

**An Investigation into the Protein Requirement of a Marine
Prawn *Fenneropenaeus indicus* (H. Milne Edwards).**

by

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Preface

The research work described in this thesis was carried out in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban, South Africa from February 1999 to December 2006 under the supervision of Professor A.T. Forbes and Professor R. Gous.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others, it is duly acknowledged in the text.



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As the candidate's supervisor I have/have not approved this thesis for submission.

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Dedication

This thesis is dedicated to my parents, Rose and John Sara, who have been a source of inspiration, encouragement, support and love. Thank you for believing in me. And to my biological father:

Carlos Luiz de Sousa

(1st October 1928 - 23rd November 2004).

You are sorely missed especially since I never got the chance to say goodbye.

Abstract

The white prawn, *Fenneropenaeus indicus* (H. Milne Edwards) (Perez-Farfante & Kensley 1997), is a species native to east-southeast Africa. Until 2004 South Africa boasted a small yet viable prawn industry which, during its prime, produced an annual harvest of approximately 130 mt of cultured *F. indicus*. At times pond yields surpassed total yearly trawls of wild stocks caught offshore, but due to high running costs the farms were forced to close. Renewed interest by investors to revive and expand the industry using large scale farming techniques is presently being addressed. However, there remains concern about the high costs incurred in manufacturing feeds since, in both natural and prepared diets, protein is the main and most expensive nutrient. To date, diets formulated for *F. indicus* have a protein content of approximately 33 - 43 %. To assess if protein requirement changes with age the survival, nutritional indices, body composition gains and growth response in approximately 2, 7 and 14 g prawns fed diets with protein concentrations ranging between ~ 5 to 50 % during 35 day growth trials, was investigated. Results indicated that dietary protein significantly influenced growth and nutritional indices and that the protein requirement for 2, 7 and 14 g prawns was 34, 35 and 41 % of the diet, expressed on a dry matter basis, respectively. Moreover the quantitative aspect of protein utilization (g nutrient/unit of mass gain) was examined by implementing the Reading model, primarily used to establish the optimal requirement of essential amino acids in laying hens based on economical terms. Results indicated that the optimum dose increased with age with protein requirements of 18, 46 and 152 mg/day recorded for 2, 7 and 14 g prawns.

In formulated diets fish meal, although the most suitable source of protein with a digestibility coefficient in excess of 90 %, remains the most expensive ingredient and due to fishing pressures may become scarce in the future. Alternative and less expensive sources of plant proteins viz. wheat gluten, sunflower oilcake, brewers yeast and soybean meal together with a slightly more expensive animal protein, casein, were investigated and found to have digestibility coefficients of 97, 96, 95, 88 and 90 %, respectively.

Moreover, a model used to describe growth in pigs and poultry was adapted to describe and predict growth in *F. indicus*. The model describes the potential rate of increase of body protein using the Gompertz equation, which estimates body protein mass at a point in time (P_t) from the mature body protein mass (P_m), the degree of maturity at birth μ where $\mu = P_t / P_m$ and from the rate of maturation (β), which is a Gompertz constant. In addition the potential rate of increase in mass of each body component viz. flesh and exoskeleton and their respective chemical fractions, expressed as a function of the current protein mass, may be calculated using allometry. Body mass of the animal at any given point in time is then determined by adding the mass of each component and their associated fractions.

In this study the procedures used to establish the relevant parameters of the Gompertz growth equation (i.e. β , P_m) under assumed non-limiting conditions involved the measurement

and comparison of the growth of prawns reared in a laboratory system and in a growout pond for ~ 400 days. Based on a series of mixed samples of pond-reared male and female prawns, the inherent growth parameters of the species, when reared at 19 – 27 °C and in 21 – 27 ‰, were as follows: P_m of 6541.00 mg, β of 0.014 per day, maximum rate of protein deposition of 32.68 mg/day, and lipid:protein, ash:protein, water:protein, carbohydrate:protein at maturity equal to 0.03, 0.02, 3.30 and 0.06, respectively.

Animals require dietary energy for maintenance and growth. In order to establish the metabolic rate of fasting *F. indicus* the oxygen consumption of 5, 7 and 14 g prawns at temperatures of 20, 25, 30 °C and salinities of 15, 25 and 35 ‰ was measured. In addition the percentage increase in metabolism caused by the specific dynamic action (SDA) for the same size animals fed diets of 21, 31 and 41 % crude protein was measured. Salinity had no significant effect on respiration rates but metabolic rates were affected significantly by temperature. Oxygen consumption ($\text{mg} \cdot \text{hour}^{-1}$) as a function of live mass of fasting animals at 20, 25 and 30 °C was described by $0.194 M^{0.783}$, $0.243 M^{0.810}$ and $0.341 M^{0.854}$, respectively, at 15 - 35 ‰. Despite SDA contributing to an increase above pre-feeding levels of about 9, 10 and 13 % for prawns fed diets containing 21, 31 and 41 % crude protein respectively, no significant differences in respiration rates between animals fed the different diets were found. Oxygen consumption of fasting prawns, together with a conversion rate of 20 kJ/l O_2 respired, provided a means of calculating the minimum digestible energy required by the species at the above-mentioned environmental parameters. For example, an individual of 14 g at 20 °C is predicted to have a maintenance requirement for energy (HE_f) of $[0.194 \times (14)^{0.782}] \times 20 = 0.031 \text{ kJ/hour} \times 0.744 \text{ kJ/day}$.

Emmans (1984) and Oldman and Emmans (1990) have shown that an estimate of metabolizable energy (ME) is not an accurate means of describing energy yields of feedstuff since the heat increment of feeding is not accounted for. A solution known as the effective energy system, proposed by Emmans (1984; 1994) and Emmans and Fisher (1986), takes these deficiencies into account and was used here in place of the ME system when calculating dietary energy content and prawn requirement. The daily energy requirement (EERQ) of prawns may be predicted using: $\text{EERQ} = \text{HE}_f + 47 \text{ PR} + 56 \text{ LR} + 17 \text{ CR}$ (kJ/day) where PR, LR and CR are the retention of body protein, lipid and carbohydrate, respectively. The desired feed intake (DFI) is calculated as $\text{DFI} = \text{EERQ} \div \text{EEC}$. Using these same principals the desired protein intake based on the amino acid requirements for maintenance and growth was established.

This study indicated that, with estimates of potential growth rate, fasting metabolic rates and changes in body composition, the nutrient requirements of *F. indicus* could be predicted. With this knowledge, especially for protein and energy, producers can make better decisions that will ultimately lead to increased production and a decrease in costs.

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1.

General Introduction**The Importance of the Prawn Industry in South Africa with Specific Reference to *Penaeus indicus* (H. Milne-Edwards, 1837).****1.1. Shallow water prawns in South Africa.**

Penaeus indicus is a tropical Indo-Pacific penaeid that is widely distributed across Northern Australia, the Philippines, India and down the east coast of Africa (Figure 1.1) (Hughes, 1970; Dall, Hill, Rothlisberg & Staples, 1991; Pérez-Farfante & Kensley, 1997). In a recent taxonomic review Pérez-Farfante and Kensley (1997) raised several subgenera to generic level and *Penaeus indicus* (H. Milne-Edwards, 1837) is now formally known as *Fenneropenaeus indicus*. From here on out reference to revised names by Pérez-Farfante and Kensley (1997) will be followed by the original in parenthesis.

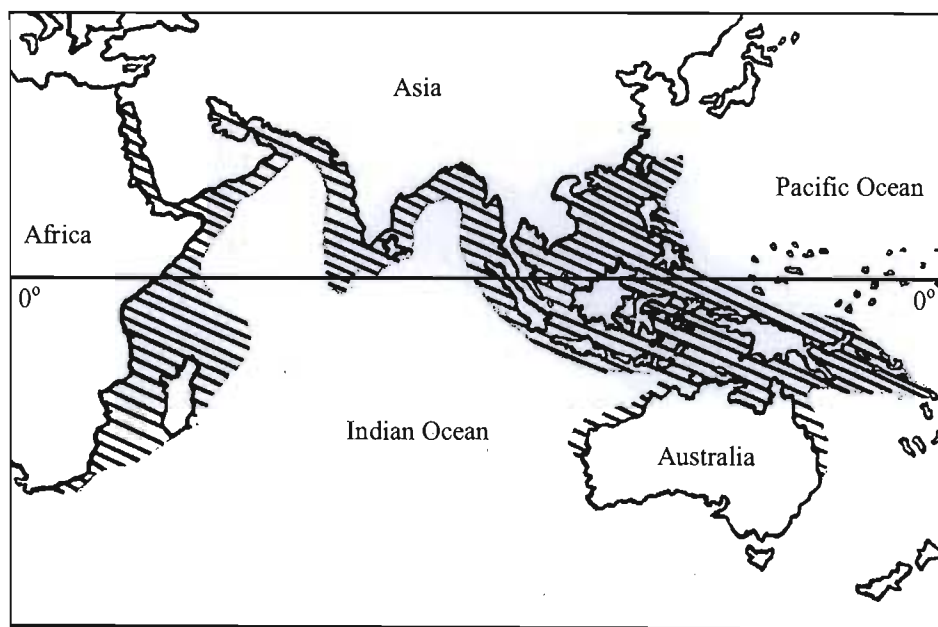


Figure 1.1: Known geographic distribution of *Fenneropenaeus indicus* (after Dall *et al.*, 1996 and modified to include Hughes, 1970).

In 2003 the total world catch of shrimps[†] and prawns was 2.6 million tonnes (mt), of which 0.4 % came from the southern African fishing grounds of Angola, South Africa and Moçambique (FAO, 2003). With the exception of Japan and the United States of America, most prawn trawling countries widely utilise this highly valued resource as earners of foreign exchange (De Freitas, 1995). Off the east coast of southern Africa, practically along the entire coast of Moçambique and isolated regions of KwaZulu-Natal (KZN), Indian Ocean shallow water species belonging to the family Penaeidae occur mainly over the continental shelf in waters 5 to 70 m deep. This resource consists of five commercially important species: *F. indicus*, *Penaeus monodon*, *P. semisulcatus*, *Marsupenaeus japonicus* (*P. japonicus*), *M. monoceros* and two of lesser importance, *M. stebbingi* and *Melicertus latisulcatus* (*P. latisulcatus*) (De Freitas 1980, 1995). Catches further offshore are, however, dependent on the pink prawn *Haliporoides triarthrus* (Forbes & Benfield, 1985).

In shallow waters off the South African coast of KZN *F. indicus* and *M. monoceros* are by far the most common species caught (Champion, 1970; Hughes, 1970; Emmerson, 1982; Forbes & Benfield, 1985; De Freitas, 1995). Proportionally by mass, annual estuarine and offshore catches of *F. indicus* have been recorded to fluctuate between 82 – 94 % and 45.5 – 73 %, respectively (Champion, 1970; Forbes & Benfield, 1985; Benfield, Bosschieter & Forbes, 1989; Demetriades & Forbes, 1993; Forbes & Demetriades, 2005). Followed by *M. monoceros* which contributes about 2 - 4 and 10 - 24 %, and *P. monodon* which makes up approximately 1 - 2 and 10 - 8 % of estuarine and offshore catches, respectively (Joubert & Davies, 1966; Champion, 1970; Emmerson, 1982; Forbes & Benfield, 1985).

Variability in annual catches is closely related to rainfall patterns in catchment areas since a high runoff is thought to bring about the introduction of nutrients into the generally oligotrophic inshore water of KZN (Forbes & Demetriades, 2005) by ensuring that rivers remain open to the sea (Morant & Quinn, 1999). Nutrient loading contributes towards the production of phytoplankton which in turn has the potential to enhance the survival of the planktonic larval stages of penaeids (Forbes & Demetriades, 2005). Alternatively KZN Rivers when subjected to major floods, as a consequence of cyclones, decrease *F. indicus* numbers considerably since very low salinities make nursery areas inaccessible and/or inhabitable for the species (Forbes & Benfield, 1986a; Forbes & Hay, 1988; Forbes & Demetriades, 2005). In contrast *M. monoceros*, being tolerant of low salinities, thrive and become proportionally more abundant (Forbes & Benfield, 1986a; Forbes & Hay, 1988)

[†] Popular terms “shrimps” and “prawns” have been used indiscriminately worldwide to describe crustaceans of the Decapod superfamilies Caridea and Penaeoidea. For the purpose of this study the term “prawn” will be used when referring to members of the superfamily Penaeoidea and “shrimp” for members of the infra-order Caridea as done by De Freitas (1995).

1.2. The life cycle of *Fenneropenaeus indicus*.

The life cycle of all known members of the family Penaeidae involves a variety of naupliar, protozoel, mysis and postlarval stages, all of which are planktonic, followed by the active swimming juvenile and adult stages. The greatest difference between species lies in the habitat of preference by post larvae, juveniles and adults, *i.e.* whether they are predominantly estuarine, inshore or offshore, and whether demersal or pelagic (Forbes & Demetriades, 2005). The life cycle of *F. indicus* is shown in Figure 1.2.

In South African waters *F. indicus* females, after attaining a mean carapace length of 33 mm and an approximate live mass of 25 g, commence breeding at an estimated age of 5 – 6½ months (Emmerson, 1980). Conversely, males are found to attain sexual maturity at a mean carapace length of 25 mm and a live mass of approximately 14 g (Champion, 1988). The life span of females is estimated to be approximately 14 months, yielding a breeding period of 7½ - 9 months with an estimate of 7 - 9 spawns per lifetime (Emmerson, 1980).

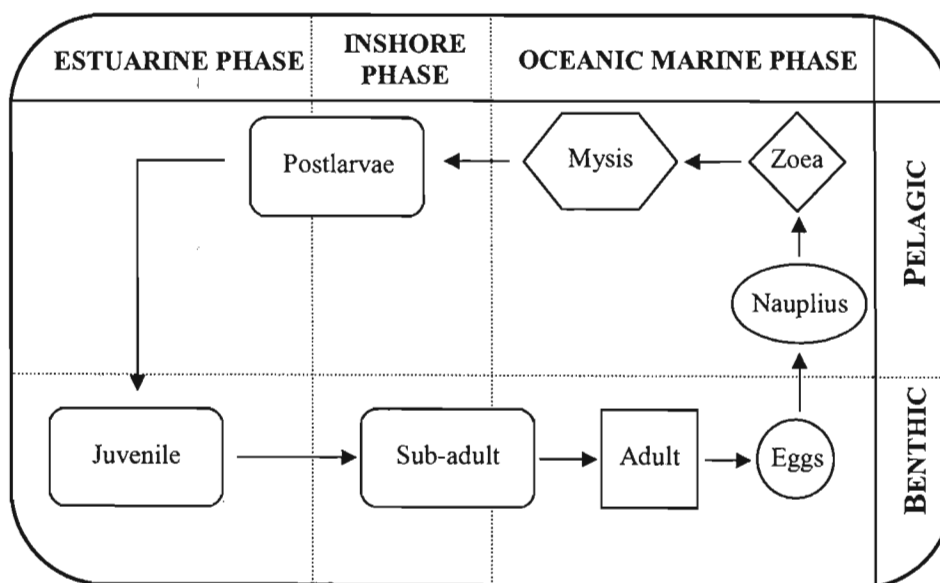


Figure 1.2: Habitat and morphological changes in the life cycle of *Fenneropenaeus indicus* (adapted after Dall *et al.*, 1991)

Peak activities of spawning occur during May and September (Champion, 1970; De Freitas, 1980; Emmerson, 1982). Once spawned the fertile eggs, which are demersal, hatch within 14 - 24 hours into the first larval stage or nauplius, which metamorphoses into zoëae, mysis and later into postlarvae. Dependent on temperature, development from egg to postlarvae takes place over a period of approximately 13 days (Muthu, Pillai & George, 1979). The development from juvenile to adult lasts a few months. Currents, wind and tides assist the late mysis or early postlarvae into the shelter of bays and estuaries. On reaching the estuarine environment the postlarvae become omnivorous bottom feeders, feeding on epiphytic algae, small worms, crustacea, molluscs and decomposing matter (Seppings & Demetriades, 1992;

Demetriades & Forbes, 1993; De Freitas, 1995; Heydorn, 1995). In estuarine backwaters postlarvae transform into juveniles and grow over a period of three to four months until they are large enough to migrate offshore where they subsequently mature and reproduce (Joubert & Davies, 1966; Hughes, 1966; Champion, 1970; De Freitas, 1980).

Along the coast of KZN, from north to south, there are four major coastal bodies that support the juvenile stages of the southernmost population of commercially exploited penaeids in the western Indian Ocean. These are Kosi Bay, the St Lucia Lake system, Richards Bay and Durban Bay with the latter two having been developed into commercial harbours (Forbes, Niedinger & Demetriades, 1994). The St. Lucia Lake system is the largest estuary in southern Africa and provides a nursery habitat for *F. indicus* and several other penaeid species (Forbes & Benfield, 1986a; Benfield *et al.*, 1989). Although densities of postlarvae were found to vary, with lower densities in tidal currents of harbours attributed to much larger flood tide-volumes, recruitments into all four coastal bodies were found to be similar in species composition (Forbes *et al.*, 1994).

The migration of sub-adults from the nursery areas occurs during late summer and early winter, between May and June, and populate the St Lucia and Tugela Banks from September until the following April where the majority are thought to survive (Forbes & Benfield, 1986; Champion 1988; Forbes & Cyrus 1991). Due to the very narrow continental shelf, the spawning grounds of St Lucia and Tugela Banks are situated within a 50 m depth contour adjacent to the Matikulu River and St Lucia River between 1.6 - 11 nautical miles (nm) offshore. The shelf is at its widest in this area with the break found to be ~ 27 nm offshore (Figure 1.3) (Forbes & Demetriades, 2005).

Recruitment into the St Lucia Lake and Richards Bay systems by penaeid larvae spawned on the St Lucia and Tugela Banks is possibly a result of gyres and counter-currents that periodically assist the invasion of larvae into adjacent estuaries where tidal currents convey them further upstream (Figure 1.3). Larvae recruitment in the St Lucia system has been dominated by *M. japonicus* in numbers up to an order of magnitude greater than *F. indicus* followed, in descending order, by *M. monoceros*, *P. monodon* and *P. semisulcatus*, respectively (Forbes & Benfield, 1986a). However, in the St Lucia system *M. japonicus* soon perish since they fail to accomplish the ~ 20 km migration upstream to reach the sandy substrata typically associated with this species (Forbes & Benfield, 1986a). Recruitment further north of St Lucia, as far as Kosi Bay, is thought to be highly unlikely due to the opposing Agulhas current which forms a natural barrier to passive northward movement of penaeid larvae (Forbes & Cyrus, 1991).

Influx of penaeids into the estuaries north of St Lucia is speculated to be a result of larvae being washed in from further north by the Agulhas current (Forbes & Cyrus, 1991). Similarly the same current is thought to facilitate distributions further south of Durban leaving the young juveniles with no alternative but to find temporary shelter in the cooler waters of eastern and

southern Cape estuaries. With the onset of winter the fate of sub-adults migrating offshore in search of breeding grounds is unknown. It is assumed that mean sea temperatures adjacent the Cape coast, which at times fall well below the species tolerance level, precludes the survival of the species (Hughes, 1970; Emmerson, 1982)

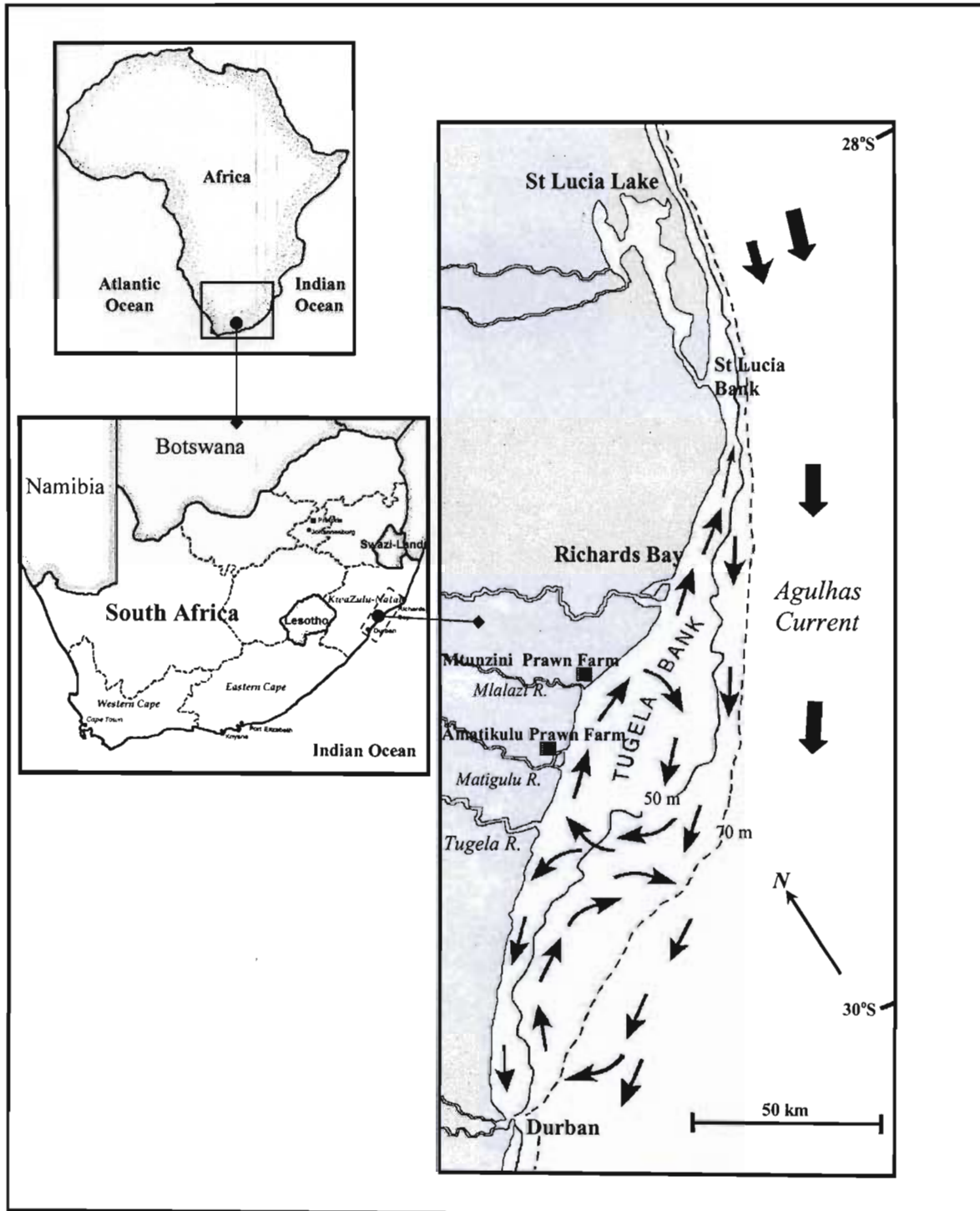


Figure 1.3: Location of the St Lucia System, St Lucia Bank, Tugela Bank, Agulhas current, Mtunzini and Amatikulu Prawn farms along the east coast of South Africa (adapted after Forbes *et al.*, 1994)

In 1996 and subsequently with the Greater St Lucia Wetland Park being proclaimed a UNESCO World Heritage site in December 1999, activities associated with the bait fishery in the St Lucia system have been terminated. As a result data descriptive of population dynamics, species composition and biomass of penaeids in these systems and other KZN Rivers is scarce. At present it is thought that species composition remain unchanged.

1.3. Ecological factors influencing the life cycle of F. indicus.

A reduction of fluvial input, siltation due to catchment degradation, coastal sand drift, impoundment and abstraction of freshwater are some of the factors that contribute towards the formation of sand berms across river mouths which exacerbate periods of closure in South African rivers (Morant & Quinn, 1999). Except for Durban and Richards Bay harbours, which provide permanently opened sheltered marine habitats, the mouth dynamics of KZN rivers are primarily driven by the occurrence and magnitude of rainfall in the catchment areas. Substantial rainfall, if unhindered by anthropogenic structures, enables berms to be overtopped or, due to the scouring mechanism of large floods, breached (Forbes, Niedinger & Demetriades, 1994; Whitfield & Bruton, 1989; Morant & Quinn, 1999). The frequency and duration of estuary mouth openings are major factors affecting the estuarine biota, particularly juveniles of marine fish (Whitfield, 1994), zooplankton (Wooldridge, 1991) and the migration of crustacean larvae (Forbes & Benfield, 1986) in and out of riverine systems. Failure by mysis and/or postlarvae of crustaceans to reach these nursery areas can potentially be fatal (Heydorn, 1995). Thus, the significance of estuaries and bays as nursery grounds for penaeid postlarvae depends on accessibility and the suitability of habitats within such systems.

Annual catches of penaeids trawled on the St Lucia and Tugela Banks during 1984 to 2000 fluctuated between 12 - 161 tonnes (mt), provided an average of 88 mt per annum (Demetriades, 2000). In 2003, total offshore catches amounted to less than 15 mt[†] and was attributed, in part, to actions taken following the running aground of a 31 kt Italian registered cargo ship named the 'Jolly Rubino' near the St Lucia mouth. For fear of pollution from the possible spillage of 1100ℓ of fuel and hazardous chemicals onboard, local conservation bodies decided to barricade the river mouth. In 2004 due to low summer rainfalls and failure by the river to breach the sand barrier the migratory patterns of inshore prawns into and out of the system were disrupted.

[†] Information supplied by Marine and Coastal Management of South Africa and Spray Fisheries (Pty.) Ltd.

1.4. A brief overview of the history of penaeid mariculture in South Africa.

Unlike trawling wild stocks, which is dependent on rainfall and river mouth dynamics, the mechanism of culturing prawns is less reliant on natural events. Mariculture requires that at least one phase of the animal's life cycle, within a simulated environment, be controlled, manipulated or enhanced in order to facilitate optimal conditions to produce maximum yields in land based ponds. There are currently two commercial prawn farms located along KZN's coastline: Amatikulu Prawns with an equivalent of 10 hectares (ha) (Figure 1.4) and Mtunzini Prawns with 53 half-hectares of ponds (Figure 1.5). The farms are located approximately 120 km and 150 km North-East of Durban Bay, respectively as shown in Figure 1.3. Amatikulu Prawns is situated adjacent to the Matigulu ($29^{\circ} + 04' \text{ S}$; $031^{\circ} + 38' \text{ E}$) while Mtunzini Prawns is located further north, along the Mlalazi estuary ($28^{\circ} + 57' \text{ S}$; $031^{\circ} + 38' \text{ E}$).

Attempts to culture prawns commercially in South Africa dates back to the early 1970's when globally, at the time, substantial knowledge pertaining to factors affecting the biology of some cultured species such as water quality, feed requirements, physio-chemical requirements and the prevention and control of prawn diseases already existed. However, potential investors had reservations and expressed concern regarding insufficient knowledge and unknown growth factors relating to local conditions that potentially could jeopardise the success of such a venture (Champion, 1971). Thus, in 1971, supported by and under the auspices of the Fisheries Development Corporation of South Africa (Ltd) (FDC), a governmental organisation, the Prawn Research Unit (PRU) was established at the Amatikulu site, known then as the Amatikulu station. Pond trials were implemented and hatchery procedures were initiated to ascertain whether prawn culture in South Africa was possible (Beggs 1978; Emmerson 1982). At the same time diatom cultures for larval feeding and larval rearing techniques were tested at the Port Elizabeth Museum and Oceanarium complex (Emmerson 1982a), located approximately 900 km southwest of Durban in the eastern Cape. Nutritional studies were also undertaken during this period at the Oceanographic Research Institute (ORI) in Durban (Colvin, 1976a, 1976b).

Additional projects focused on penaeid nutrition at the Amatikulu station during 1972 but after three years of feasibility studies financial support by the FDC ceased, halting further research at Port Elizabeth and ORI. As a result, research became centred at the PRU where facilities, with the financial assistance of Kwa-Zulu Development Corporation (KDC), were expanded to enable further larval rearing, pond and small tank trials to be pursued (Emmerson, 1982). Between 1973 and 1986, due to *F. indicus* being readily available, several higher degree theses dealing with this species were consequently produced that formed the basis of a number of scientific publications Emmerson (1977, 1981, 1982, 1983, 1984), Gerhardt (1980, 1981), Read and Caulton (1980) and Read (1981).

During this period in 1981 a separate and unrelated attempt by an investor, Mr S Myburgh, in conjunction with the KDC, originally with the intentions of farming the giant caridean fresh water prawn *Macrobrachium rosenbergii*, endemic to south-east Asia and northern Australia, was imported from a aquaculture operation in Mauritius (Forbes & Demetriades, 2005). This species was preferred since it can be reared successfully in brackish water and because it was and remains to be a food source highly sought after in the Far East. Furthermore, as opposed to penaeids, larval development in this species is shorter and less complex.

The project was established on a site neighbouring Amatikulu Station and operated under the name of Amatikulu Prawns (Pty.) Ltd. A year later, failure to cultivate *M. rosenbergii*, due to difficulties selecting and culturing suitable algal species for larval rearing and a shortage of technical expertise, the operation at Amatikulu ceased. Despite these setbacks, later that year, the same investor modified the existing infrastructure and trading under the name of Amatikulu Hatchery (Pty.) Ltd began farming fresh water ornamental fish on a site situated 5 km from the Amatikulu station.

In 1988, following financial successes, interest to develop prawn culture operations were renewed. That same year in collaboration with the KDC a pilot project involving hatchery trials, at Amatikulu station on *P. monodon* and *F. indicus* was initiated. Preference for the former was due to the high growth rate and commercial value of the species. The project was conducted in conjunction with pond trials using both species. Once a successful method had been established, funding was secured from the Industrial Development Corporation (IDC) to construct and stock 6 ha of grow-out ponds at the Amatikulu site (Forbes & Demetriades, 2005). The following year in 1989 following a successful harvest the requirements to successfully produce prawns commercially in KZN were recognised, permitting Amatikulu Prawns (Pty.) Ltd to be re-established.

In a parallel development during the 80's a separate scheme was developed at the village of Mtunzini, on an old sugar cane field on the south bank of the Mlalazi river (Figure 1.2). Funded by a consortium of shareholders with the major stakeholder being the IDC, the development to accommodate the large production of cultured prawns, gave rise to Mtunzini Prawn Farm Holdings. In 1990 hatcheries and grow-out facilities of Amatikulu Prawns and Mtunzini Prawn Farm Holdings were committed towards culturing *P. monodon* (Rosenbury, 1992). But after two years, due to difficulties in obtaining wild *P. monodon* broodstock, the Amatikulu farm resorted to culturing *F. indicus* with Mtunzini following suit a few years later.

In 1998, due to a succession of entrepreneurs and expertise from abroad Mtunzini Prawn Farm Holdings was unsuccessful at producing profitable and viable yields. The result was that Mtunzini Prawn Farm Holdings was taken over by the Amatikulu group, which by this time had diversified their freshwater fish operation after successfully culturing *F. indicus* in 10 hectares of ponds. However, facilities at Mtunzini required significant repair but with the IDC hesitant

to contribute towards further costs and without the necessary financial backup prospects to upgrade the facility were placed on hold. The result was that, alternate investment had to be sought from the private sector. Under the management of Amatikulu Prawns (Pty.) Ltd the Mtunzini site became known as Mtunzini Prawns. Over the years with the expansion of Amatikulu Prawns (Pty.) Ltd and with each hectare of pond producing between 4 - 8 mt per season, a production of 39 mt in 1993 (Hoffman, Swart & Brink, 2000) increased to approximately 150 mt in 2004.



Figure 1.4: Aerial view of Amatikulu prawn farm (sourced from Amatikulu Prawn's website: <http://www.prawn.co.za> [2004/04/30]).

Over the years, techniques used to culture *F. indicus* commercially in South Africa have been developed and refined by adapting the technology developed elsewhere. Innovative farm managers overseeing production have received advice from specialists worldwide in order to establish a protocol best suited for local conditions. Moreover, numerous technical and scientific publications including works by Tacon (1990), Treece & Fox (1993), D'Ambro *et al.* (1997), Fegan (2001) and those available on internet sites such as <http://www.enaca.org> and <http://www.was.org> have assisted in the development of commercial prawn farming in South Africa.

Apart from those produced in the late 70's and early 80's no further publications have emerged that were tailored specifically towards or comparable to conditions found locally. In recent years renewed interest by the Amatikulu Prawns to involve tertiary institutions in refining existing technology has resulted in the funding of research projects done by Wahlberg (1996), Pollard (1998) and Pillay (2000). Procedures for culturing *F. indicus* in South Africa are fundamentally similar to what is practised globally. These entail the maturation, spawning, hatchery, nursery, grow-out and harvesting phase, which will be briefly reviewed in the following sections.



Figure 1.5: Aerial view of Mtunzini prawn farm (sourced from Amatikulu Prawn's website: <http://www.prawn.co.za> [2004/04/30]).

1.4.1. Maturation and spawning.

In South Africa the mariculture procedure begins with the collection of sexually mature males and females on the Tugela Banks by commercial trawlers and comprises about 5 – 10 % of the broodstock. Once brought ashore these prawns, along with cultured prawns, are placed into maturation tanks. Maturation tanks have flat-bottoms, are black, *ca.* 1 m in depth and are illuminated by low intensity light from green overhead fluorescent tubes. Water that is continuously aerated is maintained at (mean and standard deviation) $28^{\circ} \pm 1^{\circ}\text{C}$, $35 \pm 2\text{‰}$ and $\text{pH } 8.2 \pm 2$. Each tank is stocked with twice as many females as males (Evans, 1992).

Induced spawning by ablation is no longer practiced since the average number of spawns per moult cycle (Emmerson, 1980; 1983) and hatching success (Emmerson, 1983; Makinouchi & Primavera, 1987) was found to be greater amongst unablated *F. indicus* females. Moreover, correct nutrition enhanced spawning frequency and fecundity (Emmerson, 1980; Galgani, Cuzon, Galgani & Goguenheim, 1989). Broodstocks are offered rations daily (8:00, 12:00, 18:00 & 20:00 h). These rations comprise a combination of a compound pellet (Table 1.1) and, depending on availability, portions of frozen squid, octopus, mussel, oysters and/or the cracker shrimp, *Callinassa kraussi*.

Table 1.1: The ingredients of a compound diet, on a dry matter basis, presently used by the industry.

<i>Ingredient</i>	<i>Percentage composition</i>
Fish meal ^a	26.30
Soybean meal ^b	30.00
Industrial wheat ^c	27.20
Wheat gluten ^d	2.50
Brewer's Yeast Dehydrated ^e	5.00
Soya Lecithin (Crude Oil) ^f	2.00
Fish oil ^g	2.00
Cholesterol (33% Pure) ^h	0.20
Vitamin mixture ⁱ	0.40
Vitamin C (Roche Stay-C)	0.40
Mono calcium phosphate ^j	2.00
Mineral mixture ^k	0.50
Lysine ^l	0.50
Methionine ^m	1.00
Total	100.00
<i>Nutrients on a dry matter basis.</i>	
DE (MJ/kg)	17.36
Protein (%)	41.58
Lipid (%)	5.32
Fibre (%)	4.12
Ash (%)	6.79
Total	100.00

^a Fish meal, Contra Fisheries, Cape Town, minimum 65% protein, lipid >12 %.

^b Soybean meal, Epol, minimum 44% protein.

^c Industrial wheat, Ngwane Mill (Pty) Ltd, S.A. minimum 13% protein.

^d Wheat gluten, C.J. Petrow.

^e Brewer's yeast dehydrated, South African Breweries (SAB).

^f Soya Lecithin, Crest Chemicals (Pty) Ltd.

^g Fish oil, Energy Oils, Durban.

^h Cholesterol, Salvay Pharmaceuticals.

ⁱ Thiamin, 10.5mg; riboflavin, 10mg; pyridoxine, 12.5mg; pantothenic acid, 18.5mg; Niacin, 10mg; Biotin, 0.50mg; inositol, 60mg; choline, 100mg; folic acid, 2.5mg; cyanocobalamine, 0.025mg; ascorbic acid, 250mg; vitamin A, 4200IU; vitamin D, 2500 IU; vitamin E, 300mg; vitamin K, 20mg; Calpan, 50mg; Endox, 25mg.

^j Mono calcium phosphate, Kynoch (S.A.).

^k Calcium, 246mg; phosphorus, 80mg; magnesium oxide, 6.31mg; sodium, 60mg; potassium, 90mg; iron, 3mg; copper, 0.34mg; zinc, 1.1mg; manganese, 0.2mg; selenium, 0.05mg; cobalt, 0.14mg; iodine, 0.01mg; ferrous, 0.89mg

^{l & m} Amino acid, BASF., S.A.

Healthy females kept under favourable conditions are capable of spawning on average 1.5 times per month for up to 6 - 7 months (Emmerson *et al.*, 1983). Since spawning takes place at night gravid females are selected between 15:00 and 16:00 h and placed into separate spawning tanks, which are black and conical in shape. The following morning the females are removed and placed back into the maturation tanks while the water in the spawning tanks is gently stirred until the eggs hatch. The newly hatched nauplii are harvested, disinfected in accordance with procedures described by Fegan (2001) and transferred to larval rearing tanks containing water of maturation quality (Evans, 1992).

The nauplii stages, nourished by body yolk, lasts 48 hours. When body reserves are near depletion metamorphosis takes place transforming the nauplii into zoëae larvae. This transition is regarded as a critical stage of larval development since there is a switch from yolk to phytoplankton as food (Evans, 1992; Ribeiro & Jones, 2000). The zoëa stage has three sub-

stages, each with a duration of ~ 24 hours. Zoea₁ and zoea₂ stages are fed *Chaetoceros gracilis* (*mullerii*) at densities of 100,000 cells. mL⁻¹ and *Tetraselmis suecica* at densities of above 10 000 cells. mL⁻¹. *C. gracilis* and *T. suecica* concentrations are maintained in excess of cell ingestion rates of *Thalassiosira weissflogii* previously established for *F. indicus* by Emmerson (1980) (Table 1.2).

Table 1.2: Ingestion rates for *F. indicus* larvae fed different food sources and expressed as µg dry mass. d⁻¹ per µg body mass (Emmerson 1980; Jones, Yule & Holland 1997).

Stage	Herbivorous feeding:	Carnivorous feeding:
	<i>Thalassiosira weissflogii</i>	<i>Artemia</i> nauplii
Zoea	8.6	-
Mysis	5.2	3.9
Postlarvae	-	4.2

After three days the zoëae moult into the first mysis stage (M₁) and are fed a combination of brine shrimp nauplii, *Artemia salina*, a crumble - similar in composition to that shown in Table 1.1 - and cultures of *C. gracilis* and *T. suecica* with the latter increased to 20 000 cells. mL⁻¹. The post larval (PL) stages are fed a combination of crumble and brine shrimp while maintaining algal blooms in order to sustain good water quality. From PL₁₀ – PL₁₅, i.e. 10 - 15 days after the post-larval stage has been reached, the larvae, being old enough to withstand a change in environment, are transferred into grow-out ponds throughout the period from September to November when water temperatures are around 25 °C.

1.4.2. The grow-out phase.

In South Africa prawn farming is practised in earthen ponds consisting of either sand, as found at Amatikulu, or clay, in the case of Mtunzini. Ponds range between 0.4 - 2 ha, are continuously aerated by paddle wheels, have a flow through design and are 2 m deep at their centres.

Animals, fed a compound diet (Table 1.1), remain in the ponds from November to April and are harvested after having attained a marketable mass of 25 - 35 grams. In ponds growth from PL₁₂ to 25 g, depending on pond temperatures and based on a growth rate of 0.14 - 0.25 grams per day, takes approximately 140 - 150 days resulting in a single annual harvest. The monitoring of growth in culture ponds is done using the von Bertalanffy (1957) model in the following form (Evans, 2000 *pers. com.*[§]):

$$W_t = W_{\infty} \left\{ 1 - e^{-K(t-t_0)} \right\}^b$$

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Where the fixed parameters are calculated to be $W_{\infty} = 28$ g,
 $K = 0.015$
 t = day after hatching
 $t_0 = 0$
 $b = 3$

1.4.3. Feeding and growth phase.

In conjunction with correct grow-out procedures, nutrition is considered to be the most important factor since an economically viable feed that facilitates optimum growth contributes directly towards the success and profit of the industry (Evans, 1992, 2000, 2001). The feeding regime and the provision of rations, to date, has been based upon and adapted from the feeding curve used for *L. vannamei* (*P. vannamei*), (sourced from the website of Pond Dynamics/Aquaculture CRSP

<http://pdacrsp.oregonstate.edu/pubs/technical/14tchhtml/2/2b/2b2/fig1.html> [2000/03/05]),

(Evans 2000 *pers. com*):

$$W_f = 100 \left\{ 10^{[-1.05 - 0.446(\text{Log}_{10} W_p)]^{0.8}} \right\}$$

where

W_f = feed rate as a percentage of biomass; and
 W_p = mean mass of prawn in grams.

Daily rations are divided into four portions with 35 % of the ration fed at dusk, 15 % at midnight, 25 % at dawn and 25 % at midday. The timing of the provision of the largest ration is in accordance with laboratory trials conducted by Natarajan (1989a, 1989b) whereby peak activity was found to occur at dusk and early evening, during predicated times of high water. These findings confirm results by Janakiram, Venkateswar, Reddy and Babu (1985) in which the species was found to exhibit an increase in respiration that coincided with sunset.

With the onset of winter the majority of ponds are harvested since pond water averaging 18 °C, at times 14 °C, cause a cessation in growth. Only ponds containing animals that have been selected as broodstock are left over winter. Harvesting procedures are similar in principle to those described by Lucien-Brun (2002). After harvesting and with the ponds drained the build-up of feed residue and decomposed organic matter, accumulated at the centre of the pond, is removed. At the end of August ponds are refilled and prepared to receive a new batch of PL₁₂ - PL₃₀ (Evans, 1992) whereby chicken manure is added in order to accelerate algal blooms. As a means of comparison data pertaining to best annual yields produced by the farms are provided in Table 1.3.

Table 1.3: Best production, FCR and growth data recorded for Amatikulu Prawns (Pty.) Ltd (Evans 2005, *pers. com*).

Sites	Amatikulu ^a	Mtunzini ^a
Total Yield in Kilograms	58 618	89 066
Food Conversion Ratio	2.4	2.5
Kilograms per Hectare	5 862	3 958
Hectares	10	23
Average Growout Days	223	224

^a Data for Amatikulu represent biomass harvested in 1998 while those of Mtunzini were obtained in 2001.

1.5. *Physico-chemical and biological parameters that influence the mariculture of F. indicus.*

Physico-chemical and biological parameters such as temperature, salinity, light intensity and stocking density are known to have an effect on the growth and survival of the species. The following paragraphs will briefly discuss abiotic and biotic factors that affect the growth performance of *F. indicus*. High levels of ammonia, nitrate, nitrites, phosphates and sulphide that are detrimental to the survival and growth of cultured animals is ignored since the farms flow-through design disposes and avoids the accumulation of such contaminants. Other more direct influences such as the negative effects of disease and the positive effects of an application such as genetic selection on growth is beyond the scope of this study but has been reviewed by Tacon (1990) and Treece and Fox (1993).

1.5.1. *The effect of light intensity and photoperiod on maturation and growth.*

In the hatchery, compared to diffused natural light, the maturation of gonads in *F. indicus* over long periods was found to increase under blue and green light (Emmerson, Hayes & Ngonyame 1983). Unlike red light that is rapidly attenuated, the irradiance of blue light of lower intensity is able to penetrate deeper into water and is used to approximate offshore oceanic benthic conditions (Emmerson *et al.*, 1983). Photoperiod has little or no effect on overall growth (Vijayan & Diwan, 1995).

1.5.2. *The effect of temperature and salinity on growth.*

Studies of natural stocks of prawns world-wide have demonstrated a direct relationship between maximum size and mean sea temperatures, with low rather than high temperatures limiting growth of tropical penaeids (Dall *et al.*, 1990). *F. indicus* tolerates a narrow range of temperatures and mainly inhabits warm to temperate waters with temperatures lower than 20 °C adversely affecting metabolism, nitrogen excretion, food consumption, food conversion ratios (FCR) and survival of the species (Kutty, 1969; Kutty Murugapoopathy & Krishnan, 1971;

Gerhardt, 1980; Maguire & Allan, 1992; Deering, Fielder & Hewitt, 1995; Vijayan & Diwan, 1995; Wyban, Walsh & Godin, 1995).

F. indicus, a euryhaline osmoregulator, is able to tolerate a wide range of external salinities by controlling internal osmolality (Parado-Esteba, Ferraris, Ladjja & de Jesus, 1987). Under natural conditions the species was able to tolerate 3.9 ‰ over a ten-day period (Shylaja & Rengarajan, 1993) while a study in the Philippines indicated strong osmoregulatory ability between 8 – 40 ‰ at 27 °C (Parado-Esteba *et al.*, 1987). Hatchery reared *F. indicus*, PL₂₀ – PL₆₀, displayed better growth and survival at 20 – 30 ‰. Animals larger than PL₆₀ were less tolerant of salinities ranging between 40 – 50 ‰ whereas salinities less than 10 ‰ were found to be lethal for postlarvae older than PL₄₅ (Kumlu & Jones, 1995).

Long term survival of juveniles kept at salinities of 10, 15, 20 and 25 ‰ and at temperatures of 15, 20 and 25 °C in laboratory trials were lowest at 15 °C for all salinities (Pollard, 1998). The optimum temperature and salinity for juveniles over a 20-day growth trial were 31 °C and 15 ‰ (Vijayan & Diwan, 1995) with growth decreasing at temperatures above 35 °C (Evans, 2004 *per. com.*, data from Iran).

1.5.3. The effect of stocking density on growth.

A stocking density of 50 postlarvae per m² was recommended by Emmerson and Andrews (1981) for *F. indicus*, reared in circulating water, since higher densities at 27 – 28 °C increased ammonia levels that was detrimental to the survival and growth of the species. Prior to harvesting and after 7 - 9 months in growout ponds the practice of stocking 60 – 80 PL₁₂ per m² by Amatikulu Prawns (Pty.) Ltd yields ~ 45 % survival.

1.5.4. Moulting and growth.

Tissue growth is essentially a continuous process and unlike in lower vertebrates such as fish, growth in prawns proceeds by a series of moults or ecdyses and occurs when the old integument is discarded. During this phase a rapid increase in size occurs before the new cuticle hardens and becomes inextensible. Periods separating moults are termed intermoult periods or diecdyses. Thus, in order to grow both processes need to occur.

Growth physiology and reproduction of penaeids are essentially linked to the moulting cycle (Vijayan, Mohammed & Diwan, 1997). Vijayan *et al.* (1993), described the morphological and histological structure of the Y-organ, an organ considered to be the source and release of ecdysteroids that initiates and controls the moult cycle. Investigations have primarily focused on the moulting stages and cycles of adult females *F. indicus* during ovary development. The following moult stages in *F. indicus* have been recognised and are: stage A, early postmoult; stage B, late postmoult; stage C, intermoult; stage D₀₋₁, early premoult; stage

D₂, premoult; stage D₃, late premoult and stage E, ecdysis (Read 1977; Vijayan & Diwan, 1996). In adults, the premoult stage was found to occupy a major portion of the moult cycle, followed by the intermoult and postmoult stages (Vijayan *et al.*, 1997).

Temperatures of 26 °C and lower have been reported to affect moulting frequency resulting in poor growth due to prolonged intermoult periods (Vijayan & Diwan, 1996). Emmerson (1980) found a positive correlation between an increase in temperature and a decrease in moult cycle period with the best line of fit describing the relationship at 21 – 29 °C to be exponential. The effects of temperature (Parado-Esteva *et al.*, 1987; Vijayan & Diwan, 1995), light intensity (Emmerson *et al.* 1983; Vijayan & Diwan, 1995), dissolved oxygen concentration (Kutty, 1969; Kutty, Murugapoopathy & Krishnan, 1971), injury and trauma (Dall, Hill, Rothlisberg & Sharples, 1991a) play a significant role in controlling the frequency and duration of moulting. In *F. indicus* moulting occurs primarily between 00:00 h and 04:00 h (Vijayan *et al.*, 1997).

1.6. The future of the prawn industry in South Africa.

Inconsistency of rainfall and modification of freshwater fluxes by anthropogenic structures such as dams and weirs have disrupted the natural cycle and mouth dynamics of most estuarine systems in South Africa. With the exception of harbours, expected increases in future demand and further abstraction for this finite resource may prolong periods of mouth closure in estuaries along KZN. Extended periods of mouth closure will prove detrimental for postlarvae seeking nursery habitats, which in turn will have a negative impact on wild stock populations.

In South Africa trawled and cultured *F. indicus* constitute the resource output for the industry with mariculture yields in 2003 outstripping offshore catches. The advantage of prawn mariculture is that the mechanisms for farming prawns are reliant to a lesser degree on natural events and river mouth dynamics since culturing techniques are able to manipulate, to some extent, environmental factors.

Of the 2.4 million metric tons of prawns placed annually into the world market, 25 % are cultivated (Rosenberry, 1990 *loc. cit.* Hopkins *et al.*, 1995). While the commercial harvest of wild prawn stocks can be considered to have stabilised, declining slightly due to overfishing and habitat loss, prawn farming continues to expand with cultured stocks largely stabilising the price of prawns. It is envisaged that an increase in future demand will inflate consumer prices, which in turn will intensify fishing pressure for finite wild stocks requiring additional supplies to originate from prawn cultures (Hopkins *et al.*, 1995).

Globally, prawn mariculture is not without problems, since it has been found to impact negatively on the environment and surrounding ecosystems due to the discharge of effluent and habitat destruction when constructing ponds (Clarke *et al.*, 1992; Zieman, Walsh, Saphore & Fulton-Bennett, 1992; Hopkins, DeVoe & Holland, 1995; Sansanayuth, Phadungchep, Ngammontha, Ngdngam, Sukasem, Hoshino & Ttabucanon, 1996; Sze, 1998; Tookwinas,

1998). In semi and intensive stocked ponds, feed residue and waste material accumulates faster than the natural decomposition process can assimilate. Nutrient loading is particularly severe towards the end of the growth season when biomass and feed demands are greatest. The problem of waste management is aggravated by a general tendency to overfeed, since prawns are bottom feeders whose consumption rates are difficult to estimate. In South Africa despite Pillay (2000) having found that the impact of prawn effluent in estuaries was minimal, concerns remain regarding localised oxygen depletion where the effluent canal meets the Matigulu estuary during periods of maximum production.

In mariculture the greatest expense is the manufacturing of feed. In the past diets formulated and manufactured for *F. indicus* consisted of ~ 43 % crude protein. However, in recent years the industry has manufactured a starter, grower and finishing feed comprising 40, 38 and 36 % CP, respectively formulated using the ingredients listed in Table 1.1. These values are derived from feeds produced by Seychelles Marketing Board (SMB) who, in collaboration with VDS Crustacean feeds, supply mainly to Iranian and Saudi farmers culturing *F. indicus*.

The provision of dietary protein increases costs considerably since approximately 50 % of the protein is derived from fish meal (Table 1.1). With increased costs of high quality fish meal, due to natural fish stocks being under increasing fishing pressures, future demand for this resource might ultimately exceed supply and lead to a substantial increase in production costs. This is especially true for aquaculture operations in developing countries where a weaker currency dramatically increases import costs of good quality fish meal. Furthermore, an increase of raw ingredient prices and feed manufacturing costs coupled with a static and/or decreased market value for cultured prawns, necessitates a reduction in production costs in order to increase profitability.

In the past, the practice of culturing prawns in South Africa not only proved viable but also provided much needed employment in the province. In 2000, against a strong US\$, Amatikulu Prawns (Pty.) Ltd was able to export prawns at US\$ 10.00 per kg, equivalent to 100.00 South African Rand (ZAR) per kg. However, in recent years due to the strengthening of the ZAR the industry was forced to land larger grades of about 20 - 30 individuals per kg at US\$ 5.00 per kg making it difficult to compete with export countries that fixed their currency to the US\$ such as China, Iran and Saudi Arabia. During 2004 Amatikulu Prawns (Pty.) Ltd had to compete with farmers abroad who were able to supply prawns at ZAR 30.00 per kg. The consequence was that sale prices were well below production costs, which in turn resulted in the closure of the industry at the end of 2005.

Presently managers of Amatikulu Prawns in conjunction with potential investors, both national and international, are reviewing the situation with the intention of restarting production. Development of a diet that will keep production costs to a minimum remains an obvious goal to be targeted, provided that the total biomass previously obtained is maintained or improved.

Due to these reasons, the rationale for this thesis was to establish a means of lower production costs by evaluating current feeds previously used by the industry and to assess if protein requirement in *F. indicus* changes with age. From the literature it is evident that growth is affected by a multitude of factors and since it was beyond the time scale of this study to examine all variables, trials were undertaken using parameters that were either reflective of ponds conditions or construed, based on criteria specified in the literature, as being ideal for survival and growth. Hence, the main objectives of this study were to construct a model whereby the protein requirement of *F. indicus*, during the grow-out phase, could be established and to evaluate the digestibility of feedstuffs found locally. In order to do this the following were undertaken:

- I.) Review and summarise dietary studies involving *F. indicus*.
- II.) Develop and establish a performance model whereby changes in body composition and protein retention in the body tissue of *F. indicus* can be predicted.
- III.) To determine what effect diets varying in protein content had on the metabolic rate and standard dynamic action of *F. indicus* under various environmental conditions.
- IV.) Establish the optimal protein requirement for the species based on the effective energy systems approach.
- V.) To evaluate the model approach by comparing the protein requirements in three different age classes of *F. indicus*, fed diets containing graded levels of protein during short-term growth trials, to that predicted using the model.
- VI.) To establish if endogenous crude fibre and/or acid-insoluble ash, when compared with chromic oxide, were suitable for use as dietary markers.
- VII.) To evaluate the digestibility of local feedstuff using the substitution technique.
- VIII.) To formulate new diets based on these findings.

2.

General Review:

Nutritional Requirements of *Fenneropenaeus indicus*.

2.1. Introduction.

Nutrition encompasses the chemical and physiological processes that provide nutrients to an animal for maintenance and growth. On the other hand the process of nutrition involves the ingestion, digestion, absorption and transport of nutrients as well as the removal of waste products. Since early 1970 dietary studies, undertaken to determine the nutrient requirements and formulate feeds that promoted maximum growth and survival of penaeids, have played a vital role and contributed towards the development of semi-intensive and intensive mariculture operations (Akiyama, Dominy & Lawrence, 1991). However, comparisons between past and current studies prove difficult due to differences in research methodologies and the absence of a standard and/or reference diet, despite attempts by Reed and D'Abramo (1989) and Castell, Kean, D'Abramo and Conklin (1989) to establish one.

Problems of comparison are exacerbated due to differences in research variables viz. environmental parameters, experimental design, food source, diet composition, species, size and physiological state of the animal (Akiyama, 1992). These problems are compounded by the absence of ingredient definitions, lack of international feed numbers, proximate chemical composition, quality analysis of ingredients and the particle size used in manufactured feeds (Tacon & Akiyama, 1997). Furthermore, efforts to obtain a standard/reference diet are made difficult since researchers have a preference for using local feedstuff as opposed to importing raw ingredients or diets at possibly greater cost from elsewhere. Such problems are evident in Table 2.1 where the requirement for a particular ingredient or nutrient by *Fenneropenaeus indicus* is shown to vary due to differences in experimental parameters and feedstuffs used.

The term "nutrient requirement" used in Table 2.1 is defined as the minimal percentage of the nutrient needed within the diet for a species to achieve its potential or maximum growth rate (Guillaume, 1997). The expression "true requirement", associated with the term "optimal level", refers to the minimal amount needed either per animal or per biomass per day since animals are fed quantities of nutrients and not percentages or ratios (Guillaume, 1997).

Table 2.1: The life stages, survival, growth rates, feed conversion ratio's (FCR) and nutrient requirements of *F. indicus* fed different diets and reared under various conditions. Dietary information displaying single values of growth, feed sources, inclusion level, FCR and nutrient requirement are derived from diets that yielded the best results for the species.

Life stage or mass in (g)	Trial duration (days)	Temperature (°C)	Salinity (‰)	Survival (%)	Growth mg/animal/day	Primary feed source, with the inclusion level thereof in the diet expressed as a (%)	FCR	Nutrient/ingredients investigated with the test range thereof expressed as a (%)	The range in dietary protein expressed as (%) tested.	Determined nutrient/ingredient requirement	Authors
0.90	35	29.0 (± 1.0)	34.5 (± 1.5)	80-100	105	Prawnmeal (57.5) Potato starch (60.2)	2.4	Protein (21.3 - 53.1)	21.3 - 53.1	43.0 (%)	Colvin (1976 ^a)
0.10	30	30.5 (± 2.0)	4.7 (± 2.9)	80	13.8	Prawn meal (28) Tapioca (31) Groundnut cake (38)	1.7	Protein (33.3 - 35.0)	33.3 - 35.0	35.0 (%)	Ahamad (1982)
0.20	30	31.7 (± 0.9)	18.8 (± 1.5)	100	23.0	Prawn meal (35) Tapioca (15) Groundnut cake (48)	1.3	Protein (20.6 - 46.5)	20.6 - 46.5	42.9 (%)	Ahamad (1982)
PL ₁ - PL ₄₂	10 - 15	29.44 (± 1.8)	20.8 (± 5.9)	25	-	Casein (20 - 70) Starch (54.5 - 4.45) Lipid (12)	-	Protein (20.0 - 70.0)	20.0 - 70.0	PL ₁ - 10 40 (%) PL ₁₁ - 25 30 - 50 (%) PL ₂₇ - 42 30 (%)	Bhaskar & Ahamad (1984)
PL (0.02)	30	27.4 (± 2.4)	20.0 (± 2.5)	70	3.2	Casein (36) Egg Albumin (1) Starch (21)	0.8	Protein (32.5 - 47.5)	32.5 - 47.5	35.0 - 37.5 (%)	Gopal & Raj (1990)
PL	60	28.3 (± 1.7)	26.5 (± 5.9)	70	15	Fishmeal (42) Tapioca (33) Ricebran (17)	-	Protein (15.3 - 30.6)	15.3 - 30.6	22.4 (%)	Thomas, Kathirvel & George (1991)
0.06	30	28.0 (± 0.5)	16.0 (± 0.5)	100	Albumin - 7.1 Casein - 4.2	Albumin (30) Casein (29) Cellulose (6) Cod liver oil (6) Starch (24)	3.5	Protein	29	Albumin 25 - 33 (%) Casein 29 - 37 (%)	Ahamad (1994)
0.07	30	28.5 (± 0.5)	18.1 (± 1.0)	75	-	Clam meat powder (51) Fishmeal (47) Mantis shrimp (69) Shrimp waste head (86) Silkworm pupa (50) Coconut cake (73) Gingerly (89) Ground nutcake (62) Spirulina (50)	-	Plant <i>versus</i> Animal Protein	30.3	Animal Protein	Ramesh & Kathiresan (1992)

Table 2.1: *continued.*

0.33	60	30.0 (± 1.0)	30.0 (± 1.0)	89	14.2	Squid (40) Groundnut oilcake (39) Ricebran (13) Arginine: lysine:methionine: tryptophane 1:1:1:1	3.0	Crystalline Amino Acids (0.6)	42.4	0.6 (%)	Fernandez & Sukumaran (1995)
0.04*	30	28.0 (± 0.5)	18.0 (± 0.5)	> 60	4*	Albumin (40) Cellulose (8) Cod liver oil (6) Sucrose:multose:starch 1:1:1	3.5	Carbohydrate	31.5	32.8 (%)	Ahamad (1993)
0.01*	30	17.2 (± 1.0)	28.6 (± 0.5)	90	10.0	Casein (10) Albumin (10) Fibrin (10) Gelatin (10) Sucrose:multose:starch 1:1:1	2.9	Carbohydrate (52.7 - 55.0)	34.8	32.3 (%)	Ahamad (1996)
0.60	35	29.0 (± 1.0)	34.5 (± 1.5)	90	91.0	Fishmeal (31) Prawnmeal (16) Corn Flour (37)	2.7	Lipid (8.0 - 9.9)	38.3	9.8 (%) $\omega 3 : \omega 6 = 1:1$	Colvin (1976 ^b)
0.50	30	26.0 (± 2.5)	25.0 (± 3.0)	100	45.0	Fishmeal (18) Prawn meal (31) Maize flour (28) Food yeast (10)	1.8	Lipid	34.0	Sunflower:fish $\omega 3 : \omega 6 = 1:1$	Read (1981)
0.05	30	28.5 (± 1.5)	20.0 (± 2.0)	85	18.3	Casein (31) Albumin (8) Cod liveroil (6) Soybean oil (3) Lecithin (2)	2.2	Lipid (0 - 18)	40.0	12 (%)	Chandge & Raj ^{a,b} (1997)
0.05	30	27.9 (± 1.9)	20.0 (± 2.0)	55	4	Casein (31) Albumin (8) Cod liveroil (6) Soybean oil (3) Lecithin (2)	16	Palmitic acid (9) Linoleic acid (0.5) Linolenic acid (0.5)	40.0	Linoleic: Linolenic 1:1	Chandge & Raj (1998)
N ₆ - PL ₁	11	26.0 (± 0.0)	35.0 (± 0.0)	96	-	<i>Thalassiosira weissflogii</i> <i>Brachionus plicatilis</i> <i>Artemia salina</i>	-	Natural feed	-	-	Emmerson (1980)
PZ ₁ - PL ₁	6	27.5 (± 0.5)	25.0 (± 0.0)	70	-	<i>Tetraselmis chuii</i> (25 µl ⁻¹) + <i>Skeletonema costatum</i> (35 µl ⁻¹) + 5 <i>Artemia salina</i> ml ⁻¹	-	Natural feed	-	-	Kumlu & Jones (1993)
0.07	90	24.9 (± 0.1)	17.7 (± 3.6)	95	62.1	Fishmeal (37) Ricebran (13) Tapioca Flour (31) Oilcake (37)	0.6	Protein Hormones	59.5	HCP (10 _{ppm}) + TP (1.5 _{ppm})	Sambhu & Jayaprakas (1994)
0.10	85	24.7 (± 0.4)	24.0 (± 2.7)	84	126.0	Fishmeal (37) Ricebran (13) Tapioca Flour (31) Oilcake (37)	1.3	Protein Hormones	40.0	TP (3µg.g ⁻¹)	Sambhu & Jayaprakas (1997)

Table 2.1: *continued.*

9.00	120	unknown	Brackish (salinity unknown)	97 (HCG) 92 (L-thy)	106mg (HCG) 111mg (L-thy)	Fishmeal (37) Tapioca (13) Ricebran (37) Groundnut cake (13)	1.0 HCG 1.1 L-thy	Protein Hormones	40.2	12.5mg HCG 1.1mg L-thy	Jayaprakas & Sambhu (1998)
0.13	120	24.0 (\pm 1.0)	24.9 (\pm 3.8)	88	95.0	Fishmeal (37) Tapioca (13) Ricebran (37) Groundnut cake (13.24)	1.0	L-carnitine	40.2	500 _{ppm}	Jayaprakas & Sambhu (1996)

* determined as dry mass

HCG – Human chorionic gonadotropin

L-thy - L-thyroxine

TP – testosterone propionate

ppm – parts per million.

Comparisons only become more meaningful if growth trials are performed using similar feedstuffs/ingredients and/or in which the test organisms are exposed to similar conditions. Hence, a consensus needs to be reached that establishes a protocol that standardises both experimental parameters and procedures. Due to limitations in nutritional knowledge and the problems highlighted above, a need to apply and develop innovative approaches remains. But in order to do this, an understanding pertaining to factors that constitute and encompass a balanced diet that fulfils the nutritional need of any said species, is a prerequisite and will be dealt with briefly in this chapter. The following sections are a review highlighting the dietary requirements of *F. indicus*.

2.2. Protein.

Proteins consist of bonded amino acids and are large complex molecules, which are essential in the structure and function of all living organisms. In most animal tissues, proteins are the major organic component comprising about 65 – 75 % of the total on a dry matter basis (dm) and differ in size and function due to the relative proportions of integrated amino acids. About 20 amino acids make up most protein molecules, and depending on the protein structure, some proteins may be deficient in certain amino acids while others may include all twenty.

Prawns, like most animals, do not have an absolute requirement for protein *per se* but do require a balanced mixture of essential and non-essential amino acids (Lovell, 1989). To obtain a continual supply of amino acids an animal needs to consume protein. An insufficient supply results in the reduction and cessation of growth followed by mass loss due to the withdrawal of protein from body tissues since prawns, in order to maintain vital bodily functions, are able to routinely oxidise amino acids as a source of energy. Conversely, if a prawn ingests too much protein, part thereof will be metabolised while the remainder is converted into energy or excreted as ammonia. The hydrolysis of ingested protein releases free amino acids, which once absorbed by the intestinal tract and transported into the bloodstream are distributed to the various organs and tissues wherein they are synthesised into body protein (Akiyama *et al.*, 1991).

In nutritional studies two protein variables *i.e.* the dietary protein concentration and the protein source are generally of interest (Emmerson, 1982). However, regardless of definition, protein requirement is dependent on a myriad of biotic and abiotic factors such as age, species, physiological state, including dietary characteristics *e.g.* protein quality, digestibility, biological value and/or energy levels and environmental factors *viz.*, temperature, salinity and pH. In penaeids the optimal protein level is mostly independent of temperature and moderately dependent upon inter-species differences, size and/or age (Guillaume, 1997).

Due to feeding habits, nature of the diet, source and the organisms digestive system, various species require different concentrations of protein. The amount of dietary protein required for optimal growth in penaeid prawns is found to range between 28 – 60 % of the diet

(Table 2.2). From Table 2.2, *F. indicus*, which is naturally omnivorous, has a requirement of 22 – 43 %. This is lower than the 45 – 60 % established for a carnivorous species such as *Marsupenaeus japonicus*, yet is found to be similar to the 23 – 41 % required by herbivorous *Litopenaeus vannamei*.

Table 2.2: Dietary requirement of protein by penaeids.

Species	Protein source	Protein requirement (%)	References
<i>Farfantepenaeus aztecus</i> (<i>Penaeus aztecus</i>)	Fish meal + squid meal	29 - 31	Shewbart & Mies (1973)
	Fish meal + mixture	< 40	Venkataramiah <i>et al.</i> (1975)
	Soy flour + menhaden meal + shrimp meal	51	Zein – Eldin & Corliss (1976)
<i>Farfantepenaeus californiensis</i> (<i>Penaeus californiensis</i>)	Fish meal + shrimp meal and soybean meal	> 44	Colvin & Brand (1977)
<i>Farfantepenaeus duorarum</i> (<i>Penaeus duorarum</i>)	Soybean meal	30	Sick & Andrews (1973)
<i>Fenneropenaeus indicus</i> (<i>Penaeus indicus</i>)	Prawn meal	43.0	Colvin (1976a)
	Prawn meal	35.0	Ahamad (1982)
	Prawn meal	42.9	Ahamad (1982)
	Casein	PL ₁ – 10 40 PL ₁₁ – 25 30 - 50 PL ₂₇ – 42 30	Bhaskar & Ahamad (1984)
	Casein & Egg Albumin	35.0 – 37.5	Gopal & Raj (1990)
	Fish meal	22.4	Thomas, Kathirvel & George (1991)
	Albumin Casein	Albumin – 25 Casein – 29	Ahamad (1994)
<i>Fenneropenaeus merguensis</i> (<i>Penaeus merguensis</i>)	Mussel Meal	34 - 42	Sedgwick (1979)
<i>Fenneropenaeus penicillatus</i> (<i>Penaeus penicillatus</i>)	Fish meal	22 - 27	Liao <i>et al.</i> (1986)
<i>Fenneropenaeus setiferus</i> (<i>Penaeus setiferus</i>)	Fish meal + collagen + squid meal	28 - 32	Andrew & Sick (1972)
<i>Fenneropenaeus stylirostris</i> (<i>Penaeus stylirostris</i>)	Fish meal + shrimp meal and soybean meal	> 10	Colvin & Brand (1977)
	"	30 - 35	Colvin & Brand (1977)
<i>Litopenaeus vannamei</i> (<i>Penaeus vannamei</i>)	"	> 30	Colvin & Brand (1977)
	"	30	Cousin <i>et al.</i> (1990)
	Fish meal	23 - 41	Smith <i>et al.</i> (1985)
	Casein + albumin	54	Deshimaru & Kuroki (1974)
<i>Metapenaeus japonicus</i> (<i>Penaeus japonicus</i>)	Squid meal	60	Deshimaru & Shigueno (1972)
	Shrimp Meal	> 40	Balazs <i>et al.</i> (1973)
	Casein + albumin	52 - 62	Deshimaru & Yone (1978)
	Casein + albumin	> 55	Teshima & Kanazawa (1984)
	Casein + albumin	45 - 55	Teshima & Kanazawa (1984)
	Crab protein	42	Koshio <i>et al.</i> (1993a)
	Crab protein	42	Koshio <i>et al.</i> (1993a)
<i>Metapenaeus monoceros</i>	Casein	55	Kanazawa <i>et al.</i> (1981)
<i>Metapenaeus macleayi</i>	--	27	Macguire & Hume (1982)
<i>Penaeus brasiliensis</i>	Shrimp meal + casein	55	Liao <i>et al.</i> (1986)
<i>Penaeus monodon</i>	Casein + fish meal	46	Lee (1971)
	--	40	Khanapa (1977)
	Mixture	35	Bages & Sloane (1981)
	Mixture	40	Alava & Lim (1983)
	Shrimp meal + casein	45	Liao <i>et al.</i> (1986)
	Mixture	40	Shiau <i>et al.</i> (1991)

An important source of protein used in prawn feeds is derived from marine sources, since they tend to be the natural prey and food of ocean dwelling crustaceans. Preferences for using marine sources are based on their contribution to the overall flavour of the feed, their significance as an excellent source of ω -3 polyunsaturated fatty acids (PUFA) and their assumed efficiency for energy utilisation (D'Abramo *et al.*, 1997). In recent years, due to the decline and

availability of good quality fish meal at reasonable prices it has become common practice to include vegetable protein into crustacean feeds as a substitute for a predominantly marine based diet.

In a study by Thomas, Kathirvel and George (1991) artificial feeds comprising ricebran, wheat flour, tapioca powder, coconut cake, groundnut cake, fish meal, prawn meal and mineral supplements of varied proportions were used to formulate feeds ranging from 15.3 to 30.6 % crude protein (CP). A diet comprising a combination of 33.3 % tapioca powder, 16.7 % ricebran and 41.7 % fish meal with a protein content of 22.4 % produced the highest growth rate of 15 mg/day in *F. indicus*.

In a comparative study five animal protein sources; clam meat powder (*Sunneia scripta*), fish meal, mantis shrimp (*Oratosquilla nepa*), shrimp waste, silkworm pupae were evaluated against three plant protein sources; coconut cake, gingely cake, groundnut cake including a single cell protein *Spirulina* spp. By formulating isonitrogenous diets ingredients were evaluated by comparing feed indices viz. true digestibility of protein, protein efficiency ratio (PER), net protein utilisation (NPU), biological value (BV), growth and feed:gain ratio (FGR). Among animal sources fish meal produced higher PER, NPU and BV followed, in decreasing order, by clam meat powder, shrimp waste, mantis shrimp and silkworm pupae. Digestibility of clam meat powder was significantly higher than shrimp waste, followed by mantis shrimp and silkworm pupae. Amongst plant sources groundnut and *Spirulina* produced higher growth than coconut and gingely cakes with NPU and BV of *Spirulina* being considerably better than those of groundnut cake. In general, compared to plant sources including *Spirulina*, animal protein provided for better indices viz., PER, NPU and BV (Ahamad, 1992).

2.2.1. Protein requirement.

In addition to fish meal, Colvin (1976a) used dried prawn meal, derived from the same species, to establish the protein requirement of *F. indicus*. A complete substitution of fish meal with prawn meal at similar crude protein inclusion levels gave improved growth rates. Live-mass gains at levels ranging from 21 – 53 % were greatest for a diet containing 43 % crude protein (CP) with the efficiency of protein utilisation declining with an increase in dietary protein beyond 43 % CP (Colvin 1976a). A later study by Ahamad (1982) using readily available ingredients such as fish meal and groundnut cake formulated to comprise 20.5 – 46.5 % CP confirmed Colvin's (1976a) findings, since a diet comprising 42.9 % CP produced higher growth and a better food conversion ratio (FCR) in 0.2 g juveniles. The protein requirements based on purified casein diets changed with age since dietary requirement for PL₁, found to be 40 % CP, decreased to 30 % in PL₃₀ (Bhasker & Ahamed Ali, 1984) whereas in a subsequent

study the requirement for 0.02 g individuals, using similar ingredients, was 35 – 37.5 % (Gopal & Raj, 1990).

Results from two sets of diets, comprising either albumen or casein, indicated that the optimum protein level for albumen based diets was 25 – 33 % and that of casein 29 – 37 % (Ahamad, 1994). Consequently, *in vitro* investigations on the digestibility of dietary protein using hepatopancreas tissue of *F. indicus* revealed that the prawn was able to effectively utilise 33 – 35 % dietary protein with higher levels being poorly digested (Ahamad *et al.*, 2000). With the use of albumen and casein based diets, nitrogen balance, calculated as the difference between dietary nitrogen and faecal nitrogen, indicated that a minimum of 22.5 % CP provided a zero nitrogen balance with a metabolic faecal nitrogen (MFN) of 324 mg/100g diet consumed (Ahamad, 1994).

2.3. Amino acids.

In theory the nutritional value of dietary protein depends essentially on its amino acid composition. This is certainly the case for those proteins that are highly digestible and readily assimilated. Amino acids are derived from dietary or metabolic sources. Proteins derived from dietary or endogenous origin *e.g.* from enzymes, epithelial cells and gut bacteria, are hydrolysed prior to absorption. Amino acid metabolism is concerned with the totality of reactions involving their biosynthesis and use within both anabolic and catabolic pathways. In contrast to carbohydrates and lipids, which may be stored either as hepatic and muscular glycogen and body lipid respectively, protein when supplied in excess cannot be accumulated within the prawn. Whilst protein synthesis allows for the manufacture of new proteins, degradation on the one hand leads to nitrogen excretion and, on the other, to the production of carbon skeleton molecules, or oxidised into CO₂ which is expired. The carbon skeleton may be used in metabolic pathways involving energy transfer by conversion into carbohydrates (glucogenesis) or into lipids (lipogenesis) (Larbier & Leclercq, 1994).

Prawns, like most other animals, are incapable of synthesising all amino acids and must therefore have them included in their diet. With respect to protein synthesis, all amino acids are equally essential as the absence of one will interfere with the anabolic processes. However, from the biochemical point of view and in terms of nutrition the pathways by which transamination and deamination of amino acids take place in higher vertebrates is thought to be similar in an invertebrate prawn. Thus, as in vertebrates, amino acids have been classified into three groups:

2.3.1. Essential amino acid.

They have to be provided in the diet and are subdivided into three categories (Larbier & Leclercq, 1994):

- a) Those amino acids which are strictly essential because they cannot be synthesised even from intermediary metabolites particularly 2-oxo precursors. These are lysine and threonine for which the transaminases are absent.
- b) Those which may be synthesised from their respective 2-oxo precursors, but at a rate that is insufficient to meet the demand for protein synthesis and maintenance. These are leucine, valine and isoleucine.
- c) Those that may be synthesised within the general metabolic process but at a rate far too low to meet the requirements of the prawn. These are arginine and histidine. The former may be synthesised from glutamate through utilising a metabolic pathway involving *N*-acetyl derivatives, in the urea cycle as in mammals

2.3.2. Semi-essential amino acids.

These may be synthesised from essential amino acids. They are cysteine and tyrosine, which arise from methionine and phenylalanine respectively. Cysteine is synthesised from methionine and serine, which are essential and non-essential respectively. Methionine is initially transformed into adenosyl methionine, thereafter into *S*-adenosyl homocysteine and finally into homocysteine which reacts with serine to form cysteine and homoserine.

Tyrosine is synthesised following the hydroxylation of phenylalanine, an essential amino acid, in the presence of phenylalanine hydroxylase, which is an oxygenase (Larbier & Leclercq, 1994).

2.3.3. Non-essential or ordinary amino acids.

These are easily synthesised either from intermediary metabolites or from similarly non-essential amino acids. In the former group are alanine, glycine, serine and aspartic and glutamic acids, and in the latter glutamine and asparagine. Alanine is obtained through the transamination of pyruvate in the presence of glutamate or aspartate and an amino group donor. Glycine may be synthesised from choline or serine. Aspartic acid is derived from the transamination of oxaloacetate, with alanine being the amino donor.

The same applies to glutamic acid from α -keto glutarate. The amino donor may be another amino acid or simply ammonia, a reaction catalysed by *L*-glutamate dehydrogenase in the presence of NAD^+ or NADP^+ . Glutamine synthetase catalyses the synthesis of glutamine from glutamate and NH_4 in the presence of ATP and Mg^{++} . This reaction is analogous to that

where asparagine is synthesised from aspartate through asparagine synthetase (Larbier & Leclercq, 1994).

In the case of serine, two biosynthetic metabolic routes exist which utilise the same metabolite from glycolysis, *D*-3-phosphoglycerate. Through the route based on phosphorylated intermediaries, the metabolite is initially oxidised to phospho-hydroxypyruvate, which is transaminated into phosphoserine and after losing its phosphate group, serine. In the pathway that does not involve phosphorylation, *D*-3-phosphoglycerate initially loses its phosphate group to give *D*-glycerate which is itself oxidised into hydroxy-pyruvate before being transaminated to provide *L*-serine. The amino acid proline is synthesised directly from glutamic acid, through a metabolic pathway, which is the complete reverse of the catabolic process (Larbier & Leclercq, 1992).

2.3.4. Amino acids requirements.

Early studies by Cowey and Forster (1971a) and Shewbart, Mies and Ludwig (1972) identified arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine as being essential for the *Palaeomon serratus* and *Penaeus aztecus*. A subsequent study by Kanazawa and Teshima (1981) established that the requirements for essential amino acids by *M. japonicus*, with the exception of tyrosine, were similar. The formation of tyrosine by *M. japonicus* was thought to be from non-essential phenylalanine. Numerous works on essential amino acids established in several species of prawn, cited by Guillaume (1997), are in agreement and confirm Kanazawa and Teshima's (1981) findings. Thus, indispensable amino acids in crustaceans, as in higher vertebrates, are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

Imbalances of essential amino acids are equally important since a lysine and arginine dietary relationship, known as the lysine-arginine antagonism found to occur in livestock fed excess levels of either amino acid, resulted in depressed growth. Although not yet demonstrated in prawns, it is believed that the lysine:arginine ratio should be maintained at 1:1 to 1:1.1 (Akiyama *et al.*, 1992) since Millamena, Buatista-Tereul, Reyes and Kanazawa (1998) found the lysine and arginine requirement in *P. monodon* to be 2.1 and 1.9 % of the diet, respectively. However, if a correlation between the dietary profile of essential amino acids and those found in body tissue is to be presumed, then a collagen lysine to arginine ratio of 1:1.9 in *F. indicus* (Sivakumar, Suguna & Chandrakasan, 1997), indicates that the dietary lysine:arginine ratio could be considered higher than the 1:1 specified by Akiyama *et al.* (1992).

A relationship between the branched amino acids, leucine, isoleucine and valine is also assumed to occur (Akiyama *et al.*, 1992). These essential aliphatic acids promote muscle growth when catabolised within the prawn (Murray *et al.*, 1990). The effect of the crystalline

amino acids arginine, lysine, methionine and tryptophan on the growth of juvenile *F. indicus* was tested during a 60 day growth trial by Fernandez and Sukumaran (1995). Diets comprising squid meal and fish meal with a mixture of synthetic arginine, lysine, methionine and tryptophan in a 1:1:1:1 ratio was incorporated at 0.4, 0.6 and 0.8 % or individually at 0.1 % in fish meal-based diets. Growth rate, FCR, protein efficiency ratio, consumption units, assimilation, metabolism, gross and net growth efficiency were better in *F. indicus* fed a diet based on squid meal with the amino acid mixture incorporated at 0.6 % (Fernandez & Sukumaran, 1995). These findings are in agreement with Teshima, Kanazawa and Koshio (1992) who successfully tested a method of improving the amino acid balance of protein by binding amino acids to proteins, known as plasteins. A more cost effective approach demonstrated by Chen, Leu and Roelants (1992a) indicated that the growth of *P. monodon* improved when crystalline arginine microencapsulated in cellulose acetate, phthalate or glycerol monostearate supplemented in casein based diets proved more effective than the sole addition of crystalline arginine. A subsequent study by Chen *et al.* (1992a) confirmed that feeding *P. monodon* juveniles diets containing vitamin free casein supplemented with increasing amounts of micro-encapsulated arginine improved the biological value of casein as a protein.

