remaining four were up to 90% digestible. Consumption for all plants tested was low, the majority of plants were consumed at less than 0.2% (0.04 - 0.2%) of body weight per day with the maximum at 0.87%.

b) Metabolic Rates

The mass exponent 0.6112 (b in the power function $RMR=aW^b$) determined in this study is lower than that reported for other decapod species (Ivleva, 1980; Wheatly, 1989). However, it is similar to that reported in Woodland's (1967) study of this species. He reported b = 0.6478 at 20°C in his study of a pond population of *Cherax albidus* (= *C. destructor*). Wheatly (1989) reported b = 0.790 for *Pacifasctacus leniusculus* Dana. She included this figure when deriving an interspecific mean of b= 0.964 which was based on 15 species, including freshwater and marine forms. These data were collected at or near 12°C. Ivleva (1980) determined respiration rates on a large number of marine crustaceans, including 35 species of decapod. He found a mean value of b = 0.689 among the decapods at 29°C. There were methodological differences between the present and quoted studies which may account, in part, for the different results. As discussed below, the lower metabolic rate reported in this study may also reflect differences in growth rate under laboratory conditions if metabolic rate is a reflection of growth rate.

c) Relationships between Consumption, Assimilation and Metabolic Rate

There is a common exponential decline in weight-specific consumption, assimilation and metabolic rate with increasing size (**Fig 2.12**). The close correlation between energy intake (assimilation) and energy use (metabolic rate) suggests yabbies are feeding for energy across the size range used. This is also suggested by the fact that consumption was the same on both diets, despite differences in protein level. Under these experimental conditions less than ten percent of assimilated energy was channelled into metabolism. The remainder would be available for storage, growth and the energetic requirements of foraging behaviour and perhaps moulting (Skinner, 1962). The contribution of metabolism, growth and activity to estimates of assimilation and net growth efficiency (K_2) will be discussed in Chapter 6.

The relationship between metabolic rate and assimilation rate may be explained with reference to Parry's (1983) model of energy use which included the cost of growth as a component of respiration. Brett and Groves (1979) and Parry (1983) suggested that part of a ectotherm's heat production arises from the "cost of growth" which was equivalent to the work of growth or the heat production from tissue synthesis. This concept has also been applied to plants (Thornley, 1970), microbes (i.e. Pirt, 1965, Stouthamer, 1977 - both in Parry, 1983) and

endotherms (i.e. Vleck, Vleck and Hoyt, 1980). So growth is not considered as independent and competing with metabolism for limited energy (Parry, 1983). Rather, growth has both catabolic and anabolic components, the first of these contributing to the overall cost of metabolism. Earlier, Lavigne (1982) suggested that ingestion, assimilation, metabolic rate and growth should all be proportional to each other. This is supported by the strength of the correlation in this study. The juvenile phase is the time of rapid growth, so the cost of growth would be expected to account for a large proportion of the assimilated energy used in metabolism in juvenile crayfish.

The results of this laboratory study may be applied to intensive nursery stage aquaculture that has been promoted for *C. destructor* (Geddes *et al*, in press). Application of laboratory studies to the field must be made carefully even if the 'field' is the relatively controlled conditions of intensive culture. With that in mind, I would suggest two possible practical applications of these experiments. Firstly, as it appears that the energetic requirements at 24^{o}C were represented by the line of assimilation and because of the close relationship between assimilation and metabolic rate, knowing the metabolic rate at a particular size and temperature and how that rate relates to assimilation could allow estimation of the energy requirement for growth (i.e. assimilation in so many joules/g/day) which may be extrapolated between sizes and temperatures within a diet type. Secondly, energetic requirements declined exponentially with increasing size with yabbies of 1g consuming and assimilating 2.8-3 times and respiring 3.3 times more energy than 10g animals (**Table 2.13**). Using these diets, 1 g yabbies should be fed at a rate of about 7% body weight/day and 10 g yabbies at about 2%. Such knowledge of size-specific energetic requirements could be useful in intensive culture of juveniles to avoid wasting food.

Chapter 3.

Moulting and Growth in semi intensive aquaculture conditions

3.1 Introduction

Growth in crustacea is usually described in terms of % moult increment and moult frequency (Hartnoll, 1982, Botsford, 1985). It is seen as a stepwise process where growth in length occurs only at and immediately after the moult. Once the new exoskeleton has hardened further growth is limited by the extension or unfolding of the arthrodial membranes (Botsford, 1985). Thus there are rapid increases in size at moult followed by relatively long intermoult periods during which size remains relatively unchanged (Botsford, 1985, Somerton, 1980).

However, the real growth processes of cell proliferation and protein synthesis are out of phase with changes in body length (Aiken and Waddy, 1987). Whereas increases in length and volume occur immediately after ecdysis (Aiken and Waddy, 1987) tissue growth occurs primarily between moults. In this context, growth includes muscle and exoskeleton anabolism and the associated energy and calcium storage and use. Few studies have closely considered the changes that take place during the moult cycle or have considered the effect of size on this type of growth. Changes in body composition within the moult cycle have been investigated in terms of fluctuations in individual components such as enzymes, hormones, lipids and protein within isolated organs or fluids (Passano, 1960, McWhinnie and Kirchinberg, 1962, Skinner, 1962,1966 and Skinner *et al*, 1985, Heath and Barnes, 1970, Armitage *et al* 1972, Gwinn and Stevenson, 1973a and b, Durliat and Vranckx, 1982) and in whole animals (Read and Caulton, 1980, Barclay *et al* 1983, Anger, 1988, 1991, Anger *et al*, 1989, Zhu and Wang, 1989, and Nicol *et al*, 1992). The most complete studies in decapods are those by Barclay *et al* (1983) and Anger (1988, 1989, 1991) on prawns and marine larvae respectively and freshwater crayfish studies have been undertaken by Armitage *et al* (1972), Gwinn and Stevenson (1973a and b) and Durliat and Vranckx (1982).

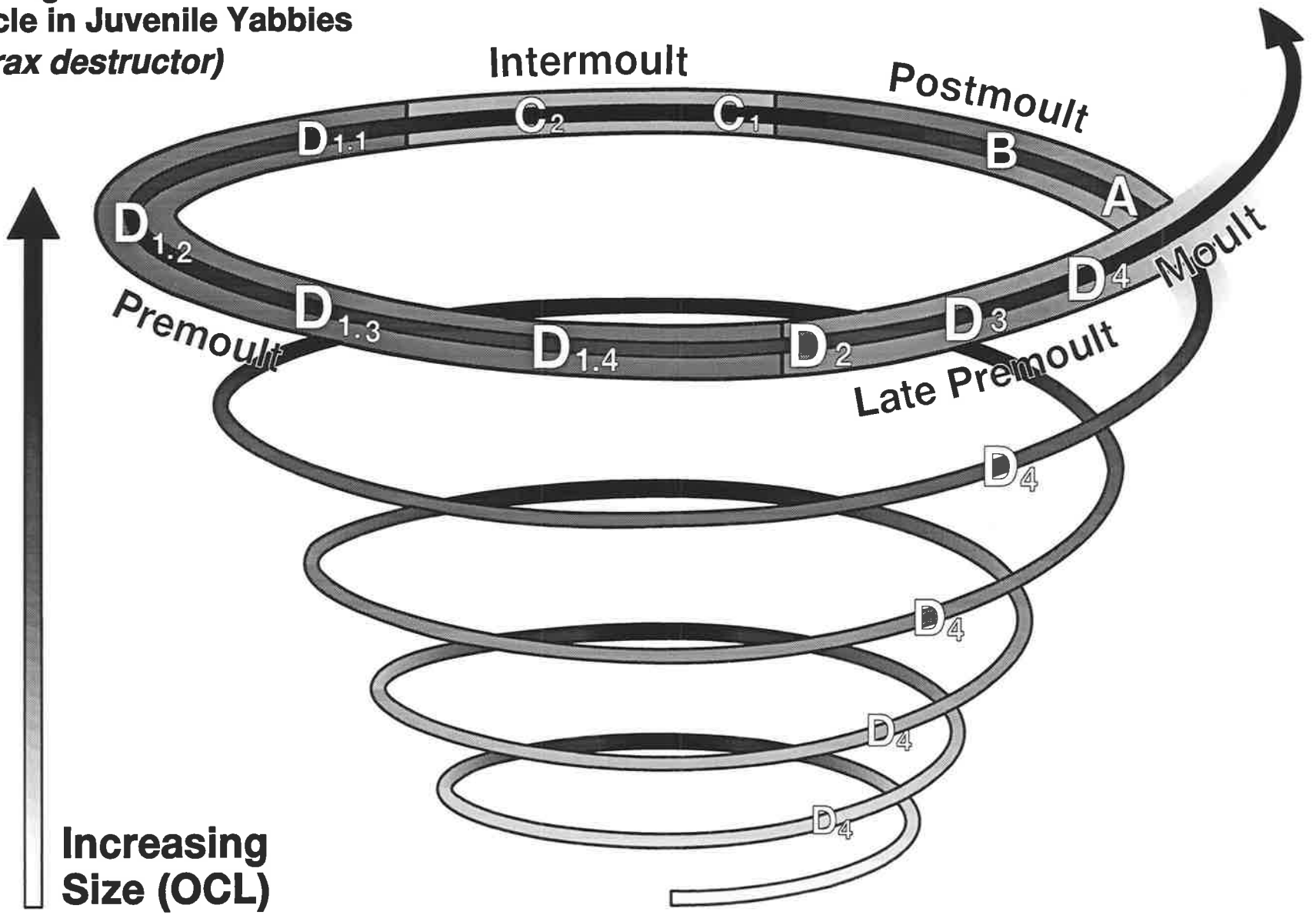
Although the moult cycle is a continuum it may be subdivided into substages by reference to morphological changes in the integument which accompany physiological events. In freshwater crayfish four general categories have been recognised. These are usually labelled A to D (Aiken and Waddy, 1987) and may be identified by reference to integument state (i.e. soft, firm, hard) and appendage setal development using light microscopy. As stated in Chapter 1, for the purposes of this study Stage E (ecdysis) (Aiken and Waddy, 1987) is referred to as an event, not a stage. In yabbies the four stages have been divided into 11 substages (Burton and Mitchell, 1987) as shown in **Fig 3.1**. The classification is based on the development of uropod setae and on the integument state. Stages A and B have been defined as postmoult, substages C_1 and C_2 as intermoult, substages $D_{1.1}$ - $D_{1.4}$ as premoult and D_2-D_4 as late premoult.

Given that the physiological events of the juvenile crustacean moult cycle have a complicated format which includes tissue growth as well as reserve accumulation, hardening of the new exoskeleton and manufacture of the next one (Aiken, 1980, Aiken and Waddy, 1987, Chapter 1 - this study), it would be expected that accumulation and use of energy would be similarly complicated. Simply measuring energy content at either end of this process would underestimate energy accumulation/use. Therefore it was decided to investigate moult stage and size-specific changes in whole body composition in terms of water, carbon, protein-nitrogen, non protein-nitrogen, ash and energy in order to better describe the nature of growth in juvenile yabbies and to better estimate energy use during the process.

The study was carried out in conjunction with a field experiment which investigated growth of stage three to 3g juveniles under semi-intensive aquaculture conditions. The growth study was intended as a indicator of potential juvenile field growth on an artificial diet for comparison with other field studies and with the experiments in Chapter 2. The 35% protein diet used in the latter was also used here in addition to naturally occurring food.

Moult stage and size-specific proximate analysis of animals grown under field conditions allowed the development of realistic models of juvenile yabbie tissue accumulation and use. Initially, regression analysis is used on a moult stage by moult stage basis to examine and produce models of size-specific wet weight, dry weight and water accumulation. The second

Fig. 3.1
The Moult Cycle in Juvenile Yabbies
(Cherax destructor)



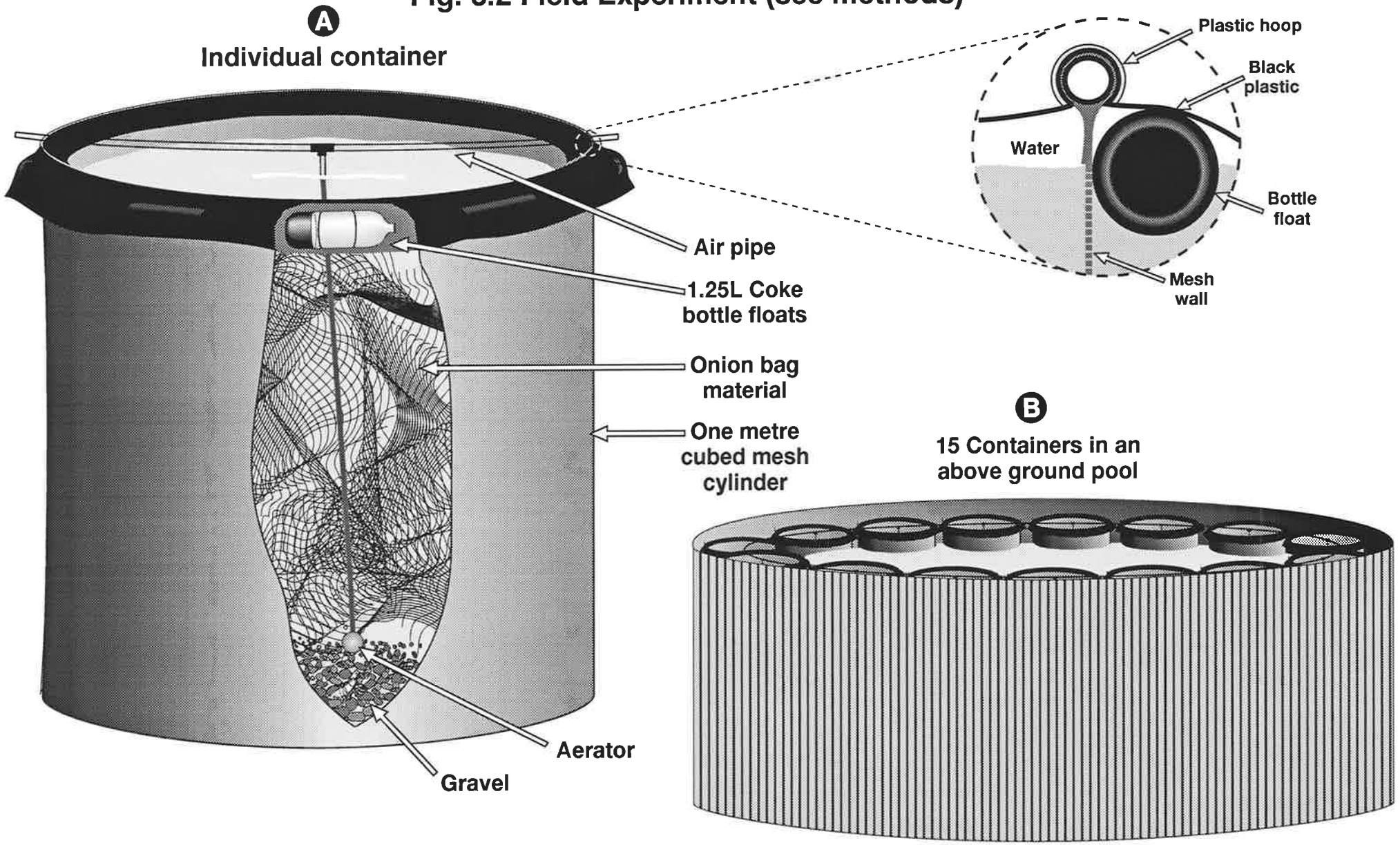
of these forms the framework for description of models of moult stage and size-specific ash and organic content. Finally, the organic content model is used in conjunction with other analyses to examine moult stage and size-specific fluctuations in carbon, protein and energy components of the tissue.

Section 3.2 details the methods and 3.3 presents the field growth results in instantaneous terms. Moult stage and size-specific variation in each tissue component is analysed and modelled for a series of consecutive size classes based on field moult increment data. Section 3.4 compares the field growth results with other studies and discusses the tissue models in terms of their contribution to the understanding of tissue and energy dynamics for juvenile crayfish. Results will be combined with those in chapters 4 and 5 to estimate the energy budget (Chapter 6) for juvenile yabbies.

3.2 Methods

Fifteen cylindrical containers were built from woven mesh shade cloth. (**Fig 3.2**) They were 1 m^3 in capacity, with a mesh bottom. All seams were double stitched and the mesh size (rated as 75%) was sufficiently small to prevent the escape of the smallest yabbies (14 mg). The shape of each container was maintained with plastic hoops sewn into the top and the bottom, so they were collapsible and easy to transport. Floats were attached to the rims of individual containers and the bottoms weighted with gravel so they could be suspended in water. To stop yabbies escaping, floats were adjusted to kept the top of each container 10 cm above the water level and a ledge of black plastic was fastened around the rim. The containers were individually aerated using airstones attached via plastic tubes to an air compressor and each was filled with 5 m^2 of plastic onion mesh to increase surface area and thereby minimise aggressive encounters among crayfish. The containers were moored around the edge of an 18 m^2 above-ground plastic-lined pool maintained at a depth of 1.5 m. The pool had been full for some time prior to the experiment and contained a community of phytoplankton, filamentous algae (*Cladophora*), aquatic insects and zooplankton.

Fig. 3.2 Field Experiment (see methods)



3.2.1 Field Growth

The stock for the experiment were obtained by establishing a small hatchery. A large number of egg-bearing females were caught from a local pond. Twenty crayfish whose eggs were at approximately the same stage of development were placed in individual compartments in an outdoor hatchery consisting of small above-ground swimming pools in an enclosed yard with a shade cloth roof. Each pool was vigorously aerated and water temperature varied between 20 and 25^{o}C during the period of incubation. Stage three juveniles were released from females over a period of about a week and a half. As each brood was released it was put into a cold room at 15^{o}C to slow further growth. All broods were left in the cold room for a further week after the last release to standardise acclimation temperature.

In the two days prior to the beginning of the growth experiment the water temperature in the cold room was raised to 20^{o}C, which was approximately the temperature in the experimental pond. On the day of the experiment 16 groups of 200 yabbies were selected from about 3600 individuals. Each group was made up of ten animals randomly selected from each of the twenty broods. Fifteen groups were randomly assigned to the containers mentioned above. The sixteenth group was quick frozen in liquid nitrogen, freeze dried and weighed. It thus acted as the initial weight and organic content sample.

The experiment lasted 53 days, during which time the animals were fed the 35% protein diet (Chapter 2) at a rate of 15% body weight every third day. The amount of food was adjusted using the average weight at each harvest. The yabbies also had access to other food in or on the surfaces of their containers. Water temperature was monitored continuously using thermistors attached to a Grant 'Squirrel' data logger. Oxygen, pH and conductivity levels were measured weekly.

A group of five containers, chosen at random, was harvested at 15, at 30 and at 53 days. After each harvest, fresh gravel was put into the empty containers and they were suspended in their original positions in the pool to minimise any disturbance effect. Aeration was also continued in these containers.

The animals from each harvest were returned to the lab where most were killed by immersion in liquid nitrogen then stored in a -80°C freezer until required for analysis. Advanced premoult (substages D_3 to D_4 - Burton and Mitchell, 1987) crayfish were separated from this group and sorted into pairs of similar sized animals. Half (i.e. one member of each pair) of these premoults were killed as above. The other half were left to moult overnight in individual tanks containing water at the same temperature as the ponds. The following day these crayfish, now at moult stage 'A', were killed as above. The shed exoskeleton was collected and similarly dried and stored. The exoskeleton was usually intact - only two out of sixty were actually consumed during the experiment. (Those two yabbies were not included in subsequent analyses)

All animals were moult staged (Burton and Mitchell, 1987) weighed to the nearest 0.1mg wet weight, and their OCL measured to the nearest 0.1mm with vernier calipers. Comparisons between moult substages determined on the same specimens before immersion in liquid nitrogen and after thawing suggested that such treatment did not affect the moult substage allocated. They were then freeze dried, weighed again and stored in sealed desiccators over silica gel at -20°C. Those crayfish which were too small to measure accurately with the callipers were measured with the binocular dissecting microscope, digitiser and computer apparatus used in Chapter 2.

Field moult increment data was collected from the yabbies which had been allowed to moult overnight in water at the same temperature as the pond at the time of harvest. They were measured before and after the moult. This was felt to be an accurate method as repeated measurements on lab grown animals at A and later substages had not shown any measurable change in OCL from A to C_1 with hardening of the carapace. If any expansion occurred, it was within caliper error ($\pm$ 0.1 mm). There is however more chance of error when measuring at A because of the softness of the carapace. Those yabbies which were already at A when harvested were not included in subsequent analyses. The crayfish used for the moult stage A weight : CL relationship were those collected at D_4 and allowed to moult. They represented the beginning of that stage more precisely than those caught from the field.

3.2.2 Tissue Accumulation and the Moult cycle

Intermoult tissue accumulation was examined using pooled data on moult substage, wet and dry weights and organic content and OCL from all harvests. Yabbies were separated into eleven moult substages ranging from A to D_4 as described above with each moult substage allocated a number (i.e. 1 to 11) to allow statistical analysis. The influence of moult substage on dry weight was examined using GM regression (Ricker, 1973, Sokal and Rohlf, 1981).

Analyses for moult substage-specific ash, and tissue (measured as AFDW) carbon, nitrogen, non-protein nitrogen, chitin and energy composition were carried out on animals whose weights fell within 1 SD of the appropriate moult substage-specific weight vs OCL relationship. In this way data on each moult substage was taken on animals that best represented that substage. Each analysis used a range of sizes between 5 and 17mm OCL for each moult substage. Data on a given moult substage is taken as representing an animal at the beginning of that substage.

a) Tissue Accumulation

Organic content was determined by ashing to constant weight (500°C, 24 h). The numbers ashed varied according to their availability within each moult substage and because carbon, nitrogen, energy and chitin determinations had to be carried out using the same groups. The size-specific relationship between organic content and dry weight was determined for each group of animals within a given moult substage using GM regression. Organic content was also calculated as a percentage of dry weight and examined for moult substage and size-specific effects using ANCOVA.

b) Carbon and Nitrogen

Five replicates within two size groups (5 - 8 mm OCL and 9 - 17 mm OCL) within each moult substage were analysed for carbon and nitrogen. Late intermoult (C_2) was not included in the analysis as resources were limited. Individual freeze-dried yabbies were finely ground, weighed into separate test tubes and analysed for Carbon or Nitrogen content using a Carbon

analyser (Leco Carbon Induction Furnace, Model CR12) and Kjeldahl method respectively. There was enough tissue in the large individuals to perform both analyses on the same animal. Composition was calculated as percentage dry weight. This data was normalised with an arcsin transformation (Sokal and Rohlf, 1981) and analysed for size and moult substage effects using ANCOVA.

c) Chitin

Non-protein nitrogen was assumed to be mostly chitin (Dall, Smith and Moore, 1991) which was analysed using a modification of Raymont`s (*et al* 1963) method as recommended by D Smith (pers comm, 1993). It was measured to allow estimation of protein content from the nitrogen data. Non-protein, non-chitin nitrogen (Anger *et al*, 1983) was beyond the scope of this analysis.

Five yabbies were analysed for each moult substage as follows. Dry individuals (20 - 250 mg) were ground to a fine powder and weighed into separate Pyrex test tubes. Five ml of 1N NaOH was added to each tube and left to stand for five minutes. Each sample was then gently mixed using a vortex mixer and a further 10 ml of NaOH added. The initial five ml dissolved most of the sample, the remaining 10 ml was used to wash the residue from the sides of the tube after mixing. After standing overnight at room temperature the samples were centrifuged for two minutes and the supernatant removed and discarded. Fifteen millilitres of distiled water was added and the sample sonicated centrifuged and the water changed again. The latter two steps were repeated until the supernatant was clear. After the final wash the supernatant was removed and the residue carefully rinsed into tared crucibles. These were oven-dried (60^{o}C) to constant weight and reweighed. This weight was taken to be chitin plus ash (Raymont *et al*, 1963). The crucibles were then ashed (500^{o}C, 12 hours) and weighed again, giving the weight of ash. The weight of chitin was determined by subtraction.

d) Energy Content

This was determined for a range of sizes within each moult substage. Individuals weighing less than about 70 mg (dry weight) were 'bombed' using a Phillipson Microbomb Calorimeter. One to three pellets were made from each individual after the dry tissue was ground to a powder. Those weighing more than about 70 mg were similarly pelleted and analysed using a Galenkamp Ballistic Bomb Calorimeter. Benzoic acid (26.454 kJ/g, Brafield, 1985) was used as a standard in all determinations.

3.2.3 Data Analysis

Data were analysed by a combination of parametric and nonparametric methods using the statistical package SYSTAT$_{TM}$ on a microcomputer. If the data were normal or could be normalised ANOVA or ANCOVA were used. For example, size-specific changes in weight or composition with moult stage were analysed with ANCOVA, whereas ANOVA was used in the analysis of chitin content because there were too few replicates (5 per moult substage) to include size as a variable. Both parametric and nonparametric methods were used in investigating moult substage-specific dry weight-OCL relationships. When the data were normal, ANCOVA was carried out but when the data could not be normalised the following method was used. Geometric mean regressions were carried out on the Log-Log transformed data and confidence limits were calculated (Sokal and Rohlf, 1981). After inspecting the separation between stages, apparent differences were tested using pair-wise Mann Whitney U tests. Analyses are described in more detail in the appropriate sections (below).

3.3 Results

Mean daily temperature varied between 15 and 24.5°C (**Fig 3.3**). The lowest temperatures were recorded in the first fifteen days. Mean temperatures from harvest to harvest (i.e. between days 0 and 15, 16 and 30 and 31 and 53) ranged from 18 to 22°C (**Table 3.1).**

Table 3.1 Mean Daily Water Temperatures (± SE) during Growth Periods (Days)

Growth Period (Days)	Temperature(± SE) (°C)
0 - 15	**18.43 (0.45)**
16 - 30	**20.67 (0.20)**
31 - 53	**21.84 (0.23)**

Mean weekly conductivity was 2.215 (± 0.100) mS/cm and mean weekly pH 7.95 ± 0.397.

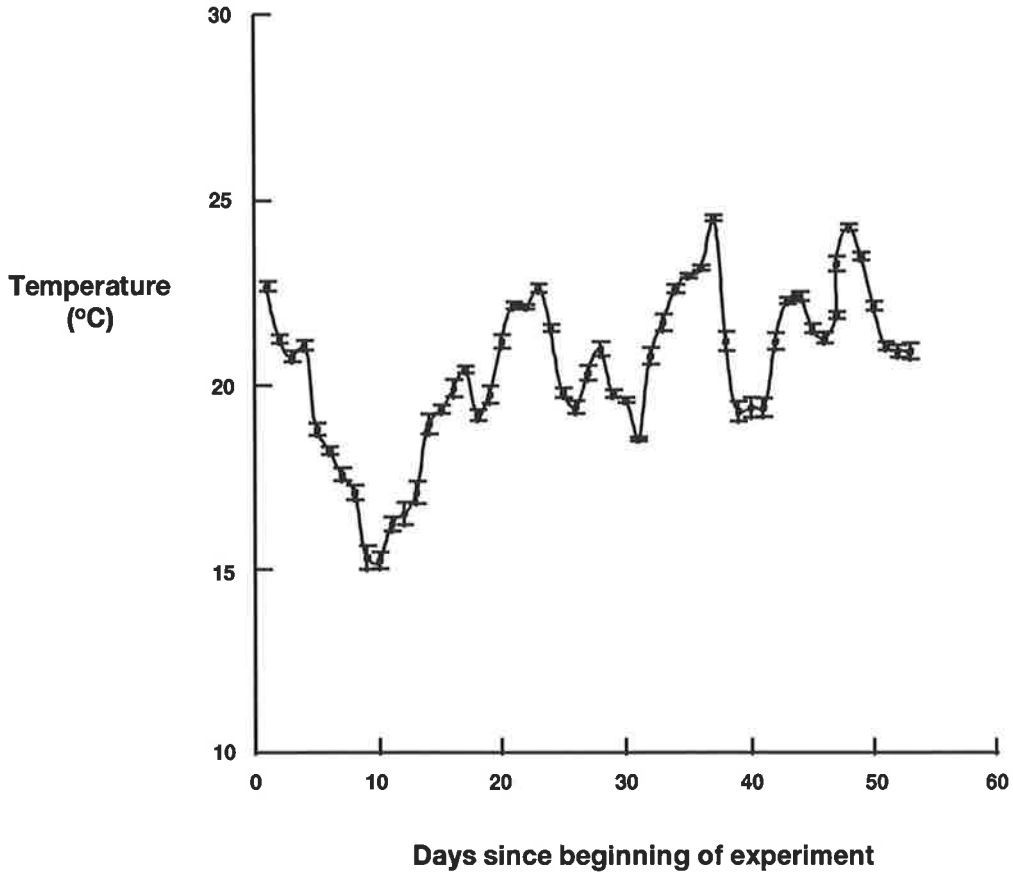
Survival ranged from 33 to 92%. Means and standard errors are presented in **Table 3.2.**

Table 3.2: Percent Survival by Container (c) and Harvest (H). (SE= standard error).

Harvest /container	% Survival	Harvest Mean	SE
H1/c1	73	69.3	8.06
/c2	83		
/c3	39		
/c4	70.5		
/c5	81.5		
H2/c1	33	69.6	9.71
/c2	76		
/c3	69.5		
/c4	92		
/c5	72.5		
H3/c1	77	74.7	6.2
/c2	78.5		
/c3	87.5		
/c4	79.5		
/c5	59.1		

The survival rate did not change between harvests suggesting that most deaths occurred during the first fifteen days of the experiment.

Fig. 3.3
Mean Pool Water Temperature
(°C) over period of experiment (± SE)



3.3.1 Field Growth

The largest yabbie grew to 19.5mm and the heaviest to 3.630g wet weight. The heaviest individual measured 19 mm. The 19.5 mm yabbie was at moult stage A and weighed 3.3g. Growth in terms of ash free dry weight was exponential over the size range used (**Fig 3.4**). As the box and whisker plots (McGill *et al*, 1978) in **Fig 3.5a-c** show there was little variation in OCL between containers within harvests. The mid line in each plot represents the median, the ends of the box enclose the upper and lower quartiles (25%), the ends of the 'whiskers' enclose the extremes and the asterisks and circles show the outliers. The position of the median within the box shows the degree of skew of the data. The plot showing the distribution of the sizes at the beginning of the experiment is shown as 'I' in **Fig 3.5a.**

Cumulative and between harvest instantaneous growth rates were estimated using the growth equation recommended by Pratten (1980) as used in Chapter 2. W_0 for harvests two and three was estimated from the mean weight (afdw) of the preceding harvest. The mean growth rates in joules were calculated in the same way from initial and final caloric content . **Table 3.3a and b** present means and standard errors for all parameters measured for each container, each harvest and the growth periods between harvests. Mean moult stage varied between 4 and 6. Note that this derives from the practice of assigning moult stages A - D_4 numbers from 1 to 11. Thus the means and the standard errors suggest that most animals in each container were between stages C_2 and $D_{1,2}$ when harvested. There was also a slight decline in mean moult stage with successive harvests. Mean growth in weight was low during the first 15 days (**Table 3.3a**: 0.087 $\pm$ 0.006 mg/mg afdw/day), increased from day 15 to 30 (**Table 3.3b**: 0.111 $\pm$ 0.005 mg/mg afdw/day) and dropped again during the last twenty days (0.063 $\pm$ 0.003 mg/mg afdw/day). Crayfish removed at Harvest 1 averaged 0.089 mg/mg afdw/day, at Harvest 2 they averaged 0.1 mg/mg afdw/day and at Harvest 3 they averaged 0.083 mg/mg afdw/day (**Table 3.3a**). In terms of wet weight, mean instantaneous growth was slightly lower at 0.083, 0.097 and 0.081 mg/mg wet weight/day for Harvests 1, 2 and 3 respectively (**Table 3.3a**) .

Table 3.3a Summary of Means (± SE) from each Harvest (H) and Container (C). Harvests 1, 2 and 3 were taken at 15, 30 and 53 days respectively. n= total number of yabbies at each harvest or in each container at harvest. IG = Instantaneous Growth Rate in terms of weight (mg) or energy content (Joules).

Harvest/ Container (n)		Moult Stage	OCL (mm)	Wet Weight (mg)	Dry Weight (mg)	AFDW (mg)	Energy Content (Joules)	IG (wet weight)	IG (afdw)	IG (energy content)
H1	**mean**	**5.30**	**4.50**	**48.431**	**9.201**	**7.064**	**158.292**	**0.083**	**0.090**	**0.089**
(647)	SE	0.10	0.04	1.558	0.331	0.255	5.626	0.001	0.001	0.001
H2	**mean**	**5.19**	**7.94**	**259.843**	**49.200**	**37.937**	**826.282**	**0.097**	**0.101**	**0.100**
(678)	SE	0.09	0.06	6.505	1.150	0.897	18.947	0.001	0.001	0.001
H3	**mean**	**4.35**	**12.36**	**1038.718**	**203.963**	**159.879**	**3329.490**	**0.081**	**0.084**	**0.083**
(727)	SE	0.09	0.09	22.434	4.678	3.704	75.724	0.0004	0.000	0.000
H1C1	**mean**	**4.82**	**4.47**	**41.718**	**8.832**	**6.749**	**153.022**	**0.073**	**0.087**	**0.087**
(146)	SE	0.21	0.06	1.478	0.723	0.548	13.560	0.002	0.003	0.003
H1C2	**mean**	**5.04**	**4.39**	**46.598**	**8.642**	**6.612**	**150.816**	**0.08**	**0.085**	**0.086**
(166)	SE	0.19	0.05	1.723	0.312	0.236	5.810	0.002	0.002	0.003
H1C3	**mean**	**5.42**	**4.19**	**41.095**	**7.705**	**5.921**	**140.375**	**0.072**	**0.078**	**0.081**
(77)	SE	0.23	0.07	2.021	0.378	0.291	7.031	0.003	0.003	0.003
H1C4	**mean**	**5.31**	**4.37**	**41.597**	**7.323**	**5.641**	**119.073**	**0.073**	**0.075**	**0.070**
(95)	SE	0.33	0.06	1.394	0.344	0.262	6.264	0.003	0.003	0.003
H1C5	**mean**	**5.95**	**4.84**	**68.167**	**11.904**	**9.177**	**201.949**	**0.106**	**0.107**	**0.105**
(163)	SE	0.23	0.10	2.496	1.035	0.807	16.599	0.004	0.003	0.003

Table 3.3a contd Summary of Means (± SE) from each Harvest (H) and Container (C). Harvests 1, 2 and 3 were taken at 15, 30 and 53 days respectively. n= total number of yabbies at each harvest or in each container at harvest. IG = Instantaneous Growth Rate in terms of weight or energy content (Joules). afdw = Ash Free Dry Weight (mg)

Harvest/ Container (n)		Moult Stage	OCL (mm)	Wet Weight (mg)	Dry Weight (mg)	AFDW (mg)	Energy Content (Joules)	IG (wet weight)	IG (afdw)	IG (energy content)
H2C1	**mean**	**5.33**	**7.35**	**199.152**	**36.809**	**28.388**	**619.204**	**0.089**	**0.091**	**0.090**
(66)	SE	0.28	0.19	17.134	2.561	2.014	41.086	0.002	0.002	0.002
H2C2	**mean**	**5.39**	**7.95**	**274.309**	**51.022**	**39.551**	**862.127**	**0.099**	**0.102**	**0.101**
(144)	SE	0.21	0.14	14.651	2.756	2.187	45.003	0.002	0.002	0.002
H2C3	**mean**	**5.00**	**8.07**	**264.309**	**49.761**	**38.324**	**836.861**	**0.0098**	**0.101**	**0.100**
(139)	SE	0.19	0.11	10.438	1.937	1.496	32.226	0.001	0.001	0.001
H2C4	**mean**	**5.07**	**8.15**	**270.457**	**53.020**	**40.802**	**884.047**	**0.099**	**0.103**	**0.102**
(184)	SE	0.19	0.12	12.011	2.234	1.717	36.569	0.001	0.001	0.001
H2C5	**mean**	**5.24**	**7.88**	**254.786**	**51.885**	**40.115**	**874.007**	**0.097**	**0.103**	**0.102**
(145)	SE	0.19	0.16	17.636	4.104	3.267	69.440	0.002	0.002	0.002
H3C1	**mean**	**4.52**	**11.42**	**840.844**	**163.186**	**126.918**	**2639.578**	**0.077**	**0.080**	**0.078**
(150)	SE	0.20	0.17	33.733	7.404	5.693	114.345	0.001	0.001	0.001
H3C2	**mean**	**4.44**	**12.34**	**1048.139**	**201.896**	**158.685**	**3315.294**	**0.081**	**0.084**	**0.083**
(151)	SE	0.22	0.20	49.396	10.279	8.187	169.448	0.001	0.001	0.001
H3C3	**mean**	**4.28**	**12.49**	**1082.154**	**222.333**	**175.553**	**3652.709**	**0.082**	**0.086**	**0.084**
(172)	SE	0.18	0.21	52.072	11.235	8.987	181.938	0.001	0.001	0.001
H3C4	**mean**	**4.38**	**12.54**	**1063.410**	**210.405**	**163.507**	**3385.151**	**0.082**	**0.085**	**0.083**
(156)	SE	0.18	0.19	47.774	9.756	7.570	150.780	0.001	0.001	0.001
H3C5	**mean**	**4.01**	**13.29**	**1218.276**	**227.067**	**178.882**	**3751.465**	**0.084**	**0.086**	**0.085**
(98)	SE	0.24	0.25	65.643	12.767	10.230	218.368	0.001	0.001	0.001

Table 3.3b Mean Instantaneous growth rates (IG) in terms of weight and caloric content (± SE) during each growth period. Growth periods were between 1 and 15, 16 and 30 and 31 and 53 days. AFDW= ash free dry weight (mg)

Growth Period	IG (AFDW)	SE	IG (Joules)	SE
1-15	0.087	0.006	0.086	0.006
16-30	0.111	0.005	0.108	0.005
31-53	0.063	0.003	0.060	0.003

Fig. 3.4
Field Growth by Container
(afdw vs. time (days)) (means ± SE)

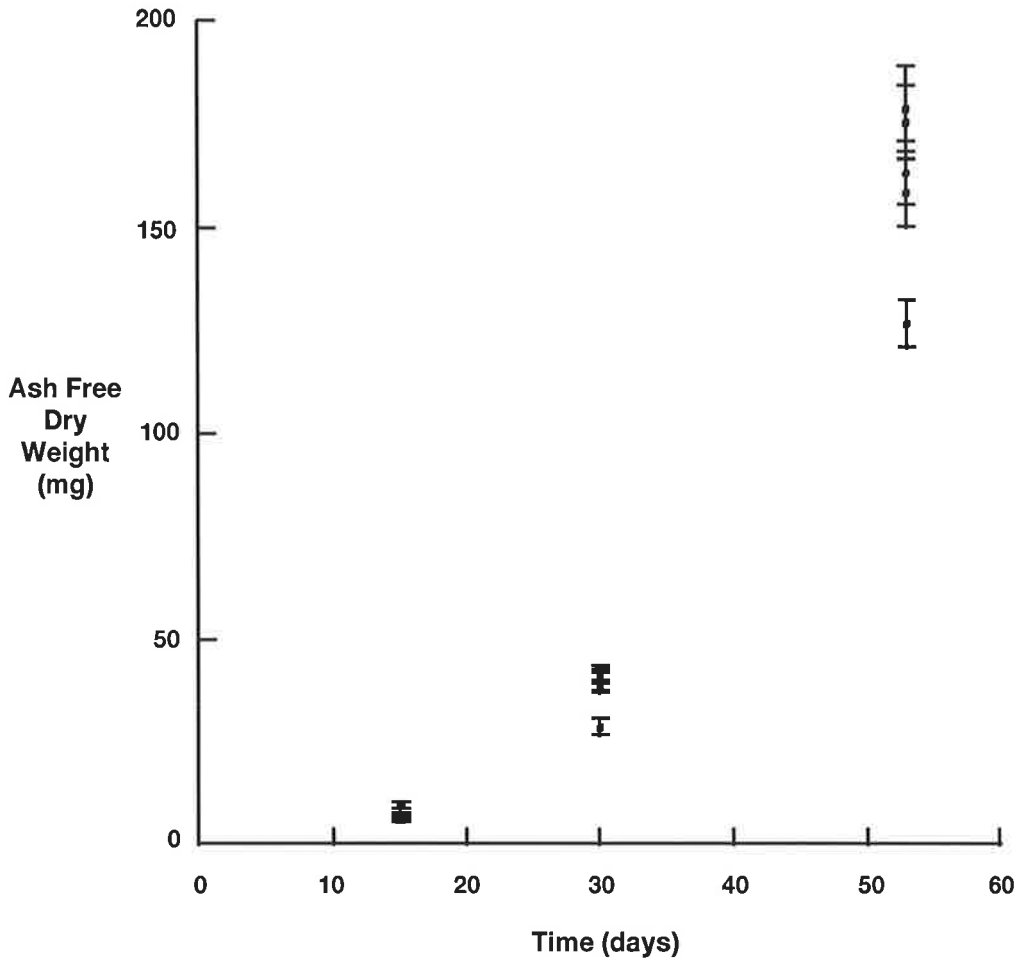
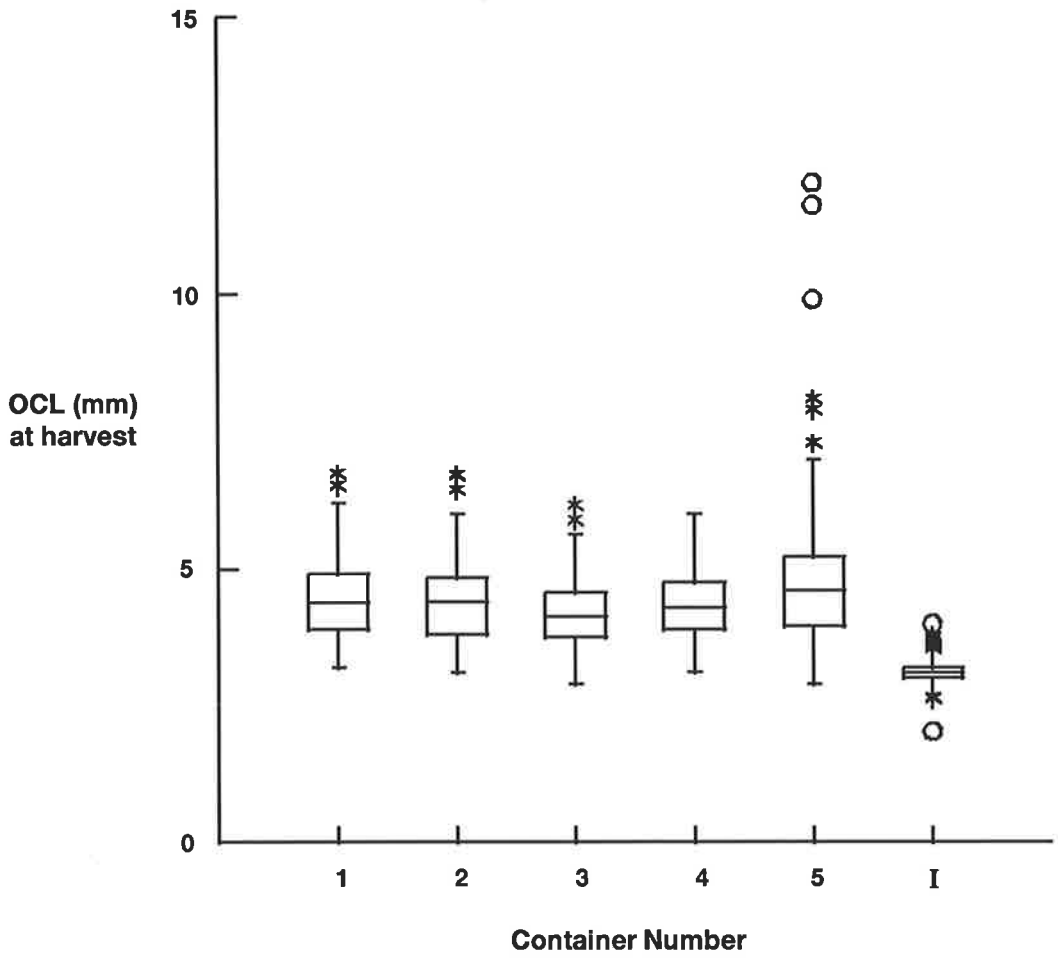


Fig. 3.5a
Harvest 1: OCL (mm) vs Container Number at Harvest
(see text for explanation of box & whisker plots)



I = initial sample

Fig. 3.5b
Harvest 2: OCL (mm) vs Container Number

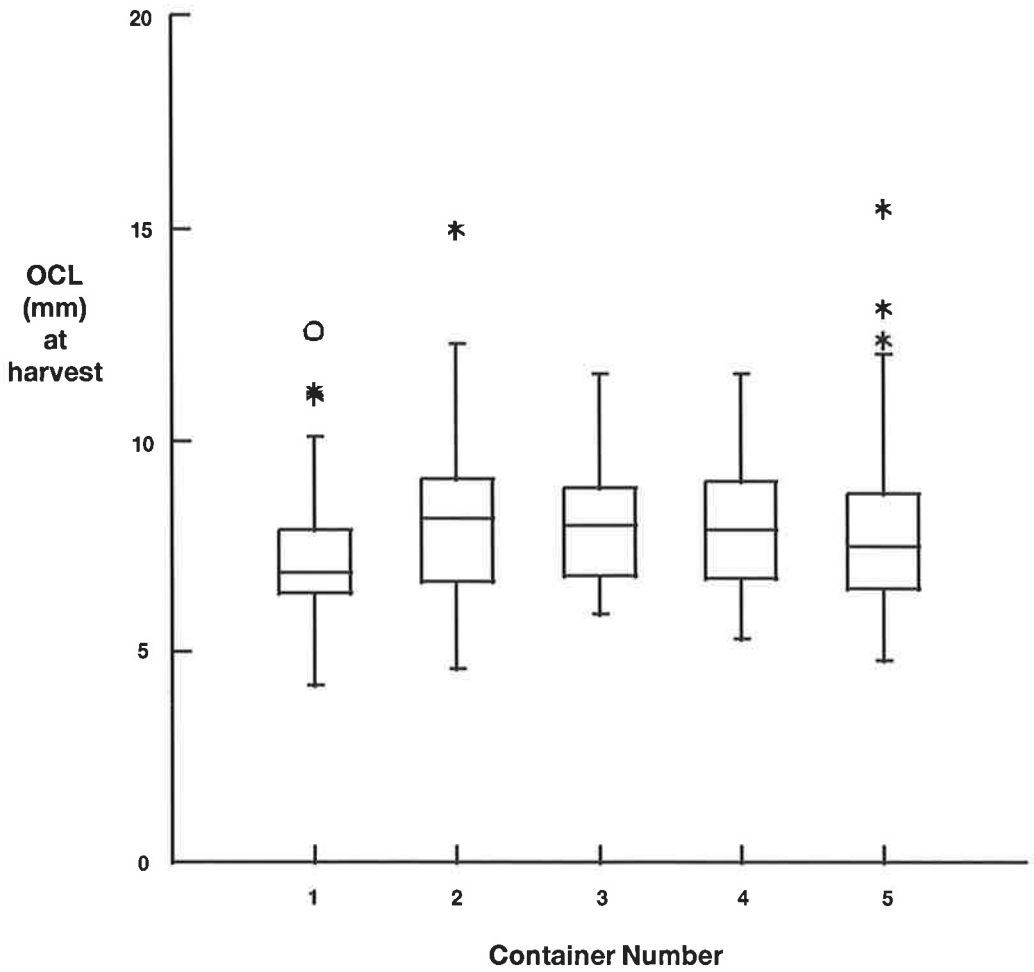
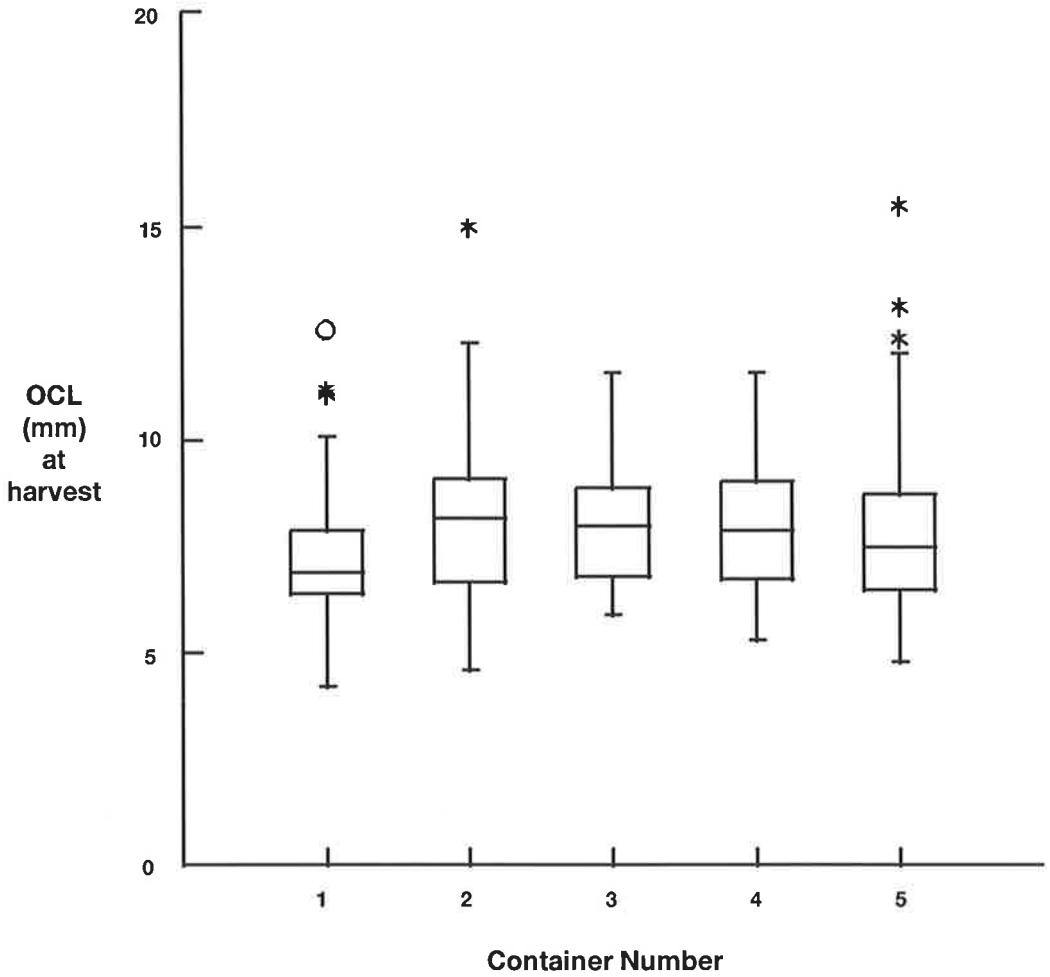


Fig. 3.5c
Harvest 3: OCL (mm) vs Container Number



a) Moult Increment

Mean percent moult increment varied from 16.2 to 17.5% of Premoult OCL, with the lowest % MI observed in the first harvest. (**Table 3.4**).

Table 3.4: Overall and Harvest-specific Mean % Moult Increment (± SE)

Harvest	Mean % MI	SE	n
1	**16.211**	**0.9805**	**14**
2	**17.539**	**1.318**	**14**
3	**16.758**	**1.165**	**20**
Overall	**16.742**	**0.574**	**48**

These differences were non significant (ANOVA, P>0.05). The pooled data gave a mean of 16.74% (± 0.578). There were also no changes in %MI with increasing size (**Fig 3.6**).

b) Exuviae Weight

Exuviae weight accounted for between 12 and 13% of dry weight and 7-8% of organic content at D_4. Exuviae contained 50.00% (± 0.781, n=15) organic content. The latter was independent of size. Weights (dry and ash free) were regressed against Premoult OCL (mm) after log-log transformation. The resulting lines were:

Log dry weight (mg) = Log -1.907 + 3.091 Log OCL

Log ash free dry weight (mg) = Log -2.208 + 3.090 Log OCL

Both lines were significant at $P < 0.001$.

c) Size Classes and Growth

Because of the similarity between containers, animals were pooled within harvests and size frequency histograms constructed (**Fig 3.7a to e**). These show the development and subsequent fragmentation of size classes with time. Histograms are also included for the initial sample and the combined harvest data.

Fig 3.6
% Moult Increment vs Size (OCL) and Harvest Number

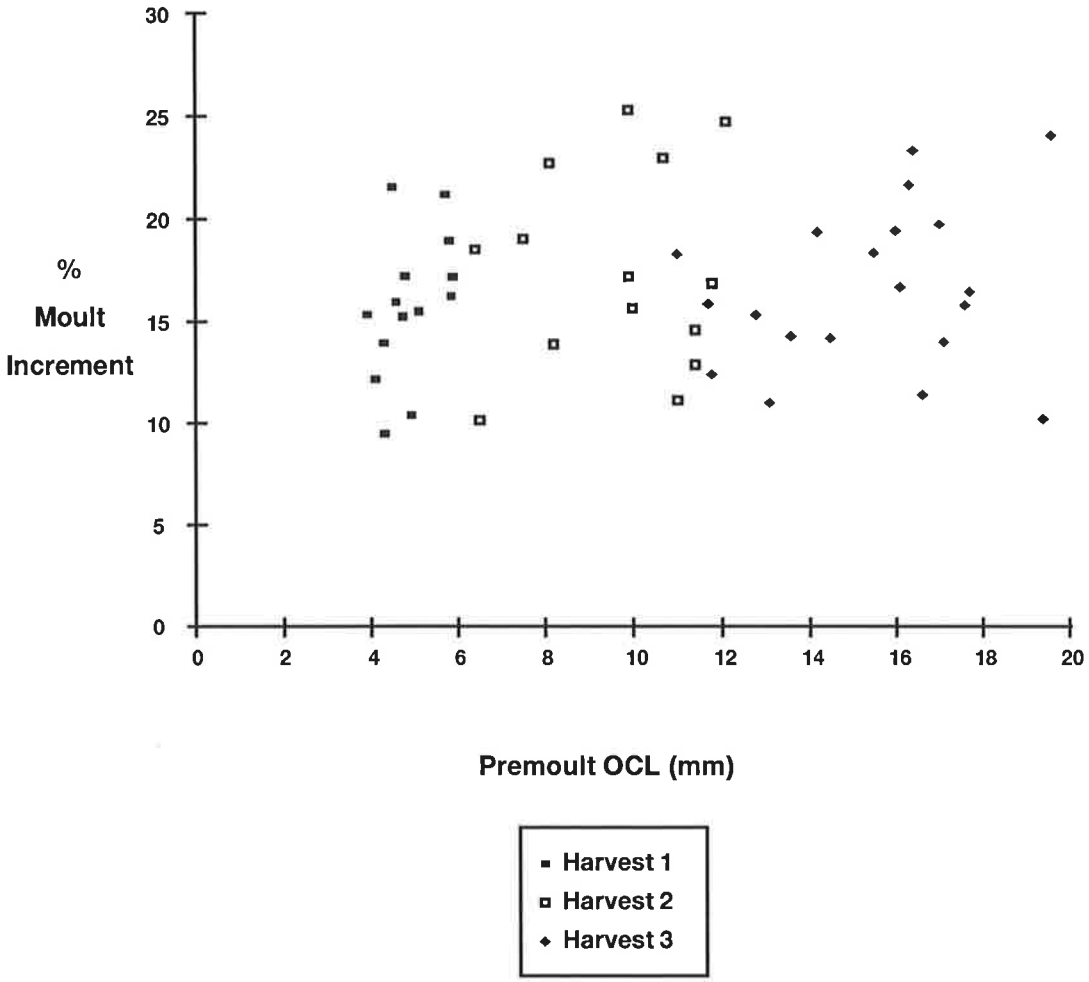


Fig 3.7a
Initial Sample
n=200

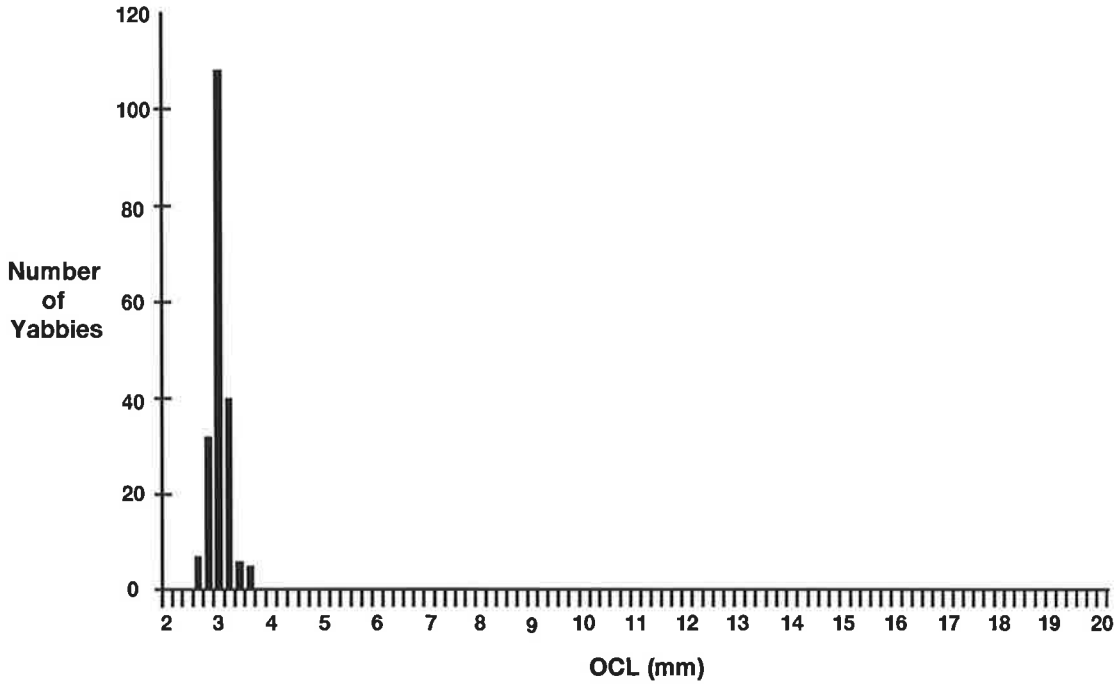


Fig 3.7b
Harvest 1
n = 647

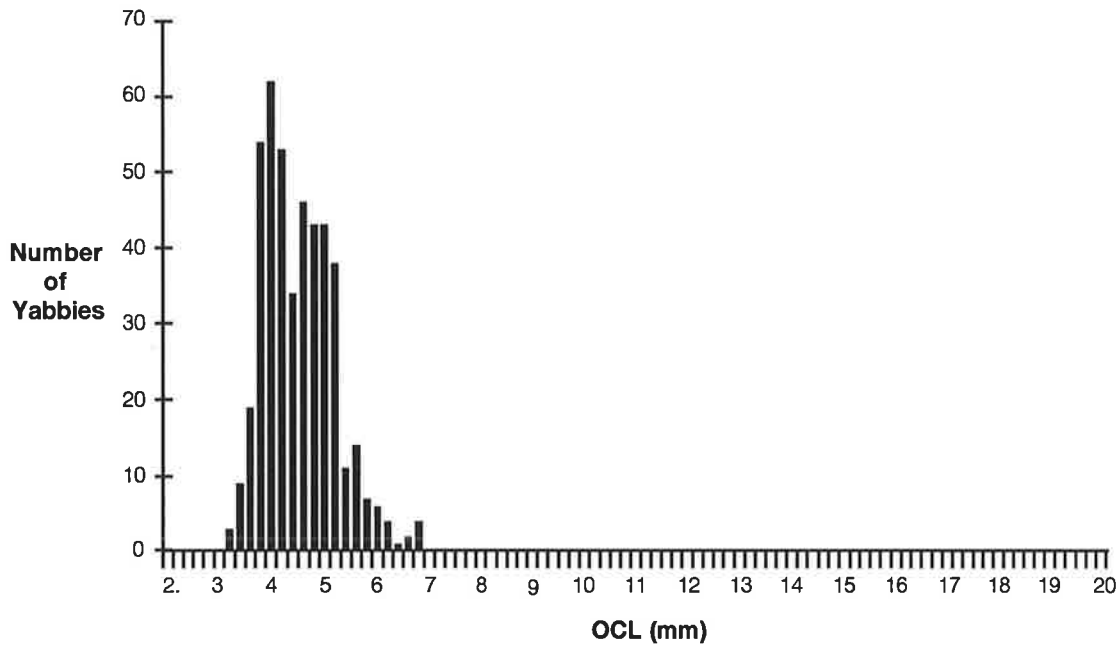


Fig 3.7c
Harvest 2
n=678

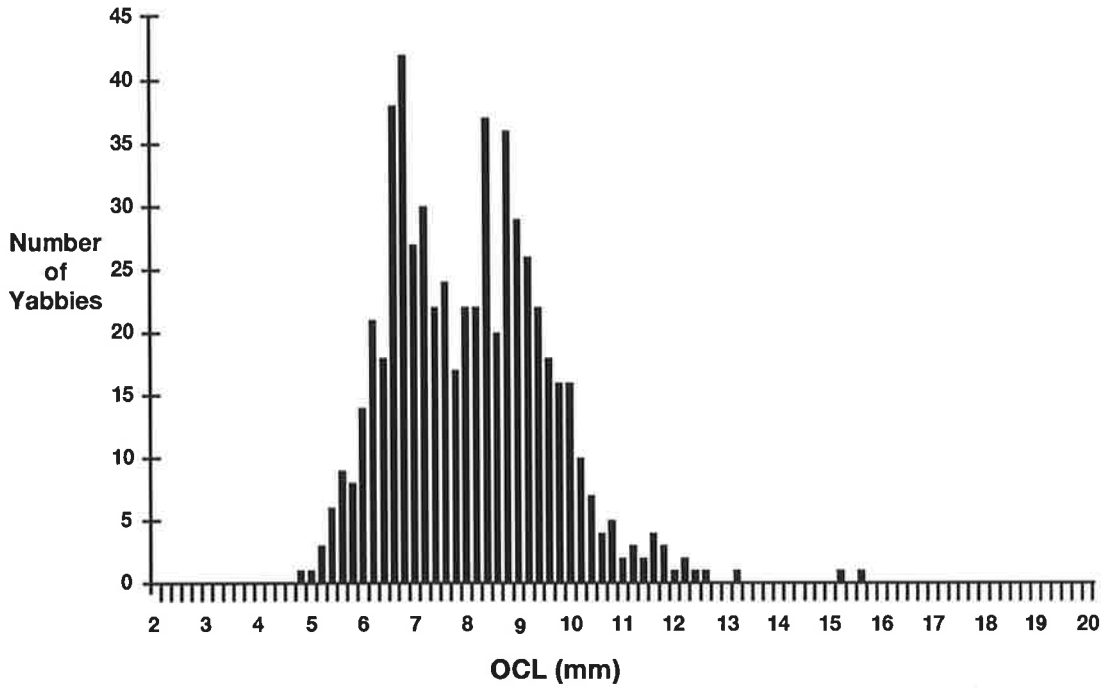


Fig 3.7d
Harvest 3
n=727

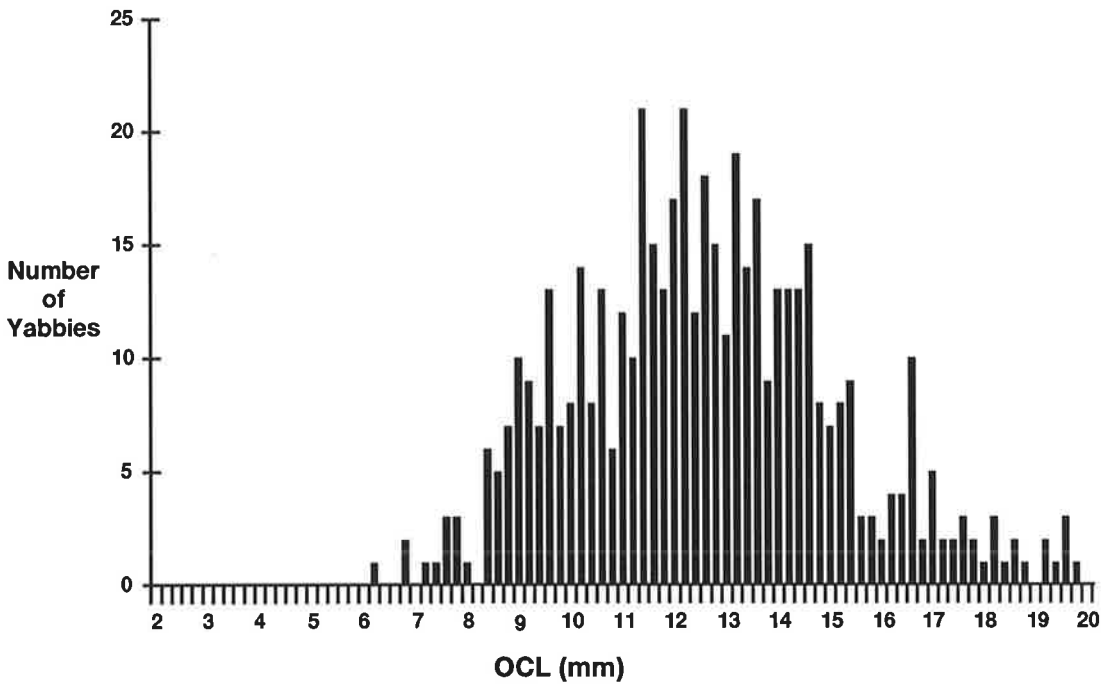
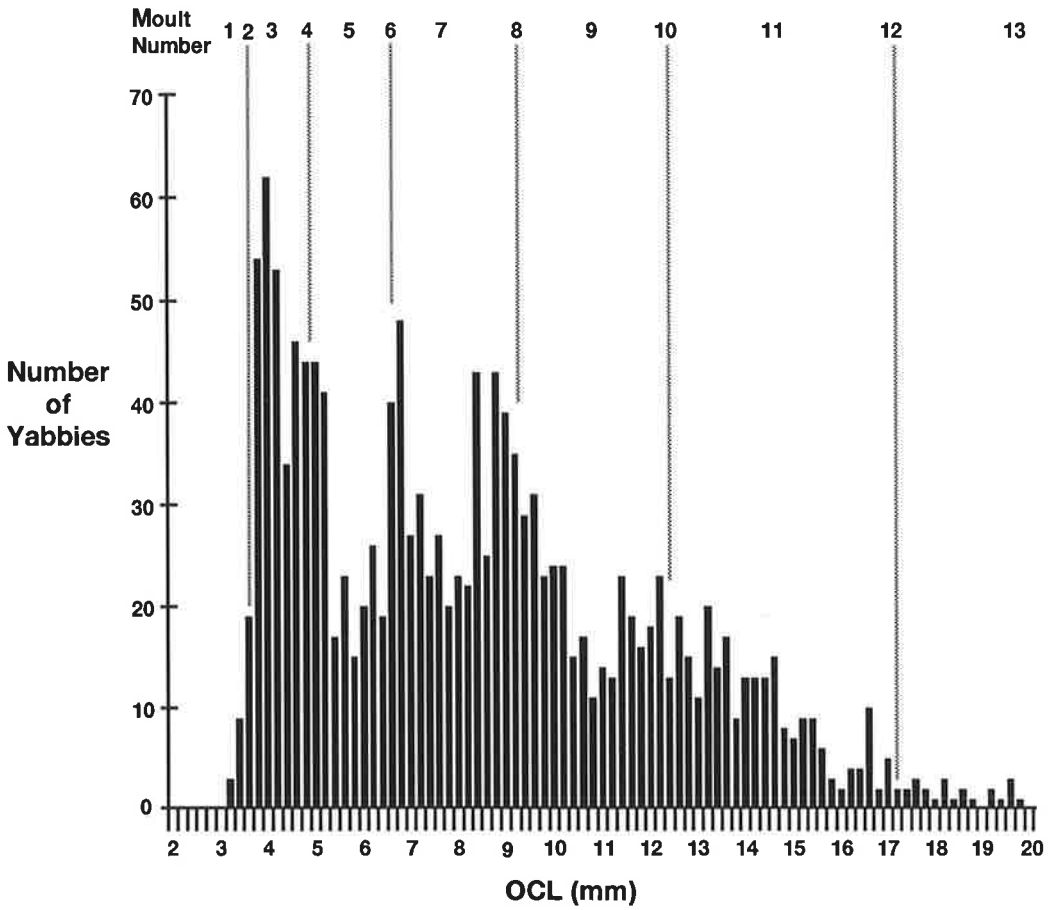


Fig 3.7e
All Harvests
n=2052



Key

Moult Number	OCL (mm)
1	3.1
2	3.6
3	4.2
4	4.9
5	5.8
6	6.7
7	7.8
8	9.2
9	10.7
10	12.5
11	14.6
12	17.0
13	19.9

Size class modes and their standard errors were to be estimated from combined within and between harvest size frequency data using MIX (McDonald and Pitcher, 1979). This program divides data into size classes by superimposing and testing the fit of normal distributions. In this case its usefulness was limited because although there were definable peaks in the data none approached normality, even when transformed.