2.4. Energy.

Energy in itself is not a nutrient, but is present in the chemical bonds that bind the nutrient molecules together. There are many different types of chemical bonds each associated with a different amount of energy. Accordingly, the amount of energy in the various components that make up a feed is of great importance. Generally prawns require energy for moulting, muscle activity, locomotion, reproduction, protein synthesis and the maintenance of bodily functions *e.g.* respiration, excretion and digestion. The biological process of utilising energy is defined as metabolism, while the rate at which energy is utilised is called the metabolic rate. Metabolic rate in prawns is influenced by biotic factors such as the species, age, body size, activity, physical condition, and body functions (Subramanian & Krishnamurthy, 1986; Kurmaly, Yule & Jones, 1989; Dall, Hill, Rothlisberg & Sharples, 1991a) and abiotic factors such as water temperature, oxygen and/or carbon dioxide concentrations, pH and salinity (Kutty, Murugapoopathy & Krishnan, 1971; Du Preez, Chen & Hsieh, 1992).

Based on similar mass to volume ratios crustaceans are believed to have lower dietary energy requirements than most terrestrial animals due to the following reasons. Firstly, prawns do not have to maintain a constant body temperature. Secondly, crustaceans require relatively less energy for locomotion since the minimum total metabolic cost of transport, which is calculated as the total metabolic expenditure divided by body weight (mass multiplied by the gravitational constant) and velocity, is inversely related to body mass for terrestrial (walking and running), swimming and flying locomotion (Withers, 1992). Thirdly, because crustaceans

are ammonotelic, excreting most of their nitrogenous waste as ammonia instead of urea or uric acid whereby the initial step involves the removal of the amino group ($-\text{NH}_2$) either by a process of transamination or deamination, less energy is required for the metabolism of amino acids (Akiyama *et al.*, 1991).

Energy requirement for the species was established by feeding *F. indicus* six iso-nitrogenous diets comprising 40 % CP in which the calorific content was formulated to range from 272 kcal/100g (1.14MJ/100g) to 462 kcal/100g diet (1.93MJ/100g). Results indicated that a progressive increase in dietary energy up to 415 kcal/100g (1.73 MJ/100g) improved the growth and FCR with a further increase providing no additional benefit (Ahamad, 1990).

2.5. Carbohydrates.

Carbohydrates include monosaccharides, disaccharides and polysaccharides. Important polysaccharides include starch, the major form of carbohydrates stored in plants, and glycogen, in animals. The primary source of energy in crustacea is derived from protein (New, 1976) but the substitution, utilisation and cost effectiveness of carbohydrates in crustacean feeds as alternative forms of dietary energy are undeniable since they assist by sparing protein for growth instead (Akiyama *et al.*, 1991).

The significance of starch in the diet of *F. indicus* was examined by Ahamad (1982) who formulated purified casein based diets comprising 30 % CP and found that growth and FCR improved with the addition of up to 40 % starch. Seven carbohydrates namely; glucose, fructose, galactose, maltose, sucrose, glycogen and starch when incorporated at 30 % in separate test diets indicated that growth and survival was the highest, with an apparent digestibility above 85 %, for individuals fed starch (Ahamad, 1993). Growth and FCR in *F. indicus* fed diets containing maltose and/or starch were superior as opposed to monosaccharides (Ahamad, 1993).

The use of maltose, sucrose and starch in a 1:1:1 mixture with albumen, casein, fibrin (blood) and gelatin in a ratio of 1:1:1:1, which provided a protein level of 35 %, revealed that the apparent digestibility was unaffected when fed 7.3 – 52.3 % carbohydrate (Ahamad, 1996). Growth was significantly higher and FCR lower for diets comprising 32.3 % carbohydrate with a total digestible energy (DE) of 388.7 kcal/100g (1.65 MJ/100g). At a constant level of 35 % CP and 6 % lipid, growth was shown to increase and FCR improve when fed diets with a concentration of 22.5 % carbohydrate and a DE of 347.6 kcal/100g (1.45 MJ/100g diet) (Ahamad, 1996). Results indicated that, for a given protein concentration, the prawn was capable of utilising carbohydrate of 22 – 40 % inclusion as a source of energy (Ahamad, 1996; 2000).

A study of the digestive gland in *F. indicus* revealed the hydrolysis of α -1 \rightarrow 4 and α -1 \rightarrow 1 bond oligosaccharides, including disaccharides, β -dextrin, and α -D-glucans *e.g.* starch, glycogen and, to a greater extent, pullan (Omondi & Stark, 1995a). However, β -type glycosidic bonds

such as the β -2 \rightarrow 1 bond in inulin and the β -2 \rightarrow 6 bond in levan including β -1 \rightarrow 4 bonds in β -D glucans like cellulose, xylan, chitin and agar tended to be resistant to enzymatic degradation. Failure to hydrolyze simple carbohydrates such as the building units, β -1 \rightarrow 4 bonds, of chitin indicated the absence of endogenous and exogenous microbial chitinase (Omondi & Stark 1995a). Results indicated the absence of β -1 \rightarrow 4 glucanase but a significant activity of α -disaccharase in the gut of the prawn. These results support Ahamad (1993) findings.

Carbohydrases from the midgut of *F. indicus* indicated that the species possesses a similar array of carbohydrate digesting enzymes found in *P. monodon* with the pH optima at 30 °C falling within a range of 5.0 - 8.0 (Omondi & Stark, 1995b). In juvenile *F. indicus* α -amylase activity, when fed purified diets containing graded levels of starch ranging from 0 – 40 % for a period of four weeks, increased to a maximum substrate turnover of 0.440 μ mol/ml/min. The highest digestibility coefficient of 81 % was established for diets with the inclusion of 20 % starch. Protease and lipase activity was shown not to vary significantly over the same period (Hemambika & Raj, 1999).

2.6. Fibre.

Fibre refers to a mixture of cellulose, hemicellulose, lignin, pentosans and other generally indigestible fractions found within feeds. A concern regarding high fibre content is that it not only increases faecal production, and as a consequence pollutes the water, but that fibre strands hinder the binding process by creating fractures that act as a conduit for water to enter and thus lower the water stability of the feed pellet. Hence, feeds are usually formulated to limit the level of fibre, notwithstanding that a strict limitation thereof significantly increases manufacturing costs (Akiyama *et al.*, 1992).

Chitin, a polymer of *N*-acetyl-*D*-glucosamine and a major structural component of the exoskeleton with linkages similar to the structure of cellulose, is believed to have a growth promoting effect. However, a minimum level of 0.5 % chitin recommended in commercial feeds by Akiyama *et al.* (1992) proved to have no benefit for *F. indicus*, other than being a source of fibre, since the species is unable to hydrolyse the building units, β -1 \rightarrow 4 bonds, that make up chitin (Omondi & Stark, 1995a).

Even though the cellulose digesting enzyme, cellulase, has been identified in *F. indicus*, cellulose was not digested in levels sufficient for consideration in diets (Omondi & Stark, 1995a). However, a study by Ahamad (1993) indicated that mixed carbohydrates of sucrose-maltose-starch in a 1:1:1 ratio gave a significantly better performance with the inclusion of up to 10 % cellulose with higher levels adversely affecting growth performance. The inclusion of cellulose was found to be necessary even at the expense of dietary energy since the FCR and survival of the species improved.

2.7. Lipids.

Lipids are the generic name for fat soluble compounds found in living matter and are broadly classified as fats, phospholipids, shingomyelins, waxes and sterols. Dietary lipids are important in the nutrition of prawns since they are a highly concentrated digestible sources of energy; are a basis of essential fatty acids necessary for growth and survival; serve as carriers of fat soluble vitamins and are a source for other compounds such as sterols and phospholipids essential for normal metabolic functions (Akiyama *et al.*, 1992).

Lipids such as phospholipids and sterols are important structural components of cell and organelle membranes. Sterols are important in the synthesis of steroid hormones and the hormone-like prostaglandins. Dietary lipids also serve as “food attractants” and can affect the texture of the feed. Recommended lipid levels for commercial feeds of prawns range from 6 - 7.5 % and should never exceed 10 % since decreased growth and increased mortality are associated with higher levels (Akiyama *et al.*, 1991).

2.7.1. Fatty acids.

High concentrations of essential fatty acids found in phospholipids are important in the activation of certain enzymes, in lipid transport and maintaining flexibility and permeability of biological membranes. Four fatty acids considered essential for prawns are: linolenic (18:3 ω 3), linoleic (18:2 ω 6), eicosapentaenoic (20:5 ω 3) and decosaheptaenoic (22:6 ω 3). Plant oils are generally high in 18:2 ω 6 and 18:3 ω 3 while oils derived from marine organisms are high in 20:5 ω 3 and 22:6 ω 3 (Akiyama *et al.*, 1991).

At 5 % inclusion the essential fatty acids linolenic (ω 3), linoleic (ω 6) and oleic (ω 9) type series derived from four plant oil sources namely, sunflower, groundnut, linseed and soybean oil produced no significant difference in growth of *F. indicus* (Colvin, 1976b). Although high tissue deposits of linoleic acid (18:2 ω 6) was associated with these diets, it was assumed that *F. indicus*, like many other penaeids, are unable to metabolise long fatty acids comprising C₂₀ and C₂₂ chains (Read, 1981).

Cod liver oil, prawn head oil, sardine oil and soy lecithin, were individually or in a mixture of equal proportions, tested in casein based diets having 40 % CP (Ahamad, 1990). Diets with prawn head oil produced the highest growth and a better FCR followed by soy lecithin, sardine oil and cod liver oil, respectively (Ahamad, 1990). Using the above oils in a 1:1:1:1 mixture, the effect of lipid concentration from 0 – 18 % was tested. Growth increased and the FCR improved at levels up to 6 %. Lower concentrations resulted in poor performance while higher levels had no beneficial effect and suppressed growth beyond 12 % inclusion (Ahamad, 1990). Working with individual fatty acids Chandge and Raj (1990, 1995a)

underpinned the importance of polyunsaturated fatty acids (PUFA), especially linoleic acid (18:2 ω -6), as being essential for growth and survival of *F. indicus*.

An inclusion of linolenic and linoleic, at a 1:1 ratio, at 1 % in the diet provided better growth rates than any other combination (Chandge & Raj, 1998). The superior performance of prawn head oil and sardine oil over other oils suggests the need for essential ω -3 fatty acids since a poor response associated with the exclusive use of vegetable oils advocates a lesser preference for a series of ω -6 fatty acids (Ahamad, 2000). Chandge and Raj (1995b) established that an inclusion of 2 % lecithin was the required dietary level for *F. indicus*.

In a subsequent study, *L*-carnitine produced significantly higher growth responses in *F. indicus* when comparing animals that were fed diets that had none (Jayaprakas & Sambhu, 1996). *L*-carnitine, a product derived from protein metabolism, transports long chain fatty acids from the cytoplasm to the mitochondrial matrix where it is metabolised by the β -oxidation enzyme, thereby performing a vital role in regulating the catabolism of fat. Feed conversion efficiency, assimilation efficiency and protein efficiency ratios were high in diets treated with carnitine, with optimum values recorded to be 500 parts per million (ppm) in diets with 40 % CP. Amylase, protease and lipase activity were highest in the mid-gut of prawns fed carnitine diets. RNA/DNA ratios in the muscle and hepatopancreas in treated prawns were significantly higher when compared to those of the control. Body lipid was found to decrease in all carnitine-fed groups, an indication of enhanced lipid catabolism, thereby sparing dietary energy and protein for growth and anabolic processes (Jayaprakas & Sambhu, 1996).

2.7.2. Cholesterol.

Prawns are incapable of synthesizing the sterol ring. Many sterols and essential components such as moulting hormones, sex hormones, bile acids and vitamin D, are synthesized from cholesterol. Cholesterol also functions as a component of cell membranes and in the absorption and transport of fatty acids (Akiyama *et al.*, 1991).

Lipid profiles of gonads, hepatopancreas and tail muscle tissue of *L. setiferus* have shown that cholesterol is the dominant sterol and that PUFA comprised a significant portion of the fatty acid fraction (Middleditch, Missler, Hines, McVey, Brown, Ward & Lawrence, 1980).

Dietary potential of cholesterol extracted from *Rhizophora mucronata* leaves was studied using *F. indicus*. The crude mangrove cholesterol was incorporated at 0.5, 1.0 and 2.0 % (w/w) in a basal diet in which prawn head waste, mussel meat, fish meal, groundnut oil cake, tapioca powder, rice bran and vitamin tablets were combined to produce a diet comprising 42 % CP, 10 % lipid and 40 % carbohydrate. FCR was found to decrease with an increase in mangrove cholesterol (Ramesh & Kathiresa, 1992). In a later study Chandge and Raj (1995b) confirmed the dietary requirement for cholesterol to be between 0.1 – 0.5 %.

The effects of steroids have been tested whereby diets containing either 3 $\mu\text{g.g}^{-1}$ synthetic androgen, testosterone propionate and diethylstilbestrol were administered to juvenile *F. indicus*. Compared to diethylstilbestrol and human chorionic gonadotropin (HCG) treatments the inclusion of testosterone propionate produced significantly superior growth ratios. Feed consumption, conversion efficiency and assimilation rates were increased after incorporation of the hormone in the diet. Activity of digestive enzymes of the stomach and hepatopancreas and the intestine of the treated prawns showed significant increases compared to that of the control while RNA/DNA ratios increased, corresponding with an increase in growth (Sambhu & Jayaprakas 1994; 1997).

2.8. Vitamins.

Vitamins are complex organic compounds and are required in minute amounts for normal growth, metabolism and reproduction. In intensive high-density culture practices where natural foods are limited, diets are supplemented with vitamins in order to promote normal growth. Vitamin requirements for prawns are affected by size, age, growth rate, environmental conditions, and nutrient inter-relationships. Water-soluble vitamins required by penaeids are known to be: thiamin (B_1), Riboflavin (B_2), pyridoxine (B_6), pantothenic acid, niacin, biotin, inositol, choline, folic acid, cyanocobalamine (B_{12}) and ascorbic acid, and the fat soluble vitamins A, D, E and K (Akiyama *et al.*, 1991).

Because they are fat soluble, Vitamins A, D, E and K are absorbed through the same mechanisms as are fats. A, D and E are subsequently thought to be stored in adipose tissue in quantities dependent upon the dietary concentration. These reserves, therefore, have the advantage of providing the prawn with regular amounts necessary to meet requirements, but have, at the same time, the disadvantage of becoming toxic should levels accumulate to where they become excessive. In terms of metabolism, those fat soluble vitamins found associated with cell membranes have a common critical role in ensuring their integrity.

Vitamins are classified according to their solubility in water or lipids. The former have closely related biochemical functions as they are all, without exception, involved in cellular metabolism as prosthetic groups within co-enzymes. Their activity is based fundamentally upon their structure, as even the smallest modification may render them completely inactive whereas fat soluble vitamins, on the other hand, have very diverse functions.

The supply of water-soluble vitamins in the diet pose a problem, since they are readily absorbed into the surrounding medium, underscoring the need and importance of the binding compound to retain these vitamins within the feed. The recommended levels of vitamins in commercial prawn feeds are summarised in Table 2.3.

Table 2.3: Recommended levels of vitamins in commercial prawn feed^a (Akiyama *et al.*, 1991).

Vitamin ^a	Quantity per kg Feed
Thiamin (B ₁)	50 mg
Riboflavin (B ₂)	40 mg
Pyridoxine (B ₆)	50 mg
Pantothenic acid	75 mg
Niacin	200 mg
Biotin	1 mg
Inositol	400 mg
Choline	600 mg
Folic acid	10 mg
Cyanocobalamine	0.2 mg
Ascorbic acid ^b / Vitamin C	1200 mg / 250 mg ^c
Vitamin A	10,000 IU
Vitamin D	5000 IU
Vitamin E	300 mg
Vitamin K	10 mg

^a As fed basis^b Coated ascorbic acid (heat stable ascorbic acid)^c Stabilised form of dietary ascorbic acid.

The requirements for water-soluble vitamins in *F. indicus* were investigated using a vitamin mixture in casein based purified diets. Absence of ascorbic acid in the diet resulted in poor feed intake, blackening of the gills, lesions in the abdomen and mortality whereas a dietary level of 0.4 – 0.8 % improved growth and survival.

Ascorbic acid and α -tocopherol acetate levels in broodstock diets of *F. indicus* affected the egg concentrations of highly unsaturated fatty acids (HUFA). A direct correlation was found between hatching success and the concentration of ascorbic acid and α -tocopherol acetate in eggs. Supplementation of HUFA in broodstock diets induced a decrease of α -tocopherol concentrations in both organs and eggs, suggesting that a large quantity of α -tocopherol was required as an anti-oxidant when dietary concentrations of HUFA increased from 1.6 – 2.6 % (Cahu, Villette, Quazuguel & Guillaume, 1991). In a later study compound diets containing different concentrations of HUFA (ω 3) at 1.5 – 2.6 % (dm) with the inclusion of α -tocopherol, maintained egg HUFA (ω 3) concentrations at a constant 3.8 % (dm) (Cahu, Cuzon & Quazuguel, 1995).

Dietary requirement of thiamine for the species was established to be 0.01 % of the diet (Gopal, 1986 *loc. cit.* Ahamad, 2001). Whereas the dietary requirement of niacin, a component of coenzymes NAD⁺ and NADP⁺ essential for lipid and carbohydrate metabolism, was found to be 0.03 % of the diet. In order to avoid poor growth and survival choline was required at 0.6 % and pyridoxine at 0.01 – 0.02 % of the diet. In the same study the dietary requirement of pantothenic acid, inositol and riboflavin was found to be 0.08 % (Gopal, 1986 *loc. cit.* Ahamad, 2000b). A similar study by Reddy, Rai and Annappaswamy (1999) demonstrated that fat soluble vitamins A, D and E, and nine water soluble vitamins thiamine, pyridoxine, cyno-

cobalamine, pantothenic acid, niacin, biotin, choline, inositol and ascorbic acid proved essential whereas vitamin K, riboflavin or folic acid did not.

2.9. Minerals.

There are approximately 20 recognised inorganic elements that perform essential functions in the body. Minerals generally contribute to the constituents of the exoskeleton, balance of osmotic pressure, structural matrix of tissues, transmission of nerve impulses and assist in muscle contractions. Moreover, minerals serve as essential components of enzymes, vitamins, hormones, pigments, catalysts and are co-factors in metabolism (Akiyama *et al.*, 1991). Some minerals are required in considerable quantities and termed macro-minerals while others, required in lesser amounts, are referred to as micro-minerals. Macro-minerals include calcium, phosphorus, magnesium, sodium, chlorine and sulphur. Micro-minerals include iron, copper, zinc, manganese, cobalt, selenium and iodine. Other micro-minerals, considered for requirement include nickel, fluorine, vanadium, chromium, molybdenum, tin and silicon (Akiyama *et al.*, 1992). The recommended supplementation levels of minerals in commercial prawn feed as suggested by Akiyama *et al.* (1992) are summarised in Table 2.4.

Table 2.4: Recommended levels of minerals in commercial prawn feed^a.

Mineral	Quantity per kg Feed
Calcium	Maximum – 2.3 %
Phosphorous – available	0.8 %
- total	1.5 %
Magnesium	0.2 %
Sodium	0.6 %
Potassium	0.9 %
Iron	300 ppm
Copper	35 ppm
Zinc	110 ppm
Selenium	20 ppm
Cobalt	1 ppm

^a As fed basis

The requirement for calcium, phosphorus and magnesium was studied by Ahamad (1999). In juvenile *F. indicus* of 0.15 g calcium levels higher than 0.53 %, suppressed growth and increased the FCR. Although the survival and body calcium levels were unaffected, dietary levels of phosphorus above 1.05 % had no beneficial outcome. A dietary Ca:P ratio of 1:1.98 provided the best growth and FCR for *F. indicus*. An inclusion of 0.1 - 0.7 % magnesium in the diet suppressed growth and increased FCR (Ahamad, 2001). Despite *F. indicus* fed diets varying in magnesium concentrations, a constant body concentration of magnesium was maintained suggesting that the requirement was satisfied through the absorption of the mineral from the surrounding medium (Ahamad, 2000).

Purified diets comprising 0 - 20.3 mg copper/100g had no affect on growth whereas a total copper content of 22.7 mg/100g provided the best FCR and survival. *F. indicus* fed manganese supplemented diets ranging from 0 - 2.4 mg/100g grew best on 2.1 mg/100g with higher levels suppressing growth. Diets containing 1.9 - 2.4 mg manganese/100g diet improved the FCR and survival of the species (Ahamad, 2000). When fed 23.6 mg zinc/100g diet and higher, growth was suppressed and body zinc concentrations were augmented, however, 38.6 mg zinc/100g, surprisingly, was found to decrease the FCR (Ahamad, 2000).

2.10. Summary.

From the literature the information pertaining to the dietary requirements of *F. indicus* is fairly extensive and well documented. On a dry matter basis the protein requirement for the species was found to be 22 – 43 % (Table 2.1). Requirement was dependent on age, protein source, feedstuffs used, digestibility and dietary composition. A higher demand for protein during the early stages of development was attributed to failure by an underdeveloped digestive system to assimilate test diets more effectively and/or due to increased synthesis of body protein.

In the wild *F. indicus* spend their early stages in estuaries and inshore nursery grounds rich in primary production where a change in preference from planktonic to benthic feeding takes place. Decreased activity of the digestive enzyme, trypsin, during the early postlarval phase coincides with morphogenic changes in the gut and hepatopancreas with the ratio between amylase and protease increasing from PL₁ onwards with amylase activity exceeding that of trypsin 2 – 3 weeks after metamorphosis (Ribeiro & Jones, 2000).

In penaeid prawns a correlation between ontogenetic changes in enzyme activity and a change in feeding pattern has been widely accepted since a high presence of protease, in the gut and digestive tract, is associated with carnivores and carbohydrases with herbivores (Ribeiro & Jones, 2000). The presence of both enzymes is found in omnivores (Ribeiro & Jones, 2000). Unlike *P. monodon* where, from PL₂₀ onwards, a shift from a herbivorous to a more omnivorous feeding nature takes place *F. indicus*, despite consuming large amounts of plant material during the early stages of development, remains omnivorous throughout its life (Jones, Yule & Holland, 1997).

In terms of protein requirement recent studies have shown a reduction in protein demand (Table 2.1). This decrease can be attributed to the implementation of different techniques and experimental approaches. An example is the study by Ahamad *et al.* (2000), which estimated the *in vitro* digestibility of protein to be 33 – 35 % of the diet as opposed to the 43 % previously determined for the species in a 30-day growth trial by Colvin (1976a). Findings by Ahamad *et al.* (2000) not only contributes towards a more pragmatic understanding of protein concentrations that can be assimilated by the prawn, but substantiates earlier findings

by Ahamed Ali (1982), Bhasker and Ahamed Ali (1984), Gopal and Raj (1990) Thomas, Kathirvel and George (1991). Alternatively a decrease in the requirement can be due to selective breeding whereby individuals that exhibit certain desirable traits are chosen. Hence, over the years it could be possible that selective breeding has lead to new strains capable of assimilating practical diets more effectively and which are well adapted for grow-out conditions.

In penaeids the partitioning of protein requirement for maintenance and production has rarely been considered. To determine the minimum requirement for maintenance Ahamad (1994) established, using a protein free diet, that faecal nitrogen losses in *F. indicus* were 324 mg N per 100 g of the diet consumed.

Unlike carnivorous species, *F. indicus* are able to utilize carbohydrate as a source of energy. Apparent digestibility of starch at 30 % inclusion was found to be above 85 % (Ahamad, 1993) with polysaccharides, as opposed to monosacchrides, providing better growth. In contrast a later study by Hemambika and Raj (1999) found juvenile *F. indicus* incapable of digesting dietary starch in excess of 20 % more effectively. However, in a subsequent study by Ahamad (2000) *F. indicus* was capable of utilising dietary carbohydrate sufficiently at 42 % inclusion (Abdel Rahman *et al.*, 1979; Alava & Pascual, 1987). Due to the various outcomes it would therefore seem that carbohydrates selection by feed manufacturers should be assessed in the light of Omondi and Stark's (1995a) findings.

F. indicus has been shown to require lipids rich in polyunsaturated fatty acids (PUFA) with a strong preference for prawn head oil, which is particularly rich in ω -3 fatty acids. Dietary tests have indicated that a series of ω -3 and ω -6 fatty acids performed better than ω -9, with linolenic (18:2 ω 3) and linoleic acid (18:2 ω 6) being essential for growth and survival. Besides the inclusion level and the source of fatty acids, the required dietary level of phospholipids and cholesterol for the prawn are also available. Phospholipids, namely lecithin, are required at a 2 % inclusion while the dietary requirement of cholesterol is considered to be 0.1 – 0.5 %. A guide proposed by Akiyama *et al.* (1992) for providing adequate energy is to maintain a protein:lipid ratio of approximately 6:1.

In terms of vitamins the values recommended in Table 2.3 by Akiyama *et al.* (1992) are well in excess of those suggested by Gopal (1986), Reddy *et al.* (1999) and Ahamad (2000) for the species. Due to the primary focus of this study and because of time constraints the requirement for minerals and vitamins was not investigated. However, it is important to note that should a vitamin or mineral be limiting then the growth response becomes a false indication of the nutrient/ingredient tested.

With regards to mineral requirements, a distinction between those necessary to maintain osmotic balance both intra- and extra-cellular, such as sodium, potassium and chloride, and those which form part of the body tissue *e.g.* exoskeleton, membrane phospholipids and

enzymes, must be drawn. Requirements for the latter are very closely linked to tissue accretion and, accordingly, to live mass gain. It can be assumed that the requirement for sodium, potassium and chloride in terms of concentration within the diet vary little with age. Alternatively those for calcium, phosphorous and trace elements are essentially considered to be dependent on growth. When expressed in terms of dietary concentrations calcium, phosphorous and trace elements are assumed to decrease with age. Due to leaching, it is common to supplement diets with high concentrations of vitamins and minerals, provided that these concentrations fall below the maximum permissible level since levels above the maximum become toxic.

2.11. Conclusion.

From this review, it is evident that nutrient requirements varies with age and are dependent on the diet, feedstuffs, experimental parameters and methods used. Since feed formulations are based largely on published literature, intuition and on the assumption that there are unknown growth factors, a more substantial means of predicting the protein requirement and feed intake in growing *F. indicus* needs to be established, especially since information regarding nutrient requirements in animals larger than 1 g is scarce. The following chapters investigates and proposes a solution.

3.

Systems Approach**Describing Growth in *Fenneropenaeus indicus*.****3.1. Introduction.**

Growth in animal production has been the topic of innumerable studies. This is especially true in aquaculture operations where a better understanding of growth represents significant benefits in terms of productivity, sustainability and profitability. However, careful examination of technical and scientific literature reveals that growth processes in cultured prawns remains to be fully understood by scientists and aquaculturists alike (Bureau, Azevedo, Tapia-Salazar & Cuzon, 2000).

In an attempt to objectively describe and predict growth in prawns culturists have used the linear, polynomial, log reciprocal, logarithmic, exponential, Von Bertalanffy and Gompertz models (Tian, Leung & Hochman 1993; Zeng & Wan, 1999). Subsequently Jackson and Wang (1998) adapted the Gompertz model, commonly used to describe the growth response of poultry and mammals, to predict the growth of *Penaeus monodon* under aquaculture conditions. Their model accounts for the effects of temperature, mortality and age and although not fully validated, appears to faithfully represent growth in *P. monodon*. Although the model signifies a significant advance it is difficult to understand how pond age and survival can be used objectively as independent variables in a generalised growth model that is widely applicable in aquaculture conditions. Consequently Bureau *et al.* (2000), Mishra, Verdegem and Van Dam (2002) compared existing models by highlighting their shortcomings and in turn suggested alternative approaches for predicting the growth in cultured prawns. Apart from the method proposed by Bureau *et al.* (2000), which makes use of the Gompertz function, current models are limited in that they describe growth as outputs only and exclude the vital input component *viz.* feed intake. Moreover, empirical approaches are rigid in their design and contribute little to the understanding of the actual nature of predicted outcomes since they are confined by parameters and conditions in which the original trial was conducted (Ferguson, 1996).

Hence, due to the hypothetical nature and the current limitation in knowledge a solution to predicting growth lies in the use of simulation models since they incorporate both empirical elements and deductive processes that are aimed at describing the casual relationships between inputs. Moreover, factorial models are more interpretive and flexible and allow for predictions beyond the circumstances in which the information is collected (Ferguson, 1997).

Central to designing simulation models is the need to keep the description of the system as simple as possible without limiting its use. Ideally, the model should be a system based on

first principles that quantify causal forces and which translate inputs into responses. However, an understanding of the mechanisms in which a system operates and a knowledge of the causes of responses that give rise for predicting new outcomes, is required (Ferguson, 1997).

This chapter examines a theory of animal growth and feed intake that was used by Emmans and Oldman (1988) to construct a simulation model. The proposed model integrates three components *viz.* a description of the genotype, feed and environment. The following sections discuss algorithms, the concepts and their functional forms which were used to develop the theory.

3.2. A description of the integrated system.

The methodology of Emmans and Oldman (1988) provides the framework to model the relationship between inputs and predicted outcomes (Figure 3.1). The underlying principle behind the model is that an animal has an innate purpose, namely, to attain its mature state as fast as it possibly can (Emmans & Fisher, 1986; Webster, 1989).

The systems approach is simplified by comprising three components, namely, an adequate description of the genotype and its current state, the feed and the environment. The model regards these components as being interconnected, whereby the feed and environment yield resources to an animal in order for it to reach its potential mature state. However, these same resources can act as constraints to prevent the animal from achieving its potential on any given day. Constraints can be dietary related *e.g.*, nutrient composition, physical characteristics, bulk density and non-nutritional factors or be due to husbandry practices and/or environmental factors *viz.*, temperature, salinity, pH and the like (Bureau *et al.*, 2000). Additional factors can be from external interventions that involve, among others, hormonal treatments, photoperiod manipulation and eyestalk ablation. Hence, given a set of environmental and nutrient sources the desired food intake will be the amount of food that satisfies the animal's requirements with actual intake and subsequent growth performance being dependent on whether the animal is able to eat the required/desired amount.

Hence, provided with adequate nutrition and a favourable environment an animal will achieve its genetic potential, a term often used to describe the upper limit of growth (Moughan & Verstegen, 1988). Emmans (1988) explains genetic potential to be an animal's potential rate of normal growth at any point in time and proposed, since Taylor (1965) found a definite relationship defining a systematic change in growth between protein and non-protein components, that growth should rather be expressed in terms of protein gain as opposed to an output of live mass. Hence, a means of predicting the potential growth rate with some degree of

confidence lies in the suitable description of the animal in terms of its genotype¹ and body composition² (Emmans & Fisher, 1986).

In contrast when the nutritional and environmental inputs are inadequate, the extent to which the potential growth is constrained will have to be predicted according to a set of rules that deal with failure (Emmans, 1988). These rules will be discussed under the relevant sections.

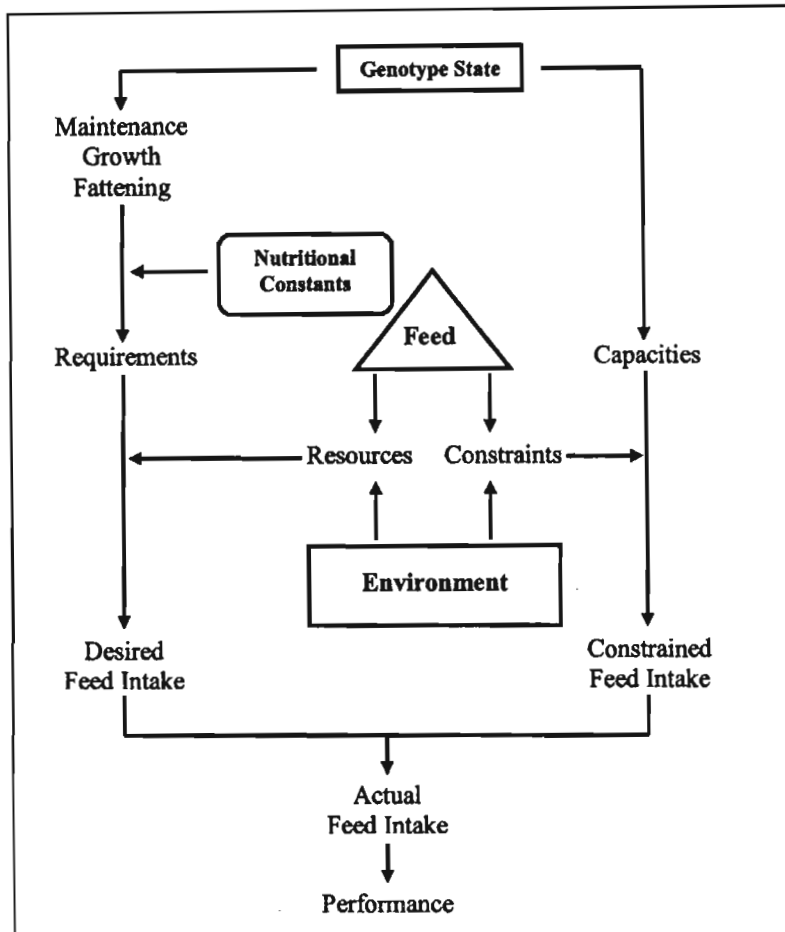


Figure 3.1. A systems approach to predicting growth and feed intake in growing animals (after Emmans & Oldman, 1988).

Changes in body composition are based on nutrient and environmental interactions as well as the current physiological state of the animal, as measured by the protein content. As there is an abundance of literature on the description of feed, as reviewed in the previous chapter, this component will not be discussed in detail but will be included, where necessary, when explaining the theory of food intake. The remaining two components are discussed below in the sections describing the genotype and environmental constraints, respectively.

¹ i.e. size, growth rate, initial and mature mass of either sex for a given species.

² The carcass of an animal can be described in terms of the gut-fill and empty body with the latter, void of blood and gut content, calculated as the sum of protein, lipid, water and ash content with carbohydrate, occurring in small amounts, being ignored (Armsby & Moulton, 1925).

3.3. Establishing genotypic characteristics.

3.3.1. Animal growth.

Growth has been used in nutritional studies, as a practical and reliable indicator, for quantitatively measuring the response of an organism to increased levels of a dietary nutrient. If live mass is plotted against time the best fitting line will be sigmoidal in shape (Brody, 1945). This pattern can be divided into an exponential growth phase, which continues to a point of inflection, where maximum rate of growth occurs, followed by a decelerating phase until a specific asymptotic mass is reached (AFRC, 1991). Although the sigmoidal pattern of growth has been well established in the literature, there are several mathematical expressions, previously mentioned, that have been used to fit growth data.

Results are generally based on gains in live mass but in order to distinguish from an accumulation of body fat and given that protein is the primary constituent of dry matter in muscle, organs and demineralised tissue the preferred option is to quantify growth by measuring gains in body protein (Lovell, 1989). A measure of body protein gains has proven to be of great merit in defining the potential growth in swine (Kyriazakis & Emmans, 1992; Ferguson & Gous, 1993; Ferguson, Gous & Emmans, 1994) and poultry (Emmans, 1981a, 1981b, 1986, 1987a, 1987b, 1988a, 1988b, 1989; 1990), including the goldfish *Carassius auratus* (Lovell, 1989), the rainbow trout *Oncorhynchus mykiss* (Lovell, 1989), the Atlantic salmon *Salmon salar* (De Silva, 1997) and a marine mollusc, *Halotis* species (Mai, Mercer & Donlon, 1995; Britz & Hecht, 1997). However, in terms of penaeids few determinations have been based on protein gains (Gopal & Raj, 1990; Guillaume, 1997).

The advantage of measuring protein as the underlying variable is that it can be used to define the current state of the animal since body fat, and hence body mass, may vary according to the diet and/or the manner by which the animal is fed. The current state of an animal is defined as the degree of maturity (μ) where $\mu = P_t / P_m$ and where P_t is the protein mass at any point in time (t) and P_m the inherent asymptote of body protein mass (Webster, 1989; Emmans, 1989; Moughan *et al.*, 1990).

The corollary to this theory is that if the first limiting nutrient and the potential protein growth rate are known or can be predicted, then by making use of the allometric relationships between protein and other body constituents *viz.*, water, fat and ash, the desired feed intake can be determined (Moughan *et al.*, 1990). However, growth in prawns, for any given stage of maturity, is dependent on the sex, genotype, physical condition, physiological state and environmental factors (Dall, Hill, Rothlisberg & Staples, 1991). For example sex-based dimorphism in penaeids, with larger sizes and faster growth rates found amongst females, have been documented in wild stocks of *Farfantepenaeus aztecus*, *Penaeus longistylus*, *L. vannamei*, *P. monodon*, *Metapenaeus endeavouri* (Primavera, Parado-Esteva & Lebata, 1998) and *F. indicus* (De Freitas, 1980; Devi, 1986; Seppings & Demetriades, 1992; Demetriades & Forbes,

1993; Heydorn, 1995). Whereas most prawn genotypes are found to have considerable inter-specific differences, *Penaeus monodon*, besides having similar feeding habits, are able to attain a larger mature size/mass more rapidly than *F. indicus* when reared under similar conditions (Dall *et al.*, 1990; Pillay, 1990).

Hence, in order for an animal to express its inherent potential it has to be reared under conditions that are non-limiting (Emmans & Oldman, 1988). This term is difficult to measure experimentally since the requirements of growing animals are changing constantly (Ferguson, 1996). Thus, a pragmatic approach would be to measure how well the animal performs under conditions that are conducive for growth before attempting to establish what effect various constraints will have on body gains (Emmans, 1986). Once the true potential has been established then a description of body composition using allometry provides a suitable means of measuring the extent or degree by which growth is constrained (Emmans, 1989).

3.3.2. A means of describing gains in:

(a) Body protein.

As discussed, a method of expressing the development of an animal is to accurately describe the growth path that the animal pursues over time from some initial state, for instance conception, towards maturity. Thus, it may be appropriate to recognize that most animals seek to attain conditions that are descriptive of an inherent asymptote of body protein, and once obtained will attempt to maintain this state (Emmans, 1989). Moulting is an integral part of growth in Crustacea and since the body of the prawn can be seen to comprise two distinct entities *i.e.*, the exoskeleton and soft body tissue, a distinction regarding the accretion of nutrients in each should be drawn.

Unlike fish, most crustaceans appear to have a finite mass specific to each species and a growth pattern comparable to that of birds and mammals (Mishra *et al.*, 2002). A method for describing potential growth is with the use of the Gompertz model since Taylor (1968) found the function useful for evaluating and comparing genotypes of different livestock based on protein gains. Although it may be argued that past studies of aquatic organisms have largely favoured the Von Bertalanffy model, the Gompertz model by comparison, besides having been found to fit data as well as other more complex growth functions (Zeng & Wan, 1999), is preferred since only three parameters, all of which have biological meaning, need be known. These are the rate of maturation (β), the protein mass of the body at conception (P_0) and the protein mass at maturity (P_m). The following is based on the procedure described in detail by Ferguson and Gous (1993). The Gompertz function (Gompertz, 1825) used to predict gains in body protein over time is described as (Figure 3.2.a):

$$P_t = P_m \times e \{-e [(\log_e(-\log_e(\mu_0))) - \beta.t]\} \text{ (g)}$$

[3.1]

where P_t = body protein mass at time t (g),
 P_m = mature body protein mass (g),
 μ_0 = degree of maturity at birth,
 β = rate of maturation (per day), a constant, and
 t = age (days).

The derivative of this equation describes the potential rate of protein growth (pPR) (Ferguson *et al.*, 1994).

$$pPR = P_m \times \beta \times \mu \times \log_e(1/\mu) \text{ (g/day)} \quad [3.2]$$

where μ = degree of maturity (P_t/P_m)

$$(\delta)pPR = \beta \times P_t \times \mu \times \log_e(P_t/P_m) \quad [3.3]$$

Unique to the Gompertz function is that growth reaches a maximum at approximately 0.369 of its mature mass, which is determined by plotting the relationship between the rate of maturation (β) and the degree of maturity (μ), where $\mu = 1/e$, an expression of protein mass calculated as $\mu = P_t/P_m$ (Figure 3.2b).

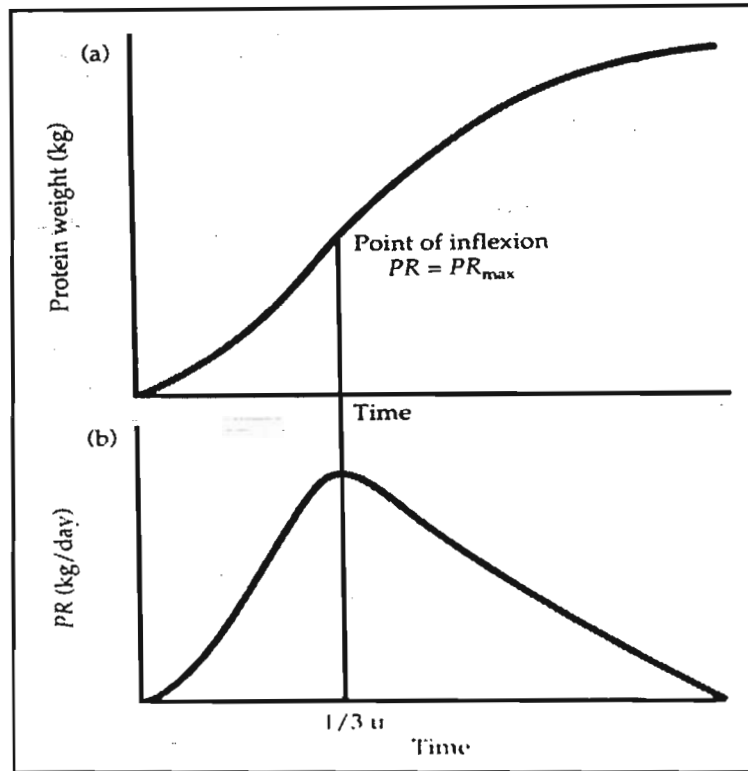


Figure 3.2. (a) Gompertz growth function over time and (b) the rate of growth, as applied to protein growth (Ferguson & Gous, 1993).

To quantify the rate of maturing (β), a modified serial slaughter experiment is required in order to plot the logarithm of body protein against the relative protein growth rate. The relative growth rate (RGR) of protein is calculated by transforming body protein mass to its natural logarithm (\log_e) along the x – axis while plotting the relative growth rate of protein along the y – axis (Figure 3.3) (Ferguson & Gous, 1993):

$$\text{RGR} = (dP_t/dt) \div \text{average } P_t \quad [3.4]$$

The result is a linear negative slope that provides an estimate of the rate of maturation (β) and is indicative of the rate of decay in growth. Rapidly growing animals are found to have a higher β value than slow-growing animals (Ferguson & Gous, 1993). The intersection of the line with the x – axis defines the natural log of mature protein mass (Figure. 3.3).

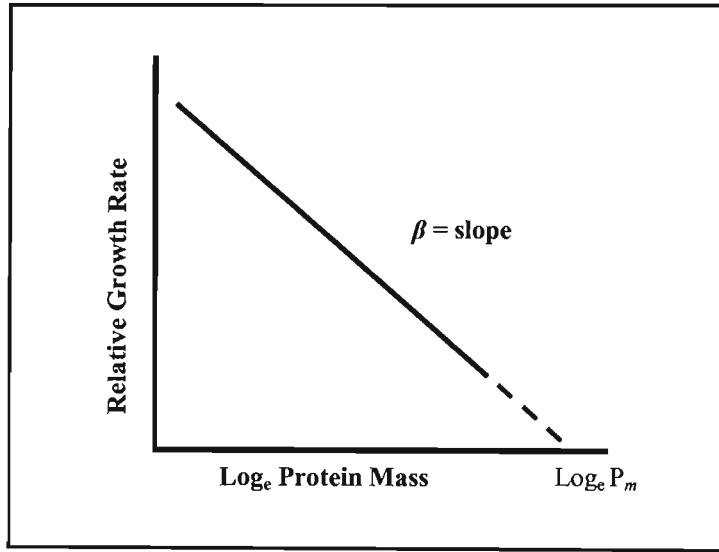


Figure 3.3: Relative growth rate as a function of the natural logarithm of protein mass (Ferguson & Gous, 1993).

This method makes it possible to test for significant differences between samples by comparing the slopes (β) using simple linear regression analysis. Parameters can therefore be calculated using the following equations (Ferguson & Gous, 1993):

$$P_m = e^{\left(\frac{a}{\beta}\right)} \quad [3.5]$$

where

$$a = \frac{(dP_t/dt)}{P_t} \times \beta \times \log_e(P_t) \quad [3.6]$$

The non-linear regressions of body protein growth rates plotted against the degree of maturity provide an estimate of maximum protein deposition rates (PR_{max}) (Figure 3.2(b)). With the maximum rate of protein deposition (PR_{max}) and mature protein mass (P_m) known, β values can be calculated using the following equation and compared with plotted values (Ferguson & Gous, 1993):

$$\beta = PR_{max} \times \frac{e}{P_m} \quad [3.7]$$

Of particular importance in livestock is that the potential protein growth (pPR_i) is only realised if sufficient quantities of the first limiting amino acid, the dietary energy and the surrounding environment permits the release of the subsequent heat produced (Ferguson & Gous, 1993). If not, then actual protein growth (PR_i) is found to be lower than pPR_i . Thus, by using equation [3.7.] the maximum retention of protein (pPR_{max}) is calculated to be (Ferguson & Gous, 1993):

$$pPR_{max} = \beta \times \left(\frac{1}{e} P_m \right) \quad (\text{g/day}) \quad [3.8]$$

(b) *Body lipid.*

One of the problems with modelling growth in an immature animal is that of determining potential gains in lipid content. In the literature, with regards to livestock, body lipid (L_i) and rate of lipid deposition (LPR_i) will depend on the quantity and quality of the food consumed, the environmental conditions and to what extent the animal is fatter or leaner than desired (Ferguson *et al.*, 1994).

The theory of the model proposed here is that this minimum desirable lipid gained is descriptive of the desired rate of lipid deposition or the inherent fatness of the animal. The relationship between protein and lipid, and the ratio that defines the amount of lipid at maturity relative to protein is known as the animal's inherent fatness or LPR_m . In livestock this ratio is species specific (Ferguson & Gous, 1993) and is found to vary between sexes (Ferguson, 1996).

In livestock an accurate determination of LPR_m and b_i , a description of the relative growth between protein and fat, has proved to be important since they serve to indicate the degree to which a species deviates from its potential growth when reared under different conditions. Associated with a required level of fatness is the extent to which the diet is balanced since animals fed a nutritionally poor diet will increase consumption in an attempt to obtain a sufficient quantity of the first-limiting nutrient (Figure 3.4). The consequence is that the deposition and accumulation of body lipid, due to inefficiency in expending the energy consumed, deviates from the inherent degree of fatness. In contrast a restricted feed intake will result in a leaner animal (Ferguson & Gous, 1993).

To illustrate this point, in pigs there is ample experimental evidence to show that previously restricted individuals with lower levels of fat than unrestricted pigs returned to normal levels of fat, for a given protein mass, whereas those previously made fatter than normal by feeding a food efficient in protein also returned to a normal lipid:protein ratio when fed a higher protein diet (Cole, Duckworth, Holmes & Cuthbertson, 1968; Stamataris, Kyriazakis & Emmans, 1991; Kyriazakis & Emmans, 1991; Kyriazakis, Stamatais, Emmans & Whittemore, 1991; de Greef, 1992; Ferguson, 1996).

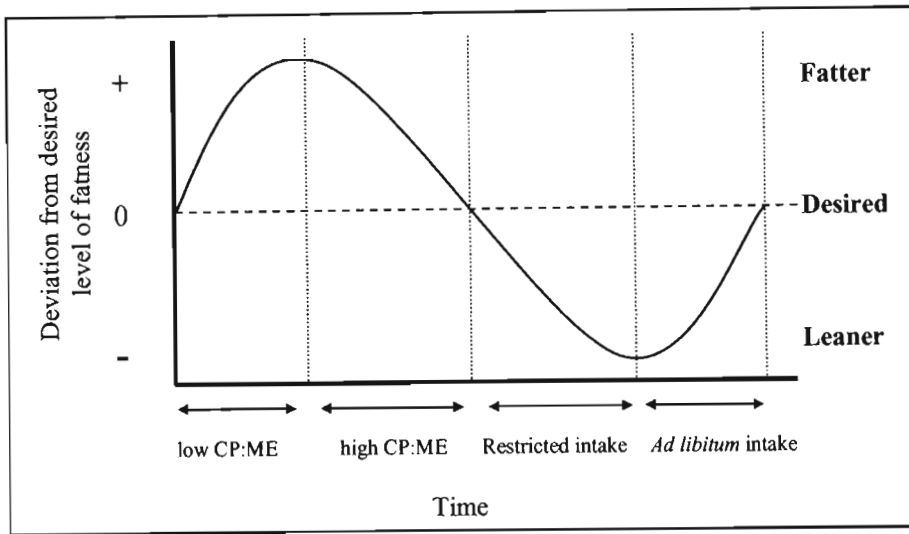


Figure 3.4: Animal responses to different nutritional stimuli where CP = crude protein and ME = metabolisable energy (Ferguson *et al.*, 1994).

Using the concept of a desired fatness compensatory growth in lipid can be determined by summation of the desired lipid growth (dL_r) and the difference between actual body fat and desired body fat content (Ferguson, 1997).

$$dL_r = pL_r + \frac{(\text{Desired } L_t - L_t)}{1000} \quad (\text{mg / day})$$

where pL_r = minimum potential lipid deposition (mg).
 $\text{Desired } L_t$ = desired body fat content (mg).
 L_t = Actual body fat content (mg).

[3.9]

Desired L_t is the expected fat content of the body based on the current protein content. If the animal is fatter than desired then it is assumed that the retention of body lipid will be less on the following day to compensate for that deposited on the previous day. This was found to be especially true in growing pigs (Kyriazakis & Emmans, 1991; Kyriazakis, Stamatais, Emmans & Whittemore, 1991; de Greef, 1992; Ferguson, 1996). Similarly, if the animal is leaner than expected, for a given protein content, then the desired L_r would be higher than the minimum genetically determined (Ferguson, 1997).

An advantage of determining the inherent fatness is that previous models assumed knowledge of food intake that was calculated using some function of body mass of which lipid was a component and as a consequence resulted in feedback effect to exist (Ferguson & Gous, 1993). For example in growing salmonids the deposition of lipid results in little or no gain in overall mass whereas associated with a gram of protein gain is three to six grams of water (Cho & Kaushik, 1990; Bureau *et al.*, 2000).

(c) *Moisture and ash.*

Since the accretion of lipid, ash and water does not require protein and in accordance with studies done on livestock, it is safe to assume that the relationship between moisture and ash will remain relatively constant across prawn genotypes. Furthermore, there is good evidence to suggest that the lipid-free dry matter, comprising protein and ash, is of constant composition (Doornenbal, 1971; 1972) and that the growth rate parameter (β) for each component is the same for a given genotype (Emmans, 1988). This means that the ash content of the carcass can be determined directly from the protein content using the isometric relationship that exists between them such that $b = 1$. Under non-limiting conditions the relative proportions of moisture and ash are less likely to vary between sexes and strains (Moughan & Verstegen, 1988; Moughan *et al.*, 1990). Given the growth rate of protein and the allometric relationship between water, lipid and ash the growth rate of the empty body could be seen to be the sum of nutrient gains in each component.

(d) *Empty body mass.*

If the protein growth rate is known or can be determined and there is a relationship between protein and the component in question, then the rate of growth of each component (dC/dt) can be predicted. Expressed in an equation form this is (Ferguson, 1988):

$$\frac{dC}{dt} = \frac{dP}{dt} \times \frac{dC}{dP} \quad (\text{g/d}) \quad [3.10]$$

where dC/dt = the growth rate (g/d) of body components (lipid, moisture, ash or carbohydrate).
 dP/dt = protein growth rate (g/d).
 dC/dP = relationship between the body's components and protein (no units).

This function forms the basis for predicting the empty body mass gains (EBMG). But in order to do this it becomes necessary to determine the growth rates of the individual component. Since C_m , P_m and b are constant for a given genotype and that components sharing the same rate of maturation, β , are said to be allometrically related (Ferguson, 1988) the mass of each component can be established using the following:

$$C = a \times P_t^b \quad (\text{mg}) \quad [3.11]$$

where C = component (mg).
 a = a scalar value.
 $B = (\ln C_m - \ln C_o) / (\ln P_m - \ln P_o)$.
 C_m = mature component mass (mg).
 C_o = component mass at birth (mg).
 P_m = mature protein mass (mg).
 P_o = protein mass at birth (mg).
 P_t = Protein mass at time (t) in (mg).

The above equation can therefore be differentiated as follows:

$$\frac{dC}{dP} = \text{CPR}_m \times b \times \mu^{b-1} \quad (\text{g/g protein}) \quad [3.12]$$

where CPR_m = is the component: protein ratio at maturity.

If the component mass at maturity, C_m , is expressed as a ratio to P_m , so that $\text{CPR} = C_m/P_m$ then the component:protein ratio at time (t) is found to be $\text{CPR}_t = C_t/P_t$. The relationship between $\ln(C)$ and $\ln(P)$ provides a slope known as b , while the slope relating to the relationship between $\ln(\text{CPR})$ and $\ln(\mu)$ is referred to as c and which is calculated as $c = (b - 1)$ such that dC/dP can be expressed as (Emmans & Fisher, 1986):

$$\frac{dC}{dP} = \text{CPR}_m \times b \times \mu^c \quad (\text{no units}) \quad [3.13]$$

where $z = \text{CPR}_m \times b \quad (\text{no units})$

$$\therefore \frac{dC}{dP} = z \times \mu^c \quad (\text{no units}) \quad [3.14]$$

By substituting equations [3.3] and [3.14] into equation [3.10] the potential growth rate of a component can be determined and expressed in terms of parameters P_m , β , the state variable (μ) and two additional parameters for each component viz. z and c (Emmans & Fisher, 1986):

$$\frac{dC}{dt} = P_m \times \beta \times \ln \frac{1}{\mu} \times z \times \mu^c \quad (\text{mg/day}) \quad [3.15]$$

With the summation of all four components, the growth rate of the empty body, $d\text{EBM}/dt$ is calculated as (Emmans & Fisher, 1986):

$$\frac{d\text{EBM}}{dt} = \{P_m \times \beta \times \ln \frac{1}{\mu} \times \mu\} \times \{\mu + (z \times \mu^c)_l + (z \times \mu^c)_m + (z \times \mu^c)_a\} \quad (\text{mg/day}) \quad [3.16]$$

where subscripts l = body lipid.
 M = body moisture.
 A = body ash.

In most animals a single allometric relationship of the physical and chemical components are descriptive of growth since they share the same rate of maturation, β . However, should the β of the physical components, *i.e.* exoskeleton and flesh, be found to be different then the growth rates of chemical constituents in each entity are to be considered separately. In order to obtain the true growth rate of the $dEBM/dt$ the exoskeleton and the carbohydrate fraction is needed to be incorporated into equation [3.17] whereby the determination of $dEBM/dt$ is derived as follows:

$$\begin{aligned} \frac{dEBM}{dt} = & \{P_m \times \beta \times \ln\left(\frac{1}{\mu}\right) \times \mu\} \times \{\mu + (z \times \mu^c)_l + (z \times \mu^c)_m + (z \times \mu^c)_a\} + \\ & \{P_{em} \times \beta_e \times \ln\left(\frac{1}{\mu}\right) \times \mu\} \times \{\mu + (z \times \mu^c)_{el} + (z \times \mu^c)_{em} + (z \times \mu^c)_a + (z \times \mu^c)_{ec}\} \quad (\text{mg/day}) \end{aligned}$$

[3.17]

where

- P_m = flesh protein at maturity.
- B = flesh degree of maturity.
- L = flesh body lipid.
- M = flesh moisture.
- A = flesh ash.
- B_e = exoskeleton degree of maturity.
- P_{em} = exoskeleton protein at maturity.
- El = exoskeleton lipid.
- Em = exoskeleton moisture.
- Ea = exoskeleton ash.
- Ec = exoskeleton carbohydrate.

Prediction of the potential growth and composition of an animal is therefore dependent on the degree of maturity or state (μ) and values z , c and P_m . As the model is dynamic in nature, the genotype and state of the animal at the beginning of the day will determine the potential rate of growth of protein on that day. In turn if this potential growth rate is added to the initial condition of the animal, the state of the animal at the end of the day can be predicted. This state becomes the initial state on the following day, and the process is repeated (Ferguson, 1996). However, the constraining effects of the feed and the environment have to be calculated in order to predict the actual growth rate and carcass composition of the animal each day.

3.4. Requirements.

Description of the genotype and state allows for the rates of maintenance, growth and fattening to be transformed into necessary rates of supply. From Figure 3.1 it is apparent that in order to predict the desired food intake the next problem is to calculate the maintenance and growth requirements of the animal in terms that are similar to those used to describe the feed.

3.4.1. Maintenance requirements.

The ability of the animal to remain in an unchanged state is referred to as maintenance (Brody, 1945). In order to maintain its current state an animal requires resources from the environment and the food eaten. The problem is to quantify the rate these nutrients need to be supplied for different genotypes of different ages. According to Emmans (1990) the problem of expressing maintenance requirements can be solved by scaling between genotypes at maturity and between degrees of maturity for a given genotype. This approach makes it possible to determine both energy and protein requirements for maintenance.

(a) Energy requirements for maintenance.

Brody (1945) recommended a size scaling rule for maintenance and found that between genotypes the energy requirement was roughly proportional to mature size raised to the power of 0.73 whereas for immature sizes the requirement was found to be a function of live mass raised by -0.27 (Taylor & Young, 1968; Taylor, 1970). In order to decrease the residual variation of the exponent, instead of live mass, Emmans and Fisher (1986) suggested that maintenance be made a function of protein mass. Incorporated into a version of Taylor's (1970) scaling rule Emmans and Fisher (1986) proposed that the energy required for maintenance (MH) be calculated as:

$$MH_{m\ ij} = m_i \times P_m^{0.73} \times \mu \text{ day} \quad (\text{MJ}) \quad [3.18]$$

or

$$MH_{m\ ij} = m_i \times P_m^{-0.27} \times P_k \text{ day} \quad (\text{MJ}) \quad [3.19]$$

where m_i is the resource required per maintenance unit (kJ/kg or mg/g), and subscripts i , j , k refer to the i th resource, the j th genotype and the k th equilibrium mass, respectively and P_m a measure of the protein mass at maturity. The estimated values of m_i for energy in livestock is $1.63 \text{ MJ}/(P_m^{0.73})$ (Emmans, 1988).

(b) Protein requirement for maintenance.

Knowledge of the food intake assists in establishing the optimum supply of dietary amino acids, since protein requirement is considered to be dependent on the amino acid content in the feed and body tissue. The preferred theory used in this study, depending on the nature of the diet and environment, is that an animal will attempt to eat sufficient food to reach its potential

growth while maintaining a desired body composition. The protein required for maintenance (MH) is assumed to be identical in composition to body protein and is scaled in the same way as energy, where m_i is the protein or amino acid required per maintenance (kJ/kg or mg/g). In livestock the value of m_i has been estimated to be 8 g. unit⁻¹. day⁻¹ (Emmans, 1988). In prawns 0.008 g. animal⁻¹. day⁻¹ is assumed.

(c) *Energy requirements for growth.*

Emmans and Fisher (1986) proposed a model to establish the energy required by an animal for maintenance and growth on a daily basis, known as the effective energy system. In short the effective energy system can be described as the metabolizable energy (ME) of a diet less the heat produced due to defecation and excretion resulting from it being eaten. The effective energy scale considers the energy lost due to an increased heat increment following the utilization of dietary protein. For more details regarding the effective energy systems, an approach which has been used successfully to establish dietary intake in livestock, it is important to refer to the work by Emmans (1994). Simply stated the effective energy in a diet is defined as:

$$EEC = ME_n - (0.16 \times w_u \times D_{CP}) - (w_d \times FOM) + z \times D_{CL} \times (w_l - w_f) \quad (\text{kJ/g}) \quad [3.20]$$

where	w_u	= 29.2	(kJ/g)
	DCP	= digestible crude protein	(g/day)
	w_d	= work done in defecating	(kJ/g)
	FOM	= faecal undigested organic matter	(g/day)
	z	= proportion of dietary fat	
	D_{CL}	= digestible crude lipid	(g/kg)
	w_l	= work done in depositing fat from dietary protein and carbohydrate sources	(kJ/g)
	w_f	= work done in depositing fat from dietary lipid	(kJ/g)

Consequently for a given ME there will be less energy available for growth as the supply of dietary protein is increased (Ferguson, 1996). The same energy scale, used to determine the energy content of the feed, is also used to measure the energy requirements of a growing animal in a given state living in a non-limiting environment. The effective energy required by an animal is calculated as:

$$EERQ = MH + 50.3 \times PR + 56 \times LR \quad (\text{kJ/d}) \quad [3.21]$$

where	PR	= crude protein retained	(g/day)
	LR	= crude lipid retained	(g/day)

Since the effective energy system is geared towards and has proven to have great merit in modelling the energy requirement of pigs and poultry (Ferguson, 1996; Emmans, 1997) subsequent chapters will examine if this approach is appropriate for describing the daily requirements in an ectotherm, such as *F. indicus*.

(d) *Protein requirements for growth.*

As previously mentioned protein requirement does not depend on the protein *per se* but on the amino acids that make up the protein. As with energy, the scale on which amino acids requirement is measured, and on which the amino acids in the feed are described would need to be the same. However, inefficiencies exist when digestible amino acids are converted into body tissue, with the result that the requirement for a reference/ideal protein is not identical to the body tissue being formed. Conventional wisdom is to express true requirements in terms of digestibility since the assimilation of amino acids depends on the source and not the quantity of dietary protein given. An 'ideal protein' is defined as the mixture of amino acids that meets the exact requirement of the tissue being formed (or the animal as a whole) for each amino acid, both essential and non-essential, with neither being in excess nor deficient (Larbier & Leclercq, 1994). Moreover, the marginal efficiency with which the first limiting amino acid is used for protein retention above maintenance is not necessarily constant, but can be modified by the supply of other amino acids and energy (Kyriazakis & Emmans, 1992).

The required amino acid intake (AAI) is defined in terms of a response function relating intake to the potential level of protein production and maintenance and can be calculated using (Emmans, 1988):

$$AAI = (a \times PR) + (b \times MP) \quad (\text{g/day}) \quad [3.22]$$

where a_i = coefficient of the first limiting amino acid for growth (mg/g protein).
 B_i = coefficient of the first limiting amino acid for maintenance (mg/g protein).
 $PR = dP/dt$ (g/day).
 $MP = 0.008 \times P_m^{0.73} \times \mu \text{ day}$ (g/day).

An important assumption is that the coefficient of utilisation of the essential amino acids for growth (a) and for maintenance (b) are constant between sexes. From this assumption the ideal amino acid balance for growth is considered to be similar to that found in body tissue. Whittemore (1983) suggests a coefficient of net utilisation of amino acids for growth of between 0.85 and 0.95, whilst Fisher (1988) *loc cit* Ferguson (1996) predicts a value of 0.75. The net efficiency of an apparently digested amino acid, which is the first limiting, is assumed to be 0.80 (Emmans, 1989) for growth and 1.04 for maintenance (ARC, 1981).

3.5. Feed intake.

3.5.1. The theory controlling food intake and the subsequent growth response.

Description of the genotype and state allows for the rates of maintenance, growth and fattening to be transformed into necessary rates of nutrient requirements. Moreover knowledge of the food intake assists in establishing the optimum supply of dietary amino acids, since protein requirement is considered to be dependent on the amino acid content in the feed and body tissue. Once the potential rate for a genotype is known the daily requirement of each nutrient for maintenance and growth can be determined.

The desired feed intake (DFI) is defined as the rate at which the animal needs to eat just to meet its requirements for the first-limiting resource (RQ), whether it be energy, an amino acid or some micro-nutrient (Emmans & Fisher, 1986). Based on the premise that an animal will eat to satisfy its needs for the first-limiting nutrient an animal will only achieve its desired purpose if it is able to eat sufficient food. The DFI will therefore be the quantity of the diet needed to satisfy the requirement (RQ) for the most limiting nutrient and is determined as follows (Emmans & Fisher, 1986):

$$DFI = \frac{RQ}{FCON} \quad (\text{g/day}) \quad [3.23]$$

where RQ = animal requirement for the first limiting nutrient (g/day).
 $FCON$ = concentration of the first limiting nutrient in the diet (g/g).

If an amino acid is the first limiting nutrient then the desired feed intake (DFI_p) will be based on the available amino acid requirement and the concentration of dietary available amino acid (Kyriazakis & Emmans, 1990; Ferguson, 1996). To determine the availability of the limiting amino acid in the diet, the quality of the protein has to be considered (Whittemore, 1983; Moughan *et al.*, 1987). The quality of protein supplied is quantified by the relative biological value (BV). The BV represents the proportion of the first limiting amino acid in the feed relative to the ideal protein balance. The DFI_p to satisfy potential protein growth (pPr) from the first limiting amino acid is determined as follows:

$$DFI_p = \frac{\left(\frac{pPR}{e_p} + \frac{MP}{e_m} \right)}{dCP \times BV} \quad (\text{g/day}) \quad [3.24]$$

where pPR = coefficient of the first limiting amino acid for growth (g/day).
 e_p = efficiency of protein utilisation for growth
 e_m = efficiency of protein utilisation for maintenance
 $MP = 0.008 \times P_m^{0.73} \times \mu \text{ day}$ (g/day).
 dCP = digestible crude protein (g/kg).
 BV = biological value of diet

The efficiency of protein utilisation for growth (e_p) and maintenance (e_m) is affected by a number of different dietary factors including the availability of amino acids after processing and the amount of energy supplied (ARC, 1981). The effect of amino acid availability is very difficult to incorporate into a model when the composition of the diet is an input variable because it is dependent on the composition and treatment of dietary protein. For this reason protein and amino acid digestibilities are considered to be input variables (Ferguson, 1996). Default efficiency values used for pigs are 0.75 and 1.00 for growth and maintenance, respectively (Ferguson, 1997).

Should energy be the most limiting nutrient then by substituting equations [3.20] and [3.21] into [3.23] the desired feed intake will be that required to satisfy energy (DFI_e) whereby:

$$DFI_e = \frac{EER}{EEC} \quad (\text{g/day}) \quad [3.25]$$

Where there are no physical constraints on food intake actual feed intake (AFI) of an animal, under non-limiting conditions, would be the larger of DFI_e and CFI_p such that (Ferguson, 1996):

$$\text{if } DFI_e > CFI_p \text{ then } AFI = DFI_e \quad (\text{g/day}) \quad [3.26]$$

or

$$\text{if } CFI_p > DFI_e \text{ then } AFI = CFI_p \quad (\text{g/day}) \quad [3.27]$$

For a perfectly balanced feed:

$$AFI = DFI_e = CFI_p \quad [3.28]$$

The logical conclusion of trying to maintain a desired body state is that the animal will always attempt to return to this inherent state, if it has deviated from it. The constraining factors will be the environment and the first limiting nutrient. A consequence of the above conclusion is that feed efficiency is automatically taken into account by the changing rates of lipid and protein deposition, as the body composition returns to its desired physiological state.

The problem arises when DFI is not attainable. Assuming that the environment is not a constraining factor, there are three possible options/paths an animal may take. Associated with these are certain rules that control the partitioning of the energy and protein resources into body protein and lipid (Ferguson, 1996).

3.5.2. Food intake = DFI_e

This is the simplest of cases where the animal consumes sufficient protein, to satisfy its maintenance requirement, that for potential growth, and energy to provide for maintenance and a certain amount of lipid deposition that is associated with 'normal' growth. Excess protein

ingested is deaminated which in turn incurs an energy cost that reduces the amount of metabolisable energy (ME) available for growth. The net result is that feed intake will have to be increased to overcome the deficiency. In turn the potential rate of protein retention, as defined by the current state of the animal, and the minimum desired lipid deposition would be realised (Ferguson, 1996).

3.5.3. Food intake = CFI_p

In this situation there is sufficient energy but food intake is constrained due to protein (CFI_p) or more specifically because of an amino acid being first-limiting. The response will be an increase in food consumption in order to obtain sufficient of the first-limiting amino acid. The energy above that used for maximum protein retention and maintenance will be deposited as fat, which will result in the animal becoming fatter than its inherent fatness (Ferguson, 1996).

3.5.4. Food intake = CFI_b

When bulkiness (CFI_b) of the food prevents the animal from meeting its potential growth rate, then a further step has to be considered viz. what is the next most limiting nutrient in the feed. This is an important consideration as it determines whether the animal can reach its potential growth rate or not. It may be possible for the animal to consume sufficient protein to enable it to reach its potential but be restricted in its energy intake. In this case energy becomes the next most limiting factor after bulk. The model is designed to ensure that sufficient energy is available for maintenance and protein growth (pPr).

3.5.5. Food intake = RFI

Restricted food intake (RFI) or controlled food intake is the amount provided to the animal that is less than if allowed to feed *ad libitum*. The approach adopted in this model assumes that the same initial rules apply that are applicable to *ad libitum* intake except that the daily feed intake is constrained by some predefined limit. If in spite of the restriction imposed, there are sufficient quantities of the most limiting nutrient, be it an amino acid and/or energy, the potential protein growth will be attained. This will have a consequential effect on energy partitioning with fat deposition being restricted to the remaining energy requirements after the requirements for maintenance and protein retention have been met. It can be considered highly unlikely and somewhat improbable that any animal irrespective of its predisposition for lean tissue, will continue to deposit protein at its maximum potential when there are insufficient fat reserves available to supplement the inadequate dietary energy. Hence, there is therefore a need to ensure that body fat reserves are not depleted beyond some realistic value (Ferguson, 1996).

3.6. Describing the feed.

The nutritional value of feedingstuff to a given kind of animal is defined by the quantities of energy and the nutrients that it yields. A feedstuff, in principle, can be described by a set of numbers in a feed composition table, such as are widely used. However, feedingstuff may also have other non-nutritional properties such as small quantities of toxins. An appreciable proportion of nutritional research has been concerned with such properties and will not be dealt with here.

3.7. Environmental constraints.

Up until this point the model has assumed that the environment has had no constraints on feed intake and growth. However, the environment has a considerable effect on voluntary food intake since it can increase or decrease the requirement for dietary energy which in turn will have an effect on the voluntary food intake of the animal. It is necessary to establish the extent of the interaction of the environment with the animal and with the diet before the voluntary food intake of the animal, and hence its actual growth rate, can be assessed (Ferguson, 1996).

To remain in thermal balance the amount of heat that an endotherm produces through various functions, such as maintenance, growth, food intake, digestion, excretion, *etc.*, must equal the amount of heat lost to the environment, as heat storage in the animal is minimal. More heat can be lost in cool than in hot environments (Ferguson, 1996). At high temperatures more heat is lost by evaporation in livestock than through convection, conduction and radiation. On the other hand, when heat production is insufficient to match the demand of the environment then the animal must generate heat. The result is that less energy is available for productive purposes. To compensate the animal will increase feed intake to satisfy both its growth requirements and the increasing heat demand of the environment. In contrast, in a hot environment the same animals will be prevented from consuming their DFI because the amount of heat produced would be greater than what could be lost.

Since ectotherms fail to maintain a constant body temperature, when exposed to a change in ambient temperatures, the problem of heat production or heat loss to the environment does not warrant a similar concern as with endotherms. However, since temperature, amongst other environmental parameters, affects the metabolic rate of poikilotherms the effects thereof and those of salinity are investigated in Chapter 5.

3.8. A summary of the model approach.

To reiterate the first step towards predicting feed intake is to establish the genotype characteristics of the animal. Since each species is unique, certain inherent characteristics, that

describe an animal's growth performance, needs to be quantified. Table 3.1 summarises the parameters that define the genotype of a species in relation to protein gains.

Table 3.1: Inherent variables that describe the growth potential of an animal (Ferguson *et al.*, 1994).

Potential protein growth is a Gompertz function with a rate parameter:	β
Mature size is described as the animals asymptotic protein mass:	P_m
Inherent fatness at maturity:	$LPR_m = L_m/P_m$
Component mass is allometrically related to protein mass with each component (x) having their own exponent value (b):	(b_x)
Mature ash is described as:	$APR_m = A_m/P_m$
Mature moisture is described as:	$WAPR_m = WA_m/P_m$

Where P_m , L_m , ASH_m , and WA_m are the mass of protein, lipid, ash and moisture at maturity.

The Gompertz equation and the derivative thereof allows the potential rate of protein growth of an animal to be predicted from only two inherent characteristic *viz.*, P_m , β , and its present body state (μ). Body components can be established using allometric relationships with that of body protein.

An animal will attempt each day to eat sufficient of a given food to achieve its potential growth rate and ultimately its mature inherent state. Associated with this asymptotic state is a required amount of nutrients. This mature state is described in terms of a single mass variable, the mature protein mass P_m , and by three ratios that describe composition, which are LPR_m , APR_m , and $WAPR_m$. Essentially when the exoskeleton is accounted for in crustacean, due to the presence of chiton, an additional mass variable describing the carbohydrate component, CA_m , and the carbohydrate to protein ratio, CPR_m , should be included whereby $CPR_m = CA_m/P_m$.

Allometric relationships between the chemical and physical components of the body can be defined in terms of β , which refers to the rate of maturation or the rate of decline of the relative growth rate of protein (Ferguson & Gous, 1993; Ferguson *et al.*, 1994). Components sharing the same rate of maturation are considered to be allometrically related. Allometry, on the other hand, provides the rate variable b , which is derived from the growth of different physical components and their respective chemical constituents in relation to body protein (Table 3.2).

Table 3.2: Functions that determine the growth path of body protein and related chemical components (Emmans & Oldman, 1988).

Variables	Time		
	$t = 0$	$t = t$	$t = \infty$
Size*	$\mu_0 = P_0/P_m$	$\mu_t = P_t/P_m$	$\mu = 1$
Functions*	Gompertz	$d_\mu/d_t = \beta \cdot \mu \log_e$	
	Allometric	$CPR_t = CPR_m \cdot \mu^b$	

* P = protein mass, C = Chemical component's mass & CPR = C:P ratio while subscripts 0 , t and m denotes time = 0, time = t and $t = m$ (maturity), respectively with μ representing the degree of maturity.

An innovative approach to answering the question of how an animal satisfies its nutrient requirements most efficiently is with the use of the effective energy systems (Emmans, 1989). This approach provides a means to predict the desired feed intake (DFI) for a perfectly balanced feed as being the effective energy required by the animal divided by the effective energy that the diet yields. Predictions are dependent on sex, strain, physiological state of the animal and the quality of the diet. The latter needs to be expounded as it contributes significantly to the rates and composition of growth and to the daily requirements for energy and protein.

3.9. Conclusion

In commercial operations extremely accurate decision-making can rarely be met by empirical means alone since the problem of predicting the growth rate, body composition and feed intake is that it involves many interactive causal forces which combine to produce a response to specific conditions. A modelling approach seems to be the only defensible means of quantifying these forces. However, in order to be successful a growth model must be able to calculate the nutritional and environmental requirements of growing animals. Furthermore, should deviations from optimum conditions occur then the model must be successful at predicting the outcome.

Based on the above principles the theory on growth by Emmans and Oldman (1988), shown to have a degree of pragmatic value, used to predict the amino acid requirements in broilers with great accuracy (Emmans & Fisher, 1986) including food intake in turkeys (Emmans, 1989) and growing pigs (Ferguson & Gous, 1993) was adapted as an effective means of predicting growth and nutritional requirements in growing *F. indicus*. The following chapters examine the feasibility of this model for use in mariculture practices.

4.

Describing the Genotype and State**Application of the Systems Approach to Model Growth in
Fenneropenaeus indicus.****4.1. Introduction.**

Part of the theory defining animal growth used by Emmans and Oldman (1988) requires that the current state of the animal be known. However, information on nutrient accretion in prawns reared under aquaculture conditions is limited since chemical composition is seldom analysed in scientific studies. A general outline of chemical fractions in cultured prawns, compiled by Bureau *et al.* (2000), is given (Table 4.1).

Table 4.1: Estimates of the chemical composition in penaeids expressed on a live mass basis.

Stage	Live mass (g)	Moisture (%)	Crude Protein (%)	Lipid (%)	Nitrogen Free Extract (%)	Ash (%)	Gross Energy (kJ/g)
Post larvae	1	79	11	1	5	4	3.8
Juveniles	5 - 20	74	12 - 16	2 - 3	2 - 7	4	5.2
Broodstock	30 - 35	70	12	4	10	4	6.6

Due to the success of Ferguson and Gous (1993) and Ferguson *et al.* (1994) in defining the genotype, chemical state, performance and optimal growth in livestock, analogous to the method suggested by Bureau *et al.* (2000) for prawns and/or shrimps, the systems approach was implemented to describe and predict the growth response and feed requirements in growing *F. indicus*.

The first step, however, was to define the genotype of the prawn before attempting to describe the nutrient requirement of the species. This study attempts to establish the growth and rate of maturation of body fractions in the prawn. The most problematic aspect when applying this approach is that non-limiting criteria for growth in *F. indicus* are poorly defined, both in terms of feed and the environment. Therefore, in the present study and in an attempt to simulate non-limiting conditions, prawns were fed an existing diet, previously used by the prawn industry, and were reared in two distinctly different environments. This allowed for separate growth trials to be undertaken whereby animals from the same brood were reared simultaneously under laboratory, in conditions stipulated in literature to be ideal, and in a grow-out pond under outdoors conditions.

Trials required that juveniles larger than one gram were grown to adults of ~ 35 g. By using the serial slaughter approach body composition was determined in order to:

- Define growth parameters in each body component *i.e.*, exoskeleton and flesh.
- Establish the maximum protein deposition rate (PR_{max}) and to predict the potential retention of protein (dPR_i) in the different components.
- Predict the accretion of body fat (dLR_i), ash (AR_i) and water retention (WR_i) in each component and to compare observed values with those predicted using the model.
- Establish the growth rate of the empty body ($dEBM_i$) based on the sum of the body fractions.
- Determine if the diet or environment or both were growth-limiting by comparing the predicted rate of maturation, β , and the potential protein growth (pPR) between mixed samples of pond and laboratory reared prawns.

4.2. Materials and Methods

4.2.1. Experimental animals.

Juvenile *F. indicus* from the same brood stock were stocked at 60 prawns per m^2 in a pond situated on the grounds of Amatikulu Prawns. A total of six hundred individuals with a mean and standard deviation of 1.4 (± 0.7) g in mass was collected and transported to the laboratory. This was done by distributing 40 animals into a single breeder bag containing 10ℓ of pond water, inflated with industrial oxygen and sealed with the aid of an elastic band. A hundred juveniles were placed into each of the six rearing tanks and were provided with the same feed and similar rations as was done on the farm (Table 1.1). Only healthy post-moult juveniles of approximately similar size and mass were used for trials.

4.2.2. Pond environment.

Cultured animals were grown to a mean mass of 35 g in a one-hectare grow-out pond. The pond was seine netted on a weekly basis and 50 animals, arbitrarily selected, were weighed to establish the average mass of pond animals. When pond animals averaged 3, 6, 9, 12, 15, 20, 25, 30 and 35 g a sample of 90 prawns was captured, weighed and subdivided into three groups for chemical analysis. One group, a third of the total, consisted of males; another comprised females, while the remaining third contained both sexes in disproportionate numbers. Animals were killed

by placing each sample into separately labelled plastic bags and into a cooler box filled with ice. On arrival at the laboratory the plastic bags were vacuum-sealed and stored at -20°C .

4.2.3. Laboratory environment.

In an attempt to minimise environmental variables a concurrent experiment was carried out in the laboratory aquaria where prawns were reared in glass tanks [Appendix A]. Mix samples of thirty prawns for a given mass class comprising uneven numbers of either sex were slaughtered after removing an equivalent number of individuals from each tank. All samples were placed into labelled plastic bags, vacuum-sealed and stored at -20°C until dissected.

4.2.4. Experimental facilities and conditions

A rearing system, explained in detail in Appendix A, housed the animals for the duration of the trial. Water conditions in laboratory tanks were maintained at: (mean and standard deviation) $29.0 (\pm 1.0)^{\circ}\text{C}$, $23.0 (\pm 3.0) \text{‰}$ and $\text{pH } 8.0 (\pm 2.0)$ with oxygen concentration kept near saturation throughout the duration of the trial. Nitrogen determinations of ammonia-N, nitrate-N, nitrite-N were taken before the animals were fed in the mornings and were found to have a mean and a standard deviation of $0.2 (\pm 0.1)$, $0.1 (\pm 0.1)$ and $0.23 (\pm 0.2) \text{ mg/l}$, respectively. Similarly, pond temperatures and salinity were measured twice daily and were found to average $23.0 (\pm 4.0)^{\circ}\text{C}$ and $24.0 (\pm 3.0) \text{‰}$, respectively.

4.2.5. The test diet and feeding regime

A single experimental diet containing $\sim 43\%$ crude protein (Table 1.1.) was fed to pond and laboratory-reared prawns. A pellete feed was produced on the farm in a resident manufacturing plant whereby the raw ingredients were steamed cooked in the preconditioner at 90°C and extruded through a die. The animals in the pond and laboratory were fed daily at 06:00, 12:00, 18:00 and 24:00 h. Pond rations were based upon the formula described in Chapter 1 whereas laboratory animals were fed a ration at a rate in excess of 15% of live mass per day. Unlike livestock that are fed *ad lib*, laboratory daily rations were provided with the aid of automatic feeders at designated times as specified above. However, in order to ensure the sufficient provision of food, every couple of hours during daily routine inspections, additional rations were offered to laboratory animals until satiation. This was done on condition that no feed residue was visible at the bottom of tanks. To determine feed allowances, every fortnight five animals from each tank were randomly caught and individually weighed to ascertain mass gains after which the food rations were adjusted

accordingly. Every morning and early evening any residue found on the bottom of tanks was siphoned to waste. Mortalities were recorded and the carcasses removed.

4.2.6. Mass determination

On initiation, during and on termination of each experiment prawns were individually weighed to the nearest 0.01 g using an analytical balance (Model: Mettler PM 400). Depending on where sampling took place live mass was determined by removing the captured animal from a bucket containing either pond or tank water, rapidly drying it using blotting paper and placing it into a tared beaker containing water of similar quality. This was done in an attempt to reduce stress during the weighing process. In the laboratory, on reaching the required mean for each mass class, five prawns from each of the six tanks, a total of 30 individuals, were removed, killed, placed into a labelled plastic bag, vacuum sealed and stored at -20°C . Trials were concluded when mean body masses of mixed groups were found to reach an asymptotic mass of approximately 22 and 35 g for laboratory and pond reared animals, respectively.

Thirty-five grams is by no means the maximum body mass since individuals of cultured *F. indicus* have on occasion attained an exceptional mass of 51 g (Laurence Evans¹ *pers com* 2003). However, in the present investigation, amongst the final sample of adult females two individuals of approximately 48 g were found. On termination of the laboratory trial, all remaining animals from each tank were killed, pooled, placed into a labelled plastic bag and stored at -20°C . Collection and storage of pond-reared animals followed similar procedures.

4.2.7. Dissection procedures

A sample of frozen prawns, once removed from cold storage, was left at room temperature for approximately 15 – 30 minutes to thaw. Once thawed, animals were thoroughly dried using blotting paper and weighed. Mass, read to two decimal places, was determined using a digital balance (Model: Mettler PM 400). Carapace length, defined as the distance between the postorbital notch and posterior mid-dorsal margin of the carapace, was used to denote specimen size throughout the study.

Exoskeleton tissue was separated from the flesh; each segment of exoskeleton was then inspected under a stereomicroscope to ensure that there were no remnants of flesh still attached and if present were removed using a scalpel blade. Thereafter each segment was rinsed in distilled water, blotted dry and placed into a petri-dish on ice. Similarly flesh, once separated, was blotted

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dry and placed into a separate petri-dish embedded in crushed ice. Contamination by gut content was avoided by separating the cephalothorax from the abdominal region. With the gut sliced open and all contents removed, the thorax and gut walls were rinsed in distilled water, dried and placed into the petri-dish containing flesh. The carapace, once removed and inspected for any adhering flesh, was placed in the same petri-dish containing exoskeleton fragments. Pleopods were discarded since attempts to separate flesh from the cuticle proved to be difficult and time consuming. Legs and external appendages such as antennules were placed with exoskeleton casts. Eye content was extruded by applying pressure on the cornea using forceps. The exuded fluid was placed with the flesh tissue while the eye membrane was rinsed in distilled water, dried and pooled with the exoskeleton fragments. After dissection the thoracic tissue containing stomach lining and flesh, for a given individual, were collectively weighed, placed into a labelled plastic bag, vacuum sealed and immersed into the crushed ice. Likewise exoskeleton derived from the same individual underwent a similar procedure. Bags containing each of the tissue samples were placed into a single plastic bag, labelled, vacuum-sealed and stored at -20°C until chemical analyses were possible. The sex, total wet mass and the mass of both exoskeleton and flesh were recorded.

4.2.8. Chemical determination of animal tissue and feed.

The exoskeleton and flesh tissue were analysed separately. Individual exoskeleton and flesh samples were freeze-dried, ground and three sub-samples from each homogenised tissue sample were taken for analysis of amino acids, crude protein, lipid, moisture and ash content. The content of nitrogen free extract (NFE) was calculated by the difference $100 - (\text{crude protein} + \text{lipids} + \text{ash} + \text{crude fibre})$ as done by Morales *et al.* (1999).

Ideally, individuals rather than groups of animals should be measured to determine population variability (Emmans, 1990). However, in order to quantify exoskeleton lipid the cuticle mass of an individual prawn was insufficient for analysis resulting in the integument from all individuals being pooled. To ensure a representative and sufficient sample for the analysis of exoskeleton lipid all tissue samples from the thirty prawns was required, especially when dealing with younger individuals.

Dry matter content was determined by freeze-drying each sample for 72 hours. Nitrogen content of the dry matter of the feed and animal tissue were determined using a LECO FP2000 Nitrogen Analyser using the Dumas combustion with protein content calculated as $\% \text{ nitrogen} \times 6.25$. Lipid content was assessed by Soxhlet extraction of the freeze-dried samples with petroleum ether at $40 - 60^{\circ}\text{C}$. Ash was determined by burning the samples in a muffle furnace at 550°C for 4 hours. Gross energy was established using a DDS isothermal CP 500 bomb calorimeter and amino

acid composition was established using a Beckman Amino Acid Analyser System 6300. Since the building blocks of chitin are nitrogenous polysaccharides, exoskeleton protein determined using the LECO FP2000 Nitrogen Analyser was compared with samples established using the Beckman Amino Acid Analyser System 6300. All the above methods were followed in accordance with the procedures stipulated by the AOAC (1990).

To ensure feed quality, food manufactured on the prawn farm was analysed for amino acid, protein, moisture, lipid, ash and gross energy content. Feed pellets were stored in a cold room at -5°C and discarded if not utilised within 60 days.

4.2.9. Determination of survival and FCR.

Since protein retention was determined as the change in body protein over the test period a measure of protein intake was therefore not necessary as described by Ferguson and Gous (1993). Average food conversion ratio (FCR) was calculated as follows (Gopal & Raj, 1990):

$$\text{FCR} = \frac{\text{Average dry mass of food consumed}}{\text{Average gain in live mass}} \quad [4.1]$$

and percentage survival as:

$$\text{Survival (\%)} = \frac{\text{Final numbers harvested} + \text{sample numbers removed}}{\text{Initial numbers stocked}} \times 100 \quad [4.2]$$

4.2.10. Statistical and data analysis

To determine if the growth of prawns reared in the experimental pond was representative of other culture ponds on the farm growth rates in grow-out ponds simultaneously stocked were compared and tested for significant differences using ANOVA. Feed conversion ratios were regarded as apparent and no correction factor was introduced for the exuviae and dead prawns eaten by the cohabitants in the pond or aquaria during this study. Data pertaining to apparent protein growth in the exoskeleton and flesh were extrapolated using the Gompertz function and in order to produce the best fit equation constants were estimated by minimising the square of the residual mean using Microsoft excel solver version 97. Predicted and observed values for each group and mass class were compared using a one-sample *t*-test. Differences were considered to be different at the 0.05 % level.

By comparing slopes between the relative growth rate and natural log of flesh protein, significant differences between β values of samples were determined using simple linear regression analysis. To establish if a linear relationship between the wet mass of the exoskeleton and chemical constituents of exoskeleton and flesh tissue existed, data once converted and expressed using natural log were plotted against the natural log of flesh protein. Determination of equation constants a and b for each of the resultant regressions were achieved using Graphpad Prism Software, Inc version 3.02. Significant differences between regressions were established in accordance with Zar (1996) multiple comparison among slopes and elevation tests. If significant differences were found then the Tukey test was applied to these data to determine where differences occurred. Similarly, chemical composition of exoskeleton and flesh in laboratory animals was analysed and the data compared with mixed sex samples of pond-reared prawns.

4.3. Results

4.3.1. Physical components of samples collected.

Data for live mass, carapace length, flesh mass, exoskeleton mass and exoskeleton to flesh ratios of pond and laboratory reared prawns are given (Table 4.2). Compared to pond-reared prawns, laboratory animals took longer to achieve a similar mean mass. During the early stages of development, upon attaining a live mass of ~ 6 g, measurements of male prawns were larger than those of females but not significantly ($P > 0.05$). By comparison, the physical parameters of female prawns at around 152 days of age and older were found to be greater ($P > 0.05$) than those of males of similar age.

Exoskeleton:flesh (E:F) ratios in adult prawns were lower but not significantly different ($P > 0.05$) when compared to juveniles of 3 g. Although the E:F in males larger than 20 g was higher than females of equivalent mass no significant differences between ratios ($P > 0.05$) were to be found. Similarly differences between E:F ratios of laboratory and pond reared prawns were not significant ($P > 0.05$).

Table 4.2: Live mass, carapace length, wet mass of flesh, exoskeleton and ratios thereof for different samples of prawns reared in the pond and laboratory. Mean values ($n = 30$) with standard deviation in parenthesis are indicated.

Mass Class	Sample	Age (days)	Live mass (g)	Carapace length (mm)	Flesh mass (g)	Exoskeleton mass (g)	Ratio Exo: Flesh
3	Male	62	3.42 (± 0.31)	15.88 (± 1.31)	1.83 (± 0.24)	0.67 (± 0.10)	2.69 (± 0.38)
	Female	62	3.18 (± 0.64)	13.55 (± 1.08)	1.64 (± 0.65)	0.49 (± 0.18)	3.32 (± 0.29)
	Mixed	62	3.33 (± 0.49)	15.01 (± 2.48)	1.77 (± 0.29)	0.64 (± 0.11)	2.86 (± 0.43)
	Laboratory	64	3.52 (± 0.84)	16.86 (± 1.58)	1.76 (± 0.45)	0.53 (± 0.13)	3.30 (± 0.48)
6	Male	79	6.86 (± 1.62)	20.99 (± 4.08)	3.71 (± 0.89)	1.05 (± 0.26)	3.56 (± 0.26)
	Female	79	5.79 (± 1.31)	18.39 (± 1.76)	3.14 (± 0.79)	0.87 (± 0.21)	3.61 (± 0.35)
	Mixed	79	6.57 (± 1.37)	20.82 (± 1.54)	3.61 (± 0.86)	1.05 (± 0.23)	3.42 (± 0.28)
	Laboratory	98	5.26 (± 0.82)	19.02 (± 1.12)	2.79 (± 0.49)	0.76 (± 0.14)	3.71 (± 0.42)
9	Male	98	8.96 (± 1.59)	22.67 (± 1.46)	4.98 (± 1.04)	1.41 (± 0.31)	3.55 (± 0.29)
	Female	98	10.16 (± 1.19)	23.74 (± 1.23)	5.53 (± 0.64)	1.62 (± 0.22)	3.43 (± 0.24)
	Mixed	98	9.69 (± 1.72)	23.24 (± 1.22)	5.39 (± 1.12)	1.51 (± 0.34)	3.60 (± 0.33)
	Laboratory	128	9.13 (± 0.87)	23.06 (± 1.02)	5.28 (± 0.67)	1.50 (± 0.18)	3.53 (± 0.33)
12	Male	111	10.65 (± 1.03)	25.02 (± 1.56)	6.02 (± 0.62)	1.57 (± 0.18)	3.87 (± 0.41)
	Female	111	13.86 (± 2.15)	21.16 (± 2.01)	7.78 (± 1.28)	2.00 (± 0.36)	3.91 (± 0.55)
	Mixed	111	12.20 (± 1.45)	25.65 (± 1.72)	7.04 (± 0.94)	1.81 (± 0.21)	3.90 (± 0.42)
	Laboratory	152	12.01 (± 0.57)	24.87 (± 0.57)	6.99 (± 0.36)	2.04 (± 0.22)	3.47 (± 0.50)
15	Male	120	12.51 (± 1.99)	26.02 (± 1.52)	7.31 (± 1.28)	1.79 (± 0.26)	4.06 (± 0.41)
	Female	120	15.60 (± 3.15)	27.88 (± 2.43)	9.29 (± 2.06)	2.34 (± 0.57)	4.00 (± 0.36)
	Mixed	120	14.04 (± 2.17)	26.84 (± 1.62)	8.26 (± 1.34)	2.11 (± 0.38)	3.92 (± 0.27)
	Laboratory	194	15.19 (± 1.17)	27.11 (± 1.35)	8.71 (± 0.82)	2.57 (± 0.27)	3.40 (± 0.31)
20	Male	152	15.15 (± 1.20)	24.91 (± 1.37)	8.69 (± 0.68)	2.10 (± 0.31)	4.22 (± 0.67)
	Female	152	19.90 (± 1.87)	29.05 (± 1.07)	11.49 (± 1.28)	3.04 (± 0.37)	3.80 (± 0.26)
	Mixed	152	18.75 (± 2.93)	28.87 (± 1.62)	10.52 (± 1.91)	2.45 (± 0.41)	4.32 (± 0.55)
	Laboratory	275	18.97 (± 1.11)	29.14 (± 1.44)	10.87 (± 0.75)	3.18 (± 0.25)	3.43 (± 0.18)
25	Male	194	16.46 (± 0.61)	27.33 (± 1.89)	10.18 (± 0.51)	2.49 (± 0.19)	4.10 (± 0.29)
	Female	194	24.22 (± 1.88)	31.74 (± 3.03)	14.22 (± 1.47)	3.73 (± 0.31)	3.82 (± 0.24)
	Mixed	194	24.93 (± 2.42)	33.82 (± 1.06)	12.83 (± 1.08)	4.12 (± 0.58)	3.63 (± 0.21)
	Laboratory	305	21.68 (± 1.02)	30.55 (± 1.19)	11.00 (± 0.42)	3.14 (± 0.73)	3.49 (± 0.20)
30	Male	250	22.63 (± 0.48)	31.38 (± 0.47)	14.24 (± 0.65)	3.50 (± 0.52)	4.14 (± 0.73)
	Female	250	31.20 (± 4.89)	36.08 (± 2.83)	18.98 (± 2.72)	5.26 (± 1.12)	3.65 (± 0.27)
	Mixed	250	28.35 (± 5.74)	34.20 (± 3.23)	17.08 (± 3.19)	4.55 (± 1.26)	3.85 (± 0.53)
	Laboratory	-	-	-	-	-	-
35	Male	397	23.16 (± 2.13)	32.04 (± 1.32)	14.62 (± 1.56)	5.11 (± 0.68)	3.00 (± 0.13)
	Female	397	40.00 (± 4.75)	39.10 (± 2.23)	25.00 (± 2.81)	8.64 (± 1.12)	2.91 (± 0.20)
	Mixed	397	30.81 (± 9.26)	35.25 (± 4.00)	19.34 (± 5.71)	6.72 (± 2.00)	2.89 (± 0.19)
	Laboratory	-	-	-	-	-	-

4.3.2. Potential growth rate, development and mature body protein.

To establish if the physical components were allometrically related the rate parameter (β) in the exoskeleton, flesh and empty body of each sample was determined [Appendix C; Figures: 1 – 12]. The parameters describing the growth response of body protein in the exoskeleton, flesh and empty body of pond and laboratory reared *F. indicus* are given (Table 4.3). In order to determine significant differences between the β of the physical components for each sample, the relative growth rate was plotted against the natural log of protein growth and compared using simple linear regression analysis [Appendix C; Figures: 13 – 24].

Slopes describing the relative growth rate and the natural log of protein mass were found to be significant between the exoskeleton ($F = 16504.9$; $df = 2, 1290$; $P < 0.001$), flesh ($F = 3833.7$; $df = 2, 1290$; $P < 0.001$) and empty body ($F = 3281.64$; $df = 2, 1290$; $P < 0.001$) of pond-reared prawns (Table 4.3). Similarly by comparing the β between samples of mixed pond and laboratory-reared prawns differences were found in the exoskeleton ($F = 88241.6$; $df = 2, 782$; $P < 0.001$), flesh ($F = 58352.3$; $df = 2, 782$; $P < 0.001$) and the empty body ($F = 218.89$; $df = 2, 782$; $P < 0.001$).

Table 4.3: Growth parameters of the exoskeleton, flesh and empty body in different samples of *Fenneropenaeus indicus* reared in a pond and in a laboratory. Mean values ($n = 30$) with standard deviation in parenthesis are indicated.

Body Component	Sample	β (per day)	P_m (mg)
Exoskeleton	Male	0.0069 ^a	1086 (± 143) ^a
	Female	0.0075 ^b	1687 (± 220) ^b
	Mixed Pond	0.0106 ^c	1545 (± 405) ^{ab}
	Mixed Laboratory [†]	0.0179 ^d	628 (± 224) ^c
Flesh	Male	0.0139 ^c	3062 (± 327) ^a
	Female	0.0121 ^a	5573 (± 627) ^b
	Mixed Pond	0.0129 ^b	5002 (± 1219) ^b
	Mixed Laboratory [†]	0.0178 ^d	2453 (± 1095) ^a
Empty Body	Male	0.0122 ^a	4148 (± 455) ^a
	Female	0.0124 ^b	7260 (± 824) ^b
	Mixed Pond	0.0135 ^c	6541 (± 1615) ^b
	Mixed Laboratory [†]	0.0179 ^d	3067 (± 1021) ^a

[†] Laboratory prawns failed to grow to an equivalent mature mass attained by pond animals. $\therefore P_m = P_t = P_{mLab}$
Significant differences between samples are shown by a change in letters a, b, c and d with ($P < 0.01$).

Higher β values are associated with rapidly maturing animals (Ferguson & Gous, 1993) and although laboratory-reared prawns were found to mature at a faster rate they failed to achieve an asymptotic mass equivalent to or higher than that attained by mature animals in mixed pond samples.

This was most evident when examining P_m values between the different samples. From Table 4.2 although differences between mature protein mass in the body tissue of mixed pond and female samples were not significant ($P > 0.05$), they were significantly higher when compared to the protein content in the exoskeleton ($F = 76.30$; $df = 3, 116$; $P < 0.001$), flesh ($F = 84.76$; $df = 3, 116$; $P < 0.001$) and empty body ($F = 103.20$; $df = 3, 116$; $P < 0.001$) of male and laboratory samples. Low P_m values in laboratory animals were attributed to constraints imposed by the environment, the diet or both.

Unlike laboratory-reared prawns, the β in pond-reared prawns was significantly different between body components. These results suggest that the different body components are not allometrically related. To establish if this were true the natural log of wet mass gain in the exoskeleton was plotted against the natural log of flesh protein gains. In all cases a linear relationship was found ($r^2 > 0.94$) whereby exoskeleton growth could be expressed as a simple power function of flesh protein gains (Figures 4.1 - 4.4). Since no significant differences between the slopes could be found these data were pooled. The result was a single equation descriptive of exoskeleton gains in relation to the accretion of flesh protein and was of the following form:

$$\text{Exoskeleton mass (EM}_t\text{)} = 1.55 P_t^{0.95} \text{ (mg)} \quad [4.3]$$

where EM_t = Exoskeleton mass (mg), and
 P_t = Flesh protein mass (mg), at any point in time.

4.3.3. The maximum deposition of tissue protein.

Maximum protein retention (PR_{\max}), expressed in mg/day, determined for the different sexes and mixed samples of pond and laboratory reared prawns are shown in Table 4.3. The point of inflexion, PR_{\max} , for each sample was determined using non-linear regressions based on observed and predicted data [Appendix C; Figures: 25 – 36]. In addition PR_{\max} values were calculated using observed data (Table 4.3).

Besides the absence of growth data for post larval stages, plots based on observed data [Appendix C Figures: 25 – 36] failed to display the curvilinear relationship depicted in Figure 3.3 (b), a result possibly caused by the discontinuous growth pattern characteristic of prawns and that shorter sampling intervals were required.

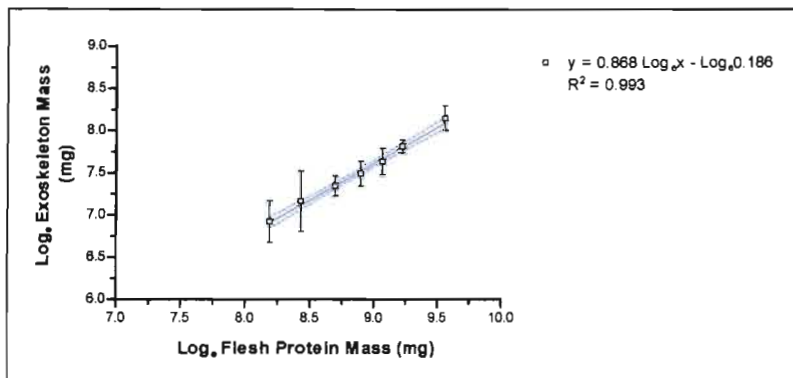


Figure 4.1: The exoskeleton mass in relation to flesh protein in male pond reared prawns.

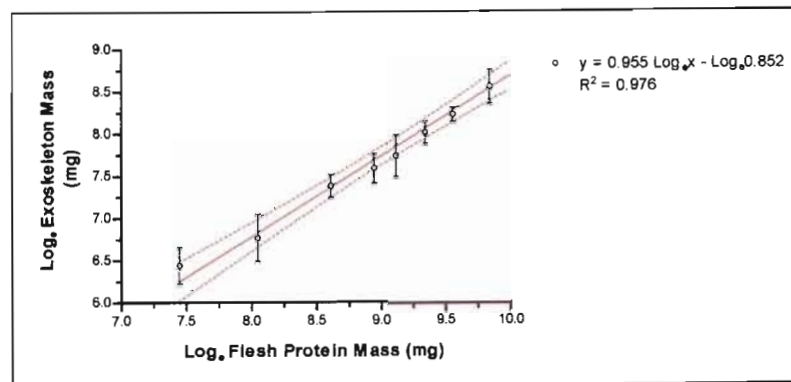


Figure 4.2: The exoskeleton mass in relation to flesh protein in female pond reared prawns.

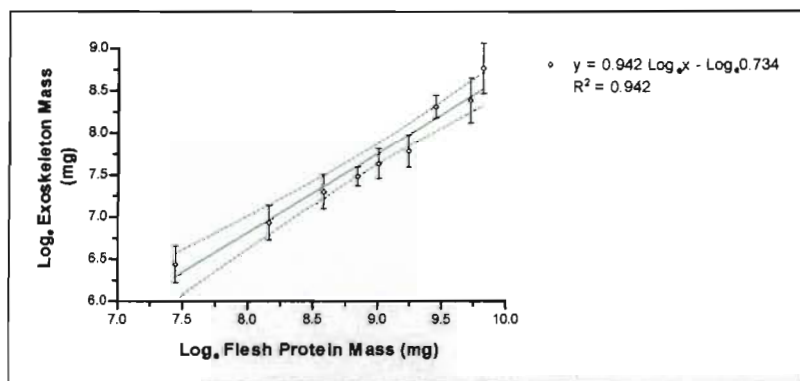


Figure 4.3: The exoskeleton mass in relation to flesh protein in mixed samples of pond reared prawns.

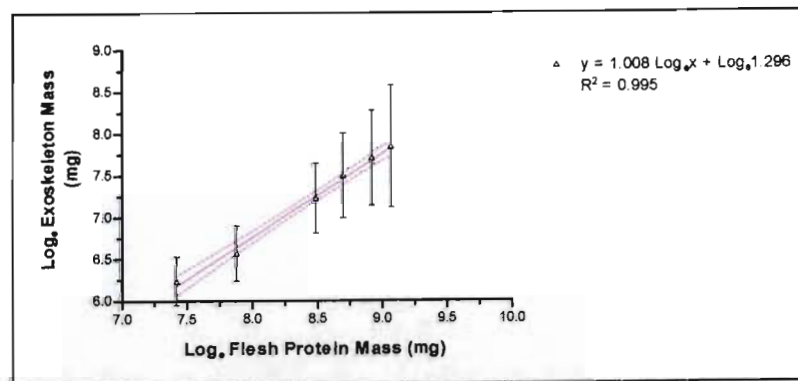


Figure 4.4: The exoskeleton mass in relation to flesh protein in mixed samples of laboratory reared prawns.

Table 4.4: Maximum protein retention in the exoskeleton, flesh and empty body of *Fenneropenaeus indicus* determined using non-linear regression of observed and predicted growth data or calculated using observed data. Mean values ($n = 3$) are indicated with standard deviation in parenthesis.

Body Component	Sample	PR _{max} (mg/day)			
		observed	predicted	#calculated	mean
Exoskeleton	Male	2.74	2.58	3.05	2.79 (± 0.24) ^a
	Female	4.63	4.67	5.49	4.93 (± 0.49) ^c
	Mixed Pond	6.02	5.85	6.32	6.06 (± 0.24) ^d
	Mixed Laboratory [†]	4.38	4.04	4.11	4.17 (± 0.21) ^b
Flesh	Male	15.67	15.66	15.98	15.77 (± 0.18) ^a
	Female	24.81	24.82	25.51	25.05 (± 0.40) ^c
	Mixed Pond	23.70	23.60	24.16	23.82 (± 0.30) ^d
	Mixed Laboratory [†]	15.88	15.91	15.97	16.01 (± 0.12) ^b
Empty Body	Male	18.57	18.55	18.48	18.53 (± 0.05) ^a
	Female	33.12	33.11	33.98	33.40 (± 0.50) ^d
	Mixed Pond	32.46	32.51	33.08	32.68 (± 0.34) ^c
	Mixed Laboratory [†]	19.96	20.16	20.02	20.13 (± 0.12) ^b

[†] Laboratory prawns failed to grow to an equivalent mature mass attained by pond animals. $\therefore P_m = P_t = P_{mLab}$

[#] Calculated using $\beta = PR_{max} \times \frac{e}{P_e}$

Based on the Tukey-test significant differences between samples are shown by a change in letters a,b,c or d with ($P < 0.001$)

From Table 4.4 the mean exoskeleton PR_{max} value of mixed pond prawns, with a maximum deposition rate of approximately 6 mg.day⁻¹, was significantly higher when compared to male ($q = 56.68$, $q_{(0.05, 60.4)} = 4.878$; $P < 0.001$), female ($q = 19.59$, $q_{(0.05, 60.4)} = 4.878$; $P < 0.001$) and laboratory ($q = 32.76$, $q_{(0.05, 60.4)} = 4.878$; $P < 0.001$) samples. Between samples, mean PR_{max} values were found to be significant in the flesh ($F = 9946$; $df = 3, 116$; $P < 0.0001$) and empty body ($F = 19820$; $df = 3, 116$; $P < 0.0001$) with the highest retention rates occurring in females.

Comparisons between apparent and predicted values of PR_{max} in empty body samples suggest that pond-reared prawns were able to attain and maintain a higher rate of gain as opposed to laboratory-reared animals.

4.3.4. The composition of mature pond reared *F. indicus*.

Variables LPR_m, APR_m, WAPR_m, and CPR_m were established using observed data (Table 4.4). Because laboratory animals failed to achieve a similar mature state, as found in pond-reared prawns, the results in Table 4.4 were derived from pond-reared animals since these data were regarded to be descriptive of chemical variables in the exoskeleton, flesh and empty body of the mature prawn.

Table 4.5: Variables describing the mature state in the exoskeleton, flesh and empty body of males, females and mixed samples of cultured *Fenneropenaeus indicus*. Mean values ($n = 30$) are indicated with standard deviation in parenthesis.

Body Component	Sample	Mature composition variables*			
		LPR _m	APR _m	WAPR _m	CPR _m
Exoskeleton	Male	0.012 (± 0.000) ^a	0.668 (± 0.000) ^a	2.549 (± 0.000) ^a	0.221 (± 0.000) ^a
	Female	0.035 (± 0.001) ^b	0.726 (± 0.010) ^c	2.770 (± 0.000) ^c	0.236 (± 0.008) ^b
	Mixed	0.035 (± 0.001) ^b	0.705 (± 0.010) ^b	2.630 (± 0.000) ^b	0.247 (± 0.000) ^c
Flesh	Male	0.033 (± 0.000) ^c	0.094 (± 0.004) ^b	3.625 (± 0.000) ^c	0.005 (± 0.000) ^a
	Female	0.027 (± 0.001) ^a	0.088 (± 0.002) ^b	3.405 (± 0.000) ^b	0.005 (± 0.000) ^a
	Mixed	0.028 (± 0.001) ^b	0.071 (± 0.000) ^a	3.285 (± 0.008) ^a	0.006 (± 0.000) ^b
Empty body	Male	0.028 (± 0.002) ^a	0.244 (± 0.008) ^b	3.343 (± 0.015) ^c	0.062 (± 0.003) ^b
	Female	0.029 (± 0.002) ^b	0.236 (± 0.008) ^{ab}	3.258 (± 0.008) ^b	0.058 (± 0.003) ^a
	Mixed	0.030 (± 0.001) ^c	0.220 (± 0.008) ^a	3.130 (± 0.008) ^a	0.063 (± 0.003) ^b

* Where APR_m, WAPR_m, LPR_m and CPR_m = Mature ratio's of lipid, ash, moisture, lipid and carbohydrate mass, respectively in relation to that of P_m.

Significant differences between samples are shown by a change in letters a,b,c or d with ($P < 0.001$).

LPR_m in the exoskeleton of female and mixed samples, although similar, were both significantly higher ($F = 7935$; $df = 2, 87$; $P < 0.0001$) than those of males. Conversely, LPR_m in the flesh of male prawns was significantly higher when compared to those of female ($q = 40.25$, $q_{(0.05, 60.2)} = 2.829$; $P < 0.001$) and mixed samples ($q = 33.54$, $q_{(0.05, 60.2)} = 2.829$; $P < 0.001$). Differences between empty body samples were significant ($F = 10$; $df = 2, 87$; $P < 0.0001$). Similarly in all cases differences between APR_m, CPR_m and WAPR_m values were found to be significant ($P < 0.001$) (Table 4.5).

4.3.5: The chemical composition of the empty body.

The chemical fractions in each of the different samples are given (Table 4.6). Compared to males of similar age results in Table 4.6 indicate that protein content in the body of female prawns ≈ 152 days and older were higher but not significant ($P > 0.05$).

Over time male prawns became leaner. Body protein remained relatively consistent at 22.05, 21.57, 22.25 and 22.19 % for males, females, mixed pond and laboratory reared prawns respectively, whereas the lipid content in the empty body of pond and laboratory samples averaged 0.68 and 0.74 %, respectively.

Table 4.6: The empty body and chemical fraction, expressed in mg, in the exoskeleton (E) and flesh (F) of pond and laboratory-reared *Fenneropenaeus indicus*. Means are provided ($n = 30$), while the standard deviation (SD) is indicated in parenthesis.

Mass Class	Age (days)	Sample	Empty Body (mg)	Protein (mg)	Lipid (mg)	Moisture (mg)	Ash (mg)	Carbohydrate (mg)
3	62	Male (F)	2464 (± 519)	388 (± 96)	13 (± 3)	1407 (± 348)	32 (± 8)	3 (± 1)
		Male (E)		119 (± 32)	1 (± 0)	384 (± 102)	90 (± 24)	25 (± 7)
	62	Female (F)	2203 (± 287)	347 (± 47)	11 (± 2)	1258 (± 171)	28 (± 4)	3 (± 0)
		Female (E)		107 (± 19)	1 (± 0)	396 (± 60)	81 (± 14)	23 (± 4)
	62	Mixed (F)	2357 (± 377)	370 (± 62)	12 (± 2)	1340 (± 222)	30 (± 5)	25 (± 5)
		Mixed (E)		115 (± 21)	1 (± 0)	372 (± 68)	87 (± 16)	3 (± 1)
	64	Laboratory (F)	2257 (± 549)	366 (± 94)	16 (± 4)	1319 (± 337)	38 (± 10)	6 (± 2)
		Laboratory (E)		99 (± 24)	5 (± 1)	326 (± 79)	65 (± 16)	17 (± 4)
6	79	Male (F)	4684 (± 1127)	820 (± 198)	26 (± 6)	2771 (± 670)	68 (± 16)	7 (± 2)
		Male (E)		189 (± 47)	3 (± 1)	610 (± 151)	150 (± 37)	40 (± 10)
	79	Female (F)	3939 (± 970)	634 (± 159)	22 (± 6)	2395 (± 599)	58 (± 15)	6 (± 2)
		Female (E)		150 (± 37)	3 (± 1)	516 (± 127)	122 (± 30)	32 (± 8)
	79	Mixed (F)	4583 (± 1088)	758 (± 181)	25 (± 6)	2739 (± 652)	63 (± 15)	6 (± 1)
		Mixed (E)		186 (± 40)	2 (± 1)	616 (± 132)	145 (± 31)	43 (± 9)
	98	Laboratory (F)	3499 (± 606)	588 (± 104)	20 (± 3)	2122 (± 375)	49 (± 9)	31 (± 6)
		Laboratory (E)		135 (± 25)	2 (± 0)	445 (± 83)	105 (± 20)	4 (± 1)
9	98	Male (F)	6268 (± 1306)	1055 (± 220)	38 (± 8)	3762 (± 784)	76 (± 16)	12 (± 2)
		Male (E)		255 (± 56)	8 (± 2)	794 (± 175)	206 (± 45)	63 (± 14)
	98	Female (F)	7010 (± 819)	1133 (± 131)	41 (± 5)	4218 (± 489)	87 (± 10)	11 (± 1)
		Female (E)		281 (± 38)	11 (± 2)	917 (± 123)	325 (± 41)	74 (± 10)
	98	Mixed (F)	6821 (± 1292)	1114 (± 208)	39 (± 7)	4105 (± 767)	81 (± 15)	13 (± 2)
		Mixed (E)		251 (± 54)	9 (± 2)	900 (± 193)	252 (± 54)	58 (± 12)
	128	Laboratory (F)	6662 (± 805)	1092 (± 138)	38 (± 5)	4022 (± 509)	80 (± 10)	12 (± 2)
		Laboratory (E)		250 (± 31)	9 (± 1)	894 (± 110)	208 (± 26)	57 (± 7)
12	111	Male (F)	7452 (± 726)	1388 (± 143)	48 (± 5)	4429 (± 455)	94 (± 10)	18 (± 2)
		Male (E)		318 (± 37)	14 (± 2)	843 (± 99)	223 (± 26)	78 (± 9)
	111	Female (F)	9593 (± 1756)	1791 (± 342)	63 (± 12)	5701 (± 1089)	123 (± 23)	28 (± 5)
		Female (E)		414 (± 75)	18 (± 3)	1079 (± 1915)	400 (± 70)	99 (± 18)
	111	Mixed (F)	5727 (± 1650)	1632 (± 219)	59 (± 8)	5162 (± 691)	123 (± 17)	18 (± 2)
		Mixed (E)		374 (± 43)	16 (± 2)	979 (± 111)	255 (± 29)	86 (± 10)
	152	Laboratory (F)	8866 (± 385)	1620 (± 83)	58 (± 3)	5123 (± 262)	122 (± 6)	18 (± 1)
		Laboratory (E)		421 (± 45)	19 (± 2)	1102 (± 117)	287 (± 31)	97 (± 10)

Table 4.6: Continued

15	120	Male (F)	8981 (± 1492)	1692 (± 297)	56 (± 10)	5401 (± 946)	121 (± 21)	9 (± 2)
		Male (E)		366 (± 54)	16 (± 2)	953 (± 140)	289 (± 41)	84 (± 12)
	120	Female (F)	11465 (± 2569)	2011 (± 446)	55 (± 12)	7014 (± 1554)	147 (± 33)	16 (± 4)
		Female (E)		454 (± 111)	19 (± 5)	1309 (± 320)	342 (± 84)	98 (± 24)
	120	Mixed (F)	10183 (± 1674)	2005 (± 327)	57 (± 10)	5962 (± 971)	143 (± 23)	25 (± 4)
		Mixed (E)		436 (± 79)	12 (± 2)	1109 (± 200)	322 (± 58)	112 (± 20)
	194	Laboratory (F)	11062 (± 986)	2115 (± 200)	61 (± 6)	6288 (± 595)	150 (± 14)	26 (± 3)
		Laboratory (E)		530 (± 56)	15 (± 2)	1350 (± 143)	392 (± 42)	136 (± 14)
20	152	Male (F)	10609 (± 863)	1994 (± 156)	58 (± 5)	6411 (± 503)	147 (± 12)	21 (± 2)
		Male (E)		424 (± 63)	14 (± 2)	1156 (± 172)	287 (± 43)	98 (± 15)
	152	Female (F)	14272 (± 1576)	2640 (± 294)	86 (± 10)	8465 (± 945)	189 (± 21)	30 (± 3)
		Female (E)		637 (± 77)	24 (± 3)	1646 (± 200)	407 (± 49)	148 (± 18)
	152	Mixed (F)	12752 (± 2207)	2432 (± 441)	74 (± 13)	7735 (± 1403)	175 (± 32)	29 (± 5)
		Mixed (E)		511 (± 85)	12 (± 2)	1336 (± 223)	330 (± 55)	120 (± 20)
	275	Laboratory (F)	13780 (± 932)	2512 (± 172)	76 (± 5)	7992 (± 548)	181 (± 12)	30 (± 2)
		Laboratory (E)		661 (± 53)	16 (± 1)	1730 (± 139)	428 (± 34)	156 (± 13)
25	194	Male (F)	12592 (± 615)	2351 (± 117)	66 (± 3)	7613 (± 378)	169 (± 8)	13 (± 1)
		Male (E)		519 (± 41)	18 (± 2)	1301 (± 102)	411 (± 32)	131 (± 10)
	194	Female (F)	17704 (± 1700)	3171 (± 326)	86 (± 9)	10668 (± 1099)	240 (± 24)	13 (± 1)
		Female (E)		728 (± 60)	26 (± 2)	2009 (± 166)	590 (± 48)	172 (± 14)
	194	Mixed (F)	16665 (± 1579)	2911 (± 244)	83 (± 7)	9564 (± 801)	206 (± 17)	18 (± 2)
		Mixed (E)		841 (± 117)	30 (± 4)	2212 (± 309)	593 (± 83)	207 (± 29)
	305	Laboratory (F)	15396 (± 788)	2757 (± 135)	83 (± 4)	8906 (± 437)	196 (± 10)	23 (± 2)
		Laboratory (E)		755 (± 69)	18 (± 2)	2002 (± 183)	437 (± 45)	174 (± 16)
30	250	Male (F)	17501 (± 629)	2982 (± 135)	99 (± 5)	10810 (± 490)	280 (± 13)	16 (± 1)
		Male (E)		744 (± 109)	9 (± 1)	1898 (± 280)	497 (± 73)	164 (± 24)
	250	Female (F)	24042 (± 3732)	4231 (± 606)	114 (± 16)	14407 (± 2062)	373 (± 53)	20 (± 3)
		Female (E)		1027 (± 216)	36 (± 8)	2845 (± 600)	746 (± 157)	243 (± 51)
	250	Mixed (F)	21303 (± 4310)	3875 (± 726)	110 (± 21)	12728 (± 2383)	273 (± 51)	24 (± 5)
		Mixed (E)		930 (± 257)	33 (± 9)	2444 (± 676)	656 (± 182)	229 (± 63)
	-	Laboratory	-	-	-	-	-	-
	-	Laboratory	-	-	-	-	-	-
35	397	Male (F)	19399 (± 2123)	3062 (± 327)	102 (± 11)	11100 (± 1186)	288 (± 30)	17 (± 2)
		Male (E)		1086 (± 143)	13 (± 2)	2767 (± 365)	725 (± 95)	240 (± 32)
	397	Female (F)	33262 (± 3778)	5573 (± 627)	151 (± 17)	18976 (± 2135)	492 (± 55)	26 (± 3)
		Female (E)		1687 (± 220)	60 (± 8)	4674 (± 608)	1225 (± 159)	340 (± 52)
	397	Mixed (F)	29095 (± 7180)	5002 (± 1219)	142 (± 35)	16431 (± 4003)	353 (± 86)	31 (± 8)
		Mixed (E)		1545 (± 445)	55 (± 14)	4062 (± 1066)	1090 (± 286)	381 (± 99)
	-	Laboratory	-	-	-	-	-	-
	-	Laboratory	-	-	-	-	-	-

Proportionally by mass, in the empty body of males, females, pond and laboratory samples body moisture content was 72.18, 72.42, 71.90 and 72.08 %; ash was 4.62, 4.87, 4.63 and 4.51 % and nitrogen free extract 1.15, 1.13, 1.23 and 1.22 %, respectively.

4.3.6. Establishing the relationships between the respective chemical constituents of the various body components and flesh protein.

In order to establish if the growth of chemical fractions in the exoskeleton and flesh could be described allometrically ($Y = aX^b$) the natural log of lipid, ash, water and carbohydrate mass in each component were plotted against those of protein in the flesh (Table 4.7). Plots of these relationships are provided in Appendix C (Figure 37 – 64).

On analysis, with the exception of exoskeleton carbohydrate and flesh ash, no significant differences in chemical fractions of the respective body components were found between samples. These data were therefore pooled and a single regression was established for each component (Table 4.7). To obtain a function that was representative of exoskeleton carbohydrate and flesh ash gains data pertaining to laboratory samples were excluded.

Table 4.7: Regression coefficients of *Fenneropenaeus indicus* derived by regressing the natural log of the mass of each chemical component with the natural log of flesh protein mass, expressed in the form $C_t = \log_e a + b \log_e P_t$. Where C_t and P_t are the chemical fraction and flesh protein mass, respectively at a point in time (t), a and b are constants while r^2 = the correlation coefficient and s.e., the standard error.

Body and chemical component	Regression variables			
	Coefficient (a)	Coefficient (b)	r^2	s.e.
Exoskeleton protein	0.250	1.005	0.972	0.123
Exoskeleton lipid	0.00008	1.603	0.791	0.412
Exoskeleton ash	0.154	1.024	0.963	0.131
Exoskeleton moisture	0.679	1.003	0.950	0.143
Exoskeleton carbohydrate	0.033	1.085	0.973	0.131
Flesh lipid	0.063	0.911	0.968	0.107
Flesh ash	0.098	0.966	0.968	0.109
Flesh moisture	4.092	0.977	0.975	0.103

From Table 4.7 the accretion of exoskeleton protein, ash and moisture was considered to be directly related to gains in flesh protein (coefficient $b \approx 1.00$), such that at any point in time the protein, ash and moisture content in the exoskeleton could be described isometrically as $PR_t = 0.25 \times PR_t$, $APR_t = 0.15 \times PR_t$ and $WAPR_t = 0.68 \times PR_t$, respectively. Similarly ash and moisture content in the flesh can be expressed as $APR_t = 0.10 \times PR_t$ and $WAPR_t = 4.10 \times PR_t$. Lipid-free dry matter in relation to body protein was constant [Appendix C; Figure 65 – 88].

4.3.7. Establishing daily gains of protein, lipid and empty body mass.

By incorporating values from Table 4.4 and 4.6 into equation [3.13], estimates of c for each component were calculated. In turn z and c values were substituted into equation [3.17] to predict the growth rate of the empty body in mixed pond samples ($pEMB/dt$) over time. Based on equations [3.3] and [3.16], the rate of protein and lipid retention (mg/day) was determined for mixed pond samples (Table 4.8).

Table 4.8: Predicted daily rates of potential mass gains of the empty body ($pEMB/dt$), protein (pPR_t) and lipid (pLR_t) in mixed samples of pond reared *Fenneropenaeus indicus* from 62 days after hatching until 397 days of age. Means are provided ($n = 30$) while the standard deviation (S.D.) is indicated in parenthesis.

Sample Period (day)	$dEMB/dt$ (mg/day)	PR_t in flesh (mg/day)	PR_t in exoskeleton (mg/day)	Total PR_t (mg/day)	LR_t in flesh (mg/day)	LR_t in exoskeleton (mg/day)	Total LR_t (mg/day)
0-62	19.94	14.97	3.87	17.03	0.11	0.08	0.20
62-79	26.27	18.90	4.98	24.67	0.15	0.10	0.26
79-98	29.75	22.06	6.05	28.87	0.17	0.12	0.29
98-111	32.43	23.29	6.62	32.01	0.18	0.15	0.33
111-120	32.75	23.74	7.05	32.48	0.17	0.16	0.33
120-152	32.05	21.49	7.26	31.73	0.16	0.17	0.33
152-250	29.28	16.25	6.33	28.15	0.14	0.16	0.30
250-305	22.27	6.84	3.41	20.01	0.09	0.15	0.23
305-397	4.59	0.13	0.10	0.00	0.00	0.00	0.00

Results indicated a gradual increase in $pEMB/dt$, pPR_t and pLR_t gains until 111 - 120 days, thereafter rates of nutrient accretion decreased rapidly until there were no further gains.

4.3.8. Predicting live mass (LM_t) from $pEMB/dt$.

Apparent and predicted values of empty body mass were established by implementing a technique whereby the sum of the chemical fractions provided an estimate of empty body mass. Predicted gains in chemical fractions were established using equation [3.17]. However, in order to predict live mass gains (pLM_t) a knowledge of the gut-fill and the portion of body fluids lost during dissection was required. Gains in live mass were derived by regressing the observed values of LM_t with EBM_t such that:

$$pLM_t = -0.000031(pEBM_t)^2 + 2.07(pEBM_t) - 2470.95 \quad (\text{mg})$$

[4.4]

Although seemingly complex when compared to that formulated for pigs (Whittemore *et al.*, 1988) pLM_t estimates were considered conservative since the equation compensates for gut and body fluid losses.

4.3.9. *Application of the model to predict changes in live and body fractions over time.*

The growth rates of the various body fractions, empty body and live mass were predicted and compared with observed values (Table 4.9). In Table 4.9 predicted values compare favourably with those observed. Similarities between estimates indicated that the potential growth of the prawn could be predicted with knowledge of a few genotype parameters. Differences were attributed to an inherent variability between individuals. The differences between estimates of body moisture were far more noticeable since predicted values were found to be higher than observed, which in turn resulted in higher values of $pEBM$. Body moisture differences were associated with losses incurred because of dissections. Thus, estimates of predicted EBM were considered a conservative representation of actual values. Live mass predictions using equation [4.4] provided good estimates.

Table 4.9: Comparison of actual and predicted empty body mass (EBM), body protein (P_t), lipid (L_t), moisture (W_t), ash (A_t) and carbohydrate (C_t) at different live masses (LM) of mixed cultured prawn samples.

Age (days)	LM, Measured (mg)	p LM, Predicted (mg)	EBM, Measured (mg)	p EBM, Predicted (mg)	Tissue Sample	Measured P_t (mg)	Predicted pP_t (mg)	Measured L_t (mg)	Predicted pLt (mg)	Measured W_t (mg)	Potential $pWrt$ (mg)	Measured A_t (mg)	Potential pAR (mg)	Measured CP_t (mg)	Potential pCP (mg)
62	3330	2234	2355	2419	Flesh	370	371	12	18	1340	1384	30	24	3	2
					Exoskeleton	115	116	1	1	372	409	87	69	25	25
79	6570	6369	4583	4586	Flesh	758	798	25	23	2739	2736	63	51	6	4
					Exoskeleton	186	179	2	2	616	637	145	114	43	41
98	9690	10214	6822	6533	Flesh	1114	1194	39	34	4105	3945	81	75	13	6
					Exoskeleton	251	268	9	3	900	839	252	112	58	57
111	12200	13206	8704	9254	Flesh	1632	1493	59	50	5162	5670	123	156	18	9
					Exoskeleton	374	337	16	5	979	1210	255	236	86	85
120	14040	15403	10183	11131	Flesh	2005	1829	57	62	5962	6894	143	138	25	12
					Exoskeleton	436	419	12	6	1109	1394	322	277	112	100
152	18750	18899	12754	13953	Flesh	2432	2697	74	75	7735	8282	175	169	29	14
					Exoskeleton	511	650	12	7	1336	1613	330	327	120	118
250	24930	23432	16665	17822	Flesh	2911	3484	83	90	9564	9825	206	203	18	17
					Exoskeleton	841	891	30	14	2212	2551	593	550	207	197
305	28350	27577	21302	22641	Flesh	3875	4439	110	119	12728	12893	273	273	24	23
					Exoskeleton	930	1251	33	16	2444	2798	656	611	229	218
397	30810	31540	29092	29399	Flesh	5002	4992	142	154	16431	16432	353	355	31	30
					Exoskeleton	1545	1538	55	31	4062	4464	1090	1037	381	368

4.4. Discussion

4.4.1. Growth in *F. indicus*.

In this study animals were reared in conditions assumed to be ideal but, under laboratory conditions, failed to attain a mass heavier than 21 g after 275 days. Emmerson (1980a; 1980b), on the other hand, was able to rear a generation from ovum to adult breeder (> 27 g), in 8000 l tanks, $\sim 27^\circ\text{C}$ and 30 ‰ within 255 days. Possible reasons for an inferior growth performance in this study were that, besides having larger rearing tanks, Emmerson (1980a; 1980b) was able to do a 30 % water exchange every couple of days and because the laboratory system was designed specifically to exclude phytoplankton, bacteria and the build-up of detritus material, commonly associated with pond and natural environments.

In natural systems juvenile *F. indicus*, being opportunistic, eat a variety of invertebrates (gastropods, bivalves, crustaceans, polychaetes *etc.*) and plant material (Dall, Hill, Rothlisberg & Sharples, 1991; Seppings & Demetriades, 1992). Over time as the prawn matures, a reduction in the consumption of microinvertebrates and plant material occurs, and preference shifts towards larger invertebrates with choice dependent on seasonality and prey availability (Dall *et al.*, 1991). A study by Wahlberg (1996) found that macrobenthic communities in Amatikulu ponds were drastically reduced during the growout phase when compared to similar communities in an adjacent estuary. Results would indicate that trophic levels commonly found in ponds provide dietary inputs essential for growth.

This view was supported by a study done on cultured *Litopenaeus vannamei* (*P. vannamei*) where approximately 40 – 60 % of carbon found in the body tissue was derived from pond biota (Anderson, Lawrence & Parker, 1987). Moss, Pruder, Leber and Wyban (1992) found that growth rates of juvenile *L. vannamei* were enhanced when cultured in pond water as opposed to when reared using filtered water since a positive correlation between growth rates and densities of particulate organic carbon and/or unicellular algae was found. Even though Moss *et al.* (1992) failed to establish a trophic link it was evident that size-fractionated pond water having solid particles between 0.5 - 5.0 μm , speculated to be microalgae and/or microbial-detrital aggregates, increased growth rates by 53 %. Moreover, Rodriguez, Le Vay, Mourente and Jones (1994) and Moss, Divakaran and Kim (2001) postulated that organically rich pond water stimulated digestive enzyme activity that promote the assimilation and accretion of nutrients in prawns.

In this study the body mass of laboratory and mix pond-reared prawns 62 - 250 days of age increased by 399 and 751 %, respectively. The overall increase in growth amongst mixed samples of pond-reared prawns, as opposed to those reared under laboratory conditions, was similar to that found by Rodriguez *et al.* (1994). Larger sizes recorded amongst pond cultured prawns would seem to suggest better nutrition and/or less competition for resources and space.

Comparisons of mean live mass and carapace length between the different sexes of pond-reared prawns, from 62 - 152 days, were relatively similar. From 152 days onwards female growth was considerably higher. Data suggested the presence of sexual dimorphism within ponds and that larger sizes were inherent of females. The same was found amongst cultured *P. monodon* (Hansford & Hewitt, 1994).

A comparison of exoskeleton to flesh ratios between 152 -194 day old prawns revealed a higher ratio amongst males. At first these results seemed contradictory since one would assume that sexually mature males require a thicker cuticle for protection against possible rivals when encountering a receptive female. On the whole, measurements of exoskeleton:flesh (E:F) were similar with differences attributed to human error and/or because selected animals were not of a similar moult stage. Since no significant differences between E:F ratios could be found an average of 3.6 ± 0.4 , was calculated using sample means. Thus, the E:F for *F. indicus* is considered to be 1:3.6.

4.4.2 Potential growth rate and P_m values in *F. indicus*.

The rate of maturation and P_m in various body tissues of female prawns were higher but not significantly different from mixed samples (Table 4.2). Conversely, with regard to pond-reared prawns, P_m values were highest amongst females. Despite being fed the same diet the maximum protein mass attained in laboratory prawns (P_{mLab}), was significantly lower than those of pond-reared animals. These differences were possibly a result of limitations imposed by the laboratory set-up.

A faster maturation rate amongst laboratory animals should not be interpreted as being due to a higher water temperature but as being indicative of animals rapidly attaining an asymptotic mass confined by laboratory conditions. More importantly β for the different components was found to be similar in laboratory-reared animals. If found different, as was the case in pond-reared prawns, the implications would be that protein and wet mass gains in the exoskeleton could not be expressed allometrically to protein gains in the flesh.

Although growth occurs *via* a series of moults, tissue synthesis in crustaceans remains essentially a continuous process which is comparable to that of birds and mammals. Physiologically the rate of maturation in each component would have to be the same since immediately after ecdysis, during the B-stage of moulting, the epidermis starts to secrete the endocuticle and continues until hardening of the cuticle occurs (Dall, 1986; Read, 1977; Vijayan & Diwan, 1996). Thus, although moulting frequency controls growth, both processes need to occur in order for the prawn to grow. Hence, the differences in β values in pond-reared prawns were

attributed to the animals not being of the same moult stage and/or because of human error during the weighing process.

Furthermore, differences in β between pond and laboratory-reared prawns was due to a decrease in observed values, as opposed to predicted, between 150 and 300 days of age [Appendix C; Figures 1-12] and was attributed to water temperatures associated with the onset of winter. These results suggest that growth rates are site specific. Although speculative and if reared in warmer tropical regions, where fluctuations in seasonal temperatures are moderate, faster growth rates are expected. However, in the South African context this period is generally of little interest to farm managers since stocks are seldom kept over winter. Should the necessity arise whereby a forecast of body mass and mortality over the winter period need be known, then the growth model modified by Jackson and Wang (1998), which accommodates changes in seasonal temperatures, could be used in conjunction with the model presented here. In future, a means to avoid confusion will be to use the β value measured for the intact carcass rather than for each body component.

Despite these differences exoskeleton mass (EM_i), in relation to flesh protein mass (P_i), could be expressed as simple power function whereby $EM_i = 1.55 \times P_i^{0.95}$ for all samples. An exponent of 0.95 is descriptive of integument deposition calculated as a constant portion of flesh protein content.

4.4.3 The maximum accretion of body protein.

In Table 4.3 maximum protein deposition/retention PR_{max} in the integument was highest in mixed samples and was attributed to a combination of growth rates between the sexes whereby a rapid increase in growth amongst males, during the initial stages, was surpassed at a later stage by those of females. Improved production and increased economic returns are often discussed in terms of sex manipulation (Hansford, 1991; Benzie, 1998). However, results indicate that females and/or mixed samples attained an optimum deposition rate of 33 mg of protein per day at ≈ 15 g i.e. $1/6$ of maturity (μ). An indication that no significant benefit would be gained by rearing a monosex culture. Theoretically, the demand for protein should decrease from the point of inflexion onwards.

Ferguson and Gous (1994) stated that differences between the actual PR_{max} and the potential deposition rate (pPR_{max}) is an indication that either the diet or the environment is limiting. In all cases comparisons between observed and predicted PR_{max} values revealed that during the early stages of the development and prior to the point of inflexion actual rates exceeded predicted [Appendix C: Figure 25 – 28]. Considering that the animals were fed the same diet results suggest that growth, in part, was influenced by temperature as higher temperatures were recorded during the first 90 days of the grow-out period.

This draws us back to the existing argument that since the laboratory animals were reared in conditions, considered ideal, a decrease in growth could be explained as being primarily due to

dietary constraints. Comparison between PR values in the flesh of laboratory-reared animals revealed that the observed rate of protein retention was lower than predicted pPR_{max} [Appendix C; Figure 25 – 28]. Due to the absence of external dietary sources and since the feed offered was the only factor that could have contributed towards the accretion of body fractions it was plausible to consider that under laboratory conditions the diet was limiting.

To illustrate pond-reared *P. monodon*, besides been given feed pellets, were found to feed selectively on plant tissue/material, introduced externally by wind from the surrounding environment, and on smaller crustaceans (Focken, Groth, Coloso & Becker, 1998). Only after the 11th week was the consumption of pellets equal to that of pond biota. Reinforcing the idea that during the early stages of development *F. indicus* supplement their diet, by feeding on a variety of sources, to meet their requirements. Hence, feeding strategies should be adjusted to consider the abundance and presence of pond biota as well as rearing conditions.

In the past trophic pathways and food sources of a number of prawn species have been identified using the stable isotopes of carbon and nitrogen (Rothlisberg, 1998). However, besides being a costly exercise, Focken *et al.* (1998) highlighted that chemical fractions within body tissues *i.e.* protein and lipid have different stable isotope ratios, which influences proximate composition of the samples and their isotopic values. This effect complicates the tracing of the food web by stable isotope ratios and leaves some doubt concerning results from studies where this phenomenon was not taken into account.

4.4.4. Growth variables describing the mature state of pond reared *F. indicus*.

Summaries of mature state variables in Table 4.4 are considered indicative of the species. From these results it was evident that due to the presence of chitin the CPR_m in the exoskeleton was higher than that in the flesh. As opposed to male exoskeleton differences in LPR_m values between components and sexes were small and consistent. The high APR_m values in the exoskeleton compared with that in the flesh and empty body is due to the high calcium content, which has been found to comprise approximately 16 % of the dry matter (Vivjayan & Diwan, 1993). With the exception of body moisture, ratios of body fractions in relation to protein were relatively consistent between samples. Variation in WPR_m values between samples was accredited to moisture losses incurred during dissection.

4.4.5. The chemical composition of the empty body.

Dry matter contents, comprising approximately 30 % of the exoskeleton and flesh, compared favourably with those of *P. vannamei* and *Litopenaeus stylirostris* (*P. stylirostris*) (Boyd &

Teichert-Coddington, 1995) while overall composition was found to be similar to those in Table 4.2. It can be assumed that dry matter of exoskeleton and flesh tissue is expected to remain constant between species. To a good order of approximation Emmans and Fisher (1986) found $c_{\text{ash}} = 0$, $c_{\text{water}} = -0.11$ and $z_{\text{water}} = 3$ to be constant across genotypes, both for plucked birds and mammals. The values of z_{ash} were found to be close to 0.19 for birds, again with the plumage excluded, and 0.23 for mammals. The values of z_{lipid} and c_{lipid} were highly correlated across genotypes since all animals when hatched or born are lean but tend to vary widely between genotypes *e.g.*, $z_{\text{lipid}} \approx 12$ for whether sheep and ≈ 0.6 for male chickens, which is a 20-fold range (Emmans & Fisher, 1986). In this study flesh values of c_{ash} , c_{water} , c_{lipid} , z_{water} , z_{ash} and z_{lipid} for *F. indicus* were found to be 0.03, -0.05, -0.12, 0.07, 3.12 and 0.03, respectively. Comparing the genotype in the present study to male chickens, results displayed a 20-fold difference in z_{lipid} whereas those of z_{water} were found to be similar.

4.4.6. The relationship between the various body components and their chemical constituents with that of flesh protein.

Allometry was used to describe the growth of each body component and their respective chemical fractions in relation to flesh protein gains. An advantage was that ash content of the carcass could be determined directly from the protein content, using the isometric relationship that existed between them. In turn, water and lipid mass were found to be related to the lipid-free dry matter of the carcass by a simple power function. However, since the allometric coefficient describing the relationship between the retention of body moisture (WR_i) and ash (AR_i) with flesh protein gains was $b \neq 1$, slightly higher values were obtained when calculated using equation [3.15].

Most noticeable amongst laboratory reared-animals was that they were often observed ingesting exoskeleton casts. This behaviour was thought to be a response by these animals to regain lost minerals since the demand for calcium has been known to increase during moulting (Shewbart *et al.*, 1973). On the other hand the requirement for phosphorous, which assists in the absorption of calcium, must be met from dietary supplies since levels found in seawater are far too low for the absorption thereof (Dall *et al.*, 1991).

Unlike the model describing feather growth in birds, which required two functions (Emmans, 1989), the relationship between exoskeleton protein and flesh protein gains was explained by a simple power function. The reason for the two functions in birds was that the maturation rate of feathers, β , is significantly higher than that for body protein, and hence these are not allometrically related. Since E:F ratios remained fairly consistent throughout, a single power function was used to describe exoskeleton growth (equation [4.3]).

Generally prawns are restricted in their capacity to store lipids and as a result are fed diets low in fats since changes in moisture and body lipid are limited when compared to fish (Bureau *et al.*, 2000). However, fatty acid and body composition of prawns are affected by environmental parameters since cold-water tolerant species are found to have a higher fat content compared with temperate and warm water species (Marco & Addison, 1993), a result noted in *Euphausia superba* (Hagen, Van Vleet & Kattner, 1996).

An advantage when using the systems approach is that the rate of lipid retention can be predicted as a function of the current state of the animals and the lipid:protein ratio at maturity. Higher body fat in laboratory animals, although not significant, was possibly a result of animals having to increase consumption in order to meet their requirements and/or due to a restriction in space normal physical activities could not be accommodated whereby the energy ingested could be expended.

On examining the allometric relationships between lipid gains and flesh protein content body fat content of males was considerably less than in females. A possible explanation was that in order to develop gonads female prawns less than 20 g had a higher requirement for dietary lipids since ripe ovaries were found to constitute more than 8 % of the total body mass in *P. monodon* spawners (Primavera *et al.*, 1998). To promote ovarian development Middleditch *et al.* (1979; 1980) suggested that certain C₂₀ and C₂₂ polyunsaturated fatty acids (PUFA) be provided in the diets of penaeid prawns. In the laboratory the amount of available dietary PUFA was possibly insufficient resulting in the cessation of growth amongst females older than 195 days of age. Results suggest that female prawns may require higher levels of PUFA at an early age, more so when reared under laboratory conditions.

4.4.7. The growth response.

It may be argued that discontinuous growth patterns, characteristic of crustaceans, do not follow the Gompertz model; however, growth between exoskeleton and flesh protein were found to be allometrically related. Moreover, Jackson and Wang (1998) were able to describe the growth of *P. monodon* successfully using the Gompertz model but in order to enhance the sensitivity of the model knowledge of the species moult cycle is essential. An attempt to observe and monitor the moulting cycle of the species under laboratory conditions proved difficult since little or no trace of the discarded exoskeleton were to be found. The data collected were therefore erratic and sparse.

In *P. monodon*, Dall (1986) reported a moulting cycle of approximately 21 days which coincided with the lunar cycle. This period was considered too general since Mishra *et al.* (2002) reported that at 27 °C, when increasing in mass from 1 - 10 g, *L. vannamei* and *L. stylirostris* were

found to moult approximately every 10 – 12 days increasing to about every 15 – 21 day between 12 – 20 g, in turn providing around 12 – 15 moults over a 4 - 5 month period.

4.4.8. FCR and survival amongst pond and laboratory reared prawns.

A comparison between growth rates of animals reared in the pond with those of three other simultaneously stocked ponds revealed no significant differences indicating that growth in the experimental pond was representative of farm conditions. Percentage survival (mean \pm standard deviation) in ponds was 43 (\pm 8) % as opposed to 78 (\pm 11) % for laboratory reared animals, and unlike the laboratory, where carcasses or remnants thereof were removed, cultured animals were able to scavenge on the dead. A contributing factor towards high survival amongst laboratory animals was that prawns removed were not replaced with individuals of similar size, mass and sex thereby decreasing the stocking density over time.

FCR established within ponds was 2.1 (\pm 0.3), while that for laboratory-reared prawns was 3.0 (\pm 0.4). A high FCR is indicative of increased consumption by an animal to sustain its nutritional requirements. The cannibalism of carcasses and consumption of pond resources, considered an additional endogenous food source within ponds, was also considered to be a contributing factor towards a better FCR.

4.4.9. Description and prediction of the EMB and LM.

Implementation of the model and the comparison between predicted and observed values of live mass and empty body gains compared favourably. However, although the data shown in Table 4.8 were based on mixed pond samples similar procedures could be used to establish those of male and female prawns.

In order to simulate non-limiting conditions, while refining and increasing the predictability of the model, trials that investigate the growth response within a narrow range of environmental conditions should be conducted. Moreover, trials performed in laboratories will require that the dietary content of essential fatty acids, vitamins and minerals be increased in order to ensure that the diet is non-limiting. Unlike the present study, due to financial constraints and limited space, the use of larger tanks *e.g.* porta pools, would have been preferred. A larger surface area would assist in reducing the stress caused by over-stocking whilst a larger volume of water would act as a buffer against slight changes in water quality.

4.4.10. Application of the model.

The method outlined in this study describes an attempt to characterise the potential growth rate of the prawn. To predict protein growth under non-limiting conditions estimates of mature protein size and the rate of maturation were required. Since temperature, salinity and pH influence the physiology and frequency of moulting (Vijayan & Diwan, 1995) further trials should be conducted to establish the effect these parameters have on growth. It is important to note that growth parameters are unlikely to represent the true potential of the species but rather reflect the response imposed by the food and environmental conditions in this study.

Nonetheless, what is encouraging is that the systems approach is capable of providing a means of describing and comparing genotypes. By implementing this approach, future studies will be able to compare inherent parameters for a variety of species reared under different conditions.

4.5. Conclusion

The Gompertz function and allometry, although having been widely used to describe and predict growth in livestock during the latter half of the 20th century, have not been readily accepted in dietary studies involving aquatic animals. Modelling animals using the approach elucidated above enables a description of the growth of body fractions in relation to body protein gains. The advantage of using protein as the driving variable is that the discontinuous/stepwise growth, characteristic of moulting prawns, could be represented realistically using allometry and the Gompertz function.

Genetic growth parameters P_m , β , LPR_m , and b , found to vary between and within sexes, are descriptive of a population and can be used to compare genotypes. Values for P_m , β , LPR_m , APR_m , $WAPR_m$, CPR_m , PR_{max} and E:F ratio in this study for representative samples of *F. indicus*, were 6541.00 mg, 0.014 per day, 0.03, 0.20, 3.30, 0.06, 32.68 mg/day and 1:3.6, respectively when prawns were reared at 19 – 27°C and in 21 – 27 ‰. Comparison of pPR_{max} values between laboratory and pond-reared prawns provided a means to establish if the growth potential was constrained. However, crucial to using this approach is that genotype variables and growth parameters should be established under non-limiting conditions. Further trials to ascertain non-limiting conditions for growing *F. indicus* are required.

In closing, the model derived and based on research in higher vertebrates was well suited for describing growth in *F. indicus*. The model proposed here provides a means of quantifying genetic parameters necessary for defining a prawn, which in turn enables the biological performance to be

predicted. Given nutritional constants and knowledge of the current state of the animal the model is able to predict the nutrient requirements of a species at any given point in time. A better knowledge of dietary requirements and feed intake will result in increased growth rates and lower production costs. The means as to how this is done will be demonstrated in the Chapters following.

5.

Environmental Effects**Effects of Size, Temperature, Salinity and Dietary Protein on the Metabolic Rate of *Fenneropenaeus indicus*.****5.1. Introduction.**

Animals require a continuous supply of energy in order to maintain bodily functions necessary for life *e.g.* blood circulation, muscular activity, repair and replacement of cells, the active transport of ions across cell membranes and excretion of bodily waste (Bureau, Azevedo, Tapia-Salazar & Cuzon, 2000). Under fasting conditions the energy released by these processes appears as heat and is referred to as the metabolic heat or H_E .

The metabolic rate is the energy metabolised over time and, theoretically, can be determined in three ways. The first requires that the difference between the dietary energy ingested and that egested in waste products *i.e.* faeces, urine and in the case of aquatic organisms, ammonia, be calculated. The second requires that the total heat produced by an organism be measured using a calorimeter while the third necessitates that the amount of oxygen used in oxidation processes be quantified (Schmidt-Nielsen, 1997).

Heat loss due to the multitude of metabolic processes is estimated most readily by measuring oxygen consumption (Jones, Yule & Holland, 1997) in fish (Morgan, Sakamoto, Grau & Iwama, 1997) and crustaceans (Dall, Hill, Rothlisberg & Sharples, 1991). Synonyms describing minimum metabolism, H_E , have been termed basal, resting, resting routine, and/or low routine metabolic rate (Jobling, 1994). These terms are based on the assumption that the animal, acclimated to the surrounding environment, is in a post-absorptive, stress free and quiescent state. However, measurements include some increase over the true minimum owing to metabolic costs in low levels of spontaneous activities *viz.*, voluntary or resting activity such as minor bodily movements. Thus, on grounds of standardising the terminology, terms defining minimum metabolism have collectively been referred to as the *fasting metabolic rate* or HE_f (Cho & Kaushik, 1990; Jobling, 1994; Cho & Bureau, 1995; Jones *et al.*, 1997).

An increase in respiration following feeding, often referred to as the apparent heat increment (AHI), also known as apparent specific dynamic action (SDA) or the heat increment of feeding (HE_i), has been associated with the calorigenic effect of food due to excited locomotory and other related activities such as the shredding, digestion, absorption and the biochemical transformation thereof (Hewitt & Irving, 1990; Jones, Yule & Holland, 1997; Rosas & Sanchez, 1996; Rosas, Cuzon, Taboada, Pascual, Gaxiola & Wormhoudt, 2001). Energy losses associated with feeding form a large percentage of the daily expenditure of aquatic animals with diets rich in protein found

to be high in AHI (Beamish, 1974). Fish and crustaceans, which are ammonotelic, have much lower AHI than uricotelic and ureotelic animals since they are more efficient in deriving energy from ingested protein than birds and mammals (Cowey, 1980).

Metabolic rates in prawns are influenced by external factors (Villarreal, Hernandez-Llomas & Hewitt, 2003) and respirometry studies on *Fenneropenaeus indicus* have dealt with the effects of mass, dissolved oxygen levels, starvation and toxins on oxygen consumption (Kutty, 1969; Bjanakiram, Venkateswarlu, Rajarami Reddy & Sasira Babu, 1985; Subramanian & Krishnamurthy, 1986; Maguire & Allan, 1992; Chinni, Khan & Yallapragada, 2000). However, to date no study has investigated the effect of salinity and temperature on the fasting metabolism and AHI of *F. indicus*, fed diets varying in protein concentrations. Hence, in the present study the effect of salinity, temperature, body mass and dietary concentration of protein on the metabolism in *F. indicus* was examined. The aims of this study were:

- To assess what effect temperatures of 20, 25, 30 °C and salinities of 15, 25, 35 ‰ had on the oxygen demand of ~ 6, 9 and 14 g *F. indicus*.
- Assess the AHI associated with diets comprising ~ 21, 31 and 41 % crude protein (CP) in prawns acclimatized to the above salinities and temperatures.

5.2. Materials and Methods.

Oxygen uptake by juvenile *F. indicus* were conducted using a 510 ℓ intermittent flow system (Figure 5.1) adapted from designs used by Marais *et al.* (1976), Talbot and Baird (1985), Du Preez, McLachlan, Marais (1986) and Sara (1998). Trials were divided into two phases, a pre-feeding/fasting phase and a post-feeding phase. The first phase measured the fasting metabolism of three mass classes of *F. indicus*, which were reared on diets varying in protein, and acclimated to the salinities and temperatures mentioned above. Phase two, under the very same conditions, measured the apparent HI after the animal was fed the diet on which it was reared.

5.2.1. Laboratory set-up, test animals and diets.

F. indicus of (mean ± S.D.): 5.62 ± 0.53 g, 9.10 ± 0.53 g, 14.10 ± 0.82 g were transported from Amatikulu prawn farm to the laboratory on various occasions. Prawns were given a minimum of 10 days to acclimate to experimental conditions. Throughout the study the handling of animals was kept to a minimum. Three 350 ℓ glass tanks, housed in the aquarium laboratory, were each stocked with 60 prawns of both sexes. Tank water of test salinity and temperature was circulated through a trickle filter with the aid of a submersible pump (Model: Aquaclear Powerhead-802). Animals in each tank were allotted a test diet.

Diets were formulated using ingredients shown in Table 1.1 and were prepared as described in paragraph 3.2.2 in order to provide diets with 21, 31 and 41 % CP (Table 5.1).

Daily feedings were done at 08:30 and 17:00 h. After 10 days five prawns from each tank, selected at random, were transferred to the environmental room where the respirometer apparatus was housed and in which the temperature was preset to test temperatures. The photoperiod in the laboratory and environmental room was set at 12:12 h (L:D) with the photophase starting at 06:00 h.

Table 5.1: Composition of test diets.

Proximate analysis (g/100g <i>dm</i>)	Test Diet		
	21 % CP	31 % CP	41 % CP
Crude protein	21.48	31.43	41.39
Lipid	6.28	5.62	4.95
Ash	5.13	7.12	9.10
Crude Fibre	1.09	1.09	1.09
Gross Energy (MJ/kg)	17.74	17.74	17.74

5.2.2. Environmental room.

Once transferred prawns were placed into a 300 ℓ glass tank that contained water of test temperature and salinity and which was partitioned into 3 sections. At any given time each section was stocked with five animal, three for use as test animals and two as backup, reared on a specific diet. Animals were fed daily rations as done in the laboratory. Overhead red fluorescent tubes were used to reduce the effect of light intensity.

5.2.3. Temperature, pH, nitrogen and salinity.

Experimental temperatures of 20°, 25° and 30 °C were representative of the range found in local ponds. In all cases trials began at 30 °C following which the next test temperature was attained by lowering the temperature at a rate of 1 °C/day. This approach, providing 98 % survival, allowed prawns to acclimate to a test temperature of 20 °C. Distilled water was used to dilute filtered (10 µm) seawater at a rate of 1 ‰ per day. Water was maintained at pH 8 ± 2 with oxygen concentrations kept near saturation throughout the trials. Dissolved oxygen, salinity, temperature, ionised ammonia and pH levels of water were monitored daily. Fluctuations of test temperatures in the 3 m³ environmental room were within 0.5 °C.

5.2.4. Design of the respirometer apparatus.

The apparatus consisted of a 250 ℓ supply tank from which water passed through a 10 µm filter and gravitated through a manifold that diverted the flow into four respirometer chambers. The cylindrical chambers of 300 ml capacity with a wall thickness of 5 mm and made from transparent perspex, were connected to the system *via* reinforced plastic tubes that had a wall thickness of 2.5 mm. The chambers, which allowed for the free movement of individual

prawns, were immersed in a water bath that contained water equivalent to test conditions (Figure 5.2). To avoid trapping air while placing animals into the respiratory chambers the chambers were sealed underwater. Stainless steel tubes of 10 mm diameter protruded from the centre of rubber bungs, which were used to seal the open ends of the respiratory chamber, and served as connectors to the outlet and inlet tubes.

Water entering the respiratory chambers was kept saturated by aerating the supply tank with two large air diffusers since oxygen consumption of fish measured in a closed system was affected when available levels dropped below 66 % saturation (Winberg, 1956). A flow rate of $60 \text{ mL} \cdot \text{min}^{-1}$, adjusted using clamps attached to the inlet tubes, varied $< 5 \%$ between readings. Flow rates were measured hourly for the first 4 hours and thereafter readings were done indiscriminately.

Three chambers were stocked with a single animal while the 4th, which always served as the control, remained unoccupied. The outflow from each of the respiratory chambers was diverted with the aid of 3/2-way universal normally open stainless steel solenoid valve (Model: Asco 8320). The systematic energising of the solenoids by 1.5 amps, generated using a step down 220V/24V transformer *via* a 4-cam timer (Model: cdc M48R), which was set to provide 15-minute intervals, diverted de-oxygenated water for a particular chamber to an oxygen probe (Figure 5.3 & 5.4). When the solenoid was de-energised, the chamber was isolated and the water was diverted to a 250 l reservoir tank. The systematic cycling through all four valves meant that a complete cycle lasted an hour.

Dissolved oxygen concentrations were measured to within $0.01 \text{ mg} \cdot \text{l}^{-1}$ using a probe and a digital meter (Model: YSI 5100, Yellow Springs Instruments, Yellow Springs, Ohio, USA). The oxygen probe was fixed to a removable cap that was part of a container specially machined from solid perspex to have 12 mm thick walls, an inlet and outlet port, an internal volume of 50 mL and threads at one end. The threaded end enabled the cap to be screwed on against an "O"-ring in order to provide a watertight seal. Water passing over the sensor membrane and exiting *via* the outlet ports, gravitated into the receiver tank.

After passing it through a 50W UV light unit water in the receiver tank was returned to the supply tank with the aid of a submersible pump (Model: Bucci 600L). The pump was activated and deactivated by float switches that were adjusted to permit a displacement of $< 3 \text{ l}$. The float switches ensured that water levels, and hence flow rates, remained constant and that cavitation, due to the suction of air, did not occur.

Dissolved oxygen readings by the YSI 5100 probe sensor, captured using the Hyper Terminal software (Microsoft 97), were downloaded using text format. By default oxygen levels were recorded every second, however, a program designed using C⁺⁺ extracted every 10th reading and sorted data from each 15-minute period into columns representative of each chamber. Oxygen uptake was calculated as being the difference between dissolved oxygen levels recorded in the control chamber with those containing prawns. The software program

automatically calculated and tabulated the mean temperature, salinity and oxygen consumption for each 15-minute period. Mean consumption was multiplied by a factor of four in order to establish the $\text{mg O}_2 \cdot \text{prawn}^{-1} \cdot \text{hour}^{-1}$. Measurements recorded for prawns that showed signs of moulting or had moulted overnight were disregarded.

5.2.5. Phase 1: Determination of fasting metabolic rates.

Metabolic rates were expressed as either rate per individual ($\text{mg O}_2 \cdot \text{prawn}^{-1} \cdot \text{h}^{-1}$) or as mass-specific ($\text{mg O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$). The allometric relationship of the former was represented as:

$$\text{QO}_2 = a M^b \quad [5.1]$$

and the latter as:

$$\text{QO}_2 = a M^{b-1} \quad [5.2]$$

with M being the live mass in grams. After log transformation equations [5.1.] and [5.2.] became:

$$\log \text{QO}_2 = \log a + b \log M \quad [5.3]$$

and

$$\log \text{QO}_2 = \log a + (b - 1) \log M \quad [5.4]$$

respectively where the value of $\log a$ indicated the intercept value and b or $(b - 1)$ the slope (Hewitt & Irving, 1990).

5.2.6. Preparation

Prior to the initiation of trials the probe was recharged with new electrolytic solution, fitted with a new membrane and calibrated according to procedures specified in the YSI manual. Saturation levels were compared with values provided by Truesdale, Downing and Lowden (1955). Preliminary 48 hr trials indicated that meter drift and bacterial activity ($< 1.2\%$ variation) had no significant effects on DO measurements. During these same trials feed pellets were placed into the chambers and left for three hours to assess if a correction factor, due to the presence of food, was necessary. Results indicated that oxygen uptake was negligible and that no correction was necessary when determining the AHI.