Despite this, some general trends are visible. There were apparent peaks at 3.1 mm OCL in the initial sample, 4, 4.8 and 5.6 mm for harvest 1 and 6.8 mm for the second harvest. Further peaks could not be distinguished because of size class overlap. There may have been three to four moults between the start of the experiment and harvest 1, although some yabbies moulted up to 5 times. This is suggested by the two peaks in harvest 1 and the difference in OCL between the initial sample and peak 1 of the harvest 1 (29%) given that the % moult increment was probably 16-17% (see Moult Increment - above). The skew of the size frequency distribution suggests that some individuals moulted four to five times. Skew increased with harvest number. The difference (42%) in OCL between the last major peak of harvest 1 (4.8) and the first of harvest 2 (6.8) also suggests at least two moults. Modal sizes were calculated by applying the mean moult increment, 16.74%, to the mean initial size (3.1 mm OCL) and calculating successive size classes iteratively up to 19.9 mm OCL. The process suggested that 13 moults were required to reach 19.9 mm OCL. These are shown in **Fig 3.7e** with the appropriate modal size estimate in mm OCL, and suggest a degree of coincidence between the generated modal sizes and the size frequency distribution. The match is reasonable at the extremes of the distribution but deteriorates in the middle because of size class overlap.

d) Length Of Moult Substages

Each of the three harvests collected all individuals, therefore the chance of catching a yabbie at a particular moult substage should be proportional to the length of that moult substage in the moult cycle. Thus, if 25% of the harvest were found to be at moult substage $D_{1.1}$ it follows that this moult substage represents 0.25 of the moult cycle.

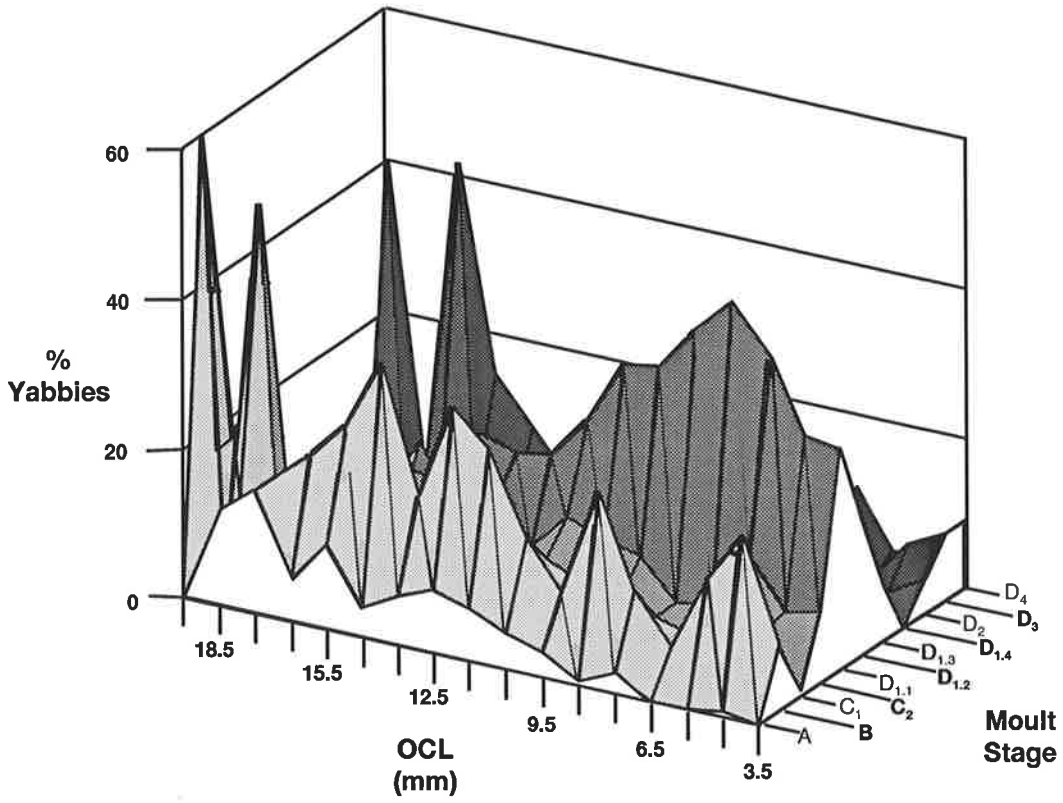
Yabbies were divided into 1mm size groups and the distribution of animals to moult substages in each size group was calculated (**Fig 3.8**). Of the sixteen size groups investigated (3mm to 19mm OCL), the first 11 groups (3mm OCL to 14mm OCL) contained 100+ crayfish each and these were used to estimate the length of the moult substages. The remaining 5 groups showed over-representation of animals at the either end of the moult cycle. **Table 3.5** shows the number of yabbies at each moult substage and its percentage of the total yabbie number as a representation of proportion of the moult cycle taken up by each moult substage in this study.

Table 3.5 Yabbies per Moult Stage from pooled data compared with % contribution of each moult stage to the moult cycle as estimated by Burton and Mitchell, 1987. A+B = postmoult, C_1+C_2 = intermoult, $D_{1.1}$-$D_{1.4}$ = premoult and D_2 - D_4 = late premoult. Refer text for explanation.

Moult Cycle phase	Moult Stage/ substage	Yabbies per Moult Stage	% Yabbies per Moult Stage	% Moult stage length (Burton and Mitchell 1987)
Postmoult	A	45	2.52	2.0
	B	301	16.87	1.7
Intermoult	C_1	147	8.24	38.3
	C_2	196	10.99	17.4
Premoult	$D_{1.1}$	580	32.5	29
	$D_{1.2}$	187	10.48	2.3
	$D_{1.3}$	105	5.88	3.4
	$D_{1.4}$	27	1.5	3.4
Late Premoult	D_2	41	2.3	1.3
	D_3	59	3.31	0.6
	D_4	96	5.38	0.6
	total	**1784**		

For comparison, the table also includes moult substage length estimates from a lab study by Burton and Mitchell (1987). The correspondence between the two studies will be discussed below.

Fig 3.8
Percentage of Yabbies per Moult Stage vs OCL (mm)



3.3.2 Tissue Accumulation and the Moult Cycle

The geometric mean regression statistics for moult stage-specific wet and dry weight vs OCL relationships are presented in **Table 3.6.** The slopes of the log OCL vs log Weight lines varied about 3 , showing the expected cubic relationship between the two parameters.

The following procedure was then carried out to identify possible size and moult stage effects on weight. The OCL vs wet and dry weight relationships for each moult stage (**Table 3.6**) were backtransformed and solved for a series of consecutive size classes to produce matrices of moult stage and OCL-specific weights as illustrated below.

	Moult Stage			
Size class	**A**	**B**	**C_1**	**etc**
3.1	X_o	X_1	X_2	
3.6	Y_o	Y_1	Y_2	
etc				

X_n and Y_n are size- specific wet or dry weights at a given moult stage. The size class intervals were determined by iteration using the mean initial size of 3.1 mm OCL and the mean moult increment (16.74%) as discussed in relation to **Fig 3.7e.** Each moult stage-specific weight was then divided by the corresponding weight at Stage A for each size class (i.e. X_1/X_o, X_2/X_o etc) to produce a model of proportional change in weight with moult stage and size as shown in **Figures 3.9a and b**. These figures show that although wet weight changed very little, dry weight changed a great deal during the moult cycle.

Both parametric and nonparametric methods were used in investigating moult substage-specific dry weight-OCL relationships. An ANCOVA was carried out on the data for Harvest 3 as OCL was normally distributed (**Fig 3.7d,** $P<0.088$, Kolmogorov-Smirnov test for Normality, Sokal and Rohlf, 1981) and dry weight could be normalised by log transformation. This analysis showed significant moult substage effects and interaction between moult substage and OCL (**Table 3.7**).

Table 3.6 Log OCL(mm) vs Log Wet and Log Dry Weight(mg) for 11 moult stages. All regressions and slopes are significant (P<0.001). The equation was Log Weight = Log a + b (Log OCL).

a) Wet Weight (mg)

Moult Stage	n	Slope(b)	Intercept (a)	SE(b)	SE(a)	r^2
A	75	2.9859	-0.3514	0.0415	0.0408	0.9859
B	360	3.0468	-0.3802	0.0273	0.0257	0.9712
C_1	191	3.0394	-0.3666	0.0348	0.0338	0.9752
C_2	216	3.0056	-0.3274	0.0245	0.0228	0.9857
$D_{1.1}$	597	3.0103	-0.3295	0.0170	0.0147	0.9810
$D_{1.2}$	212	2.9821	-0.2755	0.0291	0.0248	0.9800
$D_{1.3}$	119	2.9596	-0.2382	0.0210	0.0190	0.9941
$D_{1.4}$	38	3.0500	-0.3316	0.0329	0.0295	0.9958
D_2	43	2.9748	-0.2529	0.0331	0.0228	0.9949
D_3	60	2.8835	-0.1823	0.0502	0.0373	0.9824
D_4	64	2.9921	-0.2420	0.0467	0.0356	0.9849

b) Dry Weight (mg)

Moult Stage	n	Slope(b)	Intercept (a)	SE(b)	SE(a)	r2
A	75	3.0911	-1.3115	0.1085	0.1138	0.9286
B	360	3.0560	-1.2120	0.0313	0.0295	0.9623
C_1	191	3.2945	-1.3632	0.0501	0.0494	0.9589
C_2	216	3.1771	-1.2136	0.0444	0.0419	0.9607
$D_{1.1}$	597	3.1597	-1.1474	0.0205	0.0176	0.9741
$D_{1.2}$	212	3.1116	-1.0252	0.0349	0.0301	0.9753
$D_{1.3}$	119	3.0256	-0.9047	0.0313	0.0286	0.9883
$D_{1.4}$	38	3.1048	-0.9880	0.0404	0.0374	0.9946
D_2	43	3.1029	-1.0020	0.0554	0.0505	0.9891
D_3	60	2.9395	-0.8601	0.0609	0.0455	0.9785
D_4	64	3.0135	-0.9083	0.0787	0.0569	0.9713

Fig 3.9a

Wet Weight (mg) vs OCL(mm) over the Moult Cycle

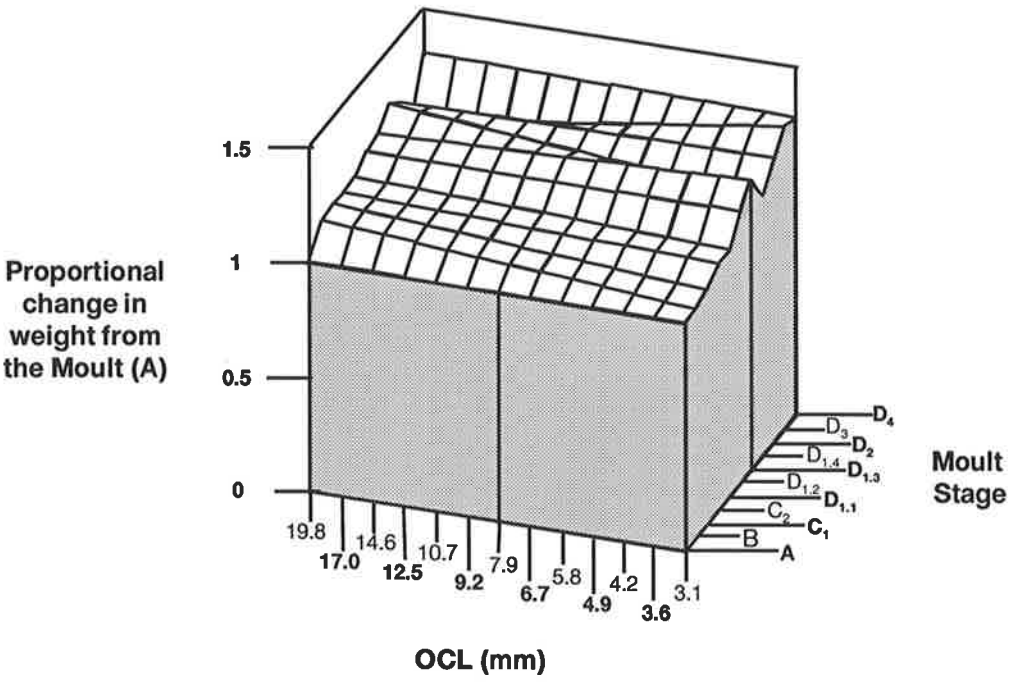


Fig 3.9b

Dry Weight (mg) vs OCL(mm) over the Moult Cycle

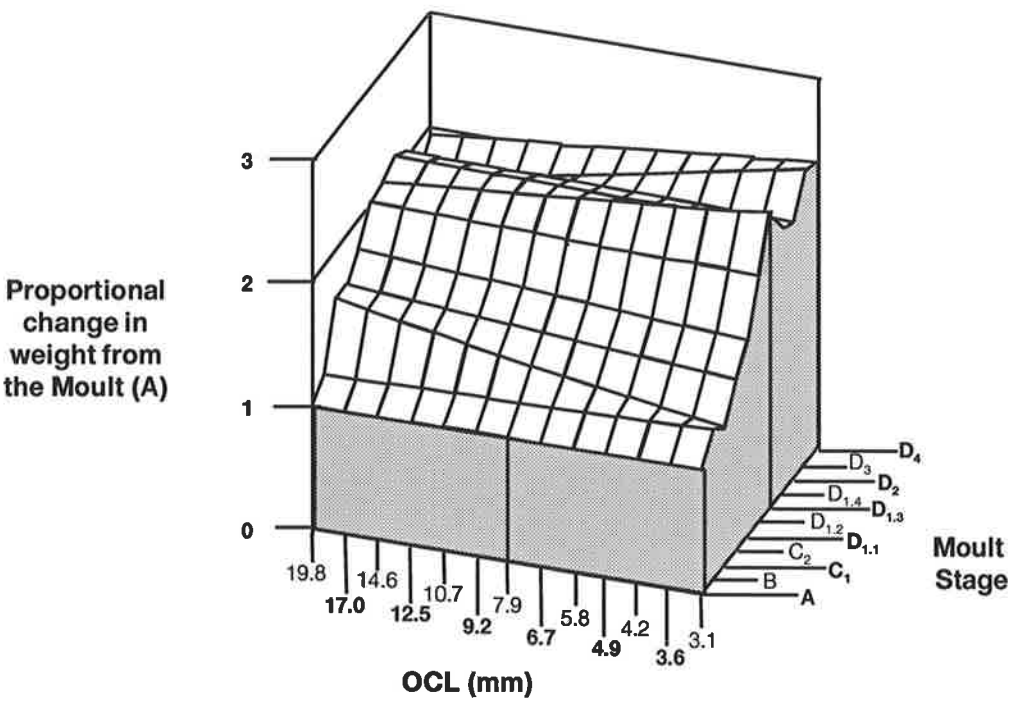


Table 3.7 ANCOVA on OCL (mm) vs Moult Stage-specific Dry Weight (mg) for Harvest 3. Data normalised using Box-Cox transformation. SS= sums of squares, DF= degrees of freedom; MS= mean square; P = probability level. r^2=0.919, n=666

Source	SS	DF	MS	F	P
Moult Stage	**0.258**	**10**	**0.026**	**4.619**	**<0.001**
OCL	**10.747**	**1**	**10.747**	**1921.268**	**<0.001**
Moult Stage x OCL	**0.402**	**10**	**0.040**	**7.185**	**<0.001**
Error	**3.602**	**644**			

Breakdown of the moult cycle into the categories postmoult (i.e. A and B), intermoult (i.e. C_1 and C_2) and premoult/late premoult (i.e. $D_{1.1}$ - $D_{1.4}$ and D_2 - D_4) showed that the interaction was confined to post moult substages (A and B) (Column 1; **Table 3.8**). Moult substage had no effect on weight between C_2 and $D_{1.2}$ and between $D_{1.3}$ and D_4 (Columns 4 and 6; **Table 3.8**). However, it was significant during postmoult and between C_1 and $D_{1.1}$ and between $D_{1.2}$ and $D_{1.3}$ (Columns 3 to 5; **Table 3.8**). The increase in dry weight during postmoult (A and B) may represent calcification while the increases between C_1, $D_{1.1}$, $D_{1.2}$ and $D_{1.3}$ represent tissue growth.

The remaining data were not normalisable so analyses were carried out on the pooled data (all harvests) using the following method. Ninety-five % confidence belts were calculated (Sokal and Rohlf, 1981) for each line in **Table 3.6** and its associated data as shown in **Fig 3.10 a, b and c.** When the lines and confidence limits are presented on the same graph, changes in weight with moult substage and their significance become apparent. Separation between the upper limit of a given moult substage and the lower limit of the next suggests differences which are significant at 95% and above. Note that differences between consecutive moult substages (i.e. $D_{1.1}$ and $D_{1.2}$) represent the weight gained during the earlier of the two substages. Further analyses were carried out using non-parametric Mann-Whitney U tests (Sokal and Rohlf, 1981) on Systat. Differences in dry weight at a given carapace length were examined for pairs of moult substages that appeared to differ in **Fig 3.10** and **Fig 3.9b.** Measurement error was taken into account by including all values within $\pm$ 0.1 mm of a

Table 3.8 ANCOVA on OCL (mm) vs Moult Stage-specific Dry Weight (mg) for Harvest 3.
Analysis within moult stages and between substages. P= Probability Level, Dependent variable = Dry Weight

	Column 1 (see text)	**2**	**3**	**4**	**5**	**6**
	A and B	**B and C_1**	**Compared C_1 and $D_{1.1}$**	**Stages C_2 to $D_{1.2}$**	**C_2 to $D_{1.3}$**	**$D_{1.3}$ to D_4**
Source	**P**	**P**	**P**	**P**	**P**	**P**
Moult Stage	**0.033**	**0.031**	**0.043**	ns	**0.006**	ns
OCL	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Moult Stage x OCL	**0.036**	**<0.001**	ns	ns	ns	ns
n	**177**	**250**	**337**	**300**	**349**	**92**

Fig 3.10a
Log OCL (mm) vs Log Dry Weight (mg)
for Moult Stages from A to D1.3

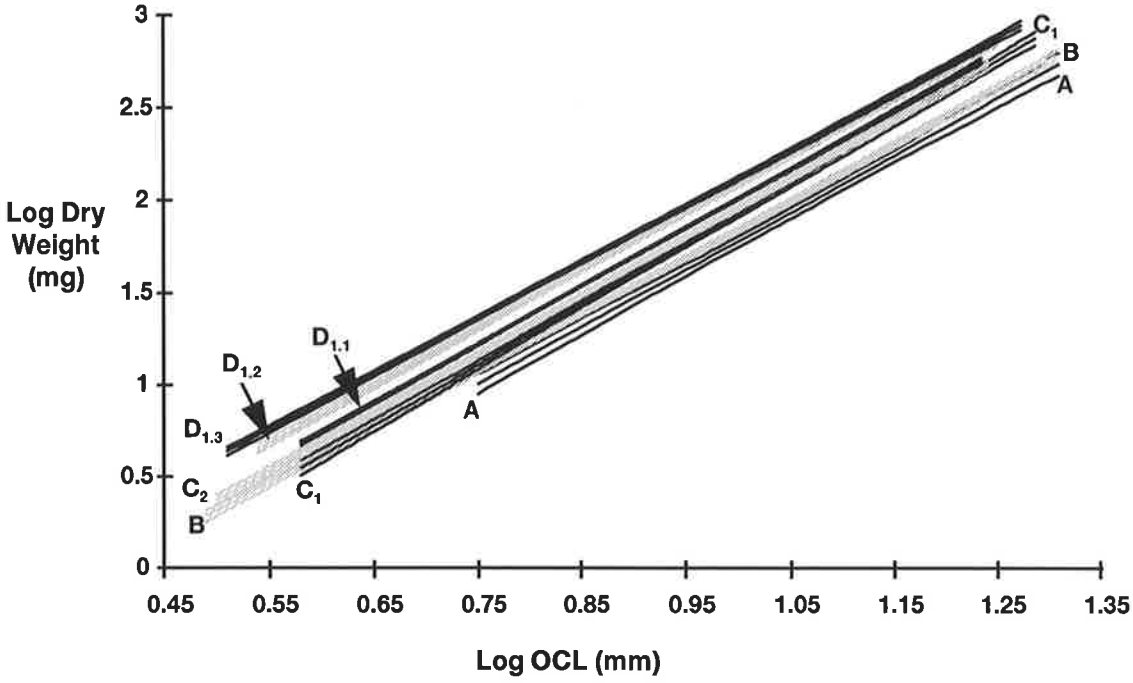


Fig 3.10b
Log OCL (mm) vs Log Dry Weight (mg)
for Moult Stages D2 and D1.3

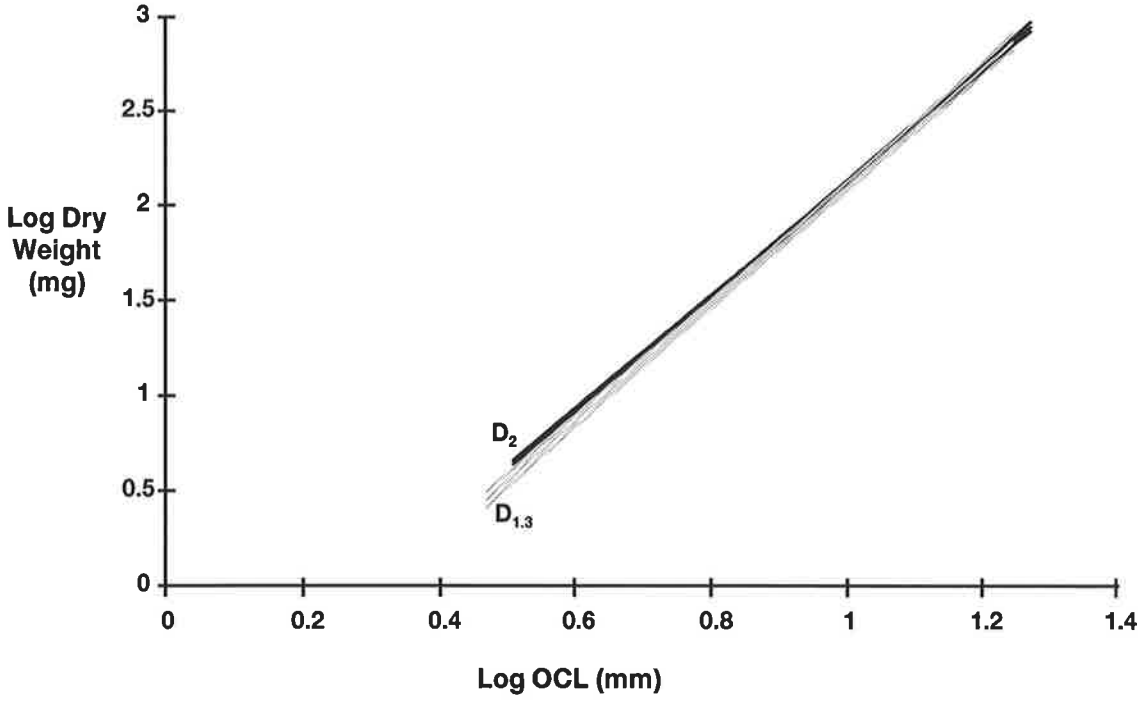
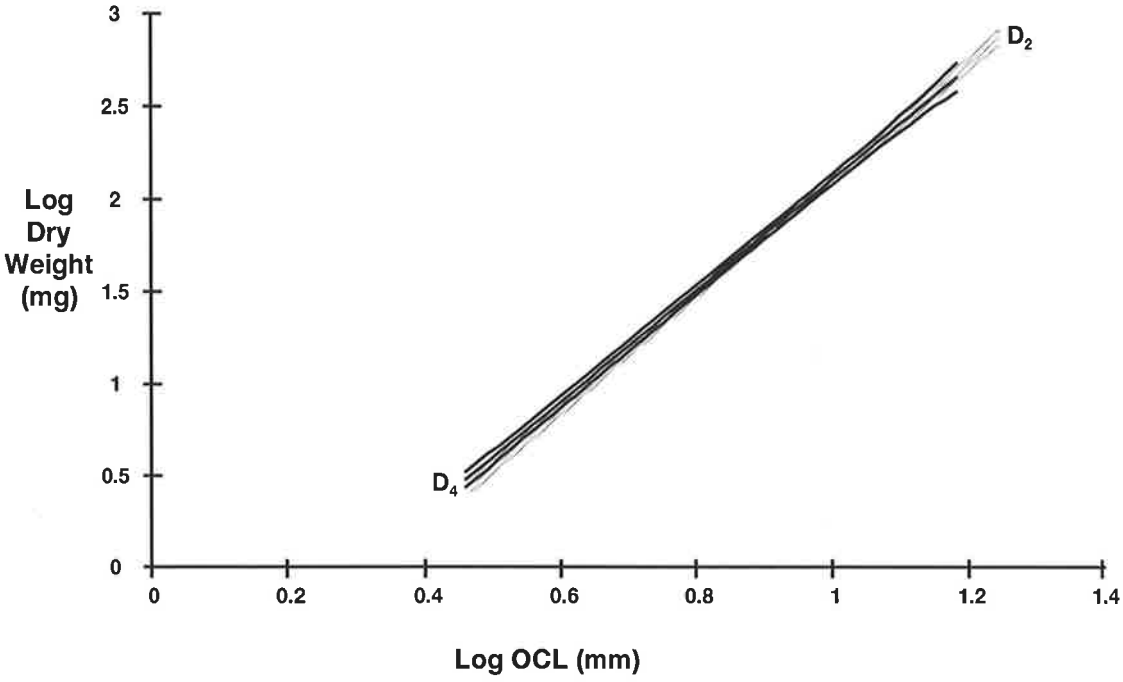


Fig 3.10c
Log OCL (mm) vs Log Dry Weight (mg)
for Moult Stages D2 and D4



given OCL as representative of that OCL. A range of carapace lengths were examined for each pair of substages dependent on availability of sufficient numbers for comparison. For example, stage A and substage $D_{1.3}$ could only be compared at 6.4, 11.1 and 14.1 mm OCL (3 pairs). Pairs of carapace lengths were tested separately within each pair of moult stages to investigate possible size effects.

Table 3.9 shows the significant results from this series of tests. Dry weight increased between A and $D_{1.3}$ (Test 1, $P < 0.05$ -0.001) over a wide size range. Smaller crayfish of about 4.6 mm OCL then declined in weight between $D_{1.3}$ and D_2 (Test 2). The subsequent increase between D_2 and D_4 (Test 3) was again limited to the smaller yabbies whereas that between B and C_1 was significant for animals of 9 mm OCL and above (Test 4). Weight also increased over a wide size range for crayfish between substages C_2 and $D_{1.1}$ (Test 5) and between $D_{1.1}$ and $D_{1.2}$ (Test 6). Finally, yabbies up to 6.5 mm OCL increased in weight between $D_{1.2}$ and $D_{1.3}$ (Test 7).

Thus, significant weight gain occurred up to the end of substage $D_{1.2}/D_{1.3}$. Gains were recorded during all preceding stages/substages especially B (i.e. between stages B and substage C_1) and $D_{1.1}$. There were also significant size-specific changes in weight between substages $D_{1.3}$ and D_4.

Organic content, measured as ash free dry weight, varied between 74 and 81.7% of body dry weight (**Table 3.10**). The organic content of the shed exoskeleton (exuvia) averaged 50.000 % ($\pm$ 0.781; n=15) and was independent of size ($P>0.05$). ANCOVA on the normalised OCL and body organic content data showed significant moult substage, OCL and interaction effects (Column 1 in **Table 3.11a**) so the data were broken down into moult cycle categories postmoult (A and B), intermoult (C_1 and C_2), premoult ($D_{1.1}$ to $D_{1.4}$) and late premoult (D_2 to D_4) and re-analysed. This showed that the interaction effect was confined to intermoult substage C_1 (Columns 2 and 3, **Table 3.11a**). Differences between stages were also tested and found to be significant in all cases except that of premoult ($D_{1.1}$-$D_{1.4}$) against late premoult (D_2-D_4) (Column 7, **Table 3.11a** : $P>0.05$). There were no moult substage or OCL effects within postmoult or premoult/late premoult (Columns 4 and 7, **Table 3.11a**) so the data within each of these stages were pooled and means derived (**Table 3.12**). ANCOVA on the

Table 3.9 Mann-Whitney U tests of Dry Weight (mg) change between Moult Stages and Substages.
Degrees of freedom = 1 in each case. n= total number of animals of a given size compared within each test (i.e. test 1, n=16 : 8 animals with a mean OCL of 6.5 mm at stage A were compared with the same number of similar sized animals at substage $D_{1.3}$). Dwt = Mean Dry Weight (mg); OCL = Mean OCL (mm). Probability (P) values shown for each OCL pair in each test. Significance accepted at $P < 0.05$.

Test (see text)	Stages compared		Statistics Significant from Tests		
1	A, $D_{1.3}$	P	0.001	0.005	0.05
		n	16	13	6
		OCL	6.5	11.1	14.1
		Dwt (A)	16.888	80.629	196.133
		($D_{1.3}$)	33.229	200.160	392.300
2	$D_{1.3}$, D_2	P	0.019		
		n	8		
		OCL	4.6		
		Dwt ($D_{1.3}$)	13.775		
		(D_2)	9.950		
3	D_2,D_4	P	0.029	0.019	
		n	10	8	
		OCL	3.5	4.6	
		Dwt (D_2)	5.550	9.950	
		(D_4)	6.700	16.075	

Table 3.9 contd Mann-Whitney U tests of Dry Weight (mg) change between Moult Stages and Substages.

Test (see text)	Stages compared	Statistics from Significant Tests					
4	B,C_1	P	0.018	0.005	0.014		
		n	11	19	9		
		OCL	9.1	11.6	15.2		
		Dwt (B)	48.000	108.346	223.725		
		(C_1)	64.033	134.300	375.800		
5	$C_2,D_{1.1}$	P	0.007	0.001	0.007	0.05	
		n	16	30	18	6	
		OCL	3.8	8.3	10.1	14.3	
		Dwt (C_2)	4.075	46.343	97.183	252.567	
		($D_{1.1}$)	5.408	64.987	115.075	379.500	
6	$D_{1.1},D_{1.2}$	P	0.002	0.001	0.001	0.013	0.013	0.023
		n	48	27	51	24	16	11
		OCL	3.8	4.8	6.7	8.8	11.4	13.4
		Dwt ($D_{1.1}$)	5.047	9.524	29.619	72.700	156.080	242.914
		($D_{1.2}$)	5.931	13.483	35.238	87.150	195.600	313.150
7	$D_{1.2}, D_{1.3}$	P	0.012	0.034				
		n	15	17				
		OCL	3.5	6.5				
		Dwt ($D_{1.2}$)	5.600	30.267				
		($D_{1.3}$)	6.650	33.963				

Table 3.10 Mean Organic Content (as % of Dry Weight) (± SE) vs Moult Stage. A and B = postmoult, C_1 and C_2 = intermoult, $D_{1.1}$ to $D_{1.4}$ = premoult and D_2 to D_4 = late premoult. Note that in C_1 % organic content also varies with size (see text).

Moult Stage	% Organic Content	SE	n
A	79.043	0.647	23
B	78.203	0.451	34
C_1	78.838	0.775	24
C_2	81.747	0.410	25
$D_{1.1}$	76.494	0.743	20
$D_{1.2}$	76.506	0.526	23
$D_{1.3}$	74.379	0.407	17
$D_{1.4}$	74.242	0.369	9
D_2	74.067	0.611	9
D_3	78.829	1.682	5
D_4	78.824	0.680	5
exuvia	50.000	0.781	15

Table 3.11 a) ANCOVA on groups of moult stages/substages to isolate significant moult stage and OCL effects on % Organic Content. Data normalised using Box-Cox transformation. Significance accepted at 0.05. P = probability, ns = nonsignificant

	Column 1 (see text)	2	3	4	5	6	7
				Compared Stages			
	All Stages	C_1 and C_2	All Stages but C_1	A and B	A,B and C_1	C_2 to D_4	$D_{1.1}$ to D_4
Source	P	P	P	P	P	P	P
Moult	0.008	<0.001	0.007	ns	0.036	0.006	ns
OCL	0.0505 (ns)	<0.001	ns	ns	<0.001	ns	ns
Moult x OCL	<0.001	0.003	ns	ns	0.011	ns	ns
n	194	49	170	57	82	112	88

b) Linear Regression : OCL vs % Organic Content for Substage C_1 (see text)

n	Slope (b)	Intercept (a)	SE (b)	SE (a)	r^2	P
24	0.0208	-0.5373	0.0047	0.0452	0.473	<0.001

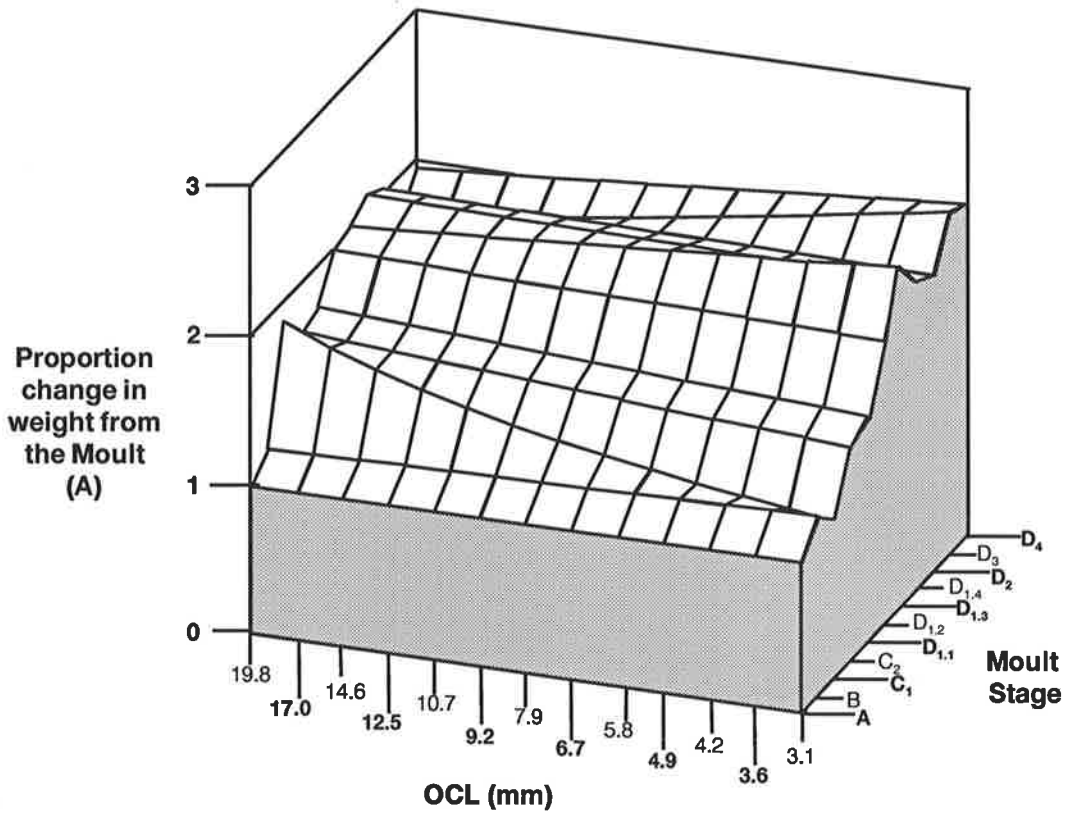
two intermoult substages (C_1 and C_2) showed significant main effects and interactions (Column 2, **Table 3.11a**) so the substages were tested separately for OCL effects. There was a significant linear regression between OCL and % organic content for Substage C_1 ($b > 0$, $P<0.001$) but not for C_2. C_2 was also significantly different from $D_{1.1}$-D_4 (Columns 6 and 7, **Table 3.11a**) so the mean derived from the data for C_2 (81.747% ± 0.410) was not grouped with any other stages. The regression statistics for C_1 are included in **Table 3.11b**. Thus % organic content was high during premoult but peaked at C_2. There were no further significant changes after an initial fall in early premoult.

Table 3.12 Pooled means for Moult Stage-specific % Organic Content .

Moult Stage or substage (n)		Mean % Organic Content	SE (b)
A + B	**(57)**	**78.403**	**0.34**
C_2	**(25)**	**81.747**	**0.41**
$D_{1.1}$ to D_4	**(88)**	**75.822**	**0.94**

Organic content in milligrams was calculated from the percentage data by applying the pooled means (**Table 3.12**) and the regression (**Table 3.11b**) to the size and moult substage-dependent dry weight data described earlier (**Table 3.6, Fig 3.9b).** These data were then used to construct a model of proportional change in size and moult stage-specific organic content from the moult (**Fig 3.11).** The model was constructed in the same way as described earlier for wet and dry weight (**Fig 3.9a and b**) and shows similar patterns of tissue accumulation and loss to that reported for dry weight. Ash free dry weight for a 3.1 mm OCL yabbie increased by 2.29 times between postmoult stage A and premoult stage $D_{1.3}$. By D_4 the weight was 2.24 times the weight at A. The organic content of a 19 mm OCL yabbie increased by 2.12 times between A and $D_{1.4}$ and the weight at D_4 was 1.94 times the weight at A.

Fig 3.11
Change in Organic Content (Ash Free Dry Weight (mg)) with OCL (mm) and Moult Stage



Mean water content declined from 85% at postmoult to 75% of wet weight during premoult/late premoult (**Table 3.13**).

Table 3.13 Mean % Water Content at each Moult Stage/Substage (± SE).

Moult Stage	n	% Water Content	SE
A	75	85.420	0.216
B	360	85.001	0.041
C_1	191	81.829	0.233
C_2	216	81.492	0.188
$D_{1.1}$	597	79.428	0.108
$D_{1.2}$	212	77.225	0.180
$D_{1.3}$	119	75.678	0.196
$D_{1.4}$	38	75.143	0.285
D_2	43	76.940	0.427
D_3	60	76.944	0.267
D_4	64	77.297	0.604

Absolute water content in microlitres was calculated from the original data (i.e. wet weight - dry weight) and moult substage and OCL effects investigated. Initially, linear regressions were carried out on moult substage specific water (ul) vs OCL (mm) relationships after log-log transformation (**Table 3.14**). These were used to calculate absolute water content (ul) which is plotted against organic content (**Fig 3.12**) to define the interactions between water uptake and changes in organic content during the moult cycle. This series of figures also show size-specific changes in that process. Variation in body composition can be followed, as movement on the horizontal plane denotes changes in water content and that along the vertical shows changes in organic content. Lines between pairs of moult substages (e.g. $D_{1.1}$ and $D_{1.2}$) represent changes in body composition during the first substage of the pair (e.g. $D_{1.1}$). This also means that any discussion of changes over the moult (i.e. D_4 to A_2) will include Stage D_4.

Several trends can be seen from this series of figures. Firstly, there are three main periods of water uptake, generally accompanied by minimal tissue accumulation or tissue loss. These occur from A to C_2, from D_3 to D_4 and from D_4 to A_2. Secondly B to $D_{1.2}/D_{1.3}$ is the period of tissue accumulation indicated by increasing organic content. Thirdly, nett growth in organic content virtually ceases during premoult substages $D_{1.2}/D_{1.3}$, with further weight gain the result of fluid uptake. Most of the water taken up in postmoult stage A is lost during B,

Table 3.14 Regression statistics for Moult Stage and substage-specific relationships between Log OCL (mm) and Log Water Content (ul). The Equation is Log Water Content = Log a + b (Log OCL). All slopes are significant at $P < 0.001$. n= number of yabbies per stage/substage.

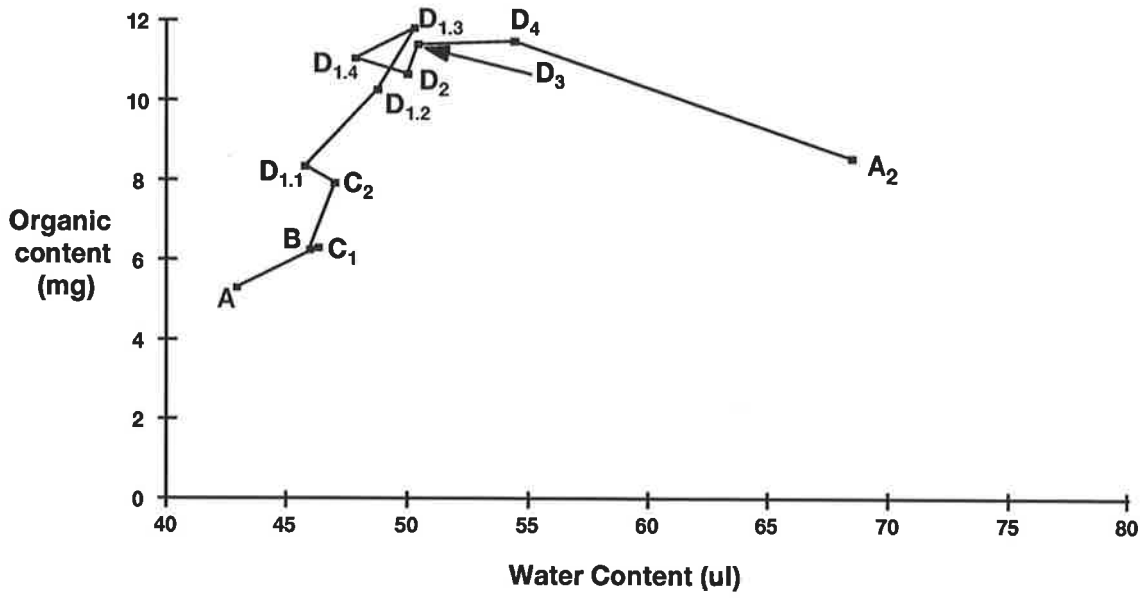
Moult Stage or substage (n)	Slope (b)	Intercept (a)	SE (b)	SE (a)	r^2
A (75)	3.0196	-0.4597	0.0461	0.0474	0.9841
B (360)	3.0125	-0.4287	0.0156	0.0148	0.9907
C_1 (191)	2.9925	-0.4111	0.0164	0.0162	0.9948
C_2 (216)	2.9545	-0.3752	0.0164	0.0153	0.9935
$D_{1.1}$ (597)	2.9645	-0.3935	0.0127	0.0110	0.9891
$D_{1.2}$ (212)	2.9265	-0.3398	0.0266	0.0227	0.9834
$D_{1.3}$ (119)	2.9205	-0.3225	0.0227	0.0204	0.9931
$D_{1.4}$ (38)	2.9752	-0.3816	0.0503	0.0488	0.9923
D_2 (43)	2.9451	-0.3420	0.0359	0.0312	0.9939
D_3 (60)	2.8640	-0.2822	0.0517	0.0384	0.9811
D_4 (64)	2.9302	-0.2949	0.0655	0.0468	0.9785

Fig. 3.12

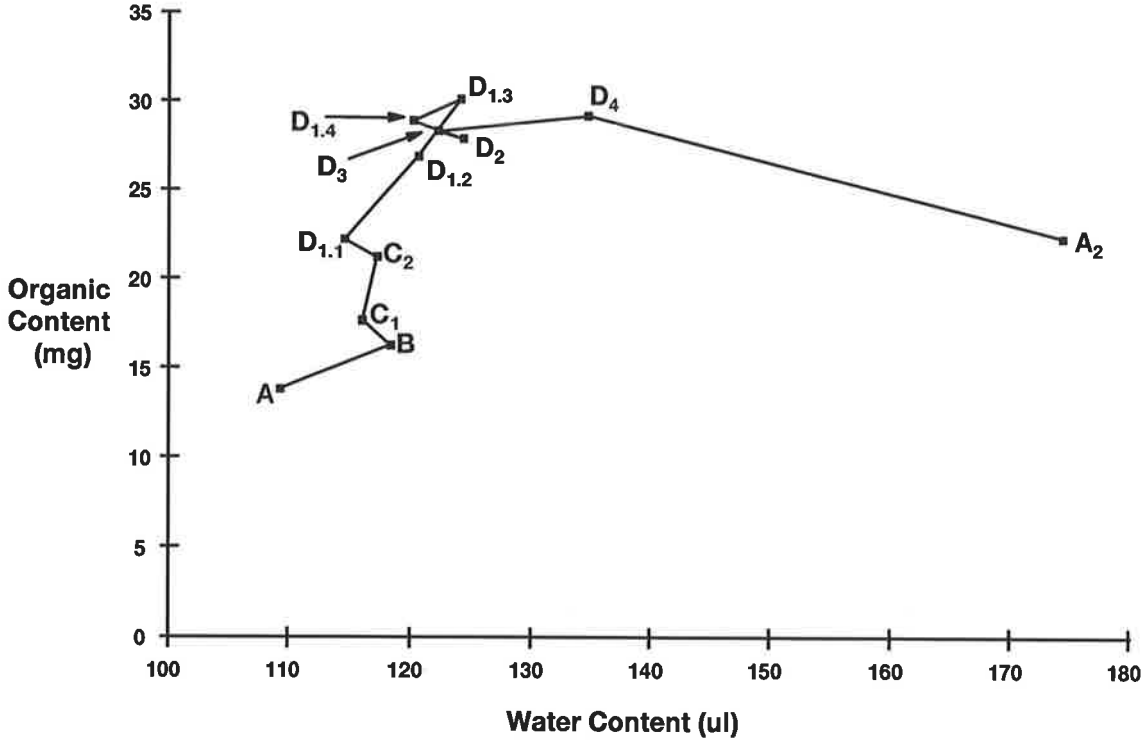
Water Content (ul) vs. Organic Content (mg) over the Moult Cycle for a series of sizes

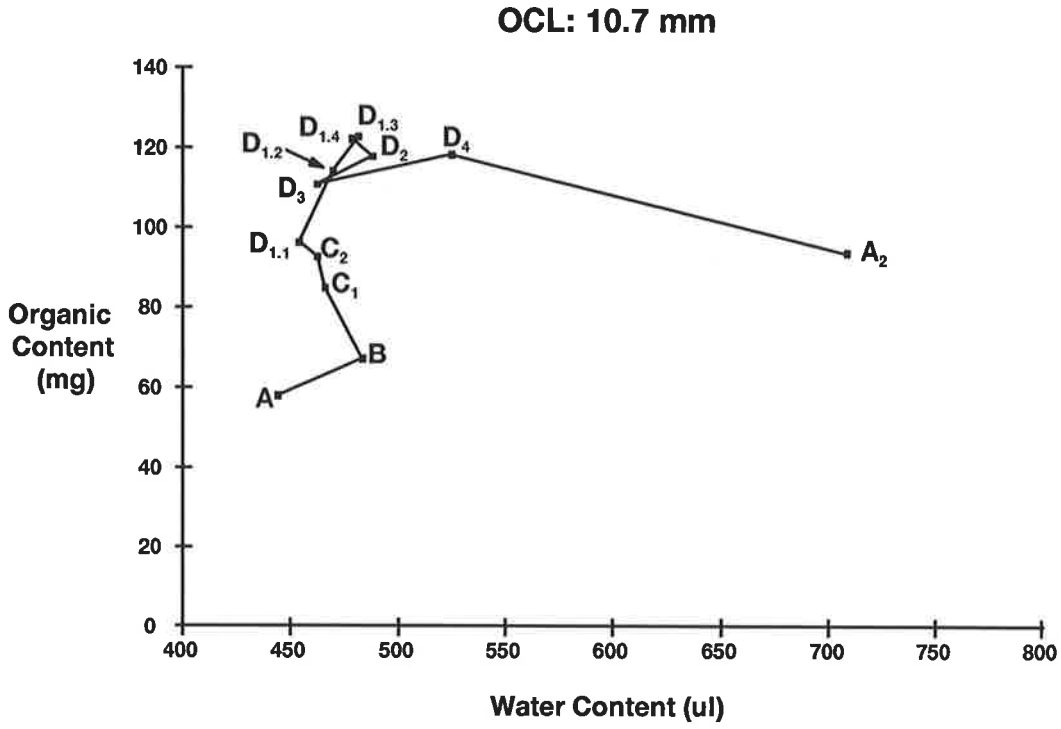
Note that D_4 to A_2 represents the next moult assuming a 16.74% moult increment (refer text)

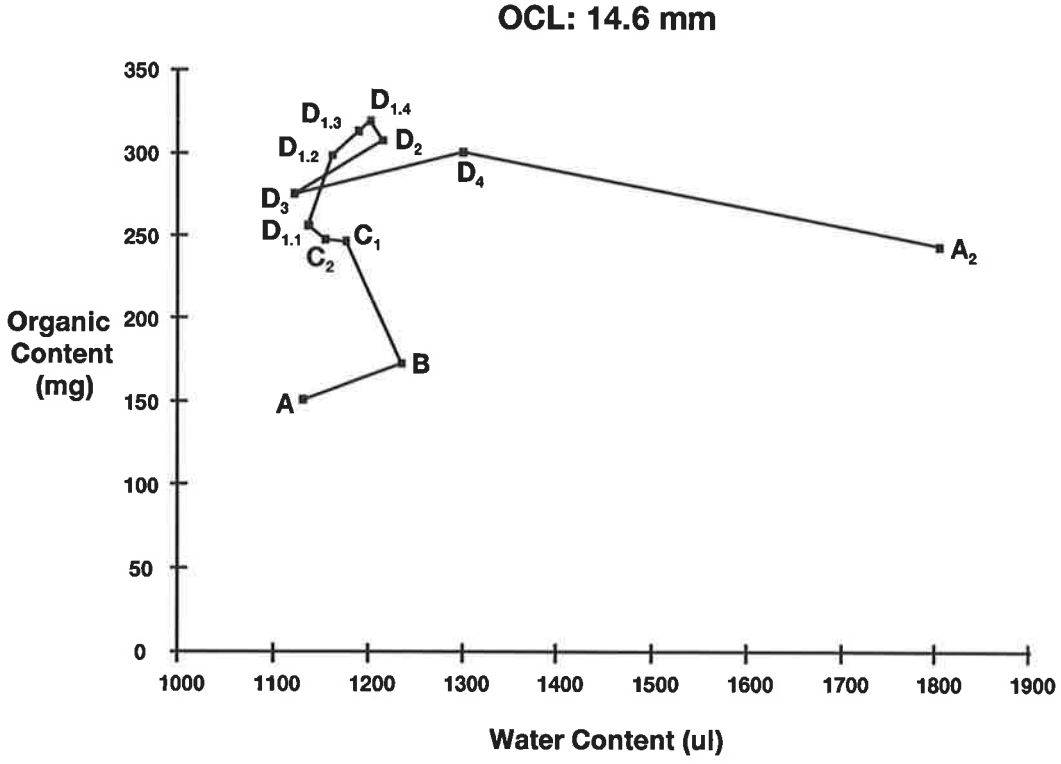
OCL: 4.9 mm



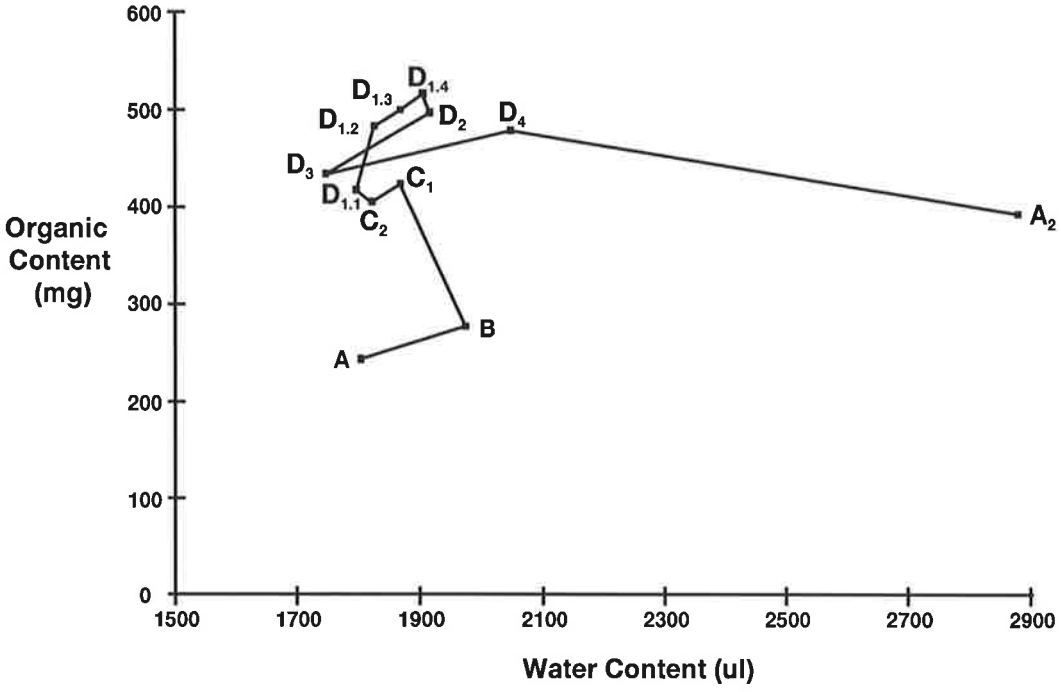
OCL: 6.7 mm




OCL: 10.7 mm
Organic Content (mg)
Water Content (ul)
140
120
100
80
60
40
20
0
400
450
500
550
600
650
700
750
800
D1.3
D1.4
D1.2
D2
D4
D3
D1.1
C2
C1
A2
B
A


OCL: 14.6 mm
Organic Content (mg)
Water Content (ul)
350
300
250
200
150
100
50
0
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
D1.4
D1.3
D2
D1.2
D4
D3
D1.1
C1
C2
A2
B
A

OCL: 17 mm


600
500
400
300
200
100
0
Organic Content (mg)
1500
1700
1900
2100
2300
2500
2700
2900
Water Content (ul)
$D_{1.4}$
$D_{1.3}$
$D_{1.2}$
D_2
D_4
D_3
$D_{1.1}$
C_1
C_2
A_2
B
A

intermoult and/or early premoult. This pattern varies with size as smaller yabbies take up water in substages A to C_1 then loose about a third of this in C_2, while yabbies from about 10 mm OCL gain most water during A, then lose it from B to C_2. In both sizes water loss is accompanied by tissue accumulation. Uptake of water in substages D_3 and D_4 and over the moult from D_4 to A_2 represents the major period of water uptake in the animals, comprising 50 to 80 % of the total water volume taken up over the moult cycle.

Ash content varied between 18 and 26% of dry weight (**Table 3.15**).

Table 3.15 Mean % Ash Content at each Moult Stage/Substage (± SE).

Moult Stage	n	% Ash	SE
A	23	20.957	0.647
B	34	21.796	0.451
C_1	24	21.162	0.775
C_2	25	18.253	0.310
$D_{1.1}$	20	23.507	0.743
$D_{1.2}$	23	23.950	0.523
$D_{1.3}$	17	25.622	0.407
$D_{1.4}$	9	25.758	0.369
D_2	9	25.933	0.611
D_3	5	21.171	1.682
D_4	5	21.176	0.680

ANCOVA on the normalised data showed moult substage, OCL and interaction effects (Column 1: **Table 3.16a**) so the data was subdivided into postmoult, intermoult and premoult/late premoult as before and differences within and between stages tested. This analysis showed that ash content changed significantly between stages (i.e. postmoult, intermoult, and premoult/late premoult, $P<0.01$). During postmoult and premoult/late premoult ash content was independent of size and changed little within stages (Columns 2 and 4: **Table 3.16a**). Means of 21.452% ± 0.359% and 24.114% ± 0.301% were derived for postmoult and premoult/late premoult respectively. However, during intermoult there was a slight but significant decline in % ash content with size and significant differences between C_1 and C_2 (Column 3: **Table 3.16a**) so linear regressions were calculated for these two substages (**Table 3.16b**).

Table 3.16a ANCOVA on groups of moult substages to isolate significant Moult Stage/ Substage effects on % Ash Content. Data normalised using Box-Cox transformation. Significance accepted at 0.05. P = probability, ns = nonsignificant

	Column 1 (see text)	2	3	4
		Compared	Stages	
	All Stages	A and B	C_1 and C_2	$D_{1.1}$ to D_4
	P	P	P	P
Source				
Moult	**0.001**	ns	**<0.001**	ns
OCL	**0.009**	ns	**<0.001**	ns
Moult x OCL	**<0.001**	ns	**0.001**	ns
n	**194**	**57**	**49**	**88**

Table 3.16b Regression statistics for linear relationship between OCL (mm) and % Ash Content for Stages C_1 and C_2. Slopes are significant at $P < 0.001$ and $P=0.004$ respectively. n= number of yabbies per substage.

Moult Substage (n)	Slope (b)	Intercept (a)	SE (b)	SE (a)	r^2
C_1 (24)	-0.0016	-0.4529	0.0004	0.0035	0.478
C_2 (25)	-0.0004	-0.4703	0.0001	0.0001	0.318

The regressions and the means were used to calculate the absolute ash content which is plotted against organic content in **Fig 3.13.** These figures show some postmoult accumulation of ash, peaking at the end of B and accompanied by rapid tissue growth. Subsequently, the major ash accumulation substages are C_1 and C_2 depending on size with C_2 showing almost no tissue accumulation. These probably represent further thickening of the exoskeleton. There was some loss in both components during D_2 which is partly made up in D_3. This last fluctuation was found to be significant for the smaller yabbies only (ref. **Table 3.9**).

Fig 3.11 and associated organic content vs OCL regressions and means were used to work out the loss in organic content at the moult which included both tissue losses and those with the shed exoskeleton (**Table 3.17**). If the net weight at A of the next size class is subtracted from the weight at D_4 the loss in weight over the moult itself may be estimated. Successive size classes were defined using a 16.74% moult increment, the overall mean derived from the field data (Table 3.4). Loss at moult as a percentage of the weight gained during the cycle preceding the moult declines with increasing size from 50.6 % at 3.1 mm OCL to 36.4 % at 17 mm OCL. The table also includes the loss as a percentage of weight gained during the next moult cycle, during which the weight must be regained. This varies from 32.1% at 3.6 mm OCL to 24.1% at 17 mm OCL. Exuvia organic content was calculated from the regression detailed previously. The shed exoskeleton accounted for between 7 and 8% of the organic content of the animals within the size range used. The exuvia also contributes between 25 and 46% of the total loss at the moult over the same size range.

b) Carbon.

Total carbon averaged between 27 and 29% of body dry weight and about 30% of the weight of the exuvia (**Table 3.18**). Total carbon as a percentage of body dry weight vs OCL (mm) is presented in **Fig 3.14**. Percentage Organic Carbon was estimated, based on the assumption that the mineral content of the integument and gastrolith of the crayfish is calcium carbonate ($CaCO_3$) (Aiken and Waddy, 1992). $CaCO_3$ has an atomic mass of 100 of which carbon contributes 12 or 12%. Thus 12% of the ash fraction should be inorganic carbon. The ash content of each animal in the carbon analysis was estimated from its dry weight using the percentage ash means and regressions detailed previously and the inorganic carbon derived.

Table 3.17 Loss of Organic content (i.e. ash free dry weight) at Moult as a function of OCL(mm). The successive carapace lengths refer to a sequence of moults at a rate of 16.74% of Premoult OCL per moult. Refer text for remainder of explanation.

OCL (mm)	Weight at A (mg)	Weight at D_4 (mg)	Total weight gain (mg)	Exuvia (mg)	Total Loss at Moult (D_4 to A)	Total loss as % of D_4	Total loss as % of gain during moult cycle	Total loss as % of gain during next moult cycle	Exuvia as % of D_4	Exuvia as a % of total loss
3.10	1.264	2.833	1.569	0.205	0.794	28.0	50.6	-	7.2	25.8
3.62	2.039	4.516	2.477	0.330	1.226	27.2	49.5	32.1	7.3	26.9
4.22	3.289	7.199	3.909	0.533	1.892	26.3	48.4	31.4	7.4	28.2
4.93	5.307	11.476	6.169	0.860	2.913	25.4	47.2	30.7	7.5	29.5
5.76	8.563	18.295	9.732	1.387	4.479	24.5	46.0	29.9	7.6	31.0
6.72	13.816	29.166	15.350	2.238	6.875	23.6	44.8	29.2	7.7	32.6
7.85	22.291	46.496	24.205	3.610	10.531	22.7	43.5	28.4	7.8	34.3
9.16	35.965	74.123	38.158	5.825	16.095	21.7	42.2	27.6	7.9	36.2
10.69	58.027	118.165	60.138	9.399	24.541	20.8	40.8	26.8	7.9	38.3
12.48	93.624	188.376	94.753	15.164	37.320	19.8	39.4	25.9	8.0	40.6
14.57	151.057	300.307	149.250	24.467	56.585	18.8	37.9	25.0	8.2	43.2
17.01	243.722	478.745	235.023	39.477	85.513	17.9	36.4	24.1	8.3	46.2

Table 3.18 Mean % Total Carbon and Mean % Organic Carbon at each Moult Stage/Substage. SE = Standard Error. n = number of yabbies analysed per moult stage. AFDW = Ash Free Dry Weight.