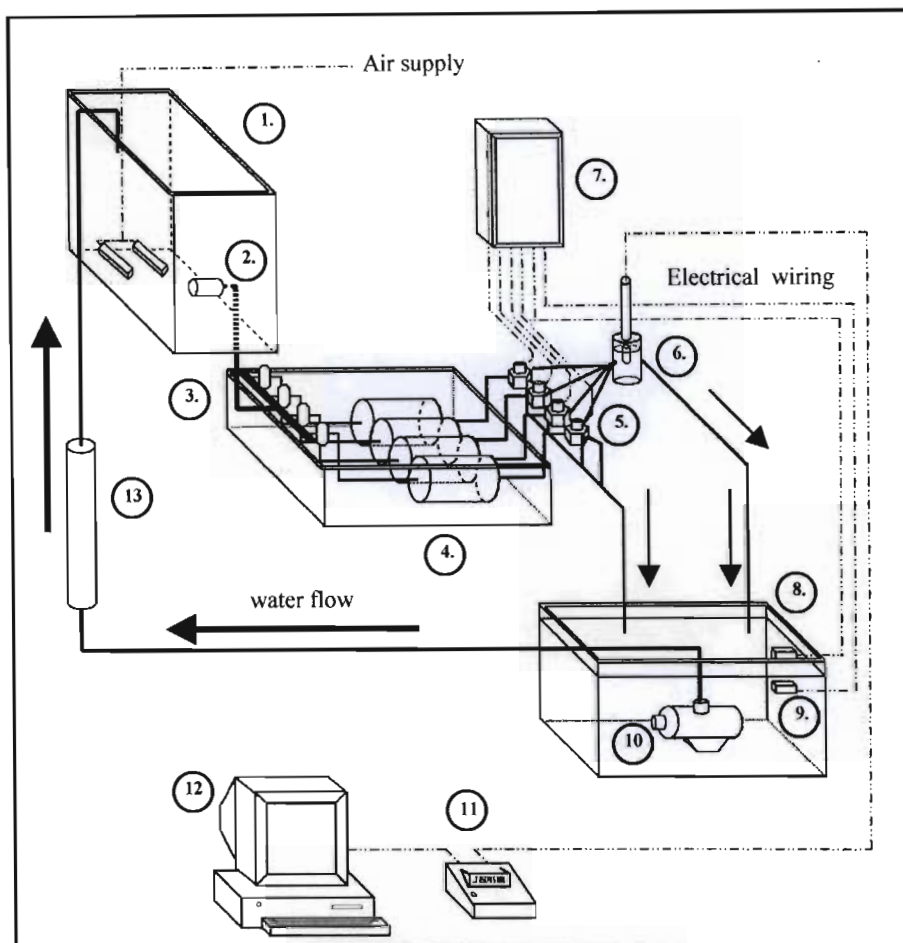


Figure 5.1: Schematic diagram of the respirometer system (not to scale) used to measure the oxygen consumption of *F. indicus* fed diets of varying protein contents where ① supply tank ② filter ③ manifold ④ water bath with immersed chambers ⑤ solenoid valves ⑥ oxygen probe ⑦ timer unit ⑧ reservoir tank ⑨ float switches ⑩ pump ⑪ oxygen meter ⑫ computer ⑬ UV unit.

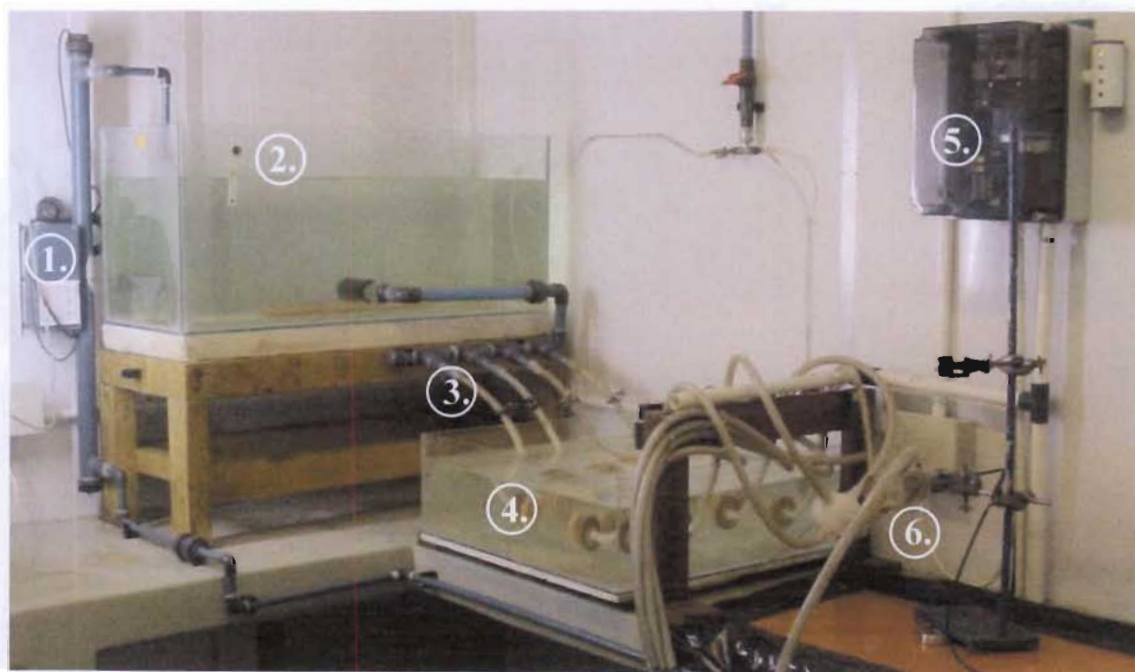


Figure 5.2: Front and side view of the respirometer with the U.V unit ①, supply tank ②, manifold ③, waterbath ④, timer unit ⑤ and probe ⑥ visible.

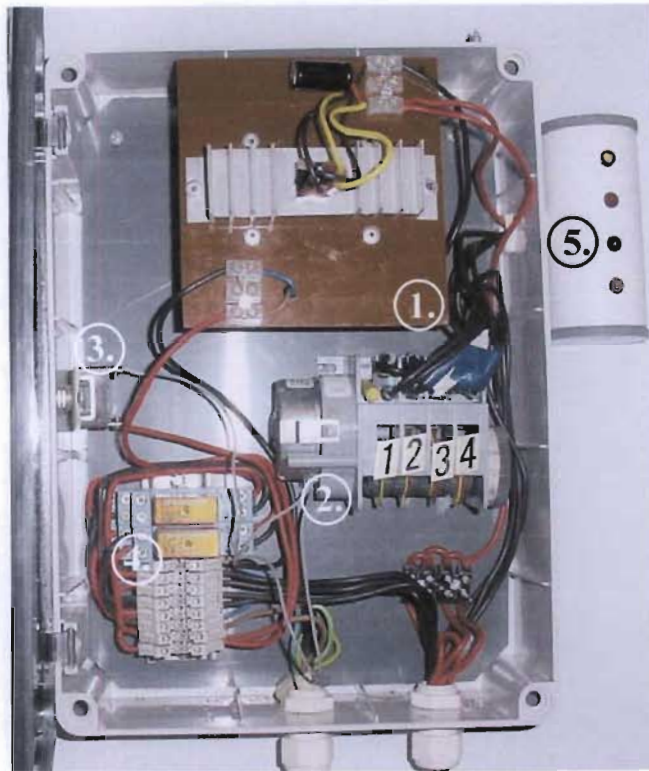


Figure 5.3: Front view of the timer unit displaying the step down transformer ①, cam-timer ②, toggle switch ③, relays ④, and L.E.D's ⑤ that indicated which solenoid valve was activated.

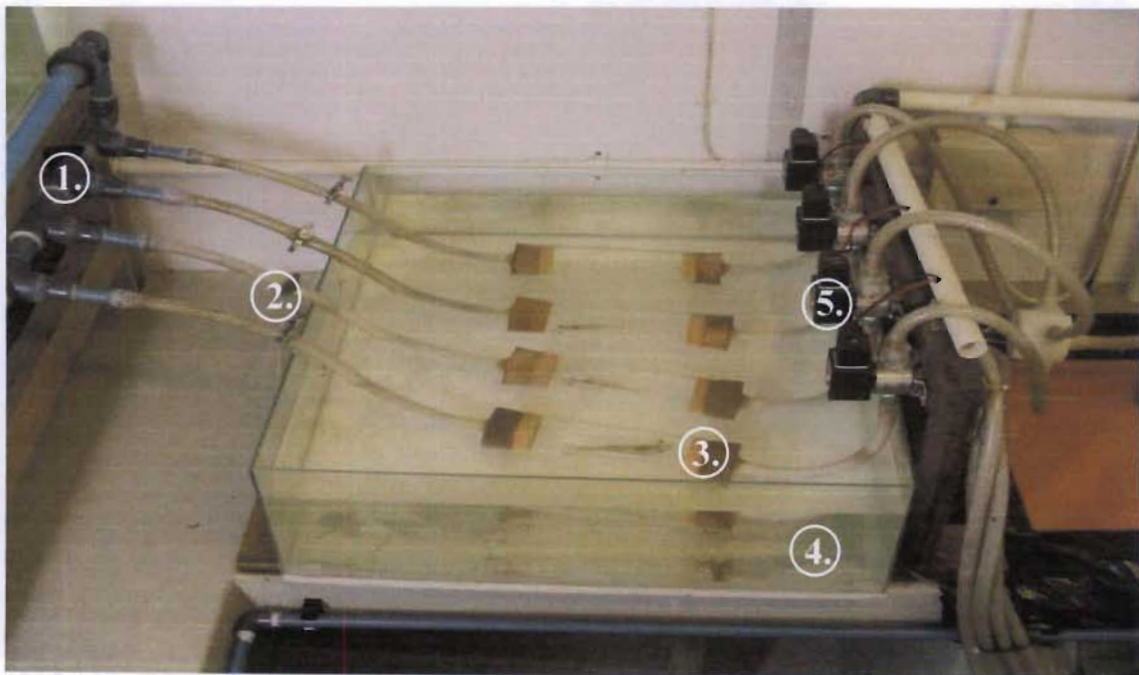


Figure 5.4: Side view displaying the manifold ①, clamps ②, the respirometer chambers ③, waterbath ④, and solenoids valves ⑤.

A day after placing the animals into the holding tank, at approximately 16:30 h, three prawns, one from each section, were removed, blotted dry with absorbent paper and weighed to the nearest 0.01 g. In order to limit fowling caused by defecation and the excretion of waste products, feeding was halted 6 hours prior to placing the animals into the chambers. Animals once placed in the chambers were left over night to acclimate to respirometry conditions. Oxygen consumption measurements were initiated at about 8:00 h the following morning and continued for 24 hours. Since oxygen consumption in starved *F. indicus* of < 1 g declined rapidly after 48 hours (Kutty, 1969) a total of forty four hours (six prior to placing them in the chambers, 14 overnight and 24 while measuring fasting rates) before recording post feeding consumption rates ensured that the animals were in a post-absorptive state.

5.2.7. Phase II: Determination of the apparent heat increment (AHI).

After twenty-four hours oxygen measurements were stopped. The post-feeding phase was initiated by introducing a feed pellet equal to 1 % of live mass, on which the prawn was reared, into the chamber. Prawns generally ingested the food presented within 20 minutes. Food not consumed within 30 minutes was siphoned onto a 63- μ m nylon mesh, and the residue rinsed with distilled water. The mass of the feed residue was determined by transferring the filtrate to pre-weighed aluminium foils, dried at 70 °C for 48 hours, placed into a desiccator to cool and thereafter re-weighed. After animals had eaten and feed residue, if any, removed oxygen measurements were resumed.

Oxygen consumption was monitored for further 7 hours thereafter since preliminary studies revealed that elevated levels caused by apparent HI occurred well within this period. To compensate for the effect of diurnal rhythms oxygen readings were taken between 09:00 and 16:00 h. In accordance with Du Preez *et al.* (1986a; 1986b; 1992) the apparent HI was calculated as the total increase in oxygen demand over fasting levels recorded 24 hours previously (Figure 5.5). Respiration was converted into energy using the equivalent: 1 l O₂ (STP) respired = 20.1 kJ consumed (Crisp, 1971; Withers, 1992; Schmidt-Nielsen, 1997). Converted values were expressed as a percentage of the energy ingested.

5.2.8. Post measurement maintenance.

Throughout the study prawns were used once only. On termination of each trial tubes and respirometer chambers were removed and replaced with a clean set. The initial set was washed with a disinfectant, a 5 % Hibitane® solution, and rinsed thoroughly with hot water, dried and set-aside for use in the following trial.

After three replicates for a given diet, salinity, temperature and mass were obtained the system was disinfected, rinsed thoroughly with fresh water and filled with water of test salinity. After three replicates for a given salinity and temperature using all three mass classes were

obtained, the supply and receiver tanks were scrubbed, flushed and rinsed clean with fresh water. Tubes were visually examined for discoloration, a sign of bacteria build-up, left to soak in a solution of 5 % Hibitane® and thereafter thoroughly rinsed and dried. Once the chambers and tubes were reassembled the system was refilled with water of requisite salinity and left to circulate while lowering the temperature to the next test temperature. The filter cartridge was cleaned and replaced after each run.

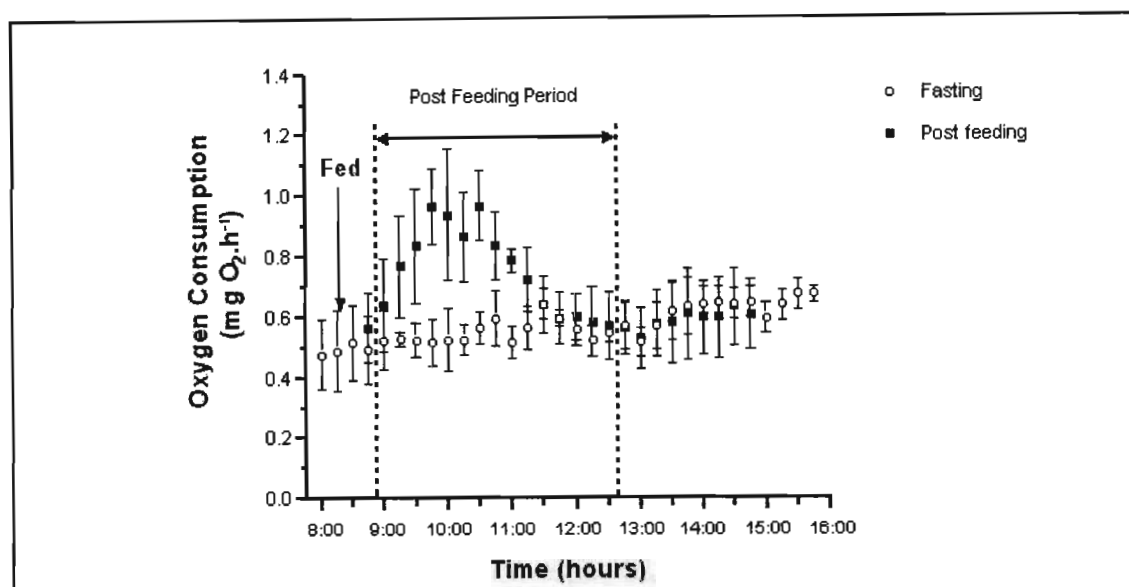


Figure 5.5: An illustration as to how specific dynamic action was determined for each of the test diets. Open circles are means of oxygen consumption while fasting, established 24 hours earlier; closed squares indicate elevated levels of consumption after feeding (↓) due to apparent specific dynamic action with bars about the mean indicating standard deviation.

5.2.9. Data handling.

All data logged by the oxygen probe and transposed by the program were transferred onto a Microsoft Excel spreadsheet. An overall mean, derived from twenty-four 15-minute means, and standard deviation was calculated for each chamber. These data were used to plot the relationship between oxygen consumption and body mass for animals fed a specific diet at the different temperatures and salinities during pre and post-feeding phases. Regression coefficient values a and b were calculated by plotting data using logarithmic scales. Differences in oxygen consumption were defined by analysis of variance. Both linear regression and analysis of variance were conducted in accordance with Zar (1996) using procedures available in GraphPad Prism version 3.02 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

5.3. Results.

5.3.1. Fasting measurements.

A period of 14 hours prior to the initiation of oxygen readings was considered sufficient for animals to acclimate to experimental conditions. However, *F. indicus* were seldom idle during trials and were often seen to gently beat their pleopods and move slowly to and fro along the respiratory chamber. In all cases the circadian rhythm of prawns exhibited a diurnal rhythm whereby oxygen consumption during the light period, between 06:00 - 18:00 h, increased gradually and was found to peak at around 18:30 h (Figures 5.6 - 5.8). Peak consumption levels in prawns 9 g and larger were maintained during the early evening hours until about 23:00 h. Thereafter oxygen uptake decreased steadily to a minimum at around 05:00 h. The oxygen consumption increased during the dark regime and was significantly higher ($P < 0.001$) than during the light regime.

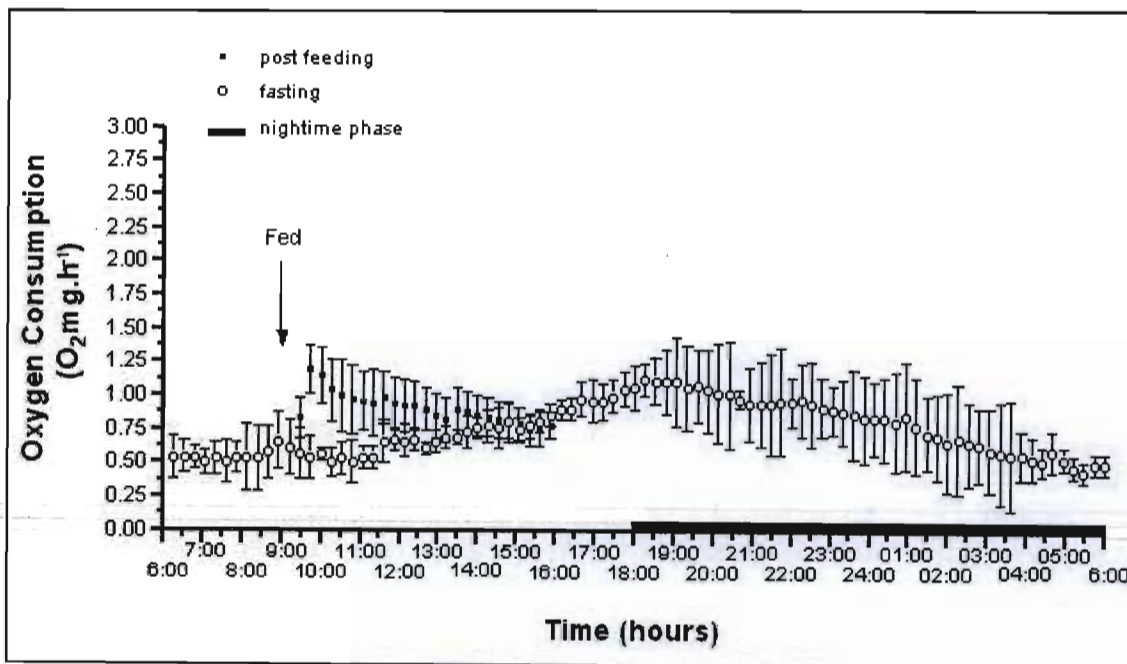


Figure 5.6: Quarter hour means with standard deviations of oxygen consumption of 5 g *Fenneropenaeus indicus* over a 34-hour period at 25 ‰ and 25 °C when reared on a diet containing 30 % crude protein. Open circles depict fasting means while closed squares indicate specific dynamic action after feeding (↓) with bars about the mean indicative of the standard deviation. The broadening of the baseline indicates the period of darkness.

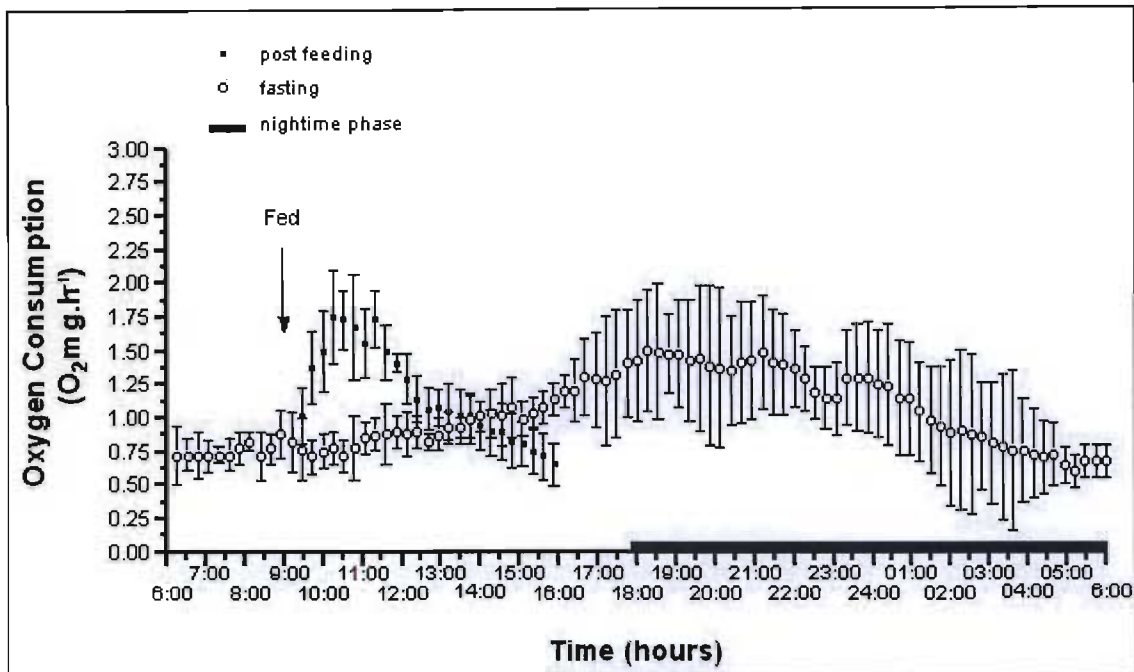


Figure 5.7: Quarter hour means with standard deviations of oxygen consumption of 9 g *Fenneropenaeus indicus* over a 34-hour period at 25 % and 25°C when reared on a diet containing 30 % crude protein. Open circles depict fasting means while closed squares indicate specific dynamic action after feeding (↓) with bars about the mean indicative of the standard deviation. The broadening of the baseline indicates the period of darkness.

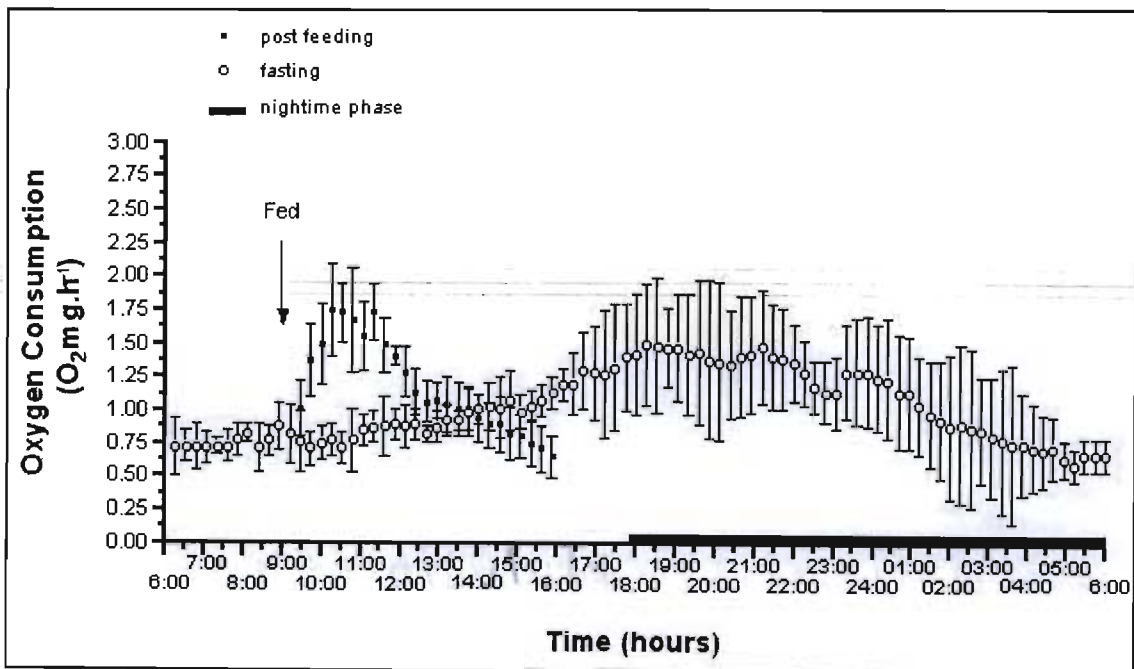


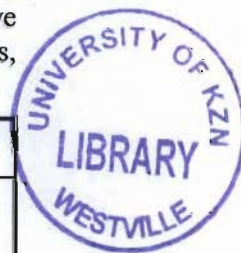
Figure 5.8: Quarter hour means with standard deviations of oxygen consumption of 15 g *Fenneropenaeus indicus* over a 34-hour period at 25 % and 25°C when reared on a diet containing 30 % crude protein. Open circles depict fasting means while closed squares indicate specific dynamic action after feeding (↓) with bars about the mean indicative of the standard deviation. The broadening of the baseline indicates the period of darkness.

5.3.2 The influence of body mass on metabolic rate.

The relationship between oxygen consumption and body mass in fasting juvenile *F. indicus* reared on 21, 31, 41 % CP at 20°, 25°, 30 °C and 15, 25, 35 ‰ are shown (Appendix D: Figures 1 – 9). Body mass significantly influenced metabolic rates with oxygen consumption per unit mass (O_2 mg. g^{-1} . hr^{-1}) decreasing as body mass increased.

Table 5.2: Values for the constants of the mass metabolism equations ($\log QO_2 = \log a + b \log M$) determined for fasting *F. indicus* at various salinities and temperatures. In the above equation QO_2 = oxygen consumption mg O_2 per hour, M is live mass (g), $a + b$ = constants, while r^2 = correlation coefficient and s.e. = standard error with $n = 9$.

Experimental parameters			Parameters of equation			
Dietary protein content (%)	Salinity (‰)	Temperature (°C)	<i>a</i>	<i>b</i>	r^2	s.e.
21	15	20	0.202	0.789	0.92	0.051
		25	0.238	0.862	0.96	0.032
		30	0.420	0.861	0.92	0.047
	25	20	0.183	0.779	0.92	0.043
		25	0.016	0.778	0.84	0.063
		30	0.339	0.860	0.91	0.057
	35	20	0.180	0.791	0.93	0.048
		25	0.213	0.862	0.96	0.034
		30	0.348	0.856	0.87	0.068
31	15	20	0.200	0.796	0.77	0.087
		25	0.231	0.833	0.93	0.046
		30	0.288	0.884	0.91	0.056
	25	20	0.174	0.783	0.76	0.088
		25	0.271	0.768	0.88	0.052
		30	0.325	0.822	0.82	0.076
	35	20	0.214	0.792	0.83	0.070
		25	0.206	0.833	0.92	0.047
		30	0.302	0.862	0.92	0.049
41	15	20	0.207	0.799	0.90	0.049
		25	0.201	0.835	0.89	0.048
		30	0.193	0.911	0.91	0.054
	25	20	0.223	0.754	0.87	0.052
		25	0.246	0.773	0.81	0.064
		30	0.390	0.809	0.87	0.059
	35	20	0.173	0.774	0.79	0.071
		25	0.179	0.835	0.89	0.049
		30	0.266	0.873	0.84	0.071



5.3.3 The influence of dietary protein on fasting metabolic rates.

For any given salinity and temperature, oxygen consumption of fasting prawns previously reared on 31 % CP was generally lower, whereas those fed 21 % CP were higher than those reared on 31 and 41 % CP (Appendix D: Figures 1 - 3). Oxygen consumption was lowest at 20 °C and 25 ‰ in all mass classes previously reared on 31 % CP (Appendix D: Figure 2a). No significance differences ($F = 0.49$; $df = 2, 48$; $P > 0.05$) in respiration rates between fasting animals reared on the different diets at a given salinity and temperature were to be found and as a result these data were pooled (Table 5.3).

Table 5.3: Pooled values of respiration rates ($\text{mg O}_2 \cdot \text{prawn}^{-1} \cdot \text{h}^{-1}$) for fasting *F. indicus*, previously fed diets containing 21, 31 and 41 % CP reared at salinities 15, 25 and 35 ‰ and temperatures of 20, 25 and 30°C. $a + b = \text{constants}$, $r^2 = \text{correlation coefficient}$ and $s.e. = \text{standard error}$ with $n = 27$.

Experimental conditions		Parameters of equation			
Salinity (‰)	Temperature (°C)	a	b	r^2	$s.e.$
15	20	0.202	0.798	0.852	0.059
	25	0.166	0.832	0.890	0.049
	30	0.245	0.884	0.879	0.059
25	20	0.193	0.778	0.835	0.059
	25	0.388	0.778	0.832	0.059
	30	0.356	0.836	0.820	0.071
35	20	0.190	0.785	0.847	0.058
	25	0.291	0.833	0.898	0.049
	30	0.218	0.850	0.790	0.081

5.3.4. Effect of salinity on oxygen consumption.

In this study salinity had no significant effect on oxygen consumption within the range tested. Although oxygen consumption was independent of salinity (15 – 35 ‰), a slight decrease at 25 ‰, for all temperatures, was noted (Appendix D: Figures 4 - 6). Since no significance differences in oxygen demand for a given temperature and mass were found between salinities these data were pooled. The result was three regressions representative of oxygen consumption at 20, 25 and 35 °C (Table 5.4).

Table 5.4: Values for the constants of the mass metabolism equations ($\log \text{QO}_2 = \log a + b \log M$) determined for fasting *F. indicus* for various temperatures. In the above equation $\text{QO}_2 = \text{oxygen consumption}$ ($\text{mg O}_2 \cdot \text{h}^{-1}$), M is live mass (g), $a + b = \text{constants}$, $r^2 = \text{correlation coefficient}$ and $s.e. = \text{standard error}$ with $n = 81$.

Temperature (°C)	Parameters of equation			
	a	b	r^2	$s.e.$
20	0.194	0.783	0.798	0.069
25	0.243	0.810	0.812	0.065
30	0.341	0.854	0.811	0.079

5.3.5 Effect of temperature on oxygen consumption.

As with other penaeid species (Dalla Via 1986; Liao & Murai 1986) temperature influenced the metabolic rate of the species significantly ($F = 116.2$; $df = 2, 242$; $P < 0.0001$) with an increase in temperature resulting in an increase in respiration (Appendix D: Figures 7

- 9). The effects of temperature on the thermal coefficient (Q_{10}) at increments of 5 and 10 degrees are shown in Appendix E. Results indicated that oxygen consumption was affected to a lesser degree between 20 - 25 °C as opposed to the temperature interval 25 - 30 °C.

From Table 5.4 the relationship between the oxygen consumption (O_2 mg. h^{-1}) of fasting *F. indicus* 5 – 15 g at 20°, 25° and 30 °C reared in salinity ranging between 5 - 35 ‰ was represented as follows:

$$QO_2 = 0.194 M^{0.783} (20\text{ °C}) \quad [5.5]$$

$$QO_2 = 0.243 M^{0.810} (25\text{ °C}) \quad [5.6]$$

$$QO_2 = 0.341 M^{0.854} (30\text{ °C}) \quad [5.7]$$

5.3.6. The effect of apparent specific dynamic action on oxygen consumption

After having eaten, a marked increase in oxygen consumption (Figure 5.3 – 5.5), which was a direct result of the feeding behaviour of individual prawns, was noted. The increase was brought about, in part, by the excited behaviour since prawns were seen to rapidly beat their pleopods while clasping food. On many occasions pleopod movement continued for two to four hours after feeding with intensity decreasing over time. Post-prandial peaks of oxygen consumption varied in magnitude and occurred any time between half to four hours after feeding. The initial increase in oxygen consumption was a result of increased activity involved in consuming food while a second peak, much lower in magnitude, occurred about 2 to 3 hours later and was attributed to absorption and digestion. This pattern was more evident in animals larger than 5 grams. Pre-feeding levels of oxygen consumption were resumed 4 to 6 hours after feeding (Figures 5.3 – 5.5). After feeding, oxygen consumption depending on the temperature and size of the prawn increased 191 – 523 % from the pre-feeding level.

Apparent HI in *F. indicus* was dependent on size and water temperature and was found to range between 1.3 and 12.2 % of the ingested energy (Table 5.5). In all cases an increase in temperature significantly influenced the apparent HI in the species when reared at 15 ‰ ($F = 95.7$; $df = 2, 75$; $P < 0.0001$), 25 ‰ ($F = 58.5$; $df = 2, 75$; $P < 0.0001$) and 35 ‰ ($F = 70.5$; $df = 2, 75$; $P < 0.0001$). Apparent HI decreased slightly with an increase in live mass. In general, although not significant, AHI was lower in prawns fed 31 % CP.

Table 5.5: Mean percentages of apparent HI, with the standard error (s.e) in parenthesis, for *F. indicus* fed diets containing 21, 31 and 41 % CP reared in 20°, 25°, 35 °C and at 15, 25 and 35 ‰.

Diet Protein (%)	Salinity (‰)	Wet Mass (g)	Wet mass of food ingested (mg)	Apparent HI (%) at temperatures		
				20°C	25°C	30°C
21	15	6	39.86 (± 0.68)	1.80 (± 0.14) ^a	5.61 (± 0.34) ^b	10.54 (± 0.46) ^c
		9	56.87 (± 0.82)	1.78 (± 0.17) ^a	5.06 (± 0.32) ^b	10.38 (± 0.42) ^c
		14	97.38 (± 1.02)	1.72 (± 0.16) ^a	4.86 (± 0.22) ^b	10.18 (± 0.46) ^c
		Mean		1.77 (± 0.04)	5.18 (± 0.21)	10.37 (± 0.14)
	25	6	38.22 (± 0.76)	1.60 (± 0.07) ^a	4.82 (± 0.32) ^b	9.25 (± 0.43) ^c
		9	61.84 (± 1.08)	1.36 (± 0.48) ^a	4.86 (± 0.27) ^a	8.67 (± 0.43) ^c
		14	104.13 (± 1.45)	1.27 (± 0.11) ^a	3.49 (± 0.28) ^b	7.97 (± 0.32) ^c
		Mean		1.41 (± 0.14)	4.39 (± 0.30)	8.63 (± 0.27)
	35	6	36.39 (± 0.51)	1.67 (± 0.15) ^a	5.13 (± 0.32) ^b	12.19 (± 0.55) ^c
		9	58.70 (± 1.03)	1.54 (± 0.16) ^a	5.01 (± 0.32) ^b	9.71 (± 0.41) ^c
		14	101.56 (± 1.48)	1.49 (± 0.15) ^a	4.64 (± 0.22) ^b	9.24 (± 0.38) ^c
		Mean		1.57 (± 0.09)	4.93 (± 0.17)	10.38 (± 0.42)
31	15	6	37.96 (± 0.73)	1.82 (± 0.21) ^a	5.58 (± 0.26) ^b	10.75 (± 0.37) ^c
		9	58.44 (± 0.86)	1.86 (± 0.20) ^a	5.00 (± 0.28) ^b	11.08 (± 0.48) ^c
		14	98.67 (± 1.06)	1.64 (± 0.21) ^a	4.67 (± 0.28) ^b	9.98 (± 0.41) ^c
		Mean		1.77 (± 0.11)	5.08 (± 0.22)	10.60 (± 0.25)
	25	6	39.12 (± 0.89)	1.71 (± 0.21) ^a	4.79 (± 0.24) ^b	9.30 (± 0.34) ^c
		9	61.64 (± 1.02)	1.41 (± 0.17) ^a	4.58 (± 0.27) ^b	8.67 (± 0.42) ^c
		14	108.39 (± 1.39)	1.30 (± 0.19) ^a	3.41 (± 0.21) ^b	7.81 (± 0.42) ^c
		Mean		1.46 (± 0.15)	4.26 (± 0.28)	8.56 (± 0.25)
	35	6	37.70 (± 0.62)	1.80 (± 0.21) ^a	4.93 (± 0.25) ^b	10.17 (± 0.32) ^c
		9	57.89 (± 0.86)	1.57 (± 0.18) ^a	4.99 (± 0.28) ^b	9.21 (± 0.43) ^c
		14	108.34 (± 1.48)	1.38 (± 0.12) ^a	4.29 (± 0.27) ^b	8.16 (± 0.36) ^c
		Mean		1.58 (± 0.15)	4.74 (± 0.21)	9.18 (± 0.33)
41	15	6	38.00 (± 0.76)	1.81 (± 0.08) ^a	5.63 (± 0.31) ^b	10.98 (± 0.44) ^c
		9	59.88 (± 0.85)	1.91 (± 0.15) ^a	5.08 (± 0.23) ^b	10.37 (± 0.34) ^c
		14	96.21 (± 1.07)	1.78 (± 0.19) ^a	4.78 (± 0.33) ^b	10.02 (± 0.44) ^c
		Mean		1.83 (± 0.07)	5.16 (± 0.21)	10.46 (± 0.23)
	25	6	40.72 (± 0.80)	1.68 (± 0.07) ^a	4.73 (± 0.28) ^b	9.31 (± 0.41) ^c
		9	64.09 (± 1.10)	1.56 (± 0.14) ^a	4.75 (± 0.23) ^b	8.59 (± 0.31) ^c
		14	104.41 (± 1.32)	1.32 (± 0.17) ^a	3.64 (± 0.29) ^b	7.89 (± 0.39) ^c
		Mean		1.52 (± 0.14)	4.37 (± 0.27)	8.60 (± 0.28)
	35	6	39.62 (± 0.54)	1.75 (± 0.08) ^a	5.03 (± 0.31) ^b	9.71 (± 0.44) ^c
		9	62.23 (± 0.93)	1.52 (± 0.16) ^a	4.72 (± 0.23) ^b	9.64 (± 0.37) ^c
		14	105.11 (± 1.49)	1.46 (± 0.21) ^a	4.30 (± 0.32) ^b	7.86 (± 0.45) ^c
		Mean		1.58 (± 0.13)	4.68 (± 0.21)	9.07 (± 0.34)

^{a,b,c} Means in each row sharing the same letter are not significantly different based on Tukey test (P < 0.05).

Essentially apparent HI was unaffected by salinity and dietary concentrations and an overall mean and standard error for *F. indicus* at 20, 25 and 30 °C was calculated to be 1.61 (± 0.05), 4.75 (± 0.12) and 9.54 (± 0.30), respectively. This amounted to a three and two fold increase in apparent HI between 20 - 25 °C and 25 - 30 °C, respectively and a six fold increase at a temperature interval of 20 - 30 °C.

5.4. Discussion

5.4.1. The circadian rhythm.

The diurnal pattern of oxygen consumption was attributed to the nocturnal behaviour of the species. A similar rhythmic pattern was recorded by Bjanakiram, Venkateswarlu, Reddy & Babu (1985) for the species. Unlike Natarajan (1989) who found that the locomotory activity of adult *F. indicus* exhibited persistent circatidal rhythmicity, with peak activity coinciding with times of high water in their natural habitat, no such correlation was found in this study.

5.4.2. The influence of body mass on fasting metabolic rates.

On a unit mass basis, smaller prawns consumed more oxygen than their larger counterparts. A decline in oxygen demand per unit body mass with an increase in body size is attributed, in part, to the ontogenetic changes in the relative sizes of different body organs. As prawns grow, highly metabolically active tissues such as the gut and hepatopancreas, gradually represent a lower proportion of body mass while the relative size of the swimming musculature, of less metabolically active tissue, increases. Moreover other factors such as the moulting frequency, which increases the metabolic demand, decreases with age.

In this study mass specific metabolic rates for animals exposed to similar conditions were higher than those recorded by Subrahmanyam (1962), Kutty (1969) and Kutty *et al.* (1971) (Table 5.6). Discrepancies were considered to be due to the difference in experimental approaches used since the above-mentioned studies were conducted over a period of 4 - 6 hours during daytime. Moreover, oxygen readings by these authors were taken manually every hour and in several instances, depending on the nature of the experiment, until the prawn died. Dall (1986), Dall and Smith (1986) and Du Preez *et al.* (1986) stress that oxygen consumption must be monitored over a period that includes both light and dark regimes.

In this study a high demand for oxygen occurred during the late afternoon and early evening and provides an explanation as to why higher metabolic rates, as opposed those previously reported by Subrahmanyam (1962), Kutty (1969) and Kutty *et al.* (1971), were to be found for the same species. However, compared to *Penaeus monodon* and *P. stylirostris* the specific metabolic rate of *F. indicus* was lower for animals of equivalent mass exposed to similar conditions (Table 5.6).

Weymouth, Crimson, Hall, Belding and Field (1944) did a comparative study between different taxa and found a mean *b* value of 0.85 to be representative of decapod crustaceans. Values varying between 0.80 – 0.90 have been found for different species of prawns (Withers 1992). For tropical forms *b* is reported to be in the region of about 0.85 while temperate forms vary between 0.50 and 1.05 (Subrahmanyam, 1962). Although *b* is considered to be species specific (Weymouth *et al.*, 1944; Beamish 1964) Rao (1958) found that, depending on the experimental conditions, this value could vary considerably.

Table 5.6: Oxygen consumption rates recorded for various penaeid species

Species	Mean Mass (g)	Temperature (°C)	Salinity (‰)	Oxygen Consumption (O_2 mg.g ⁻¹ .h ⁻¹)	Reference
<i>F. indicus</i>	5.0	28.2	14.5	0.301	Subrahamayam (1962)
	10.0	28.2	14.5	0.207	
	14.0	28.2	14.5	0.200	
	2.7	30	35	0.700	Kutty (1969)
	0.1	25 – 28	7	1.060	Kutty <i>et al.</i> (1971)
	0.1	25 - 28	21	0.970	
	0.1	25 - 28	35	0.870	
	5.9	20	15	0.128	Present study
	9.1	20	15	0.112	
	15.3	20	15	0.104	
	5.8	20	25	0.104	
	8.8	20	25	0.089	
	15.4	20	25	0.082	
	5.8	20	35	0.112	
	8.4	20	35	0.101	
	15.4	20	35	0.088	
	6.8	25	15	0.146	
	9.4	25	15	0.140	
	15.6	25	15	0.124	
	6.3	25	25	0.117	
	9.0	25	25	0.115	
	14.2	25	25	0.095	
	6.0	25	35	0.131	
	9.1	25	35	0.125	
	15.6	25	35	0.111	
	5.4	30	15	0.456	
	8.9	30	15	0.420	
	14.3	30	15	0.384	
	5.5	30	25	0.229	
	8.2	30	25	0.224	
	14.5	30	25	0.196	
	5.3	30	35	0.257	
	8.0	30	35	0.247	
	14.6	30	35	0.221	
<i>Marsupenaeus japonicus</i>	0.3	20	10	0.600	Dall Via (1986)
	0.3	20	37	0.200	
	0.2	15	15	0.293	Chen & Lai (1993)
	0.3	15	20	0.240	
	0.3	15	25	0.195	
	0.2	15	30	0.205	
	0.3	25	15	0.402	
	0.3	25	20	0.363	
	0.2	25	25	0.326	
	0.2	25	30	0.319	
	0.2	35	15	0.658	
	0.2	35	20	0.732	
	0.2	35	25	0.757	
	0.2	35	30	0.753	

Table 5.6: continued

<i>P. monodon</i>	1.5 – 8.5	17.5	30	0.194	Ting (1970) cited in Chen & Lai (1993)
	1.5 – 8.5	20	30	0.256	
	1.5 – 8.5	25	30	0.349	
	1.5 – 8.5	30	30	0.600	
	1.5 – 8.5	20 - 25	10	0.421	
	1.5 – 8.5	20 - 25	20	0.333	
	1.5 – 8.5	20 - 25	30	0.317	
	1.5 – 8.5	20 - 25	40	0.294	
	1.6	27	15	0.326	Gaudy & Sloane (1981)
	0.9	27	22	0.195	
	1.9	27	35	0.376	
<i>P. stylirostris</i>	1.7	27	5	0.275	Gaudy & Sloane (1981)
	3.9	27	10	0.809	
	2.0	27	15	0.446	
	3.3	27	22	0.687	
	3.2	27	35	0.822	

In this study *b* was influenced by an increase in temperature and was found to vary between 0.773 – 0.911 (Table 5.2). These values were considerably higher than 0.604 derived for 0.61 - 14.63 g *F. indicus* exposed to 14 ‰ and 28° – 29 °C by Subrahmanyam (1962) and 0.501 recorded for 0.1 g *F. indicus* acclimated to 28° – 29 °C and 7, 21, 35 ‰ by Kutty (1971). However, if once off measurements by Subrahmanyam (1962) were to be excluded *b* becomes 0.75 which is closer to those found in the present study under similar conditions. Furthermore, if Subrahmanyam (1962) had taken measurements over 24 hours, instead of six, the resultant slope would have been expected to fall within the range obtained in the present study. If the mean oxygen consumption of adult *F. indicus* used by Janakiram *et al.* (1985), is included the slopes then fall between 0.76 and 0.84.

5.4.3. Fasting metabolic rates.

During the first 8 - 14 hr of fasting, tissue reserves of glycogen and lipids were found to be important energy substrates in *P. esculentus* with the catabolism of body protein becoming increasingly important thereafter (Hewitt & Irving, 1990). Similarly body substrates in the order of carbohydrate, lipid and finally protein were utilised for energy in starved *Marsupenaeus japonicus* (*P. japonicus*) (Cuzon *et al.*, 1980). Since the nutritional state of an organism influences oxygen consumption (Du Preez *et al.*, 1986) and due to the absence of ammonia excretion, body composition and growth data it was assumed that slightly higher metabolic rates was a result of the rapid depletion of carbohydrates and lipid reserves in fasting prawns previously fed 21 % CP as opposed to those given 31 and 41 % CP. A higher metabolic rate was assumed to be an indication that the animal had to resort to using body protein as a source of energy with an increase in demand attributed to oxidative processes required for amino acid catabolism.

5.4.4. Effect of salinity on oxygen consumption

Salinity is one of the most important factors affecting the growth and survival of penaeids in natural nursery areas where rapid changes in environmental conditions occur (Parado-Esteva, Ferraris, Ladjá & De Jesus, 1987). When exposed to salinity extremes prawns are forced to expend considerable amounts of energy for osmoregulation at the expense of the other processes, such as growth (Bureau *et al.*, 2000; Villarreal *et al.*, 2003). Vijayan and Diwan (1995) found that the shortest moult cycle of ~ 96 and 98 hours occurred at 15 and 25 ‰, respectively with the fastest moult and growth rate occurring at 15 ‰. At 5, 35 and 45 ‰ *F. indicus* experienced extended periods of moulting.

Although salinity influenced oxygen consumption in the prawn *Palaemon serratus* and *Farfantepenaeus californiensis* (*P. californiensis*) (Villarreal *et al.*, 2003) a range of 15 – 35 ‰ had no significant effect on metabolic rates in 5 - 15 g *F. indicus* in this study. Moreover, results were in agreement with those of Subrahmanyam (1962) and Kutty *et al.* (1971). These findings were attributed to *F. indicus* of 5 - 10 g being effective osmoregulators able to handle abrupt fluctuations in external salinities of 3.9 – 40.7 ‰ (Shylaja & Rengarajan, 1993).

Tolerance towards salinity changes is due to the tissue cells of *F. indicus* being permeable to the hemolymph medium, which buffers cells from sudden and prolonged changes in salinity (Shylaja & Rengarajan, 1993). A permeable membrane signifies lower metabolic cost since energy in the form of ATP is not required for the active transport of the medium across cell walls in order to osmoregulate. However, although no significant osmoregulation costs were to be found, prawns at 25 ‰ displayed slightly lower metabolic rates for all temperatures. A possible explanation was that at 25 ‰ the ion concentration in the blood of the prawn was similar to that of the surrounding medium and that the elimination of solutes by excretory organs, however slight, was not necessary.

The metabolism of 0.1 g *F. indicus* acclimated to 7, 21 and 35 ‰ was independent of salinity and was shown to have a lower respiration in a natural range of 10 – 15 ‰ (Kutty *et al.*, 1971). Unlike Bjanakiram *et al.*, (1985) where diurnal rhythms in the oxygen consumption of 20 g adults and larger exposed to 10 – 30 ‰ induced slight phase shifts of less than 1 h, a similar phenomenon was not noted in the present study. The absence of phase shifts was a further indication that 5 -15 g *F. indicus* were tolerant of salinity changes and, unlike adults, juvenile *F. indicus* are capable of withstanding a range of salinities since the marked euryhaline habits of the prawn in the wild alone suggest the species homo-osmotic nature.

Dall (1981) mentions that euryhalinity may gradually diminish over time in generations of cultured prawns reared under stable conditions for long periods. A prime example is that due to seasonal monsoons cultured *F. indicus* in India are tolerant of low salinities than the same species originating from the Red Sea, providing the rationale for the choice of test salinities by Subrahmanyam (1962), Kutty (1969) and Kutty *et al.* (1971). In contrast *F.*

indicus derived from the Red Sea are able to tolerate salinities slightly in excess of 50 ‰ than their Indian counterparts (Kumlu & Jones, 1995). Hence in growout ponds on local prawn farms generations of cultured *F. indicus* exposed to 18 - 27 ‰ may provide an explanation for the slightly lower oxygen consumption noted at 25 ‰ as opposed to 15 ‰ reported by Vijayan and Diwan (1995). Although salinity did not influence oxygen consumption future investigations should consider examining the effect salinity has on the growth and mortality as done for *F. californiensis* by Villarreal *et al.* (2003).

5.4.5. The influence of temperature on the fasting metabolic rate

Temperature, as with other penaeid species (Dalla Via, 1986; Liao & Murai, 1986) was found to have a greater influence on respiration than salinity. In the present study a change in temperature affected the respiration of juvenile *F. indicus* substantially (Table 5.4). However, data used to obtain equation [5.5], [5.6] and [5.7] once plotted on a log-log scale and compared for significant differences ($F = 0.605$; $df = 2, 237$; $P > 0.05$) provided a pooled value of:

$$b = 0.816 \quad [5.8]$$

compared to 0.815 and 0.863 for *Penaeus esculentus* (Dall, 1986) and *P. monodon* (Liao & Murai, 1986), respectively. Differences in elevation proved to be significant ($F = 641.633$; $df = 2, 239$; $P > 0.001$), however, the intercept a in equation [5.5], [5.6] and [5.7] are directly related to water temperature (t°) and a regression of a on temperature provided a straight line:

$$a = 0.0147 \times (t^\circ) - 0.1082 \quad (r^2 = 0.964; n = 3) \quad [5.9]$$

Thus, by incorporating the b value in [5.8] and equation [5.9] into equation [5.1], the oxygen consumption (O_2 mg. prawn⁻¹. hour⁻¹) of 5 – 15 g *F. indicus* reared at 20 – 30 °C and 15 – 35 ‰ can be derived using the following:

$$QO_2 = [-0.1082 + 0.0147(t^\circ)] \times M^{0.815} \quad [5.10]$$

5.4.6. The influence of AHI on the fasting metabolic rate

In this study the AHI was largely dependent on an increase in water temperature and body mass and to a lesser extent on salinity and dietary concentrations. AHI is brought about by the excited voluntary muscular action and other related activities such the added mechanics of digestion, metabolism of the carbon skeleton of amino acids, synthesising nitrogenous waste products and the process whereby waste products are concentrated and excreted. Furthermore, after having been fed the nutrients absorbed from body tissues while fasting are replaced and the

efficiency whereby metabolisable energy is utilised is proportional to the ATP yield per unit of enthalpy of combustion of the nutrients absorbed (Blaxter, 1989; Bureau *et al.*, 2000).

Large variation of AHI estimates was attributed to a difference in feeding behaviour of individual prawns as well as a possible over estimation of the energy ingested. Once fed, elevated levels of oxygen consumption for prolonged periods up to 6 hours, depending on water temperature, was a response by the species to actively swim in search of more food. However, in some cases post-feeding levels fell below those of pre-feeding (Figure 5.7). In these instances it was speculated that prawns, once satiated, no longer desired to seek food, as was thought to be the reason for the gradual increase in oxygen demand 24 hours earlier. Moreover, animals at 20 °C did not only have a lower metabolic rate but were also found to have a lower food intake as opposed to similar sized prawns at 25 and 30 °C. Even though the AHI was generally lower amongst animals fed 31 % CP, no significant differences ($P > 0.05$) between animals of similar mass were found.

Hewitt and Irving (1990) found a link between the AHI and nitrogenous production. In *P. esculentus* glycogen and lipid were catabolised during the first 16 hours after feeding with an increase in the excretion of NH_3 being due to the utilisation of protein as the primary energy substrate. In the absence of data pertaining to ammonia excretion the same was thought to occur in *F. indicus*. Although carbohydrates represents a minor source of stored energy in prawns a study by Ahamad (1982; 2001) revealed that *F. indicus* was capable of handling substantial levels of dietary carbohydrate ($> 40\%$), as opposed to lipids, for any given level of protein. In accordance with Ahamad (1982; 2001) finding's the present results suggest, especially amongst 5 g animals, that the decrease in AHI for diets low in protein was due to carbohydrates requiring less oxygen to produce a given amount of energy than protein and lipids (Brody, 1945).

Diets formulated to have an optimal ratio of lipid, carbohydrate and protein were found to reduce protein metabolism and oxygen consumption in *P. esculentus* (Hewitt & Irving, 1990). Hence a lower demand by prawns fed 31 % protein was possibly an indication that the dietary components were metabolised more efficiently than those fed 21 and 41 % CP. A diet with 31 % CP may prove to be the optimum level for the species or that the protein:energy of the diet may be the ideal ratio for *F. indicus*. Results suggest that a dietary level other than 31 % CP will require a higher oxygen demand in order to facilitate oxidative processes.

In a study by Rosas and Sanchez (1996) the AHI and post-prandial nitrogen excretion (PPNE), measured in postlarval (PL 25 – 30) *Fenneropenaeus setiferus* (*P. setiferus*), *Farfantepenaeus duorarum* (*P. duorarum*) *Penaeus schmitti* and *P. notialis* fed a fixed ration of 3 mg/animal using purified diets with 40, 50, 60 or 65 % protein, increased with an increase in dietary protein. In this study protein concentration failed to influence oxygen demands significantly and no clear trend in relation with the calorogenic effect were found. However, results did show that a portion of the ingested energy, up to ~ 13 %, was lost as AHI. A fasting

period of 38 hours, in the present study, may be insufficient to establish significant differences or trends between AHI and HE_i , since Kutty (1969) found that *F. indicus* was able to survive for six days without food. Possibly a more detailed approach such as that done by Rosas *et al.* (2001), combined with a method proposed by Bureau *et al.*, (2000), might reveal differently since Rosas *et al.* (2001), in conjunction with calorimetric readings, measured growth rates, PPNE, haemolymph and digestive gland carbohydrates and osmotic pressure in order to establish the heat increment (HE_i).

Bureau *et al.* (2000), on the other hand, suggests that the animal should be fed intensively, as done under culture conditions, while continually measuring oxygen levels and then starved for a period no less than 2 – 4 days. Their reason was that a longer starvation period would prove sufficient for the complete depletion of readily available body reserves and, in turn, provide true estimates of the animal's fasting metabolism. HE_i is calculated as the energy required for maintenance, analogous to the fasting metabolic rate (HE_r), and that required for the retention of dietary nutrients (RE). The means as to how this is done is presented in the next Chapter since this approach proved to have merit when calculating energy requirements.

Moreover, in order to establish total metabolic costs, energy losses associated with moulting (HE_m) need to be established. Although HE_m values for some crustaceans have been estimated by several investigators (D'Ambro *et al.*, 1997) further studies that examine the energy expended while moulting will prove to be of immense benefit.

5.5. Conclusion

The effects of water temperature and salinity on the oxygen consumption of the prawn, *F. indicus* were measured. The oxygen consumption of fasting prawns was influenced significantly by temperature and body mass, whereas salinity 15 – 35 ‰ had no measurable effect on the oxygen consumption. The relationship between oxygen consumption (QO_2) and body mass (M) was expressed by $QO_2 = aM^b$ in which, a and b , signify the level of metabolism and the mass exponent, respectively. Oxygen consumption (O_2 mg. prawn⁻¹. hour⁻¹) of fasting *F. indicus* as a function of live mass at 20, 25, 30 °C and 15, 25, 35 ‰ was determined based on $QO_2 = [-0.1082 + 0.0147(t^\circ)] \times M^{0.815}$. More importantly the oxygen consumption of fasting prawns together with a conversion rate of 20 J/ℓ O_2 respired, provides a means to calculate the metabolic costs of the species at the above mentioned conditions.

Although dietary protein levels of 21 – 41 % did not significantly influence HE_i levels, results did show that a considerable portion of the ingested energy, ~ 13 %, was required for oxidative processes. When formulating diets energy losses due to HE_i costs should be taken into account to ensure that the energy requirements of the species for grown and maintenance are met on a daily basis.

6.

Effective Energy System**Establishing a Bioenergetic model to predict energy requirement and feed intake for *Fenneropenaeus indicus*.****6.1. Introduction.**

Information pertaining to the utilisation of energy is essential for formulating cost-effective diets since the provision thereof must be sufficient to allow protein to be used exclusively for maintenance and growth. The problem, however, is to quantify this supply since immature animals are rarely in a state of equilibrium. Maintenance energy (HE_m) is defined as the energy required for the stable retention of body protein and lipid so that a zero energy balance or a zero energy gain is maintained (Emmans, 1994; Bureau *et al.*, 2000). Emmans and Fisher (1986) proposed that the energy required for maintenance (HE_m) be calculated using equations [3.18] and/or [3.19].

However, in terms of growth the energy partitioning or budget for a growing animal, suggested by the U.S. National Research Council (NRC, 1981), is expressed simplistically as:

$$IE = FE + HE + WE + RE \quad [6.1]$$

where

- IE = Ingested dietary energy
- FE = Energy lost in faeces
- HE = Energy required for heat production
- WE = Energy lost due to catabolic waste
- RE = Recovery, retained or energy required for growth

Portion of dietary energy (IE) is lost in the faeces (FE) and some in the excretion of nitrogenous waste (WE). The energy available for metabolism, generally referred to as metabolisable energy (ME), is utilised for total heat production (HE), a result of metabolic and behavioural activities, and growth or tissue recovery (RE) (Cho, Slinger & Bayley, 1982; De Silva & Anderson, 1997; Cuzon & Guillaume, 1997). A measure of ME is important since it is the available energy in a diet that is not lost in the organic matter of faeces (FE), or as nitrogenous metabolites excreted in urine (UE) and/or as ammonia from the gills of aquatic animals (ZE). The available ME is defined as (Cuzon & Guillaume, 1997):

$$ME = IE - (FE + UE + ZE) \quad [6.2]$$

Since FE includes the energy from undigested food and from metabolic products *viz.*, sloughed gut epithelial cells, digestive enzymes and products of bacterial origin within the gut, the apparent digestibility energy (ADE) of the feed is slightly lower than true digestible energy (TDE) such that (Cuzon & Guillaum, 1997):

$$\text{ADE} = \text{IE} - \text{FE} \quad [6.3]$$

It is generally assumed that the ADE is absorbed completely by the animal and that ME is the balance between the ADE and the energy lost as WE where $(\text{UE} + \text{ZE}) = \text{WE}$. Hence, equation [6.3] rewritten becomes (Cuzon & Guillaum, 1997):

$$\text{ME} = \text{ADE} - \text{WE} \quad [6.4]$$

Conversely in the absence of empirically determined values of FE and WE the available ME can be determined if the apparent digestible coefficient (ADC) for the macronutrients for a given diet is known. The result is that the ME can be quantified as follows (Cuzon & Guillaum, 1997):

$$\text{ME} = 21.3 \text{ Pd} + 39.5 \text{ Ld} + 17.2 \text{ Cd (kJ)} \quad [6.5]$$

where

Pd = dietary protein \times ADC

Ld = dietary lipid \times ADC

Cd = dietary carbohydrate \times ADC

Since it is only the organic portion in the diet that yields energy on combustion, coefficients 21.3, 39.5 and 17.2 kJ/g, termed physiological fuel values, are routinely used for protein, lipid and carbohydrate respectively. Cuzon and Guillaume (1997) take it a step further by suggesting that if dietary carbohydrates are composed primarily of monosaccharides or disaccharides the coefficient 17.2 kJ/g should be replaced with 15.5 and 16.7 kJ/g, respectively.

A problem, however, is that energy demands change with age and unless different ADC's at various developmental stages have been determined the ME, for a given diet, remains fixed and as a consequence the energy requirements necessary for growth may not be realised. In addition Emmans (1984) and Oldman and Emmans (1990) showed that a measure of metabolizable energy in feedstuff is not an accurate means of describing energy yields since the dietary effect of the indigestible organic portion thereof has not been taken into account.

A solution proposed by Emmans (1984; 1994) and Emmans and Fisher (1986), known as the effective energy system, accounts for these deficiencies. In short the effective energy system can be

described as the metabolizable energy (ME) of a diet less the heat produced due to defecation and excretion resulting from it being eaten. The following section describes and discusses the effective energy system.

6.2. A systems approach for predicting the energy requirement and food intake based on the Effective Energy (EE) system.

6.2.1. A means of determining effective energy.

Theoretical and experimental evidence suggests that energy losses associated with the transformation and accretion of dietary nutrients represent a very large proportion of growth costs. Thus, a measure of total energy retained has been used to establish feed requirement models that describe and predict the deposition and growth of body substrates in various livestock and fish species (Emman & Fisher, 1986; Cho, 1992; Emmans 1994; Cho & Bureau, 1998; Cui & Xie, 1999). Based on the principle of the conservation of energy, dietary yields of ME will either be retained in the animal or be lost as heat. By combining equations [6.1], [6.2] and [6.3] ME is determined as (Emman & Fisher, 1986):

$$ME = RE + HE \quad (\text{kJ/day}) \quad [6.6]$$

where

RE = retained or energy gained (kJ/day)
HE = total heat produced (kJ/day)

All the energy retained by an animal can be considered to be in the form of the potential energy of body substrates protein and/or lipid with carbohydrate, due to such small amounts, being ignored (Emman & Fisher, 1986; Emmans, 1994). It follows that (Emman & Fisher, 1986; Emmans, 1994):

$$RE = h_p \cdot PR + h_l \cdot LR \quad (\text{kJ/day}) \quad [6.7]$$

where

PR = protein retained (dP/dt) (g/day)
LR = lipid retained (dL/dt) (g/day)

h_p and h_l are the physiological fuel coefficient of 23.8 and 39.6 (kJ/g) for protein and lipid, respectively (Emman & Fisher, 1986). However, given the performance of the animal in a given state where the values of PR and LR are known, the problem of predicting an animal's ME requirement is therefore the problem of predicting its rate of heat production, HE. The components describing the total energy lost due to heat production is as follows (De Silva & Anderson, 1997):

$$HE = HE_e + HE_j + HE_c + HE_w + HE_i \quad (\text{kJ/day}) \quad [6.8]$$

Where HE_e is the minimum heat produced due to the conduct of basal or fasting metabolism necessary to maintain life (*i.e.* cellular activity, respiration and blood circulation) and HE_j is the heat generated by minor bodily and voluntary muscular movements associated with involuntary or resting activities. In fasting animals, once the small reserves of carbohydrate have been depleted, the energy is derived from the catabolism of body protein and lipid (Emmans, 1994).

In the case of homeotherms the heat produced as a result of an animal's efforts to maintain a constant body temperature, when the environment goes above the zone of thermal neutrality, is known as heat of thermal regulation (HE_c) but the definition of basal/fasting metabolic rate for crustaceans precludes the latter condition making it necessary to specify the temperature at which the metabolic rate is measured (De Silva & Anderson, 1997). Thus, in keeping with Chapter 7 the definition of fasting metabolism HE_f for poikilotherms can be regarded as:

$$HE_f = HE_e + HE_j \quad (\text{kJ}) \quad [6.9]$$

On the other hand unassimilated energy associated with the synthesis and excretion of waste products is known as HE_w . In crustaceans 86 to 95 % of nitrogenous waste is excreted as ammonia (Claybrook, 1983). The excretion of ammonia through the gills requires no energy while the conversion of ammonia to urea, < 5 % (Claybrook, 1983), is highly unlikely. Hence losses associated with HE_w in aquatic animals are generally considered to be minimal if not negligible (De Silva & Anderson, 1997).

Heat increment (HE_i) is a measure of the increase in heat produced as a result of the metabolic processes associated with the digestion of food and the subsequent molecular transformation and assimilation of nutrients in the hepatopancreas of the prawn and elsewhere. Hence following [6.6], total heat production (HE) in a prawn can simply be written as (Bureau *et al.*, 2000):

$$HE = HE_f + HE_i \quad (\text{kJ/day}) \quad [6.10]$$

where

$$\begin{aligned} HE_f &= \text{fasting heat production} \quad (\text{kJ/day}) \\ HE_i &= \text{heat increment} \quad (\text{kJ/day}) \end{aligned}$$

By substituting equations [6.7] and [6.10] into [6.6] ME becomes:

$$ME = HE_f + h_p \cdot PR + h_l \cdot LR + HE_i \quad (\text{kJ/day}) \quad [6.11]$$

With all the parameters in equation [6.8] being quantified the problem arises in having to predict the HE_i for different diets. However, in order to do this the energy required for the metabolic processes for digestion, transformation and assimilation of food needs to be calculated. In ureotelic animals some portion of HE_f , resulting from the synthesis and excretion of nitrogen-containing compounds, is lost through work done excreting urinary nitrogen (UN_f) (Emmans, 1994).

$$ME = HE_f - w_u \times UN_f \quad (\text{kJ/day}) \quad [6.12]$$

where w_u = work done in excreting urinary nitrogen (kJ/g)
 UN = fasting urinary nitrogen (g/day)

Thus, to determine HE_i in given animals and for given diets Emmans (1988) proposed the following:

$$HE_i = w_u \times (UN - UN_f) + w_d \times FOM + w_p \times PR + w_l \times LR \quad [6.13]$$

where w_d = work done in defecating (kJ/g)
 w_p = work done in protein deposition (kJ/g)
 w_l = work done in fat deposition (kJ/g)
 FOM = faecal undigested organic matter (g/day)
 UN = urinary nitrogen (g/day)
 with $UN = 0.16 \times (DCP - PR)$

Substituting equations [6.10] and [6.11] into [6.9] ME can therefore be calculated as:

$$ME = w_d \times FOM + w_u \times 0.16 \times DCP + MH + PR \times (h_p + w_p - 0.16 \times w_u) + LR \times (h_l + w_l) \quad (\text{kJ/day}) \quad [6.14]$$

where DCP = digestible crude protein (g/day)

Studies have examined actual nutrient deposition in a range of livestock animals with the most popular approach being factorial. In this approach, first proposed by Kielanowski (1965), the energy cost of lipid and protein deposited is simply defined as the ME required to promote a defined increment in body protein or lipid. The partial efficiency of ME utilised for the growth in live mass of the whole body (k_p) and the retention of protein (PR), and lipid (LR) is the ratio of net energy retained in relation to the ME consumed *i.e.* RE/ME intake, such that (Bureau *et al.*, 2000):

$$ME = HE_m + (w_p \div PR) + (w_l \div LR) \quad (\text{kJ}) \quad [6.15]$$

Using this approach, Emmans (1994) concluded that the net energy cost for the retention of protein was 2.54 kJ per kJ of protein retained (1.54 kJ of heat expended for each 1 kJ of protein deposited) and that the energy required for the retention of protein (w_p) was calculated to be about 36.6 (kJ/g). According to Emmans (1994), when all related factors have been taken into account, energy costs for the deposition of protein do not appear to vary significantly between and across different species of cattle, sheep, pigs and poultry.

With regard to body fat the energy from lipid and non-lipid substrates was calculated to be 1.4 kJ and 1.1 kJ per kJ lipid deposited, respectively *i.e.* heat losses of 0.4 or 0.1 kJ per each 1 kJ lipid retained or alternatively ~ 15.84 and 13.96 kJ per gram retained (Bureau, Azevedo, Tapia-Salazar & Cuzon, 2000). Coefficients or heat increments for energy required for the excretion of urinary nitrogen (w_u), the retention of protein (w_p) and lipid (w_l) are 29.2, 36.5 and 16.4 kJ/g, respectively. Essentially these variables were found to be similar for single-stomach and ruminant animals (Emmans, 1994). More importantly the efficiency and energy costs for the retention of substrates in higher vertebrates are considered, with some minor exceptions, to be similar in prawns (Bureau *et al.*, 2000).

As it is common practice to include a certain amount of fat in the diet, allowance has to be made for dietary fat being used for lipid deposition. Fat is deposited more efficiently if the source of energy is from dietary lipid rather than protein or carbohydrate. A proportion (z) of the dietary lipid (D_{CL}) is retained with the remaining lipid retention ($LR - z \times D_{CL}$) arising from other sources. The variable (z) varies between zero, when no lipid is deposited, and one, when all dietary lipid is deposited as body fat. The value will vary according to diet composition and the state of the animal. Total heat production due to lipid deposition (H_{LR}) is thus (Emmans, 1988):

$$H_{LR} = w_l \times LR - (z \times D_{CL}) \times (w_l - w_f) \quad (\text{kJ/day}) \quad [6.16]$$

where

z	=	proportion of dietary fat	
D_{CL}	=	digestible crude lipid	(g/kg)
w_l	=	work done in depositing fat from dietary protein and carbohydrate sources	(kJ/g)
w_f	=	work done in depositing fat from dietary lipid	(kJ/g)

The implication is that more energy is available for other processes and as such the ME in the feed will increase proportionally to the amount of dietary fat by a value of $(k \times D_{CL}) \times (w_l - w_f)$. The heat produced by the catabolism of lipid is equivalent to the heat of its combustion whereas protein catabolism due to energy being lost in the form of nitrogenous excretory products, principally urea, is slightly less. This loss is calculated as $(h_p - a)$ where parameter “ a ” refers to the

urinary loss of about 5.44 kJ/g of protein catabolized. This corresponds to a combustion equivalent to approximately 3.54 kJ/g of protein plus the energy cost of urea biosynthesis amounting to approximately 1.46 kJ/g of protein, which in turn provides a total of 5.00 kJ/g. The difference of 0.44 kJ/g is due to nitrogenous waste, uric acid and/or creatine which constitutes ~ 10 % of the total urinary nitrogen in mammals and which has a higher calorific value than urea (Brett & Groves, 1979).

A solution to correct for nitrogen losses is to deduct the urinary energy, assuming all of the digested protein has been catabolized, from both the diet and the protein retained resulting in (Emmans & Fisher, 1986):

$$ME_n = ME - a \times D_{CP} \quad (\text{kJ/day}) \quad [6.17]$$

$$\begin{aligned} \text{where } ME_n &= \text{ME corrected for zero nitrogen retention} && (\text{kJ/g}) \\ a &= \text{heat of combustion of urine calculated to be 5.44} && \text{kJ/g.} \\ D_{CP} &= \text{digestible crude protein} \end{aligned}$$

Hence, the EE content of a feed, EEC, is determined by combining the first two terms of equation [6.14] and equation [6.17] to give:

$$EEC = ME_n - (0.16 \times w_u \times D_{CP}) - (w_d \times \text{FOM}) + z \times D_{CL} \times (w_l - w_f) \quad (\text{kJ/g}) \quad [6.18]$$

Substituting coefficient values into [6.18] the effective energy of an ingredient and/or a diet can be established using the following (Emmans, 1994):

$$EEC = ME_n - (4.67 \times D_{CP}) - (3.8 \times \text{FOM}) + (12 \times z \times D_{CL}) \quad (\text{kJ/g}) \quad [6.19]$$

In monogastric animals the EEC in the feed can be estimated from ME and digestible values of crude protein and organic matter. The heat increment of forming lipid from lipid and non-lipid, 12 kJ/g (Emmans, 1994), needs to be weighed by z , which, as explained above is the proportion of dietary lipid retained as body lipid (Emmans, 1994). Emmans (1994) established suitable values of z , which on average were found to be 1.0 for pigs and 0.3 for poultry. In crustaceans z is assumed to be 0.3, since prawns in general have a limited capacity to store lipid. With regard to prawns, since less than 5 % of the digested protein is converted into urea, the DCP portion of the diet should be multiplied by a factor of 0.05. Hence, the EEC for prawns using equation [6.19] can be calculated as:

$$EEC = ME_n - (0.23 \times D_{CP}) - (3.8 \times FOM) + (3.6 \times D_{CL}) \quad (\text{kJ/g}) \quad [6.20]$$

Equation [6.20] indicates that, in sexually immature *F. indicus*, all the assimilated energy not utilised as metabolic fuel, will be diverted towards the synthesis of body tissue.

6.2.2. Determination of the Effective Energy Requirements.

The energy system described so far predicts the dietary energy required for maintenance, growth and to a limited extent, fattening. As a corollary to this, the daily energy requirements for a growing animal, with defined growth characteristics, can be determined. The function describing the effective energy required (EERQ) for maintenance and growth consists of those variables in equation [6.14] that are absent in [6.18] such that for livestock Emmans (1988) found that:

$$EERQ = HE_m + [(h_p - a) + (w_p - 0.16 \times w_u)] PR + (h_l + w_l) LR \quad (\text{kJ/g}) \quad [6.21]$$

where	$h_p = 23.8 \text{ (kJ/g)}$
	$a = 5.63 \text{ (kJ/g)}$
	$w_p = 36.5 \text{ (kJ/g)}$
	$w_u = 29.2 \text{ (kJ/g)}$
	$h_l = 39.6 \text{ (kJ/g)}$
	$w_l = 16.4 \text{ (kJ/g)}$

By comparison in crustaceans exceptions, for a defined set of environmental conditions, are that the HE_m should be considered to be equivalent to HE_f and the h_p value of 23.8 should be replaced by 21.3 (kJ/g). In addition, Ahamad (1994) found the faecal nitrogen in *F. indicus* to be 3.24 mg N/g diet consumed. Multiplied by a factor of 6.25, to convert nitrogen into protein, and by 23.8 (kJ/g), protein losses incurred in the gut are calculated to be 0.48 kJ/g. With these exceptions in mind, equation [6.21] becomes:

$$EERQ = HE_f + \{(21.3 - 5.63) + [36.5 - 0.16 \times (29.2)] - 0.48\} PR + (39.6 + 16.4) LR + (17.2 \times 0.4) CR \quad (\text{kJ/d})$$

Rewritten the EERQ for *F. indicus* is:

$$\therefore EERQ = HE_f + 47 \times (PR) + 56 \times (LR) + 17 \times (CR) \quad (\text{kJ/d})$$

[6.22.]

6.2.3. Predicting feed intake.

The EE system considers both the requirement of an animal and the energy content of the diet, such that the desired feed intake (DFI), defined as the rate at which an animal seeks to eat (Emmans 1987), was established as:

$$\text{DFI} = \text{EER} \div \text{EEC} \text{ (mg/day)} \quad [6.23]$$

Should energy not be the first limiting resource and the DFI for the limiting nutrient is attained then excess energy ingested will be converted to body fat. The problem of determining the energy required for maintenance in animals that have a higher lipid content than 'desired' is overcome by assuming that the animal would make use of these lipid reserves if it were in a position to do so (Gous, 1991). An example would be where a fat animal was given a feed with a high nutrient to energy ratio.

6.3. *Application of various bioenergetics models to determine feed rations based on energy requirements.*

Since the energy balance in an animal is influenced by a change in temperature, mass and diet, daily requirement is generally governed by the amount of nutrients (*e.g.* protein, lipid and/or energy) the animal is able to assimilate. Following are two methods used to predict the food requirement based on the energy requirement of prawns. The first approach is an existing model, developed by Cho and Bureau (2000) (*cited in Bureau et al., 2000*) that predicts feed intake based on the energy requirements in *Litopenaeus vannamei* (Appendix F), while the second utilises the effective energy system described above.

For reason of comparison the bio-energetic model of Cho and Bureau (2000) (Appendix F) was adapted using information pertinent to *F. indicus* and compared to results obtained based on the effective energy system.

6.3.1. *Establishing the feed intake for F. indicus based in principle on the model proposed by Bureau et al. (2000).*

The following was established using observed data whereby the feed intake of *F. indicus* fed a diet (Table 6.1) was determined based on the approach used by Bureau *et al.* (2000). Energy costs for maintenance, feeding, moulting, non-faecal and exuviae were assumed to be similar for *F. indicus*.

Step 1.: DIET selection: defining feed composition:

Table 6.1: The ingredients of a compound diet used by the industry.

Ingredient	Percentage composition	Amino Acids		
		Essential	Percentage composition	Amino acids to Lysine ratios
Fish meal ^a	26.30	Arginine	2.59	85
Soybean meal ^b	30.00	Histidine	0.88	29
Industrial wheat ^c	27.20	Isoleucine	1.95	64
Wheat gluten ^d	2.50	Leucine	3.11	103
Brewer's Yeast Dehydrated ^e	5.00	Lysine	3.03	100
Soya Lecithin (Crude Oil) ^f	2.00	Phenylalanine	1.51	50
Fish oil ^g	2.00	Methionine	1.77	58
Cholesterol (33% Pure) ^h	0.20	Threonine	1.51	50
Vitamin mixture: ⁱ	0.40	Valine	2.38	79
Vitamin C (Roche Stay-C)	0.40	Tyrosine	0.93	50
Mono calcium phosphate ^j	2.00	Non-essential		
Mineral mixture ^k	0.50			
Lysine ^l	0.50	Aspartic	3.77	124
Methionine ^m	1.00	Serine	1.95	64
Total	100.00	Glutamic	6.95	229
Nutrients on a dry matter basis.		Proline	2.29	76
GE (MJ/kg)	17.75	Glycine	2.09	69
Protein (%)	41.22	Alanine	2.39	79
Lipid (%)	5.95			
Fibre (%)	4.12			
Ash (%)	6.45			
Total	100.00			

With an ADC for protein, lipid and carbohydrate calculated to be 81, 92 and 38 %, respectively (as determined in a previous chapter) dietary ME was calculated as:

$$ME = 21.3 Pd + 39.5 Ld + 17.2 Cd$$

$$ME = 21.3 (41.22 \times 0.81) + 39.5 (6.45 \times 0.92) + 17.2 (40.65 \times 0.38)$$

$$ME = (7.11) + (2.34) + (2.66)$$

$$ME = 12.11 \text{ (kJ/g)}$$

Step 2.: GROWTH prediction:

From Table 4.5 information pertaining to the period of between 98 - 111 days for a mixed batch of prawns was:

Initial mass:	9.69 g
Final mass:	12.20 g
Average water temperature:	24 °C

Growth period:	13 days
Gain in live mass per day:	0.19 g. prawn ⁻¹
Gain in live mass per week:	1.35 g. prawn ⁻¹

Step 3.: ENERGY GAIN (RE) predicted:

Unlike Bureau *et al.* (2000) the energy retained (RE) was established based on independent values of body protein, lipid and carbohydrate content. From Table 3.10 the predicted energy gained (RE), based on values relevant to the period 98 - 111 days, was calculated such that:

$$\begin{aligned} \text{protein} \quad \text{retention per day} &= [(2.406 - 1.365)] \div 13 \\ \text{lipid} \quad \text{retention per day} &= [(0.075 - 0.048)] \div 13 \\ \text{carbohydrate} \quad \text{retention per day} &= [(0.104 - 0.073)] \div 13 \end{aligned}$$

$$\begin{aligned} \text{RE} &= 21.3 (0.080) + 39.6 (0.002) + 17.2 (0.002) = 1.82 \text{ kJ prawn}^{-1} \text{ day}^{-1} \\ &= 12.72 \text{ kJ prawn}^{-1} \text{ week}^{-1} \end{aligned}$$

Step 4.: WASTE estimation:

Predicting the energy required for maintenance:

The average water temperatures (t°) in ponds over this period was 24 °C and using the following $\text{HE}_f = \{[-0.1082 + 0.0147(t^\circ)] \times M^{0.815}\}$ and a conversion of 20 J. mg⁻¹ O₂ consumed the fasting metabolic rate was calculated to be:

for 9.69 g:

$$\begin{aligned} \text{HE}_f &= 20 \times \{[-0.1082 + 0.0147(24)] \times 9.69^{0.815}\} = 0.03 \text{ kJ. hour}^{-1} \\ \text{HE}_f &= 0.03 \text{ kJ. hour}^{-1} \times 24 \text{ hours} = 0.75 \text{ kJ. day}^{-1} \end{aligned}$$

and for 11.04 g:

$$\begin{aligned} \text{HE}_f &= 20 \times \{[-0.1082 + 0.0147(24)] \times 11.04^{0.815}\} = 0.04 \text{ kJ prawn}^{-1} \cdot \text{d}^{-1} \\ \text{HE}_f &= 0.04 \text{ kJ. hour}^{-1} \times 24 \text{ hours} = 0.84 \text{ kJ. day}^{-1} \end{aligned}$$

∴ the average was considered to be:

$$\begin{aligned} \text{HE}_f &= (0.84 + 0.75) \div 2 = 0.80 \text{ kJ prawn}^{-1} \cdot \text{d}^{-1} \\ \text{HE}_f &= 0.80 \times 7 \text{ days} = 5.57 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1} \end{aligned}$$

Predicting heat increment of feeding (HE_i) was seen to be the energy required to maintain its body state (maintenance = HE_f) and the retention of nutrients in order to grow:

$$\begin{aligned} \text{HE}_i &= (\text{HE}_f + \text{RE}) = (0.80 + 1.82) = 2.62 \text{ kJ prawn}^{-1} \cdot \text{day}^{-1} \\ \text{HE}_i &= (\text{HE}_f + \text{RE}) = (5.57 + 12.72) = 18.29 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1} \end{aligned}$$

Note: since $\text{ME} = \text{ADE} - \text{WE}$ where $\text{ADE} = \text{IE} - \text{FE}$ and since the ME has been determined based ADC values which takes into account the WE and FE losses there is no need to establish non-faecal

(UE + ZE) energy losses. Moreover the 25 % of RE lost due to moulting and the 3 % as SE were used.

Predicting energy losses due to moulting (HE_x)

$$HE_x = 0.25 (RE) = 0.25 (1.82) = 0.46 \text{ kJ prawn}^{-1} \cdot \text{day}^{-1}$$

$$HE_x = 0.25 (RE) = 0.25 (12.72) = 3.18 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1}$$

Predict surface loss, exuviae (SE)

$$SE = 3 \% (RE + HE_f + HE_i + HE_x)$$

$$= [0.03 \times (1.82 + 0.80 + 2.62 + 0.46)] = 0.17 \text{ kJ prawn}^{-1} \cdot \text{day}^{-1}$$

$$= [0.03 \times (12.72 + 5.27 + 18.29 + 3.18)] = 1.18 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1}$$

Step 5.: RATION allowance:

Metabolic energy requirement was calculated as:

$$\begin{aligned} \text{ME requirement} &= (RE + HE_f + HE_i + HE_x + SE) \\ &= (1.82 + 0.80 + 2.62 + 0.46 + 0.17) = 5.87 \text{ kJ prawn}^{-1} \cdot \text{day}^{-1} \\ &= (12.72 + 5.57 + 18.29 + 3.18 + 1.18) = 40.94 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1} \end{aligned}$$

$$\text{ME content of feed:} = 12.11 \text{ kJ.g}^{-1}$$

$$\begin{aligned} \text{Feed ration} &= (40.94 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1}) \div 12.11 \text{ kJ.g}^{-1} \\ &= 3.38 \text{ g feed per prawn}^{-1} \cdot \text{week}^{-1} \end{aligned}$$

$$\therefore \text{Daily feed allowance} = \boxed{0.48 \text{ g prawn}^{-1} \cdot \text{d}^{-1}}$$

$$\text{Expected feed efficiency} = \text{live mass gained/feed consumed} = 1.35 \div 3.38 = 0.39$$

$$\text{Expected FCR} = \text{feed consumed/live mass gained} = 3.38 \div 1.35 = 1: 2.50$$

6.3.2. Use of the effective energy system to establish the daily food requirement for *F. indicus*.

Assuming $HE_m = HE_f$ the energy requirement for *F. indicus* was calculated as follows:

$$\begin{aligned} \text{EERQ} &= HE_f + 47 \text{ PR} + 56 \text{ LR} + 17 \text{ CR} \\ &= (0.80) + 47 (0.080) + 56 (0.002) + 17 (0.002) \\ &= (0.80) + (3.76) + (0.08) + (0.03) \\ &= 4.67 \text{ kJ. prawn}^{-1} \cdot \text{day}^{-1} \end{aligned}$$

Using equation [21.] and assuming that $z = 0.3$ the EE content in the feed was calculated:

$$\begin{aligned}
EEC &= ME_n - (0.23 \times D_{CP}) - (3.8 \times FOM) + (3.6 \times D_{CL}) \\
EEC &= 12.11 - 0.23 \times [(41.93 \times 0.81) \div 100] - 3.8 \times [(41.93 \times 0.19) \div 100 \\
&\quad + (6.56 \times 0.08) \div 100 + (38.65 \times 0.62) \div 100] + 3.6 \times [(6.56 \times 0.92) \div 100] \\
EEC &= 12.11 - [0.23 \times (0.34)] - \{3.8 \times [(0.08) + (0.02) + (0.24)]\} + [3.6 \times (0.06)] \\
EEC &= 12.11 - 0.08 - 1.29 + 0.22 \\
EEC &= 10.96 \text{ (kJ. g}^{-1}\text{)}
\end{aligned}$$

Hence the feed ration was calculated as:

$$\begin{aligned}
\text{Feed ration} &= EERQ \div EEC \\
&= 4.67 \text{ (kJ. prawn}^{-1}\text{. day}^{-1}\text{)} \div 10.96 \text{ (kJ. g}^{-1}\text{)} \\
\therefore \text{Daily feed allowance} &= 0.43 \text{ g prawn}^{-1}\text{.d}^{-1} \\
\text{The weekly ration} &= 0.43 \text{ g. prawn}^{-1}\text{.d}^{-1} \times 7 = 2.98 \text{ g. prawn}^{-1}\text{.week}^{-1}
\end{aligned}$$

Therefore:

$$\begin{aligned}
\text{Expected feed efficiency} &= \text{live mass gained/feed consumed} = 1.35 \div 2.98 = 0.45 \\
\text{Expected FCR} &= \text{feed consumed/live mass gained} = 3.38 \div 1.35 = 1: 2.21
\end{aligned}$$

6.3.3. Further examples.

For a mixed batch of prawns between 62 - 79 days of age energy costs were calculated as follows:

Initial mass:	3.33 g
Final mass:	6.57 g
Average water temperature:	26 °C
Growth period:	17 days
Gain in live mass per day:	0.19 g. prawn ⁻¹

From Table 4.5:

protein	retention per day = [(0.944 - 0.485)] ÷ 17
lipid	retention per day = [(0.027 - 0.013)] ÷ 17
carbohydrate	retention per day = [(0.049 - 0.028)] ÷ 17

The predicted energy retained (RE) was calculated to be:

$$\begin{aligned} RE &= 21.3 (0.027) + 39.6 (0.001) + 17.2 (0.001) = 0.63 \text{ kJ prawn}^{-1} \text{ day}^{-1} \\ &= 4.42 \text{ kJ prawn}^{-1} \text{ week}^{-1} \end{aligned}$$

Predicting the energy required for maintenance:

The average water temperatures (t°) in ponds over this period was 26°C and using the following $HE_f = \{[-0.1082 + 0.0147(t^\circ)] \times M^{0.815}\}$ and a conversion of $20 \text{ J. mg}^{-1} \text{ O}_2$ consumed the fasting metabolic rate was calculated to be:

for 3.33 g:

$$\begin{aligned} HE_f &= 20 \times \{[-0.1082 + 0.0147(26)] \times 3.33^{0.815}\} = 0.02 \text{ kJ. hour}^{-1} \\ HE_f &= 0.03 \text{ kJ. hour}^{-1} \times 24 \text{ hours} = 0.35 \text{ kJ. day}^{-1} \end{aligned}$$

and for 6.57 g:

$$\begin{aligned} HE_f &= 20 \times \{[-0.1082 + 0.0147(26)] \times 6.57^{0.815}\} = 0.03 \text{ kJ prawn}^{-1} \cdot \text{d}^{-1} \\ HE_f &= 0.04 \text{ kJ. hour}^{-1} \times 24 \text{ hours} = 0.61 \text{ kJ. day}^{-1} \end{aligned}$$

\therefore the average was considered to be:

$$\begin{aligned} HE_f &= (0.61 + 0.35) \div 2 = 0.48 \text{ kJ prawn}^{-1} \cdot \text{d}^{-1} \\ HE_f &= 0.48 \times 7 \text{ days} = 3.36 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1} \end{aligned}$$

Predicting heat increment of feeding (HE_i):

$$HE_i = (HE_f + RE) = (3.36 + 4.42) = 7.78 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1}$$

Predicting energy losses due to moulting (HE_x)

$$HE_x = 0.25 (RE) = 0.25 (4.42) = 1.11 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1}$$

Predicting surface loss, exuviae (SE)

$$\begin{aligned} SE &= 3 \% (RE + HE_f + HE_i + HE_x) \\ &= [0.03 \times (4.42 + 3.36 + 7.78 + 1.11)] = 0.50 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1} \end{aligned}$$

Metabolic energy requirement:

$$\begin{aligned} \text{ME requirement} &= (RE + HE_f + HE_i + HE_x + SE) \\ &= (4.42 + 3.36 + 7.78 + 1.11 + 0.50) = 17.17 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1} \end{aligned}$$

$$\text{ME content of feed:} = 12.11 \text{ kJ.g}^{-1}$$

$$\begin{aligned} \text{Feed ration} &= (17.17 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1}) \div 12.11 \text{ kJ. g}^{-1} \\ &= 1.41 \text{ g feed per prawn}^{-1} \cdot \text{week}^{-1} \end{aligned}$$

$$\therefore \text{Daily feed allowance} = \boxed{0.20 \text{ g prawn}^{-1} \cdot \text{d}^{-1}}$$

Therefore:

$$\text{Expected feed efficiency} = \text{live mass gained/feed consumed} = 1.33 \div 1.41 = 0.94$$

$$\text{Expected FCR} = \text{feed consumed/live mass gained} = 1.41 \div 1.33 = 1: 1.07$$

The feed efficiency and FCR based on effective energy approach were calculated as follows:

Assuming $HE_m = HE_f$ the energy requirement for *F. indicus* was calculated as follows:

$$\begin{aligned} \text{EERQ} &= HE_f + 47 \text{ PR} + 56 \text{ LR} + 17 \text{ CR} \\ &= (0.48) + 47 (0.027) + 56 (0.001) + 17 (0.001) \\ &= (0.48) + (1.27) + (0.06) + (0.02) \\ &= 1.83 \text{ kJ. prawn}^{-1}. \text{ day}^{-1} \end{aligned}$$

Hence the feed ration for pawns from 62 – 79 days of age was calculated as:

$$\begin{aligned} \text{Feed ration} &= \text{EERQ} \div \text{EEC} \\ &= 1.83 \div 10.96 \end{aligned}$$

$$\therefore \text{Daily feed allowance} = \boxed{0.17 \text{ g prawn}^{-1}. \text{ d}^{-1}}$$

$$\text{The weekly ration} = 0.17 \text{ g. prawn}^{-1}. \text{ d}^{-1} \times 7 = 1.16 \text{ g. prawn}^{-1}. \text{ week}^{-1}$$

Therefore:

$$\text{Expected feed efficiency} = \text{live mass gained/feed consumed} = 1.33 \div 1.16 = 1.15$$

$$\text{Expected FCR} = \text{feed consumed/live mass gained} = 1.16 \div 1.33 = 1: 0.88$$

Similarly for a mixed batch of prawns between 250 - 397 days of age energy costs were calculated as follows:

Initial mass:	28.35 g
Final mass:	30.81 g
Average water temperature:	24 °C
Growth period:	147 days
Gain in live mass per day:	0.017 g. prawn ⁻¹

From Table 4.5:

protein	retention per day = [(6.547 – 4.805)] ÷ 147
lipid	retention per day = [(0.197 – 0.143)] ÷ 147
carbohydrate	retention per day = [(0.412 – 0.253)] ÷ 147

The predicted energy retained (RE) was calculated to be:

$$\begin{aligned} \text{RE} &= 21.3 (0.012) + 39.6 (0.0004) + 17.2 (0.001) = 0.29 \text{ kJ prawn}^{-1} \text{ day}^{-1} \\ &= 2.02 \text{ kJ prawn}^{-1} \text{ week}^{-1} \end{aligned}$$

Predicting the energy required for maintenance:

The average water temperatures (t°) in ponds over this period was 18°C and using the following $\text{HE}_f = \{[-0.1082 + 0.0147(t^\circ)] \times M^{0.815}\}$ and a conversion of $20 \text{ J. mg}^{-1} \text{ O}_2$ consumed the fasting metabolic rate was calculated to be:

for 28.35 g:

$$\begin{aligned}\text{HE}_f &= 20 \times \{[-0.1082 + 0.0147(18)] \times 28.35^{0.815}\} = 0.05 \text{ kJ. hour}^{-1} \\ \text{HE}_f &= 0.08 \text{ kJ. hour}^{-1} \times 24 \text{ hours} = 1.13 \text{ kJ. day}^{-1}\end{aligned}$$

and for 30.81 g:

$$\begin{aligned}\text{HE}_f &= 20 \times \{[-0.1082 + 0.0147(18)] \times 30.81^{0.815}\} = 0.08 \text{ kJ prawn}^{-1}.\text{hour}^{-1} \\ \text{HE}_f &= 0.08 \text{ kJ. hour}^{-1} \times 24 \text{ hours} = 1.22 \text{ kJ. day}^{-1}\end{aligned}$$

\therefore the average was considered to be:

$$\begin{aligned}\text{HE}_f &= (1.22 + 1.08) \div 2 = 1.15 \text{ kJ prawn}^{-1}.\text{d}^{-1} \\ \text{HE}_f &= 1.15 \times 7 \text{ days} = 8.06 \text{ kJ prawn}^{-1}.\text{week}^{-1}\end{aligned}$$

Predicting heat increment of feeding (HE_i):

$$\text{HE}_i = (\text{HE}_f + \text{RE}) = (8.06 + 2.02) = 10.08 \text{ kJ prawn}^{-1}.\text{week}^{-1}$$

Predicting energy losses due to moulting (HE_x)

$$\text{HE}_x = 0.25 (\text{RE}) = 0.25 (2.02) = 0.51 \text{ kJ prawn}^{-1}.\text{week}^{-1}$$

Predicting surface loss, exuviae (SE)

$$\begin{aligned}\text{SE} &= 3 \% (\text{RE} + \text{HE}_f + \text{HE}_i + \text{HE}_x) \\ &= [0.03 \times (2.02 + 8.06 + 10.08 + 0.51)] = 0.62 \text{ kJ prawn}^{-1}.\text{week}^{-1}\end{aligned}$$

Metabolic energy requirement:

$$\begin{aligned}\text{ME requirement} &= (\text{RE} + \text{HE}_f + \text{HE}_i + \text{HE}_x + \text{SE}) \\ &= (2.02 + 8.06 + 10.08 + 0.51 + 0.62) = 21.29 \text{ kJ prawn}^{-1}.\text{week}^{-1}\end{aligned}$$

$$\text{ME content of feed:} = 12.11 \text{ kJ.g}^{-1}$$

$$\begin{aligned}\text{Feed ration} &= (21.29 \text{ kJ prawn}^{-1}.\text{week}^{-1}) \div 12.11 \text{ kJ. g}^{-1} \\ &= 1.76 \text{ g feed per prawn}^{-1}.\text{week}^{-1}\end{aligned}$$

$$\therefore \text{Daily feed allowance} = \boxed{0.25 \text{ g prawn}^{-1}.\text{d}^{-1}}$$

Therefore:

$$\text{Expected feed efficiency} = \text{live mass gained/feed consumed} = 0.18 \div 1.76 = 0.10$$

$$\text{Expected FCR} = \text{feed consumed/live mass gained} = 1.76 \div 0.18 = 1: 10.0$$

The feed efficiency and FCR based on effective energy approach were calculated as follows:

Assuming $HE_m = HE_f$ the energy requirement for *F. indicus* was calculated as follows:

$$\begin{aligned} \text{EERQ} &= HE_f + 47 \text{ PR} + 56 \text{ LR} + 17 \text{ CR} \\ &= (1.15) + 47 (0.012) + 56 (0.0004) + 17 (0.001) \\ &= (1.15) + (0.56) + (0.06) + (0.02) \\ &= 1.79 \text{ kJ. prawn}^{-1}. \text{ day}^{-1} \end{aligned}$$

Hence the feed ration for prawns from 250 – 397 days of age was calculated to be:

$$\begin{aligned} \text{Feed rations} &= \text{EERQ} \div \text{EEC} \\ &= 1.79 \div 10.96 \end{aligned}$$

$$\therefore \text{Daily feed allowance} = \boxed{0.16 \text{ g prawn}^{-1}. \text{ d}^{-1}}$$

$$\text{The weekly ration} = 0.16 \text{ g. prawn}^{-1}. \text{ d}^{-1} \times 7 = 1.11 \text{ g. prawn}^{-1}. \text{ week}^{-1}$$

Therefore:

$$\text{Expected feed efficiency} = \text{live mass gained/feed consumed} = 0.18 \div 1.11 = 0.16$$

$$\text{Expected FCR} = \text{feed consumed/live mass gained} = 1.11 \div 0.18 = 1: 6.18$$

6.4. Discussion

Reference to the literature has revealed that reliable information of nutrient accretion in prawns is limited especially with regards to energy requirements since energy demands may change due to a variety of physiological and environmental factors. Establishing the energy requirement is critical since if dietary energy levels are too high, prawns tend to limit consumption resulting in poor growth. Alternatively, if dietary levels are too low, some of the dietary protein will be used for metabolism to support vital physiological functions. However, both approaches demonstrated in this study provide a means of predicting the energy required for maintenance and growth in *F. indicus*. Both approaches were based on the assumption that the feed was non-limiting with regard to the provision of all nutrients thereby allowing only the energy requirements to be considered in this section.

The empirical approach proposed by Cho and Bureau (2000) establishes the daily ration by summing energy losses incurred due to exuviae (SE), moulting (HE_x), the formation of metabolites and costs involved for maintenance and retention (RE) divided by dietary energy yields, either digestible (DE) or metabolisable (ME). Besides a knowledge of maintenance costs for a given temperature and salinity (HE_f), the approach of Cho and Bureau (2000) necessitates that the calorific value of the feed, faeces and body tissue be known.

On the other hand, the effective energy system (EE) calculates feed rations based on the effective energy in the diet (EEC) and that required by the animal (EERQ). Although SE and HE_x costs are not taken into account the EE system calculates feed requirements based on the potential growth, which is assumed to include both processes. Moreover, despite that less than 5 % of ammonia is converted by crustaceans into urea, EER takes this cost into account. Unique to this approach is that the EEC is based on the ME and digestible portion of the diet.

In this study both approaches provide similar results for a mixed batch of prawns 62 – 79 and 98 – 111 days of age, with slightly lower rations calculated using the EE approach. Incorporating moulting (HE_x) and exuviae (SE) losses with the EERQ provided for better and, in the case of 62 – 79 day old prawns, identical results. In all cases an increase of HE_f by a factor of 0.13, established in the previous Chapter, to compensate for HE_i losses contributed little to the overall metabolic cost.

Results of daily allowances and indices amongst animals 250 – 397 days of age were, however, noticeably different. During this period, the daily ration was calculated to be 0.25 and 0.16 g prawn⁻¹.d⁻¹ based on the empirical and EE approach, respectively. Since growth, during this period approaches an asymptotic mass, which is species specific, it is highly doubtful that the prawn requires a ration of 0.25 g prawn⁻¹.d⁻¹ to sustain a growth rate of 0.017 g. prawn⁻¹. day⁻¹, providing a FCR of 1:10. Moreover, results based on empirical approach indicated an increase in feed rations over time which is contrary to what is expected since after the maximum rate of growth is attained, the point of inflection during the exponential phase of a sigmoidal growth response, growth decelerates until a specific asymptotic mass is maintained whereby daily requirements and hence feed intake decreases accordingly.

By comparison, feed intakes based on the EE systems approach decreased with age and provided for better results in prawns 250 – 397 days of age. However, the ration and a FCR of 1:6.2, established using this approach, exceeds ratios of between 1:1.8 and 1:2.5, commonly found under most farm and laboratory conditions (Bureau *et al.*, 2000). Under culture conditions a high FCR estimate was attributed, in part, to a decrease in digestibility and a low nutrient density of the diet amongst older prawns. Moreover, due to the presence of endogenous sources and warmer

temperatures during the early stages of development, better growth rates are attainable, which in turn will improve conversion ratios.

In order to obtain better feed indices water temperatures need to remain constant at ≥ 26 °C. Since this is not possible in outdoor ponds the only alternative is to formulate diets whereby the protein and energy requirements are met on a daily basis. The next Chapter examines a means to establish the dietary requirements and feed intake of prawns in order to decrease feed wastage and provide for better indices. Hence based on the findings of this study the EE systems approach is preferred for establishing energy requirements in *F. indicus*.

6.5. Conclusion

In this study both approaches established daily rations based on the energy requirement of *F. indicus* under a range of conditions *e.g.*, 20 - 30 °C and 15 - 35 ppt. Unlike the empirical approach the EE systems solves the problem of estimating daily rations as the energy necessary for maintenance and that required for the accretion of dietary nutrients. The simplicity of the EE system is by no means a limitation since, as opposed to the empirical approach, realistic feed allowances for older prawns were calculated. Moreover, an advantage when using the EE approach is that it provides a simple means to formulate diets whereby food wastage can be reduced and the energy requirement of the prawn can be met.

7.

Protein Requirements

Establishing the protein requirement in *Fenneropenaeus indicus* based on feed intake.

7.1. Introduction.

An accurate prediction of growth is critical in order to establish the food requirements for maintenance, growth and fattening since animals that grow at different rates have different requirements. While this seems obvious, careful examination of the current scientific and technical literature suggests that this concept is poorly understood by many in the field of aquaculture and prawn biology (Bureau *et al.*, 2000). However, Emmans and Fisher (1986) have shown that a suitable description of the genotype and state allows for the rates of maintenance, growth and fattening to be transformed into necessary rates of supply.

The preferred theory used in this study, dependent on the nature of the diet and environment, is that an animal will eat sufficient food to reach its potential daily growth rate while maintaining a desired body composition. The maximum rate of protein accretion is determined by the protein content of the body at that time, however, whether the intrinsic limit is attainable will depend on extrinsic factors such as energy and protein intake. In the previous Chapter feed intake was predicted based on the species daily requirement for energy whereas the method described here predicts the protein requirements in growing *Fenneropenaeus indicus* based on food intake.

7.2. The theory used to establish the daily requirement for protein.

7.2.1. Protein requirements for maintenance and growth.

Since protein requirement is considered to be dependent on the amino acid content in the feed and body tissue, the preferred option is to examine the requirement for individual amino acids. In theory, the closer the amino acid composition in the diet matches the profile found in the animal, the more effectively the dietary protein will be assimilated (Wilson & Poe, 1985). In prawns the exoskeleton contains a proportion of the total body protein, and because the amino acid composition of exoskeleton differed markedly from flesh protein, the quantity of protein to satisfy the potential rate of growth and maintenance in each component was considered separately.

The theory assumes that the coefficient of utilisation of essential amino acids for growth and maintenance are constant and that the ideal amino acid balance for growth is similar to that in the

body. As mentioned in Chapter 3, the net efficiency of an apparently digested amino acid, which is the first limiting, is assumed to be 0.80 for growth and 1.04 for maintenance. Values used in this model to describe the ideal balance of amino acids and the coefficients of utilisation are given (Table 7.1).

Table 7.1: The ideal amino acid balance and efficiency of utilisation for growth (*a*) and maintenance (*b*) of essential amino acids in the flesh and exoskeleton of *Fenneropenaeus indicus*.

Essential Amino Acids	Ideal Balance (mg/g)			Values used (mg/g)			
	Flesh	Exoskeleton	Whole body	<i>a</i> *		<i>b</i> **	
				Flesh	Exoskeleton	Flesh	Exoskeleton
Lysine	6.93	1.71	7.15	8.66	2.14	6.66	1.65
Arginine	7.39	2.10	7.90	9.24	2.63	7.11	2.02
Methionine	2.29	0.54	2.34	2.86	0.68	2.20	0.52
Threonine	3.01	1.40	3.76	3.76	1.75	2.90	1.34
Isoleucine	3.76	1.74	4.69	4.70	2.18	3.62	1.67
Leucine	6.30	1.87	6.82	7.88	2.34	6.06	1.80
Histidine	1.87	1.06	2.53	2.34	1.33	1.80	1.02
Phenylalanine	3.63	1.96	4.81	4.54	2.45	3.49	1.89
Valine	3.93	2.05	5.14	4.91	2.56	3.78	1.97

*Using a coefficient of utilisation for growth of 0.8, such that for Lysine $6.93/0.8 = 8.66$.

**Using a coefficient of utilisation for maintenance of 1.04, such that for Lysine $6.93/1.04 = 6.66$.

Moreover, the approach assumes that the dietary protein is highly digestible and that the total energy ingested is appropriate.

7.2.2. Predicting the desired feed intake (DFI).

Based on the premise that an animal will eat to satisfy its needs for the first-limiting nutrient the rate of intake of a perfectly balanced feed, defined as one where the intake of each resource is the same, the desired intake of amino acids (AAI) was determined using the following (Emmans & Fisher, 1986):

$$DFI = \frac{EER}{EEC} = \frac{AAI_i}{AAD_i} \quad (\text{mg/day}) \quad [7.1]$$

where AAI = ideal amino acid content with subscript *i*, referring to the *i*th amino acid.
AAD = dietary amino acid and subscript *i*, refers to the *i*th amino acid.

7.3. The means to establish the intake of dietary amino acids in growing *F. indicus*.

To calculate the daily protein requirement in growing *F. indicus*, it was necessary first to describe the genotype and state of the species (Chapter 4). For a mixed sample of prawns reared at 18 – 27 °C and in 21 – 27 ‰, P_m and β were found to be 6541.00 mg and 0.014 per day, respectively. With the use of these parameters, the Gompertz growth function and allometry; body protein, lipid content and growth rate could be established for any given day (Table 4.10).

Using equation [5.10] and multiplying by a factor of 20 (kJ/ℓ O₂ respired) and 24 (hours) the oxygen consumption was converted to the energy required for maintenance (HE_f). The daily effective energy requirement (EERQ) was calculated as the sum of the HE_f and the energy necessary for the retention of protein, lipid and carbohydrate (Table 7.2). The DFI_{EE} was calculated by dividing the EERQ by the EEC, the denominator being 10.96 kJ/g (Figure 7.1).

Concentrations of amino acids to sustain maintenance and potential growth were established by dividing the daily requirements, calculated using equation [3.22], by the DFI_{EE} (Figure 7.2). Although this method equips one with the knowledge of the daily requirements, it is not practical to formulate and produce feed that will meet the species' needs on a daily basis. Hence, in this study the requirement for different mass classes, previously dealt with in Chapter 4 (Table 7.2), were therefore determined.

The next step was to formulate diets using WinFeed 2[†]. Whereas the program's primary function is to devise diets based on 'least cost', formulations were done utilising the minimum protein option by stipulating the minimum concentration for all essential amino acids (Table 7.2) except cystine and tryptophan. Formulations were done using readily available feedstuff, were based on the assumption that they will be eagerly accepted and that cystine and tryptophan were non-limiting. The protein content of these diets (Appendix G) were then used to plot the species' requirement with age (Figure 7.3).

In order to reduce periods of under- and over-feeding, it is customary to divide the growth period into phases, whereby the supply of amino acid and protein in each phase is reduced as the prawns gets older. In this study the growth period was divided into four phases. These were the starter, grower, finisher and maintenance phases for animals between 0 – 7.5, 7.5 – 15, 15 – 25 and 25 – 35 g live mass, respectively (Figure 7.4).

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Table 7.2: Mean values for the daily maintenance, effective energy (kJ/day) and amino acid requirements of mixed samples of cultured *F. indicus* reared at 24 °C.

Age (days)	Mean Live Mass (g)	Maintenance [‡] Energy (kJ/day) HE _f	Physical component	RE [*] (kJ/day)	Total EERQ (kJ/day)	Daily Amino Acid Requirement (mg/day) [†]								
						Lysine	Arginine	Methionine	Threonine	Isoleucine	Leucine	Histidine	Phenylalanine	Valine
62	3	0.31	Flesh	0.59	1.23	1.75	1.93	0.57	0.92	1.15	1.67	0.62	1.18	1.24
			Exo	0.18										
79	6	0.54	Flesh	0.88	1.72	2.24	2.48	0.73	1.18	1.47	2.14	0.79	1.51	1.61
			Exo	0.23										
98	9	0.75	Flesh	1.03	2.13	2.67	2.95	0.87	1.41	1.75	2.55	0.94	1.80	1.92
			Exo	0.27										
111	12	0.90	Flesh	1.12	2.37	2.87	3.17	0.94	1.51	1.88	2.74	1.02	1.93	2.06
			Exo	0.32										
120	15	1.01	Flesh	1.12	2.53	2.99	3.31	0.98	1.58	1.97	2.86	1.06	2.02	2.15
			Exo	0.33										
152	20	1.28	Flesh	1.08	2.70	2.93	3.24	0.96	1.54	1.92	2.80	1.04	1.97	2.11
			Exo	0.34										
250	25	1.61	Flesh	0.97	2.74	2.49	2.75	0.82	1.31	1.64	2.38	0.88	1.68	1.79
			Exo	0.31										
305	32	1.79	Flesh	0.61	2.33	1.53	1.69	0.50	0.81	1.00	1.46	0.54	1.03	1.10
			Exo	0.29										
397	40	1.92	Flesh	0.00	1.93	0.73	0.80	0.24	0.38	0.48	0.69	0.26	0.49	0.52
			Exo	0.00										

[‡] HE_f = [20 × {[- 0.1082 + 0.0147(t°)] × M^{0.815}} × 24] (kJ/prawn/day)

^{*} RE = 47 PR + 56 LR + 17 CR (kJ/d)

^{*} EERQ = HE_f + RE (kJ/d)

[†] AAI = (a × PR) + (b × MP) (mg/day)

7.4. Results.

The DFI was found to be parabolic in response and was described by a third order polynomial of the form $y = 56.58 + 20.53x - 0.662x^2 + 0.006x^3$ ($R^2 \geq 0.99$) (Figure 7.1).

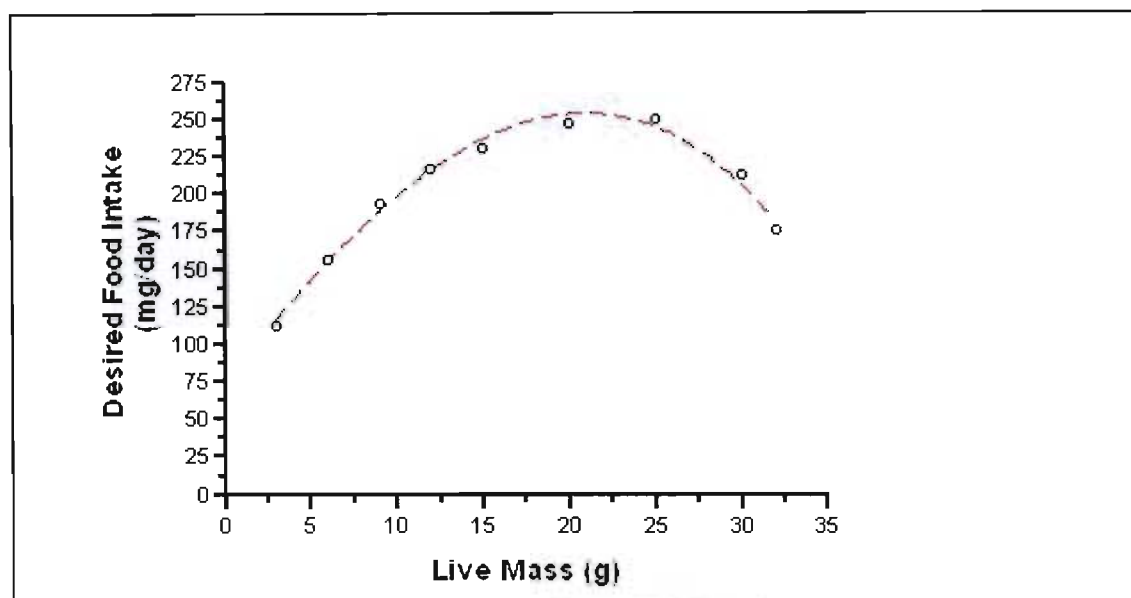


Figure 7.1. The desired feed intake (mg/day) required by *Fenneropenaeus indicus* over time.

Food consumption increased to a maximum of ~ 250 mg/day per prawn in animals 20 – 25 g and thereafter decreased to ~ 160 mg/day in adult prawns. In contrast the desired contents of dietary amino acids decreased with age, described by the relationship $Y = Y_0^{-kx}$ ($R^2 \geq 0.97$) (Figure 7.2).

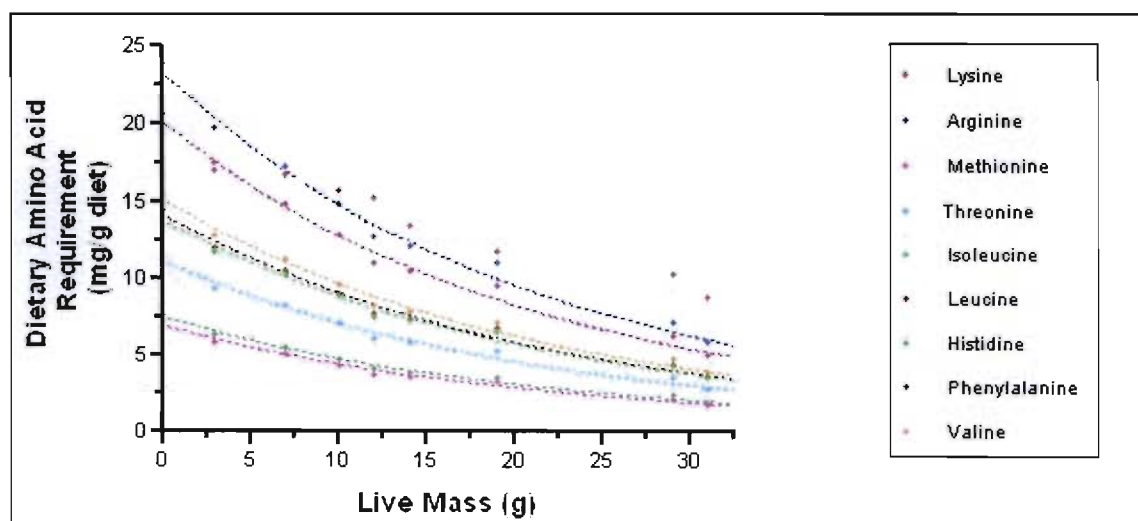


Figure 7.2. The desired amino acid requirements (mg/g diet) in *Fenneropenaeus indicus* over time.

The equation $Y = 10.48^{-0.082x} + 24.20$ ($R^2 \geq 0.99$) best described the dietary requirement for protein in relation to live mass (Figure 7.3).

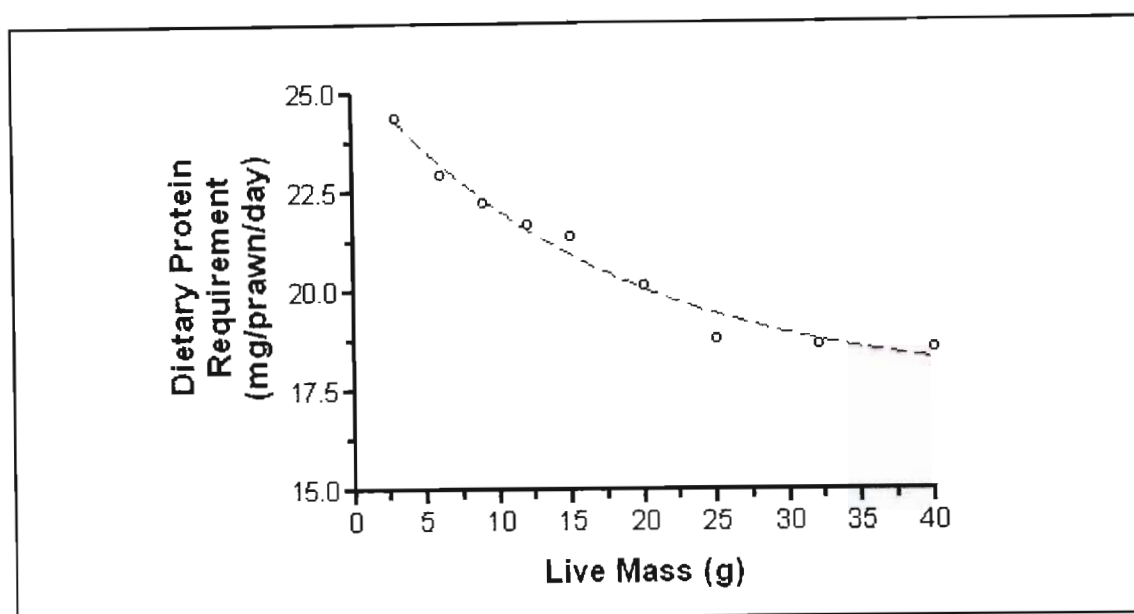


Figure 7.3. The requirement for dietary protein (mg/prawn/day) by *Fenneropenaeus indicus* with age.

From Figure 7.3 dietary protein decreased from ~ 24 – 18 % for animals 3 - 40 g. Based on these finding the growth rate was divided into four growth phases *i.e.* starter, grower, finisher and maintenance phase (Figure 7.4.)

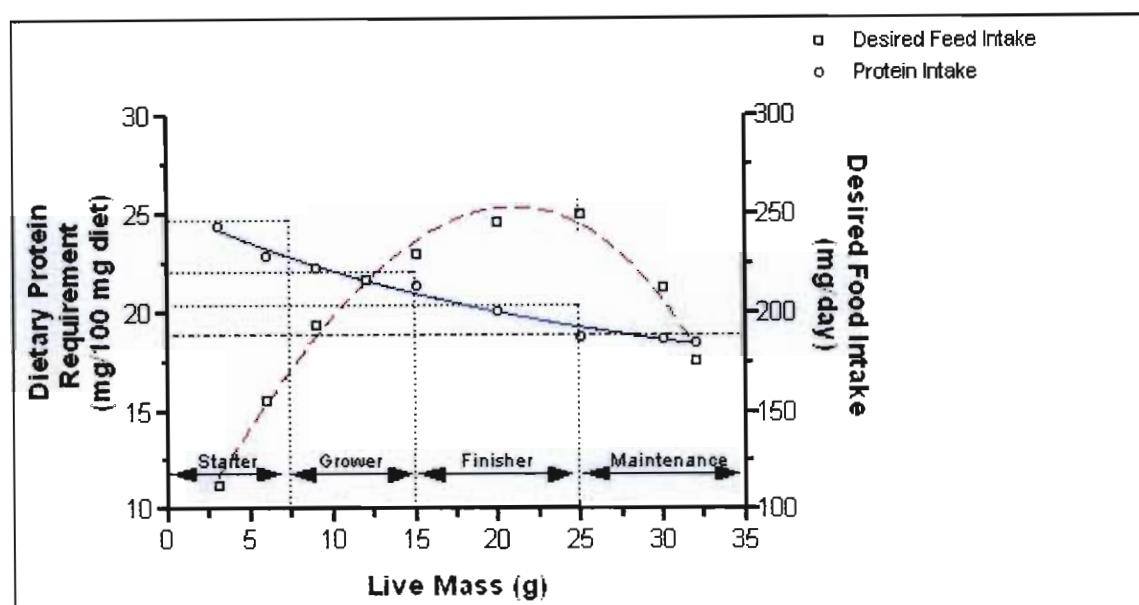


Figure 7.4. The desired feed intake (mg/prawn) and requirement for dietary protein (mg/100 mg diet) for *Fenneropenaeus indicus* over time.

From Figure 7.4 during the initial stages (Starter phase), the prawn's requirements for dietary protein were considerably higher and the DFI lower than those of subsequent phases. The reason is that during this stage a rapid increase in growth occurs where the demand for dietary protein is high and the intake is limited by morphological constraints due to gut capacity.

Depending on the presence and abundance of pond biota, the Starter and Growing phases can be divided further to compensate for periods of under-feeding. The mean amino acid requirement for each phase is given (Table 7.3).

Table 7.3: The dietary requirement for effective energy, crude protein, amino acids and food intake for *F. indicus* in grow-out ponds.

Feed	Starter	Grower	Finisher	Maintenance
Developmental stage	(0 - 7.5 g)	(7.5 - 15 g)	(15 - 25 g)	(25 - 35 g)
EERQ (kJ/day)	1.32	2.31	2.81	2.59
Crude Protein ¹ (%)	23.28	22.00	20.58	19.08
Lysine (mg/g diet)	15.21	13.49	11.61	7.73
Arginine (mg/g diet)	19.50	13.26	10.55	6.31
Methionine (mg/g diet)	5.78	3.93	3.12	1.87
Threonine (mg/g diet)	9.28	6.32	5.02	3.01
Isoleucine (mg/g diet)	11.57	7.87	6.26	3.75
Leucine (mg/g diet)	16.83	11.45	9.10	5.54
Histidine (mg/g diet)	6.24	4.24	3.37	2.02
Phenylalanine (mg/g diet)	11.88	8.08	6.42	3.84
Valine (mg/g diet)	12.68	8.63	6.86	4.11
Food intake ² (mg/day)	120	211	257	236

¹ Crude protein requirement established using WinFeed 2.

² Based on EERQ and an EEC of 10.96 kJ/g.

7.5. Discussion

Once the requirements for all the essential amino acids (EAAs) have been met, extraneous amino acids are metabolised for energy. On the other hand when fed an unbalanced feed the content of extraneous amino acids is increased as amino acids can only be utilised for protein synthesis up to the level of the first-limiting amino acid. Identifying the exact requirements of each EEA in prawn diets has proved problematic since research aimed at determining the requirement for specific amino acid is varied, inconsistent and poorly understood. Teshima, Alam, Koshio, Ishikawa, Kanazawa (2002) assessed the requirements for amino acids of *Marsupenaeus japonicus* by measuring the daily increase of each EAA in the whole body. In an attempt to avoid any dietary limitations, the prawn was fed a high quality diet containing 50 % protein based on casein and squid. Their assessment of EAA requirements, expressed as a percentage protein, was arginine (2.9); histidine (1.1); isoleucine (2.3); leucine (4.3); lysine (3.2); methonine (1.3); phenylalanine (2.6); threonine (2.3); tryptophan (0.6) and valine (2.4). In another study, using encapsulated arginine, Millamena, Bautista-Teruel, Reyes and Kanazawa (1998) estimated the requirement for lysine in *Penaeus monodon* to be 5.2 % of dietary protein. Similarly Fox, Lawrence and Li-Chan (1995)

established the lysine requirement for *Litopenaeus vannamei* to be 4.7 % of the protein when using a diet containing 45 % protein. These values and those of Teshima *et al.* (2002) are considerably lower than those expressed in Table 7.4.

Table 7.4: Recommended dietary profile of Essential Amino Acids (EAA) used to formulate prawn diets (after Conklin, 2003).

EEA	Percentage of dietary protein (%)							
	Tissue Essential Amino Acid Profile in:							
	<i>Farfantepenaeus actecus</i>	<i>Marsupenaeus japonicus</i>	<i>Penaeus monodon</i>				<i>Litopenaeus vannamei</i>	<i>Suggested Guidelines</i>
Lysine	6.09	5.78	6.23	6.54	5.53	4.04	4.84	6.14
Arginine	5.17	5.57	6.57	6.34	5.32	7.60	6.10	5.80
Methionine and Cystine	4.85	2.75	2.39	2.80	2.55	3.51	2.67	3.45
Threonine	5.38	2.98	3.25	4.76	2.87	4.11	2.52	4.36
Isoleucine	4.47	3.13	3.66	4.60	3.01	4.04	2.65	4.24
Leucine	9.75	5.49	6.29	7.76	5.25	9.09	4.69	8.16
Histidine	3.15	1.64	2.04	2.31	1.57	1.80	1.62	2.03
Phenylalanine and Tryptophan	8.43	6.17	4.37	5.75	5.85	8.28	5.39	7.21
Valine	5.07	3.03	4.24	5.69	2.90	4.63	3.10	4.00

The EEA profiles recommended in Table 7.4 were lower than those established for *F. indicus* (Table 7.1). By standardising these amino acid requirements, by making the lysine the reference amino acid and expressing the rest as a proportion thereof, the requirement for *F. indicus* compares favourably with those of *L. vannamei* and in most instances with those of *P. monodon* and *M. japonicus* (Table 7.5).

Table 7.5: Essential Amino Acids (EAA) requirement in relation to lysine (= 100 %) in various cultured species.

EEA	Percentage of dietary protein (%)								
	Tissue Essential Amino Acid Profile in:								
	<i>F. actecus</i>	<i>M. japonicus</i>	<i>P. monodon</i>				<i>L. vannamei</i>	<i>Suggested Guidelines</i> ¹	<i>F. indicus</i> ²
Arginine	84	96	105	97	96	88	126	94	112
Methionine and Cystine	89	48	38	43	46	87	55	56	-
Threonine	88	52	52	73	52	102	52	71	53
Isoleucine	73	54	58	70	54	100	55	69	58
Leucine	160	95	101	119	95	225	97	132	95
Histidine	52	24	33	35	28	45	33	33	30
Phenylalanine and Tryptophan	138	107	70	88	106	204	111	117	-
Valine	83	58	68	87	52	115	64	65	62

¹ Conklin (2003).

² As found in this study.

Unlike most current methods, whereby requirements are determined based on once-off measurements, the method expound here provides a means for predicting requirements for amino acids in growing prawns on a daily bases. Dietary intakes for amino acids to sustain maintenance and potential growth were established by dividing the amino acid requirements by the DFI, which was established based on energy requirements. With the amino acid requirement known diets based on the minimum protein or least cost basis can be formulated.

Since the dietary requirements decreased exponentially from ~ 24 – 18 % CP for 3 - 40 g animals over time, phase-feeding was recommended as a means of reducing periods of under and over-feeding in order to make the utilisation of nutrients more efficient. These findings are considerably lower than requirements of 35 - 50 % CP established and recommended by Colvin (1976a), Gopal and Raj (1990), Boonyaratpalin (1998) and levels of 25 – 33 % and 29 – 37 % for albumin (Bhaskar & Ahamad, 1984; Ahamad, 2001) and casein based diets (Bhaskar & Ahamad, 1984), respectively. Results were, however, marginally similar to a requirement of 22 % CP, established for post larvae by Thomas, Kathirvel and George (1991).

Amino acids content determined and recommended for *F. indicus* feeds (Table 7.3) will be balanced on provision that the minimum requirements are met and that the prawn is reared under favourable, extensive and/or similar conditions described in this study. However, since the objective in commercial practices is not to produce a perfectly balanced amino acid mixture, as this will prove to be prohibitively expensive, readily available feed ingredients and synthetic amino acids should be used to formulate least-cost feeds for each phase of the growth period in which the minimum bonds of the essential amino acids are set. Slight excesses in amino acid supply are unlikely to cause a problem.

7.6. Conclusion

Given the mass, body composition and growth rate of the prawn the resources needed for maintenance and growth were calculated in conditions where the mean water temperature was 24 °C. The requirement for protein and, in turn, amino acids were established based on the desired food intake for effective energy. In this study implementation and the application of the model approach proved to be a simple yet effective means of establishing the protein requirements and feed intake for the marine prawn *Fenneropenaeus indicus*.

8.

Testing the Model**Establishing the Protein Requirement and Intake of
Fenneropenaeus indicus Based on the Dose-Response Approach.****8.1. Introduction**

In dietary studies involving aquatic animals the conventional means of establishing nutrient requirements involves feeding graded levels of the test nutrient to the species under investigation. The lowest dose that elicits maximum growth is considered to be the desired concentration, normally referred to as the 'requirement'. In this way Colvin (1976a), Ahamad (1982), Bhaskar and Ahamad (1984), Gopal and Raj (1990), Thomas *et al.* (1991) and Ahamad (1994) established the protein requirement in *Fenneropenaeus indicus* to be 22 – 43 % of the diet based on live mass gains, survival and nutritional indices viz. food conversion ratio, protein efficiency ratio and net protein gains. With the exception of Gopal and Raj (1990) who used a purified diet and Colvin (1976a), test diets containing prawn meal, studies by Ahamad (1982), Bhaskar and Ahamad (1984), Thomas *et al.* (1991) and Ahamad (1994) were undertaken using either semi-purified or compound diets.

Purified diets contain refined ingredients *e.g.* lipid free casein, while semi-purified diets comprise a couple or more refined sources, usually of animal origin, and unrefined feedstuffs. Compound diets normally comprise a combination of unrefined ingredients, with protein derived from vegetable and/or animal sources. Although the use of purified diets minimises extraneous nutritional factors considerably, and allows for the precise quantification of a particular nutrient, the application thereof on a commercial scale is impractical. It is therefore rather sensible to investigate dietary requirements using semi-purified or compound diets, with a preference for the latter, whereby diets are formulated such that the amino acid profile mirrors that found in the test animal.

In dietary studies treatment means of growth data are normally compared with the aid of a standard error calculated from replicates using a variance ratio (*e.g.* F-test or Student's *t*-test) or a multiple range test (*e.g.* Duncan multiple range test or Scheffe test). However, when there is a logical structure to the set of treatments the use of 5 % probability to obtain the best estimate of some end point along the input scale is inappropriate especially since the preferred option is to establish, with a high degree of confidence, the requirement using regression analysis (Morris, 1999). Hence, dietary requirements and intakes are usually established by applying the broken line (Robbins, Norton & Baker, 1979), parabolic/second order polynomial regression (Lovell 1989), exponential or inverse polynomial models (Nelder, 1966) to these data.

Since none of the above models considers the financial implications when feeding a range of concentrations, Morris (1999) advocates the use of the Reading model, which accounts for marginal costs and financial returns. This model was first introduced by Fisher, Morris and Jennings (1973) and the statistical theory that underlies it has been published by Curnow (1973) with preference for its use highlighted in Morris (1999). Examples of the model's application for laying hens have been published for lysine (Morris & Wethli, 1978) and tryptophan (Morris & Blackburn, 1982) while Clark, Gous and Morris (1982) have used it to describe amino acid intake in growing birds.

The essential feature of this approach is that it considers the response of an individual animal as a simple factorial model, and then derives the population response as an integrated average of a large number of individual responses. Individual responses are assumed to vary only because of normally-distributed and correlated variation in output, such as the laying of eggs by hens, and maintenance requirements (Clark *et al.*, 1982). For the purpose of this study, the variable used to express the maximum output of eggs (E_{max}) was used to express the maximum accretion of body protein (BP_{max}). These responses are illustrated in Figure 8.1, where the effect of increasing protein intake on protein growth in a number of individuals is shown together with the integrated response over all animals.

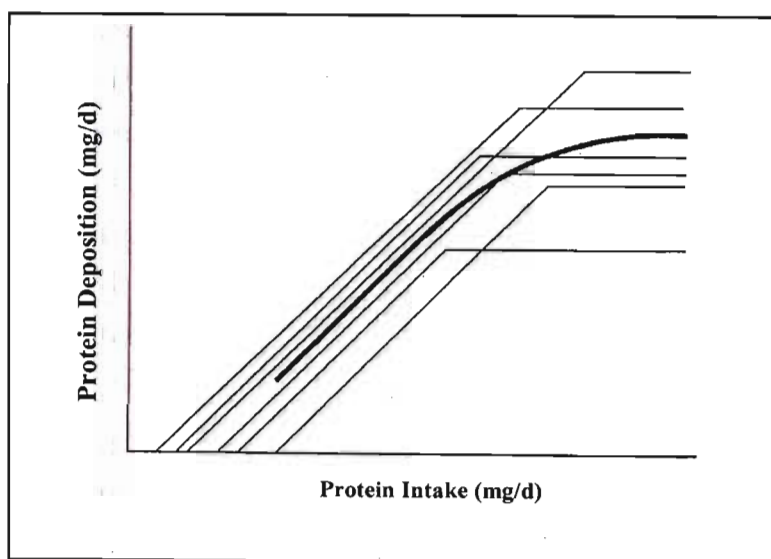


Figure 8.1. The response in protein of individuals in a population (—) and the mean response, above maintenance, (—) to increasing protein intake (adapted after Clark *et al.*, 1982).

The requirement for dietary protein is expressed in terms of mean body mass and protein gains (Fisher *et al.*, 1973):

$$PI = a \Delta \overline{BP}_{max} + b \overline{M} \quad [8.1]$$

where

- \overline{PI} = intake of protein (mg)
- $\Delta \overline{BP}_{max}$ = potential growth of protein in the average prawn (mg/prawn).
- \overline{M} = mean body mass (mg/prawn).
- a = mg protein required per mg of body mass gain.
- b = mg protein required per mg for maintenance

The optimal demand for protein, in this case, takes into account homogeneity of the population for both performance and live mass in the same way as the daily diet has to meet requirements for the least and, more importantly, the neediest animal. After having defined the relationship between the marginal cost of an additional unit of protein and the marginal revenue obtained for a further unit of protein gain the model may be used to calculate the optimum daily requirement for protein based on live mass and protein accretion together with the variance of these criteria. The response is curvilinear (Figure 8.2). According to the model the requirement for protein in a population is equal to:

$$PI = a \Delta \overline{BP}_{max} + b \overline{M} + Y \quad [8.2]$$

where

- Y = represents the additional provision of a nutrient beyond that of the mean requirement for the population (50 % of the requirements are met and 50% are not) for which the marginal costs of the nutrient are equal to marginal profits

Thus, the optimum requirement (PI_{opt}) for protein, in mg/day, may be calculated using the following (Clark *et al.*, 1982):

$$PI_{opt} = a \times \Delta \overline{BP} + b \times \overline{M} + x \sqrt{a^2 \times \sigma_{\Delta BP}^2 + b^2 \times \sigma_M^2 + 2 \times a \times b \times r_{\Delta BP \times M} \times \sigma_{\Delta BP} \times \sigma_M} \quad [8.3]$$

where

- PI_{opt} = protein intake which maximises profit.
- $\sigma_{\Delta BP_{max}}$ = variance in the maximum potential growth rate of members in a population.
- σ_M = variance in body mass.
- $r_{\Delta BP_{max} \times M}$ = correlation between maximum growth rate and body mass amongst individuals within the population.
- x = the deviation from the mean of a standard normal distribution, which is exceeded with probability ak in one tail such that k = cost per mg protein/ value per mg gained.

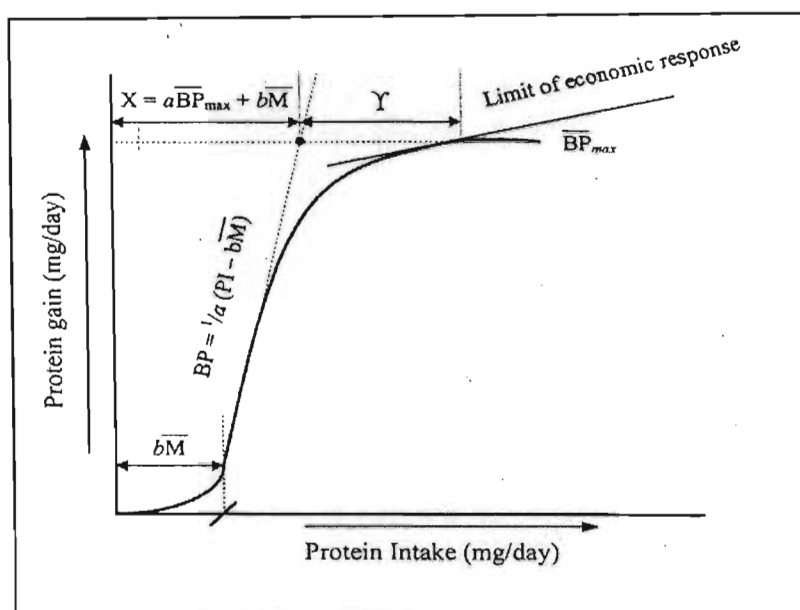


Figure 8.2. The relationship between the calculated protein requirement for the average prawn (indicated by •) and the population optimum dose based on economic terms. The line representing the limit of economic response has a slope that reflects the optimum ratio between marginal cost and marginal revenue (adapted after Clark *et al.*, 1982).

In the past *F. indicus*, cultured locally, were reared on pellets containing 43 % crude protein (CP) (Table 1.1). In recent years farm managers have resorted to using a starter, grower and finishing feed comprising 40, 38 and 36 % CP, respectively. Because of the confidential and commercial nature of the information no data validating the use of these levels has been presented in the literature. Besides the knowledge that an ontogenetic shift in the natural diet of *F. indicus* occurs with age (Bhaskar & Ahamad, 1984; Demetriades & Forbes, 1993) no investigation, prior to the systems approach presented in this study, has established if protein requirement changes during the grow-out phase.

Thus, the aims of this study were to determine the protein requirement and daily protein intake in growing *F. indicus*, using the conventional method and Reading model respectively, and compare these outcomes with those predicted based on the systems approach (Chapter 7). In order to do this the response in protein gain in the exoskeleton and flesh of three different mass categories larger than one gram, as opposed to previous studies that mainly focused on post larvae and/or juveniles < 1 g, was investigated using semi-purified diets. Hence, the objectives of this study were:

- To establish if the dietary requirement and protein intake changes with age during the grow-out phase.
- To determine what effect body lipid has on live mass gains.
- To establish the optimum level of dietary protein by assessing differences in protein gains using the Reading model.
- To compare results found in this study with those established using the systems approach.

8.2. Material and methods

8.2.1 Experimental facilities.

The laboratory set-up, described in Appendix 2, comprising three rearing systems, with each having six holding tanks, was used for 30-day growth trials. The stocking density was established by monitoring levels of contaminants, whereby a reduction in water quality necessitated the removal of one animal per tank per day until suitable parameters, stipulated by Vijayan and Diwan (1995) to be ideal, was maintained. The result was that each tank was stocked with fifteen animals.

8.2.2. Water parameters

Water was maintained at (mean \pm standard deviation): 28 ± 1 °C, 28 ± 3 ‰ and a pH 8 ± 2 with oxygen concentrations kept near saturation throughout the trials. Water temperature and salinity readings were taken twice daily while pH and nitrogen levels, measured using an ion specific meter (Model: Hanna C103) and found to have a mean and standard deviation of 0.22 (± 0.30), 0.28 (± 0.20) and 0.23 (± 0.20) mg/l for ammonia-N, nitrate-N and nitrite-N, respectively were taken before the animals were fed in the morning.

8.2.3. Experimental animals

F. indicus belonging to the same brood stock with a mass of (mean \pm standard deviation); 1.73 (± 0.24) g, 7.34 (± 0.72) g and 14.33 (± 1.37) g, simply categorised as 2, 7 and 14 g prawns respectively, were used. Prawns were transported from Amatikulu prawn farm to the laboratory on three separate occasions. In order to avoid recently moulted prawns, animals were selected based on guidelines by Peebles (1977) and Emmerson (1982). Prawns were introduced into the laboratory system following procedures described in paragraph 4.2.1. Before the initiation of trials animals, reared on a commercial pellet (Table 1.1), were allowed to acclimatise to laboratory conditions for a month. This period proved crucial since shortly after introduction 14 g prawns suffered a loss in body mass but recovered within the time provided.

8.2.4. Test diets

Six diets varying in protein content (Table 8.1) were fed to the above mentioned mass classes during three separate 30 d growth trials. Tanks were allotted a specific diet thereby ensuring three replicates per diet. The Latin square technique (after Fowley & Cohen, 1990) was used to assign a specific diet to each tank. After concluding a trial the next mass class was examined independently using the same procedures. Fish meal, soybean meal, sunflower meal and lipid free casein were used as protein sources (Table 8.1).

Test diets were formulated according to the dilution principle, which entailed the successive dilution of a concentrated protein mixture (summit diet) with a less concentrated

(basal) diet to yield six isoenergetic diets, < 2 % variation in gross energy, of approximately 5, 14, 23, 32, 41 and 50 % CP. Formulation was based on the lysine to amino acid ratios of tissue samples measured previously. Starch was used to adjust protein levels while natural oils, as done by Huner and Samuel (1979), were used to supplement dietary fat and energy levels. Alpha-cellulose was used as a filler and to increase dietary fibre.

Raw ingredients were finely ground using a mill (Model: Congent 2000) and oven dried at 25 °C for 72 hours. The dry ingredients for each diet were blended using a hand held electric mixer for 10 minutes. Fish and sunflower oil were gradually added and the mixture was blended thoroughly for a further 15 minutes. Distilled water (85 ml per 100 g dry mass) was added to the mixture to obtain a homogeneous dough, which was kneaded for an additional 10 minutes to develop the elasticity characteristic of diets high in starch. Using a conventional hand operated meat grinder the dough was forced through a die to form strands approximately 3-mm in diameter. These strands were placed in an oven and dried at 28 °C for 48 h until ~ 90 % dry. The dry strands were broken into approximately 6 mm lengths, placed into plastic bags that were vacuum sealed, labelled and stored at – 20 °C until required. Animals were fed the test diets a week prior to the initiation of trials in order to ascertain if the prawns readily accepted the diets.

Table 8.1: Percentage ingredients and amino acid composition of the basal and summit diets.

Ingredients	Percentage (%) ingredients in:		Amino Acids	Amino acids to Lysine ratios		
	Basal Diet	Summit Diet		Basal Diet	Summit Diet	Present study values of Prawn tissue
Fish meal ^a	2.5	30.0	<i>Essential</i>			
Casein ^b	0.0	6.5	Arginine	112	112	112
Soybean meal ^c	1.0	0.0	Histidine	39	41	30
Sunflower oilcake ^d	7.0	48.0	Isoleucine	80	82	58
Starch ^e	66.0	6.0	Leucine	124	124	93
Oil ^f (Fish:Sunflower 1:2)	11.5	2.0	Lysine	100	100	100
α - Cellulose ^b	4.5	0.0	Phenylalanine	66	69	58
Vitamin ^{(i)g}	5.0	5.0	Methionine	36	40	33
Mineral mix ^{(ii)g}	2.0	2.0	Threonine	63	65	46
Cholestrol ^h	0.5	0.5	Tyrosine	99	103	43
Total	100.00	100.00	Valine	40	41	62
			<i>Non-essential</i>			
On a dry mass basis (g/100g)			Aspartic	163	167	118
Protein	5.56	50.01	Serine	70	67	41
Fat	4.56	4.62	Glutamic	285	304	175
Ash	4.93	7.98	Proline	84	86	101
Fibre	5.29	5.26	Glycine	92	99	84
Gross Energy (MJ/kg)	17.30	17.27	Alanine	93	97	76

^a Contra Fisheries (Cape Town, South Africa).

^b Sigma (St. Louis, MO, USA).

^c Epol (Pretoria, South Africa).

^d Ngwane Mill (Kwa-Zulu, South Africa).

^e African Products, Lmt (Durban, South Africa).

^f Energy Oils (Durban, South Africa) ratio after Read (1981).

^g Crest Chemicals (Durban, South Africa).

⁽ⁱ⁾ Per kg of feed: Thiamin, 10.5mg; riboflavin, 10mg; pyridoxine, 12.5mg; pantothenic acid, 18.5mg; Niacin, 10mg; Biotin, 0.50mg; inositol, 60mg; choline, 100mg; folic acid, 2.5mg; cyanocobalamine, 0.025mg; ascorbic acid, 250mg; vitamin A, 4200IU; vitamin D, 2500 IU; vitamin E, 300mg; vitamin K, 20mg; Calpan, 50mg; Endox, 25mg.

⁽ⁱⁱ⁾ Per kg of feed: Calcium, 246mg; phosphorus, 80mg; magnesium oxide, 6.31mg; sodium, 60mg; potassium, 90mg; iron, 3mg; copper, 0.34mg; zinc, 1.1mg; manganese, 0.2mg; selenium, 0.05mg; cobalt, 0.14mg; iodine, 0.01mg; ferrous, 0.89mg.

^h Cholestrol, (Salvay Pharmaceuticals, South Africa).

8.2.5. Estimation of food consumption.

Animals were fed, at a rate equivalent to 10 % of the live mass, twice daily with half the ration offered at 08:00 - 09:00 h and the other at 16:00 - 17:00 h. The adjustment of feed rations was done by weighing the animals midway through the trials to ascertain live gains. Using a digital balance (Model: Mettler PM 400) animals were individually weighed to the nearest 0.01 g before being fed their morning ration. In an attempt to reduce stress, mass determinations were done by placing animals, after being dried hastily with blotting paper, into a pre-weighed beaker that contained water similar to that in tanks.

An hour and a half after feeding, uneaten food and faecal matter was collected by siphon, retained on a 63- μ m nylon mesh and rinsed with distilled water to remove adhering salts. After removing faecal strands the mass of the feed residue, for a given group, was determined by transferring the residue to pre-weighed aluminium foils, oven dried at 70 °C for 48 hours, placed into a desiccator and re-weighed. Whenever animals were removed for weighing, the plastic tubs were scrubbed clean to remove any adhering residue and bacterial growth that might have accumulated on the sides of the tanks. During morning inspections mortalities were recorded, carcasses removed and examined to determine the moult stage. Prior to the stocking of tanks thirty prawns, set aside for tissue analysis, were killed by submerging the animals into ice cold water for 10 minutes, thereafter the animals were removed, blotted dry and placed into a labelled plastic bag that was vacuum sealed and stored at - 20 °C. Similarly on termination of each trial, all surviving prawns from each treatment were slaughtered and frozen until dissection. Dissection procedures followed those described in paragraph 4.2.7.

Prior to trials losses incurred due to siphoning, expressed as a percentage, were determined in triplicate and averaged for each diet. This was done by submersing pellets, of known mass, into the tanks which were void of animals. Fifteen minutes thereafter the pellets were siphoned onto a 63- μ m nylon mesh, rinsed with distilled water, transferred to pre-weighed aluminium foils and left to dry over-night in an oven. For a specific diet the percentage lost due to siphoning was established by dividing the dry mass of the residue by the dry mass of the pellets. This provided a factor that was used to establish a close approximation for the food not eaten.

8.2.6. Proximate chemical analysis.

Tissue samples from each treatment were freeze dried, ground, homogenised and two sub-samples from each tank were used for proximate analysis of crude protein, crude fat, ash and moisture content (AOAC, 1990). Determination of exoskeleton lipid was done using a pooled sample from replicated tanks since exoskeleton material derived from animals within a single tank was insufficient for analysis. Analyses of body fractions were in accordance with procedures explained in paragraph 4.2.8.

8.2.7. Determination of food indices.

The mean growth rate (MGR), specific food consumption (SFC), average food conversion ratio (FCR) and protein efficiency ratio (PER) (Gopal & Raj, 1990) were calculated using the following:

$$\text{MGR (g/day)} = (\text{Final average mass} - \text{Initial average mass}) \div (\text{number of days animals were fed}) \quad [8.4]$$

$$\text{SFC (\%)} = [(\text{total dry mass of food offered} - \text{total dry mass of food uneaten}) \div (\text{number of animals surviving at the end of experiment} \times \text{experimental period (days)} \times \text{mean animal wet mass in grams})] \times 100 \quad [8.5]$$

$$\text{FCR} = (\text{Average mass of food consumed in dry weight} \div \text{average gain in live mass}) \quad [8.6]$$

$$\text{PER} = (\text{Final wet mass of prawns} - \text{Initial mass of prawns}) \div (\text{total protein intake}) \quad [8.7]$$

Mean gains in body protein and lipid mass were calculated as follows:

$$\text{Growth (mg/prawn)} = M_f - M_i \quad [8.8]$$

$$\text{PG}_f(\text{mg/prawn}) = M_f \times (1 - W_f) \times P_f - M_i \times (1 - W_i) \times P_i \quad [8.9]$$

or

$$\text{PG}_e(\text{mg/prawn}) = M_f \times (1 - W_f) \times P_f - M_i \times (1 - W_i) \times P_i \quad [8.10]$$

where PG = protein gain in either the flesh (f) or exoskeleton (e) tissue (mg)
 M_i = initial mass (mg)
 M_f = final mass (mg)
 P_i = initial protein mass (mg)
 P_f = final protein mass (mg)
 W_i = initial moisture level (%)
 W_f = final moisture level (%)

Similarly by substituting protein with body fat content, lipid gains and/or losses were determined using the above equations. Results were interpreted by fitting non-linear regression to these data.

8.2.8. Determination of Protein requirement.

The minimum requirement for protein was established using the broken-line model, which in principle was similar to the method used by Fisher, Johnson and Leveille (1957). A regression line ($Y = aX + b$) was fitted to the ascending portion of the response data. The position of the horizontal line, parallel to the x-axis, was established by averaging the highest observed means that did not differ statistically from each other as determined by the analysis of variance. The intercept between these two lines was regarded as an estimate of the protein requirement.

8.2.9. Determination of protein intake.

Optimum protein intake was established using the Reading model. The method described by Curnow (1973) in conjunction with Microsoft Excel Solver version 97, was used to establish the best fit, a and b constants and the optimum daily dose.

8.2.10. Statistical analysis.

Regression analyses were used to test for differences between the various mass classes. All estimates of nutritional indices *i.e.* SFC, FCR and PER were regarded as apparent and no correction factor was introduced for the exuviate and dead prawns eaten by cohabitants during this study. A two-tailed t test was used to compare initial and final mass of experimental animals. Results of body fractions, expressed as a percentage of dry matter, were subjected to one-way analysis of variance (ANOVA) with differences between means ($P < 0.05$) being tested using Tukey's multiple range test in accordance with Zar (1996).

8.3. Results.

8.3.1. Initial and final mass.

The stocking mass and final mass for the three mass classes are given (Table 8.2). In all cases body mass was influenced by an increase in dietary protein with the final mass of 7 g prawns fed 5 % CP weighing significantly less ($q = 5.422$, $q_{(0.05, 4.7)} = 4.449$; $P < 0.05$) than those given 32 % CP.

Table 8.2: Initial mass and final mass of *F. indicus* fed diets varying in protein content during a series of 30-day growth trials. Values represent means ($n = 3$) with standard error about the mean (SEM) in parenthesis.

Parameter	Mass [†] Class	Dietary Protein Level (%)					
		5	14	23	32	41	50
Initial Wet Mass (g)	2	1.68 (± 0.14)	1.77 (± 0.13)	1.78 (± 0.12)	1.70 (± 0.13)	1.73 (± 0.08)	1.71 (± 0.14)
	7	7.27 (± 0.16)	7.37 (± 0.17)	7.27 (± 0.16)	7.72 (± 0.16)	7.53 (± 0.21)	7.44 (± 0.21)
	14	14.35 (± 0.36)	14.10 (± 0.24)	14.44 (± 0.48)	14.46 (± 0.27)	14.29 (± 0.41)	14.36 (± 0.25)
Final Wet Mass (g)	2	2.05 (± 0.28)	2.42 (± 0.18)	2.50 (± 0.29)	2.71 (± 0.24)	2.50 (± 0.22)	2.84 (± 0.15)
	7	7.52 ^a (± 0.39)	7.76 ^{ab} (± 0.62)	7.94 ^{ab} (± 0.73)	8.43 ^b (± 0.13)	8.22 ^{ab} (± 0.47)	8.04 ^{ab} (± 0.47)
	14	14.37 (± 0.36)	14.14 (± 0.20)	14.83 (± 0.46)	14.81 (± 0.25)	14.80 (± 0.43)	14.67 (± 0.26)

[†] 2, 7, 14 represent the three different mass classes with means of 1.73, 7.34 and 14.33 g, respectively.

^{ab} Components with the same superscripts indicate that mean values within a row are not significantly ($P > 0.05$) different.

Differences between the initial and final mean mass were significant for 2 g ($t = 7.07$, $t_{(0.05 (2) 5)} = 2.571$; $P < 0.05$), 7 g ($t = 7.81$, $t_{(0.05 (2) 5)} = 2.571$; $P < 0.05$) and 14 g ($t = 3.42$, $t_{(0.05 (2) 5)} = 2.571$; $P < 0.05$) prawns.

8.3.2. Growth rates.

A curvilinear relationship, $y = a - bC^{-x}$, was descriptive of the growth rate in 2 and 7 g *F. indicus* with each having a goodness of fit (R^2) equivalent to 0.97 and 0.99, respectively (Figure 8.3). In contrast the growth response of 14 g animals was described by a parabolic curve obtained from the second order quadratic equation in the form of $y = a + bx + cx^2$ ($R^2 = 0.93$). Amongst 2 and 7 g prawns results indicated a concomitant increase in growth with an increase in dietary protein. In contrast results of 14 g animals indicated that a further increase in dietary protein above the 41 % level led to a reduction in growth rates. Growth rates were highest in 2 g followed by 7 and 14 g prawns.

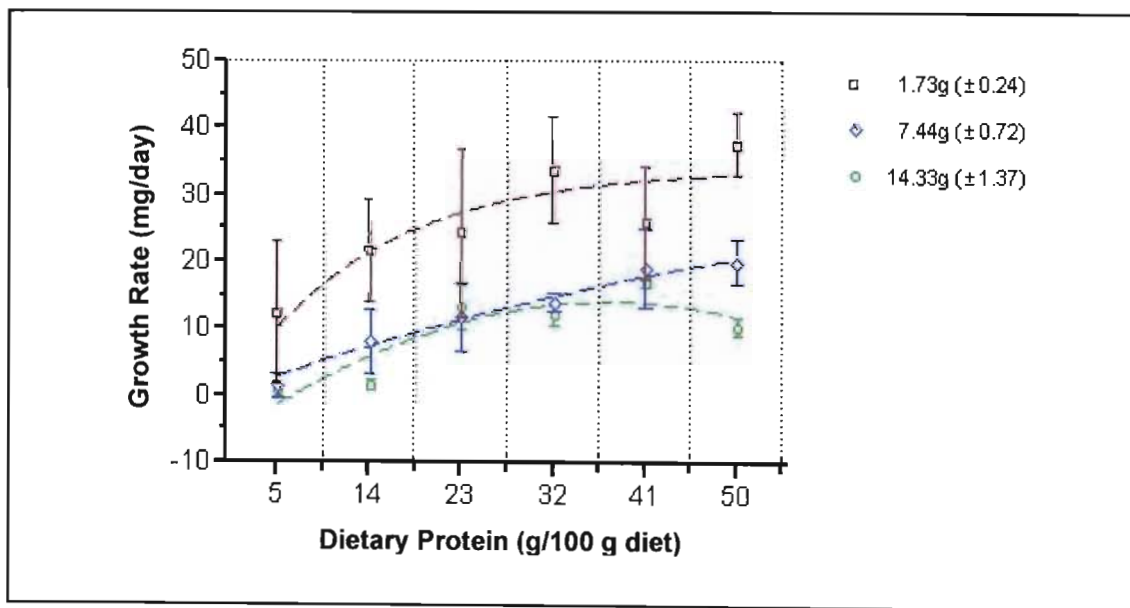


Figure 8.3: The growth rate (mg/day) of juvenile *F. indicus* fed different dietary concentrations of protein. Values are means ($n = 3$) with bars indicating standard error about the mean (SEM). Parentheses indicate standard deviation about the mean mass.

8.3.3. Survival.

A curvilinear relationship of the form, $y = a - bC^{-x}$ was used to describe survival ($R^2 > 0.97$). Survival was low for groups fed less than 23 % CP. Conversely survival became asymptotic with fed concentrations greater than 23 % CP. The highest survival of 83 % was recorded for 2 g animals fed the summit diet while the lowest, 27 %, occurred amongst 14 g prawns fed 14 % CP. In general, survival was shown to decrease with age (Figure 8.4).

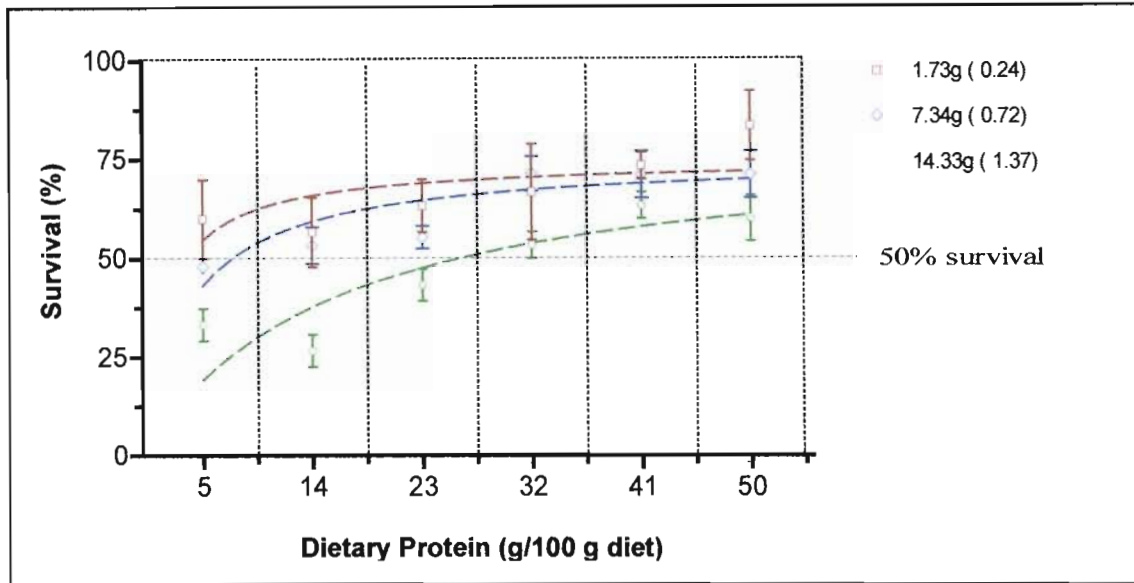


Figure 8.4: Survival of juvenile *F. indicus* fed diets varying in crude protein. Values are means ($n = 3$) with bars indicating standard error about the mean (SEM). Parentheses indicate standard deviation about the mean mass.

8.3.4. Specific food consumption (SFC).

A second order polynomial regression in the form of $y = a + bx + cx^2$ was descriptive of the SFC in all mass classes with R^2 of 0.91, 0.93 and 0.90 for 2, 7 and 14 g, respectively (Figure 8.5).

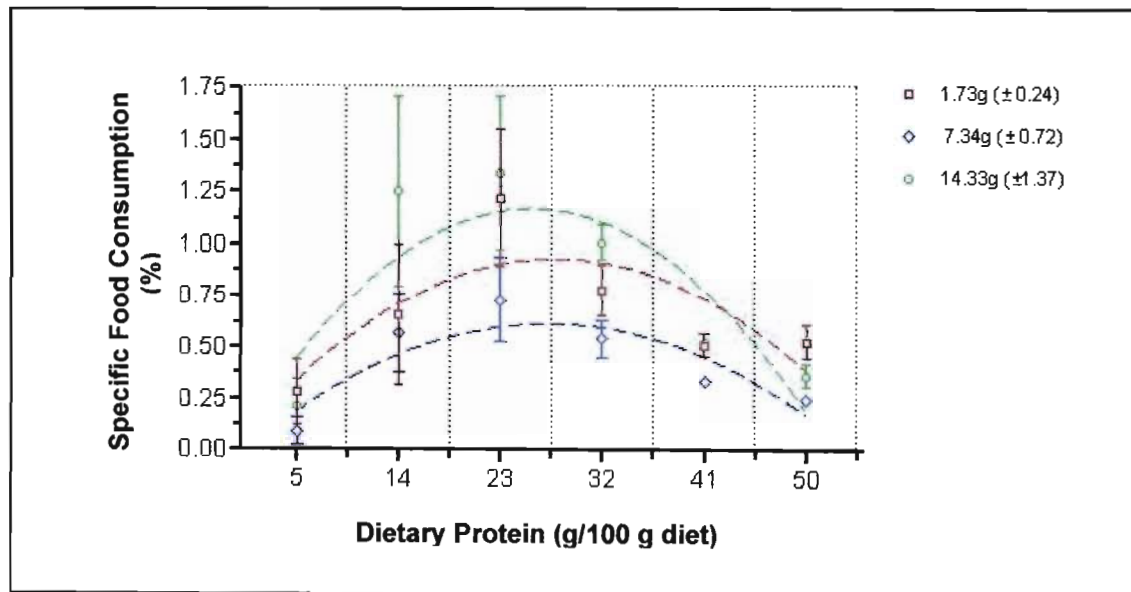


Figure 8.5: Specific food consumption of juvenile *F. indicus* fed diets varying in crude protein. Values are means ($n = 3$) with bars indicating standard error about the mean (SEM). Parentheses indicate standard deviation about the mean mass.