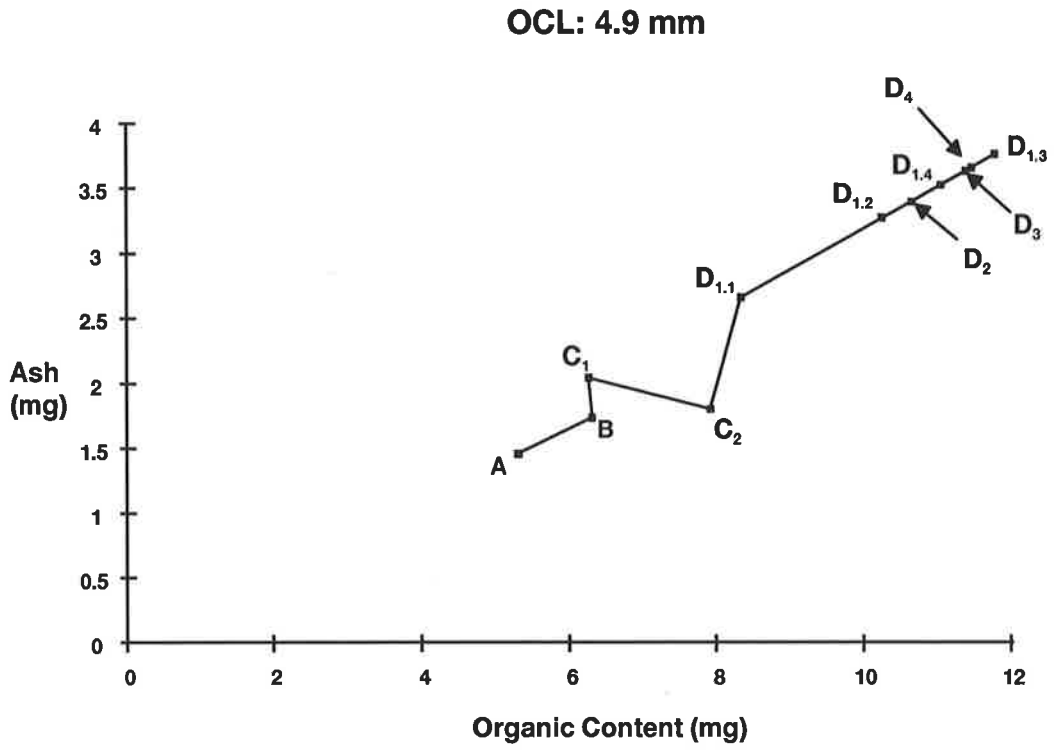
Moult Stage/Substage (n)	Total Carbon as % Dry Weight	SE	Organic Carbon as % AFDW	SE
A (9))	28.789	0.950	33.514	1.212
B (7)	29.486	1.507	34.486	1.916
C_1 (10)	28.850	1.790	32.924	1.929
$D_{1.1}$ (10)	28.880	1.598	34.372	2.108
$D_{1.2}$ (10)	28.290	1.425	33.596	1.879
$D_{1.3}$ (10)	28.970	1.307	34.157	1.724
$D_{1.4}$ (8)	28.850	1.694	33.899	2.234
D_2 (9)	29.389	1.287	34.660	1.697
D_3 (9)	27.511	0.922	32.936	1.216
D_4 (8)	27.125	1.122	32.426	1.479
Exuvia (5)	30.648	0.688	49.307	1.376

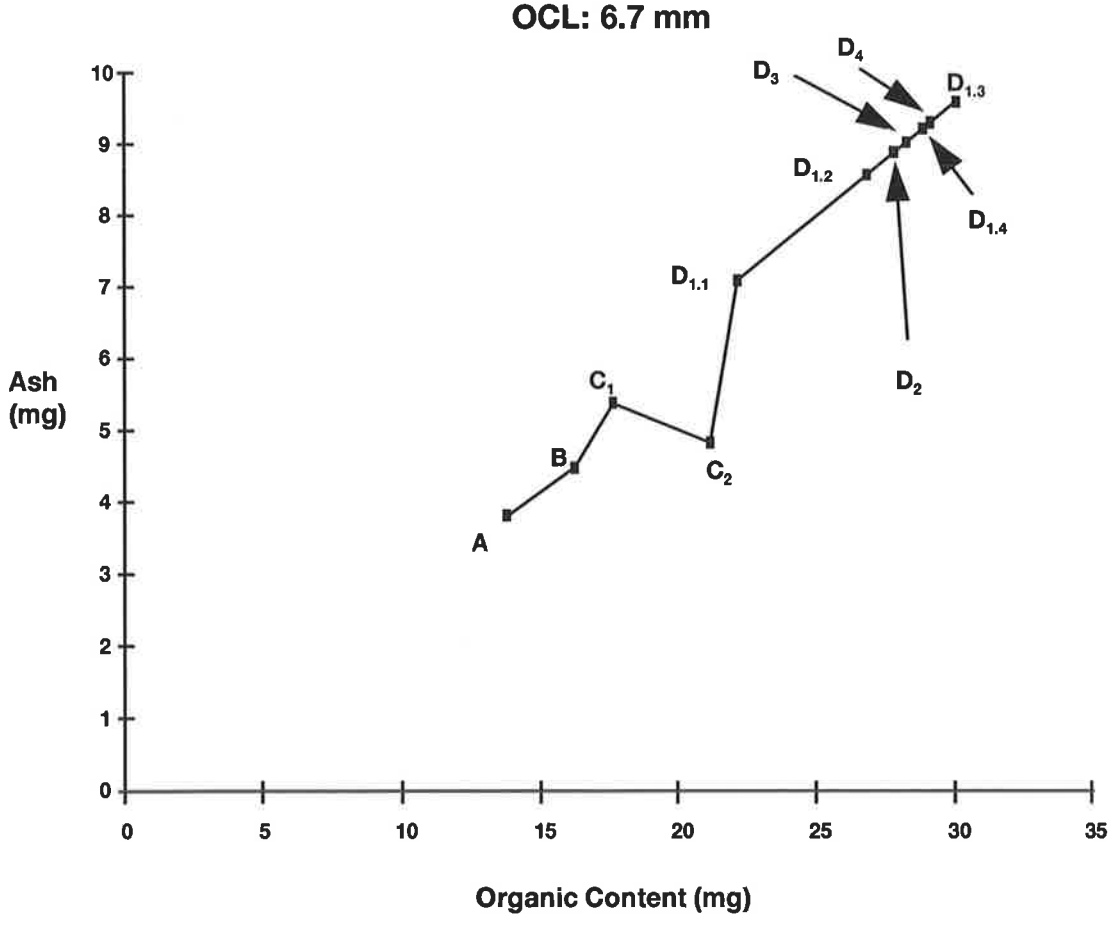
Fig. 3.13

Organic Content (mg) vs Ash Content (mg)

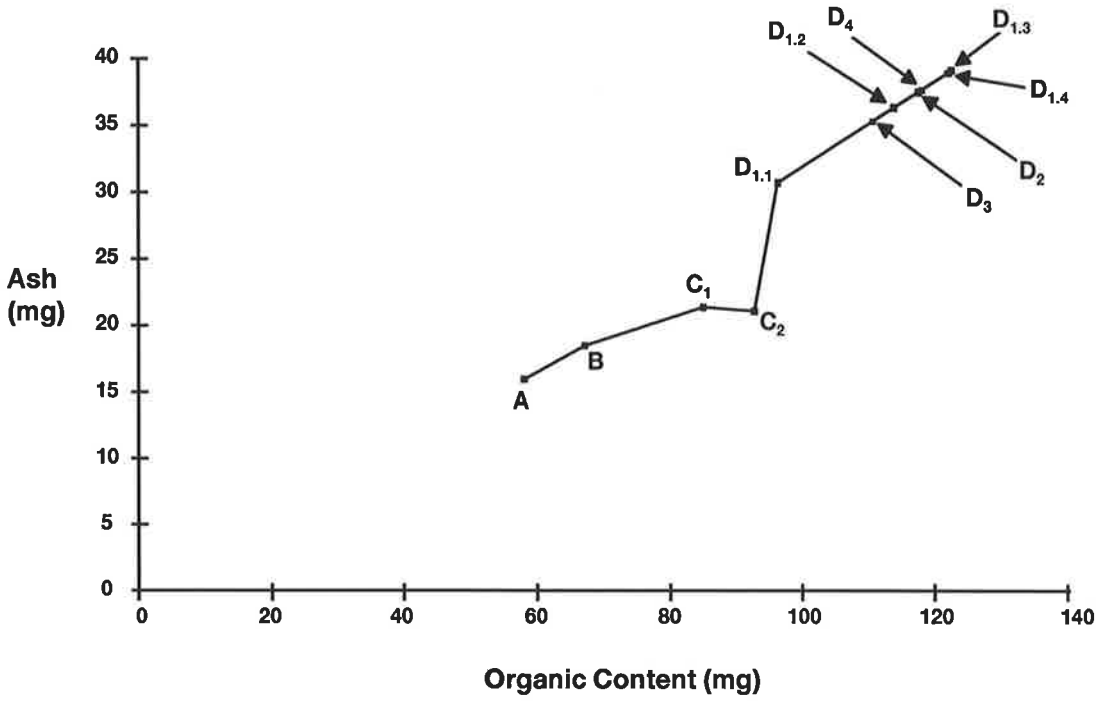
over the Moult Cycle for a series of sizes

Note that D_4 to A_2 represents the next moult assuming a 16.74% moult increment (refer text)

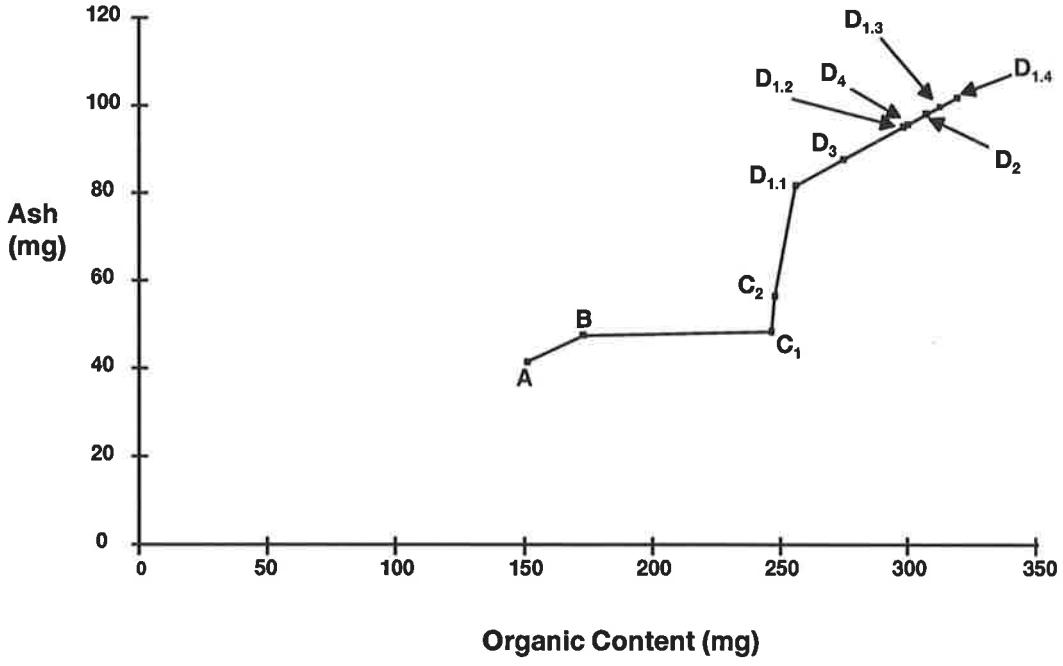

OCL: 4.9 mm
Ash (mg)
Organic Content (mg)
0
0.5
1
1.5
2
2.5
3
3.5
4
0
2
4
6
8
10
12
A
B
C1
C2
D1.1
D1.2
D1.3
D1.4
D2
D3
D4


OCL: 6.7 mm
Ash (mg)
Organic Content (mg)
0
1
2
3
4
5
6
7
8
9
10
0
5
10
15
20
25
30
35
A
B
C1
C2
D1.1
D1.2
D1.3
D1.4
D2
D3
D4

OCL: 10.7 mm



OCL: 14.6 mm



OCL: 17 mm

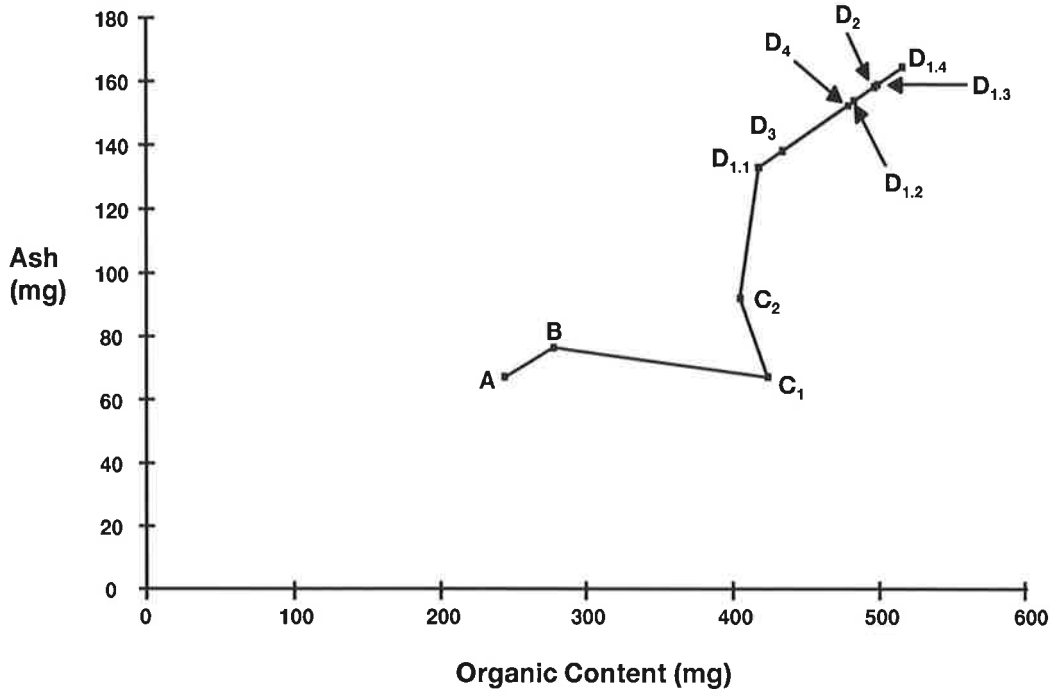
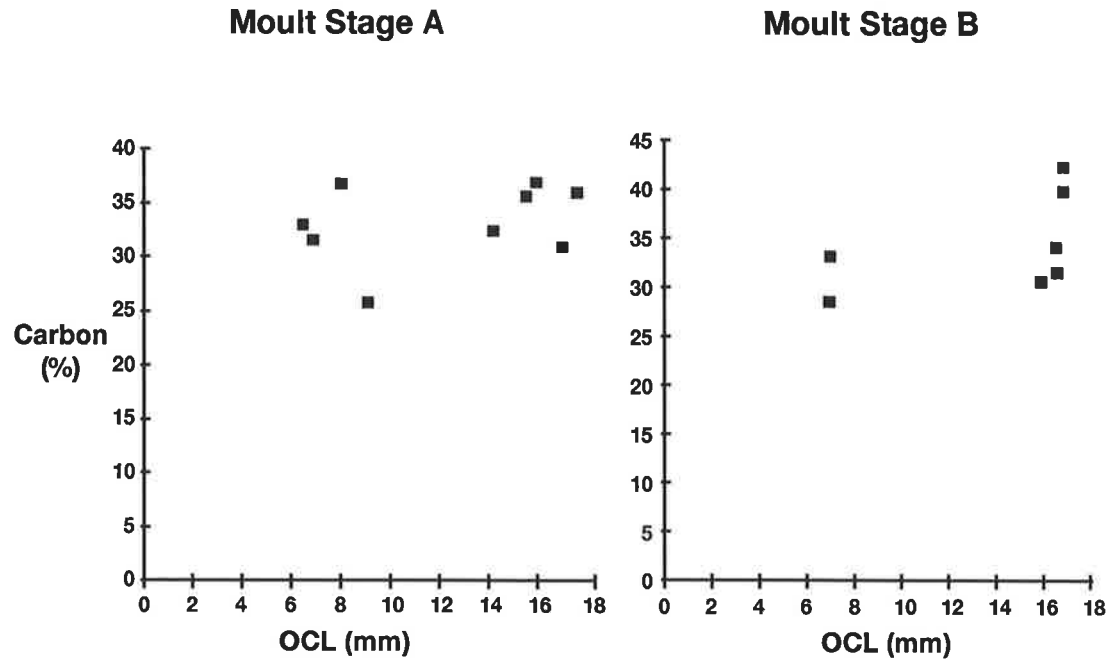
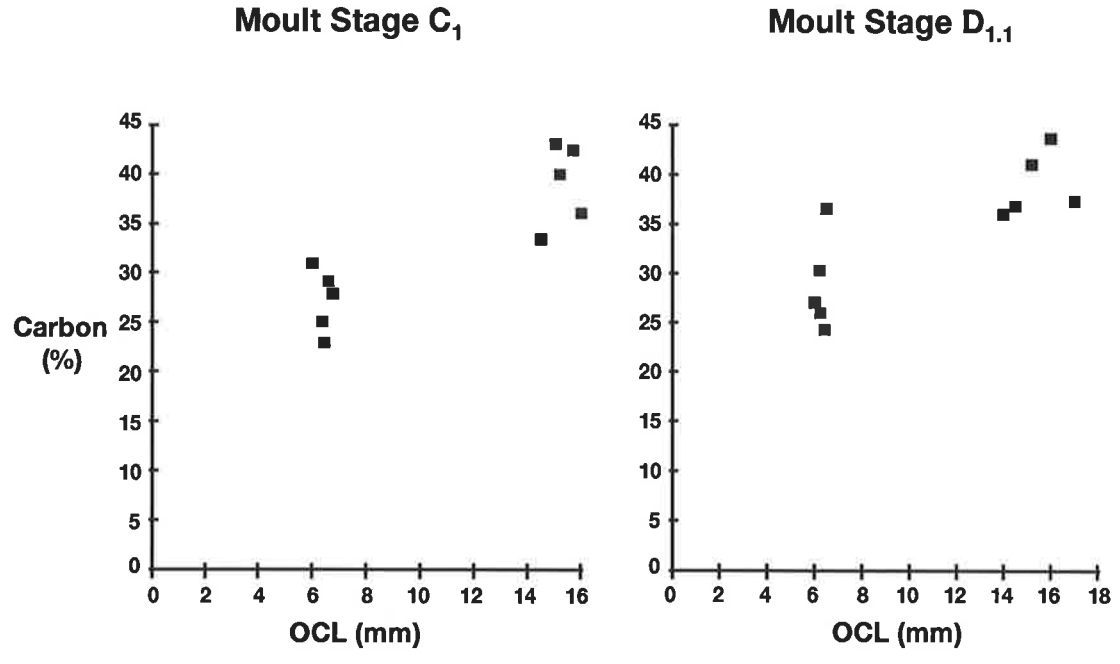
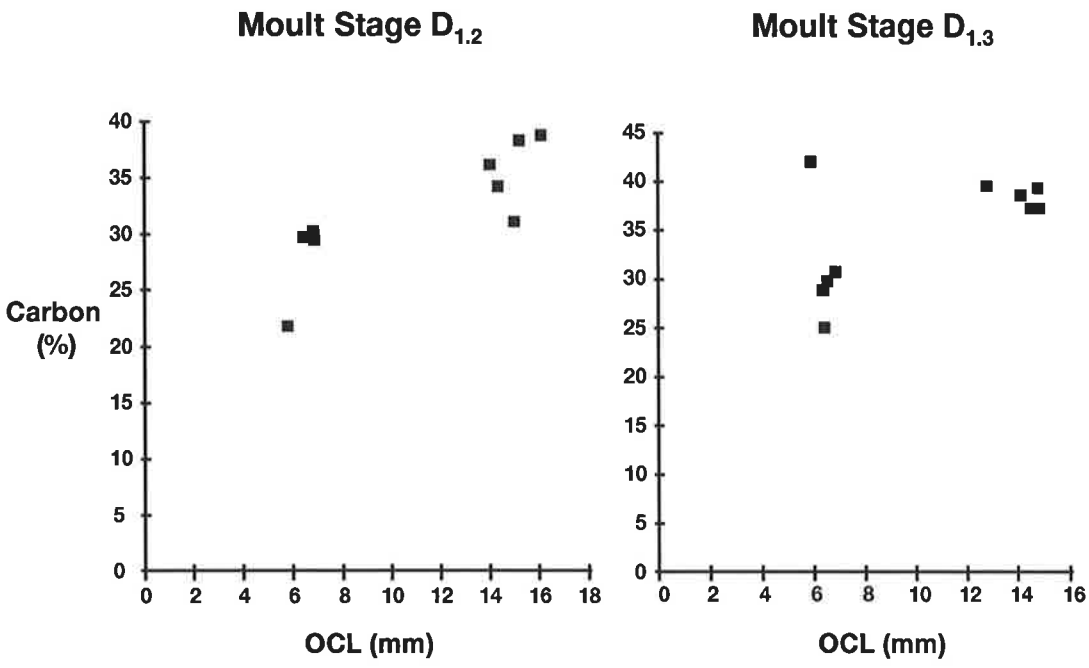
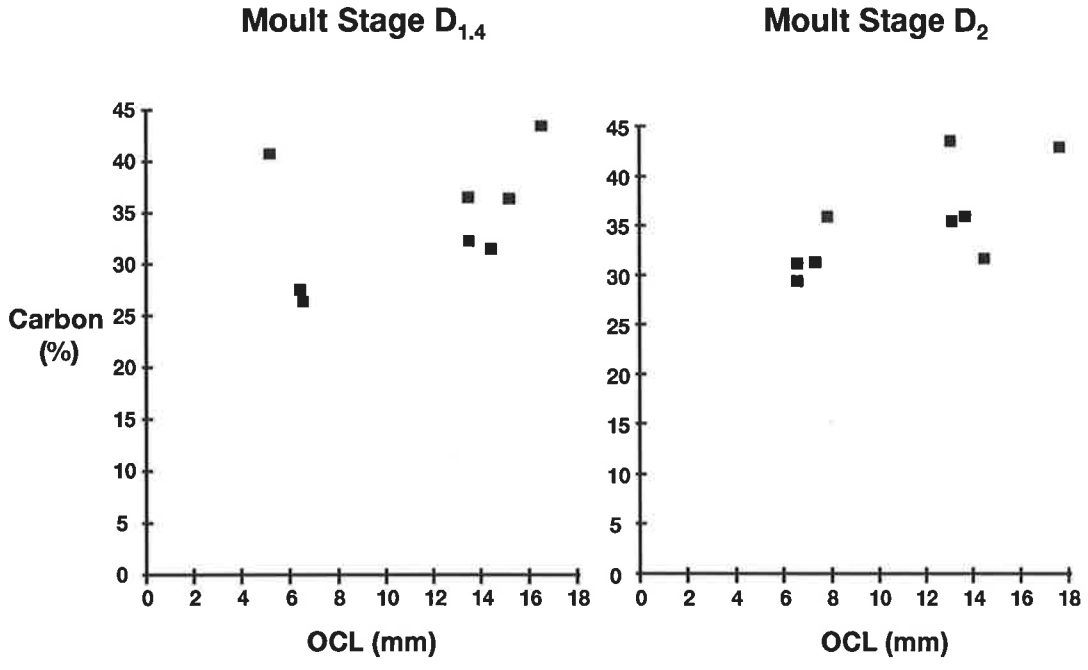

Ash (mg)
180
160
140
120
100
80
60
40
20
0
0
100
200
300
400
500
600
Organic Content (mg)
A
B
C1
C2
D1.1
D1.2
D1.3
D1.4
D2
D3
D4

Fig. 3.14
Carbon as a % of Dry Weight (mg)
vs OCL (mm) for 10 Moult Stages

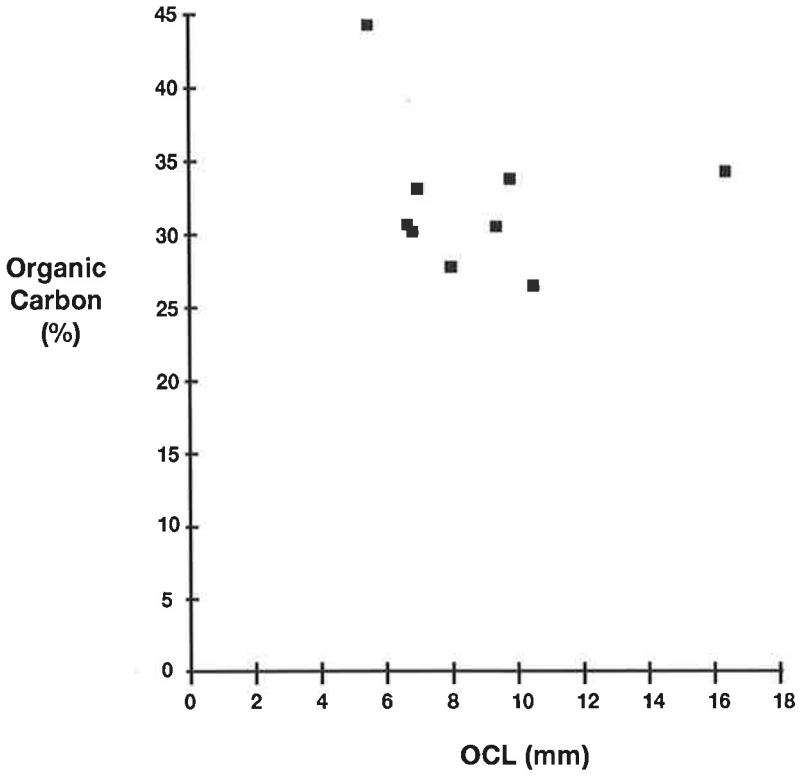

Moult Stage A
Moult Stage B
Carbon (%)
OCL (mm)
OCL (mm)


Moult Stage C_1
Moult Stage $D_{1.1}$
Carbon (%)
OCL (mm)
OCL (mm)

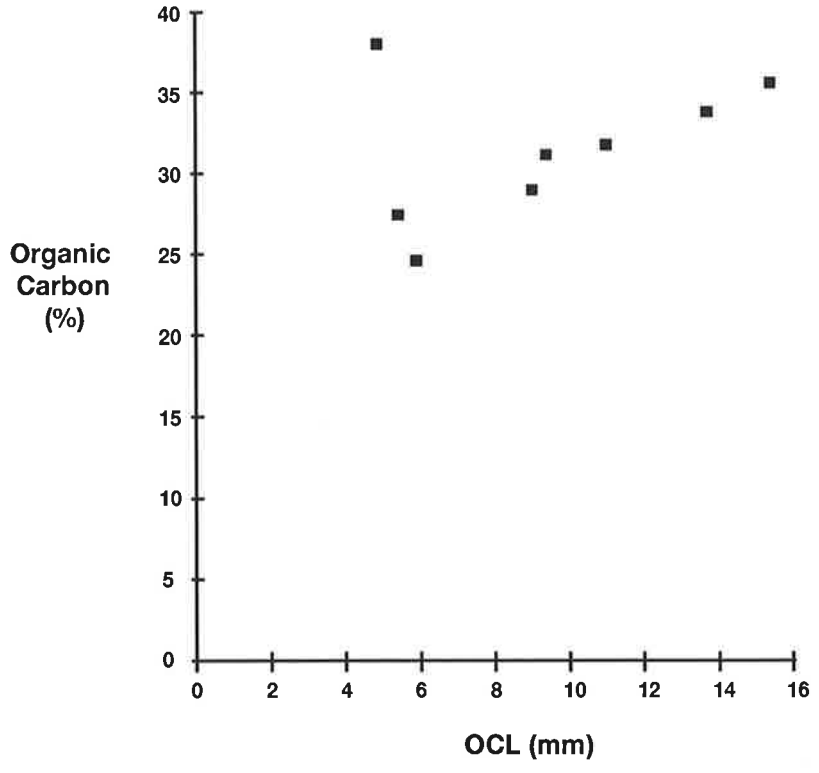

Moult Stage $D_{1.2}$
Moult Stage $D_{1.3}$
Carbon (%)
OCL (mm)
OCL (mm)


Moult Stage $D_{1.4}$
Moult Stage D_2
Carbon (%)
OCL (mm)
OCL (mm)

Moult Stage D_3



Moult Stage D_4



Organic carbon was derived by subtraction of this figure from total carbon. The latter was estimated by multiplication of each individual's dry weight by its measured % Total Carbon. Mean body organic carbon varied between 32.9% and 34.7% (**Table 3.18**) and that of the exuviae averaged 49%. Analysis for size and moult substage effects within the two original size groups (5 to 8 mm and 9 to 17 mm) showed that % Carbon was affected by OCL in the larger animals (P=0.005) but moult substage had no significant effect (P>0.05) for any of the data. A mean of 29.935 (± 0.563, n=40) was derived for the first size group and a linear regression for the relationship between OCL and % Carbon (b = 0.0007 (± 0.0002); a = 33.3936 (± 1.3097), r^2 = 0.154, P=0.005, n=49) calculated for the second group. All data were normalised with a Box-Cox transformation (Sokal and Rohlf, 1981) before analysis. Proportional change in Organic Carbon content from the moult was calculated for a range of sizes and moult substages using **Fig 3.11** as presented in **Fig 3.15**. As there was no moult substage effect on carbon content accumulation, the pattern over the moult cycle is the same as that for organic content described earlier. There was a major peak at $D_{1.2}/D_{1.3}$, a significant fall then rise in carbon for small yabbies between $D_{1.3}$ and D_4 and a significant increase during B for yabbies of 9 mm OCL and above.

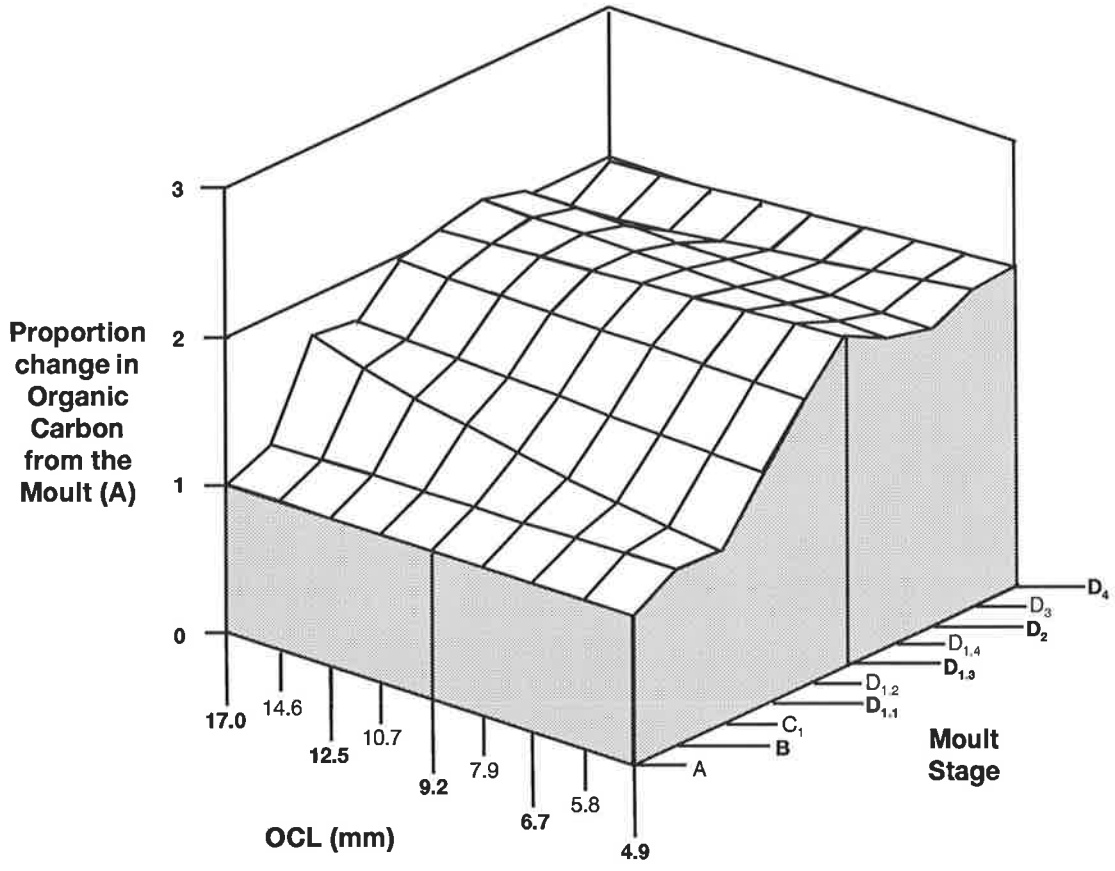
c) Nitrogen and Chitin and Protein

Mean nitrogen ranged from 9.4% to 11.3% of body organic content **(Table 3.19)**.

Table 3.19 Nitrogen as a percentage of AFDW for 10 Moult Stages/Substages. All data included. Mean ± SE. n= number of yabbies

Moult Stage	% Nitrogen	SE
A (10)	11.309	0.383
B(10)	10.430	0.409
C_1(10)	9.388	0.318
$D_{1.1}$(10)	9.700	0.356
$D_{1.2}$(9)	10.002	0.239
$D_{1.3}$(9)	9.917	0.227
$D_{1.4}$(8)	11.092	0.627
D_2(9)	10.717	0.576
D_3 (8)	10.338	0.455
D_4(9)	10.957	0.423

Fig 3.15
Change in Organic Carbon Content with Size (OCL) over the Moult Cycle



ANCOVA for size and moult substage effects within the two size groups (5 to 8 mm and 9 to 17 mm) showed that % nitrogen declined significantly with OCL in the larger animals ($P = 0.018$, $n = 46$). Moult substage had no significant effect for either group. ($P>0.05$). All data were normalised as before.

Chitin averaged between 9% and 17.7% of body organic content depending on moult substage **(Fig 3.16)**. One way ANOVA on the data showed significant moult substage effects ($P <0.001$) and the *post hoc* tests suggested significant changes during postmoult (A and B, $P<0.001$), between postmoult and intermoult (C_1 and C_2, $P = 0.018$) and between premoult ($D_{1.1}$ - $D_{1.4}$) and late premoult (D_2 - D_4, $P = 0.003$). There was also a marginally significant difference in % chitin between $D_{1.1}$ and $D_{1.2}$ ($P = 0.045$). Exuvia samples averaged 7.308% ($\pm$ 0.314%) nitrogen and 50.489% ($\pm$ 2.343%) chitin in terms of ash free dry weight.

Given that the amount of nitrogen in chitin is 6.89% (Stecher *et al*, 1968) it is possible to estimate the amount of nitrogen accounted for by the non-protein fraction and then calculate the protein component at each substage. This was carried out as shown in the example in **Table 3.20**. Note that the final non-chitin-nitrogen is multiplied by 5.8 to convert it to a value for protein. This figure was suggested for aquatic animals by Gnaiger and Bitterlich (1984) in place of the conversion factor of 6.25 suggested by Winberg (1971). They calculated the amino acid composition of 17.2% nitrogen for proteins in aquatic animals which gives a conversion of 5.8 (1/17.2)

Exuviae also appear to have a remnant protein component. This is to be expected as the chitin in the exoskeleton is embedded in a protein matrix (Aiken and Waddy, 1987). The shed exoskeleton contains 7.308 % nitrogen and 50.489 % chitin. The chitin contains about 6.89 % nitrogen. Using the method above this works out to be 47.311 ($\pm$ 2.165)% of the total exuvial nitrogen in milligrams. Assuming that the remainder is protein, exuviae contained 24.468 ($\pm$ 1.248) % protein.

Table 3.20 The calculation of % Protein from Nitrogen and Chitin data. The dry weight to organic content conversion for Moult stage A (0.79043) may be found in Table 3.10. Note that this example refers to an individual not a mean.

Step A	**B**	**C**	**D**	**E**
Moult Substage	**Dry weight (mg)**	**Organic content (mg)**	**% N (afdw)**	**Total N (mg afdw)**
A	240.600	190.177	10.510	19.988
		(**B** x 0.79043)		(**D**/100 x **C**)
Step F	**G**	**H**	**I**	
% Chitin (afdw)	**Chitin N (mg afdw)**	**Total Protein (mg afdw)**	**% Protein**	
9.040	1.232	108.785	57.202	
	((**F**/100 x **C**) x 0.0689)	(**E**-**G**) x 5.8	(**H**/**C**) x 100	

Fig. 3.16
Chitin (% afdw) vs Moult Stage

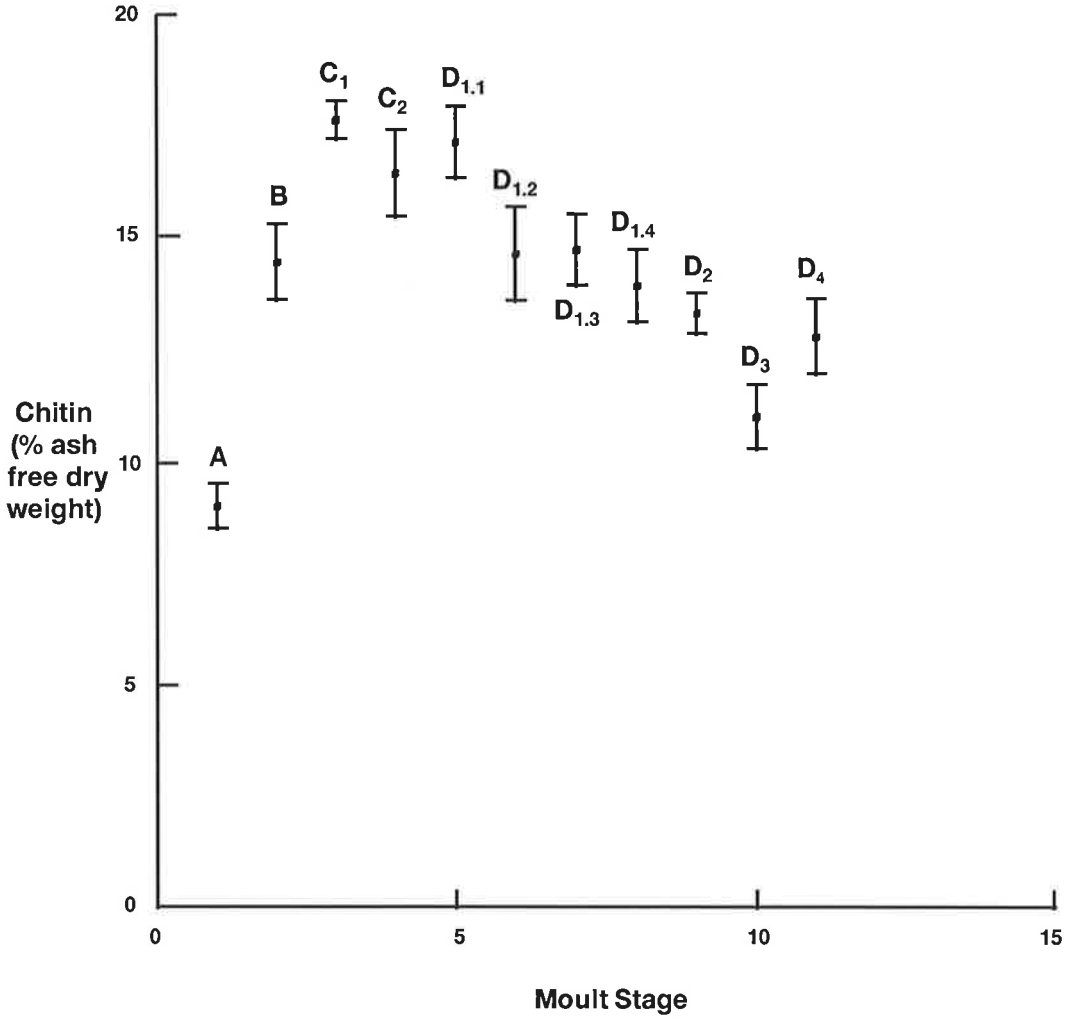


Fig 3.17 presents body protein as a percentage of ash free dry weight vs OCL. It averaged between 47 and 62% depending on moult stage (**Table 3.21**).

Table 3.21 Protein as a percentage of AFDW for 10 Moult Stages/Substages. All data included. Mean ± SE. n= number of yabbies

Moult Stage	% Protein	SE
A (10)	61.980	2.224
B(10)	54.698	2.374
C_1(10)	47.394	1.845
$D_{1.1}$(10)	49.404	2.065
$D_{1.2}$(9)	52.137	1.388
$D_{1.3}$(9)	51.615	1.319
$D_{1.4}$(8)	58.745	3.634
D_2(9)	56.817	3.338
D_3(8)	55.541	2.640
D_4(9)	58.415	2.391

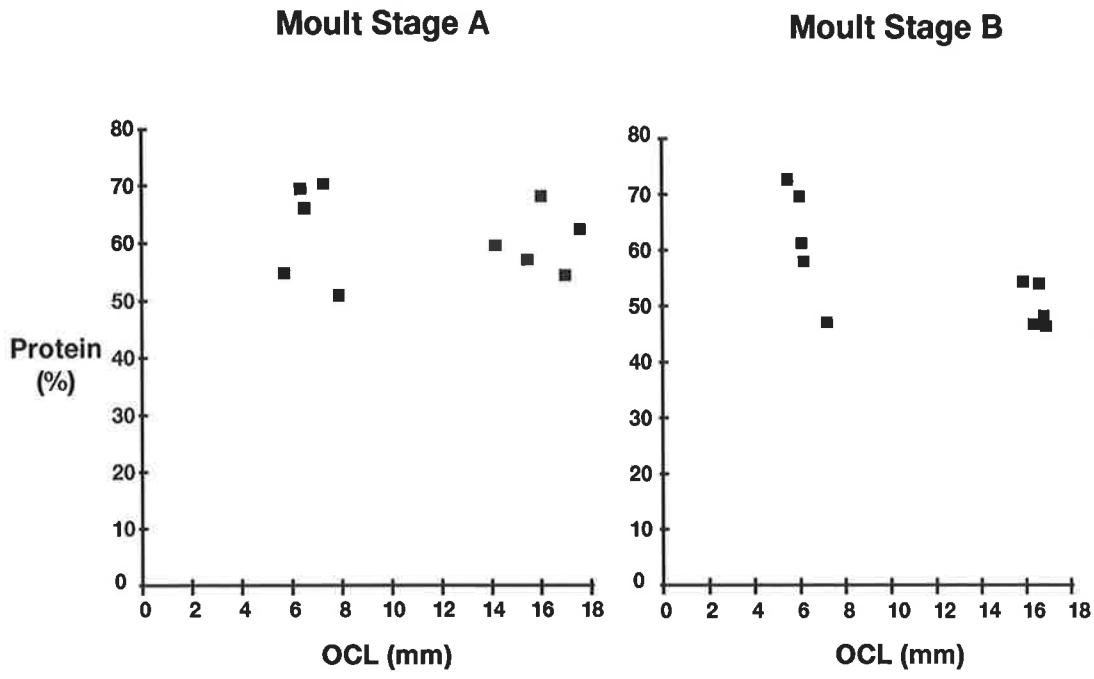
ANCOVA was carried out in the same way as reported for nitrogen. Percent protein declined significantly ($P = 0.018$) with OCL in the larger animals but moult substage had no significant effect on either group ($P>0.05$). The linear regression for the relationship between OCL and % Protein ($b= -0.142$ ($\pm$ 0.060); $a = 59.563$ ($\pm$ 3.858); $r^2 = 0.112$, $P = 0.023$; $n = 46$) for the larger animals and the mean for the smaller group (58.364 % $\pm$ 1.137; n=46) were used to calculate absolute changes in protein based on organic content as they had been for carbon. Proportional changes in total protein from the moult are presented in **Fig 3.18.** The figure shows rapid accumulation of protein in smaller crayfish to a peak at $D_{1.2}$. This peak becomes less distinct with increasing size as it spreads out to include stages between $D_{1.2}$ and D_2. Accumulation of protein during stage B also increases with size. As was the case for carbon, the patterns of change in protein largely reflect variation in organic content rather than changes in % protein.

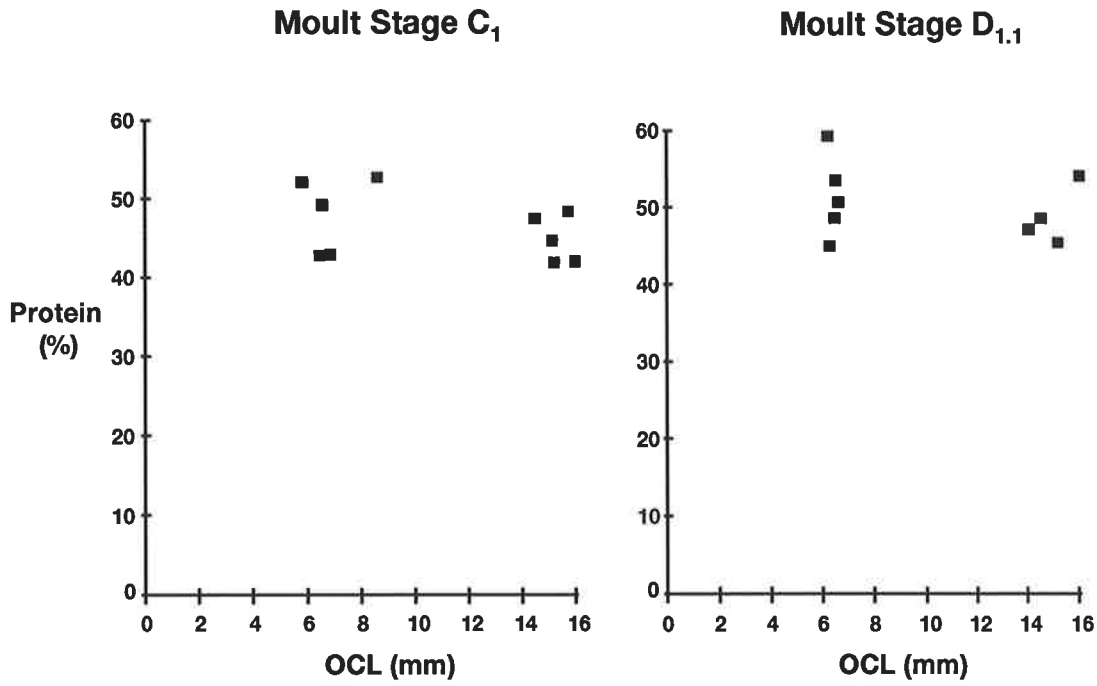
d) The Relationship Between Protein And Carbon

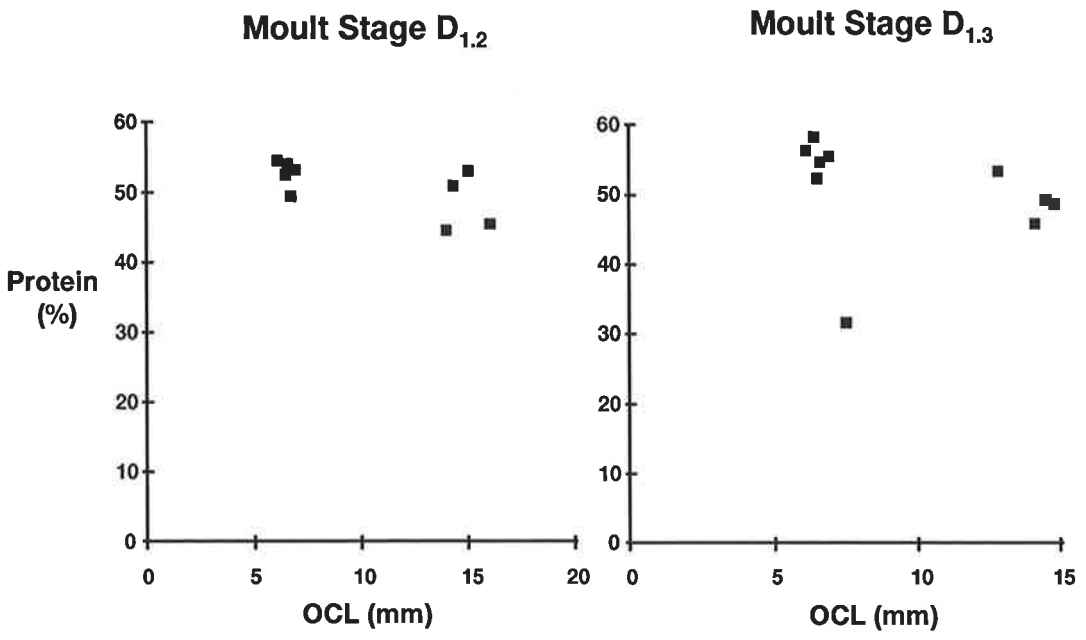
The protein : carbon relationship was further examined using the moult stage and size-specific weights of each component. **Fig 3.19** shows fluctuations in 'Remaining' carbon (mg) as a function of protein (mg) for a variety of sizes through the moult cycle. Remaining carbon was assumed to be mostly lipid as this, rather than carbohydrate, is the major energy reserve in

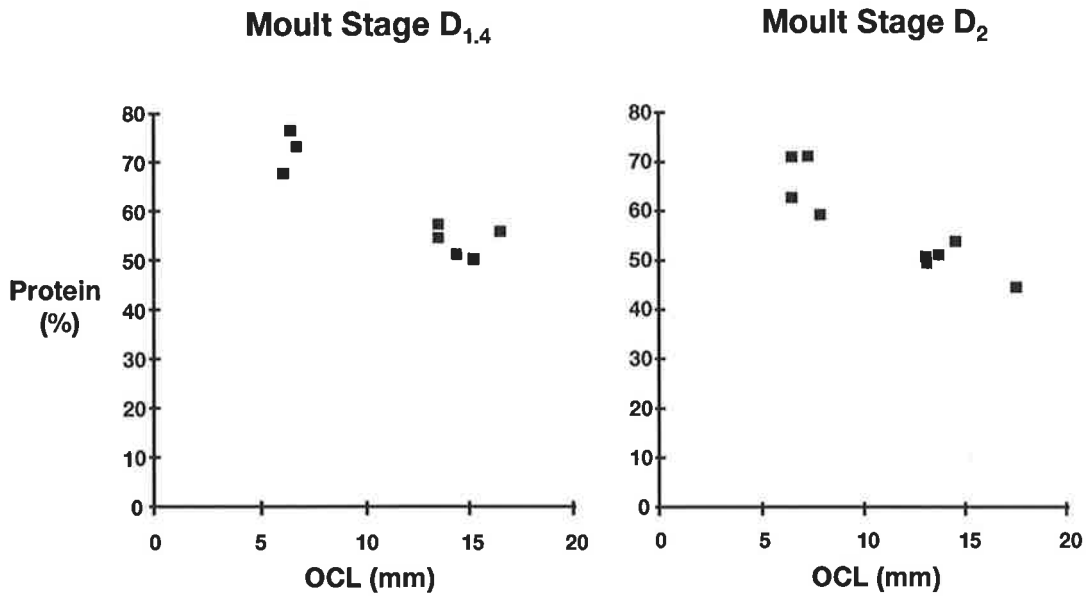
Fig. 3.17

Protein as a % of Ash Free Dry Weight (mg) vs OCL (mm) for 10 Moult Stages

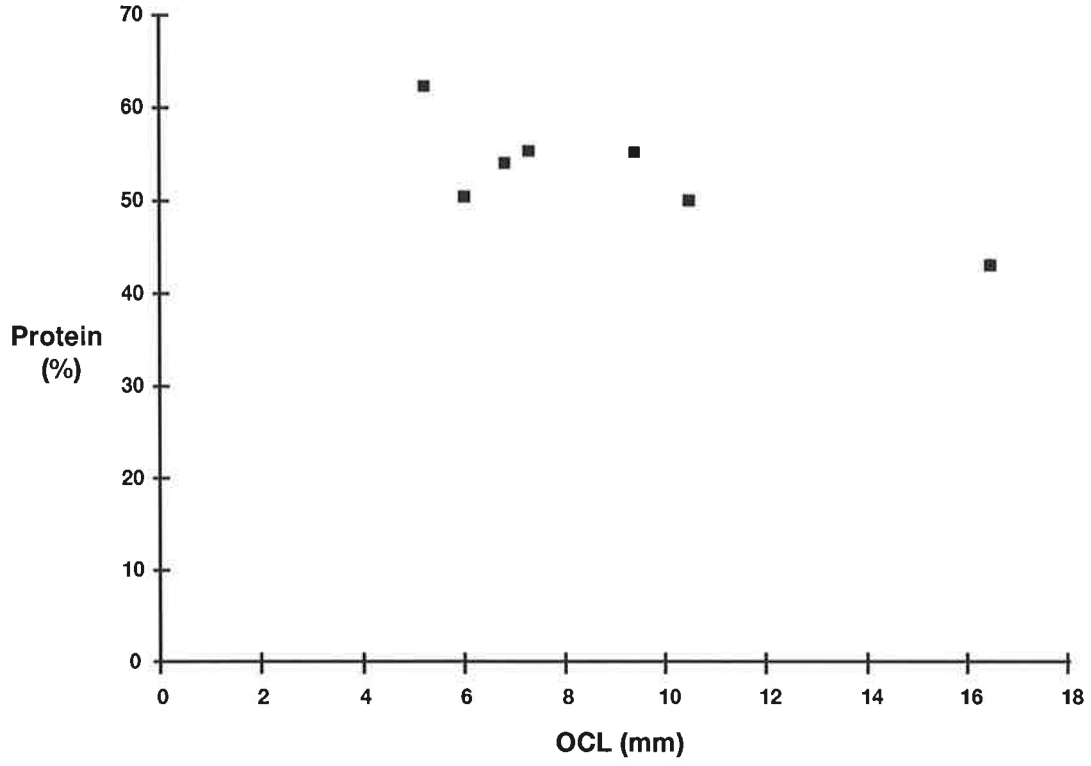

Moult Stage A
Moult Stage B
Protein (%)
OCL (mm)
OCL (mm)


Moult Stage C_1
Moult Stage $D_{1.1}$
Protein (%)
OCL (mm)
OCL (mm)


Moult Stage $D_{1.2}$
Moult Stage $D_{1.3}$
Protein (%)
60
50
40
30
20
10
0
0
5
10
15
20
OCL (mm)


Moult Stage $D_{1.4}$
Moult Stage D_2
Protein (%)
80
70
60
50
40
30
20
10
0
0
5
10
15
20
OCL (mm)

Moult Stage D_3



Moult Stage D_4

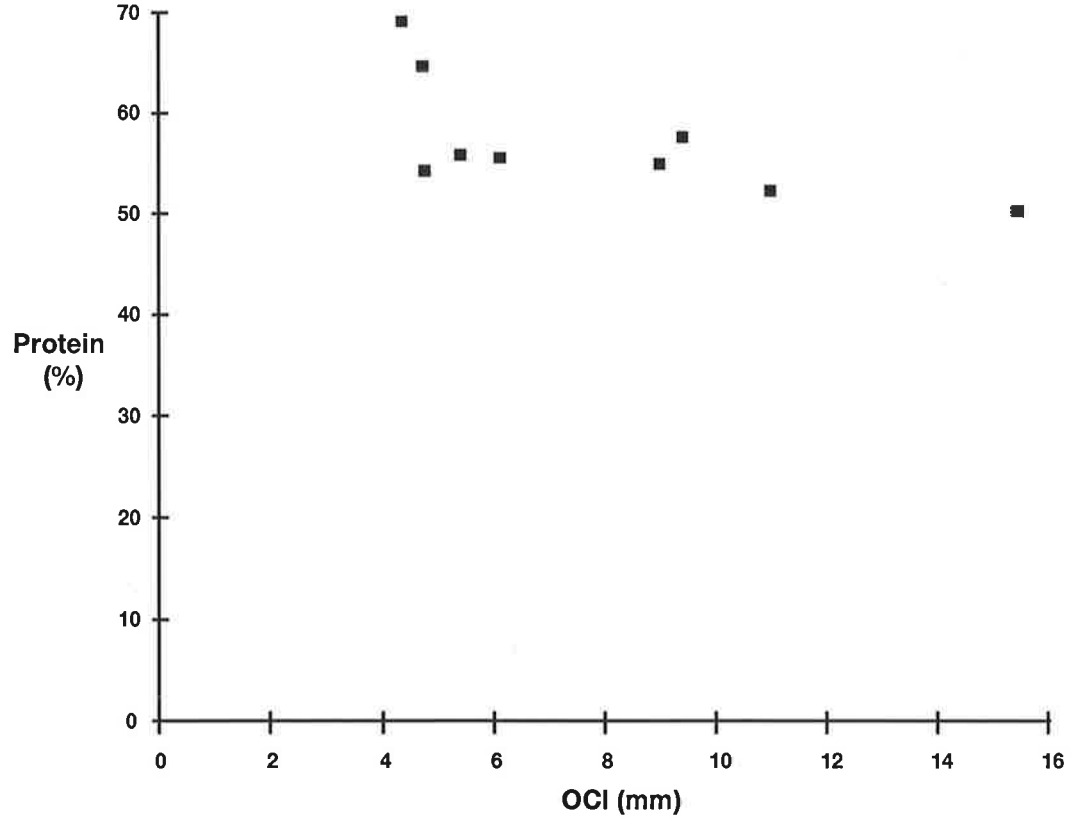


Fig 3.18
Change in Total Protein Content with Size (OCL) over the Moult Cycle

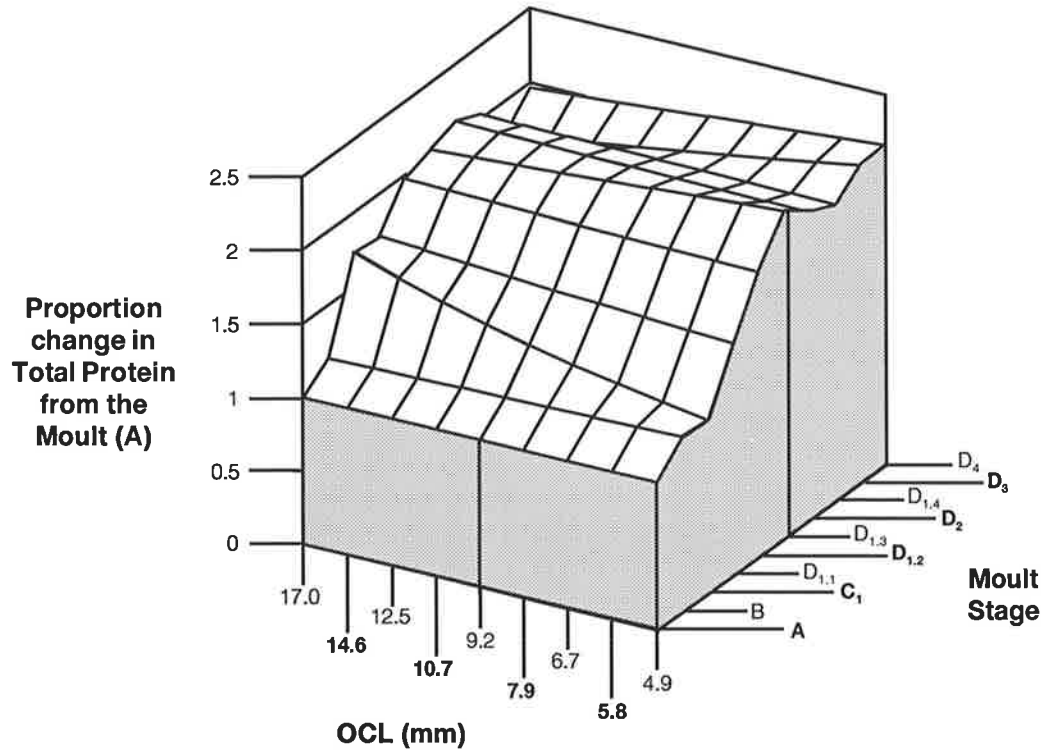
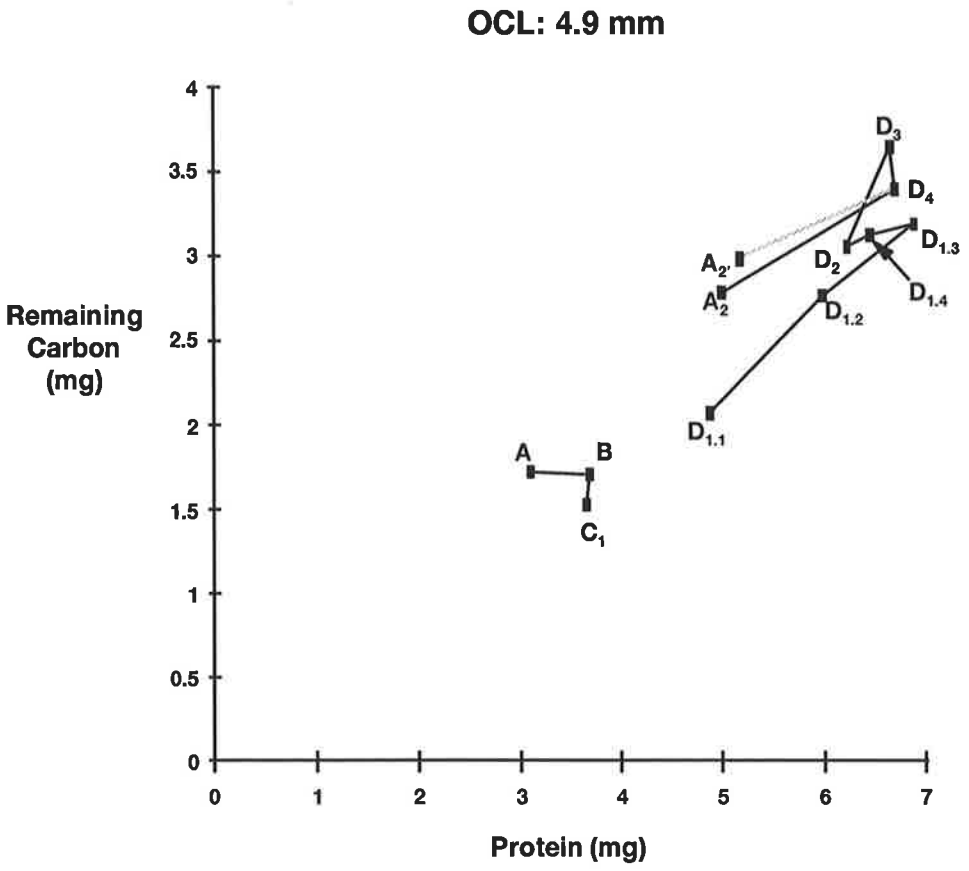
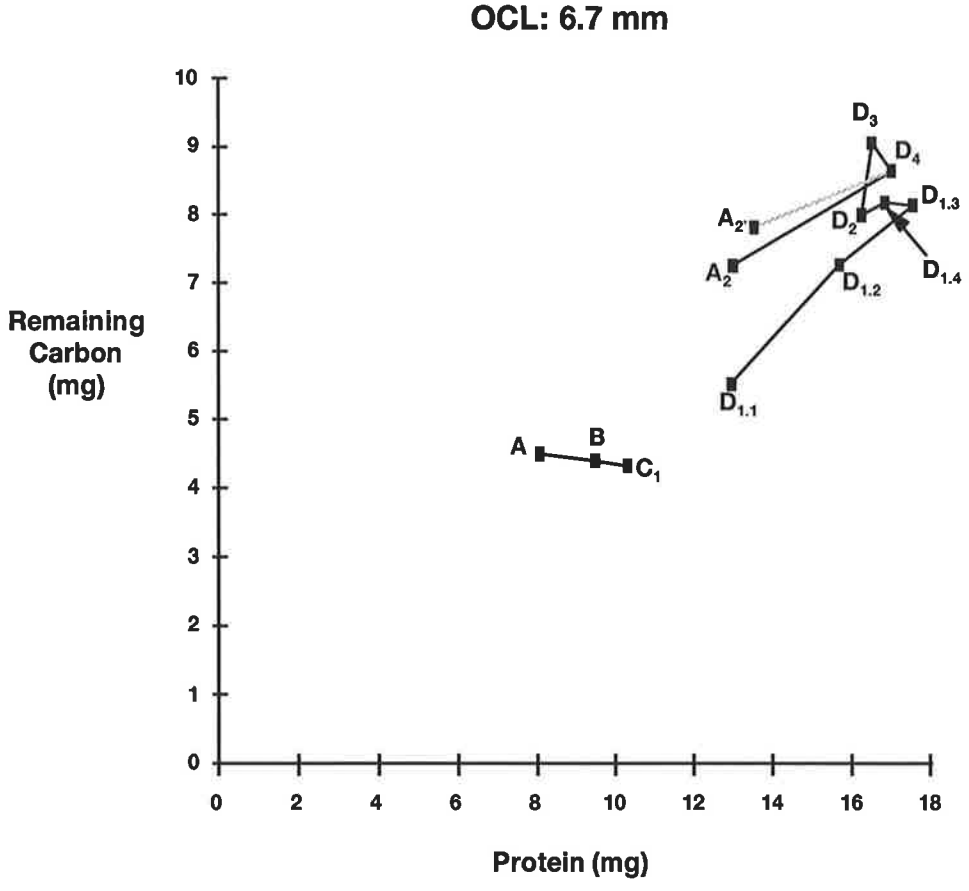


Fig. 3.19

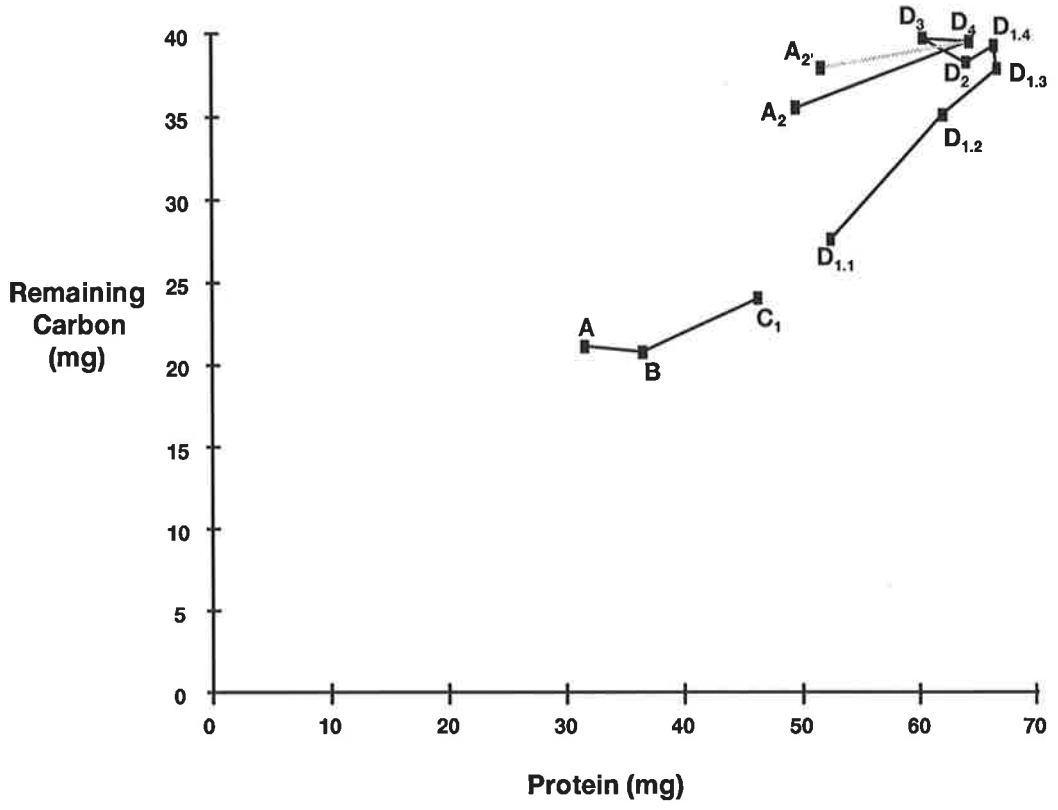
Protein (mg) vs Remaining Carbon (mg) over the Moult Cycle for a series of sizes

Note that D_4 to A_2 represents the next moult assuming a 16.74% moult increment (refer text). D_4 to $A_{2'}$ represents the same thing but without tissue lost with the exuvia

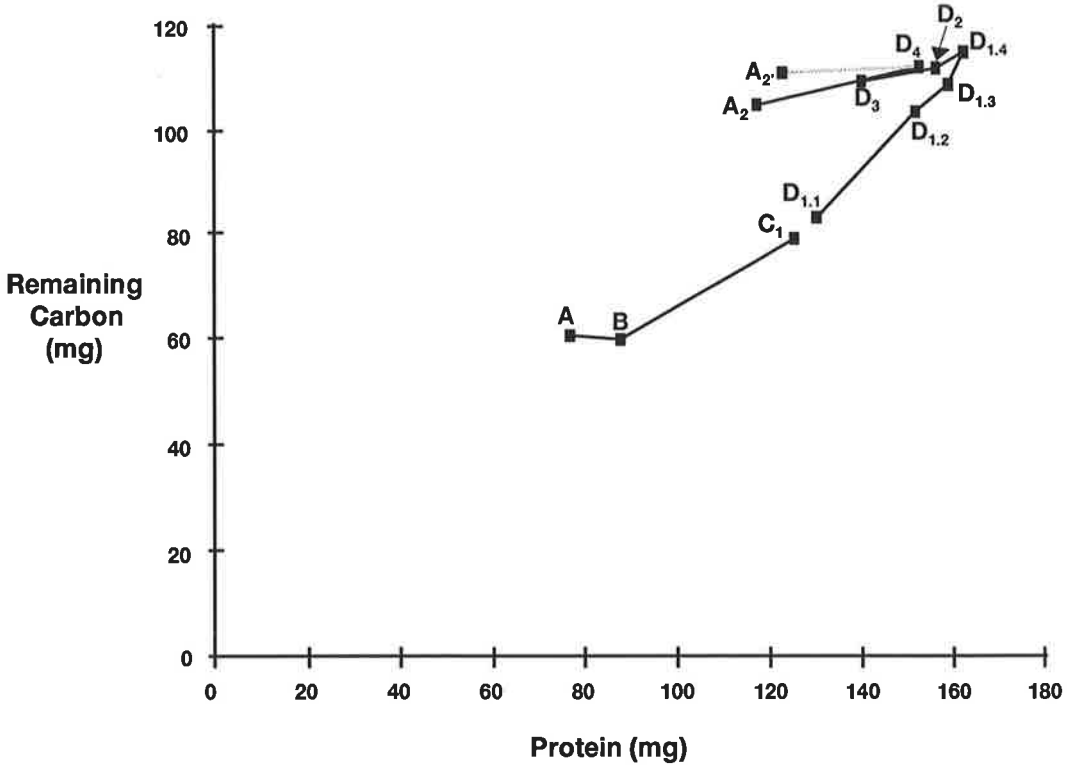

OCL: 4.9 mm
Remaining Carbon (mg)
Protein (mg)
0
0.5
1
1.5
2
2.5
3
3.5
4
0
1
2
3
4
5
6
7
A
B
C1
A2'
A2
D1.1
D1.2
D2
D3
D4
D1.3
D1.4


OCL: 6.7 mm
Remaining Carbon (mg)
Protein (mg)
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16
18
A
B
C1
A2'
A2
D1.1
D1.2
D2
D3
D4
D1.3
D1.4

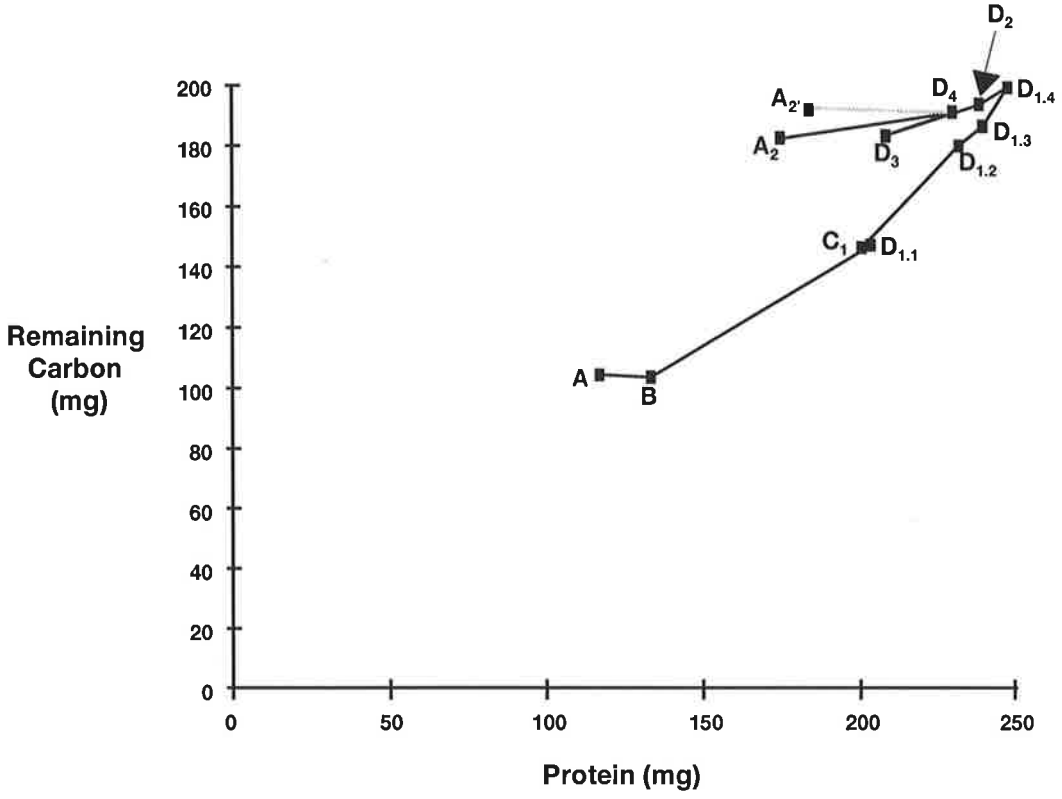
OCL: 10.7 mm



OCL: 14.7 mm



OCL: 17 mm


Remaining Carbon (mg)
200
180
160
140
120
100
80
60
40
20
0
0
50
100
150
200
250
Protein (mg)
A
B
C1
D1.1
D1.2
D1.3
D1.4
D2
D3
D4
A2
A2'

crayfish (Armitage *et al*, 1972, Barclay *et al*, 1983, Villarreal, 1989). The relationship between chitin and protein was presented separately (**Fig 3.20**) as it is primarily a structural material (Aiken and Waddy, 1987) although some chitin-glucose may be used as an energy source during late premoult (Hornung and Stevenson, 1971, Spindler-Barth, 1976).