In all cases maximum consumption was reached between 23 - 32 % CP with further increases in dietary protein leading to a substantial decrease in SFC. The highest consumption

was noted in 14 g animals followed, in decreasing order, by 2 and 7 g prawns respectively. In all cases the percentage SFC fell well below the daily ration, equivalent to 10 % live mass.

8.3.5. Feed conversion ratio (FCR).

This parameter was used to express the efficiency with which the diet is converted into body tissue. The relationship of the form $Y = Y_0 \cdot e^{-kx}$ described the feed conversion ratio (FCR) since ratios decreased exponentially with an increase in dietary protein (Figure 8.6). R^2 values of 0.89, 0.55 and 0.62 were found for 2, 7 and 14 g prawns respectively, however, when measurements on 5 % protein were excluded R^2 values improved to > 0.98 in all cases.

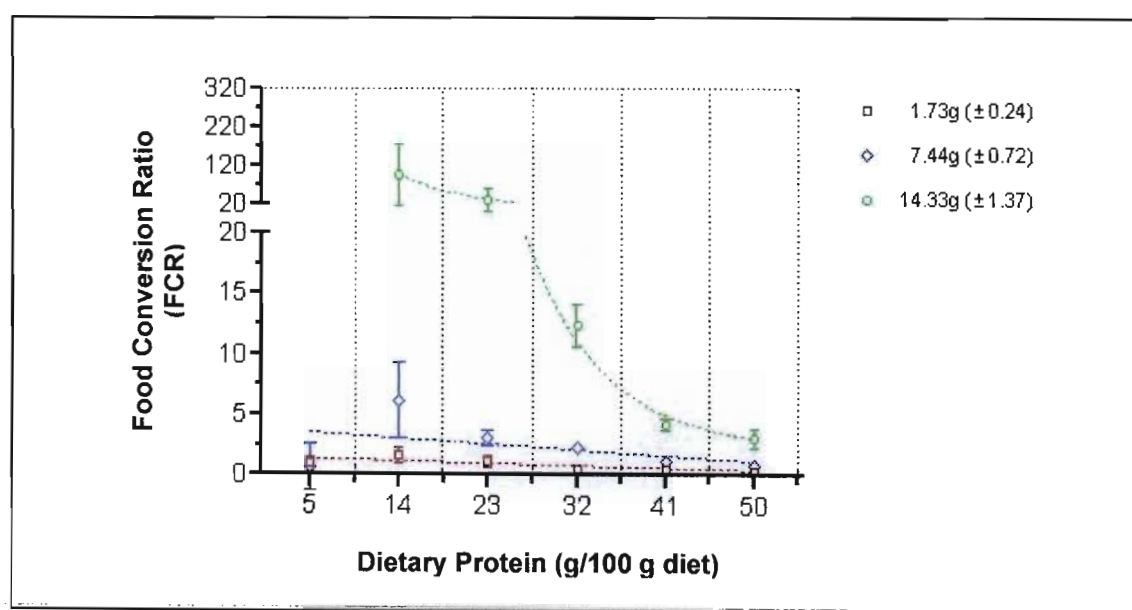


Figure 8.6: The mean food conversion ratio (FCR) of juvenile *F. indicus* fed diets varying in crude protein content. Values are means ($n = 3$) with bars indicating standard error about the mean (SEM). Parentheses indicate standard deviation about the mean mass.

Moreover, FCR for groups fed similar diets increased with age since the lowest value of 0.39, recorded for 2 g animals fed 50 % CP, increased to 0.75 and 3.02 for 7 and 14 g prawns, respectively. Except for animals fed 5 % CP, feed ratios were exceedingly higher ($P > 0.05$) amongst 14 g prawns as opposed to those for 2 and 7 g animals.

8.3.6. Protein efficiency ratio (PER).

Protein efficiency ratio (PER) is defined as the ratio of grams gained per gram protein consumed and is considered, theoretically, to be more precise than estimates of gross diet efficiency since the dietary protein consumed is the only item of the diet that is taken into account (Zeitoun, Halver, Ullrey & Tack, 1973). The response amongst 2, 7 and 14 g prawns was that the PER decreased exponentially with an increase in dietary protein ($R^2 > 0.98$).

Similarly PER for groups fed the same diet decreased with age (Figure 8.7). Amongst 7 and 14 g prawns, efficiency ratios were comparatively similar between treatments ($P > 0.05$).

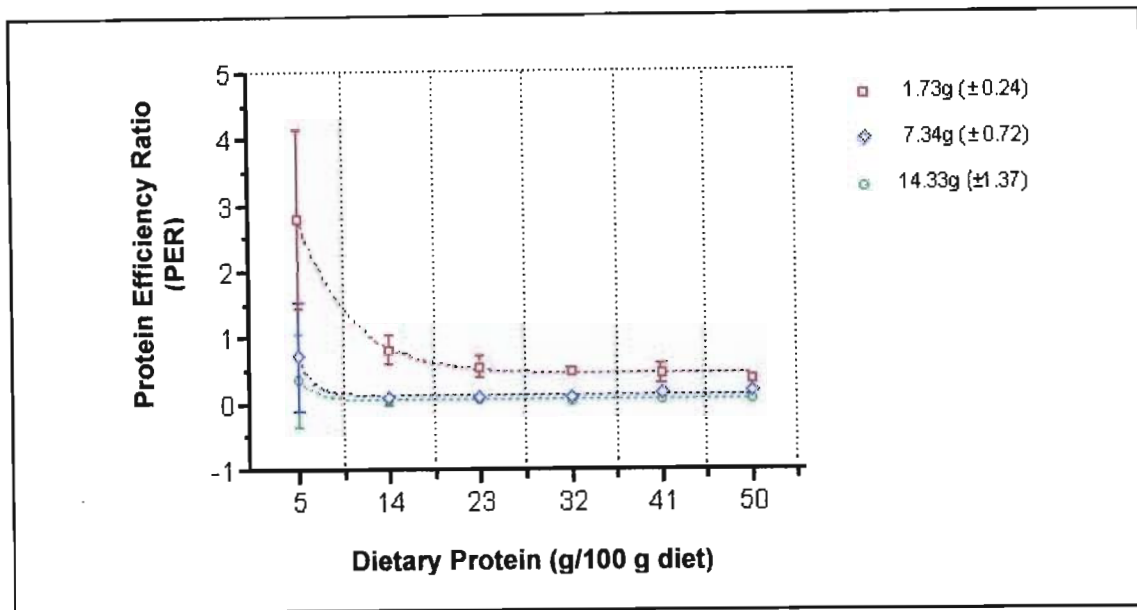


Figure 8.7: Mean protein efficiency ratio (PER) for juvenile *F. indicus* fed different dietary concentrations of protein. Values are means ($n = 3$) with bars indicating standard error about the mean (SEM). Parentheses indicate standard deviation about the mean mass.

8.3.7. Chemical composition of flesh and exoskeleton

The chemical constituents in the body tissues of the prawn are given (Table 8.3). Compared to initial values the moisture content in the separate tissues between groups of each mass class was unaffected by dietary protein. For all groups flesh moisture was considerably higher when compared to that found in exoskeleton tissue.

Compared to an initial mean of 3.3 %, expressed as a percentage of dry mass, dietary protein did not influence the deposition of fat in the flesh of 2 g prawns. Similarly, compared to initial values, crude fat in the flesh of 7 and 14 g prawns remained relatively unchanged. Body lipid decreased with age with lipid in the flesh of 2 g prawns found to be significantly higher ($F = 49.49$; $df = 2, 15$; $P < 0.001$) when compared to 7 and 14 g prawns.

Compared with the initial content the percentage protein in the flesh of 7 g animals were significantly different between groups fed 5 % and 50 % CP ($q = 6.25$, $q_{(0.05, 7-8)} = 2.92$; $P < 0.01$). The percentage flesh protein in prawns, fed similar diets, was significantly different between mass classes ($F = 24.21$; $df = 2, 15$; $P < 0.0001$).

Between treatments the percentage protein in the exoskeleton was significant between 2 g ($F = 6.15$; $df = 6, 14$; $P < 0.01$) and 7 g ($F = 7.03$; $df = 6, 14$; $P < 0.01$) prawns. The percentage differences in exoskeleton protein in prawns fed similar diets was significant between 2 and 7 g animals ($q = 4.39$, $q_{(0.05, 2-13)} = 3.06$; $P < 0.05$). In all cases flesh protein levels were considerably higher than those in the exoskeleton.

Table 8.3: The composition of moisture, lipid and protein expressed as percentages in the flesh and exoskeleton tissue of three separate mass classes of *F. indicus*, which were fed six test diets that varied in protein content. Exoskeleton to flesh ratios are provided. With the exception of exoskeleton lipid, values represented are means ($n = 3$) with standard deviation in parenthesis.

Body Component	Size [†]	Tissue [§]	Dietary Protein Level (%)						Initial* content of animals stocked
			5	14	23	32	41	50	
Moisture (%)	2	F	75.14 (± 0.60)	75.12 (± 1.43)	75.30 (± 1.44)	77.88 (± 2.24)	76.19 (± 0.09)	76.13 (± 3.57)	74.96 (± 0.61)
		E	60.53 (± 2.95)	61.79 (± 0.37)	60.76 (± 2.00)	62.07 (± 0.71)	59.69 (± 2.14)	60.82 (± 4.73)	60.54 (± 0.50)
	7	F	76.35 (± 0.65)	75.79 (± 1.02)	75.38 (± 0.85)	75.00 (± 1.65)	75.36 (± 0.97)	75.48 (± 2.05)	75.26 (± 0.27)
		E	62.75 (± 0.81)	62.36 (± 0.88)	62.72 (± 1.35)	62.99 (± 2.00)	62.63 (± 1.75)	61.82 (± 2.23)	62.25 (± 0.24)
	14	F	75.46 (± 0.57)	74.73 (± 0.68)	75.85 (± 0.61)	75.25 (± 0.86)	75.39 (± 0.69)	75.56 (± 0.78)	75.88 (± 0.53)
		E	59.67 (± 0.68)	59.24 (± 0.77)	62.05 (± 1.05)	60.61 (± 2.72)	64.86 (± 1.34)	58.88 (± 2.26)	59.24 (± 0.65)
Lipid (%) [¶]	2	F	2.95 _x (± 0.24)	3.61 _x (± 0.27)	3.01 _y (± 0.31)	3.11 _y (± 0.38)	3.74 _y (± 0.41)	3.53 _y (± 0.44)	3.33 (± 0.34)
		E*	2.82	3.74	3.21	3.59	4.43	5.37	2.46 (± 0.54)
	7	F	2.35 (± 0.32)	2.38 (± 0.28)	2.27 _x (± 0.34)	2.33 _{xy} (± 0.43)	2.40 _x (± 0.39)	2.15 _x (± 0.49)	2.65 (± 0.41)
		E*	2.09	2.09	1.81	2.18	2.02	2.04	2.28 (± 0.37)
	14	F	2.28 (± 0.19)	2.26 (± 0.22)	2.34 _x (± 0.26)	2.23 _x (± 0.37)	2.36 _x (± 0.33)	2.21 _x (± 0.45)	2.10 (± 0.24)
		E*	2.06	2.03	1.91	2.15	2.14	1.98	2.15 (± 0.22)
Protein (%) [¶]	2	F	81.55 _a (± 2.90)	82.37 _a (± 2.52)	83.23 _a (± 0.67)	84.75 _b (± 0.24)	83.16 _{ab} (± 2.16)	86.28 _{ab} (± 3.87)	83.36 _{ab} (± 0.98)
		E	43.74 _a (± 0.67)	44.90 _{xy} (± 1.46)	46.48 _{xy} (± 1.14)	38.95 _b (± 0.48)	41.70 _{ab} (± 3.42)	43.52 _{ab} (± 1.20)	43.12 _{ab} (± 1.51)
	7	F	85.59 _{ab} (± 1.85)	87.56 _{ab} (± 1.59)	86.66 _{ab} (± 0.67)	87.42 _{ab} (± 0.45)	89.22 _{ab} (± 1.22)	90.05 _b (± 1.34)	87.65 _{ab} (± 0.95)
		E	43.42 _{abcd} (± 0.23)	46.93 _{bcd} (± 0.69)	48.65 _{cd} (± 0.98)	46.97 _{bcd} (± 0.47)	45.50 _{abcd} (± 2.65)	49.07 _{abc} (± 0.84)	46.36 _{abcd} (± 1.20)
	14	F	82.54 _x (± 1.47)	81.90 _x (± 1.35)	82.43 _x (± 0.41)	82.46 _x (± 0.31)	83.13 _x (± 0.98)	82.64 _x (± 0.59)	82.44 _x (± 0.58)
		E	45.02 (± 0.18)	44.87 _x (± 0.22)	44.61 _x (± 0.61)	43.44 _y (± 0.40)	42.88 (± 1.69)	44.09 (± 0.40)	44.96 (± 0.76)
Exoskelton: Flesh Ratio	2	E:F	3.20 (± 0.36)	3.06 (± 0.20)	2.74 ^a (± 0.10)	3.53 (± 0.44)	3.02 (± 0.21)	3.43 (± 0.14)	3.16 (± 0.21)
	7	E:F	3.04 (± 0.24)	3.13 (± 0.35)	2.61 (± 0.92)	3.72 (± 0.19)	2.72 (± 0.55)	4.11 (± 0.36)	3.13 (± 0.53)
	14	E:F	3.33 (± 0.15)	3.58 (± 0.52)	2.98 (± 0.29)	3.52 (± 0.12)	3.53 (± 0.42)	3.15 (± 0.18)	3.47 (± 0.31)

- † 2, 7, 14 represents small, intermediate and medium mass classes signifying 1.73g, 7.34g and 14.33g animals respectively.
 ¶ On a dry mass basis.
 § F and E indicate flesh and exoskeleton, respectively.
 a,b,c,d Means in each row sharing the same letter are not significantly different based on Tukey test ($P < 0.05$).
 x,y,z Means in columns for a given body faction and body tissue associated with all three mass classes that share the same letter are not significantly different based on Tukey test ($P < 0.05$).
 * Insufficient material for analysis.

Besides 2 g animals fed 32 CP diets, exoskeleton to flesh (E:F) ratios between groups were similar when compared to initial values for each mass class. Differences of E:F ratios between mass classes were not significant ($P > 0.05$).

8.3.8. Protein gains in the flesh.

A curvilinear relationship of the form, $y = a - bC^{-x}$ described protein gains in the flesh of all mass classes with R^2 values equivalent to 0.97, 0.95 and 0.97 for 2, 7 and 14 g prawns, respectively. Although protein gains in the flesh of 2 g juveniles increased with an increase in dietary protein, no significant differences ($P > 0.05$) were found to occur between groups. Alternatively, as opposed to groups fed the basal diet, protein gains in the flesh of 7 g animals were significantly higher ($P < 0.05$) for groups reared on the summit diet (Figure 8.8).

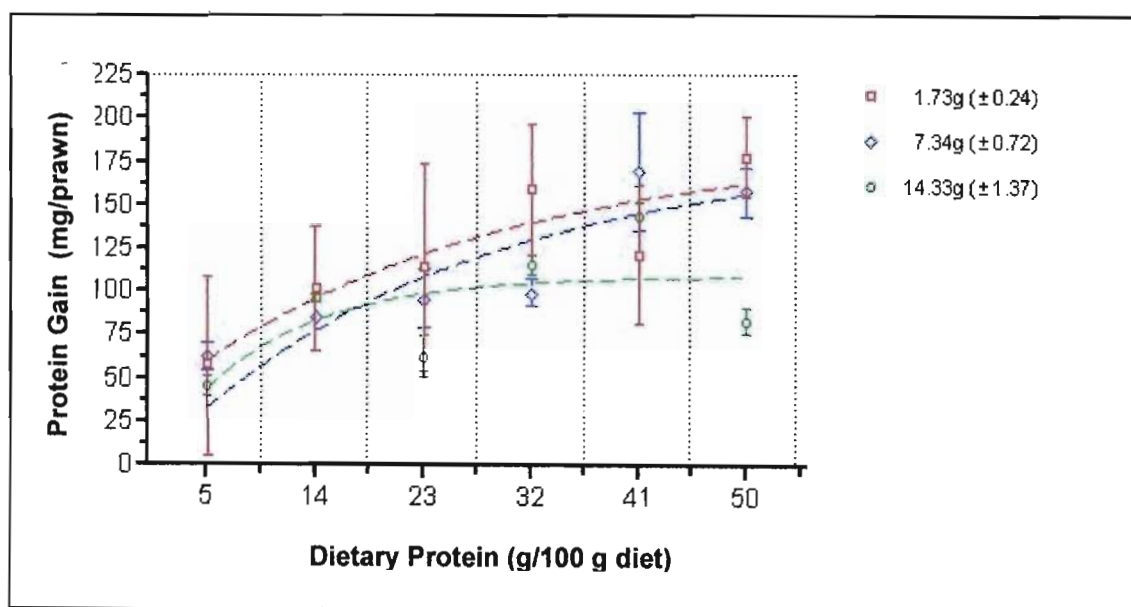


Figure 8.8: The effect of dietary protein on protein gains in the flesh of juvenile *F. indicus*. Values are means ($n = 3$) with bars indicating standard error about the mean (SEM). Parentheses indicate standard deviation about the mean mass.

An asymptotic rate of protein deposition occurred at a lower dietary protein content in 14 g (14 % CP) than in 2 and 7 g animals. Protein gain in flesh decreased with age (Figure 8.8).

8.3.9. Protein gain in the exoskeleton.

A curvilinear relationship of the form, $y = a - bC^{-x}$ was used to describe protein gains in the exoskeleton of 2 and 14 g prawns, with R^2 equivalent to 0.97 and 0.89, respectively whereas a one phase exponential association of the form $Y = Y_{\max} \times (1 - e^{-K \times X})$ described gains in 7 g animals ($R^2 = 0.99$) (Figure 8.9). In all cases protein gains in the exoskeleton were influenced by dietary protein. Protein gains in the exoskeleton of 2 g animals, although not significant amongst groups, were significantly higher ($F = 7.93$; $df = 5, 15$; $P < 0.001$) when compared to those in 7 and 14 g animals. Protein gains amongst groups fed similar diets decreased with age (Figure 8.9).

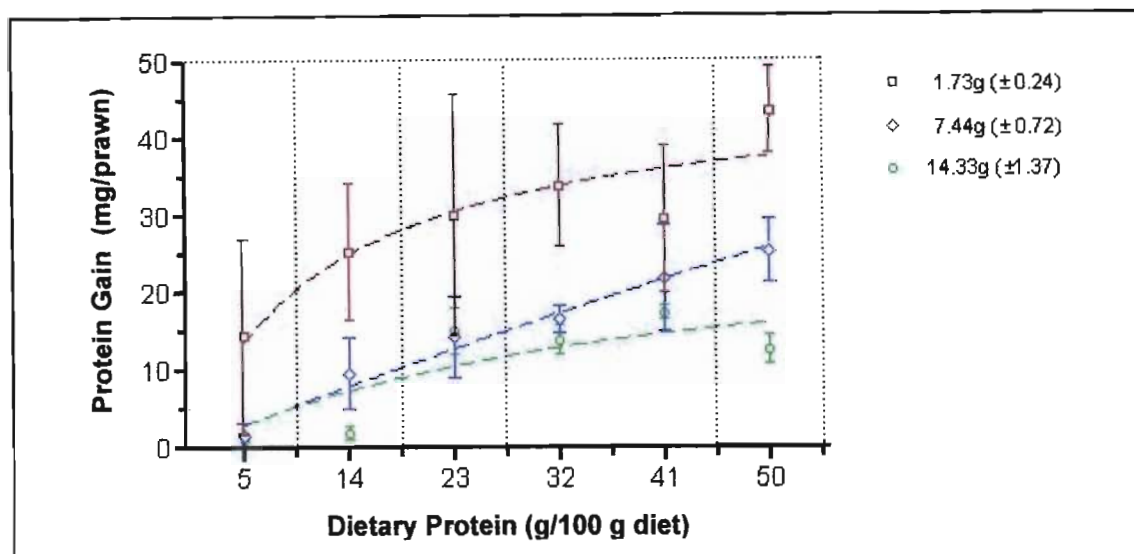


Figure 8.9: The effect of dietary protein on protein gain in the exoskeleton of juvenile *F. indicus*. Values are means ($n = 3$) with bars indicating standard error about the mean (SEM). Parentheses indicate standard deviation about the mean mass.

8.3.10. Lipid content.

In most cases lipid content in the flesh of the prawn provided no clear trend (Table 8.4), and was not significantly different ($P > 0.05$) amongst groups in 2 g prawns. However, in the flesh of 7 g prawns a significant loss of body fat occurred between groups ($F = 9.74$; $df = 5, 12$; $P < 0.001$). In 14 g animals, flesh lipid content differed significantly between groups ($F = 68.72$; $df = 5, 12$; $P < 0.0001$). In the exoskeleton of 2 g juveniles the lipid content increased with dietary protein ($P > 0.05$) (Table 8.4). The same occurred in 7 g ($F = 6.43$; $df = 5, 12$; $P < 0.01$) and 14 g prawns ($F = 5.46$; $df = 5, 12$; $P < 0.0001$).

Table 8.4: Fat gains and losses (mg/prawn) in the flesh (F) and exoskeleton (E) of juvenile *F. indicus* fed diets varying in protein content. Values in parentheses indicate standard deviation about the mean ($n = 3$).

Size [†]	Tissue [§]	Dietary Protein (%)					
		5	14	23	32	41	50
2	F	0.37 _y (± 0.48)	1.15 _y (± 0.55)	0.65 _y (± 0.76)	0.75 _y (± 0.43)	1.29 _y (± 0.61)	1.56 _y (± 0.33)
	E	0.93 _y (± 1.41)	2.11 _y (± 1.29)	2.08 _x (± 1.87)	3.12 _y (± 1.27)	3.13 _x (± 1.76)	5.38 _z (± 1.19)
7	F	-2.71 ^a _x (± 0.21)	-2.14 ^{ab} _x (± 0.18)	-2.27 ^{ab} _x (± 0.22)	-1.97 ^{bc} _x (± 0.15)	-1.41 ^c _x (± 0.21)	-2.42 ^{ab} _x (± 0.41)
	E	0.08 ^a _x (± 0.14)	0.31 ^{abc} _x (± 0.08)	0.37 ^{abc} _x (± 0.27)	0.47 ^{abc} _x (± 0.16)	1.15 ^c _x (± 0.55)	1.05 ^b _y (± 0.30)
14	F	8.71 ^a _z (± 0.88)	10.55 ^b _x (± 0.23)	6.31 ^c _z (± 0.08)	9.43 ^{ab} _x (± 0.02)	11.60 ^b _z (± 1.19)	3.35 ^d _z (± 0.39)
	E	0.05 ^a _x (± 0.09)	0.09 ^b _x (± 0.06)	0.61 ^c _x (± 0.22)	0.72 ^c _x (± 0.16)	0.81 ^c _x (± 0.07)	0.58 ^c _x (± 0.14)

[†] 2, 7, 14 indicates small, intermediate and medium mass classes and signifies 1.73g, 7.34g and 14.37g animals respectively.

^{a,b,c} Means in each row sharing the same letter are not significantly different based on Tukey test ($P < 0.05$).

^{z,y,z} Means in each column for a specific body tissue sharing the same letter are not significantly different based on Tukey test ($P < 0.05$).

Flesh lipid differed significantly amongst mass classes ($F = 54.48$; $df = 2, 15$; $P < 0.0001$) as did exoskeleton lipid ($F = 11.75$; $df = 2, 15$; $P < 0.001$). Lipid in the exoskeleton decreased with age while increasing in the flesh of the prawn.

8.3.11. Determination of protein requirement.

Minimum requirements in the flesh, exoskeleton and live mass gains in 2, 7 and 14 g prawns are given (Table 8.5).

Table 8.5: The dietary protein requirements based on protein and wet mass gains in the flesh (F) and exoskeleton (E) and overall live mass gains in *F. indicus*. Mean values ($n = 3$) and standard error about the mean (SEM), in parenthesis, are indicated.

Mass Class [†]	Body tissue [§]	Dietary Protein Requirement (%)		
		Protein gains ^a	Wet mass gains ^b	Live mass gains ^c
2	F	41.16 (0.07)	41.01 (1.71)	41.14 (2.87)
	E	41.00 (0.00)	41.00 (0.15)	
7	F	34.01 (0.01)	33.99 (0.30)	38.99 (0.48)
	E	34.00 (0.00)	34.00 (0.03)	
14	F	34.00 (0.02)	34.00 (0.19)	34.01 (0.31)
	E	34.00 (0.00)	34.00 (0.00)	

[†] 2, 7, 14 indicates small, intermediate and medium mass classes and signifies 1.73, 7.34 and 14.33 g animals respectively.

^a Values are based on a dry matter base in each body component.

^b Values are based on the wet mass for each of the body components.

^c Values are based on the wet and/or live mass of animals.

In 2 g prawns the requirements established using protein and wet mass gains for each body tissue were similar when compared to overall live mass gains. Whereas in 7 g animals the protein requirement, established using protein and wet mass gains in each component, was lower but not significantly different ($P > 0.05$) from those based on live mass gains. The protein requirement for 14 g animals was found to be similar when using all three measures. Results indicated a lower requirement for dietary protein with age.

8.3.12. Determining the optimum protein intake.

The variables used to establish the optimum protein requirement in each mass class, using the Reading model, are given (Table 8.6). Results were based on the initial mass and accretion of body protein. The optimum protein dosage was calculated to be 18, 46 and 152 mg/day for 2, 7 and 14 g prawns, respectively (Table 8.6) [Appendix F: Figures 1- 3]. Results indicated a higher intake with age.

Table 8.6: The optimum daily allowance for dietary protein based on the accretion of body protein and the initial overall protein mass for *F. indicus* fed various protein concentrations. Mean values ($n = 3$) with standard deviation about the mean (SD) in parenthesis for protein gains and body protein mass are given. Constants a and b and the optimum dosage were calculated using the Reading model.

Mass Class [†]	Mean Gains in Protein [§] (mg)	Mean Body Protein Mass (mg)	Constants				Optimum Protein Requirement (mg/prawn day)
			$a^§$	$b^§$	x^*	rw	
2	5.03 (± 3.06)	1728 (± 121)	1.56	0.005	0.30	0.00	17.54
7	4.41 (± 1.78)	7333 (± 368)	10.52	0.0000001	0.30	0.50	46.39
14	3.36 (± 1.33)	14331 (± 1023)	45.52	0.0000001	0.30	0.50	151.57

[†] 2, 7, 14 indicates small, intermediate and medium mass classes and signifies 1.73, 7.34 and 14.33 g animals respectively.

[§] Values were established using solver.

* Values are based on $k = 0.24$.

8.4. Discussion.

8.4.1. Differences between stocking and harvest mass.

Growth increments amongst older prawns were less noticeable than those for 2 g animals (Figure 8.3). The magnitude by which growth took place was attributed to the growth response of the animal with rapid gains occurring during the early stages of development.

8.4.2. The effect of dietary protein on growth.

Due to the high variability, differences in mean growth rates between protein treatments amongst 2 g juveniles were not significant (Table 8.2). Dietary protein influenced growth in 7 and 14 g animals, with the latter displaying the least variability within treatments. A decrease in variability with age was possibly due to the normal distribution about the mean decreasing over time whereas variability within tanks was attributed to sexual dimorphism.

Diets high in protein require more energy for the added mechanics of digestion, metabolism of amino acids, the synthesis of nitrogenous waste products and the process whereby these products are concentrated and excreted (Hewitt & Irving, 1990) and was the probable cause for the cessation of growth in 14 g prawns fed 50 % CP.

In general growth rates recorded in this study, as opposed to those monitored in culture ponds, were poor. In the previous study the growth rate in 2, 7 and 14 g prawns fed 43 % CP and reared outdoors in an earthen pond for the same period was 0.166, 0.122 and 0.146 g/day, respectively whereas those of equivalent mass and fed a similar protein concentration (41 % CP) was found to be 0.025, 0.019 and 0.018 g/day, respectively. These discrepancies were, in part, due to differences in the feeding regime since laboratory animals were fed twice, as opposed to four times a day as done on farms. Growth constraints amongst laboratory animals were either

due to spatial and/or nutritional factors since, spatial constraints aside, Shishenchian and Yusoff (1999) found that edible benthic organisms, hypothesised to contribute significantly towards the nutrition of cultured prawns, decreased under intensive conditions due to predation by prawns. , In this study the laboratory set-up was designed to exclude such resources.

8.4.3. Dietary protein and survival.

Low survival amongst 7 and 14 g animals was speculated to be due to an inadequate acclimation period. The handling of prawns during weighing and a restriction of space, considering that 14 g animals were confined to the same space as 2 g animals, were also thought to negatively influence growth and survival since larger individuals amongst 7 and 14 g animals were aggressive towards cohabitants when competing for bottom space.

Furthermore, crustaceans kept in small enclosures are more susceptible to being cannibalised since, immediately after moulting, they become incapacitated due to muscular contractions, flexing and swimming prior to and during ecdysis (Dall *et al.*, 1990). In this study prawns often preyed upon those that moulted prematurely since carcasses, after having been examined, were found ($\approx 90\%$) to be in the early post-moult phase. Cannibalism was evident in all groups but the frequency and extent thereof was noticeably greater in groups fed 41 and 50 % CP with the whole carcasses or a large portion thereof, with only remnants of the cephalothorax and/or abdomen remaining, eaten. In contrast carcasses in tanks allotted 5 % CP were partially cannibalised with only the eyes, pleopods or both consumed. Cannibalism was least prevalent amongst groups fed 5 - 32 % CP, which was counterintuitive since animals fed protein deficient diets were expected to resort to such behaviour.

Despite diets being isocaloric, animals reared on $\leq 14\%$ CP were less active as opposed to those fed $\geq 23\%$ CP. Moreover, it was observed that most prawns fed $\leq 14\%$ CP succumbed after moulting. Such occurrence was an indication that protein deficient diets were inefficient at providing nutrients to replace essential body tissue catabolised to maintain vital body functions and for the synthesis of new tissue, including that required for moulting and ultimately reproduction. Moulting requires a high nutrient demand since body protein in juvenile *F. indicus* was found to increase by about 29 % between stages B and D₂ (Read & Caulton, 1980) whereas ecdysis was found to utilise $\sim 26\%$ of the total energy retained during the intermoult period (Read & Caulton, 1980). It would therefore seem that in order to maintain a survival $\geq 50\%$, 7 and 14 g prawns require no less than 14 and 32 % CP, respectively in their diets.

Vijayan and Diwan (1995) found pH 7.8 – 8.2 ideal for moulting. Morning measurements of pH in this study were well within this range but unlike culture ponds where CO₂ in solution is removed by algae in suspension the effects, due to the absence thereof, was not measured. Therefore it is suggested that in conjunction with parallel trials in outdoor ponds, future investigations should consider rearing the animal for longer periods in larger holding

facilities under laboratory conditions as done by Thomas *et al.* (1991) and Jayaprakas and Sambhu (1996). A possible means of alleviating territorial behaviour would be to examine a single sex, with preference towards faster growing females.

8.4.4. The effect of dietary protein on food consumption.

The amount of food an animal consumes depends on the requirements of the animal, the nutrient content and gut capacity. The lower the concentration of nutrients the more food the animal will need to eat to satisfy its requirement, with energy cost of processing the food being reduced. The converse is true for higher nutrient density diets (Emmans, 1995). In a study involving pigs the results strongly suggested that, given a choice, pigs would rather consume a feed that allowed them to grow as efficiently as possible than eat to satisfy a certain minimum gut capacity (Ferguson, Nelson & Gous, 1999). This is also thought to be true for prawns.

In the present study the consumption by *F. indicus* was considerably lower than anticipated since the prawn was incapable of eating quantities in excess of 2 % of the daily ration. Consumption was limited by gut capacity with slower growing animals failing to eat as much as those fed higher protein concentrations.

Hill and Wassenberg (1992) reported that prior to ecdysis a change in preference for certain foods by *Penaeus esculentus* of 15.5 - 25.2 g occurred. Selective intake by the species was attributed to different nutritional requirements during this stage. Moreover pond-reared *P. monodon*, besides being given feed pellets, fed predominantly on plant tissue/material and smaller crustaceans (Focken, Groth, Coloso & Becker, 1998). Thus, unlike 2 g animals, where an increase in consumption was noted amongst animals fed diets low in protein, the cessation of growth in 7 and 14 g prawns was possibly due to older prawns finding diets low in fish meal, an essential protein source and food attractant (D'Ambramo, Castell, Conklin & Akiyama, 1997), less desirable and/or due to the inability to choose from a variety of sources. Moreover, diets high in fish oil, besides increasing the energy content and serving as an attractant, failed to increase consumption rates amongst 7 and 14 g prawns.

In the present investigation it would seem that carbohydrate was limited in its protein sparing capabilities, especially amongst older prawns, contrasting with the findings of Ahamad (1982) who found that the inclusion of 10 – 40 % of dietary starch increased FCR, growth and survival in juvenile *F. indicus*. A diet comprising 35 % CP, 6 % lipid and 32.2 % carbohydrate was found to provide the best results (Ahamad, 1996). Subsequently Hemambika and Raj (1999) revealed that α -amylase activity in the prawn digested more than 85 % of the carbohydrate in a diet containing 20 % starch with digestibility decreasing with a further increase in starch.

An increase in dietary protein decreased SFC in 2 g prawns whereas the opposite was found to occur in 7 and 14 g prawns. The SFC in 2 g prawns fed diets low in protein content

was assumed to be due to the ability of *F. indicus* to hydrolyse the α -1 \rightarrow 4 and α -1 \rightarrow 1 bonds found in starch (Omondi & Stark, 1995). Failure by 7 and 14 g prawns to assimilate high levels of dietary carbohydrates more effectively was probably due to an ontogenetic shift in feeding preference by the species. In an estuarine environment the digestive tract of juvenile *F. indicus*, an opportunistic omnivorous feeder, is capable of digesting a variety of foods. On leaving the estuary preference sways towards marine-based protein since large quantities of naturally occurring carbohydrates become scarce (Ribeiro & Jones, 2000). Although speculative, it would seem that the digestive tract of 2 g prawns and smaller is better suited to assimilate high levels of dietary carbohydrate as opposed to older individuals.

Studies that investigate digestive carbohydrases in older animals may substantiate this view since information of this nature will prove extremely useful. As the ability to digest carbohydrate in older prawns of *F. indicus* is unknown and since *P. monodon* performed better when provided with a combination of carbohydrates, as opposed to when fed a single source (Shiau *et al.*, 1992; Shiau, 1998), a combination of maltose, sucrose and starch in a 1:1:1 ratio, as done for *F. indicus* postlarvae by Ahamad (1996), should rather be considered in future.

Thus, in light of this study the carbohydrate levels suggested by Ahamad (1996) remain applicable during the early phases of development for animals younger than 2 g. Yet for older individuals a mixture of different carbohydrates should be considered instead or that dietary levels > 35 % be substituted with another nutrient or with an inert material such as silica or α -cellulose.

8.4.5. The effect of dietary protein on FCR.

Aside from possible errors incurred while establishing the amount consumed, FCR was shown to be affected by dietary protein (Figure 8.6). Ratios recorded amongst 14 g prawns was attributed to a higher portion of the food being consumed in relation to low gains in live mass. An FCR of 2 - 3 for Amatikulu and 1.9 - 4 for Mtunzini (Rosenberry, 2000; Rosenberry, 2001) compared favourably with 2 g prawns fed 41 % CP. Higher FCR values than anticipated for 7 and 14 g were attributed, in part, to portions of uneaten orts being dissolved or lost when siphoned, resulting in the gross overestimation of feed intake. A design similar to that used by Cho, Slinger and Bayley (1982) may help curb such errors. Alternatively, higher gluten levels and/or the use of the hot extrusion method when making feeds will limit orts, high in protein, from disintegrating when siphoning.

High FCR values were mainly associated with protein deficient diets with values for 14 g prawns fed 14 % CP and less speculated to be due to the inability of older prawns to assimilate large amounts of dietary carbohydrate. Pellets high in starch content (diets with 5 – 23 % CP) were harder in composition and rejected by 7 and 14 g prawns over time, especially when

considering that prior to this study these animals were accustomed to the provision of a much softer pellet on farms.

8.4.6. *The effect of dietary protein on PER.*

PER(s) were considerably higher in 2 g prawns than in 7 and 14 g animals. Groups of 2 g animals fed a diet containing 5 % CP displayed the highest PER. Results are indicative of younger animals being more efficient at assimilating protein and having a lower maintenance requirement than older animals. However, the efficiency of utilisation of protein (calculated as the amount of protein gained divided by the amount consumed, with the highest values considered favourable), which provides an idea of how well the protein is balanced and whether the ME:CP ratio is adequate was calculated and found in 2 g animals to be 0.62, 0.56, 0.36, 0.32, 0.20, 0.23; in 7 g prawns 0.59, 0.61, 0.37, 0.18, 0.12, 0.09 and in 14 g animals 0.50, 0.27, 0.09, 0.05, 0.04, 0.02 for 5, 14, 23, 32, 41 and 50 CP diets respectively. In all cases the efficiency of utilisation decreased with an increase in dietary protein fed. Results indicate that 2 g animals were generally better at assimilating diets than 7 and 14 g animals. An indication that the dietary ME, and not carbohydrate, was adequate. Therefore, low PER values were attributed to a decrease in food intake and the inability to assimilate diets efficiently.

8.4.7. *The effect of dietary protein on the accretion of chemical fractions.*

Although the accretion of body moisture and lipid does not require protein, a slight decrease in body moisture was noted with an increase in organic matter. Body fat corresponded directly with an increase in dietary protein with percentages found similar to those reported by Jayaprakas & Sambhu (1998). The lipid in the exoskeleton of 2 g animals was significantly higher when compared to that in the flesh. No such differences were found amongst 7 and 14 g animals. Although flesh lipid content decreased over time, particularly in 7 g animals, results suggest that lipid concentrations remained integrated within the exoskeleton and did not serve as a reservoir, as found with minerals by Vijayan and Diwan (1996). Compared to the initial value, higher fat deposits in the exoskeleton of 2 g animals was possibly a result caused by moulting. However, further investigations using larger tanks, so as to house more individuals, are needed in order to obtain sufficient material for replication. In general, body lipid did not occupy a significant portion of, and contributed little to, the overall body mass. E:F ratios were found to be similar between the different classes with variation attributed to human error.

8.4.8. The effect of dietary protein on exoskeleton and flesh gains.

Protein gains in the flesh of 2 g animals were related to an increase in dietary protein. Gains in flesh protein, expressed in mg/day, decreased with age. The lowest gains were recorded in 14 g prawns. A mass of 14 g is by no means close to the asymptote state of the species, as illustrated in Chapter 4. Considering the natural growth response of the species it was expected that protein gain in 7 and 14 g prawns would be less than those of 2 g prawns but higher than those reflected in Table 8.4. Low gains amongst 7 and 14 g prawns was assumed to be due to limitations imposed by the laboratory set-up and the absence of extraneous factors beneficial to the growth of the species. Proportionally protein gains in this study were 10.61, 14.66 and 14.18 % for 2, 7 and 14 g animals, respectively when compared to potential gains established using the systems approach.

In all cases dietary protein influenced gains in the exoskeleton with a positive relationship found in 2 g animals. Maximum gains in 7 and 14 g occurred in animals fed 41 % CP. However, by comparison the retention of protein in the exoskeleton and flesh, predicted using the systems approach (Table 4.12), was considerably lower. Differences illustrate the extent and manner in which system variables *e.g.* lack of endogenous sources, constrained growth.

8.4.9. The effect of dietary protein on lipid gains.

Lipid gains in both the flesh and exoskeleton were erratic with the only trend found amongst 2 g animals. In 2 g juveniles an increase in body lipid occurred with an increase in dietary protein. Results indicate that 2 g prawns, consuming diets high in protein, were able to store excess energy in the form of body fat. Conversely, 7 g animals suffered a considerable loss of body fat when fed diets low in protein. A possible explanation was that 7 g prawns were less likely to obtain energy from dietary carbohydrate and therefore were prone to assimilate body reserves, dietary protein and lipid for energy instead. Similarly lipid in the digestive-gland of *P. esculentus* was shown to deplete significantly around ecdysis (Barclay, Dall & Smith, 1984).

Higher gains in body fat were noted amongst 14 g animals and was possibly the result of animals being inactive due to spatial limitations or because at this stage in their lives they may require additional lipid to facilitate reproduction. Since the retention of dietary lipids is found to be dependent upon the source and desirability (Chandge & Raj, 1997) future studies should consider optimum levels of 5 – 8 % polyunsaturated fatty acids (PUFA), recommended by D'Ambro *et al.* (1997), since these levels are considered essential under intensive culture operations where natural endogenous food sources within ponds become depleted over time and/or in laboratory trials when using diets low in protein.

8.4.10. Protein requirement with age.

Requirements are dependent on the species, size, physiological state, experimental parameters, dietary composition and ingredients used (Gopal & Raj, 1990; De Silva & Anderson, 1995). Estimated requirements based on protein mass, wet mass and total live mass gains using regression models were 41, 35 and 34 % CP in both the flesh and exoskeleton of 2, 7 and 14 g prawns, respectively. These values are in agreement with those established by Colvin (1976) and Gopal and Raj (1990). In this study there was a definite decrease in the requirement for dietary protein with age. Protein requirements of 35 and 34 % established for 7 and 14 g prawns, respectively are in accordance with those currently offered to cultured *F. indicus* and are in agreement with those of Colvin (1976a), Gopal and Raj (1990). However, when compared to values predicted using the systems approach the protein requirement for 2, 7 and 14 g prawns was 24, 23 and 22 % CP, respectively and were considerably lower than those established using the conventional approach. This was possibly due to constraints imposed by the laboratory conditions or that the diets were found limiting.

Due to the feeding regime, as opposed to what is practised on farms and constraints due to gut capacity, increasing the protein content of the diet to almost twice that used in this study may have provided for better growth rates, similar to those found in ponds. However, under extensive conditions a protein requirement established using conventional means could probably be reduced to levels equivalent to those based using the systems approach since prawns of this age and younger are able to utilise pond biota to complement and supplement their diets. This view is supported in a study by Focken *et al.* (1998), which revealed that the consumption of pellets by *P. monodon*, with a preference for plant and animal material, only increased after the 11th week and occupied less than 50 % of the gut at all times. Focken *et al.* (1998) findings reinforces the idea that penaeids are selective and are able to supplement their diet when offered a choice.

8.4.11. The optimum requirement for protein in each mass class.

In this study the Reading model was used to describe the response in groups of prawns fed different dietary protein concentrations such that the optimum intake for protein, using data based on live mass and protein gains, was found to be 18, 46 and 152 mg/day in 2, 7 and 14 g prawns, respectively. Daily intakes for dietary protein increased whilst the requirement as a concentration decreased with age. Despite the high variability in responses the Reading model was able to produce sensible deductions from these data.

Based on the systems approach the daily intake of protein at 24 °C was predicted to be 20, 39 and 50 mg/prawn/day for 2, 7 and 14 g prawns, respectively. Daily requirements for dietary protein predicted for 2 and 7 g animals, using each approach, were relatively similar whereas

those established for 14 g prawns were considerably higher based upon conventional means. Results for the 14 g group strongly suggest that growth was constrained by dietary factors. However, whereas the Reading model may be used to determine the economic optimum intake of amino acids during growth it is difficult to place an economic value on the marginal revenue for prawns of such small sizes. Also, with the difficulty of measuring food intake in aquatic species this study has cast some doubts about the value of using this model that predicts performance based on intake rather than nutrient concentrations. Although the Reading model describes the response accurately, this may not be the best method for determining the optimum dose of amino acids or protein for prawns varying in age.

8.5. Conclusion

On the basis of the food consumption of prawns the protein requirement for the species, during the grow-out phase, was found to range between 34 - 41 % CP. Since it was not possible to ascertain the main cause of the poor growth with certainty it would be foolhardy to suggest what the protein requirement of the species should be. Were intakes or the provision of feed rations doubled, nutrient concentrations could be expected to be halved. Should this be proven true it would indicate the imprudence of describing requirements in terms of feed concentrations.

In the present study it appears that prawns do not store lipid in the body as a means of being able to over consume energy when faced with a deficiency of protein as in the case with poultry and pigs. Yet the pattern of food intake was similar to that in poultry and pigs when subjected to a range of feeds differing in protein content. It would therefore be interesting to determine what prawns do with the excess energy consumed since the lipid fraction in the flesh and exoskeleton had no significant measure on overall gains and that the quantification of protein gains, to establish dietary requirements, provided no real benefit except when using the Reading model. It is assumed that excess energy is lost to the surrounding medium.

The Reading model is based on the assumption that an individual animal responds in a bent-stick fashion *i.e.* the response to increasing intakes of the limiting nutrient is linear up to the point where the genetic potential is reached after which no further improvement in response is possible. A population curve is derived based on information about the standard deviation of body mass, maintenance and output (Morris, 1999). In this study the response was obtained from the observed normal variation in mean protein gains and body mass. The Reading model, as opposed to models that establish the requirement of dietary protein based on composition (%) *e.g.* broken stick, provided a means to establish the optimum requirement (mg/day) in a population of prawns based on financial terms. However, more thorough investigations are required to substantiate the use of the Reading model for commercial and other aquatic species, under practical conditions, on provision that food intake can be measured accurately.

9.

Digestibility Markers

Evaluation of Endogenous; Acid-Insoluble Ash and Crude Fibre as Indicators of Feed Digestibility in the Marine Prawn *Fenneropenaeus indicus*.

9.1. Introduction.

In order to formulate cost effective feeds that meet the nutritional needs of the animal the digestibility, a term used in animal nutrition, of ingredients needs to be known. Digestion involves the process by which the food is broken down by enzymes into nutrients that are rendered soluble for absorption on a cellular level. Digestibility, on the other hand, is a measure of dietary nutrients that have been digested and assimilated by an organism (De Silva, 1989). Digestibility is established by implementing either the direct or indirect approach.

The direct or gravimetric approach, commonly used in terrestrial studies, entails comparing the amount of ingredient/nutrient ingested with that which has been egested *e.g.* through defaecation and excretion. The term “apparent” is often used since a small portion of the faecal output is from endogenous sources *e.g.* mucosa cells, bacterial gut flora and digestive enzymes, which leads to a slight underestimation of digestibility. In aquatic organisms, by virtue of the medium and because attempts to directly measure ingestion, egestion and evacuation rates being at best tedious and in many circumstances impossible, the indirect approach is preferred (Ishikawa, Teshima, Kanazawa & Koshio, 1996).

This method requires that small quantities of an inert material, referred to as the external marker, be added into the feed or that the natural inert components within, known as internal or endogenous indicators, be quantified. Digestibility is calculated by comparing the ratio of the marker to a specific nutrient within the diet and faeces (Goddard & McLean, 2001). A major advantage, as opposed to using the gravimetric method, is that evacuation rates and the accurate quantification of dietary intake and egesta become irrelevant since only a small sample of faeces needs to be collected for analysis.

Two markers most commonly used in aquatic studies are chromic oxide (Cr_2O_3), an external marker (Nose, 1964; Teshima & Kanazama, 1984), and total endogenous ash (Leavitt, 1985). Cr_2O_3 has been used in digestibility studies involving *Fenneropenaeus indicus* (Colvin, 1976; Ahamad Ali, 1993, 1996), *Penaeus monodon* (Shiau, Lin & Chiou, 1992; Deering, Hewitt & Sarac, 1996), *P. stylirostris* (Fenucci, Fenucci, Lawrence & Zein-Eldin, 1982), *P. vannamei* (Akiyama,

Coelho, Lawrence & Robinson, 1989) and *Marsupenaeus japonicus* (*P. japonicus*) (Ishikawa *et al.*, 1996). Although widely used for terrestrial (McDonald, Edwards & Greenhaigh, 1977) and aquatic determinations (Furukawa & Tsukahara, 1966; Nose, 1964; Windell, Foltz & Sarokon, 1978; Sales & Britz, 2001) recent studies have raised doubts regarding the use and suitability of Cr_2O_3 as a dietary marker since the rate of passage and distribution thereof was reported to be uneven within the faeces of *Homarus americanus* (Leavitt, 1985). Moreover, the ingesta and Cr_2O_3 failed to move at similar rates along the gastrointestinal tract of the shrimps *Palaemon serratus* and *Pandalus platyceros* and the freshwater crayfish *Procambarus clarkii* (Deering *et al.*, 1996). Subsequently in a comparative study by Ishikawa *et al.*, (1996), besides requiring expensive and specialised equipment for analysis, 5α -cholestane, as opposed to Cr_2O_3 , was better suited as a dietary marker in digestibility studies of *M. japonicus*.

Hence, besides being non-toxic, a digestibility marker should ideally be inexpensive, be easily and homogeneously mixed within feeds, have the same rate of passage through the gut as the ingesta and should never be assimilated or absorbed. Furthermore, it should have no effect on the digestive metabolism and be accurately and effortlessly analysable at low concentrations (Leavitt, 1985; Ishikawa *et al.*, 1996; De Silva & Anderson, 1995; Austreng, Storebakken, Thomassen, Refstie & Thomassen, 2000). However, in digestibility studies performed using *F. indicus* and Cr_2O_3 , Colvin (1976) and Ahamad Ali (1993, 1996) make no mention of the problems highlighted above.

Nonetheless, as opposed to endogenous indicators, the use of Cr_2O_3 is restricted because the addition thereof in natural diets (*e.g.* algae and zooplankton) and in commercial feeds, due to concerns of pollution, is not possible. Conover (1966) introduced an ash-ratio technique to determine the assimilation efficiency of natural sources and practical diets in aquatic animals based on the assumption that only the organic component of the food is affected significantly during digestion. Although this technique has been applied successfully to a wide range of crustaceans including copepods (Conover, 1966) and the caridean *Macrobracium rosenbergii* (Clifford & Brick, 1979), the assumption that the ash component of a ration is not absorbed, may not hold true in all cases (Leavitt, 1985).

Endogenous markers, of appreciable amounts within the diet, such as crude fibre (CF), hydrolysis resistant organic matter (HROM), hydrolysis-resistant ash (HRA) and/or acid-insoluble ash (AIA), a natural occurring silica of no nutritional value, have been found to be suitable alternatives for Cr_2O_3 in fish digestibility studies (De Silva & Anderson, 1995). However, results based on hydrolysis-resistant ash provided estimates of protein digestibility that were highly variable for the cichlid *Etroplus surantensis* (De Silva & Perera, 1983) whereas low levels of AIA

led to an overestimate of digestibility coefficients in the trout *Oncorhynchus mykiss* (Morales, Cardenete, Sanz & de la Higuera, 1999).

When found to be low, AIA levels have been supplemented with external sources such as Celite™, an acid washed diatomaceous silica powder, or acid washed sand (Goddard & McLean, 2001). Despite being supplemented with Celite™, apparent digestibility coefficients (ADC) based on AIA in the rainbow trout *Salmo gairdneri* were significantly higher when compared to those obtained using Cr₂O₃ and CF (Tacon & Rodrigues, 1984). In general, digestibility studies in fish indicated that a measure of endogenous CF as opposed to HROM, HRA and AIA, despite having been augmented with Celite™ or acid washed sand, provided better results.

By comparison in dietary studies involving penaeids investigations of more than one endogenous component have rarely been done. AIA has been validated as a suitable marker for *P. monodon* (Deering *et al.*, 1996) but due to cellulase being identified in several species of prawns (Hood & Meyers, 1977; Dempsey & Kitting, 1987) the evaluation of dietary CF as a marker has been neglected. This despite cellulase activity having been found to be insignificant for cellulose digestion in *F. indicus* (Omondi & Stark, 1995a). Hence, in view of existing information and due to AIA and CF requiring minimal equipment and technical expertise for analysis, the objectives of this study were:

- To establish whether AIA and CF make for suitable replacements as dietary markers.
- To establish if increased levels of Cr₂O₃ and the inclusion of Celite™ and α -cellulose's effect digestibility estimates.
- To determine the ADC of dietary nutrients for the species.

9.2. Material and Methods.

9.2.1. Experimental animals

Juvenile *F. indicus*, with a mean and standard deviation of 10.14 ± 0.64 g and of the same brood stock, were transported from Amatikulu in breeder bags filled with 10 l of pond water. Bags, each stocked with 30 individuals, were inflated with industrial oxygen and sealed using a rubber band. On arrival the animals were acclimated to laboratory temperatures by placing the sealed bags into the tanks for 30 minutes and thereafter each tank was stocked with 12 prawns. Prior to the initiation of trials prawns were fed a commercial pellet (Table 1.1) for 10 days and thereafter the test diets for four days following. The four days constituted a period of purging during which all faecal matter collected was discarded.

9.2.2. Experimental facilities

Rearing tanks, described in Appendix 2, were used to house test animals.

9.2.3 Experimental conditions

Water was maintained at (mean and standard deviation): 28 ± 1 °C, 28 ± 3 ‰ and a pH 8 ± 2 with oxygen concentrations kept near saturation throughout the trials. Water temperature and salinity readings were taken in the morning and afternoon while pH was measured before the animals were fed in the mornings.

9.2.4. Diet preparations.

Experimental diets were composed of ingredients used widely in commercial and practical feeds. Raw ingredients were ground using a rotor mill (Model: Congent 2000), passed through a 250 µm sieve and oven dried at 25 °C for 72 hours. Dry ingredients were weighed, placed into a bowl and blended with a hand held electric mixer for 10 minutes. Fish oil and soylecithin were gradually added while mixing for a further 15 minutes. Approximately, 75 ml of water per 100 g of dry mass was then added to form a paste. The paste was homogenized by kneading it for an additional 10 minutes and, with the aid of a conventional meat grinder, forced through a die containing 3-mm holes. The extruded strands were placed onto oven racks and dried at 30 °C for 48 h. Thereafter the strands (< 8 % moisture) were broken into approximately 6 mm lengths and stored in air-tight containers at – 20 °C. Due to the nature of the experiments, diets differed by virtue of their ingredients and the addition of Cr₂O₃, Celite™ and α-cellulose as shown in Table 9.1.

9.2.5 Experiment diets.

The reference diet, Diet 1, contained 0.5 % Cr₂O₃ whereas Diets 2, 3 and 4 had Celite™ and Cr₂O₃ at inclusion levels of 0.5, 1 and 2 %, respectively. Diets 5 and 6, with the exclusion of Celite™, contained α-cellulose and Cr₂O₃ at 1 and 2 %, respectively (Table 9.1). The inclusion of dietary indicators was substituted against potato starch.

Table 9.1. Composition of the experimental diets (% d/m).

<i>Ingredient</i>	<i>Diet Number</i>					
	1	2	3	4	5	6
Fish meal ^a	26.30	26.30	26.30	26.30	26.30	26.30
Wheat ^b	30.00	30.00	30.00	30.00	30.00	30.00
Soybean (46) ^c	24.80	24.80	24.80	24.80	24.80	24.80
Potato starch ^d	4.00	3.50	2.50	0.50	2.50	0.50
Gluten ^e	2.50	2.50	2.50	2.50	2.50	2.50
Brewers ^f	5.00	5.00	5.00	5.00	5.00	5.00
Fish oil ^g	2.00	2.00	2.00	2.00	2.00	2.00
Soy Lecithin ^h	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin mix ⁱ	0.40	0.40	0.40	0.40	0.40	0.40
Mineral mix ^j	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin C	0.40	0.40	0.40	0.40	0.40	0.40
Cholesterol ^k	0.10	0.10	0.10	0.10	0.10	0.10
Methionine ^l	1.00	1.00	1.00	1.00	1.00	1.00
Lysine ^l	0.50	0.50	0.50	0.50	0.50	0.50
α – cellulose ^m	0.00	0.00	0.00	0.00	1.00	2.00
Chromic oxide ^m	0.50	0.50	1.00	2.00	1.00	2.00
Celite ^{TM m}	0.00	0.50	1.00	2.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Proximate analysis (g/100g dm)						
Crude protein	43.27	43.18	43.06	43.01	43.08	43.18
Lipid	6.49	6.59	6.56	6.47	6.52	6.54
Ash	9.07	9.42	10.63	11.66	10.49	10.61
Crude Fibre	1.13	1.09	1.10	1.06	2.62	3.13
NFE	29.80	30.49	28.21	26.48	27.58	26.48
Gross Energy (kJ/g)	17.91	17.89	17.70	17.42	17.73	17.45
Chromic oxide	0.49	0.49	0.99	1.97	0.97	1.98
Acid-insoluble ash	0.72	1.15	1.62	2.38	1.48	2.26

^a Contra Fisheries (Cape Town, South Africa) minimum 75 % crude protein, lipid >12 %.

^b Ngwane Mill (Kwa-Zulu, South Africa).

^c Epol (Pretoria, South Africa) minimum 44 % crude protein.

^d African Products (Durban, South Africa).

^e C.J. Petrow (Durban, South Africa).

^f South African Breweries (SAB).

^g Energy Oils (Durban, South Africa).

^h Crest Chemicals (Durban, South Africa).

ⁱ Per kg of feed: Thiamin, 10.5mg; riboflavin, 10mg; pyridoxine, 12.5mg; pantothenic acid, 18.5mg; Niacin, 10mg; Biotin, 0.50mg; inositol, 60mg; choline, 100mg; folic acid, 2.5mg; cyanocobalamin, 0.025mg; ascorbic acid, 250mg; vitamin A, 4200IU; vitamin D, 2500 IU; vitamin E, 300mg; vitamin K, 20mg; Calpan, 50mg; Endox, 25mg.

^j Per kg of feed: Calcium, 246mg; phosphorus, 80mg; magnesium oxide, 6.31mg; sodium, 60mg; potassium, 90mg; iron, 3mg; copper, 0.34mg; zinc, 1.1mg; manganese, 0.2mg; selenium, 0.05mg; cobalt, 0.14mg; iodine, 0.01mg; ferrous, 0.89mg.

^k Cholesterol, (Salvay Pharmaceuticals, South Africa).

^m Sigma (St. Louis, MO, USA).

9.2.6. Feed and faecal collection.

Thrice daily, 08:00, 12:00 and 16:00 h, each diet was fed to apparent satiation to three groups of prawns housed in different tanks within separate systems. Fifteen minutes after feeding, faeces and feed residue were siphoned to waste and the inner walls of the tanks wiped clean to prevent algal and bacterial growth. Faecal strands, siphoned onto a 63 μ m nylon mesh, were

collected half an hour after feeding and hourly thereafter. Collection continued for two hours after the last feed. The siphoned material was rinsed with distilled water and the faecal strands removed. Collected strands from each tank were dried overnight in a convection oven at 70 °C and pooled with the previous day's sample. Dried samples were placed into labelled vials and pending analysis stored at – 20 °C. Trials were conducted for 14-days in order to obtain sufficient samples for analysis.

9.2.7. Analytical protocol.

Chemical analyses of the diets and faeces were performed as follows; water content was determined by oven drying at 105 °C to constant mass with ash determined by burning the samples in a muffle furnace at 600 °C for 16 hours. Crude protein (N x 6.25) was determined by micro-Kjeldahl technique; lipid by diethyl ether extraction; crude fibre by the Weende method, gross energy by ballistic bomb calorimeter calibrated with benzoic acid and Cr₂O₃ spectrophotometrically according to Furukawa and Tsukuhara (1966). Acid-insoluble ash was established according to Atkinson, Hilton and Slinger (1984). All analyses were done in triplicate. Nitrogen free extract (NFE) content, as done by Morales *et al.*, (1999) was calculated by the difference 100 – (crude protein + lipids + ash + crude fibre).

9.2.8. Digestibility Determination

The apparent digestibility coefficients (ADC) for crude protein (CP), lipid, NFE, dry matter and gross energy were calculated according to the formula used by Maynard and Loosli (1969):

$$\text{ADC(s) of nutrients and energy of diet (\%)} = \{1 - [(F \div D) \times (D_m \div F_m)]\} \times 100$$

where	F	= % nutrient or energy in faeces	
	D	= % nutrient or energy in the diet	
	D _m	= % marker in diet	
	F _m	= % marker in faeces.	[9.1]

Digestibility of dry matter was calculated as:

$$\text{ADC of dry matter of diet (\%)} = 100 \times \{1 - (D_m \div F_m)\}$$

[9.2]

9.2.9. Statistical analysis

Analyses were performed using One-way analysis of variance (ANOVA) with Tukey multiple post-test used to compare digestibility coefficients for a given diet. Two-way analysis of

variance (ANOVA) was used to compare the interaction effect of the diet and marker on digestibility. Post hoc, Bonferroni multiple comparison test was also performed to further evaluate differences caused by marker content within diets. In all analyses, the probability value P was compared to $\alpha = 0.05$. All analyses were done with the use of GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

9.3. Results

9.3.1. Faecal composition.

Crude protein (CP), ash, fat, energy, CF and marker content in the faecal matter of prawns fed the six diets are shown in Table 9.2. Dietary marker content were considerably higher in faeces than in the feed. Faecal protein and lipid content were similar between test diets whereas in contrast the faecal content of fibre, ash, NFE and energy varied between diets.

Table 9.2: Crude protein (N x 6.25), fat, ash, NFE, GE and marker content of prawn faeces collected over a 2-week period (mean values of three samples replicates, expressed as a % on a moisture free basis, \pm S.D).

Component	Diets					
	1	2	3	4	5	6
Crude protein	23.57 (± 0.51)	22.45 (± 0.36)	23.87 (± 0.95)	22.52 (± 0.63)	23.41 (± 1.38)	26.34 (± 4.01)
Lipid	1.65 (± 0.31)	1.97 (± 0.23)	1.65 (± 0.44)	1.69 (± 0.18)	1.65 (± 0.41)	2.10 (± 0.72)
Ash	26.57 (± 0.76)	28.00 (± 1.74)	35.49 (± 0.74)	36.56 (± 0.62)	31.59 (± 0.89)	34.31 (± 0.89)
NFE	69.27 (± 2.68)	69.03 (± 3.42)	72.51 (± 1.40)	70.86 (± 0.48)	68.90 (± 5.82)	71.10 (± 5.92)
Gross energy (kJ/100g diet)	12.64 (± 0.56)	13.13 (± 1.34)	10.92 (± 0.95)	12.41 (± 0.60)	10.62 (± 0.88)	9.92 (± 0.43)
Chromic-oxide	1.34 (± 0.04)	1.33 (± 0.09)	3.45 (± 0.05)	6.83 (± 0.05)	3.05 (± 0.03)	6.74 (± 0.12)
Crude Fibre	5.14 (± 0.51)	4.56 (± 0.46)	4.63 (± 0.39)	4.96 (± 0.28)	7.96 (± 0.05)	10.52 (± 0.31)
Ash insoluble ash	2.17 (± 0.46)	3.54 (± 1.15)	6.04 (± 0.32)	8.36 (± 0.14)	4.85 (± 0.26)	7.96 (± 0.22)

9.3.2. Apparent digestibility coefficient (ADC) based on Cr_2O_3 , AIA and CF content.

Mean ADC(s) established using Cr_2O_3 , CF and AIA are shown in Table 9.3. Since the test diets were similar in composition it was anticipated that the digestibility of nutrients for each diet would be the same, but with the exception of Diets 5 and 6, mean digestibility coefficients and variation based on CF were found to be predominantly higher than those calculated using Cr_2O_3 and/or AIA (Table 9.3). In general, digestibility coefficients were more consistent with increased levels of Cr_2O_3 , α -cellulose and Celite™.

Table 9.3: Apparent digestibility coefficients for different nutrients established in prawns fed test diets that contained various levels of dietary markers. Values are means with standard error in parenthesis.

Apparent Digestibility (%)	Diet Number*					
	1	2	3	4	5	6
Crude Protein						
Chromic-oxide	79.68 ^a _x (± 0.79)	80.64 ^{ab} _x (± 1.42)	84.01 ^{bc} _x (± 0.41)	84.88 ^c _x (± 0.49)	82.82 ^{abc} (± 1.08)	82.03 ^{abc} _x (± 2.84)
Crude Fibre	88.04 ^a _y (± 0.46)	87.22 ^a _y (± 1.38)	86.74 ^a _x (± 1.41)	88.75 ^a _y (± 0.80)	82.16 ^b _x (± 0.95)	81.81 ^b _x (± 3.28)
Acid-insoluble ash	80.19 _x (± 3.57)	81.72 _x (± 2.71)	84.19 _x (± 0.82)	84.15 _{xy} (± 0.59)	82.13 _x (± 3.03)	81.61 _x (± 2.45)
Lipid						
Chromic-oxide	90.54 (± 2.35)	88.89 (± 1.31)	92.76 (± 1.80)	92.45 (± 0.78)	91.98 (± 1.99)	90.54 (± 3.24)
Crude Fibre	94.43 (± 1.38)	92.70 (± 0.86)	94.02 (± 1.48)	94.48 (± 0.58)	91.71 (± 2.06)	90.45 (± 3.22)
Acid-insoluble ash	91.63 (± 2.07)	90.30 (± 1.44)	93.26 (± 1.67)	92.55 (± 0.77)	92.48 (± 1.91)	90.48 (± 3.11)
Ash						
Chromic-oxide	-9.27 ^a _x (± 3.12)	-10.40 ^b _x (± 6.85)	3.70 ^c _x (± 0.65)	9.49 ^c _x (± 1.04)	4.31 ^c _{xy} (± 1.18)	4.85 ^c _x (± 0.81)
Crude Fibre	35.72 ^a _y (± 1.80)	27.41 ^b _y (± 4.51)	20.52 ^b _y (± 0.53)	32.84 ^a _y (± 0.77)	1.10 ^c _y (± 1.22)	3.97 ^c _x (± 0.81)
Acid-insoluble ash	3.41 _z (± 2.76)	3.61 _z (± 5.98)	10.43 _x (± 0.63)	10.64 _x (± 1.03)	7.69 _x (± 1.14)	8.43 _x (± 0.78)
NFE						
Chromic-oxide	15.31 _x (± 3.28)	15.95 _x (± 4.11)	25.91 _x (± 1.43)	22.74 _x (± 0.52)	20.62 _x (± 6.71)	20.95 _x (± 6.64)
Crude Fibre	42.64 ^a _x (± 0.69)	40.40 ^a _x (± 1.95)	36.35 ^d _x (± 1.76)	36.96 ^d _x (± 0.96)	17.96 ^c _y (± 0.21)	20.12 ^c _y (± 1.04)
Acid-insoluble ash	34.16 _z (± 6.79)	35.43 _y (± 3.93)	36.69 _y (± 1.23)	30.30 _y (± 0.47)	30.67 _z (± 5.31)	30.59 _z (± 0.75)
Energy						
Chromic-oxide	73.70 ^a _x (± 1.08)	72.75 ^a _x (± 2.78)	82.40 ^b _x (± 1.55)	79.43 ^b _x (± 0.99)	80.08 ^b _x (± 1.58)	83.23 _x (± 0.72)
Crude Fibre	84.53 ^a _y (± 0.64)	82.06 ^{ab} _y (± 1.83)	85.34 ^a _x (± 1.28)	84.74 ^a _y (± 0.73)	80.24 ^b _x (± 1.63)	83.08 ^{ab} _x (± 0.73)
Acid-insoluble ash	76.75 ^a _x (± 0.96)	76.21 ^a _x (± 2.42)	83.48 ^b _x (± 1.44)	79.69 ^{ab} _x (± 0.98)	81.61 ^{bc} _x (± 1.52)	83.86 ^{bc} _x (± 0.76)
Dry Matter						
Chromic-oxide	62.70 ^a _x (± 1.48)	62.76 ^b _x (± 2.62)	71.16 ^c _x (± 0.45)	71.13 ^c _x (± 0.19)	68.22 ^c (± 0.31)	70.56 ^c (± 0.52)
Crude Fibre	78.05 ^a _y (± 0.94)	75.42 ^a _y (± 2.44)	76.09 ^a _y (± 2.10)	78.54 ^a _y (± 1.21)	67.16 ^b (± 0.19)	70.28 ^b (± 0.88)
Acid-insoluble ash	66.16 _x (± 6.76)	67.25 _x (± 4.06)	73.13 _{xy} (± 1.40)	71.50 _x (± 0.47)	68.82 (± 5.23)	71.66 (± 0.78)

^{abcd} Components with the same superscripts indicate that mean values within a row are not significantly different ($P > 0.05$).

^{xyz} Components with the same subscripts indicate that mean values within a column are not significantly different ($P > 0.05$).

9.3.3. ADC of CP.

Apparent crude protein digestibility (ACPD) ranged between 79.68 and 88.75 % (Table 9.3). Digestibility coefficients of ACPD differed significantly between diets ($F = 19.71$; $df = 2, 36$; $P < 0.0001$) and markers ($F = 6.45$; $df = 5, 36$; $P < 0.001$). Similarly the interaction effect between diets and markers was found to be significant ($F = 6.45$; $df = 10, 36$; $P < 0.001$).

ACPD based on endogenous CF was higher than those of Cr_2O_3 and AIA, however, the inclusion of 1 and 2 % α – cellulose in Diets 5 and 6, respectively provided estimates that were similar to those established using Cr_2O_3 and AIA (Table 9.3). In all cases ACPD estimates based on AIA were found to be similar to those of Cr_2O_3 . Variations about the mean based on AIA content decreased with the inclusion of 1 – 2 % Celite™ (Table 9.3). Despite being effective at raising dietary levels of AIA, the inclusion of Celite™ had no effect on absolute values.

9.3.4. ADC of lipid.

Apparent lipid digestibility (ALD) ranged between 88.89 and 94.48 % (Table 9.3). ALD coefficients differed significantly between diets ($F = 3.83$; $df = 2, 36$; $P < 0.05$) and markers ($F = 3.30$; $df = 5, 36$; $P < 0.05$). In contrast the interaction effects between diets and markers were found not to be significant ($F = 0.75$; $df = 10, 36$; $P > 0.05$).

In all cases the apparent digestibility of lipid was found to be similar between diets (Table 9.3). Similarly digestibility coefficients based on Cr_2O_3 , CF and AIA content were similar.

9.3.5. ADC of ash.

Apparent ash digestibility (AAD) ranged between - 9.27 and 35.72 % (Table 9.3). Negative and unrealistic values for Diets 1 and 2, based on 0.5 % Cr_2O_3 , were found. In general AAD varied significantly between diets ($F = 245.09$; $df = 2, 36$; $P < 0.0001$) and markers ($F = 28.44$; $df = 5, 36$; $P < 0.0001$). The interaction between diets and markers were significant ($F = 45.26$; $df = 10, 36$; $P < 0.0001$).

Estimates based on Cr_2O_3 for Diets 1 – 4 were lower than those of AIA and CF (Table 9.3). Between diets CF estimates of AAD displayed the highest variability whereas coefficients based on AIA were found to be the least.

9.3.6. ADC of NFE.

Apparent nitrogen free extract digestibility (ANFED) ranged between 79.7 and 88.8 % (Table 9.3). Digestibility coefficients of ANFED differed significantly between diets ($F = 76.91$; $df = 2, 36$; $P < 0.0001$) and markers ($F = 12.37$; $df = 5, 36$; $P < 0.0001$). Similarly the interaction effect between diets and markers was found to be significant ($F = 10.55$; $df = 10, 36$; $P < 0.0001$).

With the exception of Diets 5 and 6, digestibility coefficients calculated based on CF content were higher when compared to those established using Cr_2O_3 . Similarly, for any given diet

estimates calculated using AIA were higher than those determined based on Cr_2O_3 . The highest variability of mean values was amongst CF based estimates.

9.3.7. ADC of gross energy.

The apparent energy digestibility (AED) ranged from 72.8 – 85.3 % with the apparent digestion varying significantly between diets ($F = 50.32$; $df = 2, 36$; $P < 0.0001$) and markers ($F = 31.51$; $df = 5, 36$; $P < 0.0001$). Moreover the interaction between diets and markers were significant ($F = 8.96$; $df = 10, 36$; $P < 0.0001$).

Except for Diets 3, 5 and 6 digestibility coefficients based on CF were higher when compared to those of Cr_2O_3 whereas, with the exception of Diets 1 and 2, results based on AIA were similar to those based on Cr_2O_3 (Table 9.3).

9.3.8. ADC of dry matter.

Digestibility of dry matter (ADMD) ranged from 62.7 – 78.1 %. ADMD values differed significantly between diets ($F = 31.51$; $df = 2, 36$; $P < 0.0001$) and markers ($F = 8.88$; $df = 5, 36$; $P < 0.0001$). Similarly the interaction effect between diets and markers was found to be significant ($F = 6.03$; $df = 10, 36$; $P < 0.0001$).

From Table 9.3, ADMD coefficients established based on AIA content were similar between diets. Digestibility coefficients based on CF content, with exception of those established for Diets 5 and 6, were found to be higher than those obtained using Cr_2O_3 . In contrast results based on AIA were similar to those of Cr_2O_3 .

9.4. Discussion

A higher concentration of marker in faeces was a result of the indigestible portion of the ingesta becoming increasingly larger due to the absorption of nutrients by the gastro-intestinal tract. Although selective rejection and uneven passage of Cr_2O_3 was reported to occur in *Palaemon serratus*, *Pandalus platyceros* (Forster & Gabbot, 1971), *P. monodon* (Deering *et al.*, 1996), *Procambarus clarkii* (Brown, William & Robinson, 1986) and *Homarus americanus* (Leavitt, 1985), with uneven colouration of faecal strands noted in *Homarus americanus* (Leavitt, 1985), similar phenomena were not observed in the present study.

In order to establish if leaching of chromic oxide from faecal strands was a cause for the difference in estimates, animals fed Diet 1 (Table 9.2) were removed after defecating. Faecal

strands were collected 1, 2, 6 and 8 hours after feeding and analysed for chromium and dry matter content (Table 9.4).

Table 9.4: The results of the apparent digestibility of dry matter based on chromic oxide content derived from faecal strands of *F. indicus* collected over time. Values are means with standard deviation in parenthesis ($n = 3$).

Apparent Digestibility Coefficient (%)	Hours after defecation			
	1	2	6	8
<i>Dry Matter</i>	66.89 (± 0.58)	67.23 (± 0.41)	66.46 (± 0.79)	65.97 (± 0.94)

Results in Table 9.4 indicate that leaching of Cr_2O_3 did not occur over an 8-hour period that would account for differences in digestibility estimates, considering that faecal matter was collected within an hour after feeding in the current investigation. Partial leaching was possible, this being attributed to permeability of the peritrophic membrane that surrounds the faecal strands of penaeids.

9.4.1. Apparent digestibility of crude protein.

ACPD based on Cr_2O_3 and AIA ranged between 79.7 – 84.9 % and 82.1 – 84.2 %, respectively. These results, although slightly higher, were similar to digestibility estimates of 79.4 – 82.6 % reported for *P. monodon* based on Cr_2O_3 , AIA and Ytterbium content (Deering *et al.*, 1996). Alternatively, in a study by Catacutan (1991) digestibility coefficients of protein for *P. monodon*, based on 1 % Cr_2O_3 were found to be 92.8 – 94.3 %.

Knowledge of the biochemical digestive capabilities of prawns is scarce (Lemos, Ezquerro & Garcia-Carreño, 2000) but the disparity between results of Catacutan (1991) and those of the current study were considered to be due to differences in dietary composition, experimental set-up, metabolic losses, size and species of experimental animals used.

Increased Cr_2O_3 concentrations in Diets 3 and 4 led to significantly higher estimates of ACPD, possibly caused by an uneven rate of passage. However, diets 5 and 6 substituted with α -cellulose and equivalent concentrations of Cr_2O_3 presented lower yet similar coefficients to those based on Cr_2O_3 and AIA. Although speculative, lower digestibility estimates were an indication that the marker(s) moved through the gastro-intestinal tract at a slower rate than the digesta.

9.4.2. Apparent digestibility of crude fat.

ALD ranged from 88.9 - 94.5 % and was similar to digestibility coefficients of 90.0 - 92.8 % reported for *P. monodon* by Catacutan (1991). The apparent lipid digestibility based on AIA content, 90.9 – 93.3 %, was similar for the six diets. Digestibility of fat calculated based on Cr₂O₃, AIA and the inclusion of 1 – 2 % α – cellulose, was found to be similar.

In general, lower coefficients based on endogenous CF were possibly due to a reduced transit time since digestion depends, in part, on the period the digesta are in contact with the absorptive epithelium. Depending on the type and amount, dietary fibre can decrease digestibility in the midgut by either hindering the absorption process and/or by accelerating the rate of passage of the digesta through the intestine thereby decreasing the contact period with the absorptive epithelium. On the other hand, dietary fibre can increase the residence time by obstructing the natural flow of the digesta through the gastro-intestinal tract and thereby prolong the contact period for the absorption of nutrients (González-Peña, Gomes & Moreira, 2002).

In this study dietary fibre, an estimate of the organic fraction of the diet that is resistant to dilute alkali and acid, was primarily of plant origin whereby the inclusion of 1 – 2 % α – cellulose proved beneficial in the absorption of nutrients.

9.4.3. Apparent digestibility of dietary ash.

A negative coefficient representing the AAD was observed using Cr₂O₃ but not with AIA and CF. Negative digestibility coefficients for ash, based on Cr₂O₃ content, have been reported in the marine mollusc *Haliotis midae* (Sales & Britz, 2001).

Moreover, a high variability of coefficients based on different dietary indicators was found between diets (Table 9.3). Ash derivatives contain nutritionally vital minerals and due to the selective absorption thereof anomalies with regard to the rate of passage was cause for high variability amongst estimates. It was for these reasons that dietary ash was considered unsuitable as a dietary marker in crustaceans (Forster & Gabbot, 1971; Leavitt, 1985).

Whilst results indicated that a small but significant fraction of total ash was assimilated in *F. indicus* digestibility coefficients based on AIA were similar for the six diets and ranged between 3.41 – 11.9 % (Table 9.3). This may be explained by the likelihood that the acid-insoluble fraction/Celite™, consisting primarily of diatom frustules and their fragments, was unable to pass through the gut wall as reported elsewhere by Bowen (1981), Goddard and Mc Lean (2001).

Results of ash digestibility in *F. indicus* provided no clear trend; however, coefficient values fell within a range of 0.7 – 37.8 % established based on dietary markers Cr_2O_3 , CF and AIA in the rainbow trout *Salmo gairdneri* (Tacon & Rodrigues, 1984).

9.4.4. Apparent digestibility for NFE.

Overall digestibility coefficients of NFE based on AIA and CF were considerably higher than those established using Cr_2O_3 . However, the inclusion of 1 to 2 % α – cellulose provided similar coefficients to those established using Cr_2O_3 . Higher estimates of ANFED based on AIA indicated once again differences in the rate of passage. Digestibility results of NFE in this study were surprisingly low since α -amylase activity in juvenile *F. indicus*, fed purified diets containing graded levels of starch ranging from 0 – 40 %, increased to a maximum of 43.97 mol maltose/ml/min which resulted in a digestibility coefficient of 81 % for diets containing 20 % starch (Ahamad Ali, 1993; Omondi & Stark, 1995b). Differences were therefore ascribed to the nature of the dietary carbohydrate since the NFE content in the present study was not derived from a single source. Moreover, failure to assimilate the NFE fraction more efficiently was possibly due to ontogenetic changes since, unlike older animals used in this study, the digestive tracts of juvenile *F. indicus* less than 1 g, which are opportunistic when feeding, are capable of digesting a variety of food sources with a preference for plant material (Ribeiro & Jones, 2000).