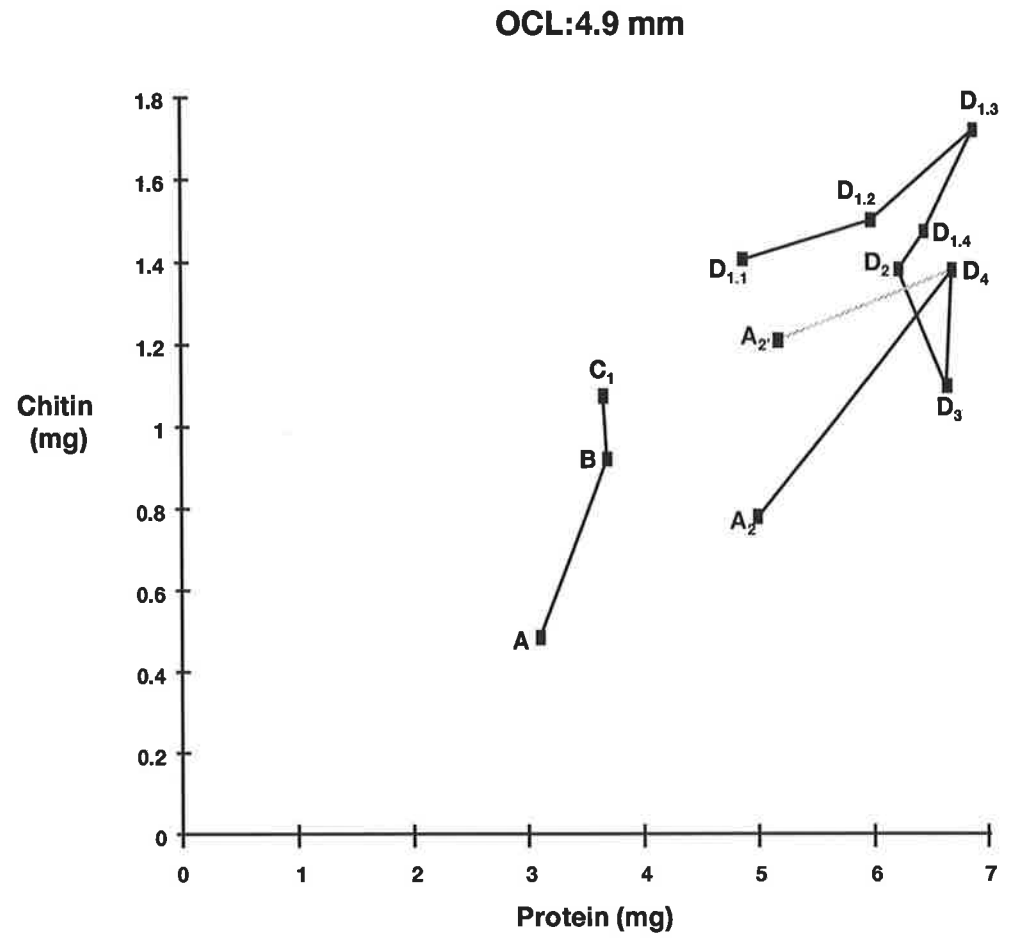
Postmoult is characterised by a slight decline in remaining carbon accompanied by an increase in chitin, which indicates rapid hardening of the exoskeleton, and in protein, which suggests that tissue growth begins immediately. The fall in remaining carbon occurs through A and B in smaller yabbies but becomes progressively confined to A as size increases. As shown previously (**Fig 3.13**) very little tissue is accumulated during intermoult (C_1 and C_2), the second and final tissue accumulation period occurring during premoult substages $D_{1.1}$ to $D_{1.3}/D_{1.4}$. Although there is no net gain in protein after this point there is a decline in chitin and an increase in remaining carbon to D_3 followed by a slight reversal in both cases to D_4. This is accompanied by a slight rise in protein content. The figures also show the loss of tissue over the moult itself (D_4 to A_2). The grey lines show the same loss without the exuvia (D_4 to $A_{2'}$). The calculated losses assumed the 16.74% moult increment in length as reported above. It is apparent that in yabbies between 10 and 17 mm OCL most remaining carbon is lost with the shed exoskeleton whereas the marked decline in protein suggests that it is catabolised during the moult. Yabbies of between 4.9 and 6.7 mm OCL also show some loss of remaining carbon during the moult but this amounts to less than 30% of the protein lost in each case. Finally, the loss of some chitin during the moult aside from that lost with the exuvia suggests its catabolism in smaller crayfish.

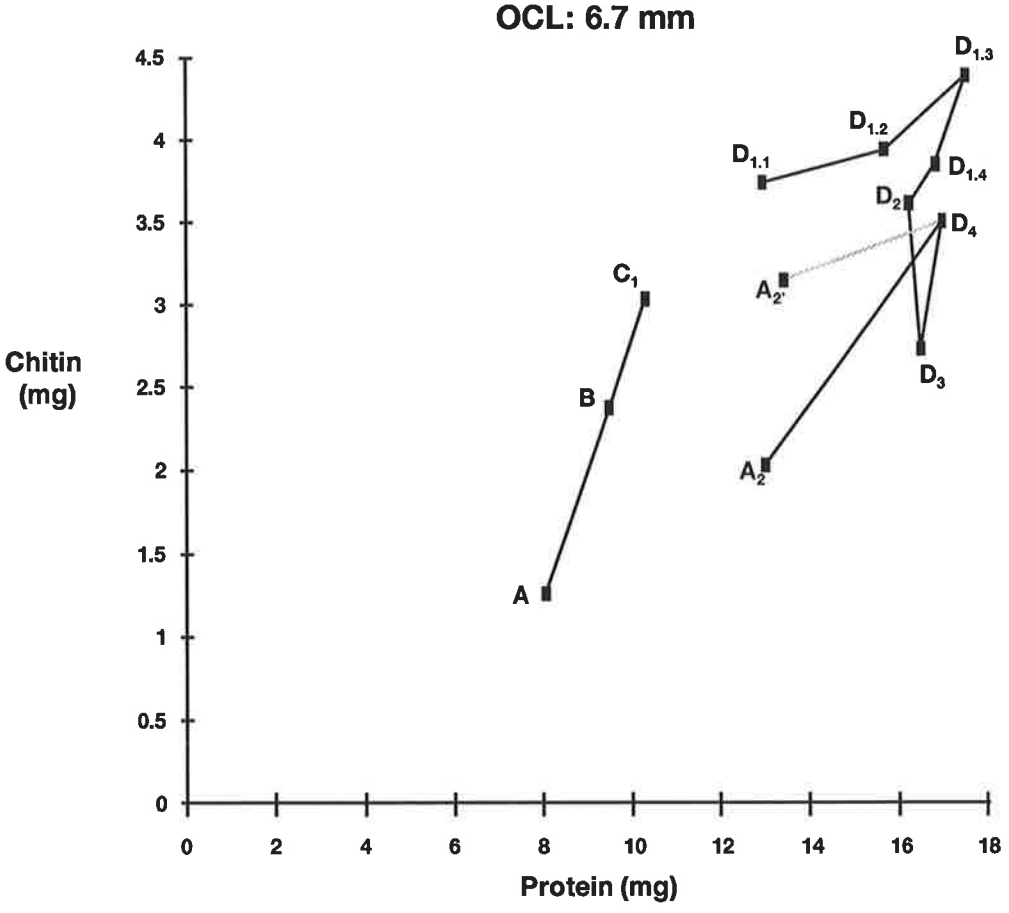
Fig. 3.20

Protein (mg) vs Chitin (mg)

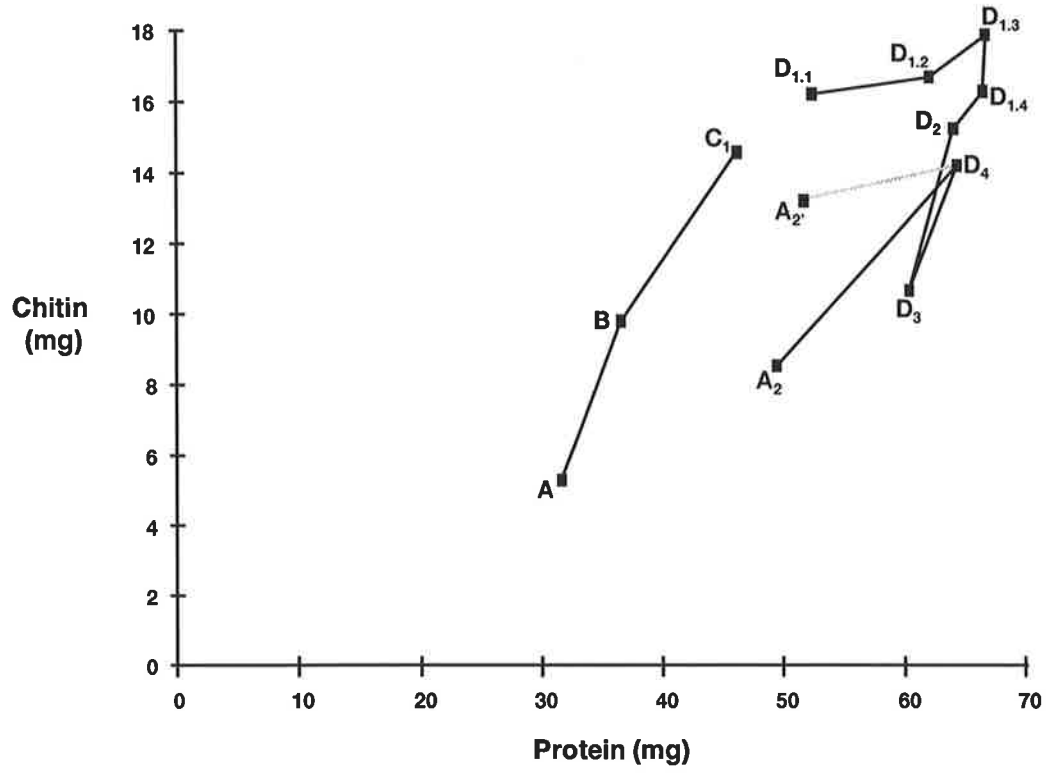
over the Moult Cycle for a series of sizes

Note that D_4 to A_2 represents the next moult assuming a 16.74% moult increment (refer text). D_4 to $A_{2'}$ represents the same thing but without tissue lost with the exuvia

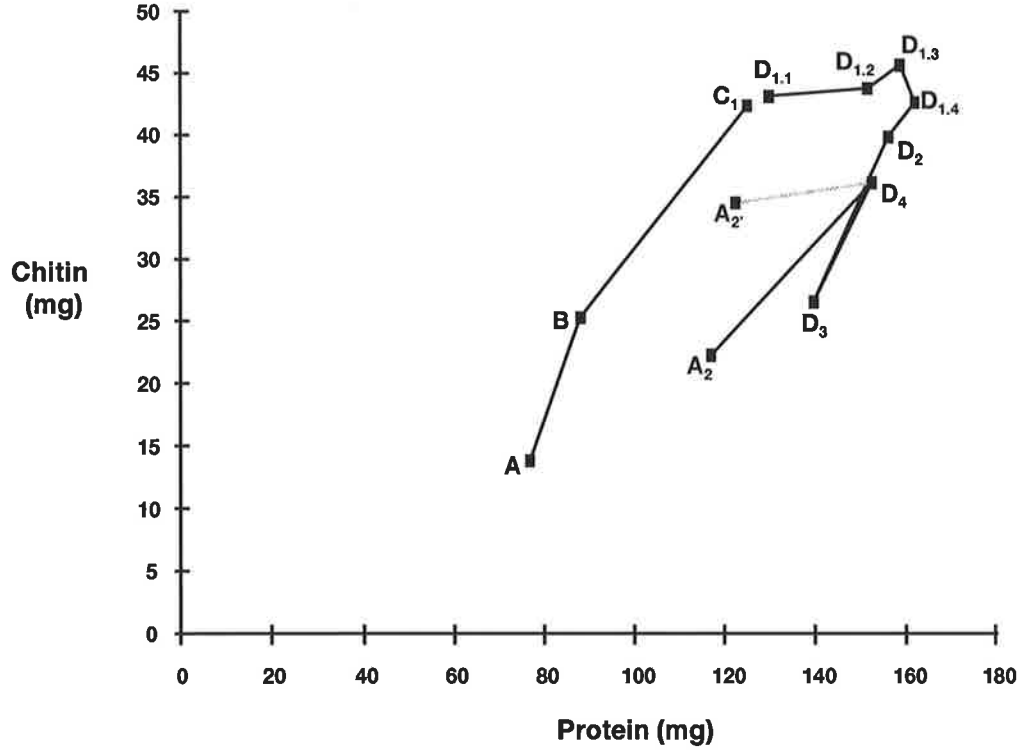

OCL:4.9 mm
Chitin (mg)
Protein (mg)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
7
A
B
C1
A2
A2'
D1.1
D1.2
D1.3
D1.4
D2
D3
D4


OCL: 6.7 mm
Chitin (mg)
Protein (mg)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
14
16
18
A
B
C1
A2
A2'
D1.1
D1.2
D1.3
D1.4
D2
D3
D4

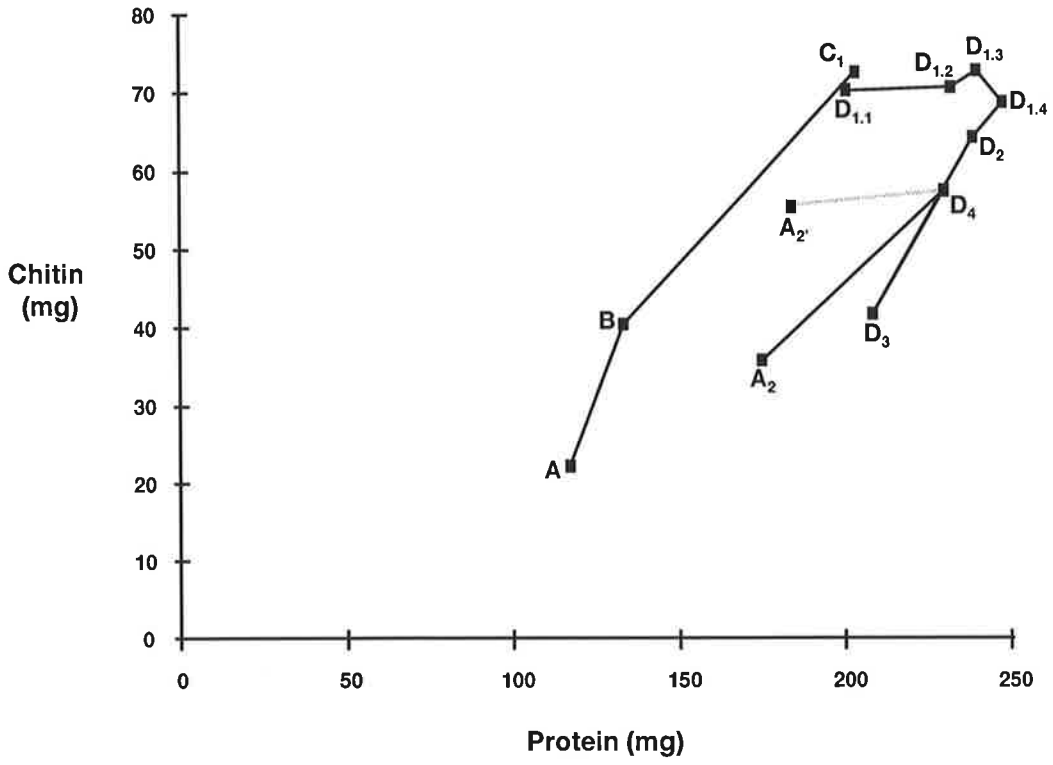
OCL: 10.7 mm



OCL: 14.6 mm



OCL: 17 mm


Chitin (mg)
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
Protein (mg)
A
B
C1
D1.1
D1.2
D1.3
D1.4
D2
D3
D4
A2
A2'

e) Energy

Mean tissue energy content for each moult substage varied between 19 and 22 joules /mg afdw (**Table 3.22**) and the overall average was 20.727 joules /mg afdw (± 0.275, n = 106).

Table 3.22 Mean Caloric content (Joules/mg) (± SE) for 10 Moult Stages/substages

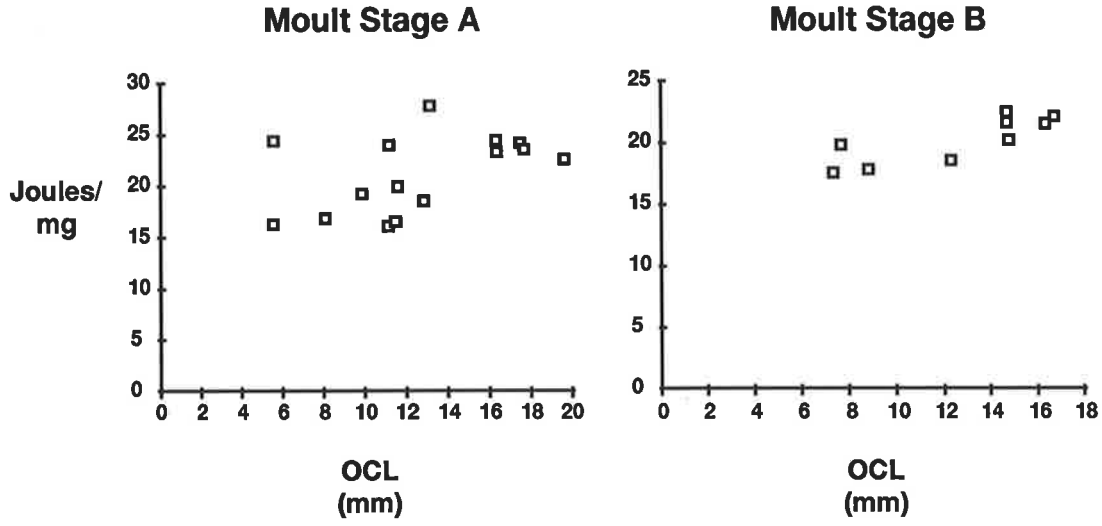
Moult Stage (n)	Joules/mg	SE
A (15)	21.379	0.9697
B (9)	20.619	0.7360
C_1(8)	19.072	0.3137
C_2 (10)	19.576	0.7931
$D_{1.1}$(11)	22.104	0.5379
$D_{1.2}$ (8)	21.896	1.1047
$D_{1.3}$ (10)	21.860	1.0139
$D_{1.4}$ (7)	19.350	1.00821
D_2 (6)	20.346	0.5914
D_4 (13)	20.393	0.7502

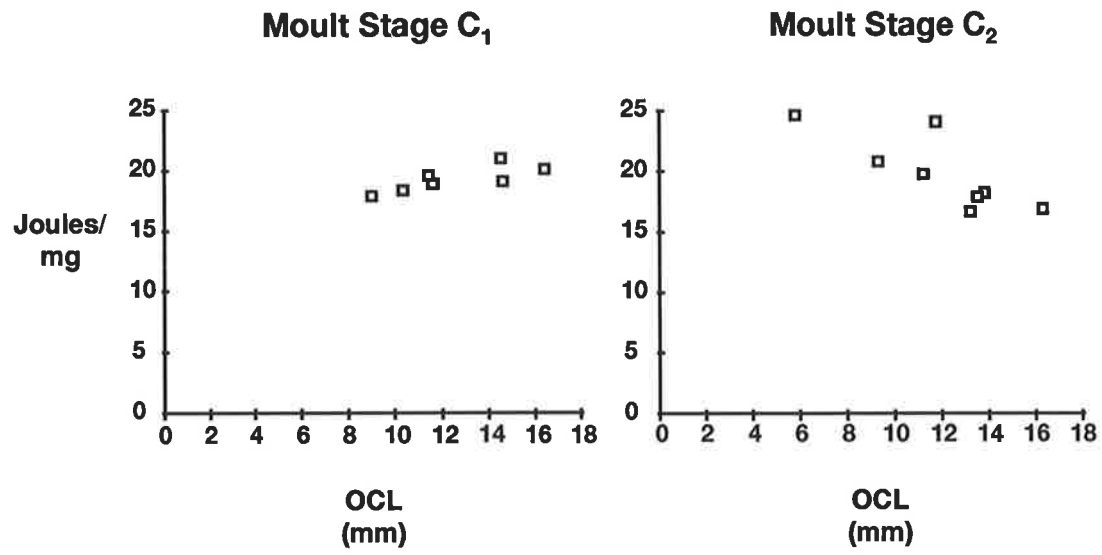
Energy content data (joules/ mg vs OCL (mm)) are presented in Fig 3.21. ANCOVA on the normalised data showed that moult substage but not size (OCL) affected overall weight-specific caloric content, although there was an interaction between OCL and moult substage (Column 1: **Table 3.23**).

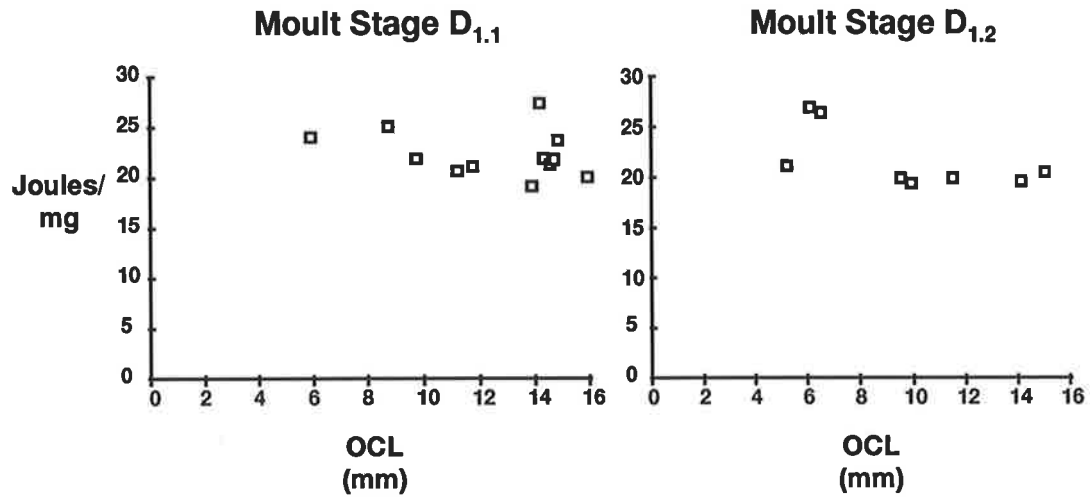
Table 3.23 ANCOVA on groups of moult substages to isolate significant Moult Stage/ Substage effects on Caloric Content (Joules/mg). Data normalised using Box-Cox transformation. Significance accepted at 0.05. P=probability, ns = nonsignificant

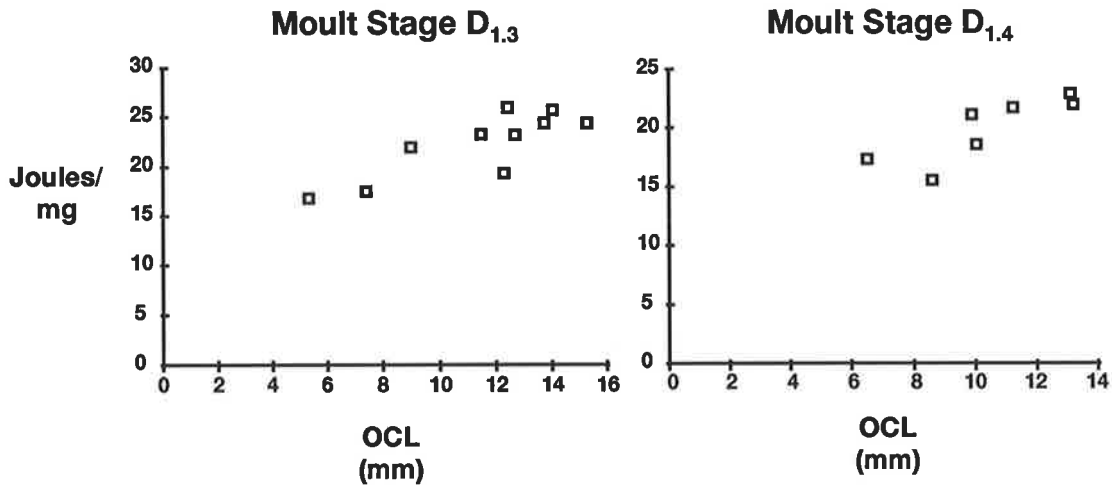
	Column 1 (see text)	**2**	**3**	**4**	**5**	**6**
			Compared Stages			
	All Stages	**A,B and C_1**	**C_1 and C_2**	**$D_{1.1}$ to $D_{1.4}$**	**C_2 to $D_{1.2}$**	**$D_{1.3}$ to D_4**
	P	**P**	**P**	**P**	**P**	**P**
Source Moult	**<0.001**	ns	**0.010**	<0.001	ns	ns
OCL	**0.204**	0.022	**ns**	0.066	0.001	ns
Moult x OCL	**<0.001**	ns	**0.011**	<0.001	ns	ns
n	**97**	**32**	**18**	**36**	**29**	**36**

Fig. 3.21
Caloric Content (Joules/mg)
vs OCL (mm) for 10 Moult Stages

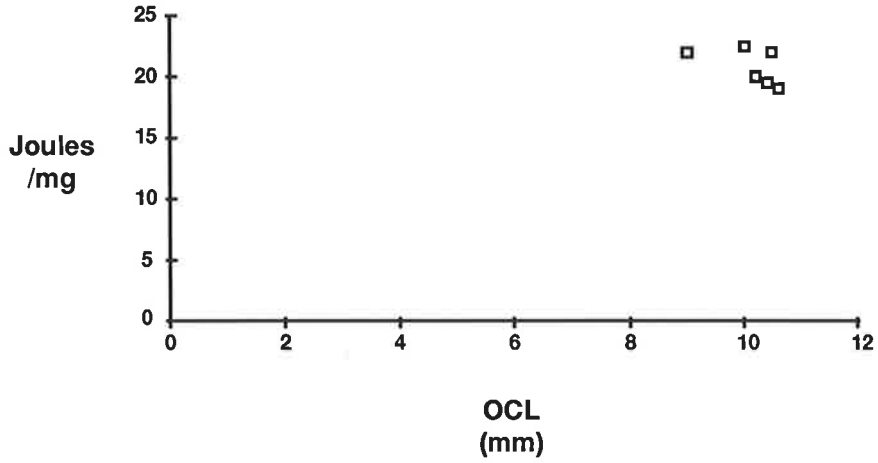

Moult Stage A
Moult Stage B
Joules/
mg
30
25
20
15
10
5
0
0 2 4 6 8 10 12 14 16 18 20
25
20
15
10
5
0
0 2 4 6 8 10 12 14 16 18
OCL
(mm)
OCL
(mm)


Moult Stage C_1
Moult Stage C_2
Joules/
mg
25
20
15
10
5
0
0 2 4 6 8 10 12 14 16 18
25
20
15
10
5
0
0 2 4 6 8 10 12 14 16 18
OCL
(mm)
OCL
(mm)

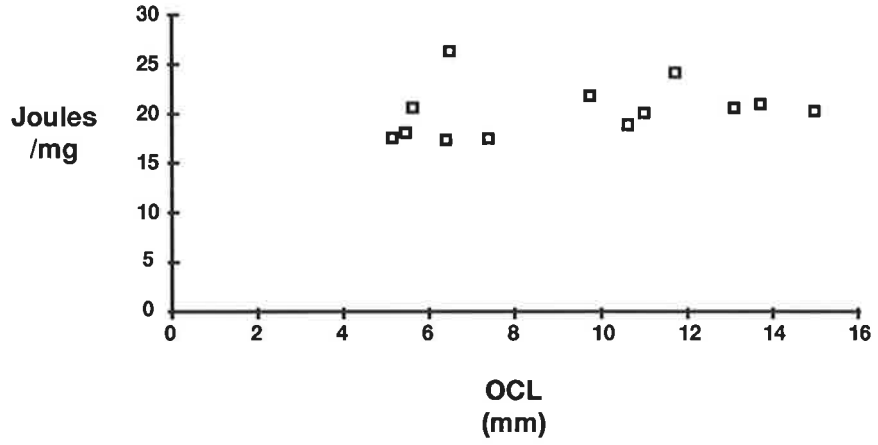

Moult Stage $D_{1.1}$
Moult Stage $D_{1.2}$
Joules/ mg
30
25
20
15
10
5
0
0 2 4 6 8 10 12 14 16
OCL (mm)


Moult Stage $D_{1.3}$
Moult Stage $D_{1.4}$
Joules/ mg
30
25
20
15
10
5
0
0 2 4 6 8 10 12 14 16
0 2 4 6 8 10 12 14
OCL (mm)

Moult Stage D_2



Moult Stage D_4



As with the carbon and nitrogen analyses the interaction term was isolated and further analyses carried out by subdivision of the data in to postmoult, intermoult, premoult and late premoult.

There were no significant main effects or interactions within substages $D_{1.3}$ to D_4 (Column 6: **Table 3.23**) so these data were pooled and a mean derived (20.590 ± 0.076, n=36: **Table 3.24**).

Table 3.24 Summary of treatment of Caloric data as a result of ANCOVA (ref Table 3.21). The regressions were of the form Y (Caloric Content) = a + bX (OCL)

Combined Moult Stages /Substages	**Action**	**Statistics**
A, B, C_1 n=32	linear regression	b = 0.410 (±0.127) a = 15.417 (±1.663) r^2 = 0.333 P=0.003
C_2, $D_{1.1}$, $D_{1.2}$ n=29	linear regression	b = -0.463 (±0.125) a = 26.525 (± 1.503) r^2 = 0.336 P=0.001
$D_{1.3}$ to D_4 n=36	Mean (± SE)	20.590 (± 0.0759)

Postmoult substages A and B and intermoult substage C_1 were similarly pooled as they showed a common increase in caloric content with size (Column 2 : **Table 3.23 and Table 3.24**). There was a significant decline in caloric content with OCL in C_2 which produced an interaction effect when intermoult substages were compared. (Column 3: **Table 3.23**). As there were no significant differences between C_2, $D_{1.1}$ and $D_{1.2}$, (Column 5: **Table 3.23**) a linear regression was calculated for this group (**Table 3.24**).

Size-specific caloric contents for A to C_1 and C_2 to $D_{1.2}$ and the mean for $D_{1.3}$ to D_4 were multiplied by the appropriate moult stage or substage-specific organic weight described earlier to produce total joules for a given size and moult stage. Proportional moult stage and size-

specific change in caloric content from the moult was then calculated as presented in **Fig 3.22.** As in previous models this figure assumes a 16.74% moult increment. The figure shows two sets of peaks. Accumulation of energy in small yabbies peaks at the end of $D_{1.1}$ and again at D_3/D_4, with the greatest caloric accumulation occurring during C_1. The latter was significant in terms of caloric content ($P < 0.01$, **Table 3.23**) but the $D_{1.1}$ and D_3/D_4 peaks reflect changes in ash free dry weight as discussed previously. Significant changes in ash free dry weight also account for the 'peak' in caloric content of larger yabbies at the end of Stage B.

Total caloric loss at the moult was calculated by subtracting the estimated caloric value of an animal at D_4 from that of the next size at A, assuming a moult increment of 16.74% as before. Exuvial energy loss at the moult was estimated using caloric equivalents as the shed exoskeleton did not combust well in the bomb calorimeter. This was done as follows. Chitin averaged 50.489% of the exuvia and Stecher *et al* (1968) state that carbon accounts for 47.29% of chitin. Furthermore, carbon took up 49.307% of the weight of the exuvia. Thus the carbon in chitin in the exuvia made up 47.29% of the 50.489% or 23.876% of the % carbon in the exuvia, leaving 25.431% non-chitin carbon. Given that the exuvia also contains 24.468% protein this gives a total of 100.388% (50.489 (chitin)+ 25.431 (non chitin carbon) + 24.468 (protein)) which rounded down evenly over the three components allows estimation of the energy lost with the exuvia at the moult. The caloric values of the components were calculated using the caloric equivalents described by Gnaiger and Bitterlich (1984) for protein (23.9 j/mg afdw) and for carbohydrate (17.5 j/mg afdw). In this study chitin is considered as a carbohydrate as suggested by Anger *et al* (1983). Non-chitin or 'remaining' carbon was converted using a factor of 47.7 j/mg (Zoutendyk, 1988). Protein, chitin and remaining carbon contributed about 22%, 33% and 45% respectively to the caloric content of exuviae. The caloric values were then summed for comparison with total energy loss at the moult, in this case estimated from caloric equivalents. The latter was derived by subtracting the calculated caloric value of an animal at D_4 from that of the next size at A, assuming a moult increment of 16.74% as before. Thus the proportional exuvial loss at the moult for sizes ranging from 4.9 to 17 mm OCL varied from 36% to 49% (**Table 3.25**).

Fig 3.22
Change in Caloric Content with Size (OCL) over the Moult Cycle

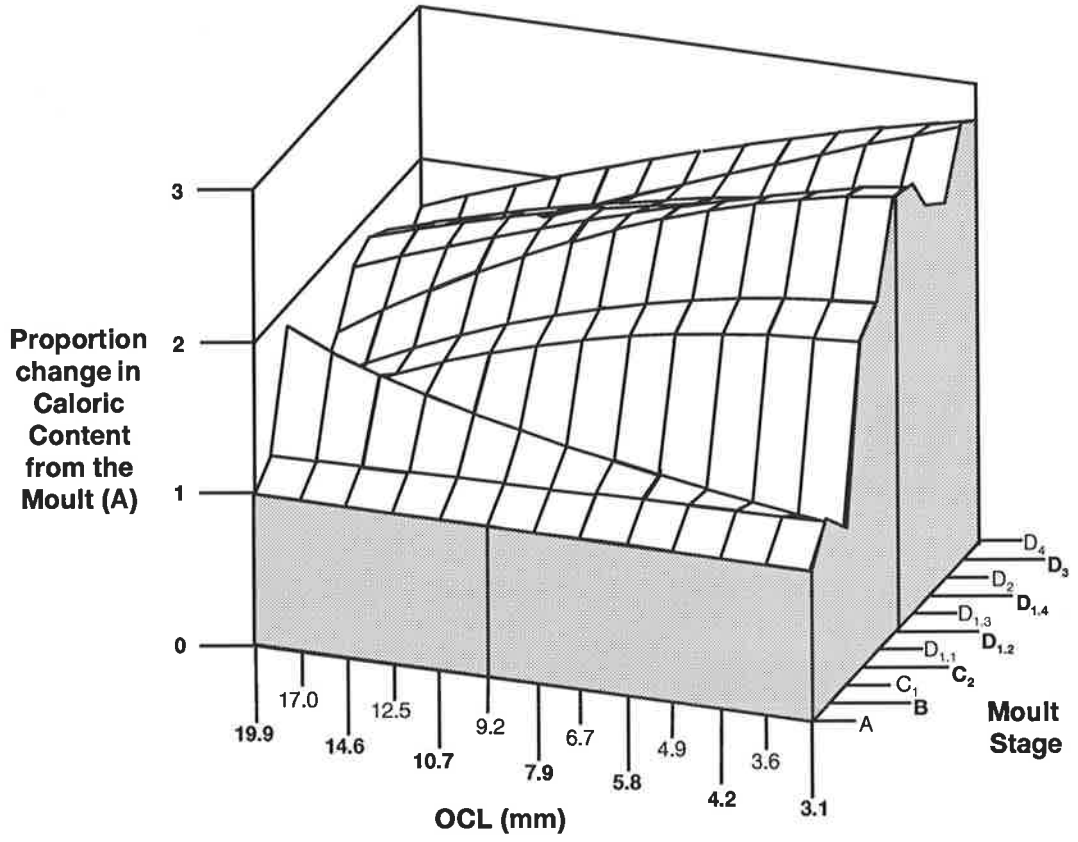


Table 3.25 Estimated Caloric loss with Exuvia as a percentage of Total Caloric loss at moult.

OCL	% Caloric Loss
4.9	35.69
5.8	37.67
6.7	39.85
7.9	40.00
9.2	41.10
10.7	43.71
12.5	46.58
14.6	49.78
17.0	48.71

Note that these estimates of total energy loss at the moult using caloric equivalents were derived only to allow the calculation of the percentage losses with the exuvia, also derived from caloric equivalents. 'Total caloric losses at the moult', used in further discussion, were based on empirical data derived from bomb calorimetry, as described above.

f) An Overview of the Moult Cycle

The following figures and table summarise the key results for the chapter. **Figures 3.23a and b** show proportional change in each individual tissue component over the moult cycle in relation to its level at postmoult stage A. Protein is not included as its pattern follows that of organic content. The data is plotted against the moult cycle with the distance between substages determined by the proportional contribution of each to moult cycle length. The stages of the moult cycle are also included on the graph and are usually grouped as A and B (postmoult), C_1 and C_2 (intermoult), $D_{1.1}$ to $D_{1.4}$ (premoult) and D_2 to D_4 (late premoult).

Table 3.26 presents a summary of significant changes in dry weight, % organic content and energy during the moult cycle. Protein and carbon are not included as analysis showed size but not moult stage effects. Significant changes between moult stages are shown by asterisks. If the change was only significant for small or large crayfish an 'S' or an 'L' is added to the asterisk. Note that dry weight was analysed using both ANCOVA and Mann Whitney U tests. The first was carried out on the crayfish from Harvest 3 and the second was carried out on all

Table 3.26 Summary of Moult Stage and Size (OCL) effects on Dry weight, % Organic Content and Energy Content (Joules/mg). Carbon and Protein are not included as analysis did not show a moult stage effect although there was a significant size effect. Note that the Mann-Whitney U test was done on all the dry weight data whereas the ANCOVA refers to Harvest 3 only. * - Significant difference between stages; S or L - only significant for either smaller or larger yabbies

Moult Stage	Dry Weight (mg)		% Organic Content	Energy Content (Joules / mg)
	ANCOVA	Mann Whitney		
A	| *			
B	| *	| * L	| *	
C_1			| *	| *
C_2		| *	| *	
$D_{1.1}$		| *		
$D_{1.2}$	| *	| * S		| *
$D_{1.3}$				
Other Comparisons				
A and $D_{1.3}$		*		
C_1 and $D_{1.1}$	*			
$D_{1.3}$ and D_2		*S		
D_2 and D_4		*S		

Fig 3.23a
Tissue Accumulation and Changes in Proximate composition over the Moult cycle for a Juvenile Yabbie of 6.7 mm OCL

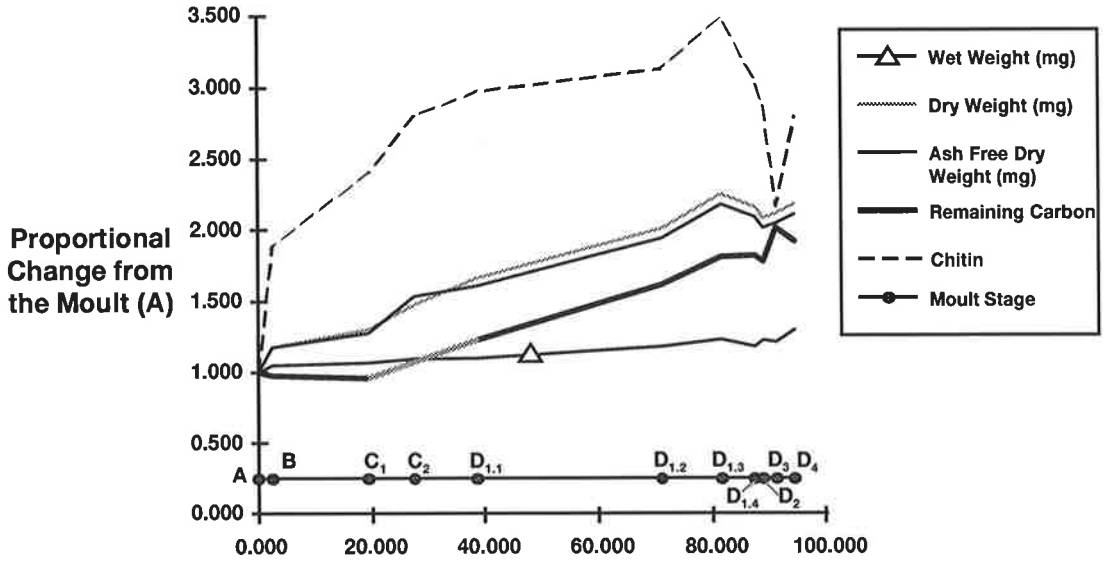
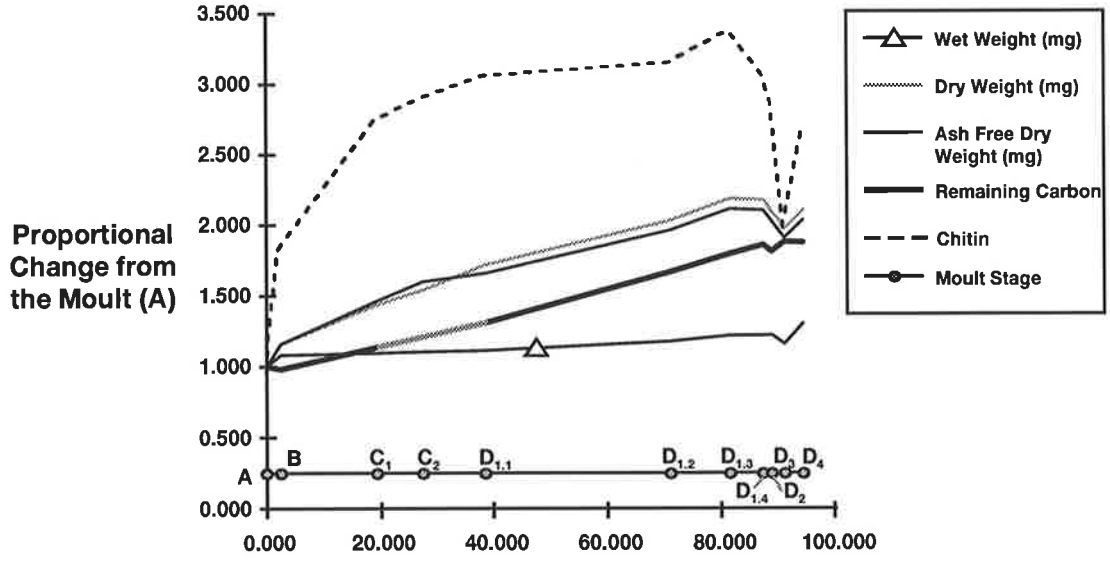


Fig 3.23b
Tissue Accumulation and Changes in Proximate composition over the Moult cycle for a Juvenile Yabbie of 10.9 mm OCL



animals. Differences were usually tested between pairs of consecutive moult stages although there were other comparisons between more distant pairs. Note that all comparisons were not significant for all three parameters. For example there was a significant change in dry weight and % organic content but not caloric content between B and C_1.

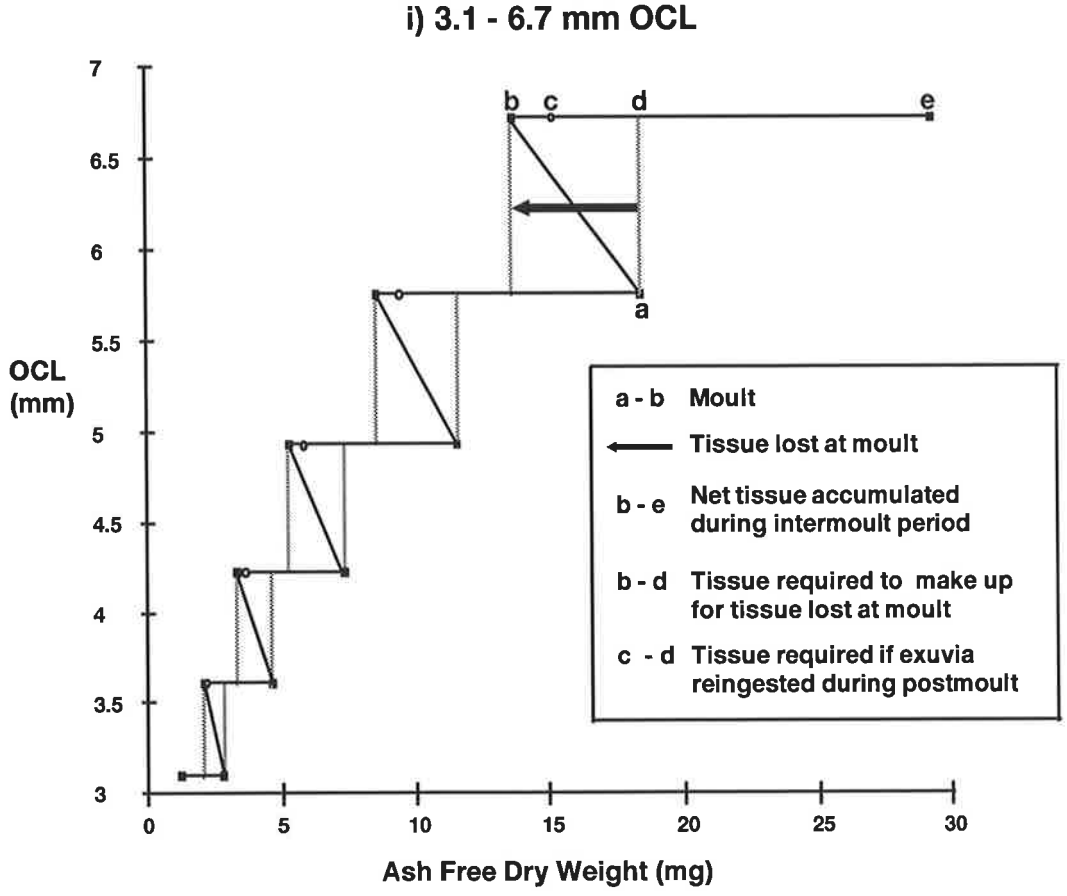
Fig 3.24a and b summarise patterns of tissue and energy accumulation as crayfish grow from 3.1 mm to 17 mm OCL. The figures include loss over the moult and net accumulation over the following moult cycle for each size. Also included are the tissue and energy accumulation necessary to make up for the energy lost at the moult and how much of this the exuvia would contribute if it was reingested following the moult. Moult increment is again 16.74% and the figures assume that all exuvial tissue/energy is completely reabsorbed on ingestion. The figures show that 40.8% of the energy and 32.1% of the tissue accumulated by a 3.6 mm OCL yabbie during its moult cycle must go toward re-establishing material losses incurred when moulting from 3.1 mm OCL. This reduces to 16.5% of the energy and 24.1% of the tissue by the time a crayfish has reached 17 mm OCL. If the exuviae are eaten these figures are reduced to 36.2% of the energy and 29.6% of the tissue for a yabbie of 3.6 mm OCL and 15.3% of the energy and 21.8% of the tissue for a yabbie of 17 mm OCL.

3.4 Discussion

3.4.1 Growth

Mean growth per container varied between 0.08 and 0.086 mg/mg afdw/day over the 53 day experimental period. Mean overall growth (0.084 mg/mg afdw/day, 0.081 mg/mg wet weight/day) was slightly lower than the best growth achieved by Geddes *et al* (in press) for juvenile yabbies under similar field conditions and at similar temperatures, ranging from 20 - 25°C. Their best growth averaged 15 mg to 800 mg over a 42 day period (M. Geddes, pers comm) which is equivalent to 0.095 mg/mg wet weight/day. The individual variability in growth was similar to that in the present study, as their animals ranged in size from 35 mg to 5 g after that period. Mills and McCloud (1983) also raised yabbies in above-ground pools and achieved similar growth rates. In their study animals grew from 13 mg to an average of

Fig 3.24a
Patterns of Tissue accumulation and loss during growth in juvenile yabbies
Yabbie size range 3.1 - 17 mm OCL . Refer text for explanation.



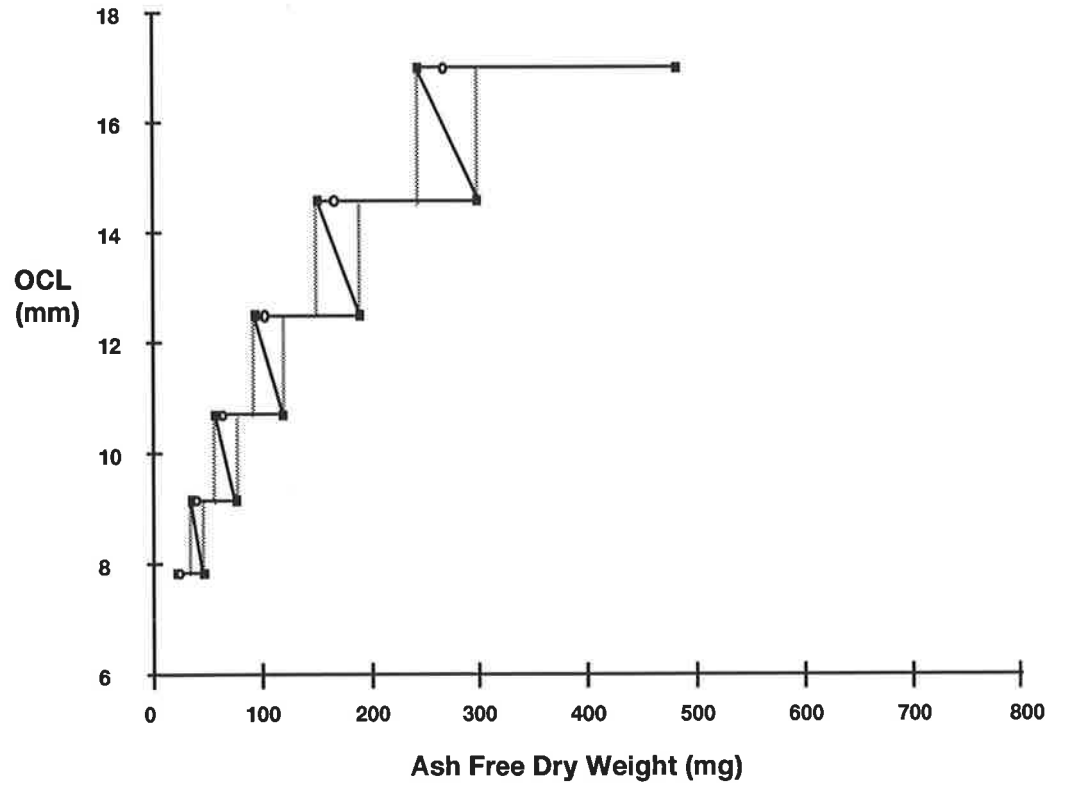
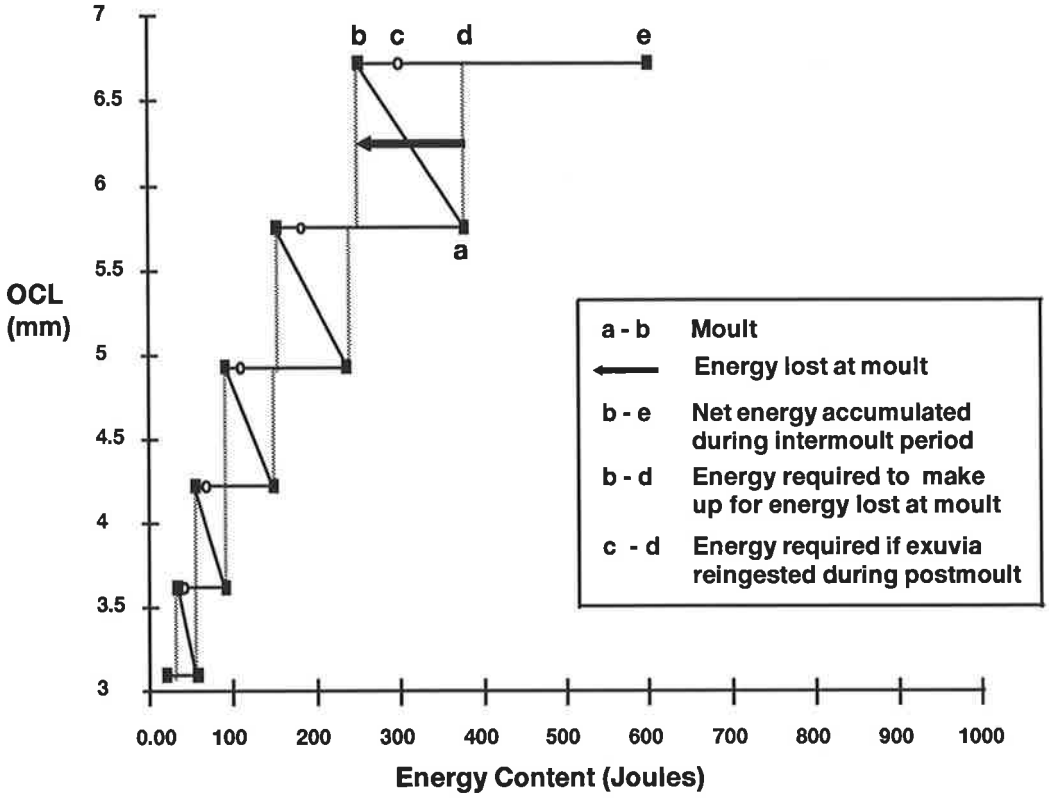


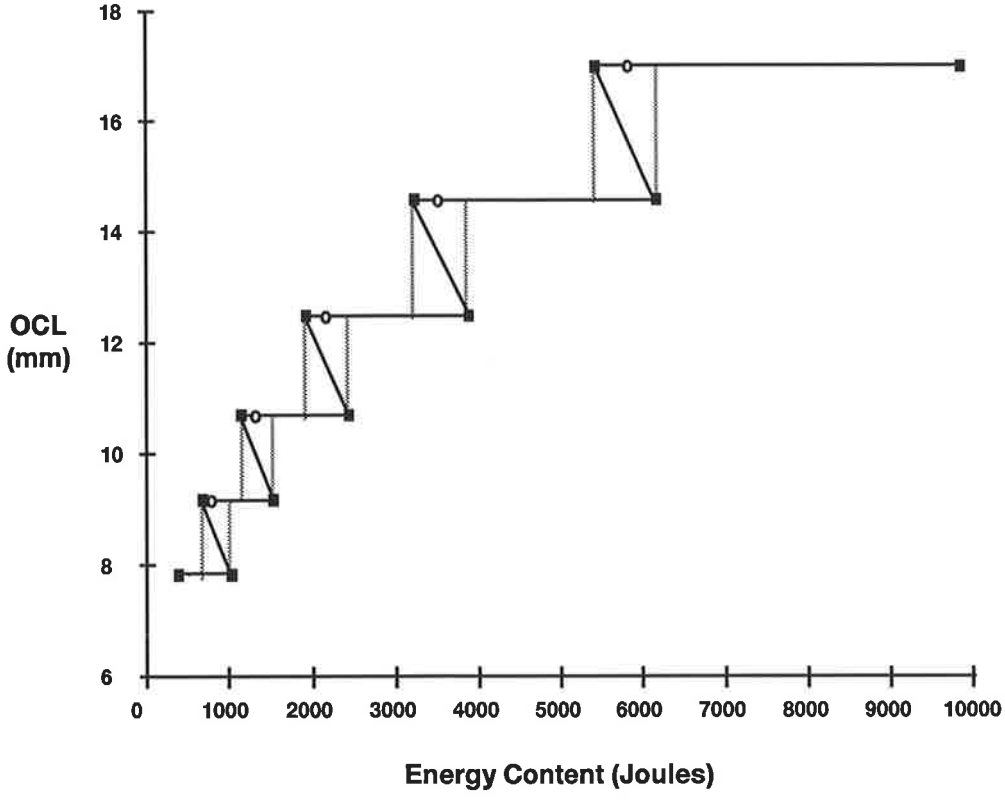
Fig 3.24b
Patterns of Energy accumulation and loss during growth in juvenile yabbies

Yabbie size range 3.1 - 17 mm OCL . Refer text for explanation.

i) 3.1 - 6.7 mm OCL



ii) 7.9 - 17mm OCL



between 1.3 g and 4 g in 8 weeks which is equivalent to between 0.08 and 0.1 mg/mg wet weight/ day. In their study temperatures ranged between 15 and 28°C. All three studies experienced average temperatures below 28°C which was described by Mills (1986) as the optimal temperature for growth. The relationship between temperature and growth will be further investigated in Chapter 4. Note that yabbies in all three studies had a similar range of food available, including commercial pellets and pond flora and fauna.

Growth rates of other crayfish species are generally lower although such comparisons are complicated by different optimal growth temperatures and nutritional environments. For example, Morrissy (1979) reported mean growth for *Cherax tenuimanus* from 0.06g to between 1.85 and 4.96g in 124 days (≡ 0.028 - 0.036 mg/mg wet weight/day). Growth rates were measured in a pond at temperatures between 13 and 20°C which are below the optimal temperature of 24°C reported by Morrissy (1990) for this species. These animals were also fed with commercial pellets and presumably had access to pond flora and fauna. Tcherkashina (1977) reported growth for *Astacus leptodactylus* from 34.6 mg to 3.34g over about four months in ponds at summer temperatures of about 20°C. Crayfish were not fed during this period but, as before, had access to abundant pond flora and fauna. Assuming a 120 day growth period the IG would be about 0.04 mg/mg wet weight/day. The optimal growth temperature is not available for this species although Cukerzis (1988) reported that *A. leptodactylus* will congregate at temperatures between 21 and 24°C in the laboratory.

3.4.2 The Moult Cycle

Length of moult substages varies greatly between studies, probably depending on size, experimental and acclimation conditions, including food sources (Passano, 1960) as well as on the method of estimation and the terminology. Although there are four generally recognised stages (A,B,C, and D; Aiken and Waddy, 1987), the number of substages and what is meant by a particular substage appears to be taxon specific, which limits comparisons between groups and in some cases, within groups (see reviews by Passano, 1960, Reaka, 1975, Aiken and Waddy, 1987). For example, in *Cherax destructor* there are two distinct substages in stage C (intermoult: Burton and Mitchell, 1987) whereas four have been reported

in stomatopods (Reaka, 1975) and other crayfish (i.e. *Orconectes sanborni* Stevenson, 1968). Comparisons using stages can be made more easily. Premoult/late premoult ($D_{1.1}$ - D_2) , for example is described as physiologically significant with respect to preparation for the next moult (Aiken and Waddy, 1987) and has been found to account for the majority of the moult cycle in other crayfish (Huner and Avault, 1976; Rao *et al,* 1977), other decapods (i.e. *Homarus* and various stomatopods, Reaka, 1975) and in this study where it accounted for 50% of the moult cycle.

The length of moult cycle substages is usually investigated in the laboratory as this allows accurate determination of the passage from substage to substage. However, it is hard to know to what extent this laboratory data reflects natural moult cycles. Although the field data collected here is not as precise it is an estimate of the moult cycle under more natural conditions. There are differences in the length of moult substages found in the present study and the laboratory study of Burton and Mitchell (1987). They found that substage C_1 was longer than B whereas this study found the reverse (**Table 3.5**). However, the length of the premoult substage $D_{1.1}$ found in both studies is similar (29%: Burton and Mitchell, 1987, 32% this study). The present finding that stage B and substage $D_{1.1}$ are the longest is consistent with the fact that these also appeared to be the stages of greatest tissue accumulation.

Although certain stages or substages share common ground with other studies, the results reported here in terms of the whole moult cycle may only be applicable to this study. Differences between this and Burton and Mitchell's study may lie in the relative sizes of the study animals and in the nutritional adequacy of the respective diets rather than the different temperatures experienced, as Freeman (1991) found that temperature affected moult cycle substages in proportion to their length. The size effect is suggested by Burton and Mitchell's (1987) report of a longer intermoult period in larger animals (40 - 50 mm total length, 15-25 mm OCL). Intermoult (C_1 and C_2) took up 55.7 % of the moult cycle for those animals, compared with 19% for the smaller animals (3.1 - 19 mm OCL) in the present study. The apparent decline in mean moult stage with harvest number (i.e. mean size) in the present study suggests the possibility that intermoult lengthens with increasing size. Finally, the observed

differences also suggest that the distribution of moult substages in a given population might reflect the nutritional health of that population.

3.4.3 Tissue Accumulation and Energy Stores

The data gathered for water, ash, carbon, nitrogen, protein and chitin in this study were generally within the ranges reported in the literature. **Table 3.27** presents a summary of the data gathered in this study.

Water content is within the ranges described for other freshwater crayfish. Villarreal (1989) reported water as 73-77% wet weight for juvenile *Cherax tenuimanus*. Hubbard (*et al*, 1986) reported 76-80% for juvenile *Procambarus clarkii*. Other crustacea show similar values (i.e. 67-79%; Dupreez and McLachlan, 1983, Dall *et al* 1991, Nicol *et al* 1992).

There were few statistically significant changes in absolute water content over the moult cycle. However, changes in dry weight/organic weight suggest that some that displacement of water may have taken place. As only 15 to 25% of a yabbie's wet weight is actually tissue, relatively small changes in total water content would signal displacement by proportionally larger changes in weight of tissue. Thus although not statistically significant the fluctuations in water content may have some biological significance. This is suggested by rescaling the plot of water content against organic weight which shows that replacement of tissue by water occurred during intermoult and early premoult after uptake of water during postmoult. However, there is no consistent rise in water content during the cycle as reported by Read and Caulton (1980) for *Penaeus indicus*. They found that absolute water content increased until late premoult (D_3). The present study suggests that most water is taken up during D_4 and at the moult itself with uptake during A, B and D_3 as part of that continuum. Thus, most new shell expansion occurs at the moult rather than during postmoult stages. Uptake in late premoult serves to rupture the ecdysal sutures at the bases of the legs and to expand the thoraco-abdominal membrane which in turn ruptures just prior to ecdysis (Aiken, 1980). This study supports Passano's (1960) suggestion that increase in size at ecdysis is the result of

Table 3.27 Summary of Mean Proximate Composition for juvenile yabbies.

Component	Mean composition as a range or single value (± SE)
Water (% wet weight)	75-85
Ash (% dry weight)	20-26
Organic content (% dry weight): body	74-81
: exuvia	50.00 % (± 0.781)
Caloric Content (J/mg)	19-22
Organic Carbon (% afdw) : body	33-35
: exuvia	49.307 (± 1.376)
Chitin (%afdw) : body	9-17
: exuvia	50.489 (± 2.343)
Nitrogen (% afdw) : body	9.2-14.5
: exuvia	7.308 (± 0.314)
Protein (%afdw) : body	47-62
: exuvia	24.468 (± 1.248)

water uptake and probably Yamoaka and Scheer's (1970) contention that "tissues grow up to the volume established by this hydration". The organic weight increase during the moult cycle represents the dry component of the growing tissue. It is assumed that water taken up at the moult is progressively incorporated into cells as growth progresses.