9.4.5. Apparent digestibility of gross energy.

In all cases estimates based on AIA, although slightly higher, were found to be similar to those based on Cr_2O_3 . In contrast results based on the exclusion of α – cellulose provided significantly higher estimates when compared to values established using Cr_2O_3 . Ultimately, digestibility depends on the physical state, age and metabolic demand/requirement of the prawn.

9.4.6. Apparent digestibility of dry matter.

Although slightly higher, estimates of 69.6 – 73.1 % based on AIA content were similar to coefficients obtained using Cr_2O_3 whereas CF estimates of 75.4 – 78.1 % were obtained for diets devoid of α – cellulose. Results in the present investigation were considerably lower than those reported for *P. monodon*, 75.7 – 86.9 % (Catacutan, 1991), and *Homarus americanus*, 92.7 – 96.1 % (Leavitt, 1985). Values established for *Homarus americanus* were obtained using the direct or gravimetric method, however, when based on dietary ash using the indirect approach the

digestibility of dry matter was found to range between 51.4 – 89.1 % for the same species (Leavitt, 1985). By comparison estimates of 53.1 – 69.5, 51.9 – 67.0 and 74.5 – 82.0 % based on Cr_2O_3 , CF and AIA, respectively were recorded for the rainbow trout *Oncorhynchus mykiss* (Morales *et al.*, 1999). The inclusion of higher levels of dietary fibre was found to facilitate the rapid passage of ingesta through the gut of tilapia *Oreochromis niloticus* and *O. aureus* (Shiau, Hwa, Chen & Hsu, 1988), which in turn decreased the residence time of ingesta for absorption. Similarly, lower estimates recorded for diets supplemented with 1 to 2 % α – cellulose indicated that higher levels of dietary fibre may have assisted in increasing the rate of passage through the gastro-intestinal tract.

In general, results in this study indicated that increased levels of α – cellulose, CeliteTM and Cr_2O_3 in the diet, influenced digestibility estimates. Digestibility coefficients based solely on endogenous CF produced significantly higher coefficients, whereas the addition of 1 - 2 % α – cellulose proved effective since results were found to compare favourably with those based on Cr_2O_3 and AIA. Thus, in view of the inability of *F. indicus* to degrade hetero polysaccharides, the inclusion or presence of α – cellulose within pelleted feeds may offer a valuable tool to nutritionist and prawn farmers alike for establishing feed digestibility, especially after considering that the inclusion of < 10 % cellulose was found, even at the expense of dietary energy, to improve the FCR and survival of *F. indicus* (Ahamad Ali, 2000).

In contrast, digestibility estimates of crude protein, crude fat, gross energy and dry matter based on AIA content, although slightly higher, provided reliable and consistent estimates similar to those of Cr_2O_3 (Table 5.3). In all cases the AIA was sufficient for analysis since a content of 0.72 % in Diet 1 was found to be adequate for measuring digestibility. The use of CeliteTM, to increase total AIA levels in excess of 2 %, reduced the variability of AIA determinations without affecting absolute values of apparent digestibility coefficients. Similarly, Thonney (1981), Atkinson *et al.* (1984) and Deering (1996) reported that the addition of an exogenous source of AIA proved reliable. The advantage of AIA as a marker is the low-cost and relative ease at which analyses were done, which makes it beneficial for use by researchers and prawn farmers in developing country.

9.5. Conclusion

Digestibility results of the chemical fraction of the six test diets were generally consistent with findings reported for other species of prawn. Results indicated that the gastrointestinal tract of *F. indicus* was effective at absorbing dietary protein and lipids and was found to be efficient at assimilating dietary energy. Differences between estimates could indicate differences in the physical and chemical composition of the marker, including density, particle size, surface area and

affinity for water that may or may not have influenced the rate of passage through the gastrointestinal tract of the species.

In closing, results of the current contribution suggest that AIA and the inclusion of Celite™ at $\leq 2\%$ proved most effective in digestibility studies involving *F. indicus*. Moreover, digestibility coefficients based on CF for diets supplemented with 1 - 2 % α – cellulose cannot be ruled out. Although results support the use of AIA and CF there remains concern that very low and variable levels in some natural sources may compromise the reliability of results. Nonetheless, due to the relative ease of analysing AIA, as opposed to chromium determination, AIA should be considered for routine digestibility studies involving *F. indicus*.

10.

Digestibility**The Apparent Digestibility of Common Feedstuff for Diets Formulated for *Fenneropenaeus indicus*.****10.1. Introduction.**

In culture operations the efficacy of feed to promote maximum growth depends on the animals' ability to ingest, digest, assimilate and metabolise the ingredients therein. Total or true digestibility are terms used to describe the degree by which diets, ingredients and/or nutrients are absorbed and assimilated. However, the extent to which dietary protein is digested is not accurately reflected since egested material will contain protein of bacterial origin, nitrogen from abraded gut mucosa and previously digested protein that has been metabolised and re-secreted into the digestive tract as enzymes (De Silva, 1989).

The term apparent feed digestibility (AFD) describes the portion of the diet absorbed without distinguishing between components derived from the feed and lost or secreted by the gut *i.e.* mucosa cells, bacteria flora and digestive enzymes (Lee & Lawrence, 1997). This fraction, expressed as a percentage based on the difference between the amount ingested and that egested, is known as the coefficient of digestibility and/or the apparent digestibility coefficient (ADC). Determination thereof is pivotal for the evaluation and formulation of least cost and balanced feeds (De Silva, 1989; Cho & Kaushik, 1990) since a major expense when culturing prawns is the costs of manufacturing of feeds, which can constitute in excess of 60 % of total production costs (Tan, 1991). Furthermore, with regards to aquatic species, knowledge of the availability and digestibility of nutrients is important in order to maintain suitable water quality and prevent the pollution thereof.

The standard and presently widely accepted approach to determine digestibility in aquatic organisms is to substitute 20 – 30 % of a reference diet, of which the total apparent and nutrient digestibility is known, with the test ingredient in a 20:80 or 30:70 ratio (De Silva, 1995). Substitution trials are based on the assumptions that no interactions or associative effects amongst the reference diet and the test ingredient takes place and that results are independent of the level of inclusion of the test ingredient. Furthermore, digestibility coefficients are assumed to be additive (Aksnes *et al.*, 1996).

Animals do not require feed ingredients *per se*, but rather the nutrients that form part of the chemical makeup of these ingredients. In both natural and prepared diets, protein is the most critical nutrient for the growth and development of prawns. A major source of protein in aqua-feeds is derived from fish meal and although the ingredient contains a well-balanced

mixture of essential amino acids and fatty acids the availability and cost thereof remains a concern among prawn farmers (Akiyama *et al.*, 1991; Sarac *et al.*, 1993; Lemos, Ezquerro & Garcia-Carreño, 2000). Thus, alternative sources of protein to formulate nutritionally sound feeds on a least cost basis remains (Reigh, Braden & Craig, 1990).

Little information pertaining to the apparent digestibility of feedstuff in prawns is known (Reigh *et al.*, 1990; D' Ambro *et al.*, 1997) and since most diets are formulated based on information from elsewhere the need to establish the digestibility of feedstuffs, obtained locally, exists. The present study was undertaken to evaluate the digestibility of fish meal, soybean meal, wheat flour, brewers yeast and wheat gluten, presently used by the local prawn industry, and two alternative sources *viz.*, sunflower oilcake and vitamin free casein. Of particular importance was establishing the digestibility of amino acids within these feedstuffs since a knowledge thereof would prove beneficial when predicting the desired food intake using the model approach elucidated in this study.

10.2. Material and Methods.

10.2.1. Experimental animals

Juvenile *F. indicus* of 12.62 ± 1.42 g (mean \pm S.D.), belonging to the same brood stock were obtained from Amatikulu Prawns and placed into rearing tanks. The juveniles were reared under laboratory conditions for 15 days and fed a commercial diet (Table 1.1). Thereafter animals were fed the experimental diets 4 days prior to the collection of faecal samples.

10.2.2. Experimental facilities.

Rearing systems, described in Appendix 2, were used to house the animals.

10.2.3. Experimental procedures.

Each tank was stocked with 12 prawns. Prior to being fed the morning ration, faeces and exoskeleton from recently moulted animals were removed and discarded. Feeding and the daily collection of samples were in accordance with procedures described in paragraph 5.2.6. Trials were terminated after 5 weeks when sufficient dry samples were obtained for analysis.

10.2.4. Experimental conditions

Water was maintained at (mean and standard deviation): 28 ± 1 °C, 28 ± 3 ‰ and a pH 8 ± 2 with oxygen concentrations kept near saturation throughout the trials. Both water

temperature and salinity readings were taken in the morning and afternoon while pH was measured before the animals were fed in the mornings.

10.2.5. Diets preparations.

For the most part diets were prepared in accordance with procedures explained in previous chapters. In this study eight experimental diets were used, with the first, the reference diet (Diet 1), formulated to comprise ingredients widely used in commercial and practical feeds. Diets 2 to 8 were prepared by substituting 20 % of the protein sources within the reference diet with the ingredient to be tested. The composition of the test ingredients and experimental diets are provided in Tables 10.1 and 10.2, respectively. All diets included 2 % Celite™. Ingredients for each diet were mixed with water in a commercial food mixer and wet extruded through a die with 3-mm holes. Extruded strands were placed onto oven racks and dried at 30 °C for 48 h. The dried strands (< 8 % moisture) were broken into approximately 6 mm lengths and stored in air-tight containers at – 20 °C.

Table 10.1. Proximate composition of the feed ingredients.

	Crude protein ^a	Lipids ^a	Ash ^a	Crude fibre ^a	Energy ^b	Dry matter ^c
Fish meal	66.57	9.43	15.88	-	19.02	92.40
Wheat flour meal	12.32	1.40	0.70	0.67	15.93	90.25
Soybean meal	49.52	2.64	6.86	4.32	17.77	90.50
Wheat gluten meal	80.92	1.53	0.86	0.41	21.40	91.65
Brewers yeast	41.39	0.39	6.49	0.64	16.92	90.22
Sunflower oilcake	47.98	2.21	6.86	10.35	17.76	91.80
Casein	92.31	0.65	2.41	0.22	21.69	91.11

^a % Dry matter

^b MJ/kg dry matter

^c % Wet matter

During this study the auto analyser broke down and at present, due to a lack of funds, has still not been repaired. As a result, the digestibility of amino acids was not examined. However, prior to the breakdown of the auto analyser the amino acid content in each of these feedstuffs was analysed and the results thereof are provided in Appendix H.

10.2.6. Digestibility Determination

ADCs in experimental diets were calculated according to Maynard and Loosli (1969):

$$\text{ADC of dry matter of diet (\%)} = 100 \times \{1 - (D_m \div F_m)\}$$

$$\text{ADCs of nutrients and energy of diet (\%)} = [1 - (F \div D \times D_m \div F_m)] \times 100$$

where

F = % nutrient or energy in faeces
D = % nutrient or energy in the diet
D_m = % marker in diet
F_m = % marker in faeces.

[10.1]

ADCs of dry matter (%) were calculated using the following (De Silva 1995):

$$\text{ADC (\%)} = \{\text{ADC of test diet} - (0.8 \times \text{ADC of reference diet})\} \div 0.2 \quad [10.2]$$

The ADC of organic matter, energy and protein in the test ingredient (%) was calculated using the following formula (De Silva, 1995):

$$\text{ADC (\%)} = \{(\text{nutrient or energy ADC of test diet}) - (0.8 \times \text{nutrient or energy ADC of reference diet})\} \div 0.2 \quad [10.3]$$

Table 10.2: Composition of the experimental diets (% d/m)

Components (g/100g d.m.)	Diets							
	D1 - RF	D2 - FM	D3 - WF	D4 - SM	D5 - WG	D6 - BY	D7 - SO	D8 - C
Fish meal ^a	36.45	49.06	28.98	28.98	28.98	28.98	28.98	28.98
Wheat flour ^b	30.97	24.62	44.70	24.62	24.62	24.62	24.62	24.62
Soybean meal (46) ^c	16.47	13.09	13.09	33.17	13.09	13.09	13.09	13.09
Wheat gluten ^d	3.87	3.07	3.07	3.07	23.15	3.07	3.07	3.07
Brewers yeast ^e	4.83	3.84	3.84	3.84	3.84	23.92	3.84	3.84
Sunflower oilcake ^f	-	-	-	-	-	-	20.08	-
Casein ^g	-	-	-	-	-	-	-	20.08
Fish oil ^h	1.27	1.27	1.27	1.27	1.27	1.27	1.27	1.27
Soy Lecithin ^c	1.27	1.27	1.27	1.27	1.27	1.27	1.27	1.27
Vitamin mix ⁱ	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Mineral mix ^j	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Vitamin C ^k	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Limestone ^h	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92
Cholesterol ^l	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Celite TM ^r	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Proximate analysis (g/100g d.m.)								
Crude protein	41.46	46.33	35.43	42.83	48.54	42.41	42.59	52.64
Lipid	6.24	6.80	5.24	5.53	5.53	5.14	5.41	5.09
Ash	10.19	11.45	8.66	9.88	8.64	9.94	9.89	9.00
Crude fibre	1.46	1.36	1.30	2.07	1.20	1.79	3.24	1.21
Acid-insoluble ash	2.33	2.78	2.34	2.69	2.39	2.23	2.54	2.19
Gross energy (kJ/g d.m.)	18.03	18.27	17.59	17.90	18.70	18.32	17.90	18.69

^a Contra Fisheries (Cape Town, South Africa), pilchard (*Sardinops ocellata*).

^b Ngwane Mill (Kwazulu, South Africa).

^c Epol (Pretoria, South Africa).

^d C.J. Petrow (Durban, South Africa).

^e SW Corp (Vierfontein, South Africa).

^f South African Breweries (SAB).

^g Sigma (St. Louis, MO, USA).

^h Energy Oils (Durban, South Africa)

ⁱ Crest Chemicals (Durban, South Africa).

^j Per kg of feed: Thiamin, 10.5mg; riboflavin, 10mg; pyridoxine, 12.5mg; pantothenic acid, 18.5mg; Niacin, 10mg; Biotin, 0.50mg; inositol, 60mg; choline, 100mg; folic acid, 2.5mg; cyanocobalamin, 0.025mg; ascorbic acid, 250mg; vitamin A, 4200IU; vitamin D, 2500 IU; vitamin E, 300mg; vitamin K, 20mg; Calpan, 50mg; Endox, 25mg.

^k Per kg of feed: Calcium, 246mg; phosphorus, 80mg; magnesium oxide, 6.31mg; sodium, 60mg; potassium, 90mg; iron, 3mg; copper, 0.34mg; zinc, 1.1mg; manganese, 0.2mg; selenium, 0.05mg; cobalt, 0.14mg; iodine, 0.01mg; ferrous, 0.89mg

^l Cholestrol, (Salvay Pharmaceuticals, South Africa)

10.2.7. Analytical protocol.

Chemical analyses were performed on the experimental diets and faeces as described previously in paragraph 9.6.7. The adjustment for nutrient loss due to leaching was established by submersing each diet, of known mass, for a period of 30 minutes, since this was the

maximum period prawns were observed to feed until satiated. Thereafter the feed residue was collected by siphon, retained on a 63- μ m nylon mesh and rinsed using distilled water. The residue was transferred to pre-weighed aluminium foils, oven dried at 70 °C for 48 hours, placed into a desiccator. Samples were analysed for nutrient and marker content. Leaching losses were determined in triplicate for each diet and averaged whereas the effect of leaching from faeces could not be determined because faecal strands that had not contacted water were unobtainable. These results were used to adjust digestibility coefficients for the leaching of nutrients as done by Reigh, Braden and Craig (1990).

10.2.8. Statistical analysis.

Results were subjected to one-way analysis of variance (ANOVA). Differences between replicated means ($P < 0.05$) were evaluated by the Tukey's multiple range test (Zar 1996).

10.3. Results.

10.3.1. Faecal composition.

Crude protein, ash, fat, energy, crude fibre and marker content of faecal matter collected from prawns fed the six test diets are shown in Table 10.3. Ash, crude fibre, phosphate and dietary marker contents were, by comparison, significantly higher in the faeces of the prawn than in the feed ingested.

Table 10.3: Mean values of faecal protein (N x 6.25), fat, ash, crude fibre, energy, phosphate and marker content, expressed as a % on a moisture free basis, with standard error (S.E.) in parenthesis ($n = 3$).

Component	Diets							
	D1 - RF	D2 - FM	D3 - WF	D4 - SM	D5 - WG	D6 - BY	D7 - SO	D8 - C
Crude protein	25.63 (± 0.37)	29.06 (± 0.29)	22.17 (± 0.30)	23.30 (± 0.31)	25.82 (± 0.54)	20.41 (± 0.12)	19.15 (± 0.39)	30.40 (± 0.70)
Lipids	1.69 (± 0.11)	4.18 (± 0.07)	1.90 (± 0.41)	1.72 (± 0.42)	2.99 (± 0.38)	1.80 (± 0.26)	1.21 (± 0.14)	2.14 (± 0.31)
Ash	40.63 (± 0.37)	38.18 (± 0.27)	40.82 (± 0.22)	42.01 (± 0.35)	39.82 (± 0.27)	44.02 (± 0.10)	37.12 (± 0.24)	34.84 (± 0.17)
Crude fibre	5.08 (± 0.21)	4.94 (± 0.34)	5.38 (± 0.18)	6.68 (± 0.24)	5.62 (± 0.33)	6.31 (± 0.37)	8.61 (± 0.91)	4.87 (± 0.56)
Energy	10.55 (± 0.36)	13.35 (± 0.12)	10.46 (± 0.25)	10.38 (± 0.33)	10.71 (± 0.28)	9.09 (± 0.11)	10.87 (± 0.32)	12.31 (± 0.22)
Phosphate	3.69 (± 1.04)	4.06 (± 0.22)	3.45 (± 0.43)	3.54 (± 0.36)	3.67 (± 0.39)	3.77 (± 0.13)	3.28 (± 0.29)	2.94 (± 0.24)
AIA	10.24 (± 0.24)	10.86 (± 0.41)	9.60 (± 0.36)	10.65 (± 0.36)	10.60 (± 0.35)	9.58 (± 0.31)	10.35 (± 0.17)	9.87 (± 0.26)

10.3.2. Apparent digestibility of dry matter.

The apparent digestibility of dry matter, crude protein, lipid and dietary energy in *F. indicus* for the six experimental diets is shown in Table 10.4. Apparent dry matter digestibility (ADMD) for the various ingredients ranged between 63.53 and 80.06 % (Table 10.4). The digestibility coefficients of the various ingredients were significantly different between ingredients ($F = 11.61$; $df = 7, 16$; $P < 0.001$).

The highest ADMD was recorded for casein followed by wheat gluten, brewers yeast, wheat flour, sunflower meal, soybean meal and fish meal, respectively (Table 10.4). Among animal sources the digestibility of dry matter in casein was significantly higher than that of fish meal ($q = 8.734$, $q_{(0.05, 15-6)} = 4.595$; $P < 0.001$). Amongst plant sources the ADMD of wheat gluten was found to be the highest followed by brewers yeast, wheat flour, sunflower oilcake and soybean meal, respectively (Table 10.4). Mean values of ADMD for animal products were less variable than those for plant products (Table 10.4).

Table 10.4: Apparent digestibility coefficients for dry matter, crude protein, lipid and energy in feedstuff consumed by juvenile *F. indicus* (mean \pm S.E; $n = 3$).

Components	Diets							
	D1 - RF	D2 - FM	D3 - WF	D4 - SM	D5 - WG	D6 - BY	D7 - SO	D8 - C
Dry Matter	77.20 ^a (± 0.32)	63.53 ^b (± 1.57)	69.21 ^{abc} (± 2.62)	64.55 ^{bcd} (± 2.56)	78.51 ^{ac} (± 1.95)	74.87 ^{acdf} (± 2.10)	68.32 ^{abcdf} (± 1.90)	80.06 ^{ade} (± 0.92)
Crude protein	85.90 ^a (± 0.23)	75.94 ^b (± 0.89)	80.02 ^b (± 1.98)	87.47 ^{ac} (± 1.66)	97.03 ^d (± 1.46)	95.58 ^{def} (± 0.67)	96.05 ^{def} (± 0.77)	90.13 ^{acde} (± 0.36)
Lipids	94.07 ^a (± 0.24)	45.85 ^b (± 1.52)	80.53 ^a (± 5.43)	85.11 ^a (± 6.36)	60.49 ^b (± 5.83)	87.28 ^a (± 1.91)	95.60 ^a (± 0.95)	87.82 ^a (± 2.16)
Energy	86.65 ^{cd} (± 0.36)	59.74 ^a (± 0.79)	80.79 ^b (± 2.03)	79.55 ^b (± 1.16)	88.86 ^e (± 0.93)	92.66 ^f (± 0.47)	81.45 ^b (± 0.68)	82.33 ^c (± 0.41)

^{abcde} Components with the same superscripts indicate that mean values within rows are not significantly ($P > 0.05$) different.

10.3.3. Apparent digestibility of protein.

Coefficients of apparent crude protein digestibility (ACPD) ranged from 75.94 to 97.03 % and were found to differ significantly ($F = 44.35$; $df = 7, 16$; $P < 0.001$) (Table 10.4). The highest coefficients were derived from plant sources, with wheat gluten having the highest ACPD followed, in descending order, by sunflower oilcake and brewers yeast. In contrast the ACPD in fish meal was the lowest amongst the foodstuffs tested (Table 10.4).

10.3.4. Apparent digestibility of lipid.

Apparent lipid digestibility (ALD) ranged from 45.85 to 95.60 % and was significantly different ($F = 29.07$; $df = 7, 16$; $P < 0.001$) between the various ingredients tested (Table 6.4). The range of ALD coefficients for fish meal and casein (45.85 – 87.82 %) was greater than for

plant products, which were between 60.49 and 95.60 %. ALD was the highest for sunflower oilcake (95.60 %) and lowest for fish meal (45.85 %) (Table 10.4).

10.3.5. Apparent digestibility of dietary energy.

Apparent energy digestibility (AED) coefficients ranged from 59.74 – 92.66 % (Table 10.4) and were significant ($F = 116.4$; $df = 7, 16$; $P < 0.001$). AED coefficients for plant products (79.55 – 92.66 %) were less variable than those for animal products, which ranged from 59.74 to 88.86 %. AED was highest for brewers yeast followed, in descending order, by wheat gluten, casein, sunflower oilcake, wheat flower, soybean meal and fishmeal (Table 10.4).

10.4. Discussion.

10.4.1. Digestion of dry matter.

Components within feedstuffs are not digested equally and the digestibility of ingredients that appear to be similar in proximate composition may vary considerably. The determination of ADMD provides a measure of the portion that is digested and absorbed (Brunson, Romaine & Reigh, 1997). However, if nutrient leaching occurs prior to the consumption and collection of faecal matter then digestibility can be overestimated. Since it was not possible to obtain faecal strands prior to coming into contact with the surrounding medium, nutrient losses due to leaching were considered to be minimal. The difference in ADMD estimates between plant and animal sources was assumed to be attributed to the indigestible components of plants *viz.*, fibrous material, being considerably higher. Thus, it was of no coincidence that the highest ADMD was found to be for casein followed by gluten since both ingredients, although high in crude protein, are low in crude fibre (Table 10.1). Overall ADMD tended to decrease as the content of fibre, ash and to a lesser extent lipid increased.

10.4. 2. Digestion of dietary protein.

Unlike ADMD, ACPD was found to be relatively high, ranging between 76 and 95 % for ingredients tested. Similar levels of crude protein digestibility have been found in *Palaemon serratus*, *Pandalus platyceros*, *Penaeus vannamei* (Forster & Gabbott, 1971; Akiyama *et al.*, 1989), *P. monodon* (Shiau, Lin & Chiou, 1992) and *P. setiferus* (Brunson, Romaine & Reigh, 1997).

In this study, ACPD coefficients greater than ~ 95 % recorded for wheat gluten and sunflower oilcake protein were significantly higher ($P < 0.05$) than those of the other foodstuff tested (Table 10.4). Information pertaining to protein digestibility of brewers yeast and sunflower oilcake by penaeids is scarce yet by comparison ACPD estimates for soybean meal,

fish meal, wheat flour and casein in this study were similar to those established for *P. mondon* (Shiau, Lin & Chiou, 1992) and *P. setiferus* (Brunson *et al.*, 1997).

An ACPD of ~ 76 % for fish meal was considered low especially since digestibility, depending on the source, is generally found to be > 80 % in prawns (Lee & Lawrence, 1997). Factors such as the presence of enzyme inhibitors within the diet, inappropriate diet formulation, the chemical makeup and the time the raw material spends in storage including the drying and processing techniques used to manufacture the feed all have an effect on the digestion (Akiyama, 1992). Due to these factors it was assumed that the fish meal used was not of fresh quality.

A higher fibre content in the diet with sunflower oilcake may possibly have facilitated a slower transit of digesta through the gut which in turn allowed for an increase in protein uptake. On the other hand, a high ACPD recorded for gluten was possibly ascribed to the chemical composition of the feedstuff which was found to be high in protein (81 %) and low in lipid, ash and fibre content of 1.5, 0.86 and 0.41 %, respectively (Table 10.1).

10.4.3. Digestion of dietary lipid.

Differences in lipid digestibility were more difficult to interpret since most of the plant products, as opposed to animal sources, had minimal amounts of lipid. This was evident when examining the apparent lipid digestibility (ALD) coefficients of brewers yeast, which had the lowest lipid content of all ingredients tested (0.39 %) whereas fish meal, which had the highest (9.49 %), was found to have the lowest ALD. Reasons for such a low ALD were once again thought to be attributed to the high ash content found in fish meal and/or the nature and composition of the fatty acids. Hence results suggest that if provided with a choice of food the species may not readily assimilate dietary fat in order to meet its metabolic requirements. The highest ALD was reported to be for sunflower oilcake and was assumed to be attributed to a decrease in transit time of digesta caused by an increased fibre content.

10.4.4. Digestion of dietary energy.

With few exceptions, the digestibility of dietary energy differed significantly between ingredients. In aquatic animals, energy digestibility of plant products tends to be inversely related to the fibre content and in some species the carbohydrate content as well (Brunson *et al.*, 1997). Fibre contains virtually no digestible energy for monogastric animals and carbohydrates may be digested with varying degrees of efficiency, depending on the chemical makeup and the digestive capability a species. Thus, apparent energy digestibility is known to vary widely among plant products, even among materials of similar proximate composition (Brunson *et al.*, 1997). Ahamad Ali (1993; 1996) reported that digestion of carbohydrate in juvenile *F. indicus*

was similar to that of protein since diets supplemented with 30 % starch had an apparent digestibility above 85 %. The efficiency to digest and assimilate carbohydrates was due to amylase activity decreasing when fed dietary starch in excess of 20 % inclusion whereas levels of protease and lipase remained unaffected by the concentration of dietary starch ingested (Hemambika & Paul Raj, 1999).

Carbohydrate digestibility was not determined in this study, but a comparison of AED coefficients for ingredients that differed in carbohydrate (NFE) content indicated that the utilization of plant starch by *F. indicus* varied amongst the ingredients consumed. Among plant products, brewers yeast (~ 25 % NFE), wheat gluten (~ 29 % NFE), soybean meal (~ 28 % NFE) and wheat flour (~ 38 % NFE) had AED coefficients greater than 79 %. All of these products, with the exception of fish meal, contained relatively low levels of lipid (< 3 %). Thus, significant differences in energy digestibility of materials that contained high levels of starch and low levels of protein, lipid and fibre (*i.e.* wheat flour as opposed to brewers yeast) appeared to be related to the nature of the carbohydrate rather as opposed to the quantity thereof.

In the present study the digestibility of the reference diet was a constant, used in calculating the digestibility of each ingredient, since diets varied only in respect of the ingredient tested. Associative effects were not examined. Non-additivity, in which the digestibility of a mixture of ingredients is found to be greater or less than the mean of the individual feedstuffs is a phenomenon that commonly occurs in terrestrial studies of herbivores (Mould 1988; Pond *et al.*, 1995) and was found also to occur in crayfish (Brown *et al.*, 1989; Reigh *et al.*, 1990) freshwater turtles (Bjorndal, 1991) and fish (Cho *et al.*, 1982; Wilson & Poe, 1985). In order to examine associative effects, future studies could establish if the above ingredients are additive by adopting the method proposed by Lee and Lawrence (1997). This method requires that a single feedstuff be evaluated by substituting the test ingredient in a reference feed, which is highly digestible, at levels of 15, 45 and 95 %. Results would entail that the feed digestibility be plotted over the percent substitution of the feed. To establish the associate effects of this feedstuff on feed digestibility the shape and slope of the curve could then be evaluated using multiple regression analyses (Lee & Lawrence, 1997). Future assessment of nutrient digestion of feedstuff, in particular protein, at different developmental stages of the species will contribute to a better understanding towards formulating feeds.

10.5. Conclusion.

Substitution of the test ingredient in a 2:8 ratio provided a reliable means for establishing the digestibility thereof. In the present study plant seed meals, obtained locally, were found to be highly digestible in *F. indicus*. These ingredients, in association with their digestibility estimates, can and were used to formulate nutritionally sound low cost feeds for the species (Appendix H).

11.

Final Remarks

On entering the 21st Century a basic understanding of growth and nutrient utilization by prawns is definitely an area scientists and producers know less than anticipated. A myriad of studies have focused primarily on diet composition (% nutrient in diet) but few have rationally looked at quantitative aspect of nutrient utilization (g nutrient/unit of mass gain). Moreover, due to the experimental design used in many studies it is very difficult to go back to published data and examine nutrient utilization *a posteriori*. Inadequate feeding practices such as feeding a fixed ration, regardless of whether or not feed wastage occurs, and failure to determine the chemical composition of the experimental animals are all too common. Furthermore, comparative studies are compounded by the absence of ingredient definitions, international feed numbers, precise descriptions of proximate chemical composition and particle size (Tacon & Akiyama, 1997).

In commercial operations accurate decision-making can seldom be met by empirical means alone since the need for more practical, meticulous and detailed approaches remain. It is assumed that empirical approaches may possibly give way to systems based approaches since simulation models are designed to determine nutrient requirements at different developmental stages and identify research that will provide for better and more effective management decisions (Ferguson, Gous & Emmans, 1994).

In order to establish a more comparative and structured means of predicting and describing potential growth and nutrient requirements in aquatic animals a plausible theory by Emmans and Fisher (1986) on growth and food intake in growing animals was used in this study. Emmans and Fisher (1986) address the problem of describing growth by assuming that growing animals have an inherent potential that can be measured under ideal/non-limiting conditions, and that an animal will attempt to attain its potential and therefore reach maturity in the shortest possible time. The potential rate of gain is determined by means of the Gompertz function whereby body protein content is used as the underlying variable. An advantage when using the above approach is that measurements of the relative growth rate at two or more intervals during the growth phase can be used to determine two components of the growth curve, namely P_m and β .

Since the amino acid composition of exoskeleton differed markedly from that in the flesh, growth in each component was examined separately. The potential growth of the exoskeleton was made a Gompertz function of time whereby the rate of maturing was found to be the same time constant as flesh protein.

Growth estimates of chemical fractions, based on flesh protein content, were predicted using allometry. Given the growth rate and the allometric relationships between the different body fractions and flesh protein, the growth rate of the empty body was calculated as the sum of the rates in each component. The retention of protein, water and ash calculated in this way represented the maximum growth under conditions described in this study.

Once the growth rate of the species was known, daily requirements for maintenance and growth, in each component, needed to be determined since in order for an animal to achieve its potential the acquisition of food and provision thereof becomes a priority. Thus, feed intake was seen to be dependent on the nutrient requirement of the animal and that in the feed. In order to predict food intake, it was necessary to predict the rate of intake of a balanced feed within a well-defined environment. This rate of intake was termed the desired food intake and was seen to be that which would allow the animal's growth potential to be realised.

In order to establish feed intake the first problem was to choose scales on which the supply of resources could be described; the scales chosen had to be consistent with those used for describing requirements. Maintenance requirement in mature livestock has been found to be proportional to mature size raised to the power 0.73, with the mature lipid-free, or protein mass being the preferred measure of mature size, considering that lipid does not have a maintenance cost. However, in growing endotherms this factor is influenced and is found to vary due to environmental conditions. In this study the fasting metabolic rate in juvenile *Fenneroenaeus indicus* reared at 20, 25, 30 °C and 15 - 35 ‰, was described by a factor of 0.78, 0.81 and 0.85, respectively. Under these conditions the oxygen consumption ($\text{mg} \cdot \text{hour}^{-1}$) together with a conversion rate of 20 kJ/mg O₂ respired and a multiple of 24 provided a means to calculate the daily energy requirement for maintenance. Although a considerable portion of the ingested energy was associated with the calorogenic effect the AHI/HE_i measured under these conditions provided no clear trend making interpretation of these data difficult. Results in this study would seem to indicate that AHI is less of an issue than in homeotherms. Thus, an alternative and plausible solution, suggested by Bureau *et al.* (2000), was used to establish the AHI. This method required that AHI be calculated by adding the energy required for maintenance (HE_r) and that for the retention of nutrients (RE).

In terms of available dietary energy Emmans (1984) and Oldman and Emmans (1990) demonstrated that metabolizable energy (ME) was an inaccurate measure of the energy content of a feedstuff because of the inability of the scale to differentiate between the energy values of the three digestible components of protein, lipid and carbohydrate, and because it fails to take into account the effect of indigestible organic matter of the diet. Instead an energy scale, proposed by Emmans (1984), which accounts for these deficiencies, known as the effective energy system, was implemented to establish the energy requirements for maintenance and growth. In turn the daily

ration was calculated based on the energy requirements of the animal and dietary yields. In this study daily rations established in this way proved pragmatic as opposed to when using the empirical approach, suggested by Bureau *et al.* (2000). Energy costs due to moulting (HE_x) and those due to exuviae (SE) losses could be incorporated into the model. However, further studies are needed to establish if this is necessary.

When comparing observed protein requirements and intakes with those predicted, based on the systems approach, results were lower than those established using conventional means. Measurements suggest that experimental conditions and/or the diet were/was limiting. Although the bent-stick model has the attraction of simplicity and an 'apparent' certainty about where the requirement lies, work by Morris (1999) highlighted the reasons why it is incorrect to measure a response from groups of animals and then apply the result to populations using this method as animals that all have the same nutrient requirements per unit body mass and per unit of output will, nevertheless, vary in response due to their size and potential.

Thus, in this study, the optimum requirement for protein was established by applying the Reading model to growth data acquired using the conventional dose-response method. Although this approach has the potential to be extremely meaningful as a management tool, since it is able to establish the optimum dose based on economical terms, interpretations are limited by individual outputs which in turn may be constrained by environmental/experimental conditions. Moreover, whereas the Reading model may be used to determine the economic optimum intake of amino acids during growth it is difficult to place an economic value on the marginal revenue for prawns small in size. With the difficulty of measuring food intake in aquatic species accurately this study has cast some doubts about the value of using this model to predict performance based on intake rather than nutrient concentrations and hence may not be the best method for determining the optimum dose of amino acids or protein for prawns varying in age.

On the other hand, the advantage when using the model was that the curvature was dependent upon the variability of experimental animals and that the response was found to be independent of the choice of dietary treatments, which is a marked contrast to fitting quadratic equations to sub-sets of data. However, for the model to be of any value an accurate means to measure feed intake is required in order to determine the optimal dosage whereby the desired feed intake (DFI), based on the daily feed requirement divided by the dietary concentration, can be calculated.

In mariculture operations protein is the primary and most expensive component of prawn diets. The bulk of feed used in the prawn industry is formulated to supply the nutrient requirements of growing juveniles to adults of market size. These feeds typically contain high levels of protein using sources such as high quality fish, prawn and squid meal with dietary protein levels ranging from 20 – 60 %, depending on the species and culture strategy. Due to these reasons the nutrient

value of feedingstuff, defined by the qualities of energy and nutrients that it yields, was studied. Sources found locally *viz.* wheat gluten, sunflower oilcake, brewers yeast, soybean meal and casein, were found to have digestibility coefficients of 97, 96, 95, 88 and 90 %, respectively for protein. In terms of dietary markers acid insoluble ash (AIA) was found to be a suitable replacement for the more traditionally used chromic oxide.

Although this study has shown that the systems approach provides a pragmatic means of predicting growth and food intake, many unanswered questions remain in order to completely understand the interactions between the animal, the diet and the environment in which it finds itself. Areas of weakness identified in the model are that it is extremely difficult to establish non-limiting conditions and feed intakes under aquaculture conditions. Another is how accurately can dietary requirements be established based on environmental constraints as these relationships are central to any theory of food intake and growth. Furthermore, unlike pigs and poultry, matters are compounded when establishing carcass composition since the exoskeleton needs to be accounted for. Within the physiological range, the maximum food intake amongst exothermic animals may be less likely to be influenced by ambient temperature. In this study the species, and possibly prawns in general, appear not to deposit fat to the same extent as do poultry and pigs. Since the body fat in laboratory reared prawns was found to be significantly different when compared to pond reared prawns, questions arise as to whether the species has a requirement to deposit fat and if so to what extent might they use this as a source of energy. Moreover, compared to chicken and pigs, juvenile prawns proportionally have a much lower growth rate which makes measurements difficult and in turn allows for inaccuracies to occur.

Nevertheless it would be incorrect to disqualify a theory on the basis of techniques that fail to quantify the concepts behind the theory since trying to prove a concept that is almost impossible to measure should not be used as a reason to discredit it. What is therefore required is some indication whether there is evidence contrary to the basic philosophy that one could disprove the theory, which should be transparent enough to be able to explain observed experimental results, rather than attempt to prove it (Ferguson, 1996).

Knowledge gained and the models derived from such research can be applied and translated into recommendations for the industry. The development of site or situation-specific feed requirement models may require some efforts by producers and feed manufacturers, by comparing current performances *e.g.* feed conversion ratios, with what is estimated to be biologically attainable.

In closing although the results of these trials could not establish the requirements or the optimum method of feeding prawns with absolute certainty, since the species failed to attain their true potential, the approach expounded in this study considers systems interactions that influence

the growth outcome. The method used here is considered to be better than techniques currently used, but requires that prawns grow at or near their potential and that food intake is accurately measured. Once these constraints are overcome the systems approach should yield results of great value to the prawn industry since it provides a simple and pragmatic means of predicting growth and feed intake in *F. indicus*. The approach requires that the growth response, the animal, nutritional constants, resource requirements, maintenance, fattening and feed intake be described in suitable terms. This not only increases the complexity of the model but is also thought to enhance the predictability with regard to the animal's response to a known treatment within a defined environment. The description of nutrient accretion in *F. indicus* is similar to that in higher vertebrates and further studies are needed to establish if the implementation of this approach remains relevant for other commercial species of prawns since the mean amino acid requirements, based on lysine ratios, were comparable to those of *Litopenaeus vannamei*, *Penaeus monodon* and *Marsupenaeus japonicus*.

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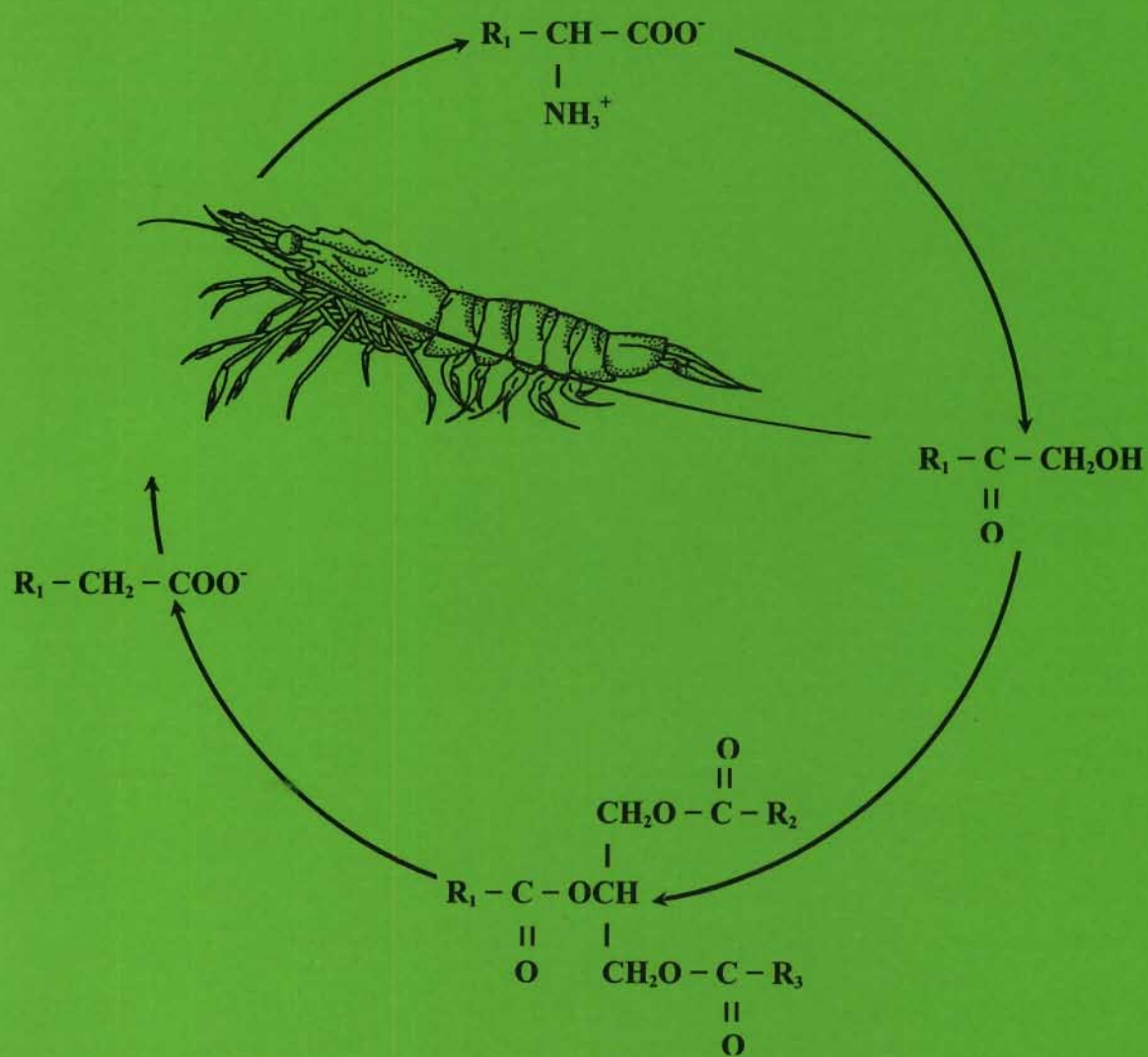
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Appendix A



Appendix A.

The Design and Construction of a Marine Re-circulating System to Facilitate Long Term Growth Trials.

A.1. Introduction.

Aquaculture/mariculture systems are broadly classified into two categories: flow through designs whereby water is continually being replenished by a fresh source and/or re-circulating designs in which water is constantly being recycled (Ng, Kho, Ho, Ong, Sim, Tay, Goh & Cheong, 1992). As opposed to re-circulating systems, flow through designs have the advantage of being able to stock higher numbers of individuals. For example, using a flow through system and a water exchange rate of 6 % per day, *Litopenaeus setiferus* postlarvae were able to be stocked at densities in excess of 350 prawns per m² (Palomino, Contreras, Sanchez & Rosas, 2001). However, flow through designs are not always practical or logistically possible since a reliable water source may not be readily available. Moreover, the costs involved for treating water borne diseases and maintaining certain parameters *e.g.* temperature and pH, may be substantial. By comparison re-circulating systems, although requiring lower stocking densities, prove ideal for controlling water parameters and are better suited in preventing the introduction of pathogenic micro-organisms associated with excessive water exchange (Tseng, Su & Su, 1998; Menasveta, Panritdam, Sihanonth, Powtongsook, Chuntapa & Lee, 2001).

The design of re-circulating systems is dependent on stocking rates, flow rates, exchange rates, feeding rate, production rate, diet composition and waste production. Re-circulating systems have been known to maintain good water quality and have been successful at rearing *Penaeus monodon* (Tseng *et al.*, 1998; Menasveta *et al.*, 2001), *P. setiferus* and *Litopenaeus vannamei* (Davis & Arnold, 1998; Velasco, Laurence & Castille, 1999). In re-circulating systems the maintenance of good water quality is vital for the health, survival and growth of the cultured organism since it is considered to be the first line of defence against environmental stress and/or the imposition of any adverse side effects that the cultured organism may experience.

Water maintenance involves the control and/or regulation of water pH, temperature, oxygen levels and the removal of animal metabolites such as faeces, total ammonia-N, nitrite-N and carbon dioxide since these factors adversely affect and lead to a deterioration in water quality. Of concern when developing a filtration system for a re-circulating system, that encompasses both nitrification and denitrification processes, is the removal of particulates and nitrogen from the recycled water

(Menasveta *et al.*, 2001). With the biological elimination of organic matter, the accumulation of total ammonia is regarded as the first limiting factor in a re-circulating system (Zhu & Chen, 1999; Bovendeur, Zwaga, Lobee & Blom, 1990) as problems encountered with the release and accumulation of metabolic waste may lead to potentially toxic conditions and significant fluctuations in water parameters such as pH, ammonia, nitrite, nitrate and dissolved oxygen. Moreover, poor water quality, although not bad enough to cause acute toxicity, may contribute to severe stress and eventually lead to the cessation in growth and the demise of the cultured organism. Thus, in a re-circulating design the main processes within a filtration system are considered to be clarification and biofiltration.

A.1.1. Clarification.

Clarification is the process whereby suspended solids are removed. Suspended solids are generally small particles ($< 100 \mu\text{m}$) of undigested or partially digested food, bacteria and algae, which may remain suspended in the water for an extended period of time. Fine suspended solids tend to reduce water clarity; whereas larger organic particles are problematic as they create a serious waste-load by consuming tremendous amounts of oxygen that adversely affects the dynamics of the system's ecology.

A.1.2. Biofiltration.

Biofiltration depends on the formation of a filter bed or filter matrix, which encourages the attachment and growth of beneficial bacteria that extract dissolved chemicals from the water and converts them to particulate biomass or harmless dissolved compounds. Given a proper environment bacteria grow and form a thin film that covers the surface of each bed medium (Zhu & Chen, 2002). There are literally hundreds of different species of bacteria at work in a biofilter. Most of the bacteria are classified as heterotrophic, which actively break down organic materials into carbon dioxide and water (Jardine, 1997).

If food and fecal matter accumulate in culture water, high molecular compounds will be proteolysed and deaminated to ammonia. The overall saprophytic process by which ammonia is released from organically bound nitrogen (*N*) is called ammonification with further mineralization to nitrite known as nitrification (Hagopian & Riley, 1998). Depending on the concentration high levels of un-ionized ammonia (NH_3) and nitrite (NO_2^-) are known to be toxic to most aquatic organisms. At $0.2 \text{ mg. } \ell^{-1}$ NH_3 becomes toxic for salmonids whereas nitrite toxicity varies greatly between species and their life stages. By comparison, the permissible nitrate level for *Penaeus monodon* is more than a 100 orders of magnitude higher whereby optimum levels for juveniles were

estimated to be 145, 158, 232 mg. ℓ^{-1} nitrate - N in salinities of 15, 25 and 35 ‰ (Tsai & Chen, 2002) and it is for this reason that it is imperative that ammonia and nitrite do not accumulate but be fully mineralised to relatively benign nitrate (Hagopian & Riley, 1998). For a given total ammonia concentration in an aqueous solution the proportion of un-ionized to ionised ammonia depends on temperature, pH, salinity (Zhu & Chen 1999; 2002), feeding regime and feed utilization (Kamstra, Heul & Nijhof, 1998). Thus, total ammonia nitrogen (TAN) concentration is often of key importance limiting water quality in intensive aquaculture systems (Bovendeur *et al.*, 1990; Zhu & Chen, 1999).

Within a biofiltration process there are phylogenetically two distinct groups of bacteria that, collectively, are responsible for nitrification. Ammonia-oxidizing bacteria catabolise un-ionized ammonia to nitrite (nitritation), while nitrite oxidizing bacteria mineralise nitrite to nitrate (nitration). The most critical, however, are broadly described as nitrifying bacteria, consisting primarily of the genera *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus* and *Nitrosovibrio*, which participate in the first stage of nitrification. The second stage involves the genera *Nitrobacter*, *Nitrococcus*, *Nitrospira* and *Nitrospina* (Hagopian & Riley, 1998) and are responsible for the conversion of the toxic nitrogen forms, ammonia and nitrite, to nitrate.

Management of biofiltration is critical at high loadings typical of re-circulating systems since the nitrification efficiency of a biofilter is normally inhibited by the existence of organic matter and heterotrophic bacteria which compete with the autotrophic nitrifiers especially in a fixed film filtration for oxygen, nutrients and space (Zhu & Chen, 2001). In terms of total nitrogen management the two major biological processes are considered to be nitrification and involves the bioconversion of ammonia to nitrate and denitrification, which is the bioconversion of nitrate to nitrogen gas (Menasveta *et al.*, 2001).

A.1.3. Nutrients.

Although non-toxic excessive nitrate levels need to be removed from the medium since they tend to stress both fish and invertebrates. In a natural system nitrates are assimilated by plants (Jardine, 1997). In a fresh water aquaculture systems, the floating water lettuce *Pistia stratiotes* was used to remove nitrogen (Prinsloo, Roets, Theron, Hoffman & Schoonbee, 1999), while marine macroalgae *Gracilaria chilensis*, *G. conferta* and *Ulva lactuca* were utilised to purge nitrogen metabolites from a polyculture of fish, oysters, clams, abalone and sea orchins (Neori, Ragg & Shipigel, 1998; Chow, Macchiavello, Santa Cruz, Fonck & Olivares, 2001).

Moreover, colonies of chemoheterotrophic bacteria are able to reduce nitrates considerably, and require an anaerobic environment in which to grow. These bacteria firstly reduce nitrates to nitrite, then to nitrous oxide and finally to nitrogen gas which is then lost from the system (Jardine

1997). Ideally a filtration system should be divided into regions where aerobic and anaerobic bacteria are able to propagate. The nitrification process may be regarded as the key factor when designing water treatment installations. Nitrification reaction based on a bacterial film attached to a fixed bed matrix, e.g. trickling filters and submerged filters are generally used in intensive aquaculture/mariculture systems (Bovendeur *et al.*, 1990).

Therefore, in this study to economise on water usage a semi-closed re-circulating system was adapted to maintain and rear *Fenneropenaeus indicus*. Various types of mechanical and biological filters were developed, tested and implemented while substantial aeration was supplied to facilitate the breakdown process of nitrogenous wastes as done by Provenzana & Witfield (1987) and Prinsloo *et al.*, (1999). In the present study a semi-closed recirculating system was constructed with the following criteria in mind:

- The system had to be able to house *F. indicus* for a minimum of 500 days.
- The system had to maintain a water quality, regarded as deal, that would allow for optimal growth.
- The system had to allow for high stocking densities.
- The system had to eliminate metabolites and high ammonia loads due to the feeding regime.
- The system had to be designed to ensure that there were no other sources of food available other than that presented.

A.2. The design of the re-circulating system.

A.2.1. Description of the closed seawater re-circulating system.

The closed re-circulating system consisted primarily of a supply tank, which supplied six existing glass aquarium rearing/culture tanks. Effluent water was filtered with the use of a mechanical filter and biofilter and pumped back into the supply tanks whereby the whole process was repeated (Figure A.1.).

A.2.2. The housing facility.

Besides funds, the limiting factor that dictated the size and design of the system was space since construction was confined to a 3.5 × 3.5.0 × 2.0 m room. A couple of extractor fans were used to ensure that there was a sufficient exchange of fresh air since all windows were kept closed and painted black to eliminate natural light. All overhead fluorescent tubes were covered with blue transparent sleeves to reduce light intensity, as opposed to diffused natural light, since the maturation and growth of *F. indicus* were found to increase under blue and green light (Emmerson,

Hayes & Ngonyame, 1983). Moreover, unlike red light that is rapidly attenuated according to the Lambert–Beer law, the irradiance of blue light of lower intensity is able to penetrate deeper into water (Emmerson *et al.*, 1983). This greatly decreases the spectrum range, which algae can utilise as photosynthesis in algae occurs mainly in the visible range of the spectrum between 400 and 700 nm, and photomorphogenic responses may extend the physiological effects of light into the far red from 700 to 760 nm (South & Wittick, 1987) and hence eliminating part of the spectrum responsible for photosynthesis.

The use of blue lighting ensured that algal growth and colonies of the photosynthetic bacterium *Rhodopseudomonas palustris*, found to be a viable source of food in a closed system (Kim & Lee, 2000), was not possible. Furthermore, it was assumed that darkened surroundings would assist by reducing stress levels. This view was supported during periods when cleaning and maintenance was performed on the system under normal lighting conditions when animals were found to be attracted to their reflection and would constantly bump themselves against the glass. An underwater quantum sensor (Model LIC-COR-189) registered the light intensity to be >1 nm.

Unlike *P. merguensis* the moulting frequency and growth rate of *F. indicus* were unaffected by photoperiod (Hoang, Barchiensis, Lee, Keenan & Marsden, 2003) since Vijayan and Diwan (1995) reported that light/dark regimes of 24L:0D, 12L:12D and 0L:24D had no effect on moult intervals and growth of the species. A light timer was used to maintain a photoperiod of 12L:12D.

A.2.3. *The mechanical filter.*

Thin shavings and off-cuts of polyvinylchloride (PVC) were placed into nylon mesh bags and stacked inside a 450 mm-diameter PVC tube that had been equally partitioned. The filter was positioned to receive the effluent of the six holding tanks *via* a 50-mm PVC pipe. The purpose of the filter, unlike the lamella separator used by Tseng *et al.* (1998), was to retard the flow of effluent by allowing the water to gravitate through these stacks, thereby trapping large suspended solids. Thereafter the water passed through a gap at the bottom of the filter and permeated upwards through an additional set of stacks that were situated within a second compartment.

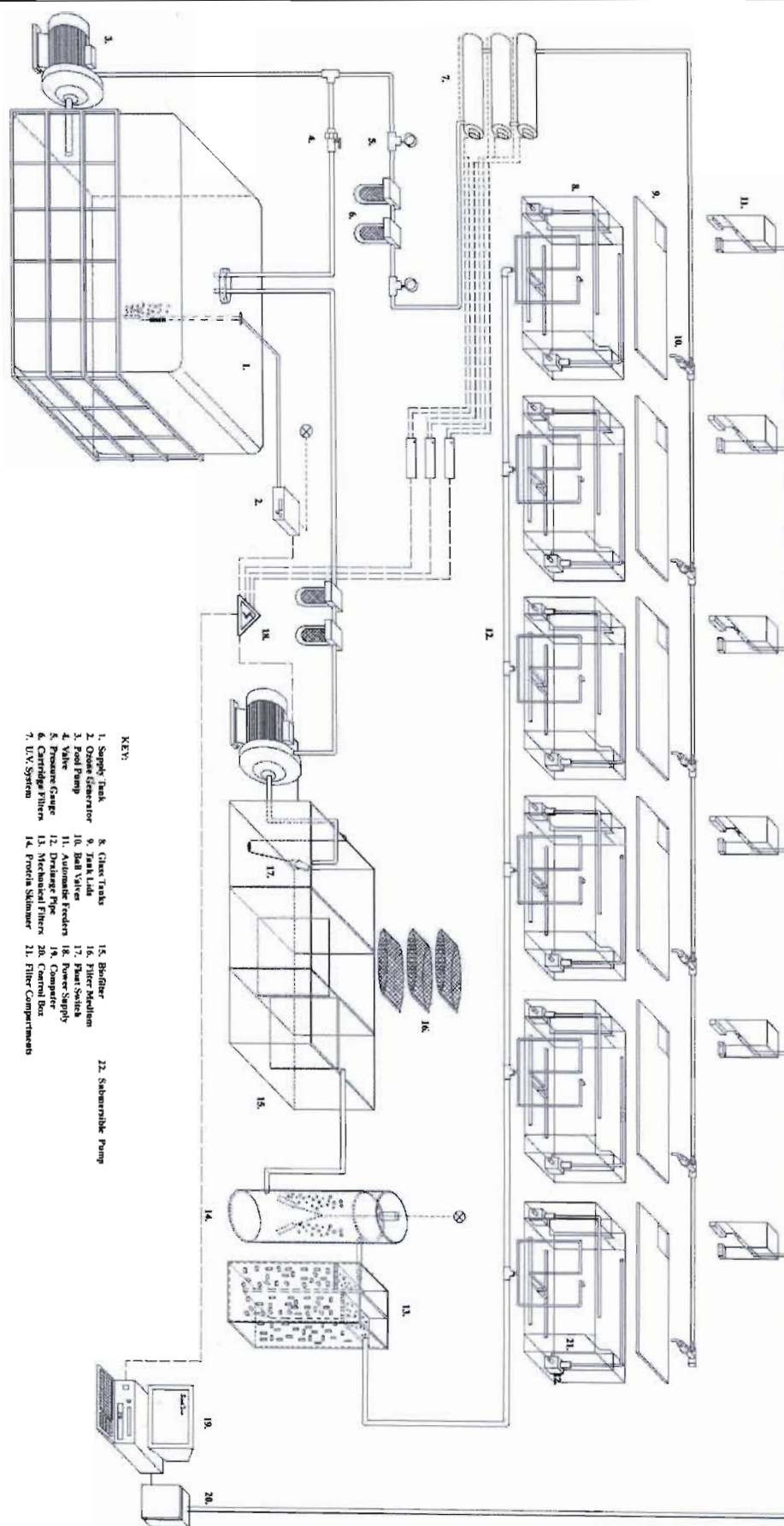


Figure A.1: A schematic diagram of the system.

Water exited the filter by trickling into a protein skimmer. Batting, a material made of coarse cotton, was placed onto makeshift shelves situated at the inlet and outlet of the unit and functioned as scrubbing screens. The mechanical filter was flushed of trapped material every fortnight by opening valves fitted to outlet ports situated at the base of the columns.

A.2.4. The protein skimmer.

The protein skimmer was a 300 mm-diameter PVC pipe that was 1 metre in height. A plastic funnel 10 mm larger in diameter was inverted and siliconed into a 400 mm long off-cut that served as the skimmer's collection cup (Figure A.1). Three large diffusers were submerged into the open end of the funnel. Generated bubbles percolated the fine suspended organic matter into a foam that was forced upward and out through the funnel nozzle and into the collected cup. The collection cup was cleaned when deemed necessary. The outlet of the protein skimmer was situated lower than the intake to allow the bubbles to trap small-suspended particles. The outflow was directed into the biofilter.

A.2.5. The biofilter.

The biofilter was a $1.16 \times 0.75 \times 0.64$ m and 5 mm thick fibreglass tank that was partitioned into seven compartments with the seventh being the largest and served as a 190 l collection tank. The compartments were designed to allow the water to flow downward into the 1st, 3rd and 5th and upward into the 2nd, 4th and 6th compartment with the water finally flowing over a trickle mat into the collection tank.

Sand pebbles, after having been sieved to obtain a size range of 6 - 10 mm, were used as the filter medium and placed into nylon mesh bags that were stacked approximately 10 high inside all six compartments. The bags made it possible to remove the medium when cleaning the biofilter. However, at a later stage these pebbles were replaced with lighter polypropylene beads as done by Ridha and Cruz (2001). The total surface area provided by the filter medium was calculated to be in excess of 480 m². Water accumulated within the collection tank was pumped into the supply tank through cartridge wound filters of 100 and 10 µm by means of a 0.45 kW Aquarius pool pump. The pump was fitted with a float switch that was activated and deactivated by the fluctuation in water levels within the collection tank.

Owing to different environments, thriving colonies of aerobic and anaerobic bacteria are not normally found together (Jardine, 1997). To compensate the mechanical filtered was void of aeration while sections of the biofilter remained well aerated.

A.2.6. The supply tank

The supply tank was a 1000 ℓ plastic tank housed within a 25 mm square tubing galvanized cradle (Figure A.2.). A temperature sensor and thermostat regulator coupled to a contactor activated a submersed electric heater (Model: Omega, 220 V, 3 kW) and ensured that the temperature did not fall below 27 °C. However due to the absence of a cooler unit water temperature, on occasion, increased to a maximum of 31 °C. Although maximum ambient temperatures were high the effect of environmental factors, namely that of temperature on the protein requirement for growth by seabass was shown to increase above the optimum temperature (Peres & Oliva-Teles, 1999). Hence it was reasonable to assume that a 2 – 3 °C above the optimum of 28 °C for *F. indicus*, suggested by Kumlu and Jones (1993), would not adversely affect the growth rate of the animal. Although variation in temperatures (24 – 31 °C) were found to significantly affect the growth, survival and biomass of *L. japonicus*, with the lowest production and survival recorded at the highest temperatures (Coman, Crocos, Preston & Fielder, 2002) in the present study the occurrence of water temperatures in excess of 30 °C were seldom attained.

Another factor in terms of maintaining a constant water temperature was that mass specific ammonia excretion rates of fasted individuals of two species of fish; *Epinephelus areolatus* and *Lutjanus argentimaculatus* showed an inverse relationship with body mass but a positive relationship with water temperatures (Leung, Chu & Wu, 1999). Temperature fluctuations may ultimately effect ammonia levels and filtration dynamics within the system promoting periods of high and low ammonia loads.

Ozone acts as a flocculent of small particulates and therefore assists in the removal thereof. Moreover, ozone is a powerful oxidizing agent capable of lowering the level of dissolved organics and destroying bacteria, viruses and numerous micro-organisms in suspension (Jardine, 1997). Due to these reasons an ozone generator (Model: AquaZone-200 Plus) combined with a redox controller was used to inject ozone into the incoming water through a silicon tube and diffuser. Ozone levels were kept conservative at dosages of 5 - 10mg/hr ozone per 100 ℓ to avoid the by-product, hyperbromous acid, which can cause mortalities amongst fish and prawns. The redox potential or oxidation-reduction potential range was set between 360 – 390 mV.

Water from the supply tank was pumped through two cartridge wound filters and a UV unit with the aid of a 0.45 kW Aquarius pool pump fitted with a float switch. The cartridge wound filters, 100 and 10 µm, were used to remove suspended particles larger in size. Cartridges were replaced when fowled.

After passing through these filters the water flowed over a series of three 30 W UV lights since UV assists in the sterilization of sea water by destroying free-swimming pathogens that

become exposed to the light (Jardine, 1997). The quartz sleeves of the ultraviolet unit were kept clean and attended to during scheduled maintenance.

Water samples were taken once a month from three randomly chosen outlets and were tested for bacteria content using the procedure described by Ingraham and Ingraham (1995). The analyses of the water indicated that the ozone and UV were adequate in supplying bacteria free seawater since high density bacteria cultures have been used as a source of food (McIntosh, Samocha, Jones, Lawrence, McKee, Horwitz & Horwitz, 2000).

A.2.7. *The rearing tanks.*

After passing through the UV unit the water was supplied to the rearing tanks *via* a ring feed system at a rate of 1.5 litres per minute providing a 12.4 times turnover per tank during a 24 hour period. The six rearing units, $0.92 \times 0.44 \times 0.43$ m glass tanks, were modified by incorporating two additional compartments of 0.15 m wide on either side that served as 28 l trickle filters (Figure A.3). The biological filter medium used was Siporax and Bio-chem beads. Initially these trickle biofilters served as a backup if NH_3 levels were found to be in the excess of 1.5 mg. l^{-1} . A submersible pump (Model: Project PJP-800), was used to circulate water back into the tanks through a spray bar.

The surface water from each of the tanks was skimmed off with the aid of a modified overflow pipe and fed into a drainage pipe while simultaneously permitting water to be drawn in from the bottom of the tank and discarded through a second opening *via* a network of pipes (Figure A.3). The effluent water was directed and disposed of using a 50 mm PVC pipe that facilitated as a drainage pipe. Once in the drainage pipe the water gravitated towards the mechanical filter.

Originally under gravel filters were used but were found to be ineffective as dead spots, with regards to water flow, resulted in the build-up of feed residue which in turn caused the water to become extremely anoxic. It was later decided that the bottom of the tanks would remain free of substrate, to allow for the easy siphoning of faecal and feed residue. The regular removal of waste material assisted in maintaining good water quality.

A.2.8. *Water quality analysis.*

Kumlu and Jones (1993) found that the optimum conditions for rearing *F. indicus* larvae in laboratory conditions were 25 ‰ and $27 - 28 \text{ °C}$. A later study indicated that the best performance of growth, survival and total biomass for *F. indicus* larvae (PL₆₀) was observed at salinities of between 20 and 30 ‰ (Kumlu & Jones, 1995). Since a change in salinity was found to negatively affected the growth and survival of *Metapenaeus monoceros* post larvae (Kumlu, Eroldogan &

Saglamtimur, 2001) a salinity of 27 ‰ was decided upon and maintained by diluting seawater with mature municipal tap water, that was left to stand for a week in order to mature. A water exchanged of 15 % was done every fortnight but due to evaporation and system losses, water levels were normally topped up every 3rd or 4th day.

Ammonia-*N*, nitrate-*N*, nitrite-*N*, pH and oxygen were measured using a multiparameter ion specific meter for aquaculture (Model: Hanna instrument C103). During trials the average values for ammonia-*N*, nitrate-*N*, nitrite-*N*, phosphate and pH levels were 0.22 ± 0.12 , 0.22 ± 0.12 , 0.03 ± 0.01 , 0.40 ± 0.02 mg/l and 8.1 ± 0.2 , respectively. During growth trials daily evaluations of dissolved oxygen and total ammonia-*N* levels were established in order to adjust aeration, water flow and exchanges rates.

Air was supplied from an industrial compressor through a number of diffusers connected by silicon tubing to outlet ports, the biofilter and the supply tank ensured that the water maintained a dissolved oxygen content of $\sim 4.8 \pm 0.3$ mg/l. pH was maintained by either using crushed oyster shells, water replacement and a crude makeshift scrubber to eliminate excess CO₂, unlike the method utilised by Menavesta *et al.* (2001) who used sources of carbon for denitrification or Wepner, van Vuren and Du Preez (1992) who used a pH controller (Model: Black Stone BL 7916).

A.2.9. Stocking densities.

In all cases healthy prawns, obtained from Amatikulu prawn farm, were used. Prawns were stocked at between 50 and 60 animals per m² as recommended by Emmerson and Andrews (1981) as opposed to 40 m⁻² and 75 m⁻² for *P. setiferus* and *P. vannamei*, respectively (Williams, Davis & Arnold, 1996). Overstocking was avoided since increased densities of 40 to 160 prawns per m² were shown to decrease the average growth rates and survival of *P. monodon* (Tseng *et al.*, 1998) and was found to impact negatively on the growth of *P. stylirostris* due to increased nitrogen levels (Martin, Veran, Guelorget & Pham, 1998).

The lack of surface area was overcome by placing makeshift platforms into the tanks in order to increase the bottom surface area by an additional 0.3 m². The grid platforms allowed for the free movement of water within the tanks, thereby avoiding the occurrence of dead spots. Increased surface area was an attempt to eliminate or minimise territorial behaviour and the formation of hierarchies that could exist within tanks between dominant and subordinate individuals since mass differentiation within groups have been known to increase inter-individual variation of food consumption, social interactions and the competition for space.

A.2.10. Automatic feeders.

Due to the feeding regime, which aim was to ensure that feed availability was not a limiting factor, automatic feeders were built. Transparent perspex containers of 1 ℓ capacity were constructed and mounted onto the edge of each tank. The containers had a 20 × 40 mm sliding door, which acted as a shutter and was operated using a central car door-locking unit fitted with an extension arm. The locking units were activated and deactivated by a series of 12 V relays, a step down transformer (220 V/12 V) using a FESTO Programmable Logic Control (PLC) (Model: E.FEC 20 - AC) (Figure A.4). The PLC was programmed to open and close the feeder units every six hours for a period that allowed for the desired amount of feed to be discharged into the tanks.

Although it is generally believed that increasing feeding frequency has immediate benefits, including reduced nutrient leaching and increased prawn growth, the growth rate of *Litopenaeus vannamei*, exposed to different feeding frequencies and regimes and reared under zero-water exchange were found not to be significantly different (Velasco, Lawrence & Castille, 1999). Moreover overfeeding was shown to be ineffective at accelerating prawn growth (Martinez-Cordova, Porchas-Cornejo, Vilarreal-Colemnares, Calderon-Perez & Naranjo-Paramo, 1998; Burford, Smith, Tabrett, Irvin & Ward, 2002). The reasoning being that the feeding rate and a diet high in protein, that isn't effectively utilised, has been shown to have a significant effect on Total Ammonia Nitrogen (TAN) and Total Organic Carbon (TOC) content (Singh, Ebeling & Wheaton, 1999) due to the leaching and contribution of nutrients into the surrounding medium (McIntosh, Samocha, Jones, Lawrence, Horowitz & Horowitz, 2001).

Prawns were fed seven days a week after every six-hour intervals (6:00, 12:00, 18:00 and 24:00 h) and was done to mirror the feeding regime practiced on the prawn farms. During the day if it was observed that there was no feed at the bottom of the tank additional food was introduced until the prawns were satiated. The feeding rates were adjusted according to the intake rates of the animals in order to avoid excessive feed residue accumulating on the bottom of tanks. Depending on the nature of the trial, the total amount of feed was adjusted weekly, fortnightly or on a monthly basis following mass determination of the animals in each tank.

A.2.11. Collection of feed residue and faecal matter.

Initially siphoning was done every day but this seemed to stress animals as they would not eat for two to three hours after siphoning. In order to remedy this problem the tanks, being of glass, were modified by installing faecal traps along the outlet pipes, down stream of the tanks (Figures A.6 & A.7). Ideally, future studies should implement and install faecal traps as done by Cho *et al.* (1987).

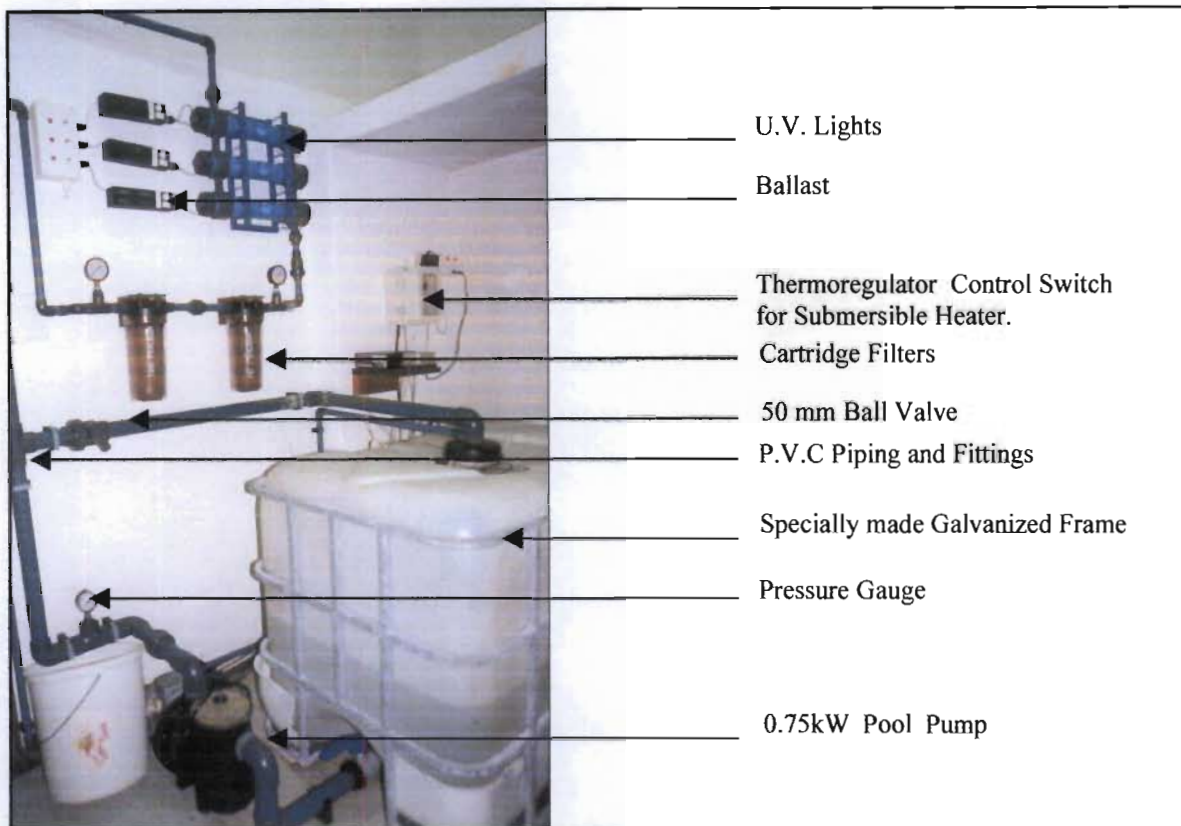


Figure A.2: View of the supply tank fully equipped with pump, plumbing, fittings, filters, UV lights and ballasts.

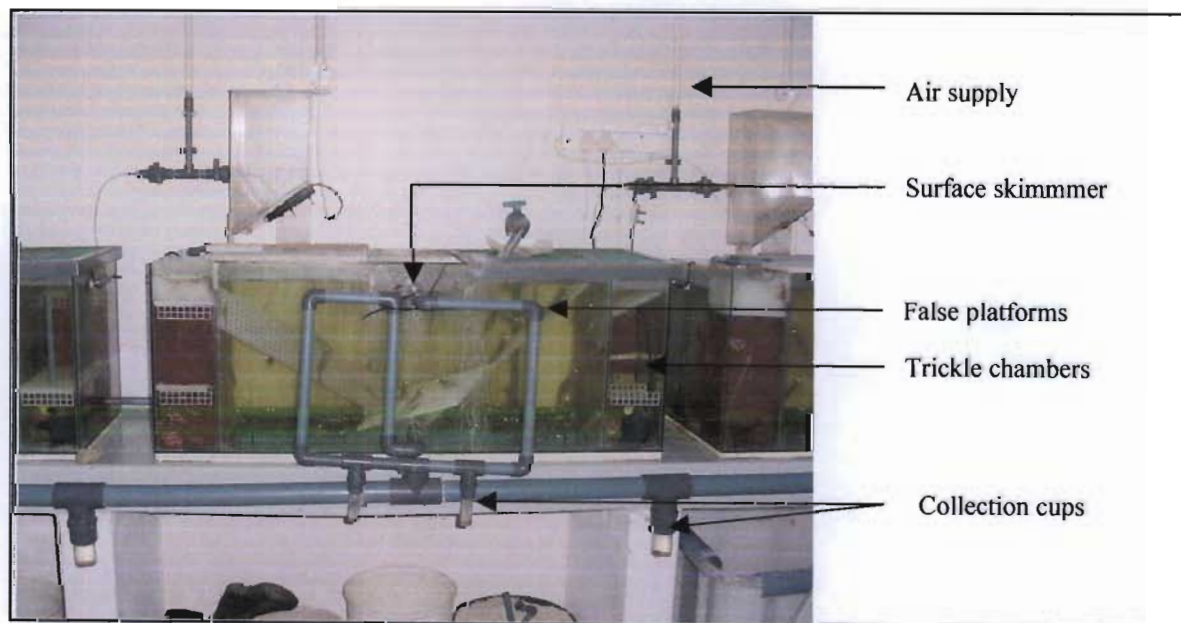


Figure A.3: View of rearing tank indicating trickle filters on either side of the tanks as well as the false platforms and collection cups.

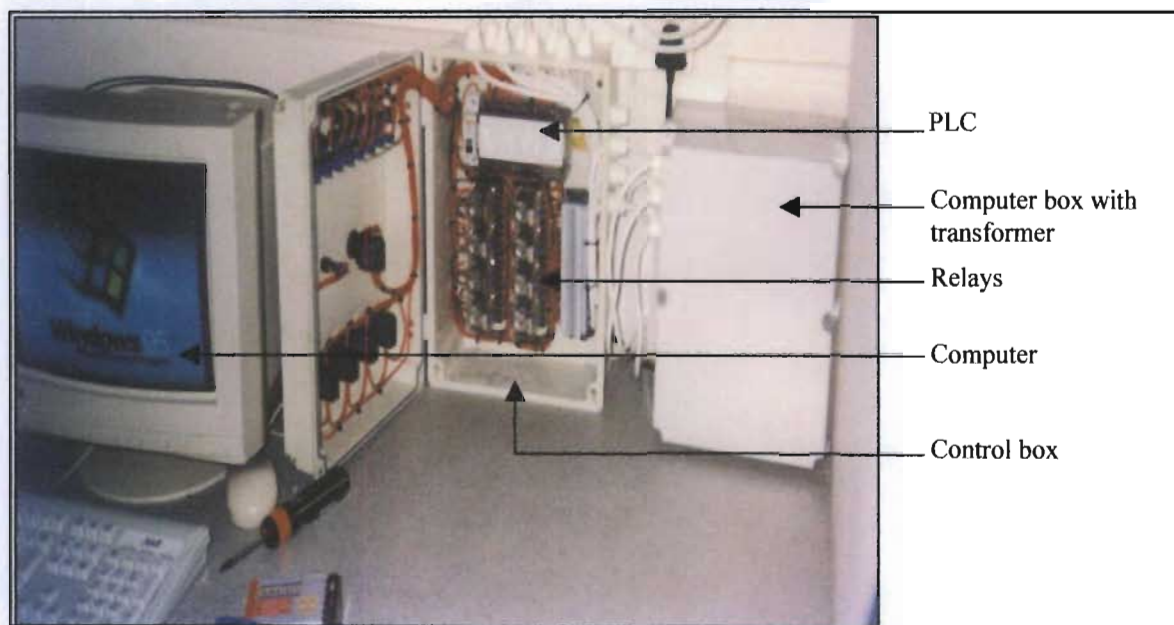


Figure A.4: View of the control box and computer.

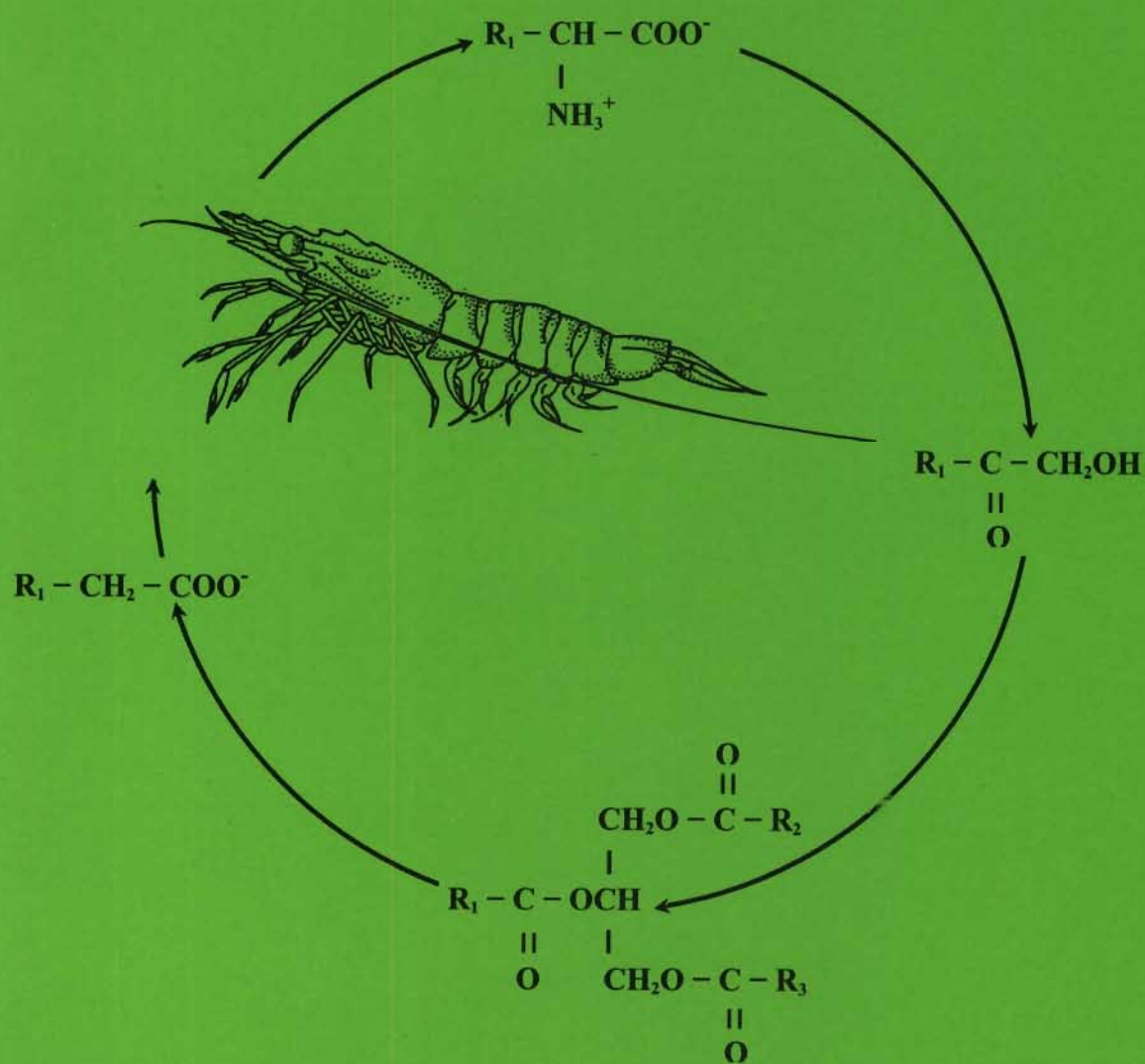


Figure A.5: View of the aquarium glass tanks complete with automatic feeders, drainage pipe and bio-filter.