Caloric values were generally within those reported in the literature. Woodland (1967) reported an average of 5040 cal/g afdw (21.084 J/mg afdw) in an earlier study on yabbies. Stephenson and Knight (1980) reported 5048 cal/g afdw (21.1 J/mg) for Stage 8 larvae of the prawn *Macrobrachium rosenbergii* and Nelson (1977) reported 4777 cal/g afdw (19.985 J/mg) for juveniles of the same species. Read and Caulton (1980) found a mean of 24 kJ/g afdw for immature prawns (*Penaeus indicus)*, Dupreez and McLachlan (1983) reported annual caloric data which averaged 21.181 J/mg afdw ($\pm$ 0.441, n=31) for the swimming crab *Ovalipes punctatus* and Anger (*et al*, 1983) reported a caloric content of 20 J/mg for juvenile spider crabs (*Hyas araneus*). Given this range of data, Villarreal's reported value for *C. tenuimanus* (3398.7 cal/g $\equiv$ 28.11 kJ/g with 49.42% ash) is unusually high.

Percent nitrogen is similar to that reported for the spiny lobster *Jasus lalandii* (9.9% dwt $\equiv$ 13.4 % afdw : Zoutendyk, 1988) as is caloric content (16.94 kJ/g dwt $\equiv$ 22.9 kJ/g afdw) although % organic carbon is lower (35.5 % dwt $\equiv$ 48% afdw). The conversions were done by the present author based on Zoutendyk's report that ash free dry weight was 74% of dry weight. Protein levels in this study were comparable with those for *C. tenuimanus* as Villarreal (1989) reported values of protein between 27-30% of dry weight (49-67% afdw). His ash content was higher (45-54%) than in the present study partly because of the larger sizes used ($\equiv$ 5g). Ash is also higher in juvenile *Procambarus clarkii* (Hubbard *et al* 1986) at about 30% of dry weight as is the protein level (70% of afdw), although in the latter case the authors did not subtract non-protein nitrogen when calculating protein levels from Kjeldahl-N. Protein levels reported in this study also encompass the range (50-56%) described for four marine crustaceans, including a decapod (Dall *et al*, 1991). Although Dall *et al* did not describe moult stage specific fluctuations they gave chitin levels of 26-27% which are higher than the maximum achieved by the present study (17%) but to be expected given a higher ash content (30-38% of dry weight). Finally, protein is higher than that reported for Antarctic krill *Euphausia superba* by Nicol *et al* (1992). They reported a moult stage-specific range of 31-

43% of dry weight which with a reported ash content of 8-10% gives an range of 33.7-48% protein (afdw). Chitin varied from 2-3% afdw.

This study indicates that carbon and protein levels undergo marked changes in association with the moult cycle in juvenile yabbies. These are reflected by an increase in ash free dry body weight of 2 to 2.3 times between postmoult and mid to late premoult. Except for chitin, the changes are evident as tissue expansion rather than density variation as percentage data are independent of moult stage. As percentages are also influenced by concomitant variation in water and ash content, absolute (i.e.mg) ash free dry values were considered appropriate when making comparisons between tissues. In this way variation in the component in question is not influenced by change in other tissue.

Within the moult cycle, carbon is used both as an energy source and a structural material. Crustaceans are unable to feed during late premoult and early postmoult (Read and Caulton, 1980, Nicol *et al*, 1992). In the first case this is presumably because of the transfer of nerve and muscular connections between the old and the new exoskeleton and during early postmoult the exoskeleton is too soft to allow manipulation of food. The late premoult - early postmoult period is further complicated by elevated metabolism (Passano, 1960, Vonk, 1960, Skinner, 1962, Aiken and Waddy, 1987). The present study suggests that non-chitin carbon declines in postmoult accompanied by increases in both chitin and protein. At least some of this carbon may be lipid as this material also declines immediately postmoult both to fuel growth and because it is a precursor in chitin synthesis (Chang and O'Connor, 1983, Aiken and Waddy, 1987). In premoult the process is more complicated. In yabbies over 11 mm OCL, remaining carbon increases during premoult to $D_{1.3}/D_{1.4}$ where feeding stops. However in smaller yabbies remaining carbon increases to D_3, although there is no net increase in organic content after $D_{1.3}$. There was a small but significant increase in tissue content from D_2 to D_4 which may be a reflection of this carbon increase as there was no further protein accumulation. As the increase has no apparent effect on net organic content it may be balanced by the decline in chitin content. Chitin is a acetyl-glucoseamine polymer (Meenakshi and Scheer, 1961) and it may be that glucose from the degraded chitin is responsible for the increase in carbon. The assumption is that the decline in chitin occurred as it and other elements of the old exoskeleton were reabsorbed to be used in the formation of the

new one. Gwinn and Stevenson (1973a and b) reported that over half the postmoult chitin in the freshwater crayfish *Orconectes obscurus* was reabsorbed and re-incorporated into premoult chitin (the new exoskeleton). This also suggests that some quantity was not reused. The process of degradation and re-incorporation is suggested for small yabbies in this study by the decline in chitin from $D_{1.3}$ to D_3 and the subsequent increase from D_3 to D_4. Both processes are mirrored by changes in remaining carbon. The difference in total chitin between D_4 and $D_{1.3}$ appears to represent that chitin which was not re-incorporated into the new exoskeleton prior to the moult. The effect may be less evident in larger animals because the proportion of chitin declines relative to stored carbon as a result of the progressive decrease in contribution of the exoskeleton to body weight.

Lipid is the major energy store in many crustaceans (Chang and O'Connor, 1983), including the freshwater crayfish *Orconectes limosus* (Collatz, 1969 - in Armitage *et al*, 1972), *O. nais* (Armitage *et al*, 1972) and *C. tenuimanus* (Villarreal, 1989). There are exceptions as Barclay *et al* (1983) found that protein not lipid was the primary energy source during starvation in the prawn *Penaeus esculentus*. Villarreal (1989) reported the reverse of this in similar experiments on *C. tenuimanus*. The role of lipid in *C. destructor* is still open to debate although this study suggests that it may be important during postmoult. It is probable that lipid plays a similar role to that in described for other freshwater crayfish. However, it is also suggested that protein, not lipid, is catabolised at the moult itself for crayfish larger than 11 mm OCL. Most of the carbon lost at the moult is shed with the exuvia which may or may not be reingested by the postmoult animal. When this is subtracted from the total carbon losses at the moult (D_4 to $A_{2'}$) there is little remainder. Protein losses are much greater as between 47 and 48% of the protein accumulated between moults is lost at the moult. It appears that carbon not lost with the exuvia is conserved for the metabolic needs of growth during the cycle, although in smaller animals some carbon is lost in addition to that with the exuvia. This is probably the result of increasing metabolic requirements with declining size. It may be that protein catabolism alone is not sufficient to supply energy needs. It is not suggested that all protein catabolised goes to supplying metabolic requirements, as part of the reduction in protein may be the result of chela muscle atrophy during D_4 (Mykles and Skinner, 1985). They suggested this was necessary to allow the withdrawl of the chela muscle from the claw

through the narrow basi-ischial joint. The remaining protein would presumably be used to fuel the moulting process itself as there appears to be no other available energy source.

Chapter 4.

Temperature, Size and Growth

4.1 Introduction

Temperature is the major extrinsic factor affecting growth in crustacea (Hartnoll, 1982, Lowery, 1988), although its contribution to growth patterns is usually influenced by synergism with population density and food quality and supply (Holdich and Lowery, 1988). Growth is usually proportional to temperature between a zero growth point and an optimum which generally occurs near the upper end of the range normally experienced within the habitat of a species (i.e. Mason, 1979; Mills, 1986 ; Morrissy, 1990). For example, Mason (1979) found that optimum biomass accumulation in *Pacifastacus lenuisculus* occurred at 18^{o}C, Morrissy (1990) suggested 24^{o}C for *C. tenuimanus* and 28^{o}C was reported for *C. destructor* (Mills, 1986). Apart from such work, temperature's effect is also described in terms of its extremes, above or below which growth declines or ceases. The low temperature limit to growth in freshwater crayfish is species and acclimation-temperature specific but usually lies around the 10^{o}C mark (Aiken and Waddy, 1987). Studies on *Orconectes virilis* (Momot, 1984) and *Austropotamobius pallipes* (Pratten, 1980) suggest limits of about 10^{o}C while juvenile *Cherax tenuimanus* stop growing between 11 and 13^{o}C (Morrissy, 1990) and Mills (1986) found that growth in *C. destructor* did not occur below 15^{o}C.

This chapter investigates the influence of temperature and size on length increase and tissue growth in the laboratory. These experiments will allow more accurate estimation of size-specific energetic requirements and expenditure in the laboratory and will assist in the explanation of observed patterns of field growth (Chapter 3).

Growth in tissue or organic content will be measured as ash free dry weight. Temperature rather than photoperiod was investigated here as the first has a major effect on growth (Hartnoll, 1982) whereas the second may only be important in the seasonal sense, acting as a zeitgeber (Armitage *et al*, 1972, Rice and Armitage, 1974).

Growth in length will be described in terms of moult increment and intermoult period. Yabbies were grown at five temperatures, three of which (15,20 and 25°C) covered the range experienced in the field. An additional two temperatures (27.5 and 30°C) were added to permit comparison with existing models (Mills, 1986). A growth rate model (growth rate vs size and temperature) will be derived to allow comparison of growth rate with metabolic rate data collected under the same conditions (see Chapter 5).

Four questions were asked in the present experiments:

1. How do size and temperature affect the components of growth, moult increment and intermoult period, and to what extent?

2. How does size affect instantaneous growth rate?

3. How does temperature affect instantaneous growth rate?

4. Can instantaneous growth be adequately described and predicted by size and temperature?

4.2 Materials and Methods:

a) General

Yabbies were collected from a local dam at the end of winter when the water temperature was about 10°C. From this population two size groups of individuals were selected for the growth experiments, one approximately 6mm -7mm OCL and the other between 11 and 13 mm OCL (**Table 4.1**). The sizes were chosen to provide growth data for the ranges 100-1000 mg and 1000 to 5000 mg wet weight. All animals were acclimated to 15°C and 12:12 Light:Dark for two weeks. Five groups of about forty crayfish, made up of twenty animals from each size group and ranging in moult stage from C_1 to $D_{1.1}$, were randomly selected and placed in a temperature controlled room designated to be 15, 20, 25, 27.5 or 30°C (12:12 LD). The rooms were then raised to the appropriate experimental temperature (from 15°C) at a rate of five degrees every three days, a rate of temperature variation which may be experienced in

the field (pers obs). Once the experimental temperature was established yabbies were measured (to 0.1mm), weighed (to 0.1 mg) and moult staged.

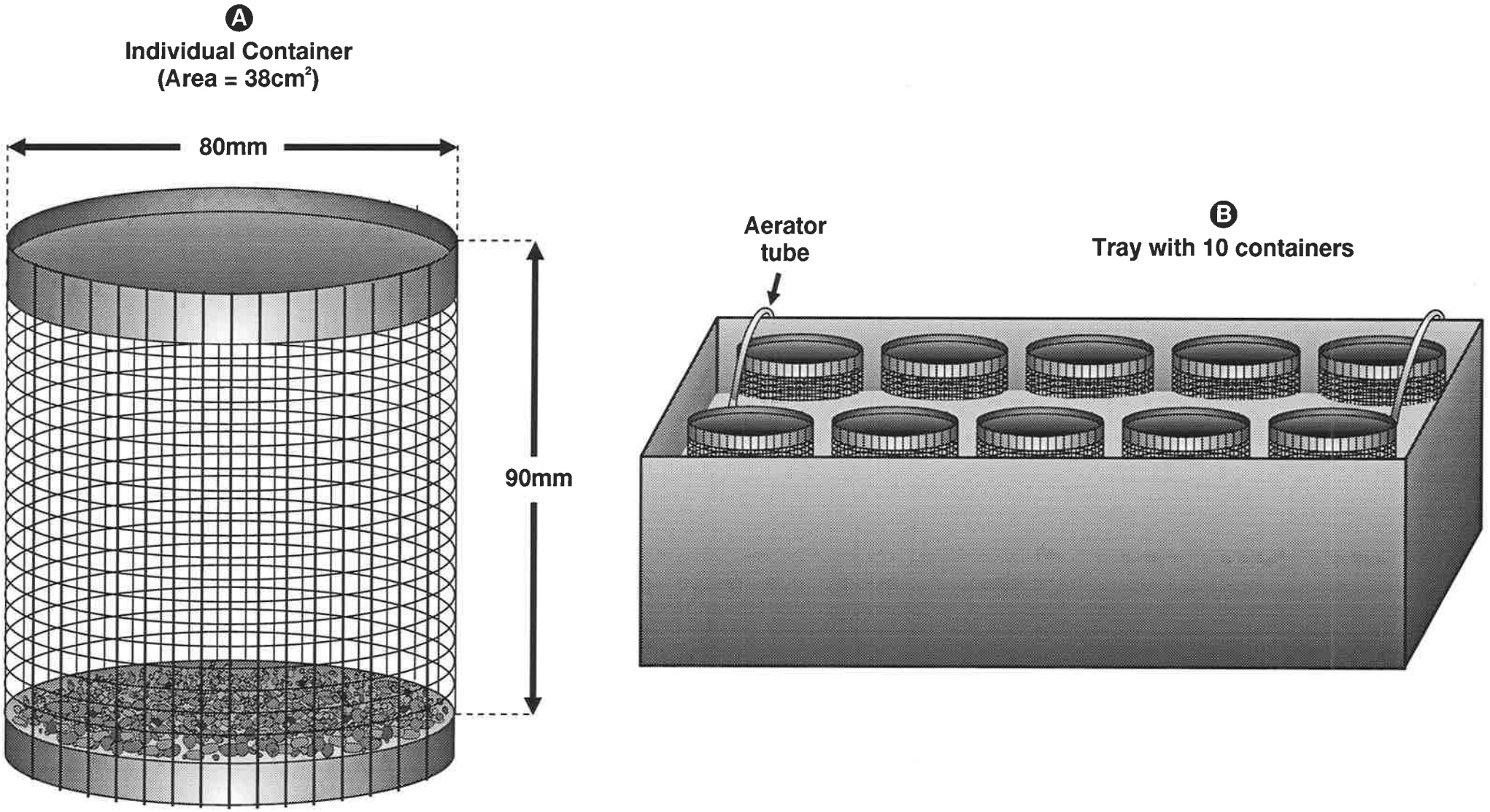
Table 4.1 Mean initial OCL (mm) , Wet Weight (mg) and Ash Free Dry Weight (mg) for yabbies in two size groups at five temperatures (T°C). Standard errors of the means are in brackets.

T°C	OCL (mm)	WetWeight (mg)
Group 1		
15	6.8 (0.16)	160.5 (10.93)
20	7.1 (0.10)	200.3 (8.13)
25	6.5 (0.10)	155.1 (6.54)
27.5	6.8 (0.07)	158.7 (5.43)
30	6.5 (0.09)	146.5 (5.75)
Group 2		
15	13.2 (0.04)	1221.0 (24.56)
20	12.63 (0.27)	1201.5 (69.4)
25	11.09 (0.22)	811.8 (44.85)
27.5	12.12 (0.74)	849.9 (78.60)
30	11.94 (0.23)	798.8 (49.80)

Yabbies were fed *ad libitum* every second day on a combination of filamentous algae (*Cladophora*) and the 35% protein diet used in the laboratory diet study (Chapter 2) and in the field study (Chapter 3). The same diet had been fed to animals in the field study. Excess food was removed at the same time. Within each room subgroups of twelve animals were randomly assigned to rectangular plastic trays (15cm x 35cm x 55cm) filled with dechlorinated tapwater. Animals were housed individually within the trays in cylindrical plastic mesh containers with solid bases (**Fig. 4.1**) as described by Geddes *et al* 1988. Each tray was continually and vigorously aerated using airstones and the water was replaced every second day with clean water at the same temperature. Oxygen levels were monitored regularly and remained above 75% saturation at all temperatures.

Growth was monitored by checking for the presence of moults each morning and by measurement of OCL and wet weight and determination of moult stage (Burton and Mitchell, 1984) at 10 to 14 day intervals. The experiment was terminated after three to four moults of each size group within each treatment. The 15°C treatment had to be curtailed after three moults because growth was so slow. The maximum size reached within each treatment was

Fig. 4.1
Equipment for Growth Experiment



about 20mm OCL which was approximately the maximum size reached in the field experiment (Chapter 3). At the end of the experiment animals were weighed, moult staged (Burton and Mitchell, 1987) then killed by freezing at -20°C overnight. They were then dried to constant weight in an oven at 60°C, cooled overnight in a desiccator containing silica gel and reweighed. Organic content was determined by subtraction after ashing to constant weight (500°C, 24 h) and cooling overnight in a desiccator. The relationship between wet weight and ash free dry weight was used to derive organic content from wet weight data gathered at each sampling interval.

b) Data analysis

Within-treatments relationships between premoult OCL and Log intermoult period were established using geometric mean regression (Ricker,1973, Sokal and Rohlf, 1981). Differences between treatments were then tested using ANCOVA. To investigate the relationship between moult increment and size, % moult increment vs premoult OCL was studied. This description of moult increment is recommended by Botsford (1985) in preference to the relationship between postmoult and premoult size (Hiatt, 1948, Mauchline, 1977) as it improves resolution when observing changes with size. Futhermore Hiatt`s method introduces a high degree of autocorrelation (Geddes, 1988) as post moult size consists mainly of premoult size. Before analysis all data were tested for normality and transformations made if required.

Individual temperature-specific instantaneous growth rates (mg/mg ash free dry weight/ day) were calculated using a method suggested by Pockl (1992). Weight data was log transformed and plotted against time. The equation was of the form :

$$\text{Ln } W = \text{Ln } a + bt \qquad \text{... } \textbf{Equation 4.1}$$

where W = ash free dry weight (mg) and t = time (days). The slope (b) gives the 'average' instantaneous growth rate (IG) of an individual over the time period. The equation assumes an exponential increase in weight with time. Such an increase was apparent at all temperatures (see 4.3). Several values of weight at time during the growth of an individual were used to derive the regression coefficients a and b.

Changes in IG with size within treatments were investigated using repeated measures ANCOVA. Temperature-specific growth rates were compared with ANOVA (Sokal and Rohlf, 1981).

A model was then developed to estimate the relationship between growth rate and size at the different temperatures. IG for this model was estimated (Pratten, 1980, Chapter 2) as follows:

$IG = Ln(W_t/W_o) /t$ **Equation 4.2**

where W_t = postmoult OCL as ash free dry weight

W_o = premoult " " " " " "

t= intermoult period

Finally, a multiple regression model was derived describing growth in terms of weight and temperature.

4.3 Results

Survival ranged between 33 and 96% with a mean of 70.4 (± 5.1) (**Table 4.2**). Most of the mortality was caused by localised failures in the aeration system.

Table 4.2 Survival within groups and temperatures. T°C = temperature, Group1 = 5-7 mm OCL, Group 2= 11-13 mmOCL

T°C	Group	Initial number	Final number	% Survival
15	1	18	13	72
	2	18	13	72
20	1	25	24	96
	2	25	15	52
25	1	21	15	71
	2	25	19	76
27.5	1	25	20	80
	2	15	5	33
30	1	25	19	76
	2	15	10	67
				Mean: 70.4 ± 5.1

4.3.1. The Components of Growth

Moult increment (MI) data were expressed as a percentage of the premoult carapace length (%MI). The data for each size group was then plotted against premoult OCL to investigate the effect of size within temperature (**Fig 4.2a to e**). One randomly selected %MI and its corresponding premoult OCL was plotted per individual for each temperature. The figures indicate that there is no change in %MI with increasing size. The slope of the GM regression describing this relationship at each temperature was not significantly different from zero ($P > 0.05$) in all cases.

The size effect was also investigated by looking at the % moult increment for successive moults, indicated as moult number. **Figures 4.3a to e** show means and ranges for % MI plotted against moult number within each temperature. The ranges and outliers are provided by box and whisker plots (McGill, *et al* 1978). In the figures, outliers are indicated by circles and asterisks. These figures suggest that although moult increment did not change with moult number for the first four temperatures there is one significant difference in comparing the moult increments at 30°C.

Fig 4.2a
% Moult Increment vs Premoult OCL at 15°C

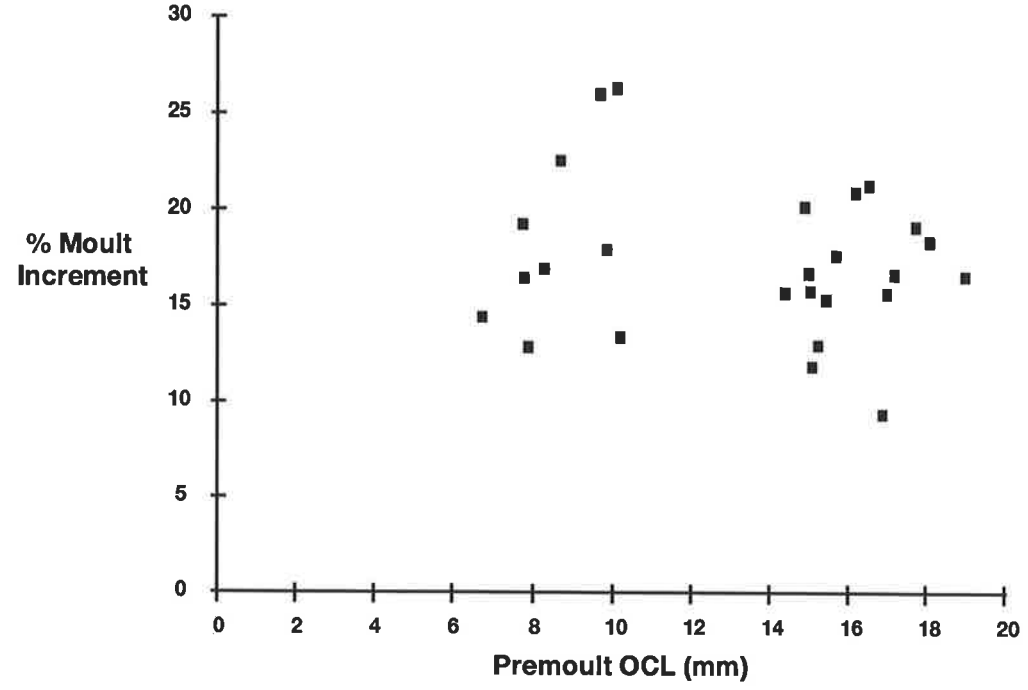


Fig 4.2b
% Moult Increment vs Premoult OCL at 20°C

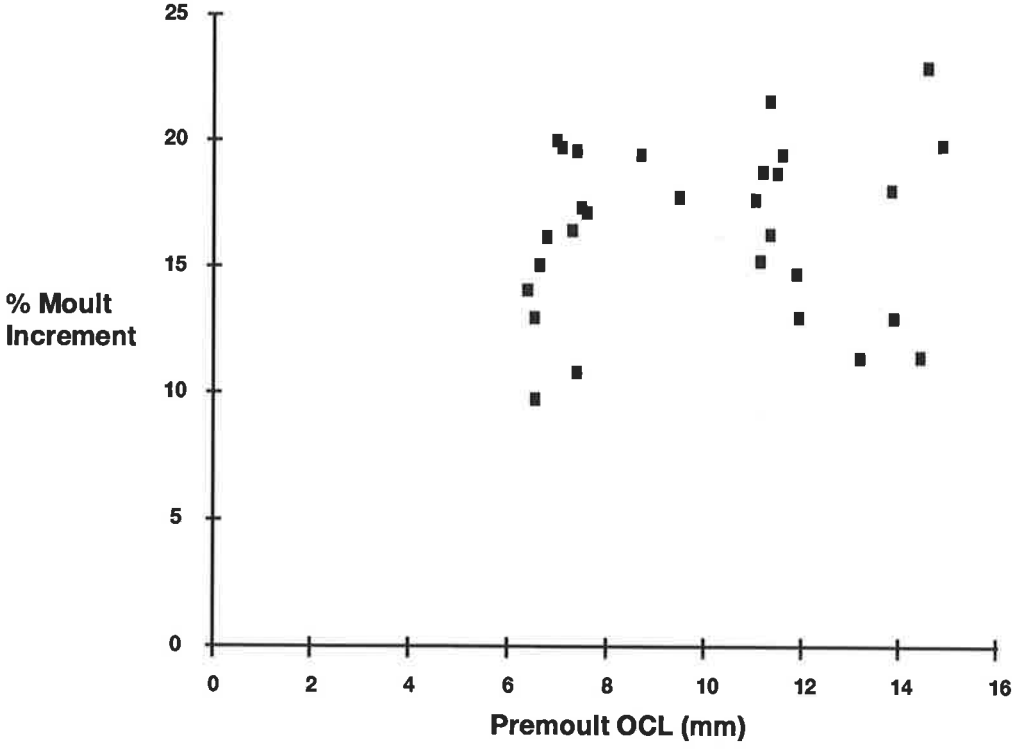


Fig 4.2c
% Moult Increment vs Premoult OCL (mm) at 25°C

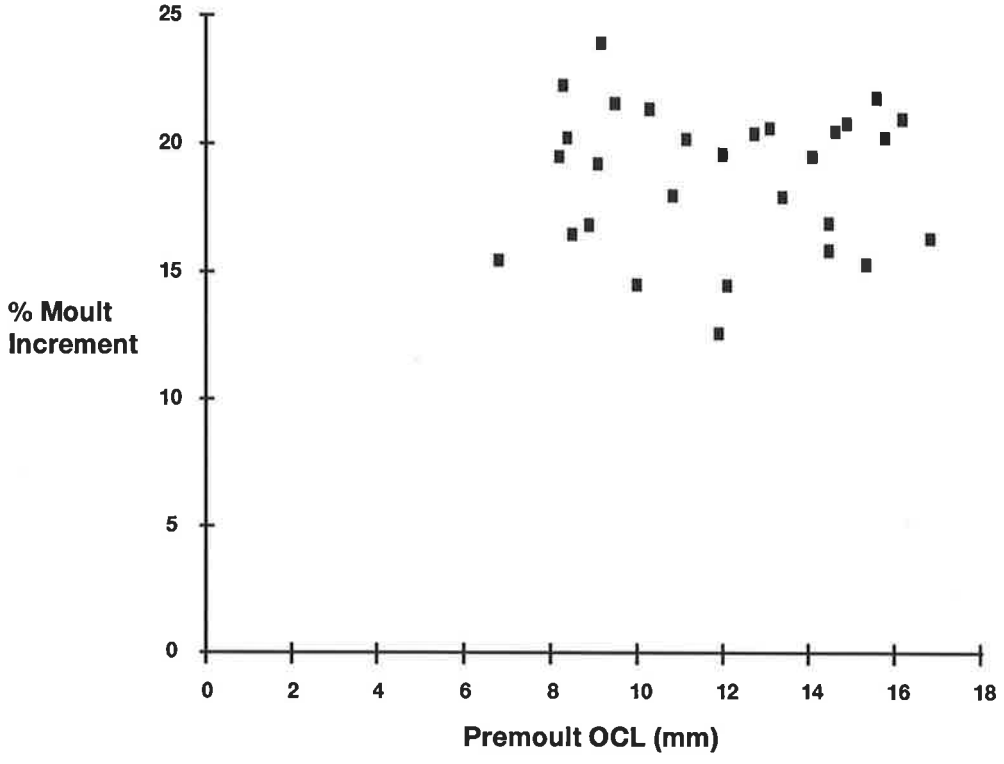


Fig 4.2d
% Moult Increment vs Premoult OCL (mm) at 27.5°C

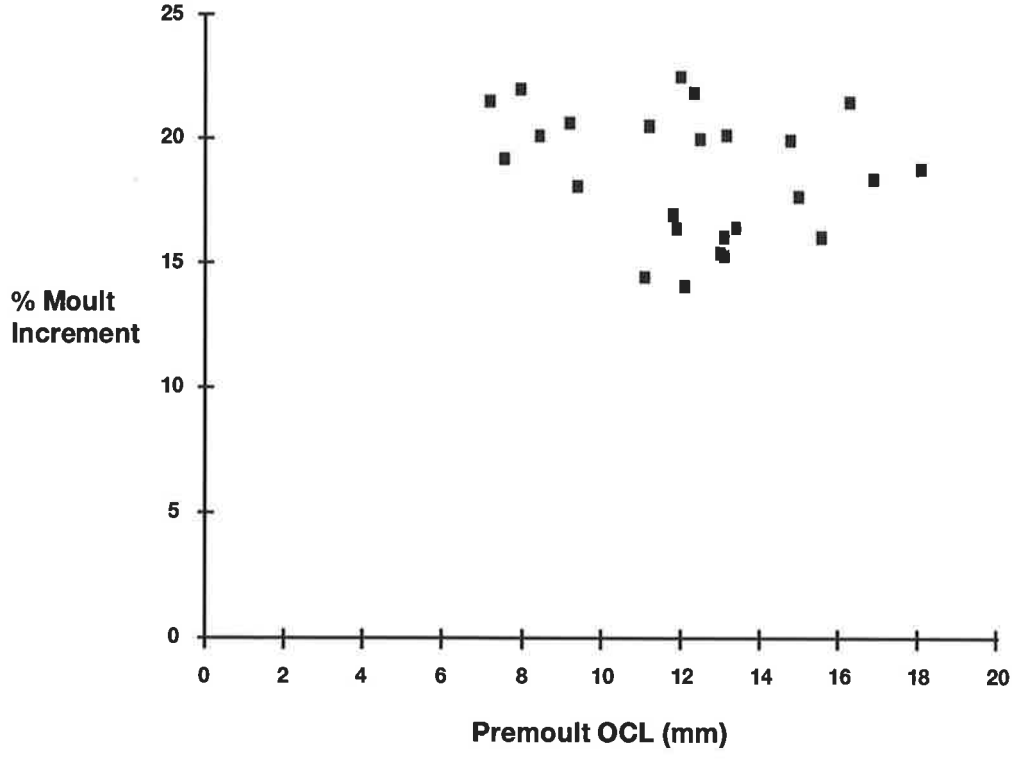


Fig 4.2e
% Moult Increment vs OCL (mm) at 30°C

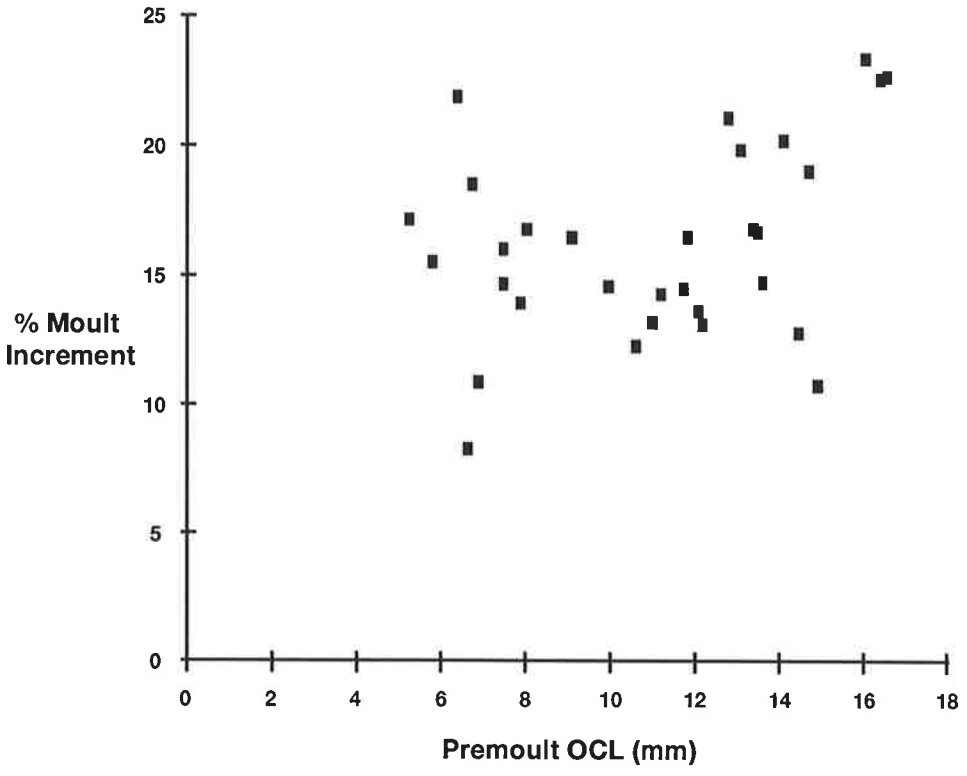


Fig 4.3a
% Moult Increment vs Moult Number at 15°C

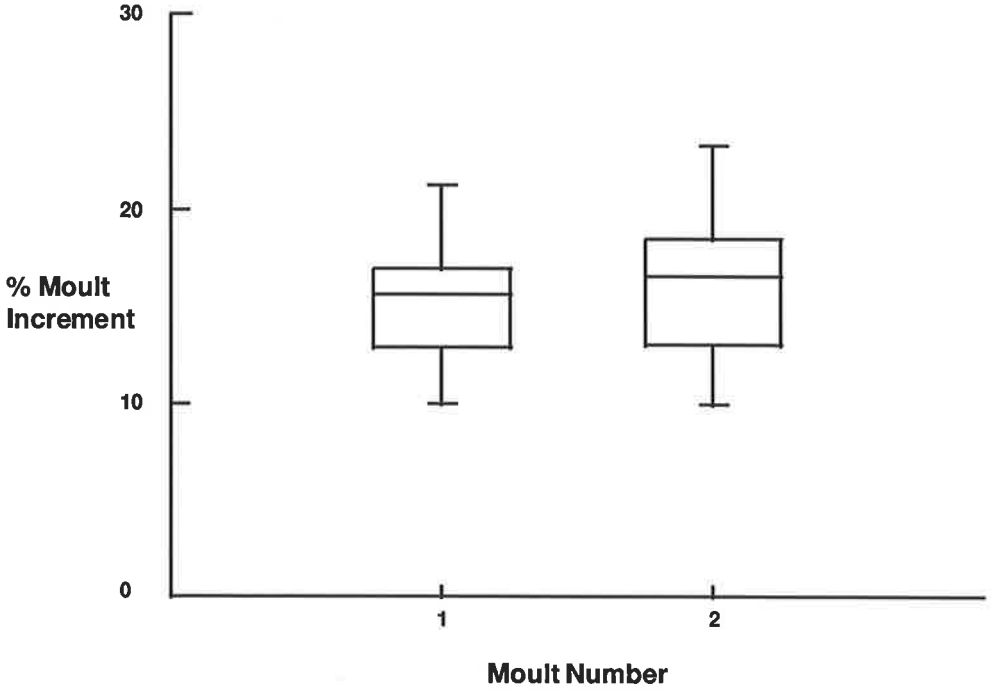


Fig 4.3b
% Moult Increment vs Moult Number at 20°C

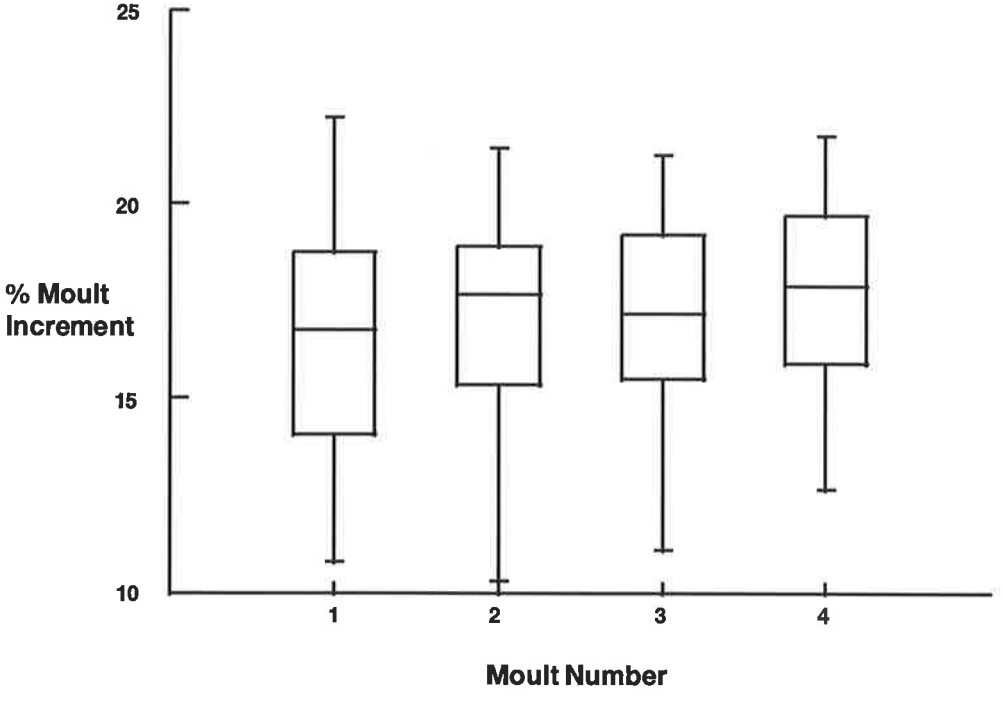


Fig 4.3c
% Moult Increment vs Moult Number at 25°C

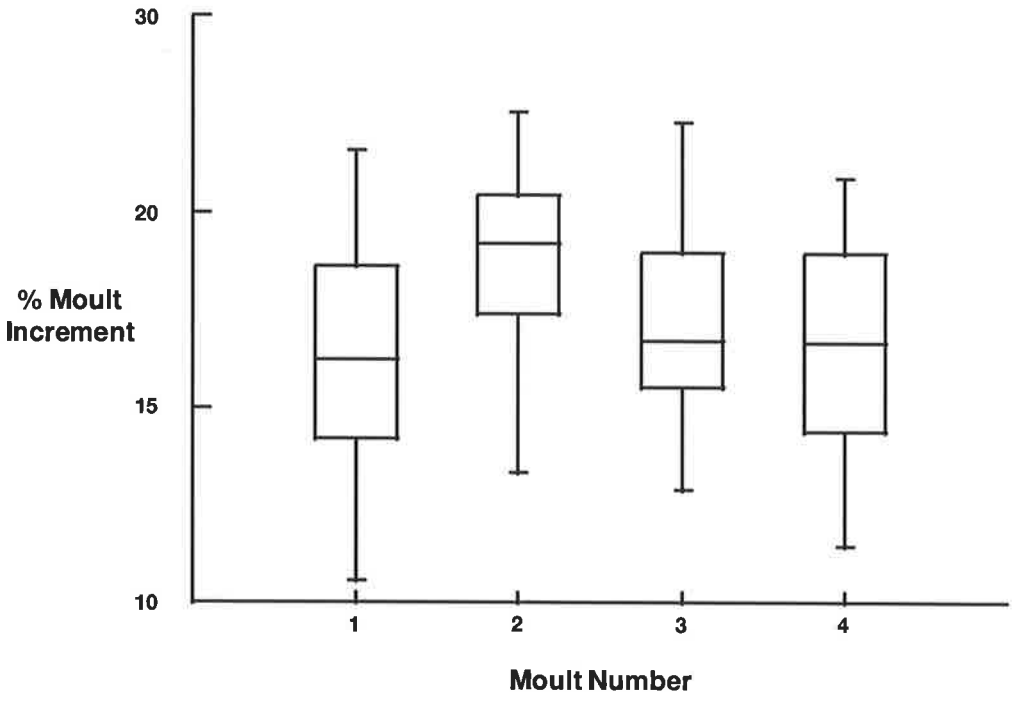


Fig 4.3d
% Moult Increment vs Moult Number at 27.5 °C

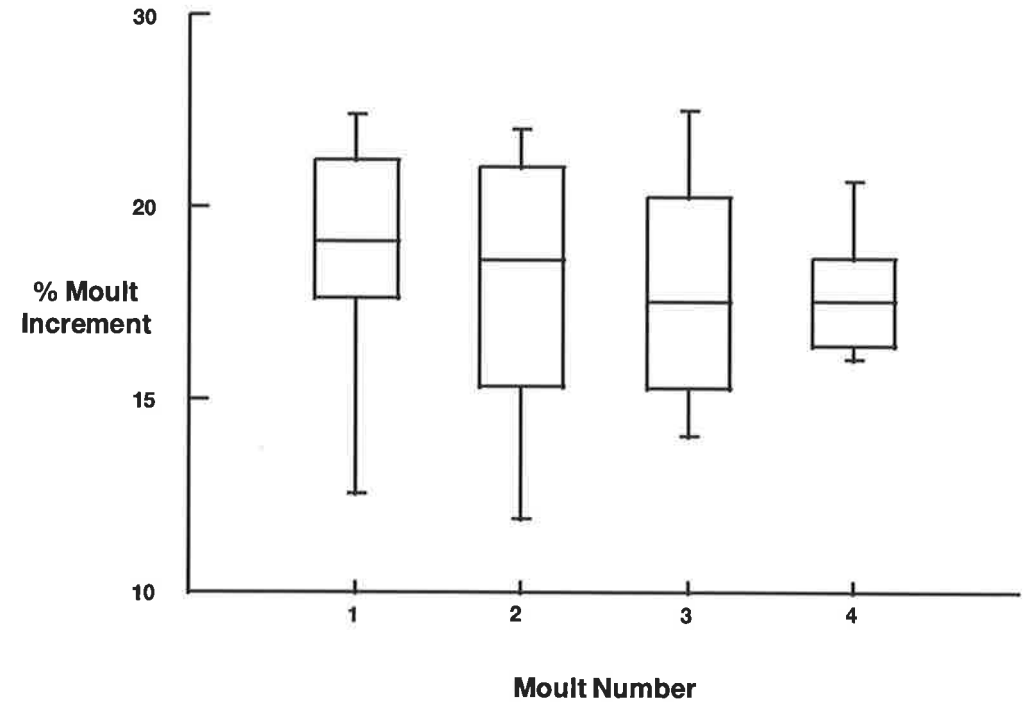
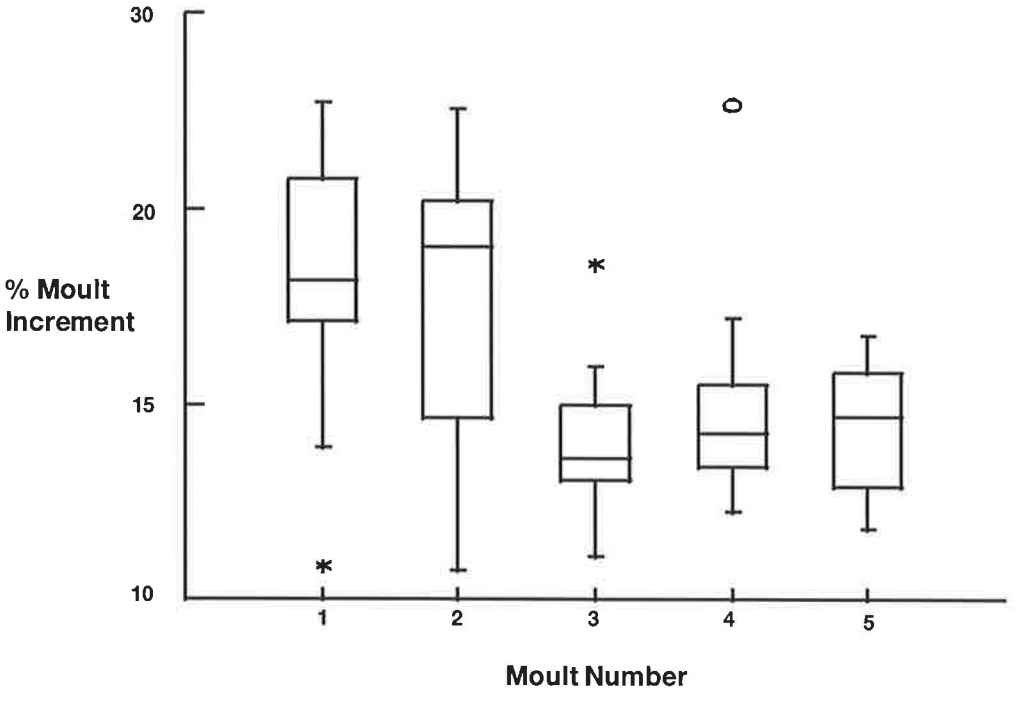


Fig 4.3e
% Moult Increment vs Moult Number at 30°C



As %MI showed homogeneity of variances between moults within temperatures from 15 to 27°C (Fmax, P>0.05), the data were combined within each of these temperatures and means and standard errors derived (**Table 4.3**).

Table 4.3 Mean % Moult Increment (+ SE) at four temperatures (T°C)

T(°C)	n	%MI	SE
15	43	15.57	0.511
20	97	16.96	0.287
25	99	17.15	0.277
27.5	57	18.22	0.363
20+25	196	17.094	0.201

The lack of homogeneity within the 30°C treatment precluded any such pooling. This treatment also showed a progressive decline in %MI with moult number (**Fig 4.3e**). Percent MI data for the first four temperatures were normalised with an arcsin transformation (Sokal and Rohlf, 1981) prior to analysis using ANOVA on Systat. The ANOVA table showed that temperature had a significant effect on %MI (P<0.001, **Table 4.4**).

Table 4.4 Comparisons between % Moult Increment at four Temperatures (T°C) using ANOVA. n=299,T (oC) = Temperature, P= Significance level

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	P
T°C	0.045	3	0.015	9.764	<0.001
Error	0.454	295	0.002		
Comparisons	**F**	**P**			
15°C,20°C	7.435	0.007			
20,25	0.217	ns			
25,27.5	9.388	0.002			
20,27.5	11.956	0.001			

The same table shows the contribution of individual temperatures. Thus mean % moult increment was proportional to temperature between 15 and 20°C and between 25 and 27.5°C. There was no significant effect between 20 and 25°C.

The relationship between intermoult period and premoult OCL was then investigated. Intermoult period was logged and plotted against Premoult OCL (Mauchline, 1976; Pratten, 1980; Hartnoll, 1983; Mohamedeen and Hartnoll,1989). **Figure 4.4** shows this relationship for all five temperatures together with the appropriate GM regressions, the regression statistics are given in **Table 4.5**.

Table 4.5 Log Intermoult period (days) vs Premoult OCL (mm) at five temperatures (T°C): Regression statistics. All regressions were significant at the 0.001 level (ANOVAR, F test, Sokal and Rohlf, 1981).

T°C	n	Slope (b)	SE(b)	Intercept (a)	SE(a)	r^2
15	13	0.0223	0.0044	1.2475	0.0587	0.5634
20	23	0.0472	0.0045	0.7474	0.0554	0.8063
25	17	0.0515	0.0082	0.5404	0.0944	0.6242
27.5	12	0.0374	0.0056	0.6128	0.0742	0.7770
30	9	0.036	0.0056	0.5702	0.0759	0.8276

All regressions were significant at the $P<0.001$ level. The data relate to one intermoult period for each animal at each temperature. The number of points is because only those animals that moulted at least twice and whose intermoult period was positively known to the day were used. Comparisons between lines made with ANCOVA, (**Table 4.6**) suggest that intermoult period is inversely proportional to temperature in the range experienced in the field study. There was also a significant increase in slope (b) between 15 and 20°C. Analysis of pooled regressions from 20 to 30°C inclusive suggested no further significant change in slope so the pooled slope derived for this interval by the ANCOVA ($b = 0.0393 \pm 0.0030$) may be used to describe the relationship.

Fig 4.4
Log Intermoult Period vs Premoult OCL (mm)
(GM regression lines shown for the
relationship at each temperature)

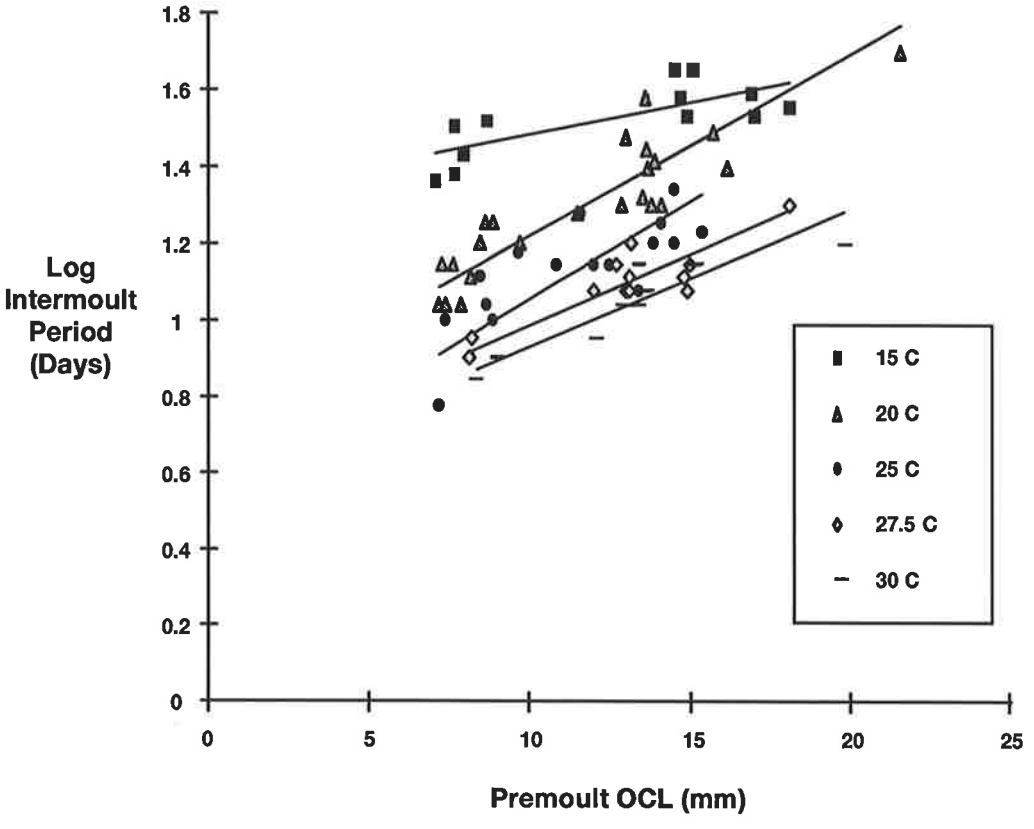


Table 4.6 Log Intermoult period (days) vs Premoult OCL (mm) at five temperatures (T°C): Comparisons between regression lines (ANCOVA, F - test, Sokal and Rohlf, 1981). df= degrees of Freedom. ** = P<0.025, *** = P<0.001

Compared T°C	Slopes (b) F statistic	df	Intercepts (a) F statistic	df
15,20	14.7367***	1,32	42.8242***	1,35
20,25	0.0036	1,36	36.7498***	1,39
20,27.5	1.1565	1,31	96.4215***	1,34
25,27.5	0.5040	1,25	8.2409***	1,28
25,30	0.5080	1,22	18.6283***	1,25
27.5,30	0.0009	1,17	7.4753**	1,20
All five	4.0925***	4,64	81.2158***	4,70
Four (20 to 30 C incl.)	0.6090	3,53	51.7010***	3,58

4.3.2. Growth rates

Ash free dry weight, tissue weight, was estimated from wet weights. The data on wet weight versus ash free dry weight were analysed for size, moult stage and temperature effects. There was no significant relationship between wet weight and % organic content (GM regression, P>0.05), but ANOVA carried out on the transformed data (Box-Cox transformation: Sokal and Rohlf, 1981) showed moult stage and temperature effects as well as interaction (**Table 4.7a**). Examination of the data (**Fig 4.5a**) and that for the proportional contribution of dry weight to wet weight (**Fig 4.5b**, **Table 4.7b**) and organic content to dry weight (**Fig 4.5c, Table 4.7c**) suggested that this effect was the result of temperature-specific changes in water content in the first two moult stages. The sample sizes for these moult stages were low, generally less than 4. Most animals were at moult stages C_1 to D_3 when weight data was collected and re-examination of the proportional contribution of organic content to wet weight (**Fig 4.5a**) using data from stages C_1 to D_3 showed neither temperature nor moult stage effects (**Table 4.8**). The mean across these moult stages and temperatures was 20.416% ($\pm$ 0.2637%; n=149) and this value was used to convert wet weight to organic content at each sampling interval. Data for moult stages A and B was combined and the data converted using the temperature specific mean % organic content (last column, **Table 4.9**) for

Table 4.7 ANOVA tables for moult stage and temperature effects on a) Organic content as a proportion of wet weight, b) Dry weight as a proportion of wet weight and c) Organic content as a proportion of dry weight. All moult stages used. T°C = temperature, Moult = moult stage, P= probability level.

a) Organic content as a proportion of wet weight (n=147)

Source	Degrees of Freedom	F	P
T°C	4	8.2562	< 0.001
Moult	1	19.8309	< 0.001
T°C x Moult	4	4.6971	< 0.001

b) Dry weight as a proportion of wet weight (n=147)

Source	Degrees of Freedom	F	P
T°C	4	10.0900	< 0.001
Moult	1	28.1767	< 0.001
T°C x Moult	4	7.1547	< 0.001

c) Organic content as a proportion of dry weight (n=141)

Source	Degrees of Freedom	F	P
T°C	4	2.0185	ns
Moult	1	2.9412	ns
T°C x Moult	4	0.3819	ns

Table 4.8 ANOVA table for moult stage and temperature effects on organic content as a proportion of wet weight. Moult stages A and B not used. T°C = temperature, Moult = moult stage, P = probability level, ns = non significant, n =121.

Source	Degrees of Freedom	F	P
T°C	4	0.8544	ns
Moult	1	1.1185	ns
T°C x Moult	4	0.3395	ns

Fig 4.5a
Organic Content as a percentage of Wet Weight (mg) vs Moult Stage and Temperature (°C)

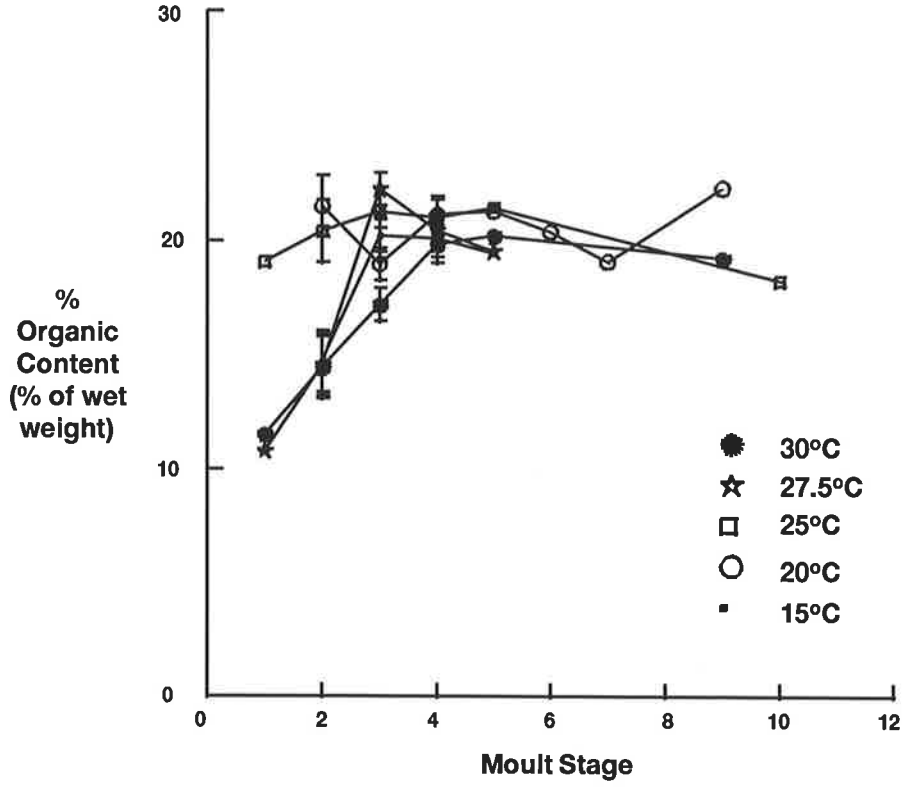


Fig 4.5b
Dry Weight as a percentage of Wet Weight (mg) vs Moult Stage and Temperature (°C)

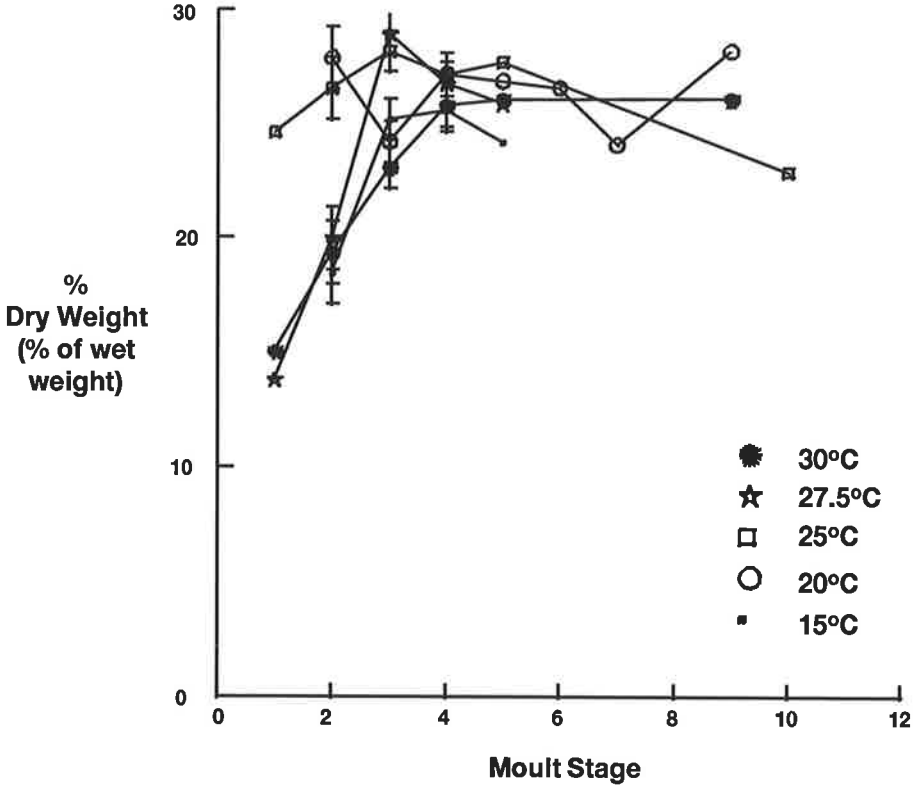
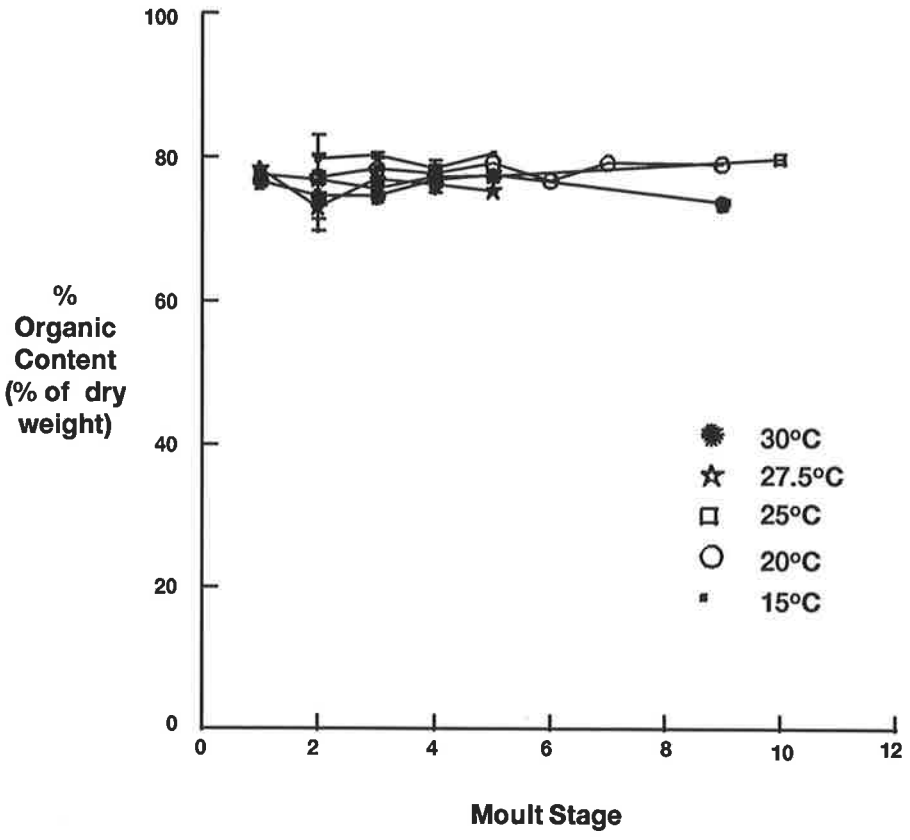


Fig 4.5c
Organic Content as a percentage of Dry Weight (mg) vs Moult Stage and Temperature (°C)



25, 27.5 and 30°C. The mean described above (20.416%) was used to convert wet weights for animals at stages A and B in the 15 and 20°C treatments.

Table 4.9 Mean organic content (± SE) as a proportion of wet weight for moult stages A and B at each temperature. n = number of animals for a given moult stage and temperature.

Temperature (oC)	Moult stage A	n (A)	Moult Stage B	n (B)	Pooled mean (A + B)
15	-	0	14.743 (0.084)	3	-
20	-	0	21.500	2	-
25	19.091	2	20.419 (0.586)	3	19.887 (0.508)
27.5	10.812	2	14.587 (0.499)	9	13.901 (0.613)
30	11.539	2	14.471 (1.347)	3	13.298 (1.089)

Instantaneous growth rates were then calculated from the organic content data. Coefficients of determination for ash free dry weight vs time regressions for the individual crayfish varied between 0.9 and 0.999. Growth was exponential at all temperatures, as illustrated by **Fig 4.6** which shows typical growth patterns of representative individuals. There was no significant relationship between initial weight and growth rate within each group at each temperature ($b = 0$, $P > 0.05$) which suggests that the slight differences in initial weight between temperatures had little effect on growth rates calculated for the whole period. Growth rates were pooled and group-specific mean instantaneous growth rates calculated (**Table 4.10**) for each temperature. This table suggests that the optimal growth temperature for the smaller crayfish was about 25°C whereas that for the larger animals was about 27.5°C. Mean IG was the same for both groups at 15°C suggesting that growth rate was independent of size at this temperature.

Fig 4.6
Typical Individual Growth Patterns at Five Temperatures (AFDW (mg) vs Time (days))

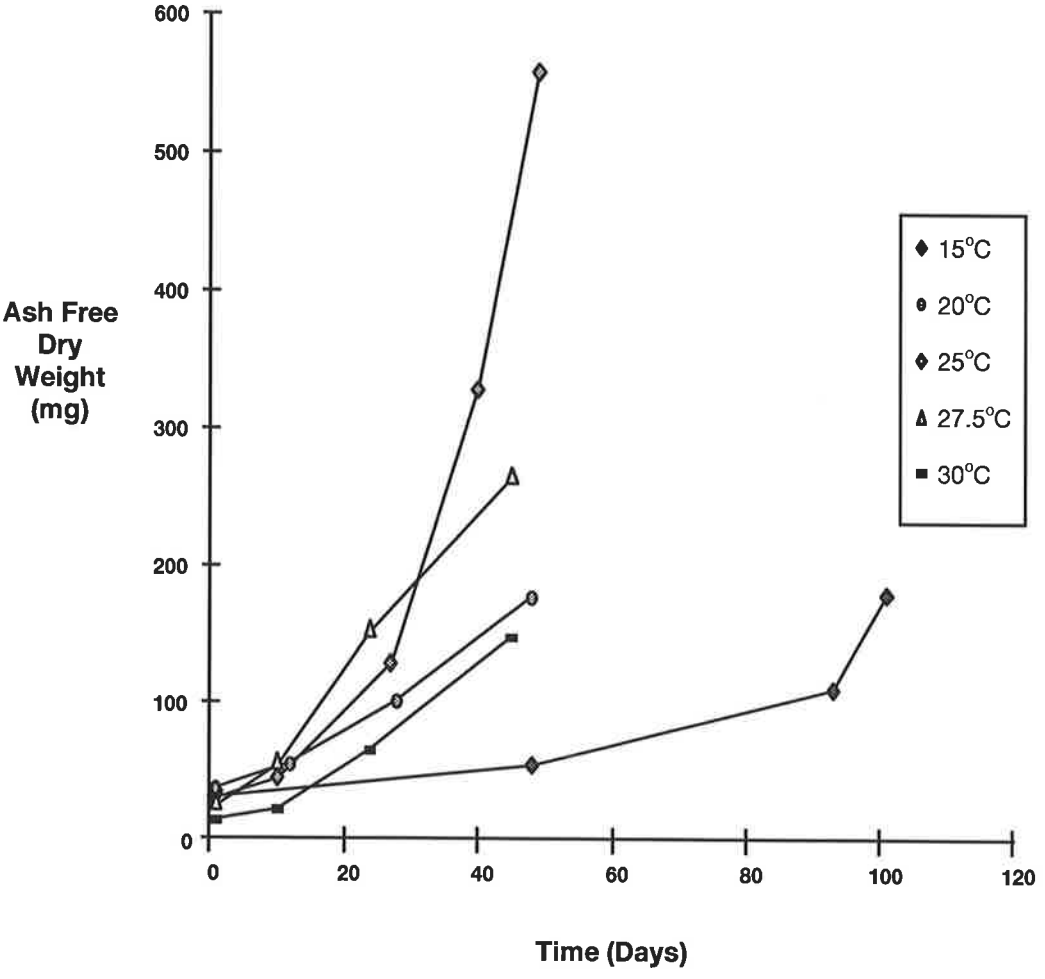


Table 4.10 Instantaneous growth (IG) rates of Group 1 and Group 2 yabbies at five temperatures (T°C). Growth rates are in mg/mg ash free dry weight/day.

T(°C)	n	Size	IG	SE
15	13	1	0.0103	0.0014
	13	2	0.0103	0.0012
20	24	1	0.0344	0.0014
	15	2	0.0214	0.0017
25	15	1	0.049	0.0024
	19	2	0.041	0.0024
27.5	20	1	0.046	0.0015
	5	2	0.043	0.0038
30	19	1	0.0419	0.0016
	10	2	0.036	0.0022

These points are illustrated by the mean IG (± SE) and spline curves for each size group shown in **Fig 4.7.** Data were normalised using a Box-Cox transformation (Sokal and Rohlf, 1981) and the effect of temperature and size on growth tested using ANOVA **(Table 4.11).**

Table 4.11 Analysis of the effect of Temperature and Size Group on Growth Rate using ANOVA. 'Size' = initial size groups (ref **Table 4.1** and text). n=160, P= Significance level, T °C = Temperature

Source	Degrees of Freedom	F_{calc}	P
T°C	4	106.525	<0.001
Size	1	21.510	<0.001
T°C x Size	4	3.515	0.009

The factor 'size' refers to the two initial groups. Both temperature and size had highly significant effects on IG and there was a significant interaction between temperature and size group. The size groups were then analysed separately as shown by the ANOVA tables in **Table 4.12**. *Post hoc* comparisons between the temperatures within size group 1 showed that growth peaked between 25°C and 27.5°C. Growth at 25°C was not significantly greater than that at 27.5°C (P>0.05) although it fell significantly between 25 and 30°C. Growth within size group 2 also appears to have peaked later than that of group 1 (**Fig 4.7**) but there were no significant differences between growth rates at 25, 27.5 or 30°C. Comparisons between size

Table 4.12 Analysis of the effect of Temperature and Initial Size (mg afdw) on IG and comparisons between growth rates at each temperature. Data transformed using Box Cox (Sokal and Rohlf, 1981). df = degrees of freedom.

a) Size Group 1 n = 91

Source	df	F	P
T°C	4	5.7617	<0.001
Weight	1	1.4801	ns
T°C x Weight	4	1.4574	ns

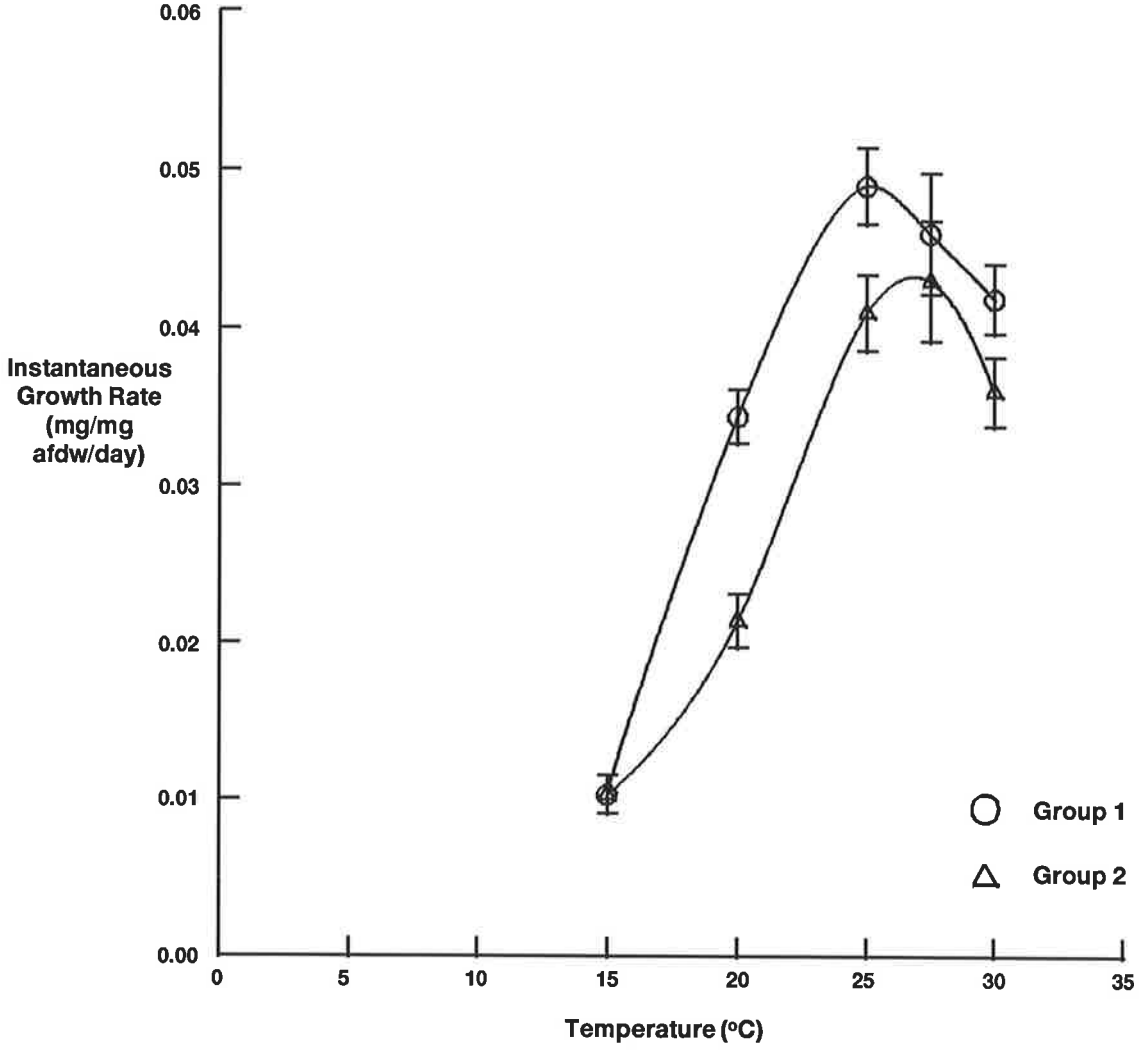
Comparisons	F	P
15°C,20°C	64.3797	<0.001
20,25	36.1189	<0.001
25,27.5	1.5860	ns
27.5,30	3.1441	ns
25,30	8.3523	0.005

b) Size Group 2 n = 63

Source	df	F	P
T°C	4	3.4225	0.0147
Weight	1	0.6569	ns
T°C x Weight	4	1.257	ns
Error	52		

Comparisons	F	P
15°C,20°C	8.0746	0.006
20,25	62.9728	<0.001
25,27.5	0.2718	ns
27.5,30	2.4925	ns
25,30	2.3800	ns

Fig 4.7
Instantaneous Growth Rate (mg/mg afdw/day) (± SE)
vs Temperature (°C) for two size groups
(ref text and Tables 4.1 and 4.10)



groups within temperatures (**Table 4.13**) showed that growth in group 1 was significantly greater than that of group 2 at 20 and 25°C.

Table 4.13 Comparisons of Growth Rates between Size Groups within Temperatures using ANOVA. Each F value represents the comparison between groups 1 and 2 at each temperature.

Temperature	F (calc)	Significance level
15	0.0002	ns
20	47.8129	<0.001
25	5.126	0.031
27.5	0.9193	ns
30	3.9779	ns

The lack of significant difference at 27.5°C suggests that growth of group 1 peaked before that of group 2 and that larger animals had a broader range of temperatures giving near optimum growth. Extrapolation to the X axis to lower temperatures suggests that growth would stop at about 13°C.

Changes in growth with time and size within treatments were then investigated. The growth periods for the first four temperatures were each subdivided into two or three subperiods on the basis of one moult per animal per growth period. This was done because growth rate changes over the moult cycle (Aiken and Waddy, 1987) thus it is important to include all moult stages in a growth rate estimate.

Growth rates were calculated for each period and plotted against initial weight (afdw) for that period as shown in **Figures 4.8a to e.** Each point represents a growth rate for a particular period and the lines join growth rates measured on the same individual over time. The figures show that individual growth rates decline with size except at 15°C where IG is independent of size (ref **Table 4.10**). Repeated-measures ANOVA (on Systat - MGLH) on period-specific IG against initial weights at the beginning of the experiment showed no significant change in growth rate between periods ($P>0.05$) suggesting that IG was independent of the time spent in the system.

A model was then developed to estimate the relationship between growth rate and size at the different temperatures. Premoult and post moult OCL for one moult per crayfish per treatment were converted to ash free dry weights using regressions based on the relationship

Fig 4.8 a
Individual Instantaneous Growth Rates (IG) at 15°C

(points refer to IG for preceeding period, lines join sucessive IG measured on the same individual)

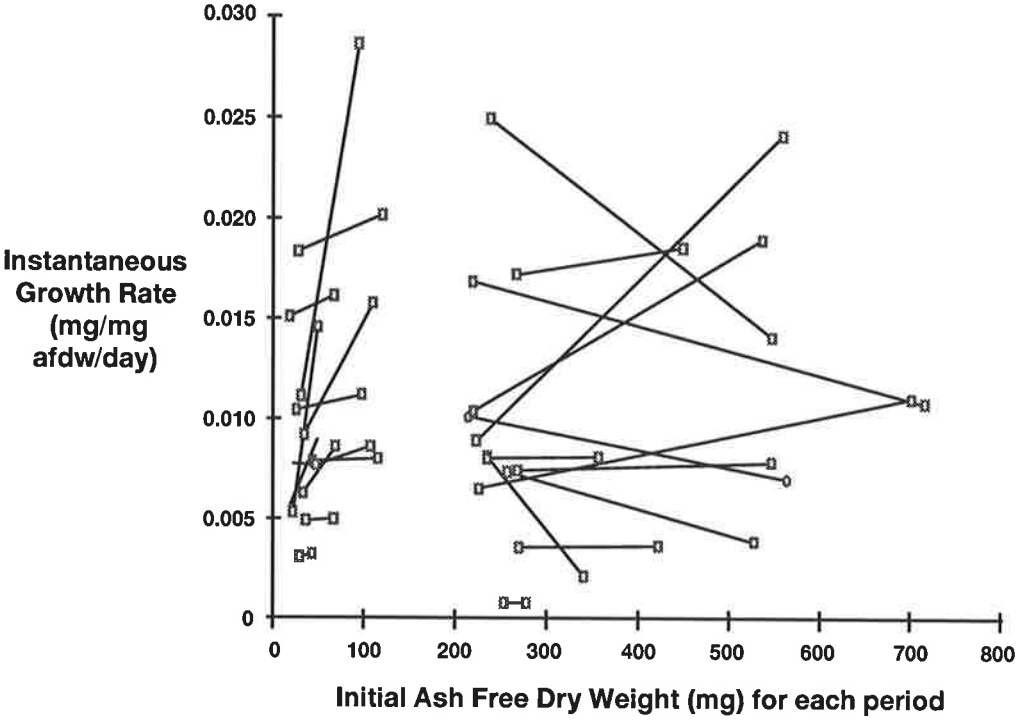


Fig 4.8 b
Individual Instantaneous Growth Rates (IG) at 20°C

(points refer to IG for preceeding period, lines join sucessive IG measured on the same individual)

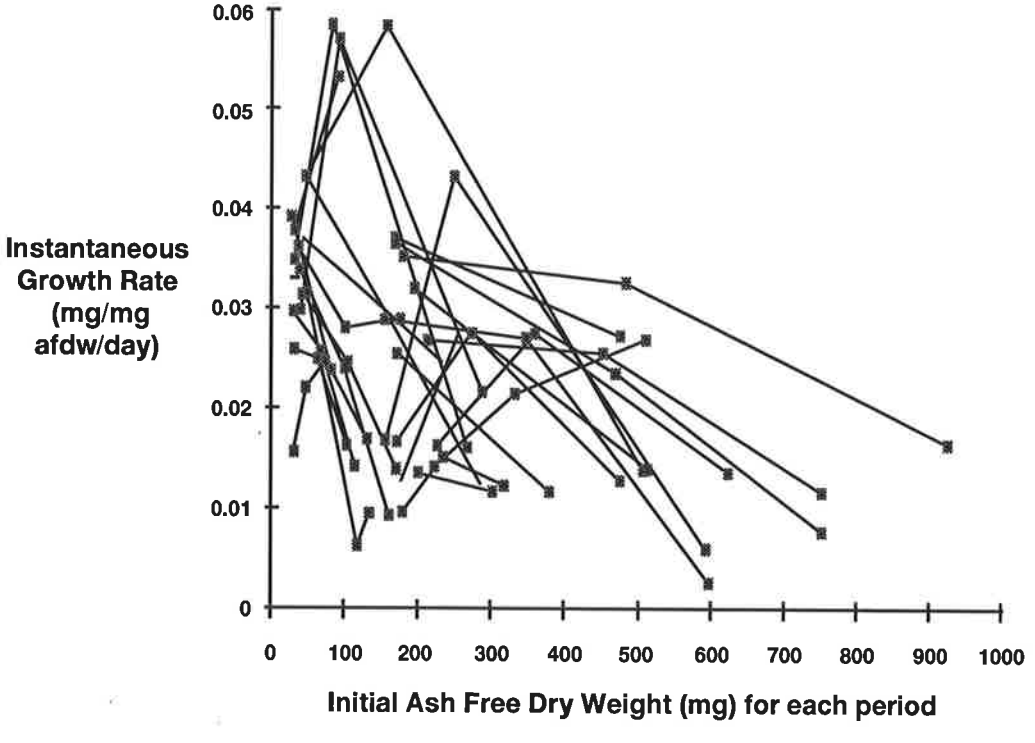


Fig 4.8 c
Individual Instantaneous Growth Rates (IG) at 25°C

(points refer to IG for preceeding period, lines join sucessive IG measured on the same individual)

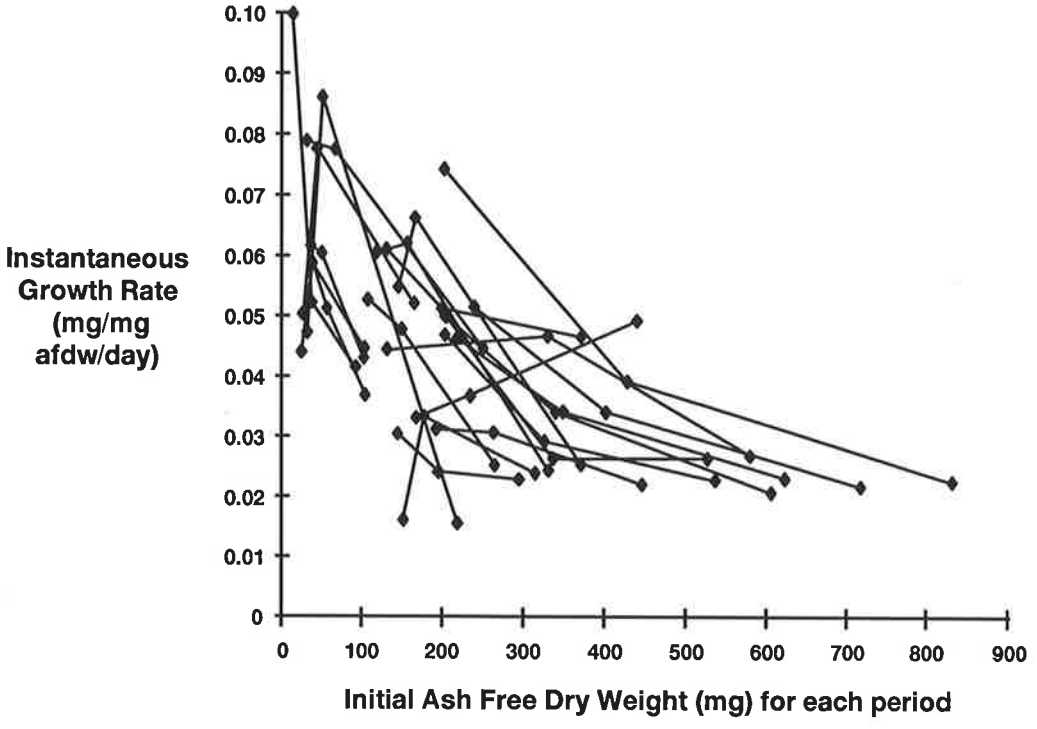


Fig 4.8 d
Individual Instantaneous Growth Rates (IG) at 27.5°C

(points refer to IG for preceeding period, lines join sucessive IG measured on the same individual)

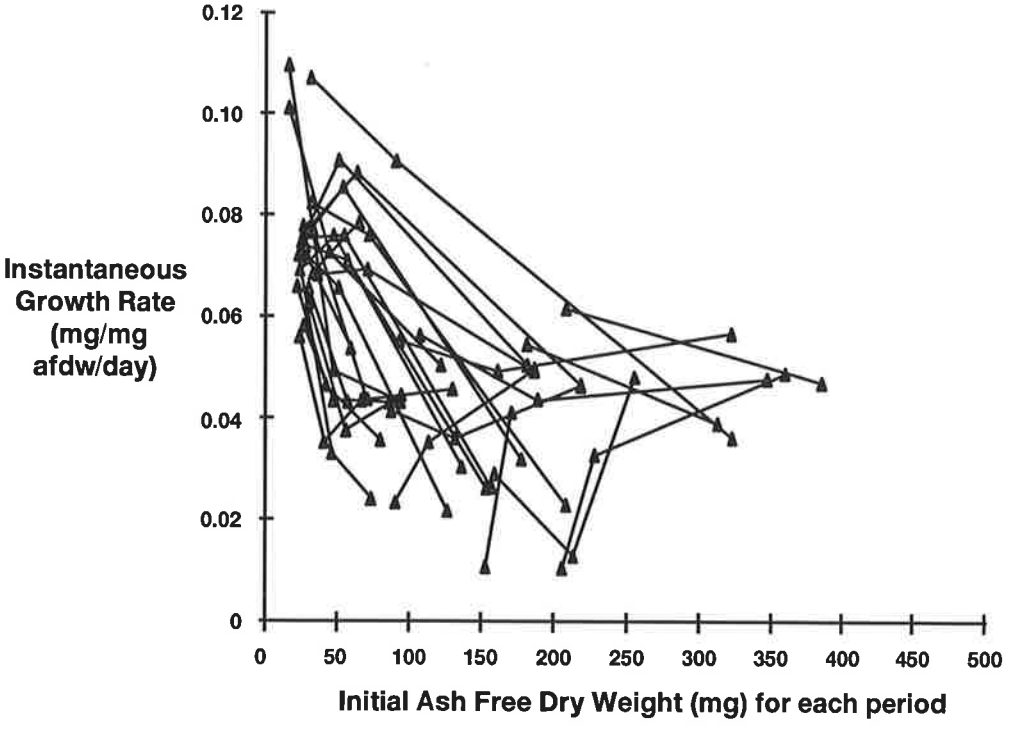
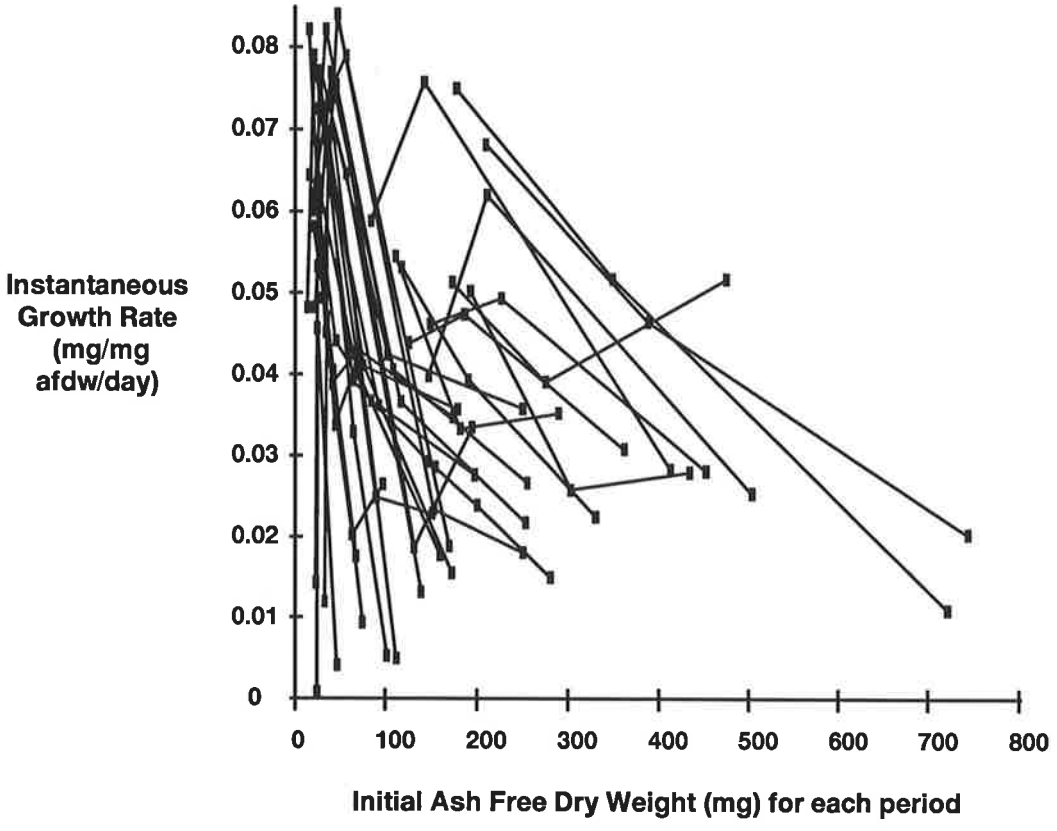


Fig 4.8 e)

Individual Instantaneous Growth Rates (IG) at 30°C

(points refer to IG for preceeding period, lines join sucessive IG measured on the same individual)



calculated for moult stage C_1 (Chapter 3). The weights were then used in combination with the associated intermoult period (in days) to generate growth rates from intermoult to intermoult for a range of sizes. Growth rates were estimated for all temperatures except 30°C which had insufficient data. Only animals whose intermoult periods were known to the day were used. Instantaneous growth rates were calculated as described by **Equation 4.2** in the methods. Linear relationships were derived between instantaneous growth rates and the associated premoult ash free dry weight (mg) at each temperature by log transformation and the data analysed using ANOVAR on Systat-MGLH. The regression statistics are presented in **Table 4.14.**

Table 4.14 Regression statistics for the relationship between IG and Size at at four temperatures. Regressions for 20 to 27.5°C are significant at $P< 0.001$, that for 15°C is significant at $P< 0.038$. T°C = Temperature.

T°C	Slope (b)	SE (b)	Intercept (a)	SE (a)	r^2	n
15	-0.1870	0.080	-1.352	0.1830	0.334	13
20	-0.4995	0.0520	-0.5350	0.1147	0.7723	23
25	-0.4494	0.0569	-0.4424	0.1230	0.7596	17
27.5	-0.4559	0.0987	-0.3634	0.2327	0.5315	12

The lines were then backtransformed and are presented on **Fig 4.9a to d.** For comparison the figures also include the period-specific growth rates shown in **Figures 4.8a to d.** In this case the lines are removed for clarity. The figures suggest that the growth curves provide good predictive functions for average growth between 20 and 27.5°C

The logged data for three temperatures (20 to 27.5°C) were used to generate a multiple regression model describing growth in terms of weight (afdw) and temperature. The curve for 15°C was not used because of the low coefficient of determination and the poor fit with the growth data in **Fig 4.9a**. At this temperature growth rate appeared to be independent of size (**Table 4.10**). The multiple regression model has the form:

$$\log IG = b \log W_o + cT + \log a$$

where IG = Instantaneous Growth Rate (mg/mg afdw/day)

W_o = Initial Ash Free Dry Weight (mg)

T = Temperature (°C)

Fig 4.9a)
Instantaneous Growth Rate (IG - mg/mg afdw/day)
and Estimated IG at 15°C

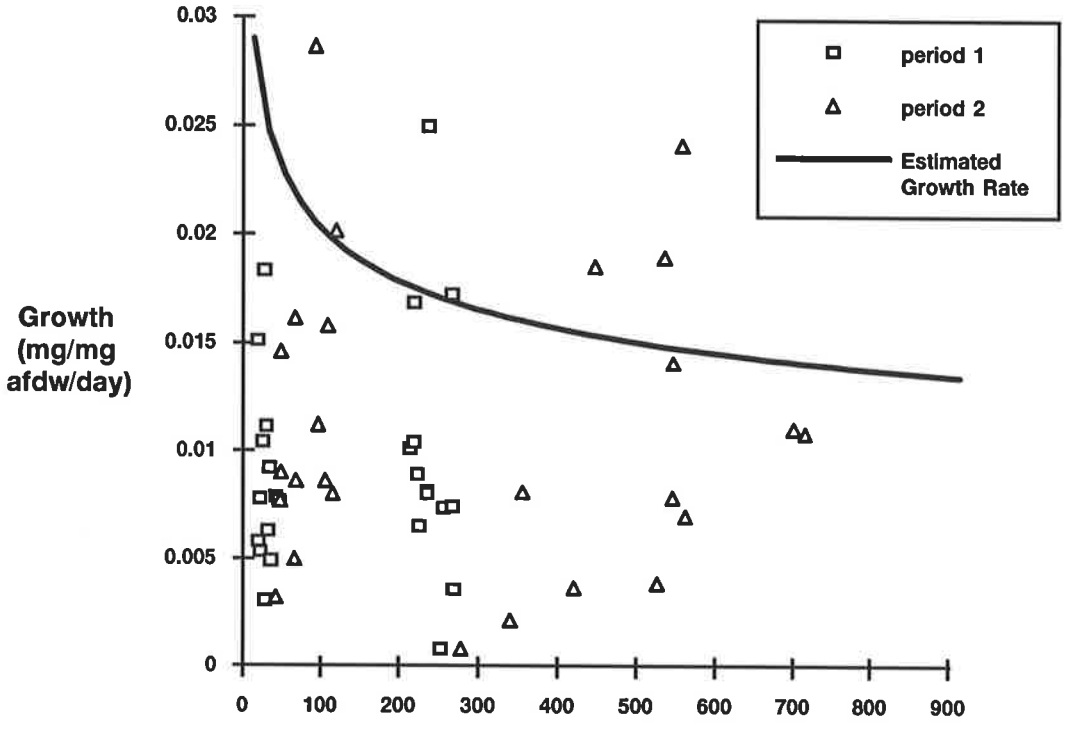


Fig 4.9b)
Instantaneous Growth Rate (IG - mg/mg afdw/day)
and Estimated IG at 20°C

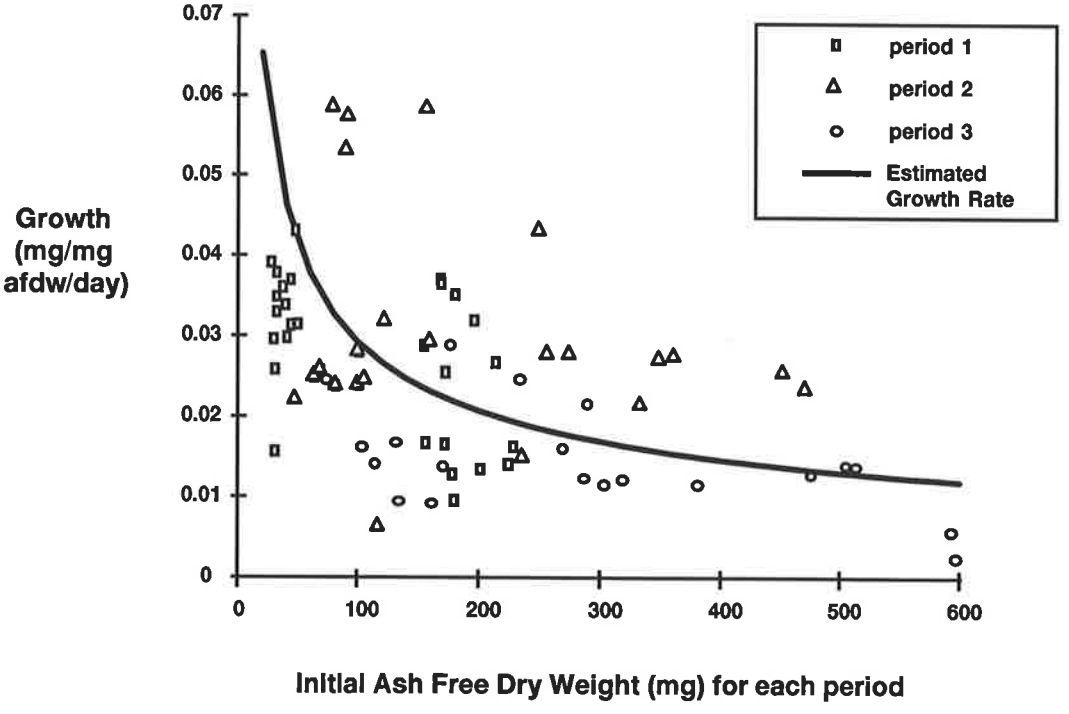


Fig 4.9c)
Growth Rate (IG - mg/mg afdw/day) and Estimated IG at 25°C

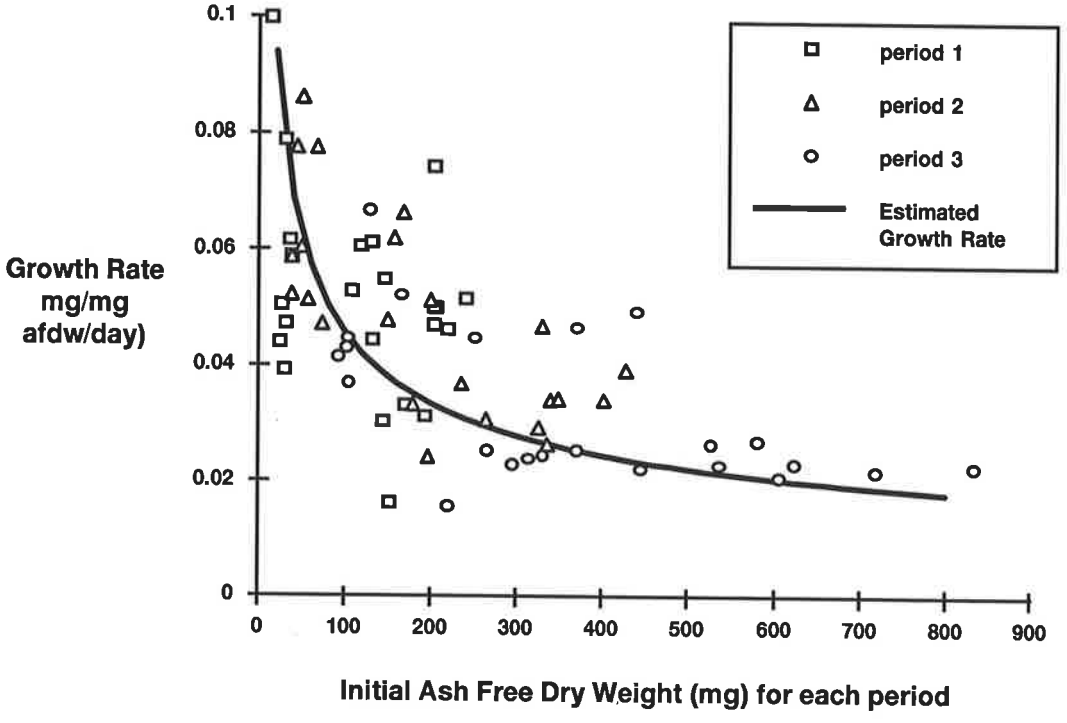
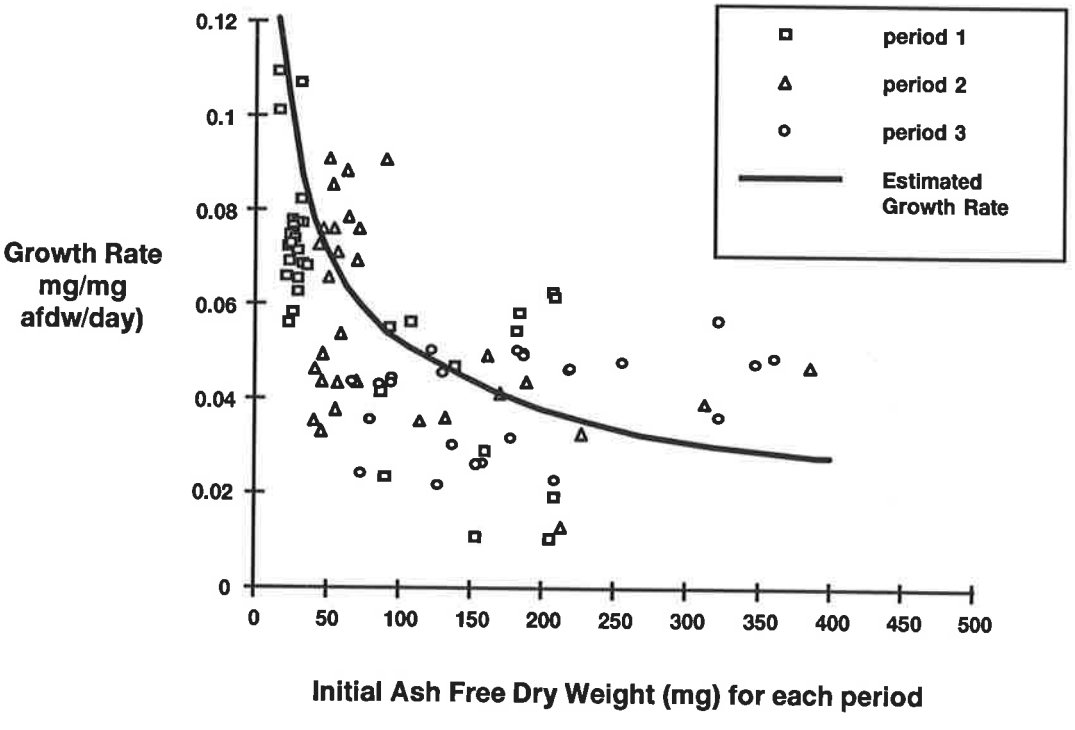


Fig 4.9 d)
Growth rate (IG - mg/mg afdw/day) and Estimated IG at 27.5°C



and a, b and c are constants determined by the regression.

The data were analysed using SYSTAT- mglh which produced the following relationship.

Log IG(mg/mg afdw/day) = - 0.415 LogW_o+ 0.036T - Log 1.437.**Equation 4.3**

This back-transforms (Dall, 1986) to :

IG (mg/mg afdw/day) = 0.0366 ($10^{0.036}$)^T x $W_o^{-0.415}$ **Equation 4.4**

or Growth (mg afdw/day) = 0.0366 ($10^{0.036}$)^T x $W_o^{0.585}$ **Equation 4.5**

where 0.585 = 1-b

The slope of the regression (**Equation 4.3**) is significant (P<0.001) and the equation explains 79.5% of the variability in the data. The rate of decline in growth rate with increasing size is equal to the slope in Equation 4.4 (i.e. -0.415) with the units mg/mg afdw/day per mg increase in afdw. This model and that derived for metabolism in the following chapter (Chapter 5) will be used in the derivation of an energy budget for laboratory growth (Chapter 6).

4.4 Discussion

The conditions under which growth was studied in this chapter fall between that in the clean system in Chapter 2 and those in the field in Chapter 3. In Chapter 2 at 24°C growth on the 35% protein diet averaged 0.0266 mg/mg afdw/day for smaller yabbies and ranged between 0.014 and 0.033 for larger animals; whereas mean growth at 25°C in this chapter averaged between 0.041 and 0.049 mg/mg afdw/day. In the field study (Chapter3) mean growth varied between 0.075 and 0.100 at an average temperature of about 20°C compared with 0.021 to 0.034 mg/mg afdw/day at the same temperature in this chapter. Variations on growth rates are related to the decrease in 'cleanliness' of the system and the associated improvement in food quality and variety.

Growth in this chapter was comparable with other laboratory studies on yabbies. For example, Geddes *et al* (1988) reported data from which may be calculated an instantaneous growth rate of 0.044 mg/mg wet weight/day for sizes between 1 and 6g. This rate is similar

to that found in the present study although the current units are ash free dry weight. The 17.1-17.8% moult increment found by Geddes *et al* at 26°C also falls within the range (25 C: 17.150% ± 0.227; 27.5 C: 18.220% ± 0.363) found here.

The slope for the log intermoult period vs premoult OCL relationship was affected by temperature below 20°C but not above. The common slope for these juvenile crayfish for the range 20 - 30°C (b: 0.0393 ± 0.0030; $P<0.001$) is close to that of smaller decapods such as the crab *Cyclograpsus punctatus* (0.036) and the freshwater crayfish *Cambaroides japonicus* (0.033) (Kurata, 1962) but higher than that of larger species such as the crab *Cancer anthonyi* (0.023: Anderson and Ford, 1976) and the lobsters *Homarus americanus* (0.013: Templeman, 1948) and *Panulirus argus* (0.004: Travis, 1954).

The common slope derived here is also higher than that reported by Geddes *et al* (1988) both for small crayfish (b: 0.01 ± 0.003) over a similar size range and for a broader range (0.1-49.3 g) of animals from three wild populations (b: 0.019-0.023). This may be partly explained by differences in precision and size between the two experiments. Geddes *et al* checked all animals for the presence of exuviae at three day intervals whereas yabbies in the present study were checked every day. Where the range of animals was broader than the present study this difference may have been exacerbated by the progressive decline in growth rate of the larger individuals. As growth slows with size the progressive increase in intermoult period at each moult within a species would decline, thus lowering the slope of the relationship between Log intermoult period and OCL.

Several factors beside temperature and size may have influenced the observed growth patterns. These include diet adequacy, container size, and ammonia levels. The adequacy of the diet for the first four temperatures is suggested by the stability of % moult increments per moult within each temperature and by the fact that there was very little loss of pigmentation as the animals grew. However, %MI declined with time in the 30°C treatment although pigmentation was retained. It is possible that the diet was unable to supply energy needs at this temperature. There is a slight decline at 27.5°C so this process may have been progressive. Container size may also have influenced growth (Morrissy, 1990, Goyert and Avault, 1978). However, the containers used in this study had been used by Geddes *et al*

(1988) and they reported no growth suppression in yabbies less than 20 mm OCL. As this was approximately the length reached in the present study (22 mm OCL) and as % moult increments and intermoult periods were consistent between sizes within treatments at most temperatures it is felt that such growth suppression would have been negligible. Ammonia levels were not monitored but as the water was vigorously aerated and changed every second day it is unlikely they have affected growth.

Although laboratory growth in this study compares well with that in other studies it was still below that achieved in the field growth experiments. As discussed above, growth in the field over similar size and temperature ranges was between two and five times that in the laboratory experiments. The similarities in mean % moult increment (Field: 16.74% cf Laboratory: 15.57 - 17.15%) between the two experiments suggest that the differences relate to changes in intermoult period. Similar results have also been documented in other studies (Goyert and Avault, 1978, Hartnoll, 1982, Morrissy, 1990).

Instantaneous growth rates declined with size at all temperatures except 15°C. Declining size-specific growth rates have also been reported in field studies on crayfish (*Austropotamobius pallipes*; Pratten, 1980, Brewis and Bowler, 1982), amphipods (*Gammarus pulex*; Sutcliffe *et al* 1981) and in laboratory studies of marine decapods (Anger, 1991) . Sutcliffe also refers to this phenomenon as Minot's Law (see Medawar, 1945, Richards and Kavanagh, 1945) which argues that the specific growth rate of organisms is maximal at or near birth and declines with age. Furthermore, Parry (1983) suggested that metabolic rate in ectotherms is partially a consequence of the cost of growth. Thus the negative exponential form of the weight-specific metabolic rate curve (Chapter 2, and Dall, 1986) might be expected to hold for the weight-specific growth rate. Suppression of growth by laboratory conditions would have the effect of increasing the rate of decline such that the growth rate would approach zero at a smaller size than would occur in a more natural system (Morrissy, 1990).

It appears that the size-growth relationship changes with temperature. At 15°C growth was independent of initial size and the weight-specific growth relationship based on growth between successive moults showed a poor fit ($r^2 = 0.334$) to growth rates calculated for each

period. The uncoupling of growth and size may result from a relative increase in intermoult period in the smaller individuals. There was a relatively greater increase in growth rates of smaller yabbies compared to larger individuals between 15°C and 20°C. Given that % moult increment was independent of size, this may be explained by a decreased q10 effect with size on intermoult period (Hartnoll 1982). This suggests that the effect of temperature on intermoult period should decline with size or, conversely, that temperature's effect on growth should be more pronounced in smaller yabbies. This finding was also reported for marron (*Cherax tenuimanus*) by Morrissy (1990). The size-temperature effect is also evident in the temperature at which maximum growth occurred. Growth appears to peak earlier in smaller yabbies. Thus the statement that yabbie growth peaks at 28°C (Mills, 1986) needs to be qualified. The effect of size on growth at high and low temperatures suggests some size-specific differences in temperature optimum for growth (*sensu* Freeman, 1991). The higher weight-specific metabolic demand in smaller individuals at high temperature may not have been met by the available oxygen thus reducing IG. Growth rather than maintenance is likely to be disrupted if energy needs are difficult to meet. Given that the growth/maintenance ratio declines with size so does the weight specific energetic cost of metabolism. This suggests that smaller animals are more likely to be stressed at a lower level of a given stressor than larger individuals. For example, animals are feeding for energy (Sedgewick, 1979, Chapter 2) and energy requirements increase with temperature. If food is of a constant energy value then ingestion rate and therefore the cost of feeding must increase with temperature. This would progressively decrease the energy available for growth which must have an effect on IG in smaller animals first as their specific growth rates are higher.

The model, describing instantaneous growth rate as a function of size and temperature, is one of the few describing such a relationship for crayfish (Mills, 1986, Morrissy, 1990). For yabbies, the only previous contribution to this subject was made by Mills (1986) who described a temperature growth function for yabbies as a smooth curve peaking at 28°C but did include the effect of size or describe the mathematical function associated with his curve. Other work on Parastacids was done Morrissy (1990) who derived a polynomial function between growth rate, temperature and initial weight for *Cherax tenuimanus*. His model comprehensively describes the relationship for the range between upper and lower limiting

temperatures but does not include the effect of changing growth rate with size. Thus this model is the first contribution describing the instantaneous growth rate as a function of temperature and size in yabbies. It will be used in combination with a similar function described for metabolism in Chapter 5 to investigate the relationship between size-specific growth and metabolic cost.

Chapter 5.

Metabolic rate and temperature; a basis for estimating field metabolism

5.1 Introduction

The average daily temperature in the field experiment varied between 15 and 24°C. In order to account for the metabolism component within both field and laboratory energy budgets the relationship between temperature and metabolic rate had to be investigated. Routine metabolic rate (RMR, Lampert, 1984) was estimated following his suggestion that metabolism should be measured on fed (i.e. growing) animals under conditions which were as natural as possible. RMR was considered to be the appropriate measure of metabolic rate in the present study as the energy budgets were on fast growing juvenile crayfish and growth accounts for much of the expenditure on metabolism in juveniles (Parry, 1983). Note that 'natural' in this work is defined by closeness to conditions in the concomittant laboratory growth study (Chapter 4).

The relationship between metabolic rate (MO_2) and temperature is usually described in one of two ways. The most common of these is the 'Q_{10}' which has been used in studies on copepods (Epp and Lewis, 1979), shrimps (McNamara *et al*, 1985), crabs (Leffler,1972), lobsters (Whiteley *et al*, 1990; Zoutendyk, 1989) and various intertidal organisms (Newell, 1979)

A Q_{10} is a ratio describing the acceleration of metabolic rate resulting from a temperature increase of 10°C (Lampert, 1984). It is described by the following formula

$$Q_{10} = [R2/R1]^{[10/T2-T1]} \quad \textbf{...Equation 5.1}$$

where R1 and R2 are rates of oxygen consumption (MO_2) at temperatures T1 and T2 respectively (Lampert, 1984). Once the Q_{10} is known for a given temperature range the respiratory rate at a different temperature within that range may be estimated by:

$$R2 = R1 \cdot Q_{10}^{[T2-T1/10]} \quad \textbf{...Equation 5.2}$$

A major constraint in using Q_{10} values is that they can only be used to compare animals of the same mass, as metabolic rate is extremely weight dependent. The use of mass-specific data does not solve this problem. The mass exponent for weight-specific MO_2 may be derived by subtraction of 1 from the slope (b) of MO_2 (Oxygen consumption vs Weight, Sutcliffe, 1984). If oxygen consumption was directly proportional to weight, b would equal 1, b-1 would equal 0 and weight-specific MO_2 would be independent of weight for a given stage or species. However, b is more commonly between 0.6 and 0.8 (see Chapter 2 where b = 0.6112), therefore b-1 is usually negative and so weight-specific MO_2 declines with increasing weight (Sutcliffe, 1984).

A more general method combining temperature and weight was used by Hart (1980) and Dall (1986) to derive a predictive equation in which MO_2 was described by the interaction between weight and temperature. Their approach involves the assumption that the MO_2 - weight relationships at different temperatures have the same slope but have different elevations and intercepts. The logged intercepts (a) (Eqn 5.3 below) from the equations for MO_2 vs Weight at each temperature are plotted against temperature. The relationship is described by the equation:

$$\text{Log } a = \alpha + \beta \cdot T \qquad \textbf{...Equation 5.3}$$

where T = temperature and β and α are the slope and the intercept of the line relating the MO_2 vs weight intercepts and temperature. This equation is combined with the common slope from the MO_2 vs temperature equations to give a multiple regression of the form:

$$\log MO_2 = B \cdot \log W + \beta \cdot T + \alpha \qquad \textbf{...Equation 5.4}$$

or as an exponential function:

$$MO_2 = \alpha \cdot (10^{\beta})^T \cdot W^B \qquad \textbf{...Equation 5.5}$$

Where B is the common slope of the MO_2 vs weight relationship at each of the temperatures. The equation is then used to predict the metabolic rate of different sized animals in the field at a variety of temperatures.

This chapter will evaluate the usefulness of the Q_{10} and multiple regression approaches with respect to the temperature and size-specific metabolic rate relationship in juvenile yabbies. Several studies have found that Q_{10} decreases with temperature or that there is a decline, usually described as a 'plateau', at the modal habitat temperature. For example, Newell (1979) described a sigmoid response curve in MO_2 for various intertidal organisms where Q_{10} increased rapidly up to the modal habitat temperature, declined to a plateau then increased rapidly again, possibly as the animals became stressed. The same curve was evident in *Jasus lalandii* (Zoutendyk, 1989), where MO_2 increased at a greater rate between 8 and 10°C (Q_{10} = 2.2) than between 10 and 13°C (Q_{10} = 1.7). The curve then increased rapidly and thus assumed a sigmoid appearance . Zoutendyk also suggested that the 'plateau' between 10 and 13°C corresponded to the modal habitat temperature.

The multiple regression approach is more general as it integrates temperature and weight, however its accuracy depends on the r^2 of the pooled slope for the MO_2 vs weight relationships at the different temperatures and the linearity of response of the logged intercepts (refer **Eqn 5.3** above). A highly variable relationship between temperature and metabolism, such as the plateau discussed above, would reduce linearity and lower the fit between observed and expected values.

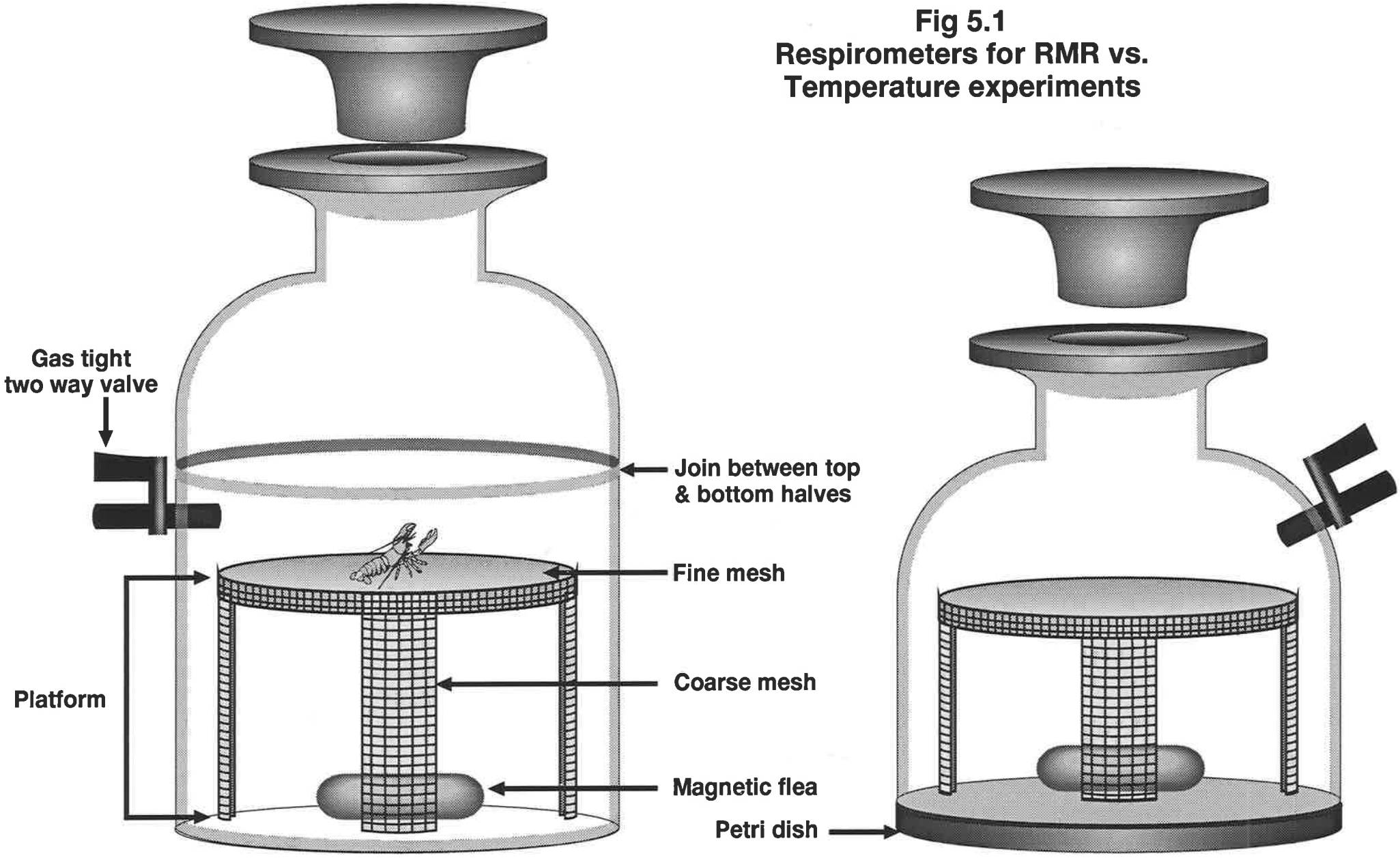
The relationship between temperature, size and metabolic cost derived in this chapter will be used to estimate metabolic costs within the laboratory and field budgets as discussed in Chapter 6.

5.2 Materials and Methods

Routine metabolic rate (RMR) was measured on yabbies grown at three temperatures (15, 20 and 25°C) in association with the growth experiments described in Chapter 4. Animals ranging in size from 100 mg to 2.3 g were tested at their respective growth temperatures and under the same light conditions (i.e. 12:12 LD). Before testing, yabbies were fed the diet described for the growth experiments, 35% protein diet plus filamentous algae.

Respirometry was conducted in a semi-closed system (**Fig 5.1**). Respirometers of two sizes were constructed from stoppered glass bottles (BOD bottles). These held 120 ml and 300 ml,

Fig 5.1
Respirometers for RMR vs. Temperature experiments



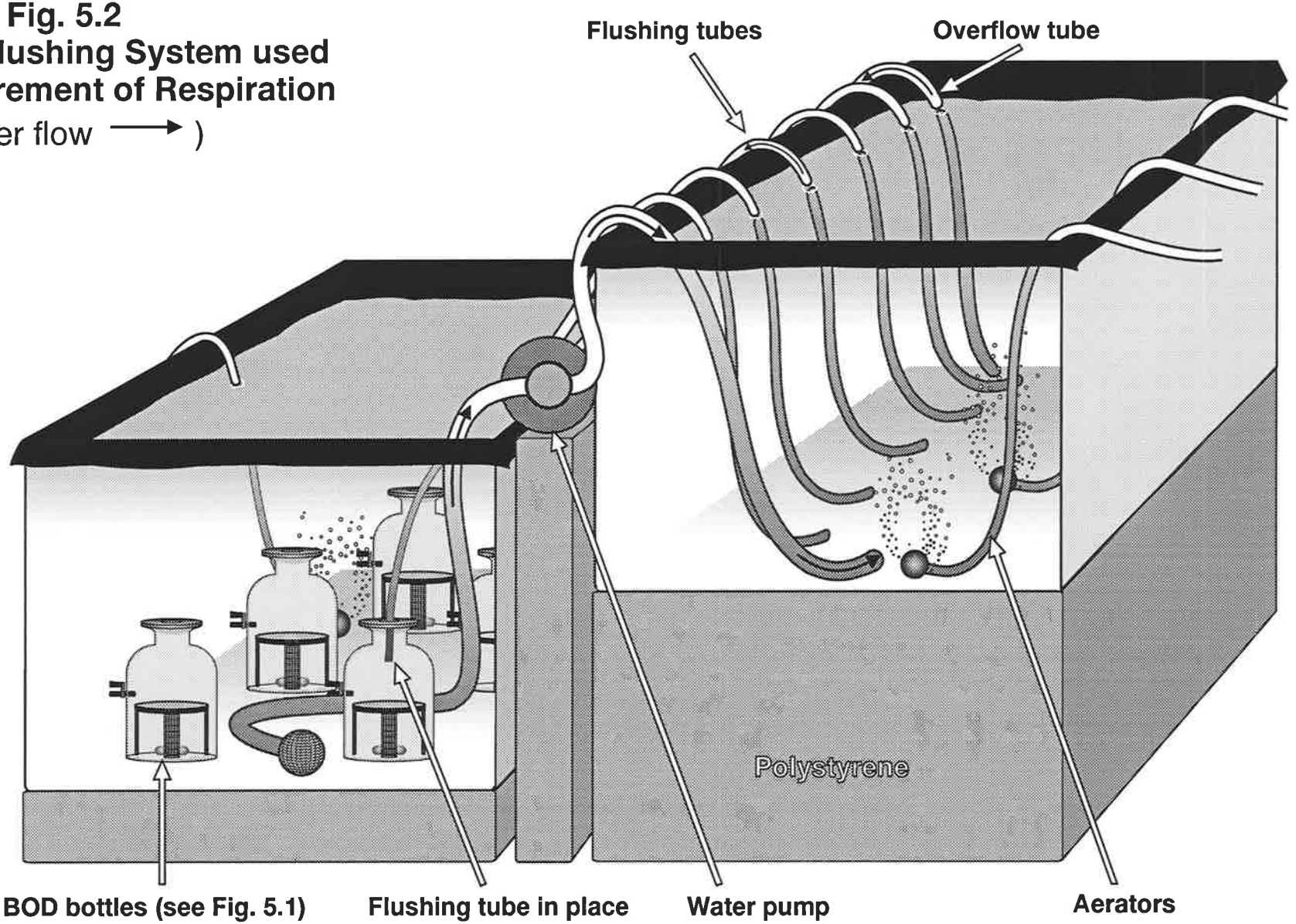
for less than 500 mg and greater than 500 mg yabbies respectively. For the 300 ml respirometers, bottles were cut in half and a magnetic flea and a plastic mesh platform inserted into the bottom half. A hole was drilled near the top of the bottom half for insertion of a gas tight two way valve. Thus water samples could be drawn from just above the platform containing the yabbie. The top half was then replaced and sealed with silicon sealant. Both joining surfaces were ground to ensure a tight fit. For the 120 ml respirometers, the platform and flea were placed on a glass petri dish and the top half of the bottle sealed to it. A hole was drilled in each respirometer in the same place relative to the platform. Ground glass stoppers were used to cap all respirometers and the body of each respirometer was wrapped in black plastic to provide shelter for the enclosed yabbie. All respirometers had approximately the same floor area as the growth chambers used in Chapter 4. Five respirometers of each size were built.

During trials all respirometers were submerged in a bin lined with black plastic and filled with aerated water. The respirometers were flushed with aerated water between trials. Water exchange took about 10 minutes (determined with dye) and flushing was carried out for an hour each time. **Figure 5.2** shows the water aeration and flushing system used in the trials. Water was pumped to the upper bin from the lower bin, containing the respirometers, then returned passively through individual tubes to each respirometer. Both bins were vigorously aerated. Three respirometers were used for yabbies and two were used as controls in each trial.

Twenty-four to twenty-seven animals were tested at each temperature. Each animal was tested once. Crayfish were weighed (to the nearest mg) and moult staged before and after each trial and only intermoult animals (stages C_1 to $D_{1.1}$ - Burton and Mitchell, 1987) were used.

Five days prior to each batch of trials, yabbies were selected randomly from one of the two size groups in the growth experiments (Chapter 4) and moved into a temperature controlled cabinet set to the same temperature and lighting conditions. During this acclimation period they were fed every second day, including the day of the experiment. Yabbies were not removed from their growth chambers (see Fig 4.1) during transfer and acclimation. Three

Fig. 5.2
Aeration & Flushing System used in the Measurement of Respiration
(Water flow ⟶)



hours before the start of each trial yabbies were carefully placed in individual respirometers which were left to flush. A small piece of mesh was attached to the top of the bottle to prevent the yabbie escaping. This also served to hold the flushing tube in place (**Fig 5.2**). Respirometers were then sealed at random over about thirty minutes and left in that condition for a period appropriate to the size of the yabbie and the temperature. At 15°C that time varied between two and four hours whereas at 25°C this was reduced to between 30 minutes and 1 hour. The time was selected to prevent more than 25% oxygen depletion within the respirometer. Three measurements of respiration rate were taken over a period of twelve hours and the respirometers flushed between trials.

The mean RMR (MO_2) was calculated from the three measurements (minus controls) and the relationship between MO_2 and wet weight derived for each temperature. The equations were of the form:

$MO_2 = a \cdot W^b$ ...**Equation 5.6**

Where MO_2 is Metabolic rate (mg/h), a = a constant (the y intercept), b = the slope of the line and W = wet weight (mg). Data are initially presented in terms of wet weight (mg) to allow easier comparison with the literature then converted to ash free dry weight to improve the accuracy of metabolic cost estimates. The relevant relationship between wet weight and ash free dry weight was developed in Chapter 3. Growth and body composition results are also presented in the terms of AFDW (Chapters 3 and 4).

The data were logged to derive linear relationships between respiration and weight at each temperature so Equation 5.6 becomes:

$\log MO_2 = \log a + b \cdot \log W$ ...**Equation 5.7**

Lines were compared using ANCOVA and the F statistic. Temperature coefficients (Q_{10}) were calculated using Equation 5.1:

$Q_{10} = [R2/R1]^{[10/T1-T2]}$

where R1 and R2 are rates of oxygen consumption (MO_2) at temperatures T1 and T2 respectively.

5.3 Results

Oxygen consumption in mg/h was positively correlated with wet weight for all temperatures ($b>0$, $P<0.001$). **Figs 5.3a to c** show plots of oxygen consumption ($MO_2 \pm SE$) against temperature. The data were logged and geometric mean regressions derived for the weight and oxygen consumption relationship at each temperature. GM regressions are recommended by Ricker (1973) where data are non-normal and open ended and where the variance increases in proportion to the size of the variate. Although Dall (1986) suggests that such a regression may give anomalous results when applied to metabolic data, GM regressions generally gave the lower proportional standard errors of the two methods.

Coefficients of determination for MO_2 varied from 0.8292 to 0.9721 depending on temperature (**Table 5.1**).

Table 5.1 Log Oxygen Consumption (ug/h) vs Log Wet Weight (mg).
The equation is of the form: Log MO_2= log a + b·log W. T (^oC) = Temperature.
All slopes are significant ($P<0.001$)

T (^oC)	Slope (b)	Intercept (a)	SE (b)	SE(a)	r2	n
15	0.6685	-0.0913	0.0223	0.0657	0.9721	27
20	0.7061	-0.0432	0.0495	0.1375	0.8872	25
25	0.7206	-0.0239	0.0635	0.182	0.8292	24

Slopes or mass exponents were 0.67, 0.71 and 0.72 at 15, 20 and 25^oC respectively. Wet weight was then converted to ash free dry weight (**Table 5.2**).

Table 5.2 Log Oxygen Consumption (ug/h) vs Log Ash Free Dry Weight (mg). The equation is of the form: Log MO_2 = log a + b· log W. T (^oC) = Temperature. All slopes are significant ($P<0.001$)

T (^oC)	Slope (b)	Intercept (a)	SE (b)	SE(a)	r2	n
15	0.6685	0.3700	0.0223	0.0505	0.9721	27
20	0.7061	0.4439	0.0495	0.1037	0.8872	25
25	0.7206	0.4733	0.0635	0.1386	0.8292	24

Confidence limits for the logged data were calculated according to Sokal and Rohlf (1981, pg 473). The logged data, GM regressions and calculated confidence limits are presented in **Figs 5.4a to c**. The 'estimates' presented in these figures were derived from the multiple regression approach of Dall (1986) as outlined in the present study and are discussed in 5.4.

Fig. 5.3a
Oxygen Consumption (ug/h)
vs Wet Weight (mg) at 15°C (+-SE)

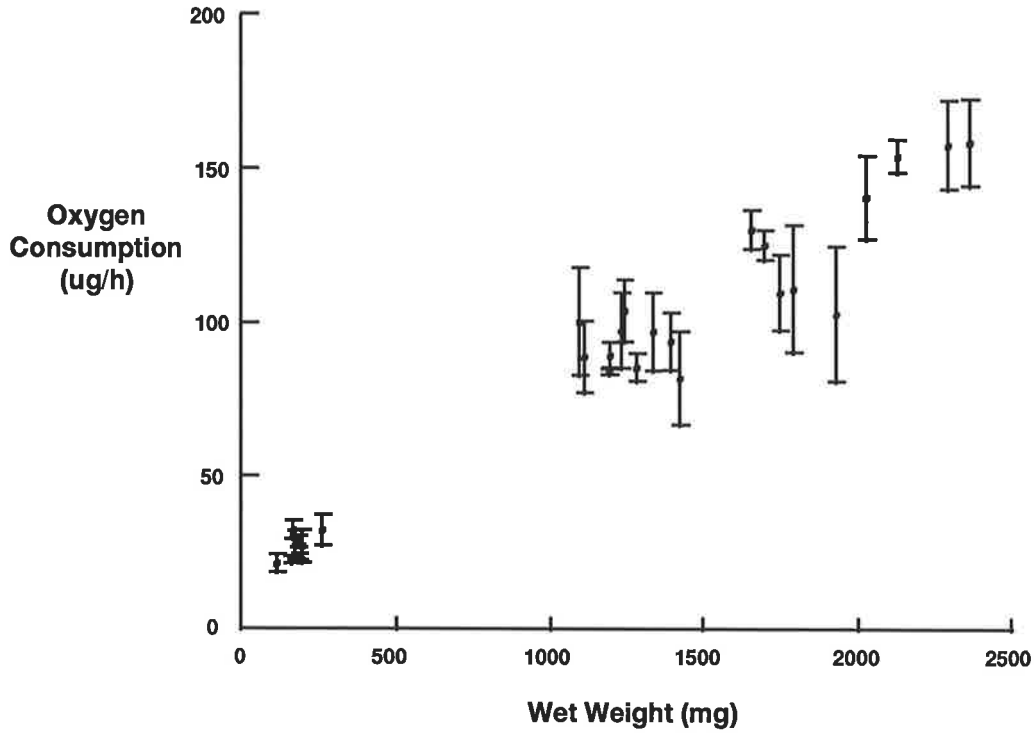


Fig 5.3b
Oxygen Consumption (ug/h)
vs Wet Weight (mg) at 20°C (± SE)

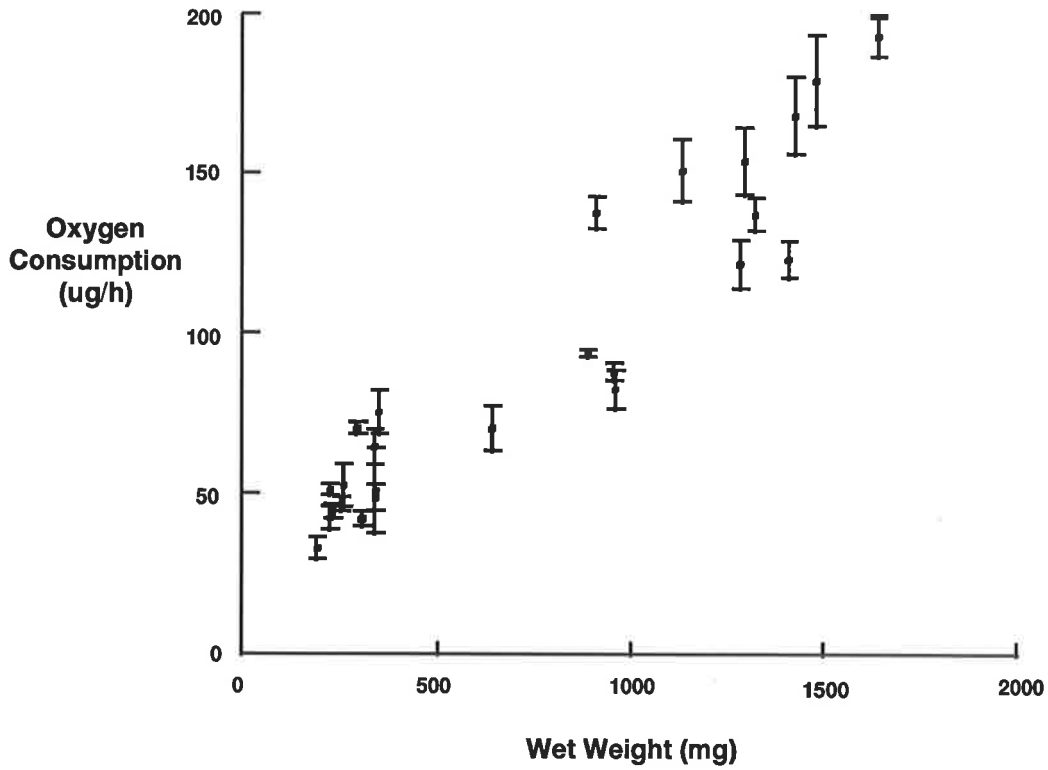


Fig. 5.3c
Oxygen Consumption (ug/h)
vs Wet Weight (mg) at 25°C (+-SE)

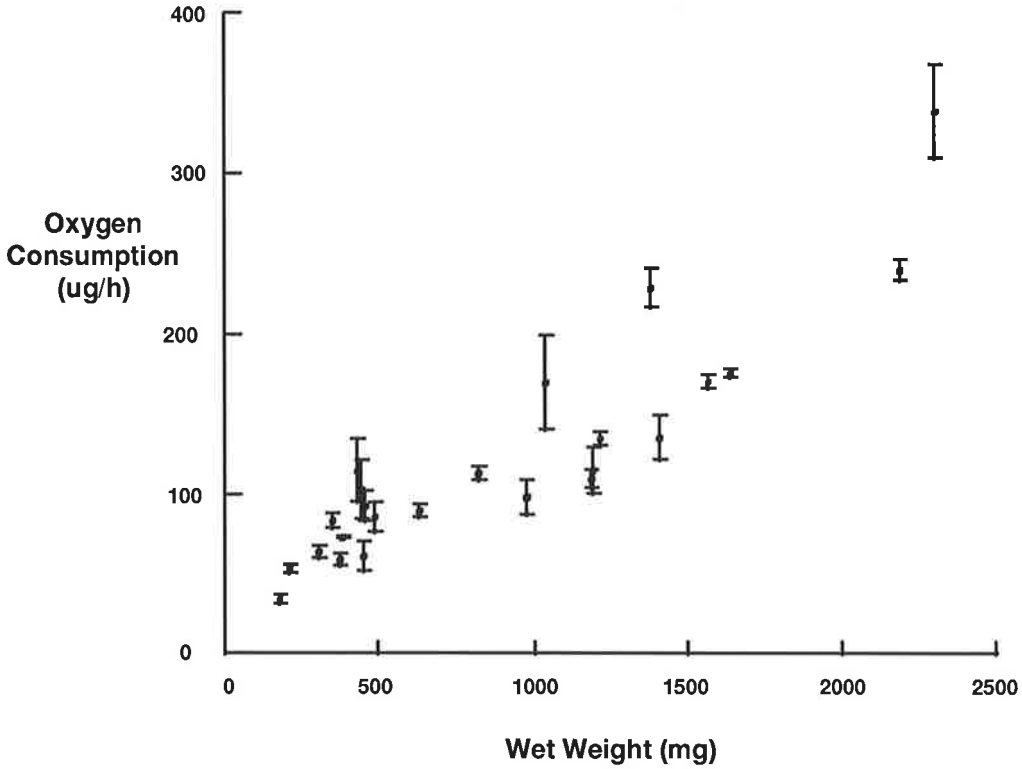


Fig 5.4a
Log Oxygen Consumption (ug/h) vs Log Weight (mg afdw) at 15°C

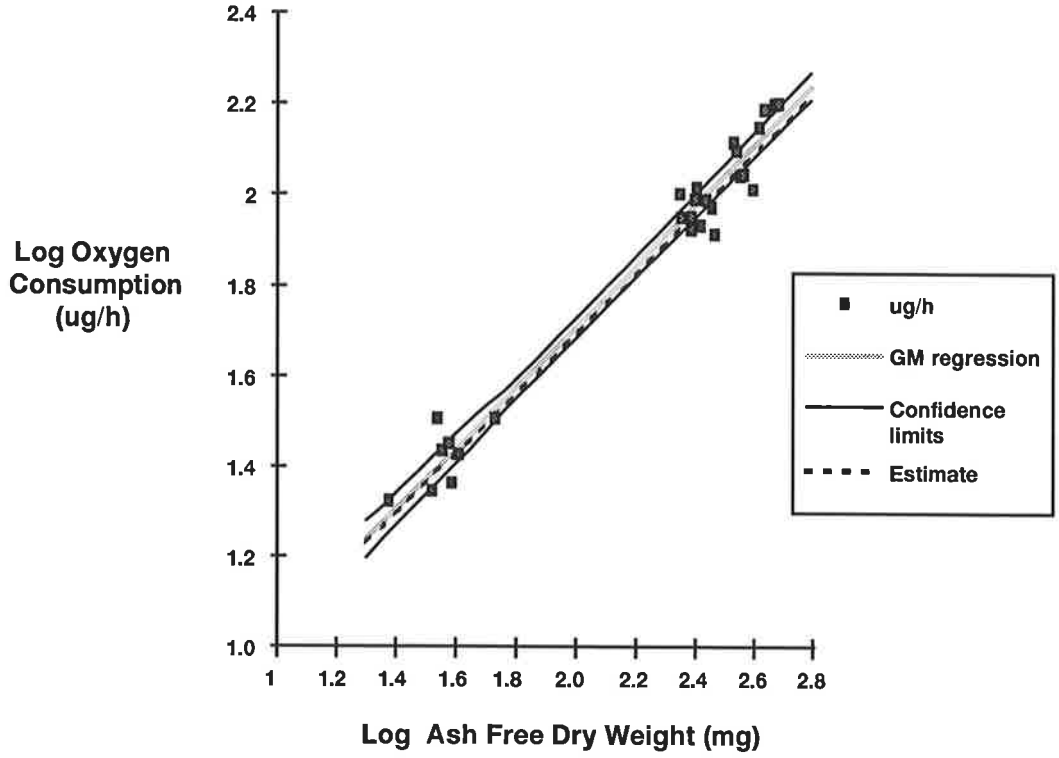


Fig 5.4b
Log Oxygen Consumption (ug/h) vs Log Weight (mg afdw) at 20°C

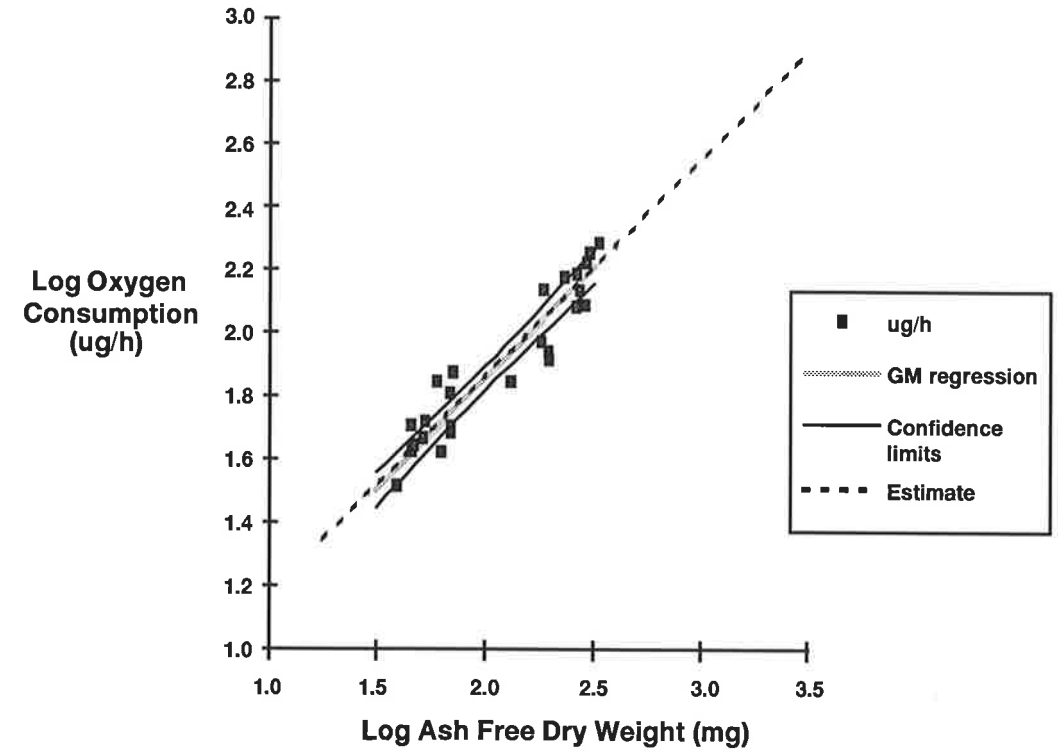
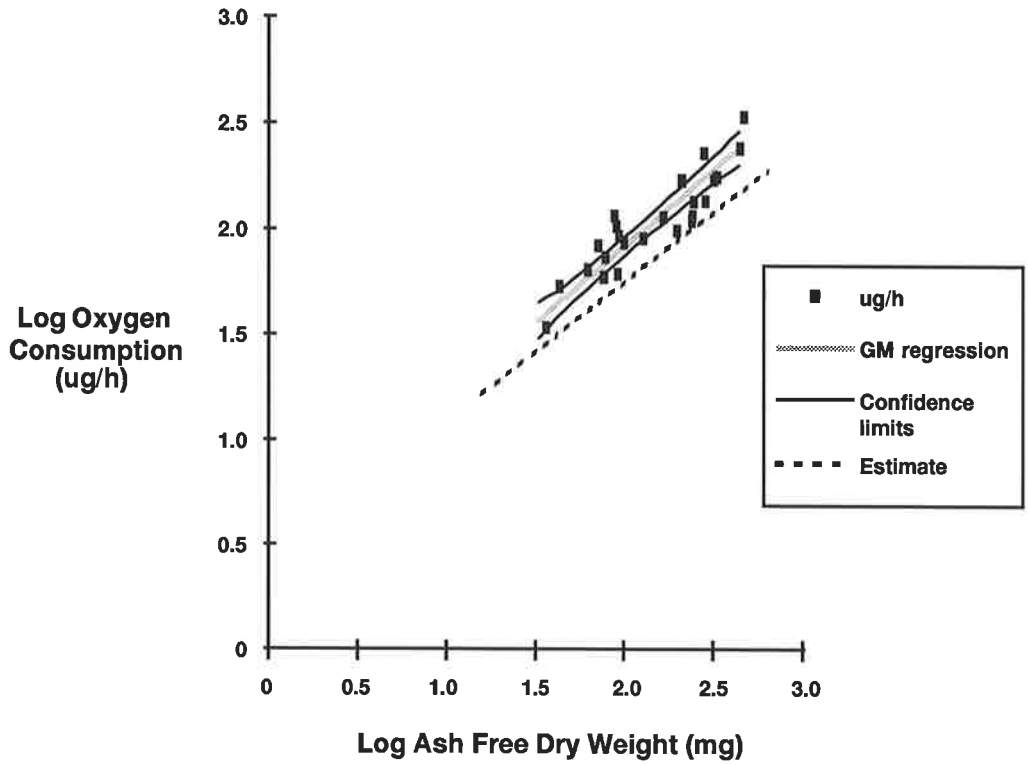


Fig 5.4c
Log Oxygen Consumption (ug/h) vs Log Weight (mg afdw) at 25°C



There were significant changes in metabolic rate between 15 and 20°C (P<0.001) and 20 and 25°C (P<0.0025). Comparisons between temperature-specific weight : metabolic rate equations were made using ANCOVA and the F statistic. The F values and significance levels are presented in **Table 5.3**.

Table 5.3: Comparisons between Metabolic rate (MO_2 in ug/h) vs weight (AFDW, mg) at three temperatures (15, 20 and 25°C) using ANCOVA.
** P<0.025, ***P<0.001

Temperatures compared	Slope (b) or Intercept (a)	F value	Degrees of Freedom	P
All	b	0.0087	2,70	ns
	a	53.4724	2,74	***
15,20	b	0.0137	1,48	ns
	a	65.2004	1,51	***
20,25	b	0.01226	1,45	ns
	a	6.5488	1,48	**

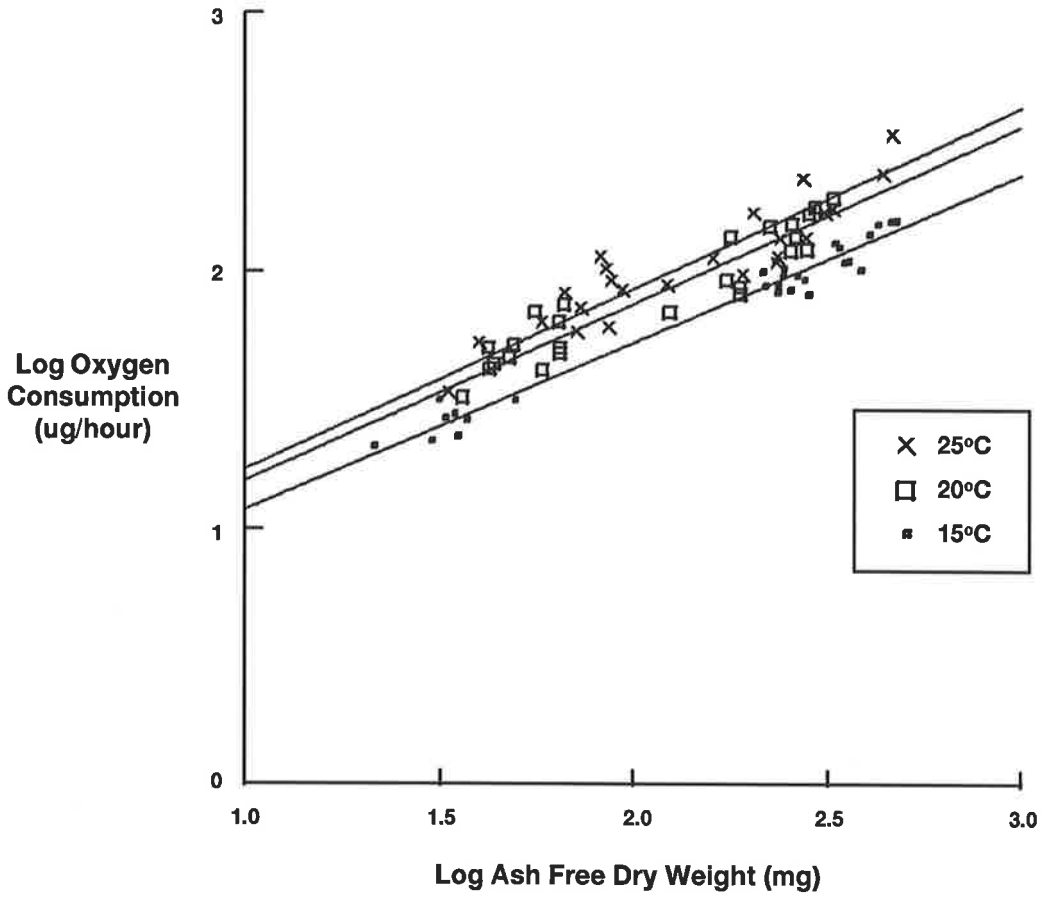
This shows significant changes in 'a' (y intercept) while the slopes of the lines were not significantly different (P>0.05). GM regressions and logged data for all temperatures are presented in **Fig 5.5**.

Q_{10} values were estimated over temperature ranges 15 to 20°C and 20 to 25°C for representative crayfish weights using the equations in **Table 5.1.** The resulting values are presented in **Table 5.4**.

Table 5.4 Q10 vs size for five degree temperature increments. Values based on MO_2 (mg/h) vs Wet Weight (g)

	Temperature Range (°C)	
Wet Weight (mg)	15-20	20-25
500	**2.24**	**1.37**
1000	**2.36**	**1.40**
1500	**2.44**	**1.42**
2000	**2.49**	**1.43**
2500	**2.53**	**1.44**
3000	**2.57**	**1.44**

Fig. 5.5
Log Oxygen Consumption (ug/h)
vs Log AFDW (mg) at three temperatures



The data are presented in terms of wet weight and show a decline in Q_{10} with temperature. This is also suggested by the relative size of the F values for each comparison in the ANCOVA table **(Table 5.3)** in that the F value for the 15 to 20°C interval is much larger than that for the 20 to 25°C interval.

Equations 5.3 and 5.4 were then used to derive predictive weight-specific temperature-metabolic rate equations (Dall, 1986; Hart, 1980). The equations produced were:

$$\text{Log } MO_2 = (0.106 \cdot T + 0.2162) + (0.6600 \cdot \text{Log AFDW}) \quad \textbf{..Equation 5.8}$$

or, backtransformed:

$$MO_2 \text{ (ug/h)} = 1.6451 \cdot (10^{0.0106})^T \cdot AFDW^{0.6600} \quad \textbf{..Equation 5.9}$$

which gives a weight specific form:

$$MO_2 \text{ (ug/mg/h)} = 1.6451 \cdot (10^{0.0106})^T \cdot AFDW^{-0.3400} \quad \textbf{..Equation 5.10}$$

Equation 5.8 was used to derive the 'estimates' of metabolic rate for a range of sizes at each temperature as presented in **Figs 5.4a to c.**

5.4 Discussion

The range of mass exponents (b = 0.6685-0.7206) found in this study was lower than the average reported for 36 other species of decapod crustacean (0.751 - Wheatly 1989). However, Wheatly extracted a large number of these from Ivleva (1980) who reported a range between 0.6-0.79 for marine crustaceans.

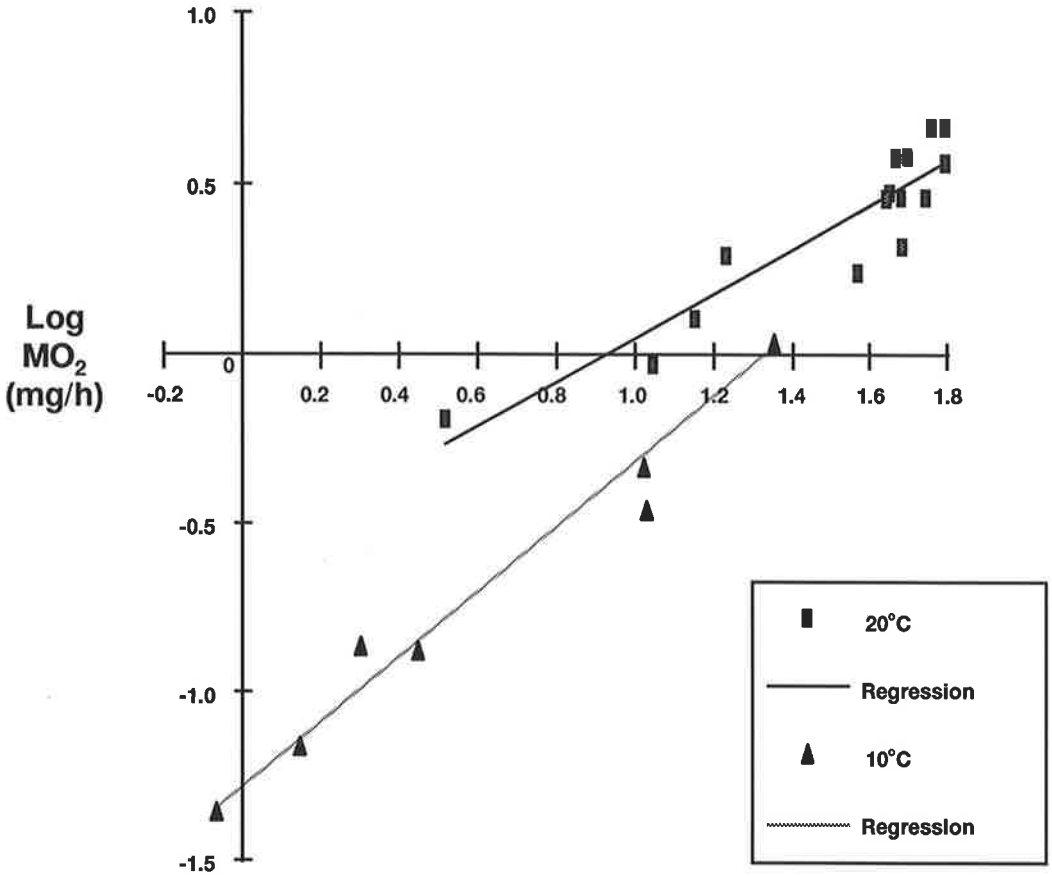
The Q_{10} (2.24 -2.57) calculated here for the interval 15-20°C agrees with those reported for similar temperatures in studies on other aquatic decapods (i.e. McMahon *et al*, 1978, Q_{10} = 2.3; De Fur and Mangum, 1979, Q_{10}=2-2.2) and for crustaceans in general (Ivleva, 1980, Q_{10}=2.17-2.45). Q_{10} also decreases according to the weight variable used - in the order Wet Weight>Dry Weight>Ash Free Dry weight (Ivleva, 1980). In this regard McMahon *et al*

(1978) and De Fur and Mangum (1979) used wet weight and Ivleva used all three. The first of the range reported above by Ivleva relates to AFDW and the last to wet weight. In the present study the figure for the interval 20-25°C (1.3-1.4) is lower than that found by Ivleva (2.45 for wet weight) and Zoutendyk (1989; Q_{10} =1.7, Wet Weight) for lobsters but similar to that reported by McNamara *et al* (1985; Q_{10} =1.19, Dry Weight) for freshwater shrimps.

Other studies on respiration rates on *Cherax* species include those described in Chapter 2 of the present study as well as work on *C. tenuimanus* (Villarreal, 1989) and *C. destructor* (Woodland, 1967). The mass exponents in the present study are all higher than that derived in Chapter 2 (b = 0.6112) at 24°C but have a similar magnitude but narrower range than those previously reported for yabbies (0.615-0.966, Woodland, 1967) and for the marron, *Cherax tenuimanus* (Villarreal, 1989; b= 0.572-1.092). Woodland (1967) studied the ecological energetics of a wild yabbie population and used laboratory measurements of metabolic rates at various temperatures to estimate field metabolism. There are several problems with this part of his study. He used small numbers of yabbies (n = 7 and 15 for 10° and 20°C respectively) to derive his field temperature-metabolic rate relationship. Also, as the data and regression lines for MO_2 (**Fig 5.6**) show, there is little overlap in size between the two data sets used. The mean size for the 10°C treatment was 7.25g ± 1.13 and that for 20°C was 40.22g ± 1.29. Furthermore, about half the data from the 10°C treatment was from animals tested in groups. Woodland extrapolated these lines and used them to calculate field metabolism but the accuracy of the resulting estimates is questionable.

The present study clearly shows a decline in Q_{10} with temperature. The reduction in slope of the metabolic rate-temperature curve between 20 and 25°C raises the question as to whether a plateau or an asymptote exists and what the physiological significance of a 'plateau' might be. Wieser (1973) suggested that a plateau may be an energy saving device to allow organisms with limited energy reserves, such as intertidal molluscs, to survive non-feeding periods at low tide. Such independence from broad temperature fluctuations would confer obvious advantages. Alternatively, the drop in Q_{10} may suggest a curvilinear response between Q_{10} and temperature (Dall, 1986). Dall reported a negative curvilinear relationship between Q_{10} and temperature for a penaeid prawn (*Penaeus esculentus*) when Q_{10} was measured at 2.5°C intervals. A similar response was found by Bottrell (1974) when

Fig 5.6
Data from Woodland (1967) : Log MO_2 (mg/h) vs Log Wet Weight (g) plus regressions



Log Weight (g)

describing the relationship between egg development and temperature in nine species of cladocerans and copepods. Brett and Groves (1979) and Parry (1983) suggested that there is a cost to growth which is reflected in metabolism in poikilotherms and thus metabolic rate is a reflection of growth rate. Lavigne (1982) came to a similar conclusion when he suggested that production was proportional to respiration. By this reasoning as growth in yabbies shows a curvilinear response to temperature (Morrissy, 1990, present study - Chapter 4) it would be expected that metabolic rate would show a similar pattern. Furthermore, as the asymptote for growth in yabbies is between 25-28^{o}C (Mills,1986 and Chapter 4 of the present study) it would be expected that growth and the associated metabolic rate would slow progressively as that peak is approached possibly accounting for the apparent 'plateau'. The term 'plateau' may be misleading as it could be an artefact of the distance between temperatures at which metabolic rate is measured. It appears that Q_{10} is a linear approximation to a curvilinear phenomenon, the accuracy of which is dictated by the distance between measurement temperatures and by the relative metabolic responses to those temperatures.

Despite the decrease in Q_{10} with temperature the multiple regression equations do provide useful estimates for MO_2 as a function of temperature and size as they are generally in agreement with observed values. This is shown by comparison of the estimated MO_2 values to the MO_2 data presented in **Fig 5.4.** The estimates from the multiple regression equation are especially close to measured MO_2 values at 15 and 20^{o}C. The multiple regression approach will be used and metabolic costs derived for laboratory and field energy budgets as presented in Chapter 6.

Chapter 6.

A Partial Energy Budget

6.1 Introduction

This Chapter presents a partial energy budget which synthesizes the work in preceding chapters. Net growth efficiencies (K_2, Calow, 1978) and models of daily energy use are calculated for the laboratory growth experiments at each of three temperatures (Chapter 4) and for the field growth experiments (Chapter 3).

The simplest form of an energy budget is Production = Assimilation - Metabolism - Excretion. A more extensive form of the budget that is particularly related to the energetics of freshwater crayfish will be used in this chapter. The budget takes account of the different metabolic energy demands and incorporates a term related to energy involved in moulting. The energy budget (Brett and Groves, 1979, Capuzzo, 1983, Jones and Momot, 1983, Parry, 1983) for crayfish may be written as

$$Q_c - (Q_u - Q_v) = Q_p + Q_g + Q_s + Q_d + Q_a + Q_m \quad \textbf{Equation 6.1}$$

Thus ingested energy (Q_c) may be lost from the individual in the form of faeces (Q_u) or assimilated but subsequently catabolised and lost as nitrogenous waste products (Q_v). Assimilated energy in the form of carbohydrates, lipids and protein may be converted to tissue (Q_p) or used to fuel the conversion process (Q_g). Q_g is the 'cost of growth' or the 'cost of synthesis of new protoplasm' (Brett and Groves, 1979, Parry, 1983, Wieser and Medgyesy, 1990) as discussed in Chapter 2. Q_d is known as the specific dynamic effect (SDE, Brett and Groves, 1979) and has been equated with the increase in metabolic rate immediately after feeding (Wieser and Medgyesy, 1990), presumably associated with digestion and assimilation. Other costs include Q_s which refers to the energetic cost of maintenance, Q_a, the cost of activity and Q_m which introduces the caloric losses at the moult and includes energy lost with the shed exuvia plus losses of energy reserves metabolised during the moult (Capuzzo, 1983). For the purposes of this study Q_m is further subdivided into E_v and E_r which refer to exuvial and metabolic losses respectively. For crustaceans the exuvia may be considered as part of

production (Kurmaly *et al*, 1989) as although shed it has been part of the body of the organism.

The terms in this budget may be grouped as assimilation (Q_c - Q_u), production (Q_p, including E_v), metabolism (Q_g, Q_s, Q_d, Q_a and E_r) and excretion (Q_v) and so related to the simple form of the energy budget:

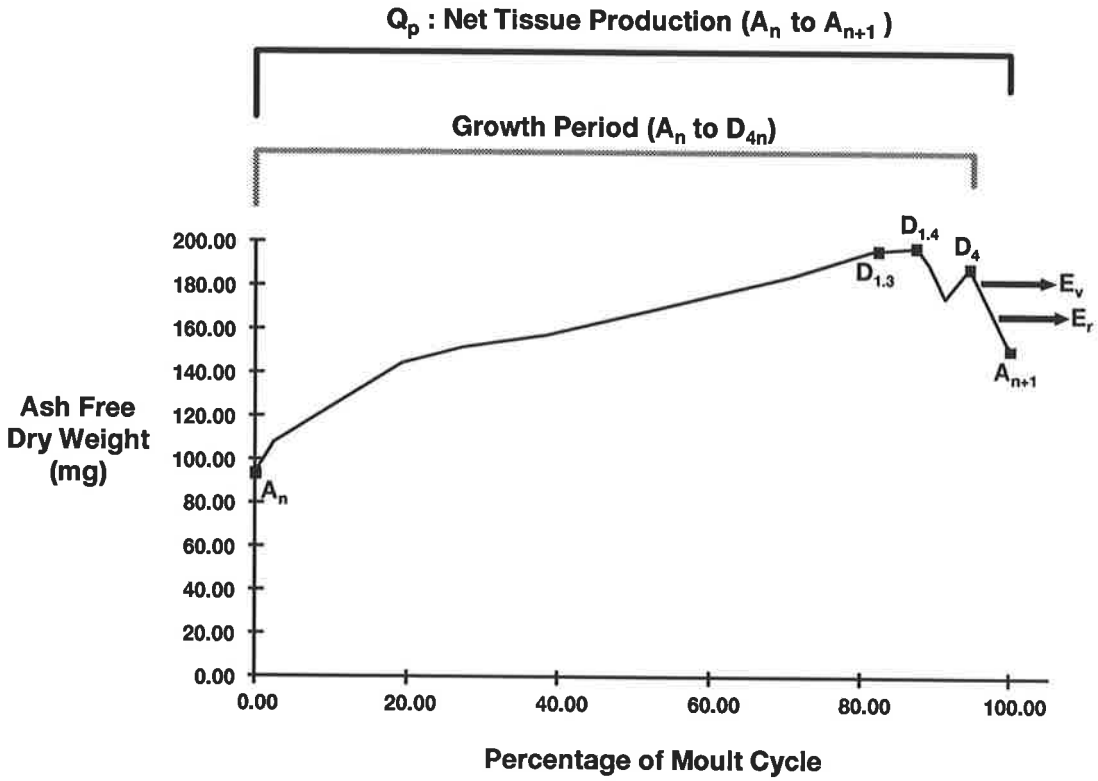
Production (P) = Assimilation (A) - Metabolism (M) - Excretion (E)

In this chapter the parameters production, assimilation and metabolism were used to calculate net growth efficiency or K_2 (Capuzzo, 1983) which is equivalent to (Production / Assimilation) x 100. A = P + M + E so K_2 also equals P/(P + M + E). Excretion, Q_v, was not considered in this study, therefore the net conversion efficiency can be written as K_2 = P/(P + M). Metabolism is measured via RMR (which includes Q_g, Q_s and Q_d plus some component of Q_a) plus the metabolic cost of moulting (E_r).

Figure 6.1 shows a typical growth profile for a juvenile yabbie as discussed in Chapter 3. Tissue accumulation occurs from A to $D_{1.3}$ - $D_{1.4}$ followed by a net loss of tissue to D_4 and another loss during the moult from D_4 to A_{n+1}. For the purposes of the laboratory budget the 'growth period' is defined as that between A_n and D_{4n} (**Fig 6.1**), for the field budget the growth period is defined as that between Day_0 and Day_n. In both cases the 'growth period' refers to 'net' growth as there are tissue losses during the period. For both budgets Q_p is defined as net tissue production between A_n and A_{n+1} (**Fig 6.1**). Assimilation is also calculated for the period A_n to A_{n+1} and is thus defined as $Q_p + E_v + E_r$ + RMR.

The multiple regression equation describing the effect of temperature and size on RMR (Chapter 5) was used to estimate metabolism (M) for laboratory and field growth experiments. However complete calculations of energy budgets require the elements E_r and Q_a to complete the estimate of metabolism. The moult is a time of high metabolic rate in crustaceans (e.g. *Geocarcinus lateralis*, Skinner, 1962; *Hyas araneus*, Anger, 1991). Thus the energy used during the moulting process, from D_{4n} to A_{n+1} of the moult cycle, should also be calculated as E_r, and included as part of the metabolic component of the energy budget. A component to account for extra activity beyond that undertaken in the respirometer (Q_a) must be added to

Fig 6.1
Growth profile for a juvenile yabbie of 12.5 mm OCL from Moult Stage A_n to A_{n+1}



RMR to provide a better estimate of M during the period of the growth experiments in the field. In the field budget in this study an estimate of an additional 50% of RMR was applied to account for the extra activity that yabbies could have undertaken. The relationships between respiration and assimilation and respiration and growth in the laboratory and in field are also calculated for comparison with those in Chapter 2 and in the literature.

6.2 Methods.

The analysis was divided into a laboratory-based budget at three temperatures to investigate the relationship between growth efficiency (K_2), temperature and size and a field budget which estimates growth efficiency for each container and harvest. Field energy use is presented with and without a field activity component which is assumed to increase RMR by 50%. Losses with the exuvia (E_V) and metabolic losses at the moult (E_r) are taken into account in the calculations.

6.2.1. The Laboratory Budget.

RMR, net growth and loss at ecdysis (E_v and E_r) were estimated for a series of moult cycles (A_n to A_{n+1}) relating to consecutive size classes for yabbies between 5 and 15 mm OCL. Temperature-specific budgets were calculated for growth at 15, 20 and 25°C. In each case the size increment at moult was determined by the temperature-specific % moult increment. The length of the growth period, the intermoult period for each size class, was estimated using the temperature-specific Log intermoult period vs Premoult OCL regression equations derived in Chapter 4 (Table 4.5).

a) Growth

The temperature and size-specific growth equation (Eq. 4.5 below) developed in Chapter 4 was used to generate net growth at 20 and 25°C for each growth period over the interval A_n to D_{4n}.

$$\text{Growth (mg/day)} = 0.03656\,(10^{0.036})^{T} \times W_o^{0.585} \quad \textbf{Equation 4.5}$$

where T = Temperature and W_o = Initial weight (mg afdw)

As the growth rate for 15°C was independent of size, the mean pooled instantaneous growth rate (IG = 0.0103 mg/mg afdw/day) was used to generate a model of weight accumulation for each growth period with IG substituted for b in Equation 4.1 after backtransformation (i.e. Ln W = Ln a + Bt becomes $W = a \times e^{bT}$). In this case W = weight (mg) at the end of the period (i.e. at D_4), a = weight at A, b = the instantaneous growth rate and T = the intermoult period.

Growth over the intermoult period for each size class was estimated by subtraction of the ash free dry weight at A from that at D_4. These weights were estimated using the relationship between moult stage, OCL and organic content described for the field data (Chapter 3); the % moult increment was set at the mean found at each laboratory growth temperature (i.e. 15.6% at 15°C and 17.09% at 20 and 25°C: Chapter 4). In each case consecutive size classes were estimated from a starting point of 3.1 mm OCL which is equivalent to that of a stage three juvenile. Insertion of these moult increments into the moult stage-specific OCL vs ash free dry weight relationships for stages A and D_4 generated a range of initial and final weights for each size class. These weights were converted to joules using the moult stage-specific energy values described in Chapter 3.

b) Routine Metabolic Rate

RMR was estimated for the growth period (A_n to D_{4n}) of each size class using the temperature and size-specific equation derived in Chapter 5.

$$RMR\ (ug/h) = 1.6451 \cdot (10^{0.0106})^{T} \cdot AFDW^{0.6600} \quad \textbf{...Equation 5.9}$$

As the animals used to measure RMR were in intermoult, daily metabolic rate was calculated for the estimated organic weight at C_2 and multiplied by the number of days of the intermoult period.

RMR in ug/h was converted to joules/day by multiplication of the value by 0.01414. This was based on the general conversion factor of 14.14 joules/ mg O_2 respired as reported for ammonoteles by Elliot and Davidson (1975). The result was multiplied by 24 to estimate the daily metabolic cost.

c) Energy Losses at the Moult

The total energy lost at the moult (Q_m), comprising the loss of the caloric content of the exuvia (E_v) and the metabolic cost of the moulting process (E_r), was calculated by subtracting the caloric content of an animal of a given size at the beginning of a moult cycle (A_{n+1}) from the final caloric content of a yabbie at the end of the previous cycle (D_{4n}) (refer Chapter 3). The caloric content of the exuvia (E_v) was then subtracted from the total loss of caloric content to estimate the metabolic cost of moulting (E_r).

d) Assimilation

Daily assimilation (A_d) was estimated for each intermoult period and the subsequent moult (i.e. for the period A_n to A_{n+1}) by taking the total value for that period, $Q_p + RMR + E_v + E_r$, and dividing it by the number of days over which it was calculated to yield joules assimilated per day. The estimate was also divided by the calculated weight at C_2 for each size class to yield joules/mg afdw/day.

e) Net Growth Efficiency

Temperature and size-specific net growth efficiency (K_2) values were calculated for the period A_n to A_{n+1} including metabolic loss at the moult (E_r) and with either the inclusion of the exuvia (E_v) as part of growth or its exclusion. If the exuvia is included, K_2 is estimated as:

$$K_2 = \frac{Q_p + E_v}{Q_p + RMR + E_r + E_v} \times 100 \quad \textbf{..Equation 6.2}$$

where $Q_p + E_v$ = production for the period A_n to A_{n+1}, RMR = Routine Metabolic Rate and E_r = metabolic losses at the moult. If the exuvia is not included as part of growth the equation becomes:

$$K_2 = \frac{Q_p}{Q_p + RMR + E_r + E_v} \times 100 \quad \textbf{..Equation 6.3}$$

6.2.2 Calculation of Field Energy Budget.

a) Growth

The mean instantaneous growth rates in terms of both weight and caloric content (Chapter 3, Table 3.3a and b) were used to generate average daily weight (W_f) and energy accumulation (J_f) for each container at each harvest. The models were generated in the same way as described for the laboratory budget, using the equation $W = a \times e^{bT}$. In this case W = ash free dry weight (mg) after T days, a = initial weight and b = instantaneous growth rate. Solving this equation for day_1 to day_f, where f = the last day of the growth period, gives the cumulative daily weight gain over the period with the final weight equal to that recorded in Chapter 3 (Table 3.3a) for the container and period in question. Subtracting the weight at day_{n+1} from that at day_n gives the weight W_f or caloric gain J_f during day_n. The models were estimated for each day of the period from establishment of the experiment to harvest, 0 - 15 days for harvest 1, 0 - 30 days for harvest 2, and 0 to 53 days for harvest 3. The initial organic weight was 1.835 ± 0.0356 mg and the initial caloric content was 41.557 joules. Daily growth was also calculated for the growth periods 16-30 and 31-53 days using the final ash free dry weights or caloric contents at harvests 1 and 2 as initial weights/caloric contents for the second and third growth periods respectively. Temperature was set at the average for the relevant period (ref Chapter 3).

b) Routine Metabolic Rate.

Daily metabolic cost for day_n was calculated using Equation 5.9 after conversion from micrograms per hour to joules per day as described for the laboratory budget. The appropriate weight for the calculation (i.e. weight at day_n) was derived from the daily growth model described above. Daily metabolic expenditure was summed for the relevant time period to

give the total metabolic cost related to the period from establishment to harvest at 15, 30 or 53 days or for the periods 16-30 and 31-53 days.

c) Energy Losses at the Moult

The loss of energy at moult as a result of caloric loss in exuvia (E_v) and energy losses resulting from elevated metabolic rates (E_r) also had to be calculated. Total loss at the moult (D_{4n} - A_{n+1}) was based on the effect of OCL and moult stage on caloric content (Chapter 3). As before, size class interval was determined by a mean moult increment of 16.74%. Because the final mean weight at each harvest was known, it was possible to estimate the mean number of moults that would have occurred to that point. The caloric loss associated with this number of moults was then summed for each container within each harvest and further subdivided into loss with the exuvia (E_v) and metabolic loss (E_r). Percent loss with the exuvia was calculated using caloric data based on the proximate components as described in Chapter 3. E_r was again calculated by subtraction of E_v from the total loss.

d) Models of Daily Energy Assimilation

In the field budget, P (production) was the net tissue accumulation during the growth period which covered a number of moults. In effect P was measured over a series of moult cycles (i.e. A_n to A_{n+1}) with the end of the growth period occurring some time during the last moult cycle for each individual. Net tissue production (P) = Q_p. Total assimilation throughout the period to harvest is equal to $Q_p + E_v + E_r + RMR$ where Q_p is the tissue contained in the crayfish at harvest, E_v and E_r are the sum of the energy losses at the moults during the growth period and RMR is the sum of the daily RMR values. Three models of mean individual daily energy assimilation (A_d) were derived. The first was estimated for the growth periods 0 to 15, 16 to 30 and 31 to 53 days. The second estimated mean weight-specific daily assimilation over the growth periods by dividing daily assimilation at Day_n by the weight at Day_n. The percentage contribution of RMR to daily assimilation was also examined. The last model examines the effect of different growth rates on assimilation. Daily caloric assimilation was estimated for the largest, smallest and average sized individual at harvest after 53 days. In all models daily energy assimilation was calculated with and without an added activity component (RMR x 1.5).

Metabolic loss at the moult (E_r) is a discrete event, not a daily rate. However, it needs to be expressed as part of a continuous function within respiration for the purposes of the model. This was achieved for E_r by distributing the energy lost at moult throughout the days of the moult cycle, thus elevating the daily respiration rate. E_r was converted to a daily rate by allocating it to each day throughout the growth period in the same proportion as energy accumulation for that day (J_{fi}). The equation is of the form:

$$Er_i = \frac{\text{Energy accumulated for day}_i\ (J_{fi})}{\text{Caloric increment at the end of growth period}_i} \times E_r \quad \textbf{..Equation 6.4}$$

where Er_i = daily rate for day_i, J_{fi} = Caloric accumulation during day i which equals joules accumulated by day_{i+1} minus those by day_i, and the Caloric increment at the end of the growth period equals final minus initial caloric content. There was an increase in E_r added to each day's assimilation as daily caloric increment increased with size.

e) Net Growth Efficiency

Net growth efficiencies (K_2) were calculated for each container for each of the five growth periods (i.e 1-15, 1-30, 1-53, 16-30 and 31-53 days) by using estimation of field growth rates and field metabolic rates in the formula K_2 = P/P+M. The net growth efficiency was investigated taking account of the growth rates that included or did not include the exuvia as part of growth. The equations used were those described for the laboratory budget **(Equations 6.2 and 6.3).**

Further estimates of K_2 were made by adding the affect of activity which was assumed to increase RMR by 50%. Thus four estimates of K_2 were calculated for each of the five periods, involving inclusion or not of E_v and estimates made with and without the activity component.

6.3 Results

6.3.1 Laboratory Energy Budget

Fig 6.2a to c show the relationships between RMR and growth, total loss at the moult and growth and the components of moult loss, E_r and E_v, and growth. Each point in the figure represents the relationship between the given parameter and growth for individual growth periods related to successive size classes defined by the OCL measurements on the X axis. In the laboratory, joules spent on RMR declined with size from 180 to 64% of growth between A_n and D_{4n} at 15°C but increased with size from 33 to 41% of growth at 20°C and from 24 to 30% of growth at 25°C. Percent total caloric loss at the moult showed a consistent pattern at all three temperatures in declining steeply with size from 54 to 60% of growth at about 5mm OCL to 21 to 28% at approximately 15mm OCL. E_r/G increased slightly with declining temperature between the 15°C and 20°C treatments and showed the greatest size-specific decline of the two components contributing to the total loss. The latter suggests that E_r was more dependent on size than was E_v. E_v also showed the same size-specific decline for all three temperatures. Finally, E_r was greater than E_v in smaller yabbies but values for E_v and E_r were similar for yabbies from 12 to 15mm OCL.

In the laboratory daily energy assimilation increased with temperature from 15 to 25°C, as yabbies grew from 5 to 15 mm OCL (**Fig 6.3a**). Energy assimilation over the period A_n to A_{n+1}, for successive size classes, varied between 6 and 70 joules per day at 15°C, between 18 and 111 joules per day at 20°C and between 27 and 156 joules per day at 25°C. **Fig 6.3b** shows weight-specific daily energy assimilation declined with size from 0.72 to 0.31 joules/mg afdw/day at 15°C, from 1.6 to 0.5 joules/mg afdw/day at 20°C and from 2.3 to 0.72 joules/mg afdw/day at 25°C

Net growth efficiency (K_2) for each moult cycle, A_n to A_{n+1}, changed with temperature and size (**Fig 6.4**). K_2 values were calculated for each temperature using **Equation 6.2** which includes exuvia in production and **Equation 6.3** where exuvia are not included in production. When exuvia are included, K_2 increases from 21% at 5 mm OCL to 51% at 15 mm OCL at

Fig 6.2a)
Relationship between Growth and i) Metabolic cost (RMR), ii) Total Loss at Moult, iii) Exuvial Loss (E_v) and iv) Metabolism associated with the Moult (E_r) during growth from 5 to 15 mm OCL at 15°C

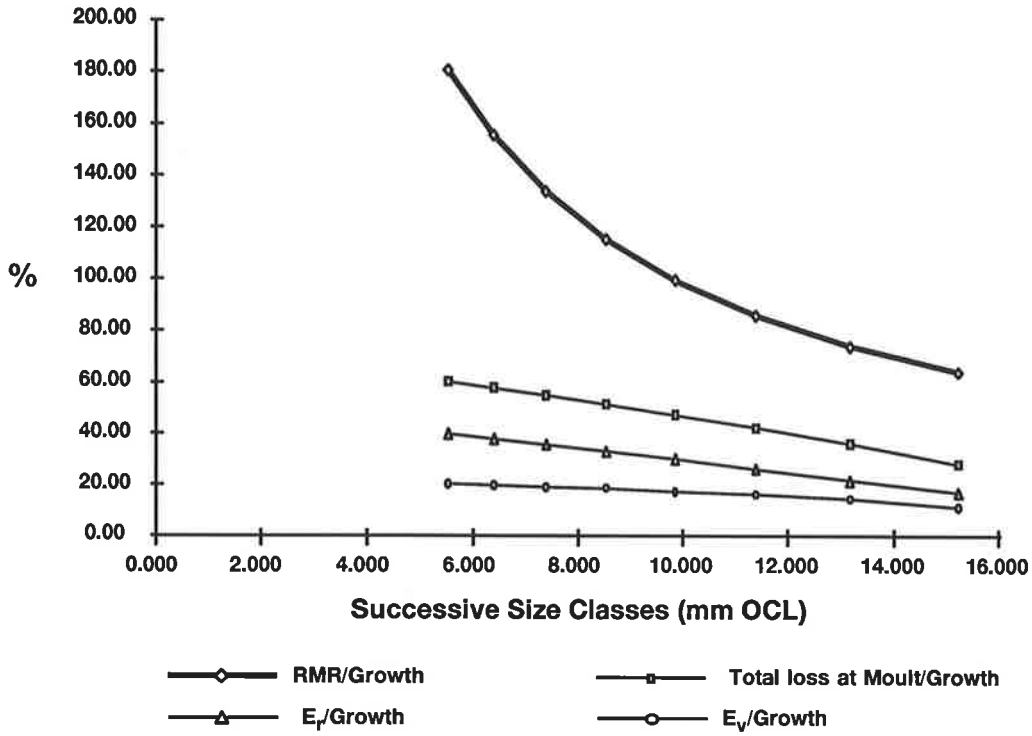


Fig 6.2b)
Relationship between Growth and i) Metabolic cost (RMR), ii) Total Loss at Moult, iii) Exuvial Loss (E_v) and iv) Metabolism associated with the Moult (E_r) during growth from 5 to 15 mm OCL at 20°C

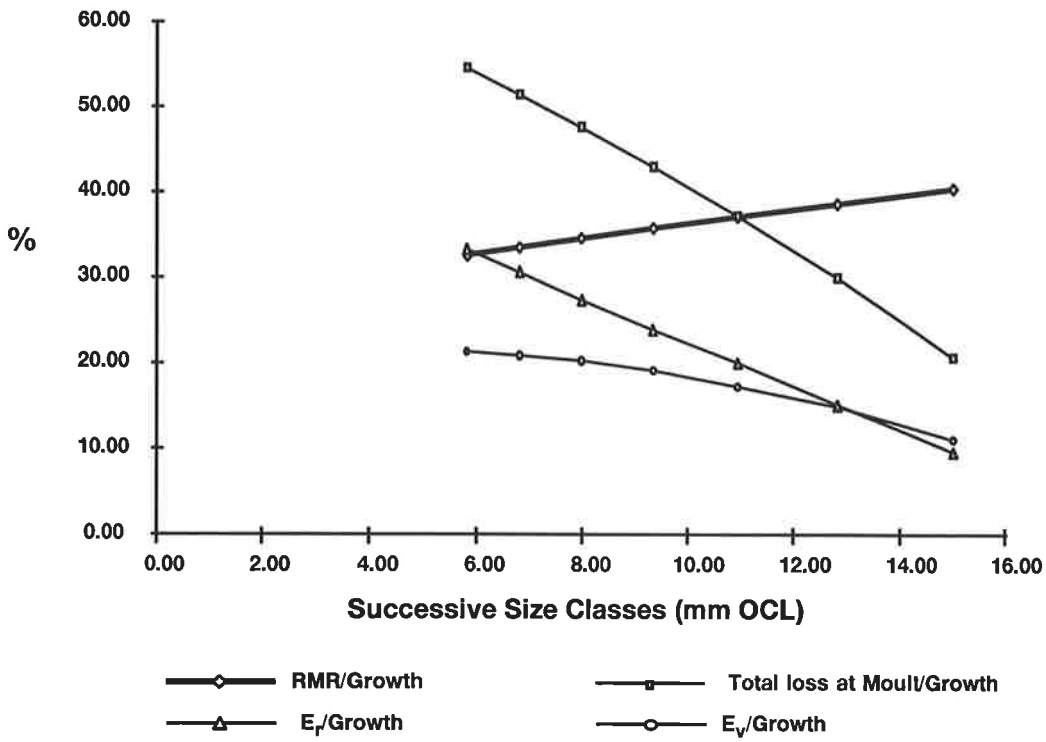


Fig 6.2c)
Relationship between Growth and i) Metabolic cost (RMR), ii) Total Loss at Moult, iii) Exuvial Loss (E_v) and iv) Metabolism associated with the Moult (E_r) during growth from 5 to 15 mm OCL at 25°C

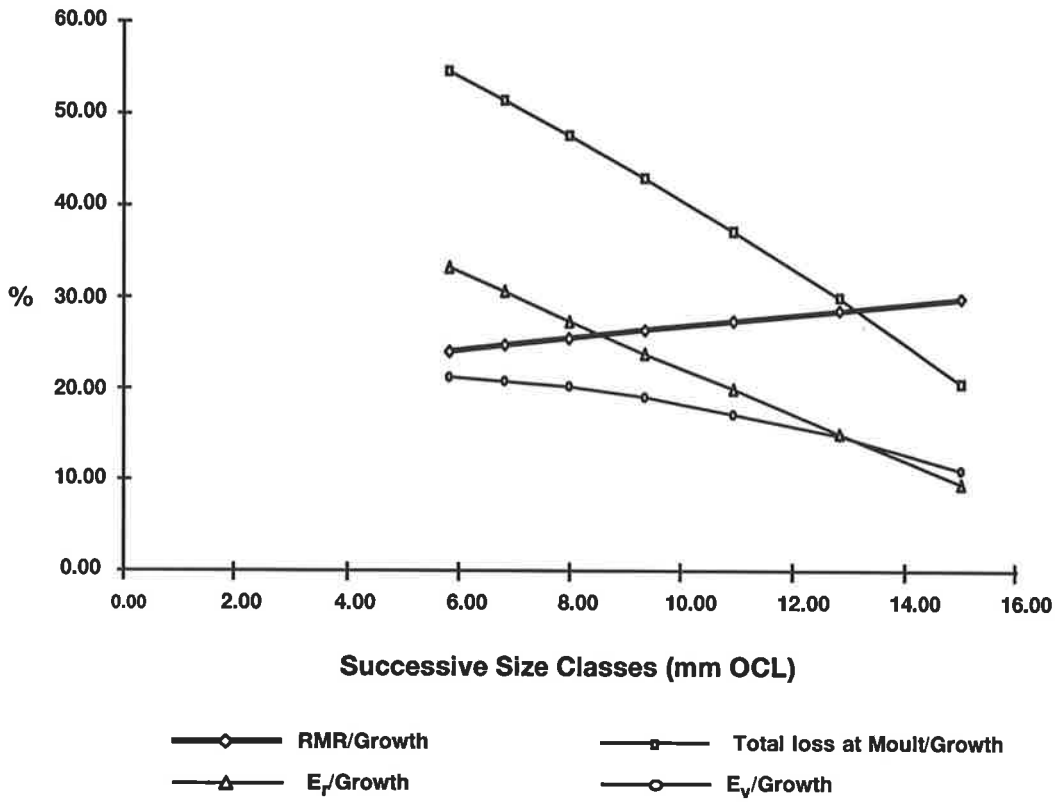


Fig 6.3a
Daily Energy Assimilation (Joules/day) during Growth from 5 to 15 mm OCL at three Temperatures

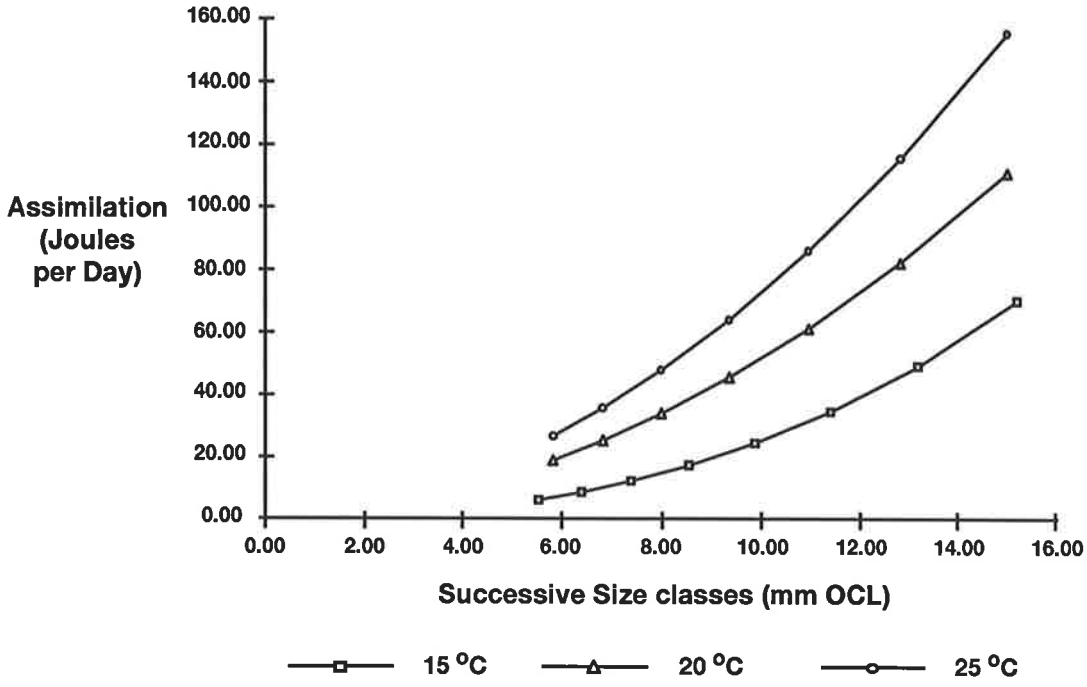


Fig 6.3b
Weight-Specific Daily Energy Assimilation (Joules/mg afdw/day) during growth from 5 to 15 mm OCL at three Temperatures

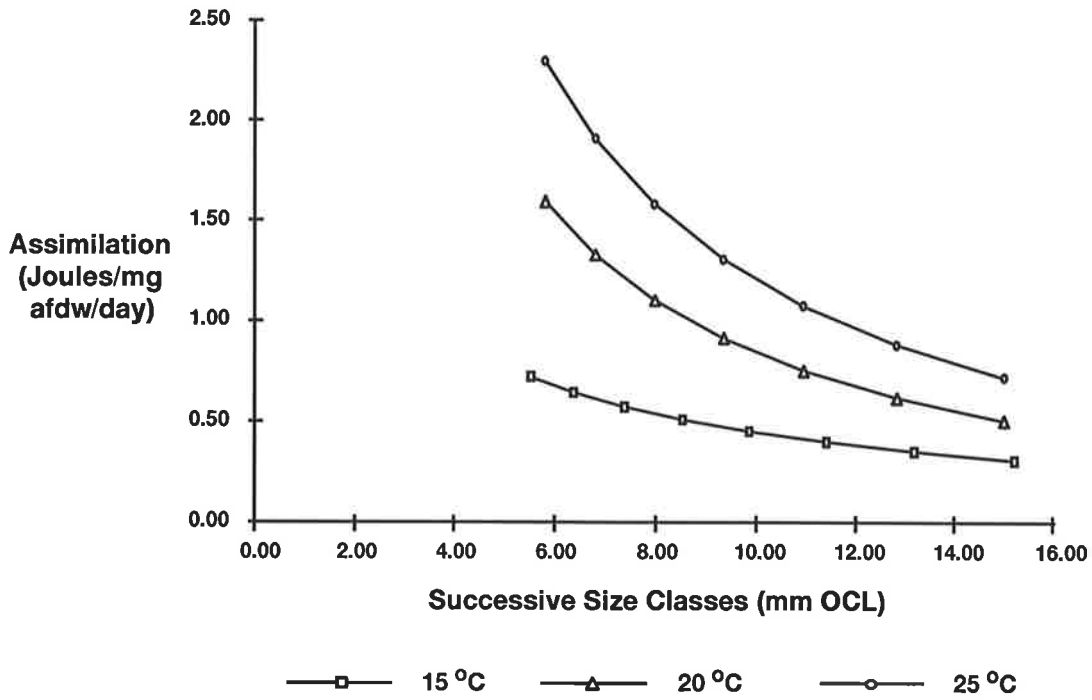
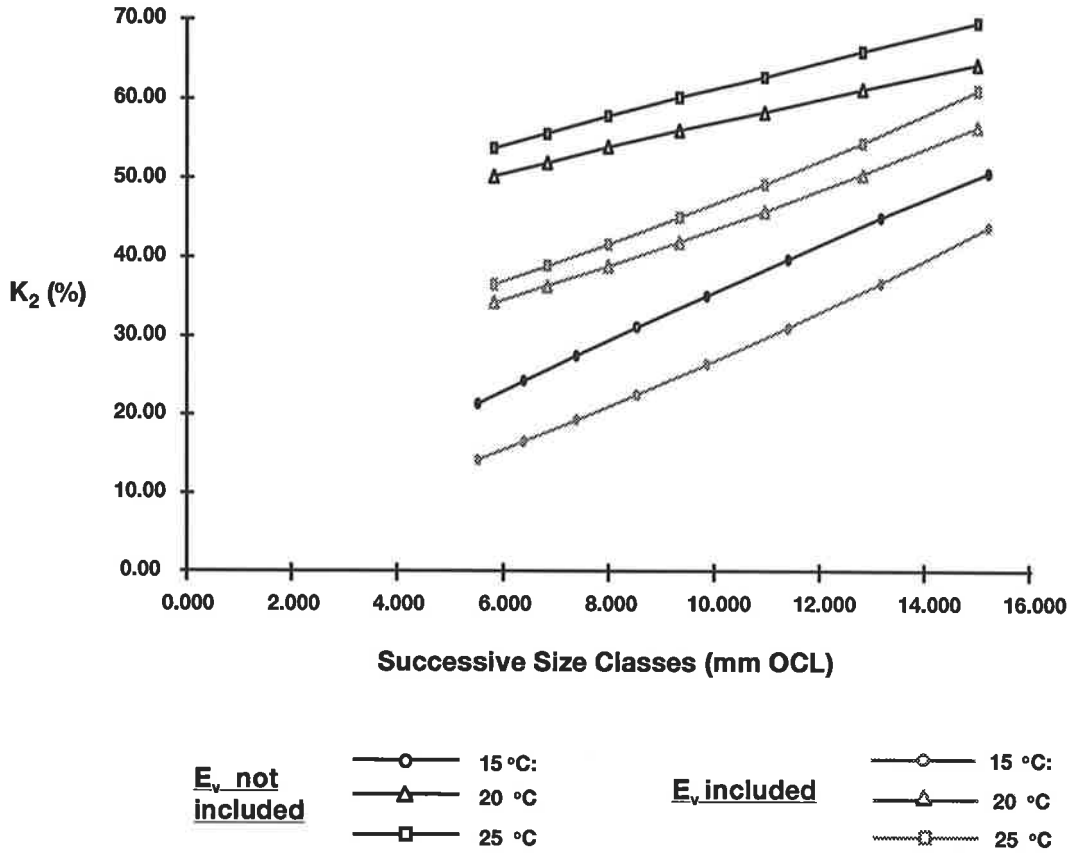


Fig 6.4
Net Growth Efficiency (K_2) vs OCL (mm) with and without Exuvia (E_v). Black symbols and lines = with E_v, grey symbols and lines = without E_v



15°C. K_2 also increases with size from 50 to 64% at 20°C and from 54 to 70% at 25°C over the same size range. If K_2 is calculated without the exuvia the values are substantially lower but show the pattern of increase with size from 14 to 45% at 15°C, from 34 to 57% at 20°C and from 37 to 61% at 25°C.

6.3.2 Field Energy Budget

Table 6.1a and b describe net growth efficiency for each container for the harvests after 15, 30 and 53 days and for the growth periods between 16 to 30 days and 31 to 53 days respectively. The tables contain four estimates of K_2 for each of the five periods, involving inclusion or not of E_v and estimates made with and without the activity component. Mean net growth efficiency per container varied from 36 to 75%, depending on whether or not exuviae were included in growth and on whether an activity component was added to RMR. The inclusion of different components such as the exuvia as production, and extra metabolism related to field activity, have substantial effects on K_2. If metabolism is restricted to the laboratory RMR and the exuvia is included in growth, then mean K_2 varied between 40 and 75%. However, if RMR is increased by 50% and the exuvia not included then mean K_2 per container ranged between 18 and 59%. If E_v is included and RMR increased by 50%, mean K_2 at Harvest 1 (1-15 days) is 48%, at Harvest 2 (1-30 days) it is 59% and at Harvest 3 (1-53 days) it is 68%. No matter how K_2 is calculated mean K_2 increases with successive harvests (**Table 6.1a**). Mean K_2 also increases when the values from the periods 0 to 15, 16 to 30 and 31 to 53 days are considered (**Table 6.1a and b**). If E_v is included and RMR increased by 50%, mean K_2 is 48% for the first period, 62% for the second period and 68% for the third period.

Fig 6.5a presents a model for daily caloric assimilation based on average individual growth calculated for the periods 0-15 days, 16-30 days and 31-53 days. The figure shows estimated mean individual daily energy use from 3.1mm (14 mg wet weight) to 12.5 mm OCL (1 g) calculated using the mean growth rates (Chapter 3, Table 3.3b) and mean temperatures, 18.43°C ± 0.45, 20.67°C (± 0.20) and 21.84°C (± 0.23) for the relevant periods. Daily energy use ranged from 7 Joules/day on day 1 to 348 Joules/day on day 53. Increasing RMR

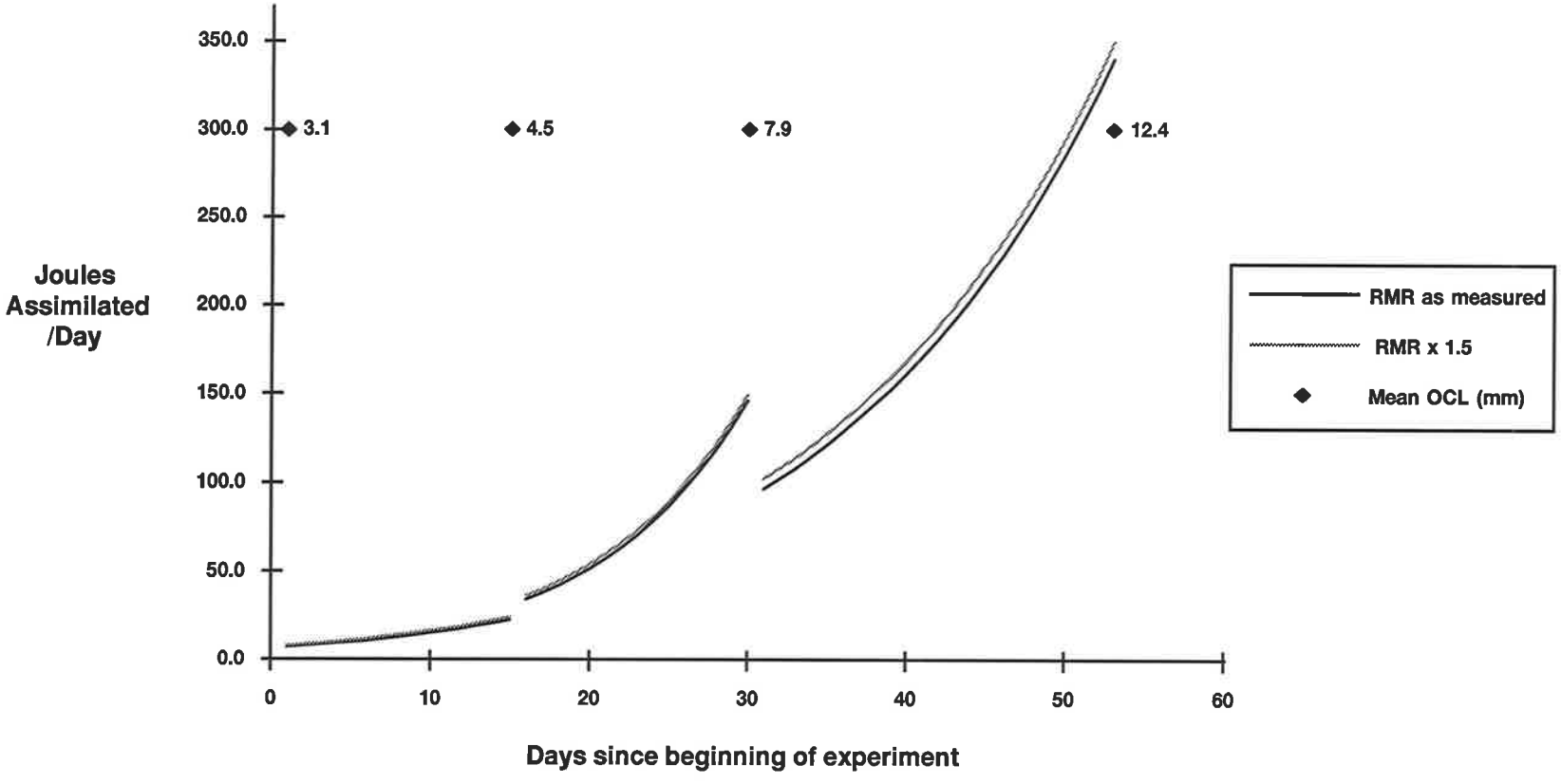
Table 6.1a Mean Net Growth Efficiency (K_2) for each Container (C) and Harvest (H)(±SE). Harvests 1,2, and 3 were carried out after 15, 30 and 53 days respectively. Metabolic cost (RMR) has been multiplied by 1.5 in the second K_2 estimate to approximate activity cost (refer text). E_v = Exuvia

	Mean K_2 for each Container				Mean K_2 for each Harvest			
Harvest/ Container	E_v included in growth and RMR as measured	E_v included and RMR x 1.5	E_v lost and R as measured	E_v lost and RMR x1.5	E_v included and RMR as measured	E_v included and RMR x 1.5	E_v lost	E_v lost and RMR x 1.5
H1C1	60.56	55.56	50.64	46.46				
H1C2	57.89	48.14	47.76	39.72				
H1C3	50.06	45.64	40.37	36.81				
H1C4	40.00	36.41	20.40	18.57	53.28	47.98	40.67	36.58
H1C5	57.91	54.15	44.19	41.32	(3.76)	(3.43)	(5.35)	(4.77)
H2C1	56.83	53.78	38.39	36.33				
H2C2	56.98	54.78	50.27	48.07				
H2C3	64.45	61.57	49.25	47.05				
H2C4	65.75	62.92	51.11	48.91	61.90	59.14	47.95	45.78
H2C5	65.48	62.64	50.73	48.53	(2.05)	(2.00)	(2.41)	(2.38)
H3C1	65.55	62.58	48.46	46.26				
H3C2	70.57	67.81	55.96	53.78				
H3C3	72.56	69.92	58.95	56.80				
H3C4	71.25	68.54	56.99	54.82	70.61	67.86	56.02	53.85
H3C5	73.09	70.48	59.75	57.61	(1.34)	(1.40)	(2.01)	(2.02)

Table 6.1b Mean Net Growth Efficiency (K_2) for each Container (C) for the growth periods between days 16 - 30 and 31 - 53 (±SE).
The period day 1- day 15 is described by data for Harvest 1 above. Metabolic cost (RMR) has been multiplied by 1.5 in the second K_2 estimate to approximate activity cost (refer text). E_v = Exuvia

	Mean K_2 for each Container				Mean K_2 for each Growth Period			
Harvest/ Container	E_v included in growth and RMR as measured	E_v included and RMR x 1.5	E_v lost and R as measured	E_v lost and RMR x 1.5	E_v included and RMR as measured	E_v included and RMR x 1.5	E_v lost	E_v lost and RMR x 1.5
Day 16 - 30								
H2C1	56.26	53.75	35.70	34.11				
H2C2	66.26	63.96	50.41	48.66				
H2C3	65.44	63.12	49.20	47.45				
H2C4	66.94	64.67	51.41	49.66	64.31	61.97	47.53	45.82
H2C5	66.63	64.35	50.95	49.21	(2.03)	(2.07)	(2.98)	(2.95)
Day 31 - 53								
H3C1	61.80	58.39	42.64	40.28				
H3C2	71.17	67.85	57.16	54.49				
H3C3	73.71	70.56	60.93	58.33				
H3C4	75.28	72.25	63.26	60.71	71.27	68.06	57.18	54.63
H3C5	74.37	71.27	61.91	59.33	(2.46)	(2.53)	(3.78)	(3.73)

Fig 6.5a
Mean Individual Daily Assimilation (Joules) over 53 days (12mg to 1g)



by 50% elevated these figures to 8 and 361 Joules/day respectively. In order to relate daily assimilation to size, **Fig 6.5a** also includes mean initial OCL and successive means for each harvest.

Total respiration, assimilation, net growth and losses at the moult were also calculated for the above growth periods. Total respiration and assimilation were calculated by summing their respective daily values for the periods 0 to 15, 16 to 30 and 31 to 53 days. Net growth in joules (caloric increment) was calculated by subtracting joules at day 53 from joules at day 1. After 53 days the caloric increment for an average sized individual was estimated as 3242.8 joules and the joules respired were 538.8 when RMR values were used and 808.2 when RMR was increased by 50%. Over the same period there were nine moults and E_v totalled 677.5 joules while metabolic losses at the moult (E_r) totalled 988.9 joules. Thus assimilation was 5448 joules if laboratory RMR was used or 5717 joules if RMR was increased by 50%. Total RMR was 10% of assimilation and 17% of growth. Addition of the activity component to RMR increased these figures to 14 and 25% respectively. Addition of E_r to RMR increased the contribution of respiration to between 28% and 31% of assimilation and between 47.11 and 55.42% of growth. The figures including the cost of moulting (E_r) give a more realistic partitioning of assimilated energy to metabolism and growth than the use of RMR alone.

Fig 6.5b shows the data in **Fig 6.5a** in weight-specific terms. The data were converted by dividing daily assimilation at Day_n by the weight at Day_n. Estimated weight-specific daily assimilation declined over the 53 day growth period from 4.4-4.6 joules/mg/afdw/day to 2.1-2.2 joules/mg afdw/day. Weight-specific daily assimilation for the first growth period varied between 4.0 and 3.4 joules/mg afdw/day, for the second period it varied between 4.6 and 4.0 joules/mg afdw/day and for the third period it varied between 2.5 and 2.1 joules/mg afdw/day. The elevated assimilation rate during the second growth period is the result of a higher mean growth rate, 0.11 mg/mg afdw/day, during that period compared with 0.087 mg/mg afdw/day for the first period. Increasing RMR by 50% had only a modest effect on weight-specific assimilation.

Figure 6.6 shows the daily RMR in joules for the three growth periods as a percentage of daily assimilation. This is presented for comparison with similar data reported in Chapter 2.

Fig 6.5b
Weight-specific Assimilation (Joules/mg afdw/day) over 53 Days

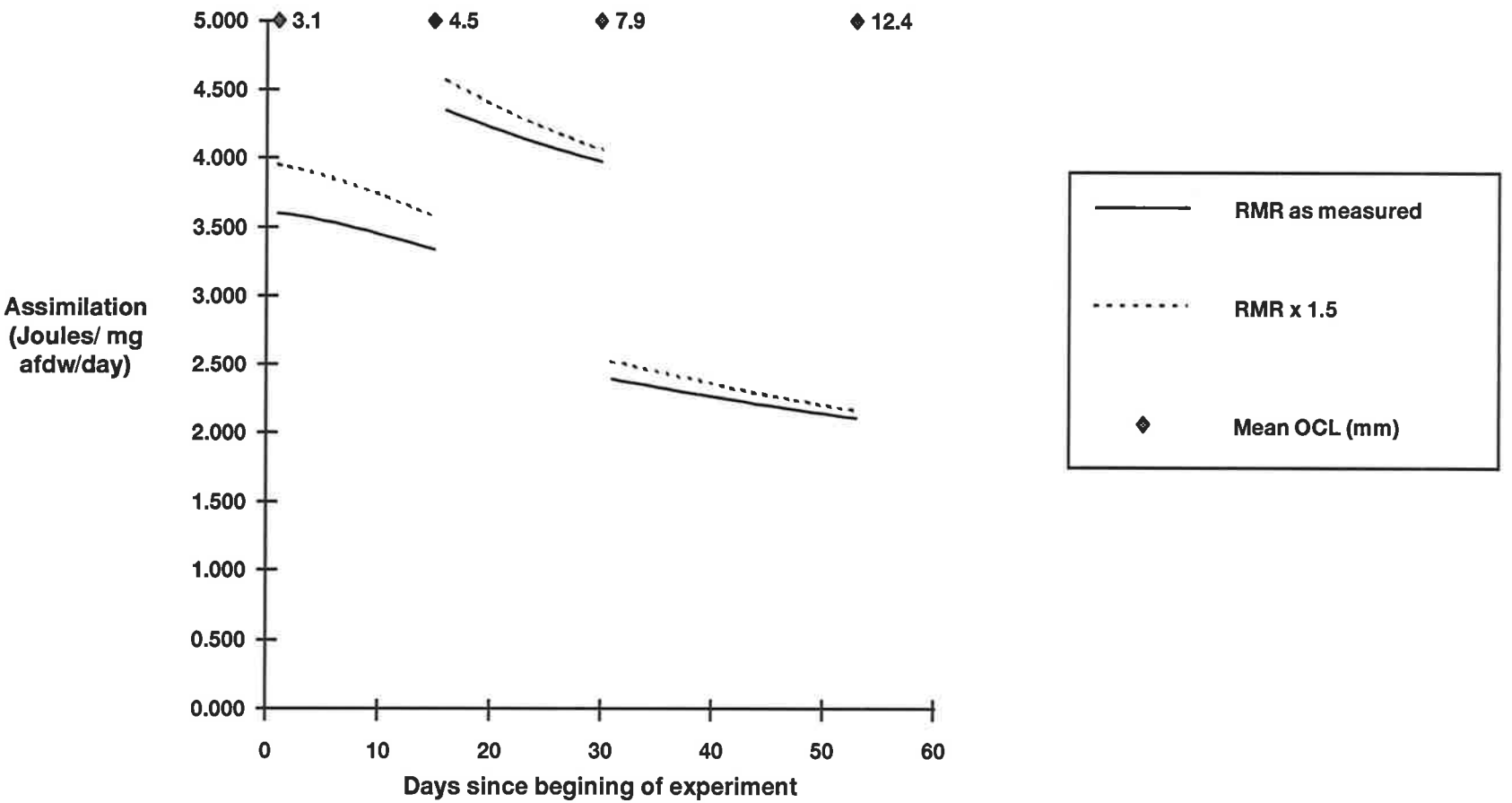
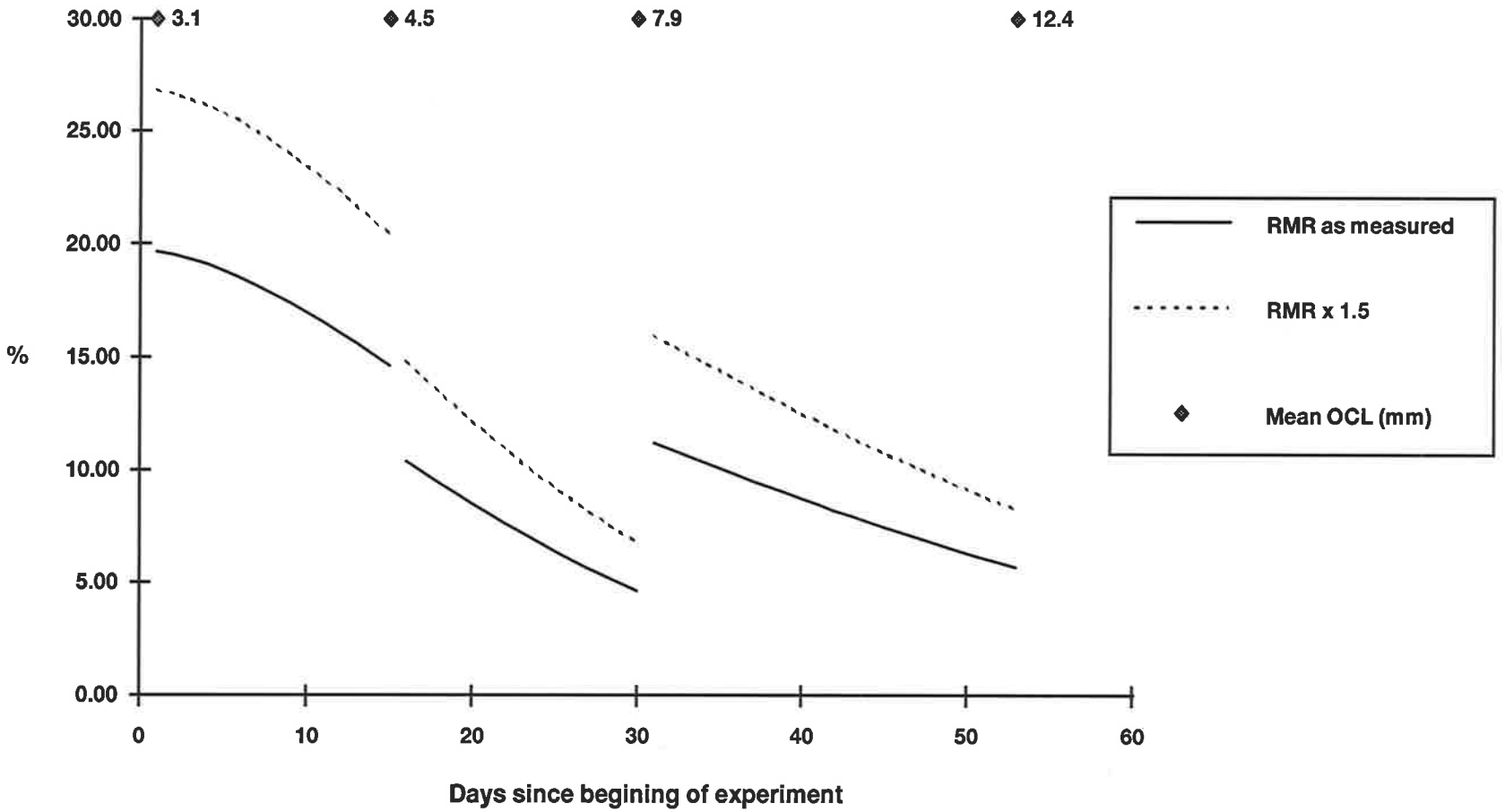


Fig 6.6
Routine Metabolic Rate (RMR) (Joules) as a percentage of Assimilation (Joules)



(see discussion). RMR declined from 19-26% to 6-8% of daily assimilation over the 53 day period and was also affected by the period-specific mean growth rate. Without the activity component RMR decreased from 19 to 15% of assimilation for the first growth period, from 11 to 5% for the second period and from 12 to 6% for the third period. If metabolism is increased by 50% to account for activity RMR declines from 26 to 21% for the first period, 15 to 7% for the second period and 16 to 8% for the third period.

Fig 6.7 shows the extent of individual variability in assimilation that relates to individuals with different growth rates. Daily caloric assimilation was estimated for the largest, smallest and average sized individual at harvest after 53 days. The average temperature for the whole period, 20.54°C (± 0.3) was used in the calculations. Growth rates calculated from these extremes of final size, 19 mm and 7 mm final OCL, were about 0.11 mg/mg afdw/day and 0.04 mg/mg afdw/day respectively, compared to the mean growth rate of 0.084 mg/mg afdw/day (Chapter 3, Table 3.3a). By the end of the growth period a yabbie growing at 0.11 mg/mg/day was assimilating energy at a rate of 1778.9 joules/day, one growing at 0.084 mg/mg/day was assimilating at 429.7 joules/day and one growing at 0.04 mg/mg/day was assimilating at 34.5 joules/day. Thus individual growth rates produce widely differing energy requirements. Increasing RMR by 50%, to account for activity, has little effect on these estimates.

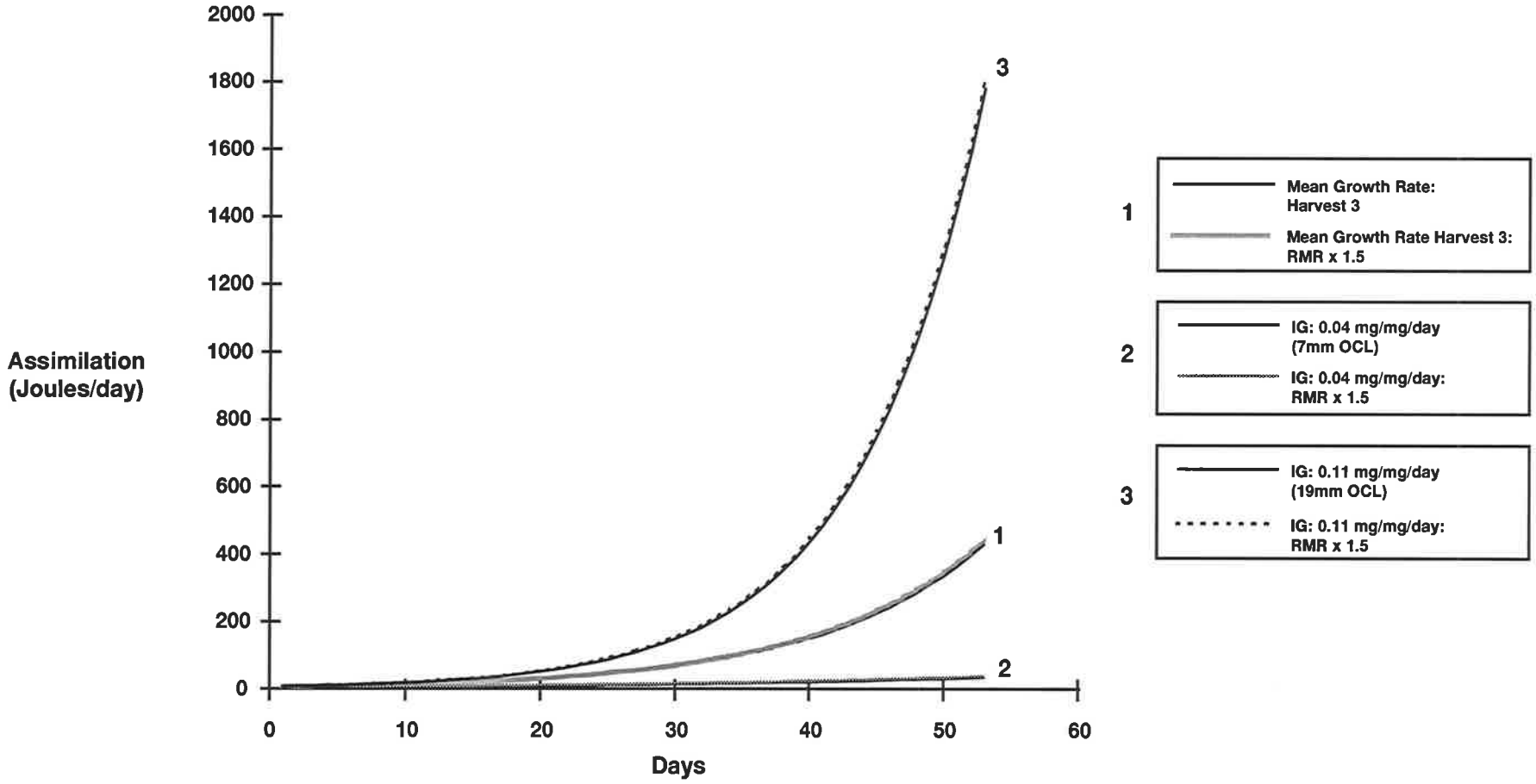
6.4 Discussion

Note that the estimates for growth efficiency and the models of energy use calculated in this chapter relate to an 'average' juvenile yabbie as they were based on previously derived equations describing average RMR, growth, E_v and E_r.

6.4.1 Routine Metabolic rate and Growth in the Laboratory

In the laboratory, the 20° and 25°C treatments showed an increase in the RMR:Growth ratio with size. This is to be expected as maintenance costs increase as tissue accumulates. Metabolic heat production measured as RMR results from new tissue synthesis (= growth), 'established' tissue maintenance, activity and the cost of digestion (SDE) (Brett and Groves,

Fig 6.7
The effect of Mean, Maximum and Minimum Growth on Mean Individual Daily Assimilation (Joules/day) (see text)



1979, Parry, 1983). In juveniles growth is responsible for much of the heat production but as size increases growth rate, and therefore the cost of growth, declines while the amount of established tissue, and therefore the cost of maintenance, increases (Parry, 1983). Thus the ratio of RMR to growth increases with size as maintenance costs increase while growth rate and therefore the relative amount of new tissue production declines with size.

In the present data set there is a decline in RMR:growth ratio with size in the 15°C treatment. This variation is probably an artifact of the data set. Although the instantaneous growth rate at 15°C was independent of size, RMR showed a size-specific decline. Thus tissue appeared to accumulate at a constant rate whereas metabolism was limited by size. The resulting decline in the RMR:Growth ratio with size may have been an artifact of the variability within the growth rate data.

As discussed in Chapters 4 and 5, metabolism and growth were depressed at 15°C. However the RMR:growth ratio calculated in this chapter shows that temperature limited growth more than metabolism. This suggests that metabolic expenditure at this temperature was more the result of tissue turnover (maintenance) than new tissue synthesis. The small contribution of growth to the energy budget at 15°C also accounts for the relatively low net growth efficiencies calculated for this temperature.

6.4.2 Net Growth Efficiency

If the exuvia is included as production in the estimate of net growth efficiency, and RMR is considered without the activity component, mean K_2 ranged from 53 to 71% for the harvests after 15, 30 and 53 days. It was also equal to 64% and 71% for growth periods from 16 to 30 days and 31 to 53 days respectively. If activity is added to RMR, K_2 ranges from 48 to 68% for the harvests and was equal to 64% and 71% for the two growth periods. This range of growth efficiencies places juvenile yabbies close to the range of calculated best possible conversion efficiencies for growing heterotrophs (70 and 80% : Calow, 1978) and generally within that (50 to 80%) suggested for young post-natal poikilotherms (Calow, 1978). Similar values have been reported for other crustaceans, although none of the following studies considered E_r. For example, Klein Breteler (1975) reported net growth efficiencies of 56 to

76% for juvenile shore crabs (*Carcinus maenas*) and Harms (1987) reported 57 to 83% for larval barnacles (*Elminius modestus*).

High growth efficiencies have also been reported for juvenile freshwater crayfish. The mean values estimated in the present study which include E_v and RMR as measured (53 to 71%), were generally higher than similarly derived values calculated for *C. albidus-destructor* in an earlier study by Woodland (1967) but lower than those reported for sub-yearlings of the freshwater crayfish *Astacus leptodactylus* by Tcherkashina (1977). Woodland calculated net growth efficiencies varying from 26 to 61% and a mean of 54% for a dam population of *C. albidus-destructor* with the highest values corresponding to high juvenile biomass. Woodland (1967) did not consider E_r or the metabolic cost of activity so the actual values were probably lower than those reported. As the present study concentrated exclusively on juveniles which were raised in warm mean temperatures with plentiful food the slightly higher values appear reasonable. Tcherkashina reported a value of 75% for sub-yearlings raised in culture ponds and a decline with increasing size to yearling crayfish which showed a K_2 of 21%.

Other workers have reported much lower values for crayfish production. For example, Jones and Momot (1983) reported an average K_2 of 23.5% for a lake population of *Orconectes virilis* and Mason (1975) estimated an average of 29% for a creek population of *Pacifastacus leniusculus*. Neither value can be compared directly with this study as they refer to whole populations but both are much lower than Woodland's mean of 54% where the methods were comparable. As suggested by Jones and Momot (1983), *C. destructor* is one of the more efficient crayfish species. This is particularly obvious when comparing the ratios between respiration and growth. Jones and Momot (1983) reported that growth was 13% of respiration (their Table 4) for young of the year. As discussed in relation to the field energy budget this study found that RMR was between 17 and 25% (with E_r: 47 to 55%) of growth or in other words, growth was between 401% and 602% (with E_r: 181 and 212%) of respiration. Both studies recorded similar temperatures (19-20^{o}C). The estimated percentage contribution of respiration to assimilation in this chapter is similar to that measured in Chapter 2. The mean size after 53 days in the present experiments was about 1 g which was the minimum size used in the experiments in Chapter 2. In the present model at 1 g RMR was estimated to be

between 6 and 8% of assimilation which is very similar to the figures recorded in Chapter 2 for similar-sized animals, 7.6% and 8.0%.

This study suggests that K_2 increases as juvenile yabbies grow. Previous reviews on a wide variety of organisms (Calow, 1977, Parry, 1983), including crustaceans, suggest that postnatal growth efficiency should decline as the system matures and reaches the non-growing phase. K_2 falls because progressively more energy is diverted to maintenance as an animal grows. While this certainly would apply to yabbies over a broad size range from egg to adult, this study suggests a modification to the pattern of declining efficiencies with size as follows. Embryonic growth efficiency would presumably be extremely high as most of the yolk appears to be converted to tissue. However, once a crayfish begins to moult, K_2 declines considerably because of losses associated with Q_m, and especially the component E_r. This is followed by an increase in K_2 with size which is a result of a decline in the percentage of tissue lost at each moult. Q_m is inversely proportional to size, therefore as the loss per moult declines with size, K_2 increases. The laboratory budget for yabbies suggests that between 60 and 21% of tissue accumulated between moults may be lost at the moult within the size range 5 to 15 mm OCL. Between 21 and 11% may be lost with the exuvia (E_v) and the remainder probably represents tissue catabolism, E_r (ref Chapter 3). This decrease in Q_m must reach a point where it is balanced by the declining size-specific growth rate so that K_2 will begin to fall with increasing size.

Tissue which is lost at the moult must be replaced during the subsequent intermoult period. For example Chapter 3 suggested that 40.8% of the energy accumulated in the field by a 3.6 mm OCL yabbie during its moult cycle must go toward re-establishing losses incurred when moulting from 3.1 mm OCL. It is suggested that the energy lost at the moult is a function of the energetic requirements of the moulting process and is independent of growth rate. Thus the need to re-accumulate moulting losses will contribute to the lengthened intermoult period of a slow growing animal. This slow growth rate will be exacerbated by the need to re-accumulate a fixed size-specific loss at the moult. The re-establishment of tissue losses will reduce the energy available for the accumulation of protein and carbon for tissue growth and the next moult. Given that there are substantial losses at the moult within the juvenile phase it is easy to see how a suboptimal diet will rapidly effect growth of juvenile crayfish.

b) Growth and growth efficiency

The conditions described in the field experiment produced a great variation in growth rates and final size (7 mm to 19 mm OCL). This suggests widely differing assimilation rates and perhaps differences in growth efficiencies. Such variability in growth rate within a cohort is common in yabbies (Mills and McCloud, 1983, Geddes *et al*, in press). It is suggested that refinement of feeding regime, nutrition, and container design may lessen the variability, although it does appear to be an innate feature of the species. Such variability may even be advantageous with respect to population survival in a natural system where different sized yabbies may exploit slightly different microhabitats (i.e. food sources, burrows etc). It may also be fostered by diversity within a particular habitat or even within an apparently 'homogeneous' culture system.

Variation in growth efficiency must come about as a result of a change in either Q_p, E_v, E_r or RMR. It is suggested that Q_p is the variable element, affected by innate differences in growth rate. It is also suggested that the energy in Q_m (E_v and E_r) is relatively constant, determined by size but not growth rate effects. If Q_m is constant the relationship between it and Q_p will affect growth efficiency. Thus the K_2 of a fast growing yabbie of a given age must be higher than that of a slow growing individual of the same age because of the contribution of Q_m relative to the growth rate.

Variation in the balance between maintenance and the cost of growth within RMR also may affect K_2. As growth accounts for a large proportion of metabolic activity in juveniles (Parry, 1983) it would be expected that faster growth would produce a higher metabolic rate. However, it is possible that faster growers are more efficient than average and conversely that slower growers are less efficient. Improvements in efficiency leading to faster growth may be achieved by suppression of energy consuming functions of maintenance (Wieser and Medgyesy, 1990). Wieser and Medgyesy (1990) discussed this possibility, called 'sequential energy allocation', in relation to the larvae and juveniles of the roach (*Rutilus rutilus*) and suggested that it may apply to other small poikilotherms. They suggested that some metabolic energy could be re-allocated from maintenance to somatic growth. They also suggested that at the highest growth rates there may also be increases in 'efficiency of synthesis and deposition

of body proteins'. If maintenance energy was re-allocated to growth then the net effect would be smaller than expected differences between the metabolic expenditure of fast and slow growers. This remains to be tested for freshwater crayfish.

c) Metabolism and growth efficiency

There is a surprisingly large fraction of total metabolism involved in the moulting period with E_r values as high as 989 Joules, representing up to 183% of total RMR (without the activity component) over the growth period. The conditions under which RMR was measured may have influenced its relationship with the other energy budget parameters. As is evident from the methods in Chapter 5, RMR was measured under conditions which were intended to be as 'stress-free' as possible. All animals were fed prior to testing and kept in low light, low vibration conditions during acclimation and experimental periods. It may be that this reduced the level of metabolism to near SMR (standard metabolic rate) + Q_s + Q_g. Although it was anticipated that extrapolation to the field would underestimate field metabolism, the conditions in the laboratory may have increased the degree of underestimation. The low RMR may also account for the relatively small impact that metabolic costs resulting from activity had on K_2.

Other workers have used laboratory-derived metabolic data to estimate field metabolism (i.e. Klein Breteler, 1975, Jones and Momot, 1983, Paul and Fuji, 1989) and as suggested by Lampert (1984), such extrapolation may be the best way to estimate such data. He suggested that the alternative *in situ* measurements often do not truly represent field metabolism because of the need to handle animals then enclose them in clean water without food. Thus such measurements are really laboratory experiments in the field but with many more variables contributing to the resulting data. Lampert (1984) concluded that it is better to measure metabolism in the laboratory where such variables can be controlled and their effects separated before extrapolation to the field. Although this does appear to be the best procedure available, a problem may arise because of the relationship between metabolic rate and growth rate. Juvenile yabbies and other young ectotherms (Jones, 1976, Calow, 1977, this study) have high growth efficiencies which indicate that much heat production is due to growth not maintenance (Parry, 1983). Thus metabolism is largely a reflection of growth rate and

because growth is generally slower in the laboratory than in the field, metabolic rates measured in the laboratory may under-estimate field metabolism.

It is not known to what extent this may have affected field estimates of RMR in the present study. One way of correcting lab estimates for field conditions may be comparison of the proportional relationship between growth and metabolic cost in the laboratory then application of that ratio to field growth measurements to estimate field metabolism. However, this assumes that the relationship between metabolism and growth does not change with growth rate, nutritional conditions or temperature regime. In the absence of such information it is suggested that, for comparative purposes, metabolic rates measured in the laboratory remain the best way to estimate field metabolism given the number of variables acting on field populations.

Both E_v and E_r may have a significant impact on K_2 in juveniles. The impact of E_r was generally higher than that of E_v within the size range used in this study. With respect to other relevant components of the equation, the field energy budget model suggests that E_r may be 183% or 122% higher than metabolic costs between moults (RMR) depending on whether activity is considered as part of RMR. The contribution of E_r to the energy budget appears extremely high when compared with RMR although other studies have suggested elevations of 50 to 1900% over the late premoult to postmoult period (Skinner, 1962, Aiken and Waddy, 1987). The high values of E_r probably represent tissue catabolism in the chelipeds to allow muscle withdrawal at the moult (Mykles and Skinner, 1985) as well as other metabolic costs associated with the shedding of the exuvia.

6.4.3 Conclusion

The effect of the components of Q_m (E_r and E_v) on the parameters of the energy budget suggests that these should be included when studying larval or juvenile crustaceans. Although E_v is usually included in budgets (i.e. Klein Breteler, 1975, Harms, 1987, Zoutendyk, 1988, Kurmaly *et al* 1989, Paul and Fuji, 1989), E_r has not been considered in such work, presumably because of the difficulty with its direct measurement. The indirect methods and

inferences used in the present study are informative but further direct measurements of metabolism during the moult should be undertaken.

The variability in K_2 estimates produced by inclusion of the exuvia in production or its absence, and the inclusion or absence of the activity component, suggests that growth efficiencies for juvenile crayfish should be presented as a range of values with and without activity and Q_m effects (i.e. E_v and E_r). Considering the assumptions that must be made in the calculation of a K_2, a range of values such as this would be more useful and more accurate than the usual single figure.

Chapter 7

Summary and Conclusions

This study has investigated the effect of temperature, size and diet on growth and metabolism in juvenile *Cherax destructor*. Growth under a range of conditions has been compared and the process of tissue and energy accumulation and use during the moult cycle described. It is suggested that the models constructed here are of use in better understanding the processes of growth and energy use in juvenile freshwater crayfish as well as the interactions between temperature, size, growth and metabolism. The following summarises the main points discussed within each chapter.

In Chapter 2 three coarse isocaloric diets were investigated to determine their suitability in growth and energy use experiments in *C. destructor*. The effect of dietary protein content on consumption and the effect of size on consumption and energy requirements was also examined. It was suggested that an unrefined diet containing 35% crude protein with a digestible Protein:Energy ratio of at least 99 mg protein /kcal and sufficient carotenoids is adequate for studies of laboratory growth in this species. It is also suggested that juvenile yabbies feed for energy, as protein level had no effect on consumption. Size-specific consumption and assimilation declined exponentially with size, providing a model describing how feeding levels should be adjusted for size within the juvenile phase. There was a close correlation between size-specific metabolic rate and assimilation so knowledge of the metabolic rate at a particular size and temperature may allow estimation of the total energy use (i.e. assimilation in so many joules/g/day) and thus the energy requirements for growth which may be extrapolated between sizes and temperatures within a diet type.

Chapter 3 investigated field growth of stage three to 3 g juvenile *C. destructor* under semi-intensive aquaculture conditions for comparison with other studies. The 35% protein diet used in Chapter 2 was also used here in addition to naturally occurring food. Yabbies from the experiment were subjected to moult stage-specific and size-specific proximate analysis which allowed development of realistic models of juvenile yabbie tissue accumulation and use. These models included moult stage-specific and size-specific wet weight, dry weight and water accumulation. The dry weight models were extended to models of moult stage and size-

specific ash and organic content. Finally, the organic content model was used in conjunction with other analyses to examine moult stage and size-specific fluctuations in carbon, protein and energy components of the tissue.

Mean growth per container varied between 0.08 and 0.086 mg/mg afdw/day over the 53 day experimental period and variation in temperature appeared to have a significant effect on growth rates. Mean overall growth (0.084 mg/mg afdw/day, 0.081 mg/mg wet weight/day) was slightly lower than the best growth achieved by Geddes *et al* (in press) for juvenile yabbies, under similar field conditions and at similar temperatures. Their best growth rates averaged 0.095 mg/mg wet weight/day, but individual variability in growth was similar to that in the present study as their animals ranged in size from 35 mg to 5 g at the end of the experiment. Mills and McCloud (1983) also raised yabbies under similar conditions and achieved growth rates between 0.08 and 0.1 mg/mg wet weight/day. In their study temperatures ranged between 15 and 28^{o}C.

Proximate composition was similar to that reported in other decapods although there were marked changes in composition over the moult cycle. For example, water content ranged between 75 and 85% which is similar to the range of 67 - 80% described for other freshwater crayfish and other decapods (Hubbard *et al*, 1986, Dupreez and McLachlan, 1983, Villarreal, 1989, Dall *et al* 1991, Nicol *et al* 1992) and caloric content (19 - 22 Joules/ mg afdw) is also close to the range of 20 - 24 Joules/mg reported in the literature (Woodland, 1967, Nelson, 1977, Read and Caulton, 1980, Stephenson and Knight, 1980, Anger *et al*, 1983, Dupreez and McLachlan, 1983, Zoutendyk, 1988). Mean % protein levels reported in this study (47-62%) encompass the range (50-56%) described for four marine crustaceans, including a decapod (Dall *et al*, 1991) but were higher than that reported for Antarctic krill *Euphausia superba* by Nicol *et al* (1992), 33.7-48%.

Ash free dry weight for a 3.1 mm OCL yabbie increased by 2.29 times between postmoult stage A and premoult stage $D_{1.3}$. By D_4 the weight was 2.24 times the weight at A. The organic content of a 19 mm OCL yabbie increased by 2.12 times between A and $D_{1.4}$ and the weight at D_4 was 1.94 times the weight at A. Dry weight showed similar patterns. Water uptake was stage-specific with most occurring between late premoult stage D_3 and postmoult

stage A with the majority taken up over the moult itself. Percent carbon content increased and percent protein content decreased slightly with size during the moult cycle, with most change reflecting changes in total organic content. Energy content (Joules/mg afdw) increased with size during postmoult (A and B) and early intermoult (C_1) declined in late intermoult (C_2) and early premoult ($D_{1.1}$ and $D_{1.2}$) then showed no size effect for the remainder of the moult cycle. Most changes in energy content appeared to have occurred in response to size not moult stage effects. Tissue losses over the moult were significant and it is suggested that this represents the catabolism of protein and some carbon to fuel the non-feeding period prior to, during and just after the moult as well as the energetic requirements of the moult itself. The tissue and energy losses are such that 40.8% of the energy and 32.1% of the tissue accumulated by a 3.6 mm OCL yabbie during its moult cycle must go toward re-establishing material losses incurred when moulting from 3.1 to 3.6 mm OCL. Losses decline with size so by the time a crayfish has reached 17 mm OCL they amount to 16.5% of the energy and 24.1% of the tissue during the intermoult period.

Chapter 4 investigates the effect of temperature on growth and the components of growth in the laboratory. These experiments allowed more accurate estimation of size-specific energetic requirements and expenditure in the laboratory and assisted in the explanation of observed patterns of field growth (Chapter 3). Yabbies of two initial size groups were grown at five temperatures ranging from 15 to 30°C.

Growth rates in these experiments were between two and five times slower than those in the field experiments over the same temperature range (Chapter 3). As percent moult increment was similar in both cases it is suggested that the differences in growth resulted from changes in the length of the intermoult period.

Mean instantaneous growth rates (IG) increased with temperature from 15°C and peaked at about 25°C (0.049 mg/mg afdw/day) for smaller yabbies and about 27.5°C (0.043 mg/mg afdw/day) for larger yabbies. At 20°C IG was 0.0344 mg/mg afdw/day for smaller yabbies and 0.0214 mg/mg afdw/day for larger yabbies. IG also declined with size at all temperatures, except 15°C where it was independent of size (0.0103 mg/mg afdw/day). This independence from size at 15°C and the lower temperature for the growth peak for the smaller yabbies show

that the size-growth relationship changes with temperature, with the effect of temperature on growth more pronounced in smaller individuals. Finally, a multiple regression model was derived describing growth as a function of size for temperature's ranging from 20 to 27.5°C.

Chapter 5 presents work on the effect of temperature on the respiration rate of the individuals in the growth experiment (Chapter 4). The range of mass exponents (b = 0.6685-0.7206) for the relationship between MO2 (ug/h) and weight (mg) was lower than the average reported for 36 other species of decapod crustacean (0.751 - Wheatly, 1989) but within the range reported for marine crustaceans (0.6 - 0.79 - Ivleva, 1980).

The Q_{10} (2.24 -2.57) calculated for the interval 15-20°C agrees with those reported for similar temperatures in studies on other aquatic decapods (2 - 2.5, McMahon *et al*, 1978, De Fur and Mangum, 1979) and for crustaceans in general (Ivleva, 1980, Q_{10}=2.17-2.45). In the present study the figure for the interval 20-25°C (1.3-1.4) is lower than that found by Ivleva (1980, Q_{10} = 2.11) and by Zoutendyk (1989, Q_{10} = 1.7) for lobsters but similar to that reported by McNamara *et al* (1985, Q_{10} = 1.19) for freshwater shrimps.

A multiple regression equation was derived to describe the relationship between size and metabolic rate for temperatures between 15 and 25°C. Coincidence between observed values and estimates derived from this equation suggested that the multiple regression approach was adequate when calculating metabolic energy expenditure in laboratory and field studies.

Chapter 6 presents a partial energy budget which synthesizes the work in preceding chapters. The energy budget equation used is:

$$Q_c - (Q_u - Q_v) = Q_p + Q_g + Q_s + Q_d + Q_a + E_r + E_v$$

where Q_c = ingested energy, Q_u = faeces and Q_v = nitrogenous waste products. Assimilated energy in the form of carbohydrates, lipids and protein may be converted to tissue (Q_p) or used in metabolism which includes Q_g, the cost of growth, Q_s, the cost of maintenance, Q_d, the cost of digestion and assimilation, Q_a, the cost of activity and E_r, the metabolic cost of moulting. Assimilated energy may also be used in production of Ev, the exuvia. E_r and E_v may be grouped as Q_m, the caloric losses at the moult.

Net growth efficiencies (K_2, Production/Assimilation) and models of daily energy assimilation are calculated for the laboratory growth experiments at each of three temperatures (Chapter 4) and for the field growth experiments (Chapter 3). Daily growth and metabolic cost was estimated for the laboratory budget using the multiple regression equations from Chapters 4 and 5 respectively. Metabolic cost for the field budget was estimated using the laboratory-derived metabolic rates but with a component added for increased field activity.

The laboratory budget suggests that growth efficiency increases with size which modifies the accepted idea that postnatal growth efficiency should decline as the system matures (Calow, 1978). It is shown that this anomaly arises because juvenile crayfish maintained fast growth over the size range studied while the contribution of metabolic losses at the moult (E_r) declined markedly with size. Both loss of exuvia (E_v) and the metabolic cost of moulting (E_r) have a significant impact on K_2 in juveniles. The impact of E_r was generally higher than that of E_v and may also be considerably higher than the RMR measured between moults. This suggests that measurement of metabolic costs for an energy budget for juvenile crayfish should include E_r so as not to under-estimate this component of the budget and thus over-estimate growth efficiency.

In addition to E_r, the variability in K_2 estimates produced by the presence or absence of E_v and, to a lesser extent, activity, suggests that growth efficiency estimates for juvenile crayfish should be presented as a range of values. Considering the assumptions that must be made in the calculation of a K_2, a range of values such as this would be more useful and more accurate than the usual single figure.

References

Ackefors,H., Castell,J.D., Boston,L.D., Raty,P., Svensson,M. (1992): Standard experimental diets for crustacean research II. Growth and survival of juvenile crayfish *Astacus astacus* (Linne) fed diets containing various amounts of protein, carbohydrate and lipid. Aquaculture 104, 341-356.

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

Aiken,D.E., Waddy,S.L. (1992): The growth process in crayfish. Reviews in Aquatic Sciences 6, 335-381.

Anderson,W.R., Ford,R.F. (1976): Early development, growth and survival of the yellow crab *Cancer anthonyi* Rathburn (Decapoda:Brachyura) in the laboratory. Aquaculture 7, 267-279.

Anger,K. (1988): Growth and elemental composition (C,H,N) in Inachus dorsettensis (Decapoda: Majidae) larvae reared in the laboratory. Marine Biology 99, 255-260.

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

Anger,K., Harms,J., Puschel,C., Seeger,B. (1989): Physiological and biochemical changes during the larval development of a brachyuran crab reared under constant conditions in the laboratory. Helgolander Meeresunters 43, 225-244.

Anger,K., Laasch,N., Puschel,C., Schorn,F. (1983): Changes in biomass and biochemical composition of spider crab *Hyas araneus* larvae reared in the laboratory. Marine Ecology Progress Series 12, 91-101.

Armitage,K.B., Buikema,A.L., Willems,N.J. (1972): Organic constituents in the annual cycle of the crayfish *Orconectes nais* (Faxon). Comparative Biochemistry and Physiology 41A, 825-842.

Barclay,M.C., Dall,W., 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.

Bordner,C.E., D'Abramo,L.R., Conklin,D.E. (1983): Assimilation of nutrients by cultured hybrid lobsters (*Homarus sp.*) fed experimental diets. Journal of the World Mariculture Society 14, 11-24.

Botsford,L.W. (1985): Models of growth. In: Crustacean issues 3 : Factors in adult growth. (Ed: Wenner,A.M.) A.A. Balkema, Rotterdam, 171-188.

Bottrell,H.H. (1974): The relationship between temperature and duration of egg development in some epiphytic Cladocera and Copepoda from the River Thames, Reading, with a discussion of temperature functions. Oecologia 18, 63-84.

Brafield,A.E. (1985): Laboratory studies of energy budgets. In: Fish Energetics (Eds P. Tytler and P. Calow) Croom Helm, London, 257-280.

Brett,J.R., Groves,T.D.D. (1979): Physiological energetics. In: Fish Physiology. Vol. 8. (Eds: Hoar,W.S., Randall,D.J., Brett,J.R.) Academic Press, New York, 279-351.

Brewis,J.M., Bowler,K. (1982): The growth of the freshwater crayfish *Austropotamobius pallipes* in Northumbria. Freshwater Biology 12, 187-200.

Brown,P.B., Tazik,P., Hooe,M.L., Blythe,W.G. (1990): Consumption and apparent dry matter digestibility of aquatic macrophytes by male and female crayfish (*Orconectes virilis*). Aquaculture 89, 55-64.

Brown,P.B., Williams,C.D., Robinson,E.H., Akiyama,D.M., Lawrence,A.L. (1986): Evaluation of methods for determining *in vivo* digestion coefficients for adult Red Swamp Crayfish *Procambarus clarkii* Girard. Journal of the World Aquaculture Society 17, 19-24.

Burton,E.M., Mitchell,B.D. (1987): Moult staging in the freshwater crayfish *Cherax albidus* Clark and *Cherax destructor* Clark via uropod setal development. Australian Journal of Marine and Freshwater Research 38, 545-552.

Calow,P. (1977): Conversion efficiencies in heterotrophic organisms. Biological Reviews 52, 385-409.

Calow,P. (1978): Life as an energy transforming system. In: Life Cycles: An evolutionary approach to the physiology of reproduction, development and ageing. (Ed: Calow,P.) Chapman and Hall, London, 20-36.

Capuzzo,J.M. (1983): Crustacean Bioenergetics: the Role of Environmental Variables and Dietary Levels of Macronutrients on Energetic Efficiencies. In: Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological approaches to Shellfish Nutrition. (Eds: Pruder,G.D., Langdon,C.J., Conklin,D.E.) Louisiana State University, Division of Continuing Education, Baton Rouge, 71-86.

Castell,J.D., Kean,J.C., D'Abramo,L.R., Conklin,D.E. (1989): A standard reference diet for crustacaen nutrition research. I. Evaluation of two formulations. Journal of the World Aquaculture Society 20, 93-99.

Chang,E., O'Connor,J.D. (1983): Metabolism and transport of carbohydrates and lipids. In: The Biology of Crustacea. Vol. 5. Academic Press, New York, 263-287.

Clifford,H.C., Brick,R.W. (1979): A physiological approach to the study of growth and bioenergetics in the freshwater shrimp *Macrobrachium rosenbergii* . Proceedings of the World Mariculture Society 10, 701-709.

Cowey,C.B., Tacon,A.G. (1983): Fish Nutrition - Relevance to Invertebrates. In: Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological approaches to Shellfish Nutrition. (Eds: Pruder,G.D., Langdon,C.J., Conklin,D.E.) Louisiana State University, Division of Continuing Education, Baton Rouge, 13-30.

Cukerzis,J.M. (1988): *Astacus astacus* in Europe. In: Freshwater Crayfish: Biology, Management and Exploitation. (Eds: Holdich,D.M., Lowery,R.S.) Croom Helm, London, 309-340.

D'Abramo,L.R., Robinson,E.H. (1989): Nutrition of crayfish. Reviews in Aquatic Sciences 1, 711-727.

Dall,W. (1986): Estimation of routine metabolic rate in a penaeid prawn, *Penaeus esculentus* Haswell. Journal of Experimental Marine Biology and Ecology 96, 57-74.

Dall,W., Hill,B.J., Rothlisberg,P.C., Sharples,D.J. (1990): The Biology of the Penaeidae. In: Advances in Marine Biology. Vol. 27. (Eds: Blaxter,J.H.S., Southward,A.J.) Academic Press, London, 1-489.

Dall,W., Smith,D.M., Moore,L.E. (1991): Biochemical composition of some prey species of *Penaeus esculentus* Haswell (Penaeidae: Decapoda). Aquaculture 96, 151-166.

De Fur,P.L., Mangum,C.P. (1979): The effects of environmental variables on the heart rates of invertebrates. Comparative Biochemistry and Physiology 62A, 283-294.

Dejours,P. (1975): Principles of Comparative Respiratory Physiology. Elsevier, New York. 253 pages.

Devcich,A. (1979): An ecological study of *Paranephrops planifrons* White (Decapoda : Parastacidae) in Lake Rotoiti, North Island, New Zealand. D.Phil. Thesis, University of Waikato, New Zealand.

Dowgiallo,A. (1975): Calorimetry and body composition: chemical composition of an animal's body and of its food. In: Methods for Ecological Bioenergetics, IBP Handbook No. 24. (Eds: Grodzinski,W., Klekowski,R.Z., Duncan,A.) Blackwell Scientific Publications, Oxford, 160-185.

Drach,P. (1939): Mue et cycle d'intermue chez les Crustaces Decapodes. Annales Institute Oceanographie Paris 18, 103-391.

DuPreez,H.H.D., McLachlan,A. (1983): Seasonal changes in biochemical composition and energy content of the three-spot swimming crab *Ovalipes punctatus* (De Haan) (Crustacea:Brachyura). Journal of Experimental Marine Biology and Ecology 72, 189-198.

Durliat,M., Vranckx,R. (1982): Proteins of aqueous extracts from the hepatopancreas of *Astacus leptodactylus* -1. Changes in proteins during the molt cycle. Comparative Biochemistry and Physiology 71B, 155-163.

Elliot,J.M., Davison,W. (1975): Energy equivalents of oxygen consumption in animal energetics. Oecologia 19, 195-201.

Epp,R.W., Lewis,M.L. (1979): Metabolic responses to temperature change in a tropical freshwater copepod *Mesocyclops brasilianus* and their adaptive significance. Oecologia 42, 123-138.

Fellows,F.C.I., Hird,F.J.R. (1979): Nitrogen metabolism and excretion in the freshwater crayfish *Cherax destructor*. Comparative Biochemistry and Physiology 64B, 235-238.

Flint,R.W. (1975): Growth in a population of the crayfish *Pacifastacus leniusculus* from a subalpine environment. Journal of the Fisheries Research Board of Canada 32, 2433-2440.

Fradd,P.J. (1974): The effect of temperature on the respiration of the yabbie, *Cherax destructor*. B.Sc.(Hons) Thesis, University of Adelaide, South Australia.

Freeman,J.A. (1991): Growth and Morphogenesis in Crustacean Larvae. Memoirs of the Queensland Museum 31, 309-319.

Frost,J.V. (1975): Australian Crayfish. Freshwater Crayfish 2, 87-96.

Geddes,M., Smallridge,M., Clarke,S. (in press): Potential for a nursery phase in the commercial production of the yabbie, *Cherax destructor*. Freshwater Crayfish 8.

Geddes,M.C., Mills,B.J., Walker,K.F. (1988): Growth in the Australian freshwater crayfish, *Cherax destructor* Clark, under laboratory conditions. Australian Journal of Marine and Freshwater Research 39, 555-568.

Gnaiger,E., Bitterlich,G. (1984): Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. Oecologia 62, 289-298.

Goyert,J.C., Avault,J.W. (1978): Effect of stocking density and substrate on growth and survival of crawfish (*Procambarus clarkii*) grown in a recirculating system. Proceedings of the World Mariculture Society 9, 731-735.

Growns,I.O., Richardson,A.M.M. (1988): Diet and burrowing habits of the freshwater crayfish *Parastacoides tasmanicus tasmanicus* Clark (Decapoda:Parastacidae). Australian Journal of Marine and Freshwater Research 39, 525-534.

Gwinn,J.F., Stevenson,J.R. (1973a): Role of acetylglucosamine in chitin synthesis in crayfish - 1. Correlation of C-Acetylglucosamine incorporation with stages of the molting cycle. Comparative Biochemistry and Physiology 45B, 769-776

Gwinn,J.F., Stevenson,J.R. (1973b): Role of acetylglucosamine in chitin synthesis in crayfish - 2. Enzymes in the epidermis for incorporation of acetylglucosamine into udp-acetylglucosamine. Comparative Biochemistry and Physiology 45B, 777-785.

Halcrow,K., Boyd,C.M. (1967): The oxygen consumption and swimming activity of the amphipod *Gammarus oceanicus* at different temperatures. Comparative Biochemistry and Physiology 23, 233-242.

Harms,J. (1987): Energy budget for the larval development of *Elminius modestus* (Crustacea: Cirripedia). Helgolander Meeresunters 41, 45-67.

Hart,R.C. (1980): Oxygen consumption in *Caridinea nilotica* in relation to temperature and size. Freshwater Biology 10, 215-222.

Hartnoll,R.G. (1982): Growth. In: The Biology of Crustacea Vol 2 (Ed: Academic Press), 111-196.

Hartnoll,R.G. (1983): Strategies of crustacean growth. Memoirs of the Australian Museum 18, 121-131.

Heath,J.R., Barnes,H. (1970): Some changes in biochemical composition with season and during the moulting cycle of the common shore crab, *Carcinus maenas* (L.). Journal of Experimental Marine Biology and Ecology 5, 199-233.

Hiatt,R.W. (1948): The biology of the lined shore crab, *Pachygrapsus crassipes* Randall. Pacific Science. 2, 135-213.

Hobbs,H.H. (1988): Crayfish Distribution, Adaptive Radiation and Evolution. In: Freshwater Crayfish: Biology, Management and Exploitation. (Eds: Holdich,D.M., Lowery,R.S.) Croom Helm, London, 52-82.

Holdich,D.M., Lowery,R.S. (1988): Crayfish - an Introduction. In: Freshwater Crayfish - Biology, Management and Exploitation. (Eds: Holdich,D.M., Lowery,R.S.) Croom Helm, London, 1-10.

Hornung,D.E., Stevenson,J.R. (1971): Changes in the rate of chitin synthesis during the crayfish molting cycle. Comparative Biochemistry and Physiology 40B, 341-346.

Hubbard,D.M., Robinson,E.H., Brown,P.B., Daniels,W.H. (1986): Optimum ratio of dietary protein to energy for the Red Crayfish *Procambarus clarkii*. Progressive Fish Culturist 48, 233-237.

Huner,J.V., Avault,J.W. (1976): The moult cycle of the subadult Red Swamp Crayfish *Procambarus clarkii* (Girard). Proceedings of the World Mariculture Society 7, 267-273.

Huner,J.V., Meyers,S.P. (1979): Dietary protein requirements of the red crawfish, *Procambarus clarkii* (Girard) (Decapoda, Cambaridae), grown in a closed system. Proceedings of the World Mariculture Society 10, 751-760.

Ivleva,I.V. (1980): The dependence of crustacean respiration rate on body mass and habitat temperature. Internationale Revue gesamten Hydrobiologie 65(1), 1-47.

Jones,P.D., Momot,W.T. (1985): The bioenergetics of *Orconectes virilis* in two pothole lakes. Freshwater Crayfish 5, 192-209.

Klein Breteler,W.C.M. (1975): Food consumption, growth and energy metabolism of juvenile shore crabs, *Carcinus maenas*. Netherlands Journal of Sea Research 9, 255-272.

Kossakowski,J. (1974): Crayfish (*Orconectes limosus*) in Poland. Freshwater Crayfish 2, 31-47.

Kurata,H. (1962): Studies on the age and growth of Crustacea. Bulletin of the Hokkaido Regional Fisheries Research Laboratory 24, 1-115.

Kurmaly,K., Jones,D.A., Yule,A.B., East,J. (1989): Comparative analysis of the growth and survival of *Peneaus monodon* (Fabricius) larvae from PZ1 to PL1 on live feeds, artificial diets and on combinations of both. Aquaculture 81, 27-45.

Lake,P.S., Sokal,A. (1986): Ecology of the yabby *Cherax destructor* Clark (Crustacea:Decapoda:Parastacidae) and its potential as a sentinel animal for mercury and lead pollution. Australian Water Resources Council Technical Paper 87. Australian Government Publishing Service. 185 pages.

Lampert,W. (1984): The measurement of respiration. In: A manual on methods for assessment of secondary production in fresh water. (Eds: Downing,J.A., Ryler,F.H.) (IBP Handbook, 17.) Blackwell, Oxford, 413-468.

Lavigne,D.M. (1982): Similarity in energy budgets of animal populations. Journal of Animal Ecology 51, 195-206.

Leffler,C.W. (1972): Some effects of temperature on the metabolic rate of juvenile Blue crabs, *Callinectes sapidus*, in the laboratory. Marine Biology 14, 104-110.

Lowery,R.S. (1988): Growth, Moulting and Reproduction. In: Freshwater Crayfish: Biology, Management and Exploitation. (Eds: Holdich,D.M., Lowery,R.S.) Croom Helm, London, 83-113.

Mason,J.C. (1979): Effects of temperature, photoperiod, substrate, and shelter on survival, growth and biomass accumulation of juvenile *Pacifastacus leniusculus* in culture. Freshwater Crayfish 4, 73-82.

Mauchline,J. (1976): The Hiatt growth diagram for Crustacea. Marine Biology 35, 79-84.

Mauchline,J. (1977): Growth of shrimps, crabs and lobsters - an assessment. Journal Conseil perm internationale Explorer Mer 37 , 162-169.

McClain,W.R., Neill,W.H., Gatlin,D.M. (1992a): Nutrient profiles of green and decomposed rice-forages and their utilisation by juvenile crayfish (*Procambarus clarkii*). Aquaculture 101, 251-265.

McClain,W.R., Neill,W.H., Gatlin,D.M. (1992b): Partitioning the contributions of forage based production system components to weight gain of juvenile crayfish (*Procambarus clarkii*). Aquaculture 101, 267-281.

McDonald,P.D.M., Pitcher,T.J. (1979): Age groups from size frequency data : a versatile and efficient method of analysing distribution mixtures. Journal of the Fisheries Research Board of Canada 36, 987-1001.

McFarland,W.N., Pickens,P.E. (1965): The effects of season, temperature and salinity on standard and active oxygen consumption of the grass shrimp, *Palaemonetes vulgaris* (Say). Canadian Journal Zoology 43, 571-585.

McGill,R., Tukey,J.W., Larsen,W.A. (1978): Variations of box plots. The American Statistician 32, 12-17.

McMahon, B.R., Sinclair,F., Hassall, C., DE Fur,P.L. and Wilkes,P.R.H. (1978) Ventilation and control of acid-base status during temperature acclimation in the crab *Cancer magister*. Journal of Comparative Physiology 128, 109-116.

McNamara,J.C., Moreira,G.S., Moreira,P.S. (1985): Thermal effects on metabolism in selected ontogenetic stages of the freshwater shrimps *Macrobrachium olfersii* and *Macrobrachium heterochirus* (Decapoda, Palaemonidae). Comparative Biochemistry and Physiology 80A, 187-190.

McWhinnie,M.A., Kirchenberg,R.J. (1962): Crayfish hepatopancreas metabolism and the intermoult cycle. Comparative Biochemistry and Physiology 6, 117-128.

Medawar,P.B. (1945): Size, shape and age. In: Essays on growth and form presented to D'Arcy Wenworth Thompson. (Eds: Le Gros Clark,W.E., Medawar,P.B.) Oxford University Press, Oxford, 157-187.

Meenakshi,V.R., Scheer,B.T. (1961): Metabolism of glucose in the crabs *Cancer magister* and *Hemigrapsus nudus*. Comparative Biochemistry and Physiology 3, 30-41.

Mills,B.J. (1986): Aquaculture of Yabbies. In: Proceedings of the First Aquaculture Workshop NSW Department of Agriculture, Sydney. February 1983. 89-98

Mills,B.J., McCloud,P.I. (1983): Effects of stocking and feeding rates on experimental pond production of the crayfish *Cherax Destructor* Clark (Decapoda: Parastacidae). Aquaculture 34, 73-83.

Mohamedeen,H., Hartnoll,R.G. (1989): Larval and postlaval growth of individually reared specimens of the common shore crab *Carcinus maenas* (L.). Journal of Experimental Marine Biology and Ecology 134, 1-24.

Momot,W.T. (1984): Crayfish production: a reflection of community energetics. Journal of Crustacean Biology 4, 35-54.

Momot,W.T. (in press): Redefining the role of crayfish in aquatic ecosystems. Canadian Journal of Fisheries and Aquatic Sciences

Momot,W.T., Gowing,H., Jones,P.D.(1978): The dynamics of crayfish and their role in ecosystems. The American Midland Naturalist 99, 10-35.

Morrissy,N.M. (1979): Experimental pond production of marron, *Cherax tenuimanus* (Smith) (Decapoda: Parastacidae). Aquaculture 16, 319-344.

Morrissy,N.M. (1984): Assessment of artificial feeds for battery culture of a freshwater crayfish, Marron, *Cherax tenuimanus* (Decapoda : Parastacidae). West Australian Department of Fisheries and Wildlife Report 63, 1-44.

Morrissy,N.M. (1989): A standard reference diet for crustacean nutrition research. IV. Growth of the freshwater crayfish *Cherax tenuimanus*. Journal of the World Aquaculture Society 20, 114-117.

Morrissy,N.M. (1990): Optimum and favourable temperature for growth of *Cherax tenuimanus* (Smith 1912) (Decapoda: Parastacidae). Australian Journal of Marine and Freshwater Research 41, 735-746.

Moshiri,G.A., Goldman,C.R. (1969): Estimation of assimilation efficiency in the crayfish, *Pacifastacus leniusculus* (Dana) (Crustacea: Decapoda). Archiv fur Hydrobiologie, Suppliment 66, 298-306.

Musgrove,R.J. (1988a): The diet, energetics and distribution of the freshwater crayfish *Paranephrops zealandicus* (White) in Lake Georgina, South Island, New Zealand. M.Sc.(Hons) Thesis, University of Canterbury, Christchurch, New Zealand.

Musgrove,R.J. (1988b): Digestive ability of the freshwater crayfish *Paranephrops zealandicus* (White) (Parastacidae) and the role of microbial enzymes. Freshwater Biology 20, 305-314.

Mykles,D., Skinner,D.M. (1985): Muscle atrophy and restoration during molting. In: Crustacean Issues 3 : Factors in Adult Growth. (Ed: Wenner,A.M.) A.A. Balkema, Rotterdam, 31-46.

Nelson,S.G., Hiram,W., Knight,A.W. (1977): Calorie, carbon and nitrogen metabolism of juvenile *Macrobrachium rosenbergii* (DE Man) (Crustacea, Palaemonidae with regard to trophic position. Comparative Biochemistry and Physiology 58A, 319-327.

Newell,R.C. (1979): Biology of intertidal animals. 3rd ed. Faversham, Kent. 781 pages.

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

Parry,G.D. (1983): The influence of the cost of growth on ectotherm metabolism. Journal of theoretical Biology 101, 453-477.

Passano,L.M. (1960): Molting and its control. In: The Physiology of Crustacea. Vol. 1. (Ed: Waterman,T.H.) Academic Press, New York, 473-536.

Paul,A.J., Fuji,A. (1989): Bioenergetics of the Alaskan crab *Chionectes bairdi* (Decapoda:Majidae). Journal of Crustacean Biology 9, 25-36.

Pockl,M. (1992): Effects of temperature, age and body size on moulting and growth in the freshwater amphipods *Gammarus fossarum* and G. *roeseli* . Freshwater Biology 27, 211-225.

Pratten,D.J. (1980): Growth in the crayfish *Austropotamobius pallipes* (Crustacea: Astacidae). Freshwater Biology 10, 401-412.

Rao,K.R., Mohrherr,C.J., Reinschmidt,D., Fingerman,M. (1977): Gastrolith growth during proecdysis in the crayfish *Faxonella clypeata* (Hay 1899) (Decapoda: Astacoidea). Crustaceana 32, 256-264.

Raymont,J.E.G., Austin,J., Linford,E. (1964): Biochemical studies on marine zooplankton. I. The biochemical composition of *Neomysis integer*. Journal Conseil perm internationale Explorer Mer 28, 354-363.

Read,G.H.L., Caulton,M.S. (1980): Changes in mass and chemical composition during the moult cycle and ovarian development in immature and mature *Penaeus indicus* Milne Edwards. Comparative Biochemistry and Physiology 66A, 431-437.

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

Reigh,R.C., Braden,S.L., Craig,R.J. (1990): Apparent digestibility coefficients for common feedstuffs in formulated diets for red swamp crayfish, *Procambarus clarkii* . Aquaculture 84, 321-334.

Rice,P.R., Armitage,K.B. (1974): The effect of photoperiod on oxygen consumption of the crayfish *Orconectes nais* (Faxon). Comparative Biochemistry and Physiology 47A, 261-270.

Richards,O.W., Kavanagh,A.J. (1945): The analysis of growing form. In: Essays on growth and form presented to D'Arcy Wenworth Thompson. (Eds: Le Gros Clark,W.E., Medawar,P.B.) Oxford University Press, Oxford, 188-230.

Ricker,W.E. (1973): Linear regressions in fisheries research. Journal of the Fisheries Research Board of Canada 30, 409-434.

Sedgwick,R.W. (1979): Influence of dietary protein on growth, food consumption and food conversion efficiency. Aquaculture 16, 7-30.

Skinner,D.M. (1962): The structure and metabolism of crustacean integumentary tissue during the molt cycle. Biological Bulletin 123, 635-647.

Skinner,D.M. (1966): Macromolecular changes associated with the growth of crustacean tissues. American Zoologist 6, 235-242.

Skinner,D.M.; Graham,D.A.; Holland,C.A.; Mykles,D.L.; Soumoff,C.; Yamaoka,L.H. (1985): Control of Molting in Crustacea. In: Crustacean Issues 3 : Factors in Adult Growth. (Ed: Wenner,A.M.) A.A. Balkema, Rotterdam, 3-14.

Sokal,R.R., Rohlf,F.J. (1981): Biometry - The Principles and Practice of Statistics in Biological Research. 2nd ed. W.H. Freeman and Co, San Francisco. 859 pages.

Sokol,A. (1988): The Australian Yabby. In: Freshwater Crayfish: Biology, Management and Exploitation. (Eds: Holdich,D.M., Lowery,R.S.) Croom Helm, London, 401-425.

Somerton,D. (1980): Fitting straight lines to Hiatt growth diagrams: a re-evaluation. Journal Conseil perm. internationale Explorer Mer 39(1), 15-19.

Spindler-Barth,M. (1975): Changes in the chemical composition of the common shore crab, *Carcinus maenas* during the molting cycle. Comparative Biochemistry and Physiology105, 198-205.

Stecher,P.G., Windholz,M., Leahy,D.S. (Eds.) (1968): The Merc Index. 8th ed. Merc and Co., New Jersey.

Stephenson,M.J., Knight,A.W. (1980): Growth, respiration and caloric content of larvae of the prawn *Macrobrachium rosenbergii* . Comparative Biochemistry and Physiology 66A, 385-391.

Stevenson,J.R. (1968): Metecdysial moult staging and changes in the cuticle in the crayfish *Orconectes sanborni*(Faxon). Crustaceana 14, 169-177.

Stevenson,J.R., Guckert,R.H., Cohen,J.D. (1968): Lack of correlation of some proecdysial growth and development processes in the crayfish. Biological Bulletin 134, 160-175.

Sutcliffe,D.W., Carrick,T.R., Willoughby,L.G. (1984): Effects of diet, body size, age and temperature on growth rates in the Amphipod *Gammarus pulex* . Freshwater Biology 11, 183-214.

Tcherkashina,N.Y. (1977): Survival, growth and feeding dynamics of juvenile crayfish *Astacus leptodactylus cubanicus* in ponds and the river Don. Freshwater Crayfish 3, 95-100.

Thornley,J.H.M. (1970): Respiration, growth and maintenance in plants. Nature 227, 304-305.

Tierney,A.J., Atema,J. (1987): Behavioral responses of crayfish *Orconectes virilis* and *Orconectes rusticus* to chemical feeding stimulants. Journal of Chemical Ecology 14, 123-133.

Titulaer,W.A. (1991): A respirometer of flexible design. Comparative Biochemistry and Physiology 99A, 347-350.

Travis,D.F. (1954): The molting cycle of the spiny lobster *Panulirus Argus* Latreille. I. Molting and growth in laboratory maintained individuals. Biological Bulletin 107, 433-450.

Villarreal,C.H. (1989): Feeding, growth and energetics of the freshwater crayfish *Cherax tenuimanus* (Smith) with special emphasis on its potential for commercial culture. Ph.D. Thesis, University of Queensland.

Vleck,D., Vleck,C.M., Hoyt,D.F. (1980): Metabolism of avian embryos: ontogeny of oxygen consumption in the Rhea and Emu. Physiological Zoology 53, 125-135.

Vonk,H.J. (1960): Digestion and Metabolism. In: The Physiology of Crustacea. Vol. 1. (Ed: Waterman,T.H.) Academic Press, New York, 291-316.

Vranckx,R., Durliat,M. (1978): Comparisons of the gradient of setal development of uropods and of scaphognathites in *Astacus leptodactylus*. Biological Bulletin 155, 627-639.

Wheatly,M.G. (1989): Standard rate of oxygen uptake and body size in the crayfish *Pacifastacus leniusculus*: intra versus interspecific relations in crustacea. Journal of Crustacean Biology 9, 212-216.

Whiteley,N.M. (1990): The effect of temperature, aerial exposure and disturbance on oxygen consumption in the lobster, *Homarus gammarus* (L.). Marine Behaviour and Physiology 17, 213-222.

Wiernicki,C. (1984): Assimilation efficiency by *Procambarus clarkii* fed elodea (Egera densa) and its products of decomposition. Aquaculture 36, 203-215.

Wieser,W. (Ed.) (1973): Effects of Temperature on Ectothermic Organisms - Ecological Implications and Mechanisms of Compensation. Springer-Verlag, Berlin. 298 pages.

Wieser,W., Medgyesy,N. (1990): Aerobic maximum for growth in the larvae and juveniles of a cyprinid fish, *Rutilus rutilus* (L.): implications for energy budgeting in small poikilotherms. Functional Ecology 4, 233-242.

Wilber,D.H., Payson,T., Wilber,J. (1989): The effects of holding space and diet on the growth of the West Indian spider crab *Mithrax spinosissimus* (Lamarck). Journal of Experimental Marine Biology and Ecology 131, 215-222.

Winberg,G.G., Duncan,A. (Eds.) (1971): Methods for estimation of production of aquatic animals. Academic Press, London, 175 pages.

Wolcott,D.L., O'Connor,N.J. (1992): Herbivory in Crabs: Adaptations and Ecological Considerations. American Zoologist 32, 370-381.

Woodland,D.J. (1967): Population study of the freshwater crayfish *Cherax albidus* Clark. Ph.D. Thesis, University of New England, New South Wales, Australia.

Yamoaka,L.H., Scheer,B.T. (1970): Chemistry of growth and development in crustaceans. In: Chemical Zoology. Vol. 5. Arthropoda, Part A. (Eds: Florkin,M., Scheer,B.T.) Academic Press, New York, 321-341.

Zoutendyk,P. (1988): Carbon and nitrogen losses through moulting in the Cape Rock Lobster. South African Journal of Zoology 23, 173-175.

Zoutendyk,P. (1989): Oxygen consumption by the Cape rock lobster *Jasus lalandii*. South African Journal of Marine Science 8, 219-230.