Figure A.6: View of aquarium overflow pipe leading into the drainage pipe.

Appendix B



Appendix B.

The Design and Construction of a Marine Re-circulating System to Facilitate Short Term Growth Trials.

B.1. Introduction.

Since the mid-1970's there has been a trend to move away from the conventional fixed bed matrix biofilters as floating plastic media have been employed in the biofiltration components of high density aquatic re-circulating systems.

Generally classified as expandable granular biofilters (EGB) or bead filters, bead filters are distinguishable by the use of a buoyant granular medium. The packed bed of plastic beads, through which water is passed, captures solids and simultaneously provides a large surface area for cultures of nitro-bacteria. Bead filters dramatically simplify aquatic operations since they are capable of maintaining quality water at high waste loading rates by providing clarification and biofiltration within a single unit. An advantage is that they occupy less space than the conventional filter. Physical straining is probably the most dominant mechanism for the larger particles ($>80\text{ }\mu\text{m}$) with suspended particles between $20 - 80\text{ }\mu\text{m}$ removed by interception, a subtle process caused by collisions between the particle and the bead media surface. Bead filters are also able to remove finer particles ($<20\text{ }\mu\text{m}$) at a slower rate. It is believed that bio-absorption, the capture of particles by the bacterial biofilm, is the dominant removal process for such fine particles.

Due to the nature of the study, three identical re-circulating systems were constructed to facilitate short-term growth trials using EGB filters. The following is a description of the re-circulating design used.

B.2. A description of a semi-closed seawater re-circulating systems.

Dose-response trials of between 30 - 40 days required that three additional systems be constructed to accommodate such trials. The bead filter system, as opposed to conventional fix bed filters, was implemented due the spatial limitations. In brief each system consisted of 6 rearing tanks that were stocked with 15 prawns per tank. Continuous flowing preheated water of $28\text{ }^{\circ}\text{C}$ was filtered and passed through a protein skimmer, a EGB and supplied to the experimental tanks at a rate of 2 l per minute *via* a ring feed design. Effluent from each tank exited *via* a 20-mm diameter outlet that was modified to skim the waters surface. Exiting water flowed through a 25 mm

diameter PVC pipe into a 75 mm wide gutter that was fitted with a down pipe, which channelled the water back into the protein skimmer.

B.2.1. The protein skimmer/foam fractionator

The protein skimmer was a 700 mm diameter, 0.6 m high PVC pipe, which was adapted and placed into the reservoir tank. The nozzle of a silicon cartridge was fitted into a 19-mm pipe and altered to serve as a ventury. The ventury was situated between the bottom of the skimmer and a “T”-piece from the delivery side of the pump. The ventury generated very fine bubbles, which acted as a barrier against suspended organic particles. These particles were forced to percolate upward and outward through a hole in the skimmer’s collection cup and disposed of *via* a drainage pipe. The ventury’s efficiency in removing suspended particles proved to be far more effective as opposed to when using air diffusers since these would periodically become clogged. Water, after having passed through the barrage of air bubbles, exited through a 50 mm outlet and into the reservoir tank.

B.2.2. The reservoir tank.

Each reservoir tank, was a $1.5 \times 0.5 \times 0.4$ m asbestos tank. The water within each tank was pumped with the use of a 0.45 kW Aquarius pool pump, which was fitted with a float switch, through separate bead biofilters. The water was maintained at around 28 °C, which at times reached a temperature of 32 °C due the absence of a chiller unit.

B.2.3. Bubble washed bead filters.

The bubble washed bead filters had the following dimensions: a diameter of 0.7 m, a height of 0.7 m and casing walls 0.01 m thick that was made of PVC. Although appearing to be cylindrical externally, the filters were modified to have a hourglass internal geometry with a constricted washing throat (Figure B.1). The bead beds comprised of 2 - 3 mm spherical polyurethane beads, which collectively provided an approximate surface area of $\sim 125 \text{ m}^2$ per filter. During continuous filtration, water pumped from the reservoir tanks entered the units at the bottom through a perforated inlet pipe, passing through the bead beds that floated in the upper filtration chamber, and exited the biofilter through a 50 mm PVC outlet that was modified using pool pump filter screens to ensure that none of the beads were lost.

Bead filters contained no moving parts for the agitation of the bead bed but used air instead, and for this purpose the filter was equipped with an air inlet below the centre of the washing throat.

Three valves namely the; inlet, air and sludge or discharge valve controlled the filter's operation (Figure B.1). Washing the bead bed was accomplished by opening the air inlet, which in turn forced the beads to collide vigorously against one another. Cavitation and agitation by the rising bubbles provided a scrubbing effect.

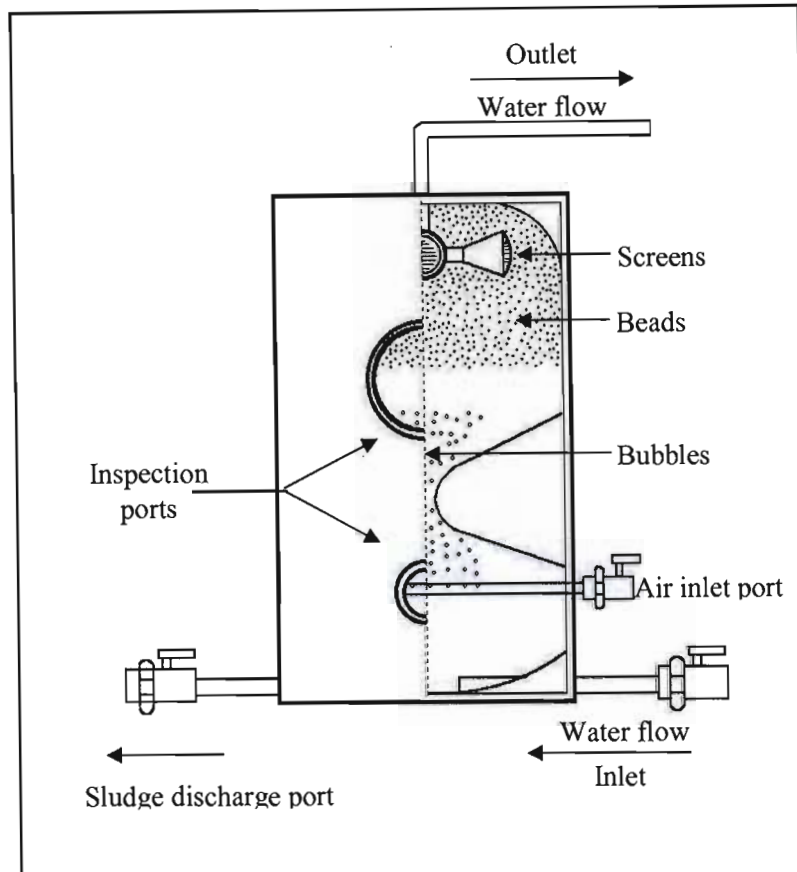


Figure B.1: A side view showing the internal workings of a bead bio-filter.

The inlet pipe had a "T"- piece connected to it with an additional piece of pipe that was fitted with a ball valve, which served to discharge sludge once the system was flushed during the backwashing process. Backwashing was accomplished by draining the fluid to the low water mark, which was a small 50 mm inspection port. This caused the bead bed to collapse and be sucked through the washing throat. The advantage of the backwashing process, despite removing solids captured by the bead bed, was that it allowed the beads to retain their delicate films of nitrifying bacteria. Backwashing was done on a weekly basis.

Water was pumped to the holding tanks through a ring feed system containing six 25 mm ball valves that regulated the flow rate at 2 l per minute. Pressure within the system was regulated by opening and closing a 50 mm ball valve, placed in a line, which acted as a bypass and which was situated between the biofilters and holding tanks. An added advantage of the bypass line was that

the water being returned into the reservoir tank would be pumped through the biofilter a number of times, thereby repeating the filtration process, before entering the holding tanks.

B.2.4. Sizing rationale for the bead filters

The organic loading rate is the primary method for the sizing of floating bead filters (bead volume, V_b). Based on the assumption for the sizing rationale, that in a re-circulating system the major source of organics is the feed, the bead filter sizing criterion (V_b) was determined by the rate of feeding (W) and the bead sizing criterion, v_b as recommended by Malone and Beecher (2000) such that:

$$V_b = L(f)v_b (P \div 35) \div 100$$

$$V_b = Wv_b (P \div 35) \div 100$$

where L = maximum mass of the aquatic organism in the system (kg);
 f = feedrate (percent of body mass fed per day)
 v_b = floating bead sizing criterion (m^3 per kg of feed per day)
 W = peak feed application rate (kg/day)
 and P = protein content of the feed (%).

Bead filter sizing criteria are dependent on the filters application, stocking density and feed rate. Generally, bead filters are sized according to the maximum amount of feed that will be placed into the system per day and/or the maximum anticipated load of animals in the system. Generally it has been found that $0.31 m^3$ of beads ($124 m^2$ of surface area) provide complete solids capture and nitrification for up to 0.5 kg of feed per day, corresponding to approximately 50 kg of fish fed 1 % body mass per day (Malone & Beecher, 2000).

B.2.5. The rearing tanks.

The rearing tanks were eighteen white 75 ℓ polypropylene tubs that were adapted and used as experimental tanks. The advantage of having white coloured tubs was that it made the faecal strands highly visible for collection. Water supply of 2 ℓ per minute, ensured a 38.5 times change-over per day (Figure B.2). The lids of the tubs were adjusted by cutting out the inner portion and gluing a screen onto the underside of the lid. Each tank was supplied with air *via* a diffuser. The outlet was situated near the top of the tank and was a 20 mm pipe which was adapted by gluing a 50 mm end cap to serve as a surface skimmer. The air supplied by the diffusers caused a current, which forced light material at the bottom of the tank to be discharged *via* the outlet. Effluent water from the tanks was discharged into a 75 mm wide drainage gutter that led into the protein skimmer/fractionator that in turn was situated in the reservoir tank (Figure B.3).



Figure B.2: View of the arrangements of rearing tanks.

B.2.6. Water parameters.

A constant pH was essential since low values (< 7.0) inhibit nitrification processes. Alkalinity on the other hand assists in controlling the pH level, and the bicarbonate ion (the principal alkalinity component $\text{CaCO}_3 > 150 \text{ mg/l}$) is a preferred source of carbon for nitrifying bacteria. Furthermore, temperatures $25 - 30^\circ\text{C}$ control growth rates of heterotrophic and nitrifying bacteria and in turn, the ammonia conversion rates. Due to these reasons water parameters were maintained at 28°C , 25 ‰ , pH $7.6 - 8.0$ and oxygen concentration near saturation throughout the trials.



Figure B.3: View of the rearing tanks, reservoir and fractionator.

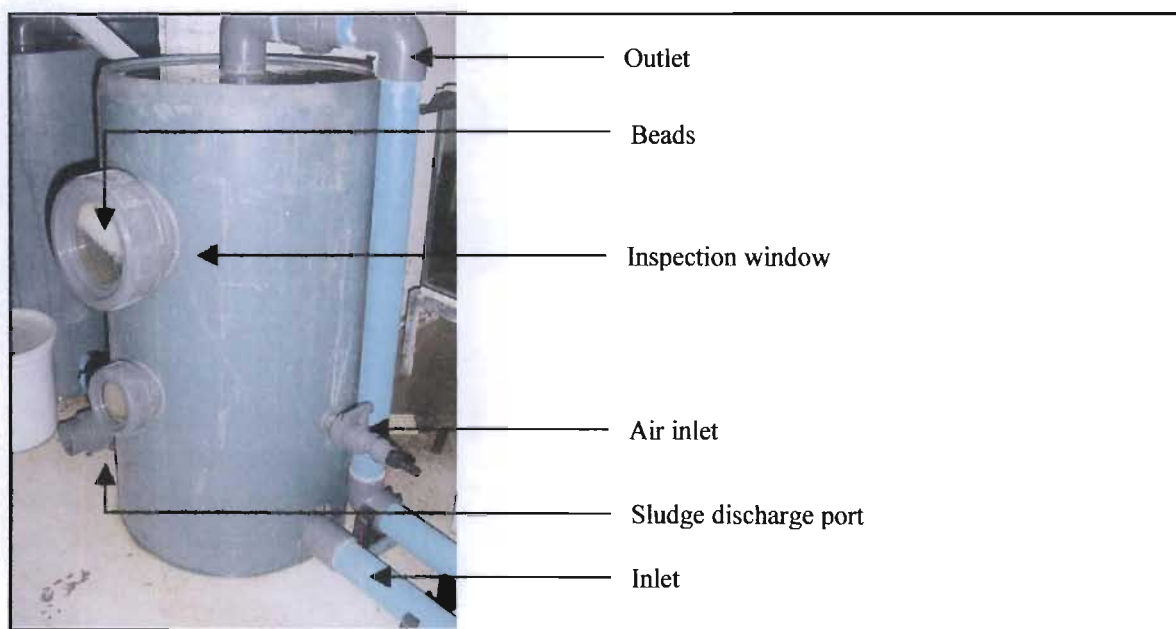
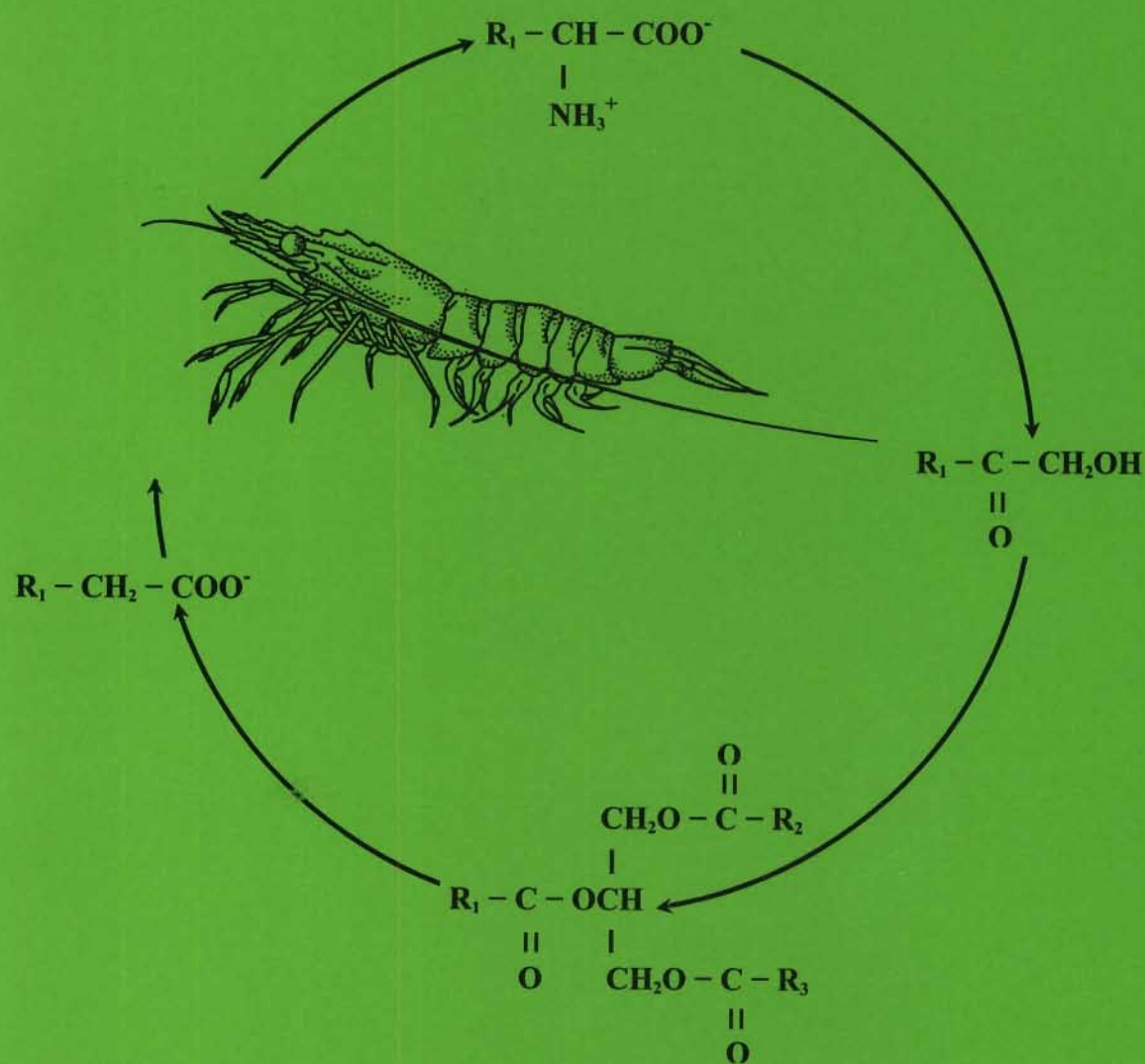
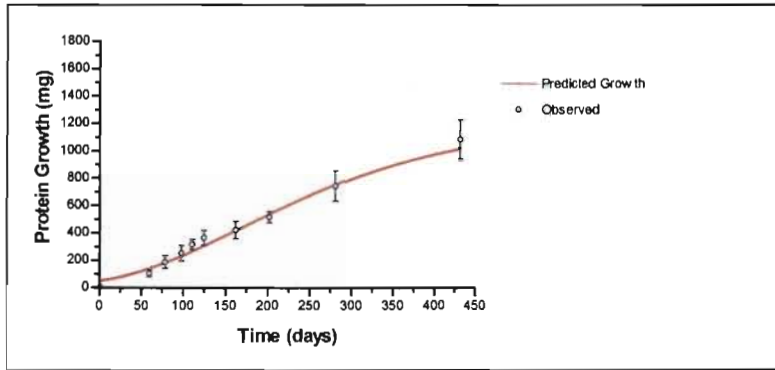


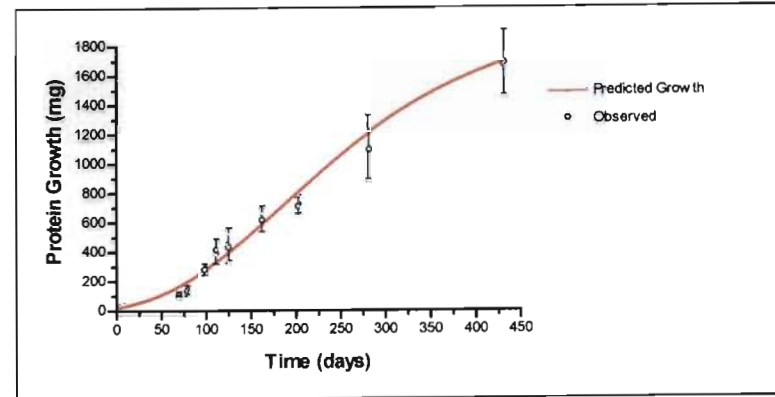
Figure B.4: View of the bead filter.

Appendix C

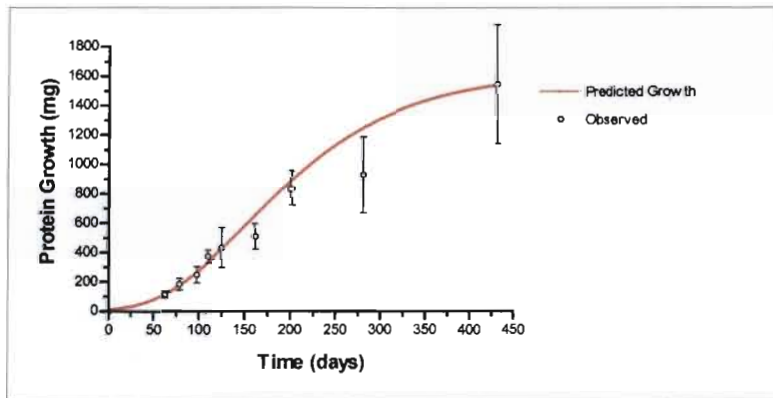




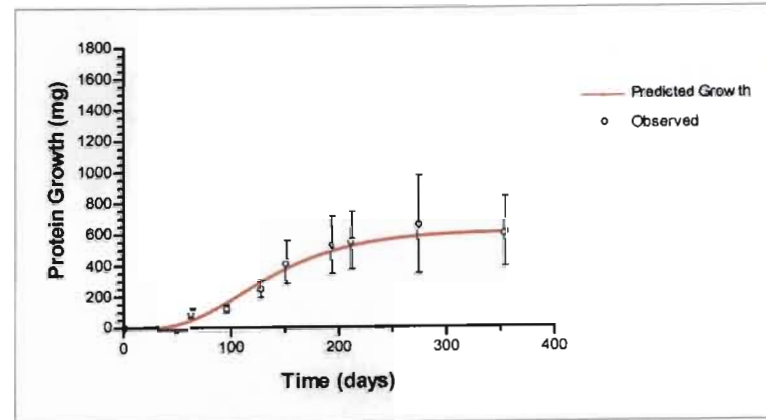
App C Figure 1: Protein gains in the exoskeleton of pond-reared male prawns with observed $P_o = 0.001$ mg, $P_m = 1086$ mg and predicted $P_o = 48$ mg, $P_m = 1023$ mg while $\beta = 0.0069$ day⁻¹.



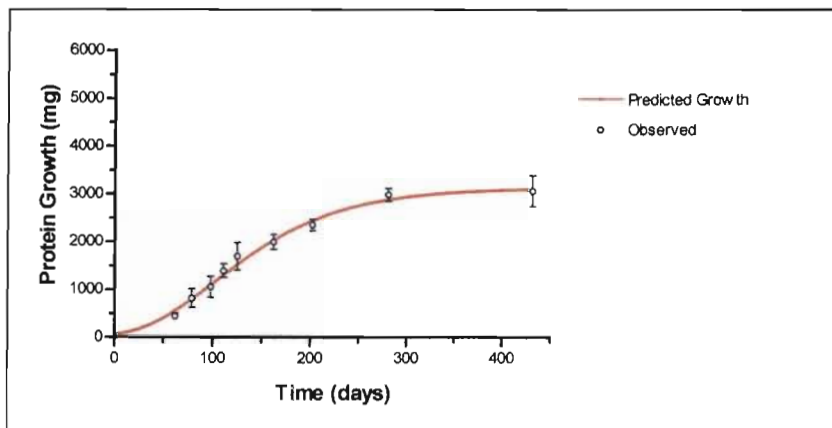
App C Figure 2: Protein gains in the exoskeleton of pond-reared female prawns with observed $P_o = 0.001$ mg, $P_m = 1600$ mg and predicted $P_o = 36$ mg, $P_m = 1662$ mg while $\beta = 0.0075$ day⁻¹.



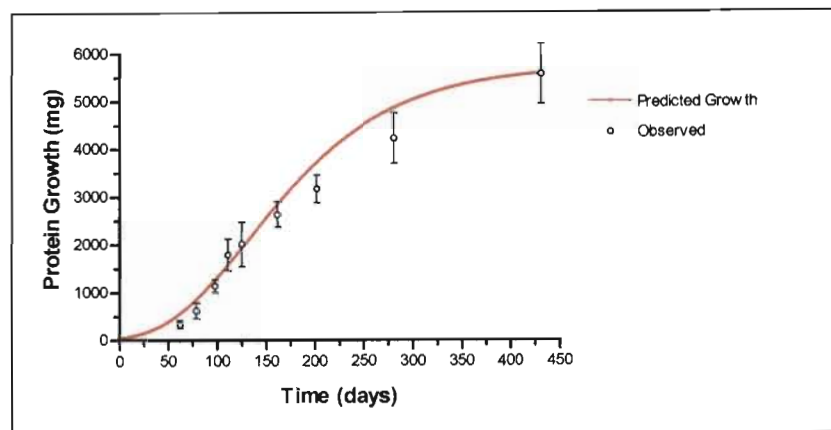
App C Figure 3: Protein gains in the exoskeleton of mixed samples of pond-reared prawns with observed $P_o = 0.001$ mg, $P_m = 1545$ mg and predicted $P_o = 10$ mg, $P_m = 1538$ mg while $\beta = 0.0106$ day⁻¹.



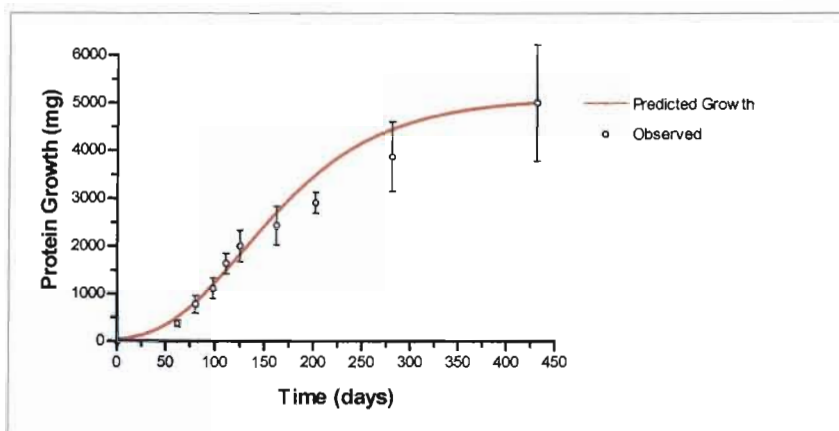
App C Figure 4: Protein gains in the exoskeleton of mixed samples of laboratory-reared prawns with observed $P_o = 0.001$ mg, $P_m = 628$ mg and predicted $P_o = 0.391$ mg, $P_m = 620$ mg while $\beta = 0.0179$ day⁻¹.



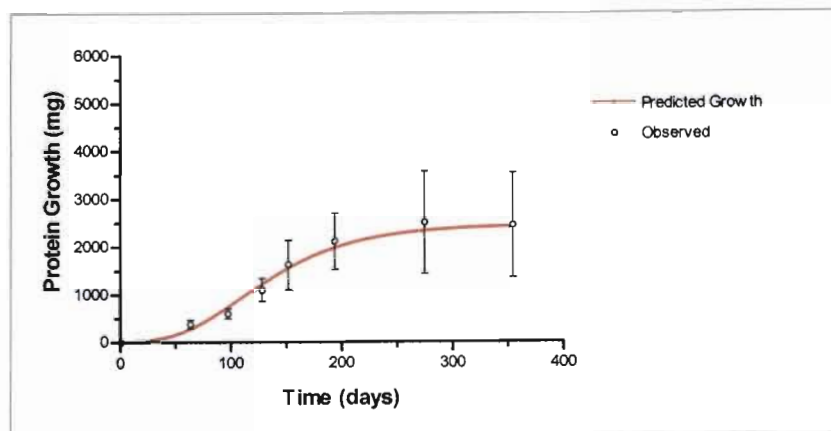
App C Figure 5: Flesh protein gains in pond-reared male prawns with observed $P_o = 0.001$ mg, $P_m = 3062$ mg and predicted $P_o = 55$ mg, $P_m = 3094$ mg while $\beta = 0.0139$ day⁻¹.



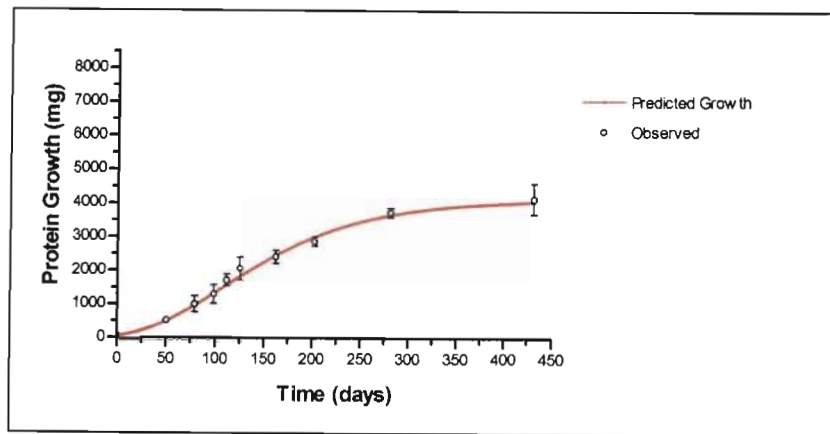
App C Figure 6: Flesh protein gains in pond-reared female prawns with observed $P_o = 0.001$ mg, $P_m = 5573$ mg and predicted $P_o = 40$ mg, $P_m = 5579$ mg while $\beta = 0.0121$ day⁻¹.



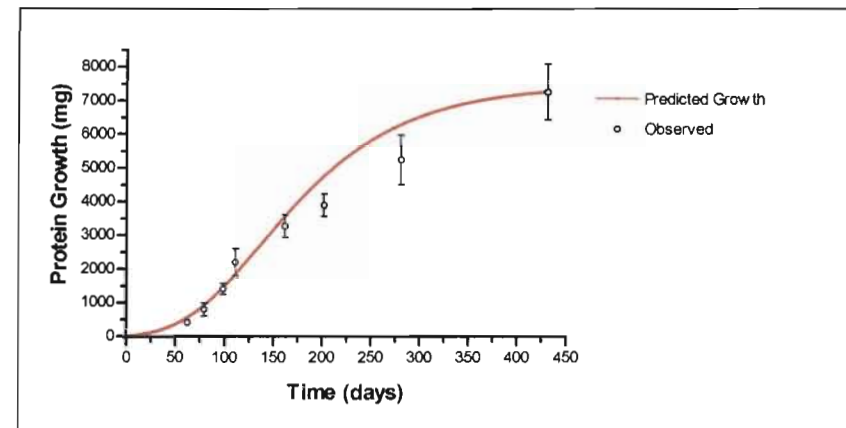
App C Figure 7: Flesh protein gains in mixed samples of pond-reared prawns with observed $P_o = 0.001$ mg, $P_m = 5002$ mg and predicted $P_o = 30$ mg, $P_m = 4992$ mg while $\beta = 0.0129$ day⁻¹.



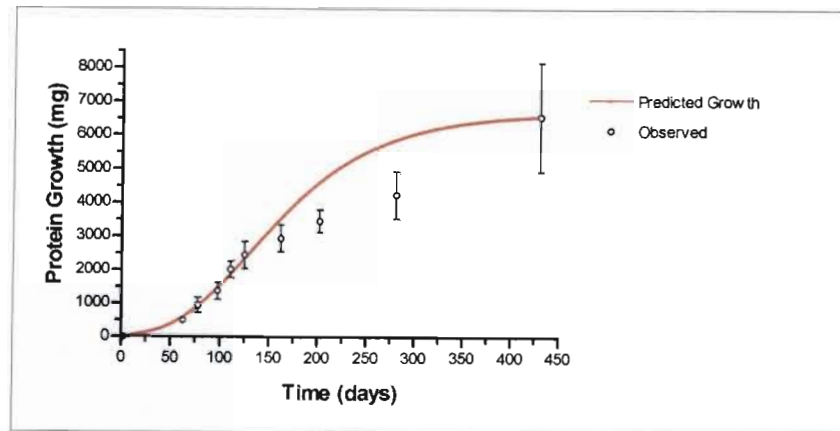
App C Figure 8: Flesh protein gains in mixed samples of laboratory-reared prawns with observed $P_o = 0.001$ mg, $P_m = 2453$ mg, predicted $P_o = 2.28$ mg, $P_m = 2411$ mg while $\beta = 0.0178$ day⁻¹.



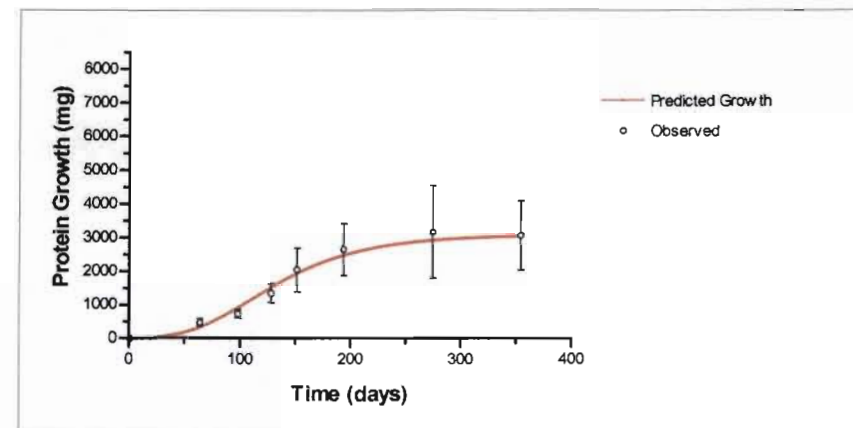
App C Figure 9: Empty body protein gains in pond-reared male prawns with observed $P_o = 0.001$ mg, $P_m = 4148$ mg and predicted $P_o = 97$ mg, $P_m = 4048$ mg while $\beta = 0.0122 \text{ day}^{-1}$.



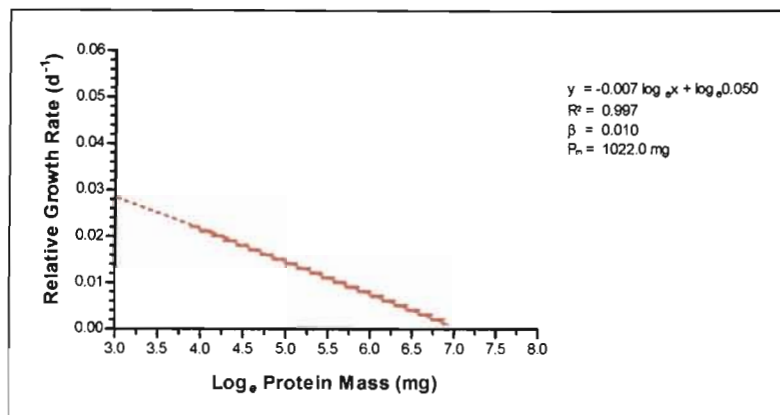
App C Figure 10: Empty body protein gains in pond-reared female prawns with observed $P_o = 0.001$ mg, $P_m = 7260$ mg and observed $P_o = 30$ mg, $P_m = 7259$ mg while $\beta = 0.0124 \text{ day}^{-1}$.



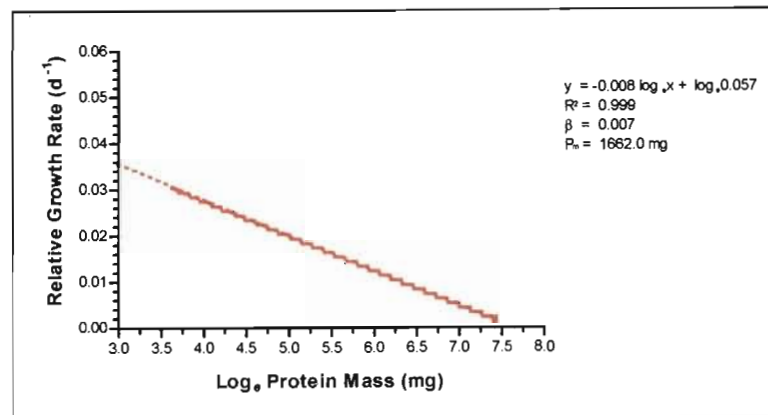
App C Figure 11: Empty body protein gains in mixed samples of pond-reared prawns with observed $P_o = 0.001$ mg, $P_m = 6547$ mg and predicted $P_o = 24$ mg, $P_m = 6551$ mg while $\beta = 0.0135 \text{ day}^{-1}$.



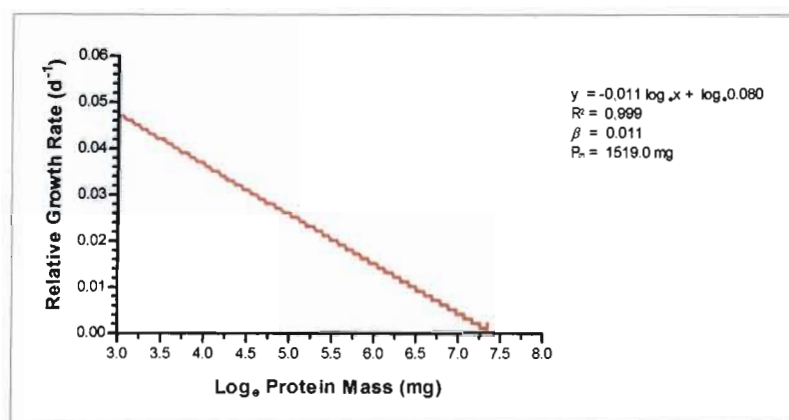
App C Figure 12: Empty body protein gains in mixed samples of laboratory-reared prawns with observed $P_o = 0.001$ mg, $P_m = 3074$ mg and predicted $P_o = 3$ mg, $P_m = 3035$ mg while $\beta = 0.0179 \text{ day}^{-1}$.



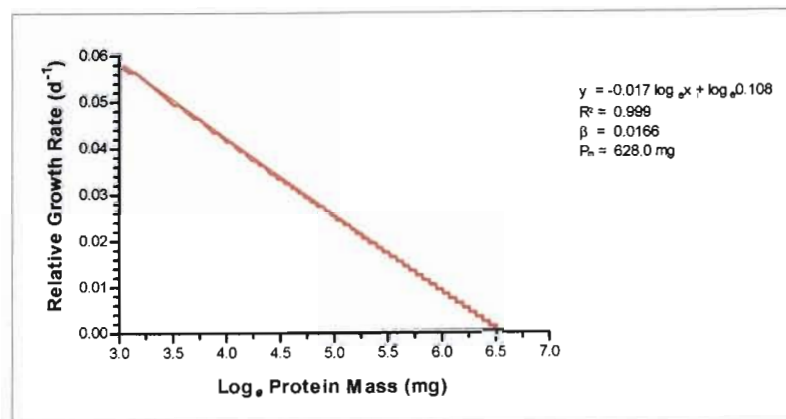
App C Figure 13 : Relative growth rate as a function of the natural logarithm of protein mass in the exoskeleton of pond-reared male prawns.



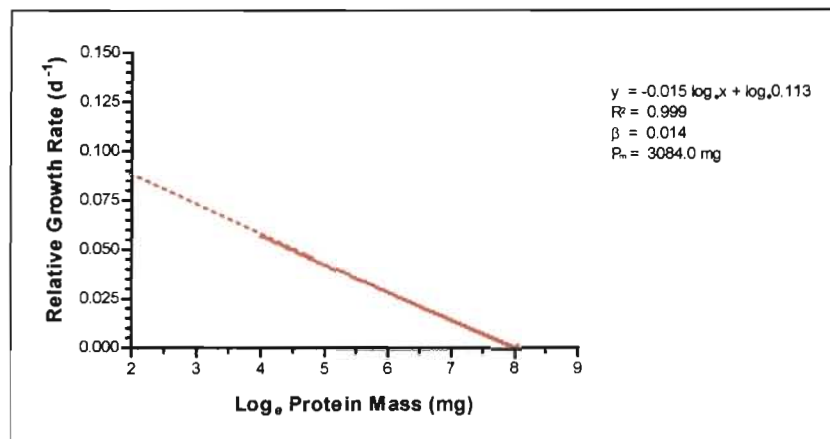
App C Figure 14 : Relative growth rate as a function of the natural logarithm of protein mass in the exoskeleton of pond-reared female prawns.



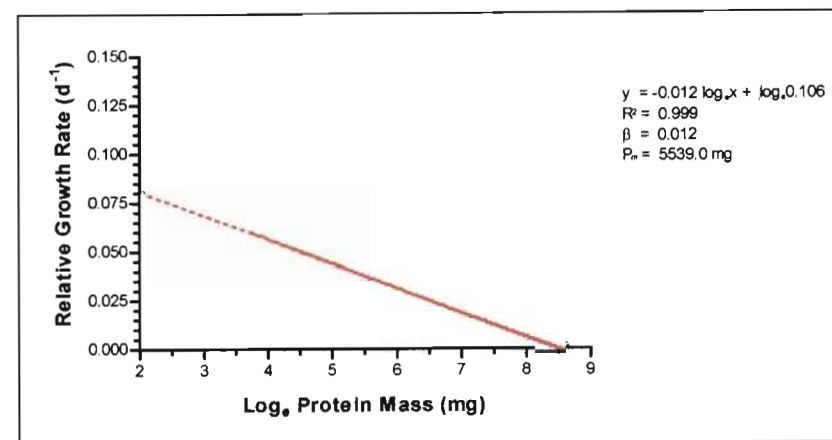
App C Figure 15 : Relative growth rate as a function of the natural logarithm of protein mass in the exoskeleton of mixed samples of pond-reared prawns.



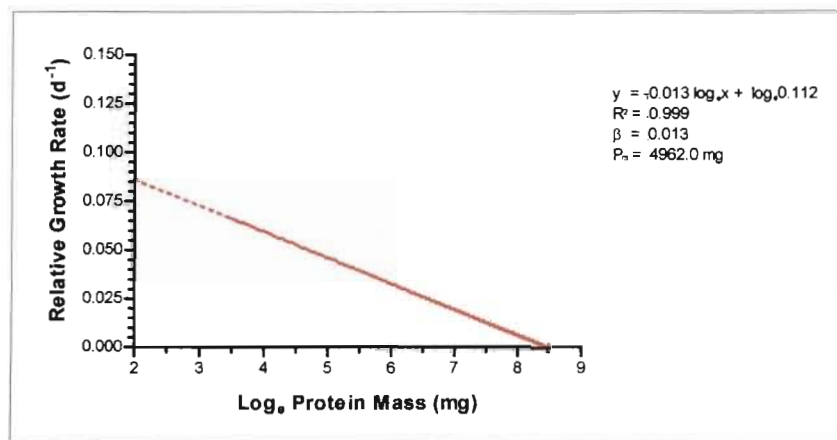
App C Figure 16: Relative growth rate as a function of the natural logarithm of protein mass in the exoskeleton of mixed samples of laboratory-reared prawns.



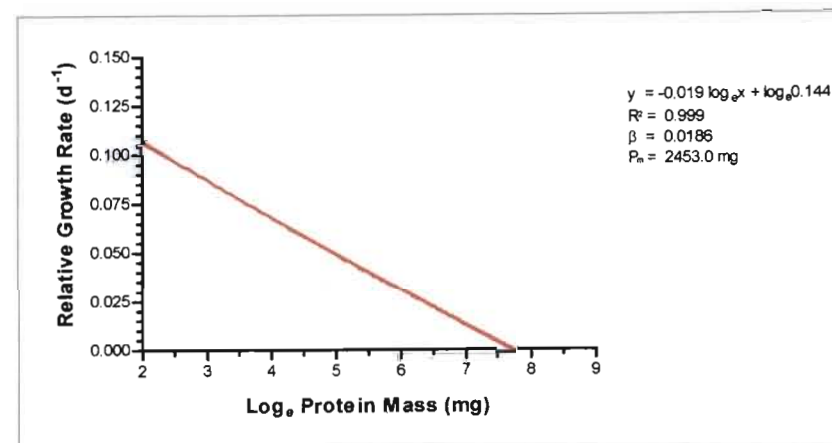
App C Figure 17 : Relative growth rate as a function of the natural logarithm of protein mass in the flesh of pond-reared male prawns.



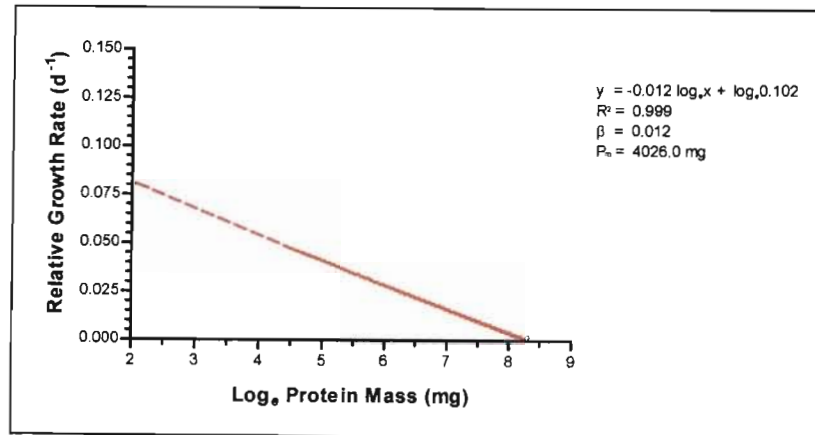
App C Figure 18 : Relative growth rate as a function of the natural logarithm of protein mass in the flesh of pond-reared female prawns.



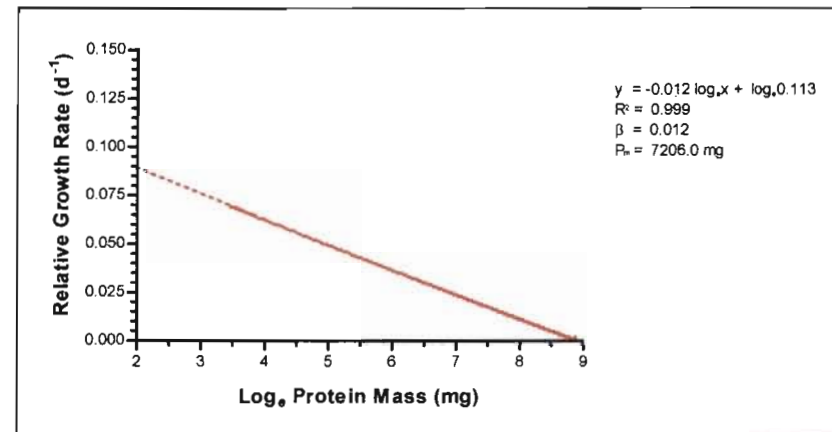
App C Figure 19 : Relative growth rate as a function of the natural logarithm of protein mass in the flesh of mixed samples of pond-reared prawns.



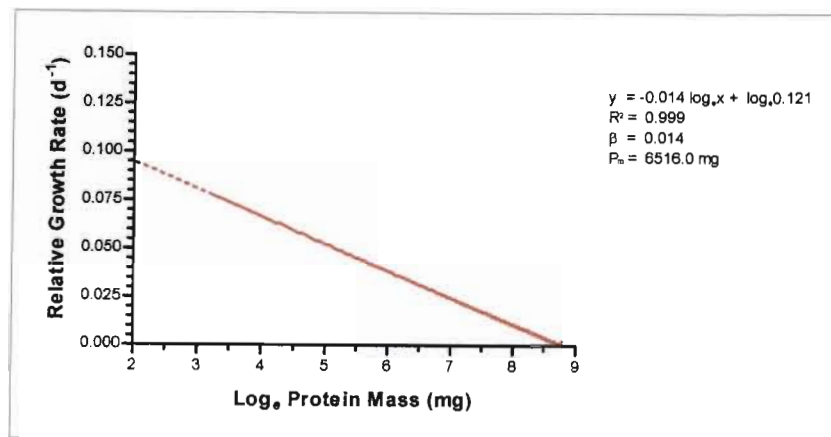
App C Figure 20: Relative growth rate as a function of the natural logarithm of protein mass in the flesh of mixed samples of laboratory-reared prawns.



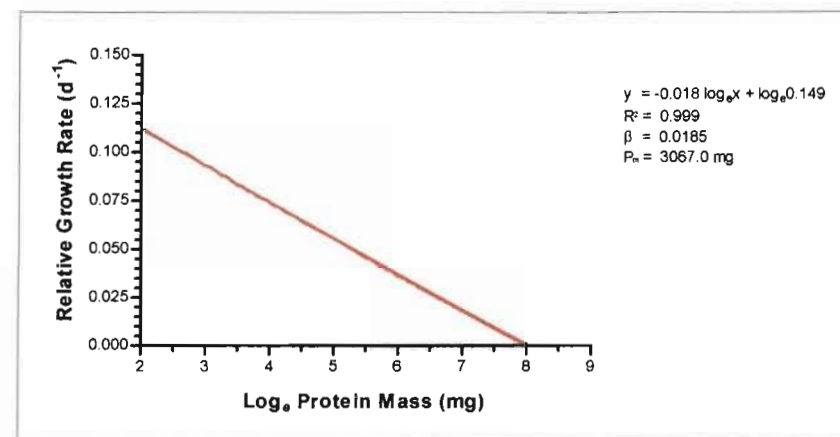
App C Figure 21 : Relative growth rate as a function of the natural logarithm of protein mass in the empty body of pond-reared male prawns.



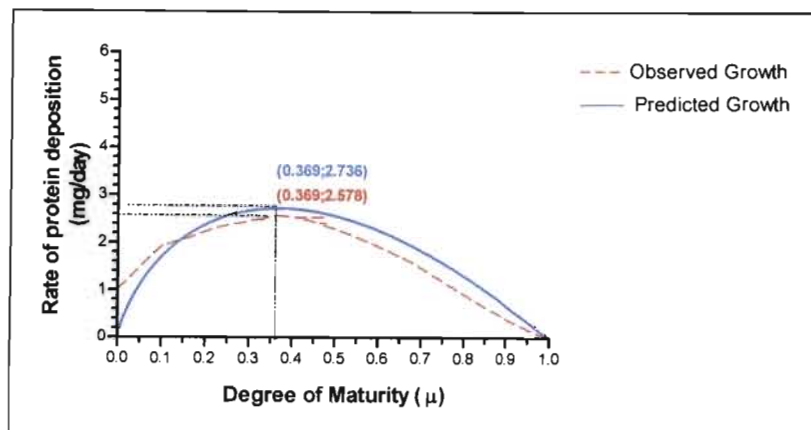
App C Figure 22 : Relative growth rate as a function of the natural logarithm of protein mass in the empty body of pond-reared female prawns.



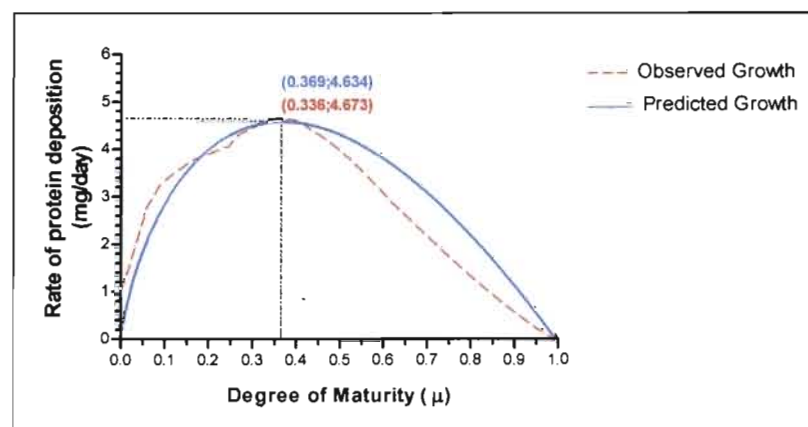
App C Figure 23 : Relative growth rate as a function of the natural logarithm of body protein mass in the empty body of mixed samples of pond-reared prawns.



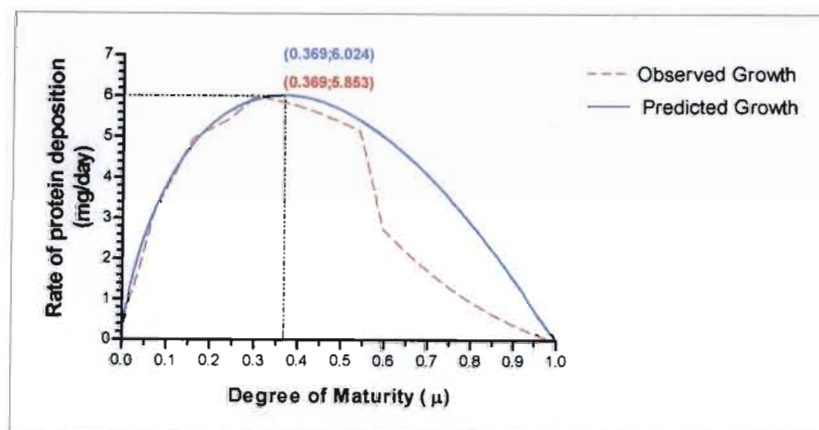
App C Figure 24: Relative growth rate as a function of the natural logarithm of protein mass in the empty body of mixed samples of laboratory-reared prawns.



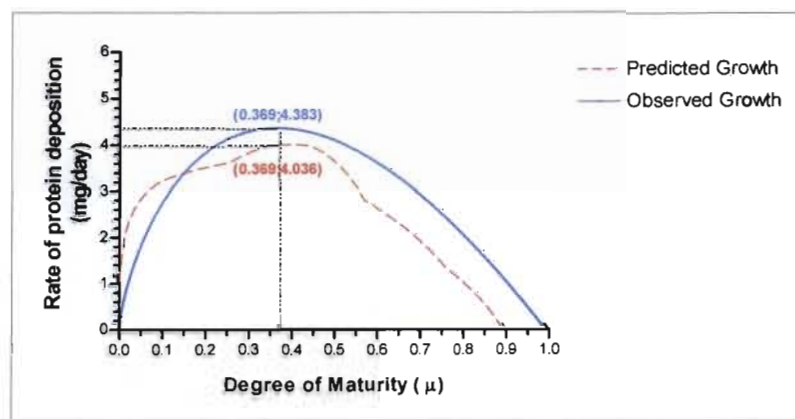
App C Figure 25: Rate of exoskeleton protein deposition in pond-reared male prawns.



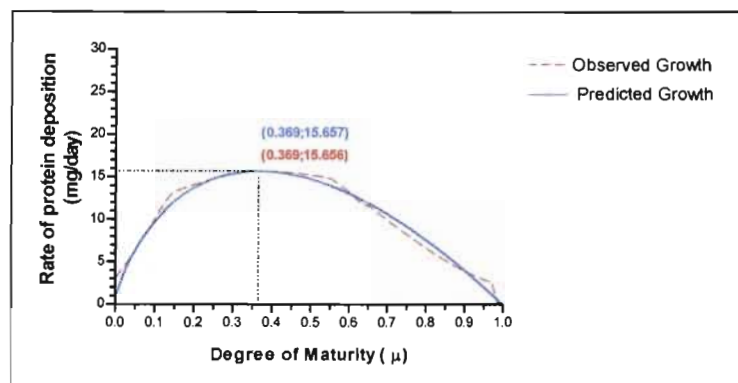
App C Figure 26: Rate of exoskeleton protein deposition in pond-reared female prawns.



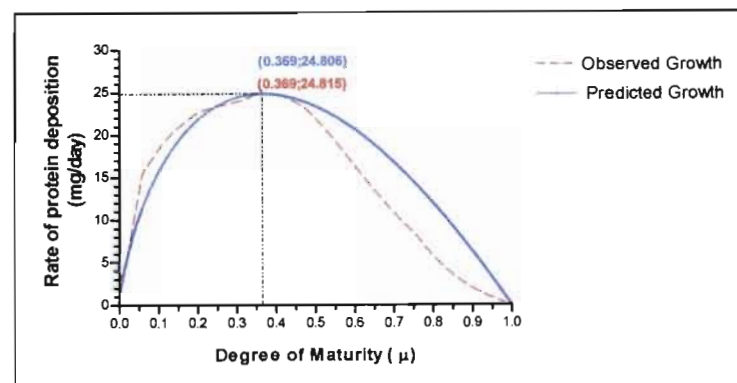
App C Figure 27: Rate of exoskeleton protein deposition in mixed samples of pond reared prawns.



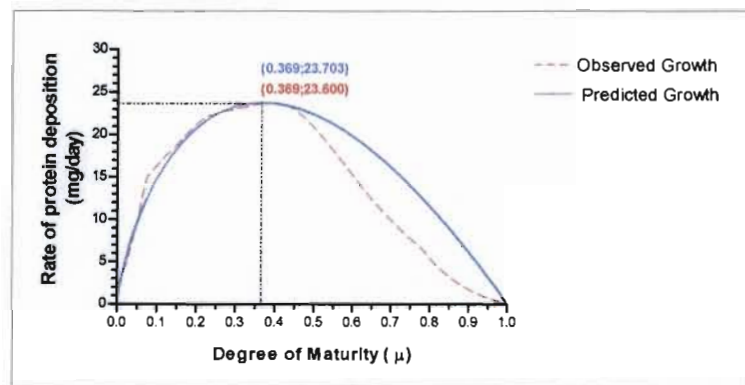
App C Figure 28: Rate of exoskeleton protein deposition in mixed samples of laboratory-reared prawns.



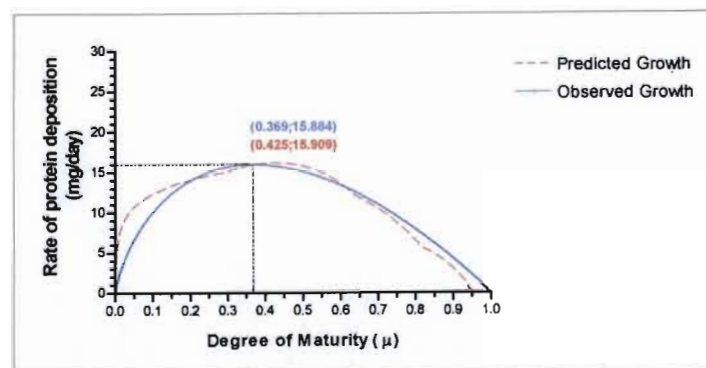
App C Figure 29 : Rate of flesh protein deposition in pond-reared male prawns.



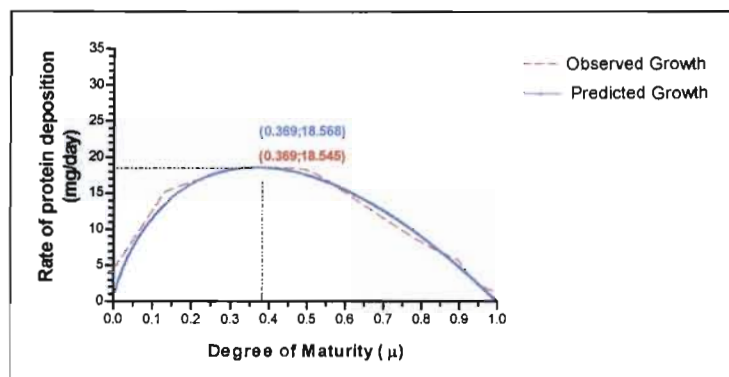
App C Figure 30: Rate of flesh protein deposition in pond-reared female prawns.



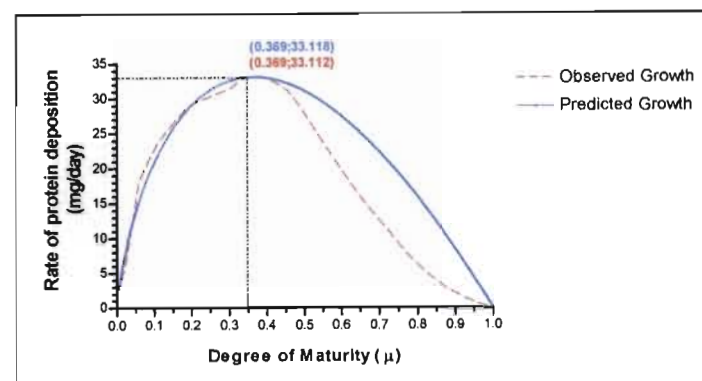
App C Figure 31 : Rate of flesh protein deposition in mixed samples of pond-reared prawns.



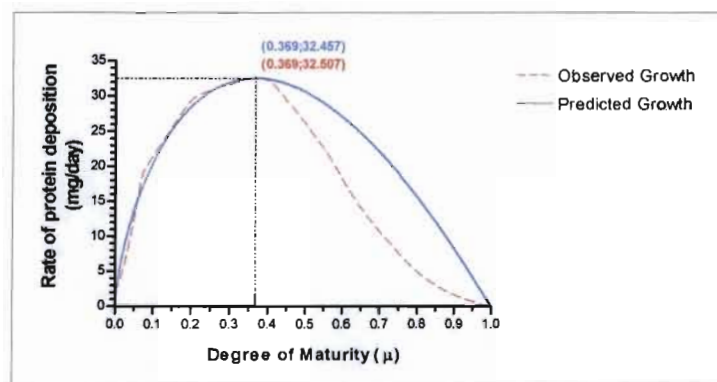
App C Figure 32 : Rate of flesh protein deposition of mixed samples of laboratory-reared prawns.



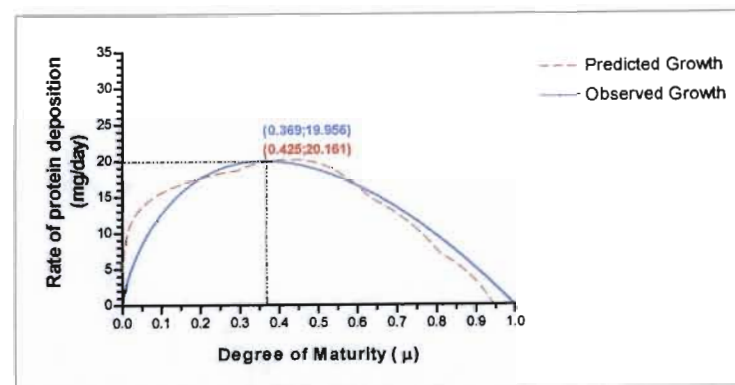
App C Figure 33: Rate of body protein deposition in pond-reared male prawns.



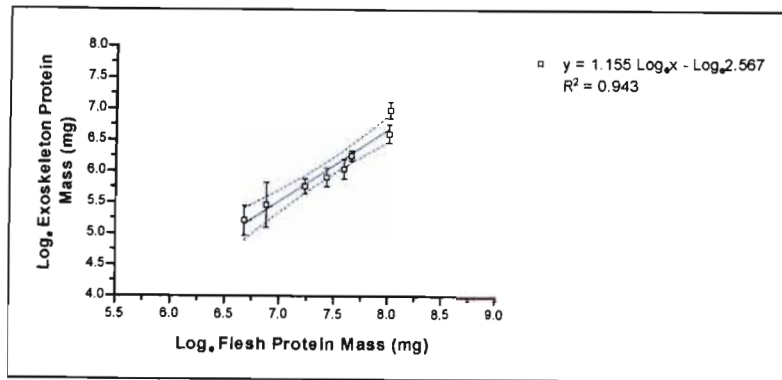
App C Figure 34 : Rate of body protein deposition in pond-reared female prawns.



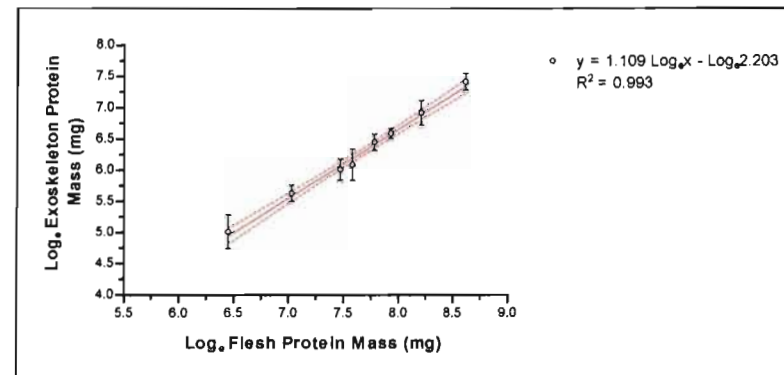
App C Figure 35 : Rate of body protein deposition in mixed samples of pond-reared prawns.



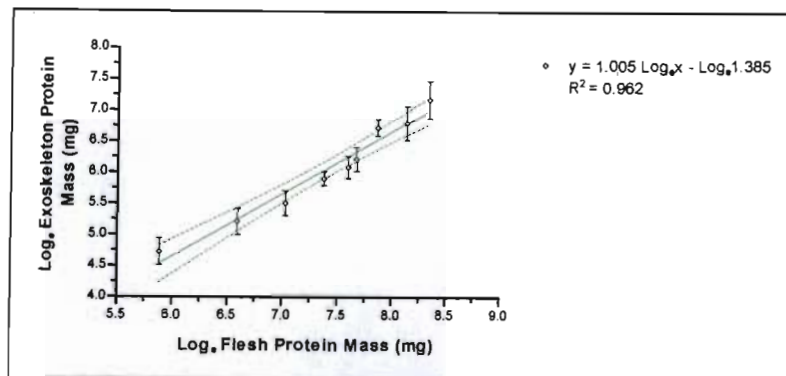
App C Figure 36 : Rate of body protein deposition in mixed samples of laboratory-reared prawns.



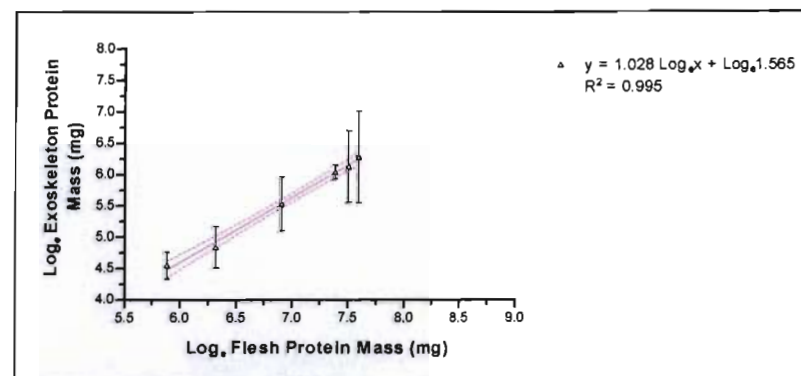
App C Figure 37: The relationship between exoskeleton protein and flesh protein mass in pond reared male prawns.



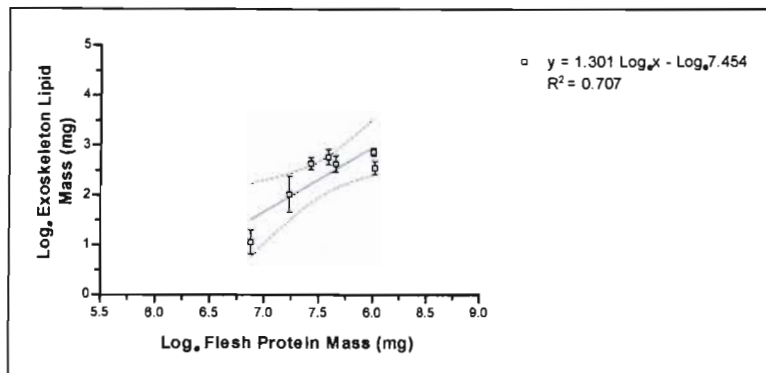
App C Figure 38: The relationship between exoskeleton protein and flesh protein mass in pond reared female prawns.



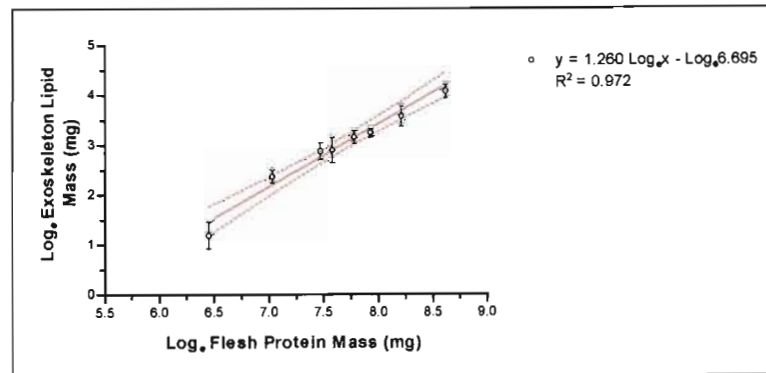
App C Figure 39: The relationship between exoskeleton protein and flesh protein mass of mixed samples in pond reared prawns.



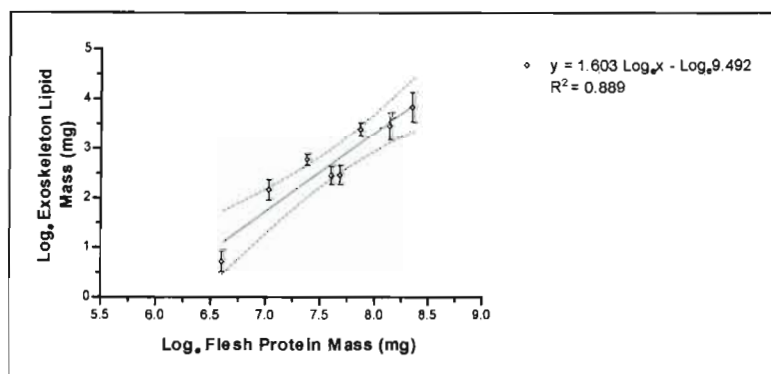
App C Figure 40: The relationship between exoskeleton protein and flesh protein mass in mixed samples of laboratory reared prawns.



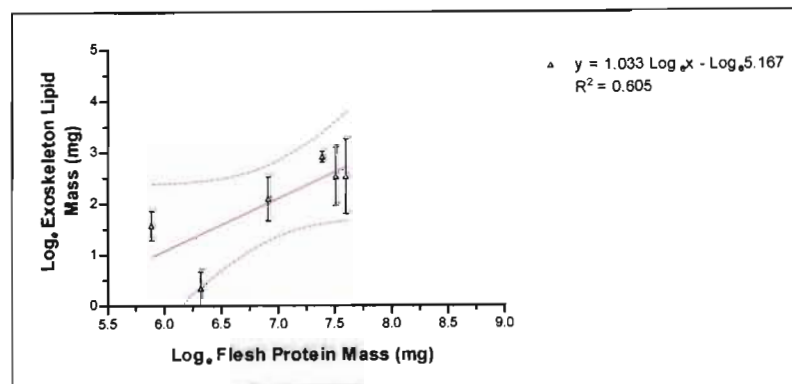
App C Figure 41: The relationship between exoskeleton lipid and flesh protein mass in pond reared male prawns.



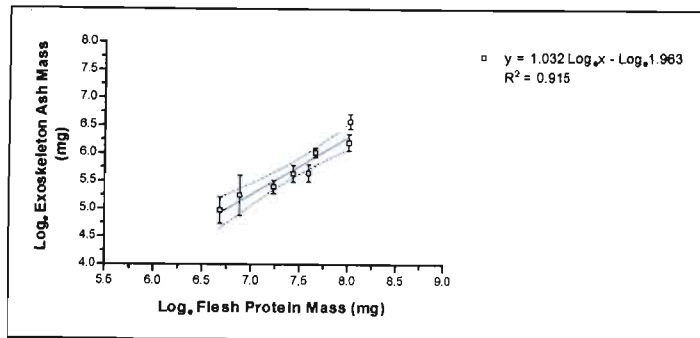
App C Figure 42: The relationship between exoskeleton lipid and flesh protein mass in pond reared female prawns.



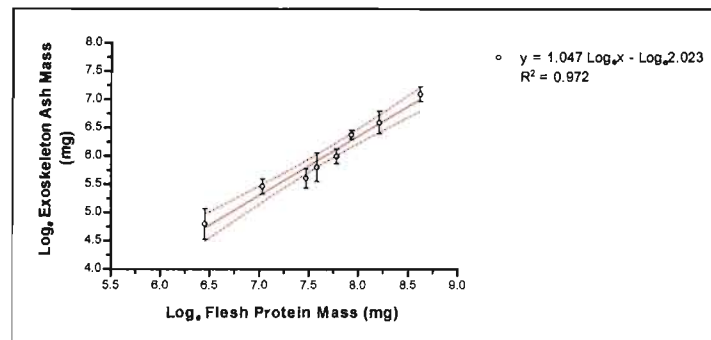
App C Figure 43: The relationship between exoskeleton lipid and flesh protein mass in mixed samples of pond reared prawns.



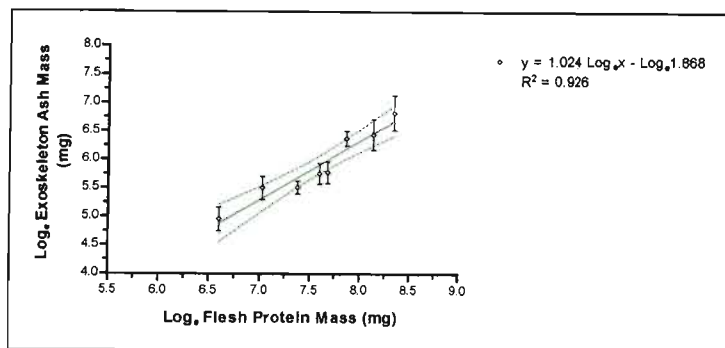
App C Figure 44: The relationship between exoskeleton lipid and flesh protein mass in mixed samples of laboratory reared prawns.



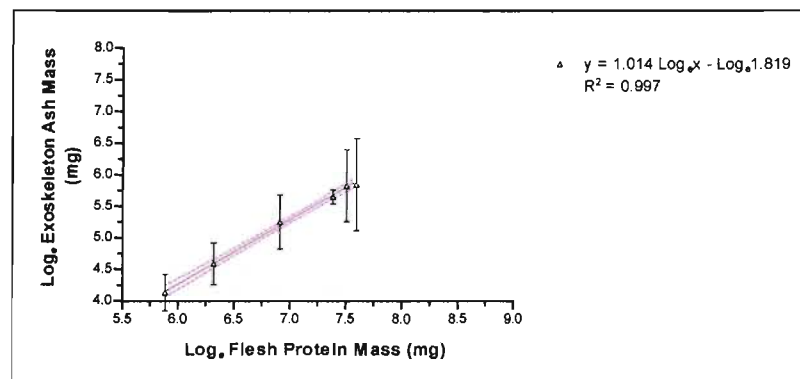
App C Figure 45: The relationship between exoskeleton ash and flesh protein mass in pond reared male prawns.



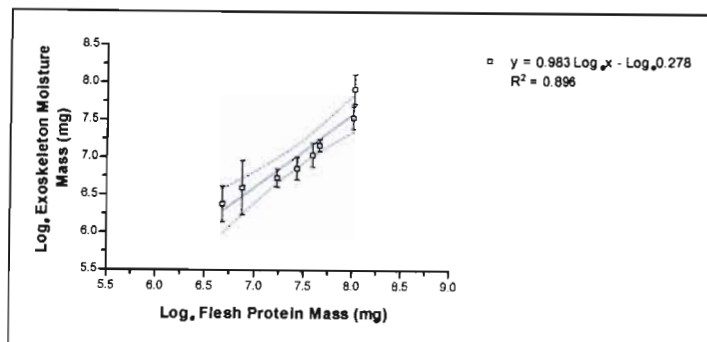
App C Figure 46: The relationship between exoskeleton ash and flesh protein mass in pond reared female prawns.



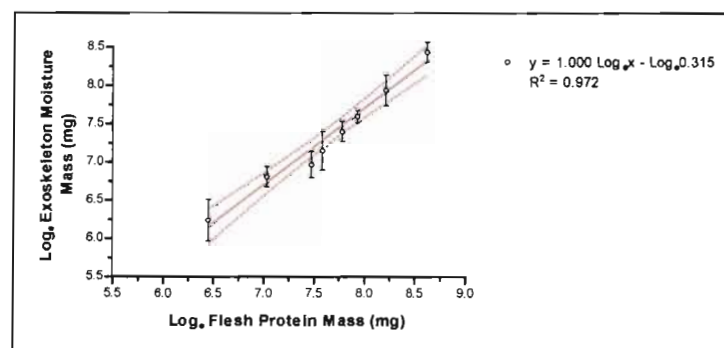
App C Figure 47: The relationship between exoskeleton ash and flesh protein mass in mixed samples of pond reared prawns.



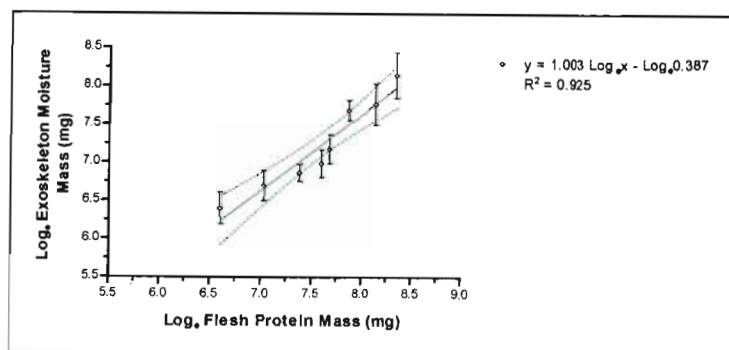
App C Figure 48: The relationship between exoskeleton ash and flesh protein mass in mixed samples of laboratory reared prawns.



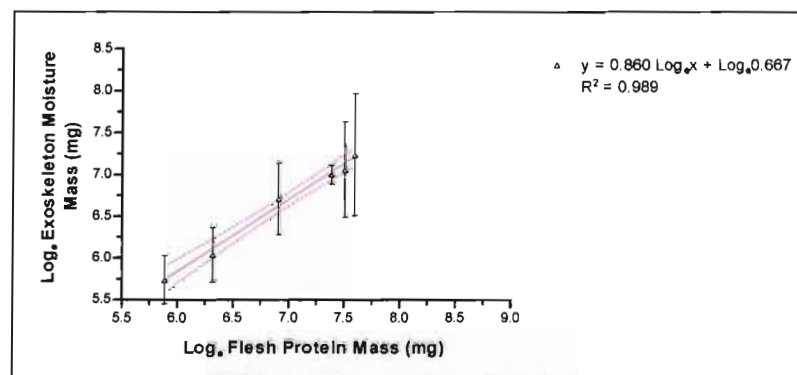
App C Figure 49: The relationship between exoskeleton moisture and flesh protein mass in pond reared male prawns.



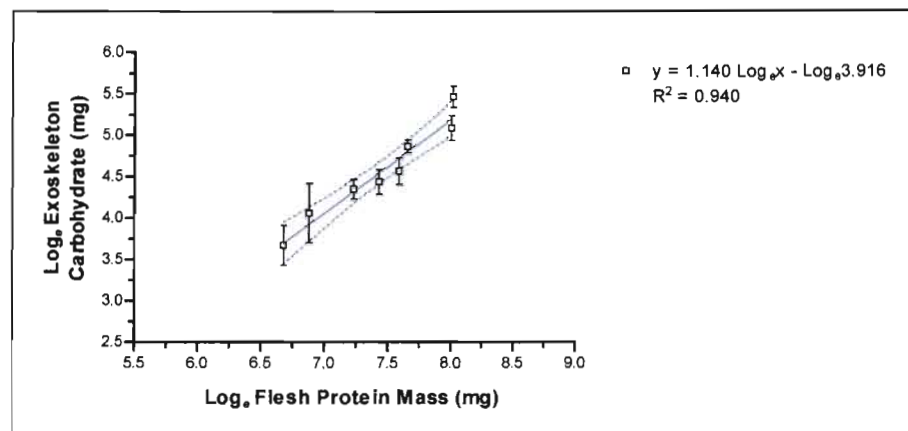
App C Figure 50: The relationship between exoskeleton moisture and flesh protein mass in pond reared female prawns.



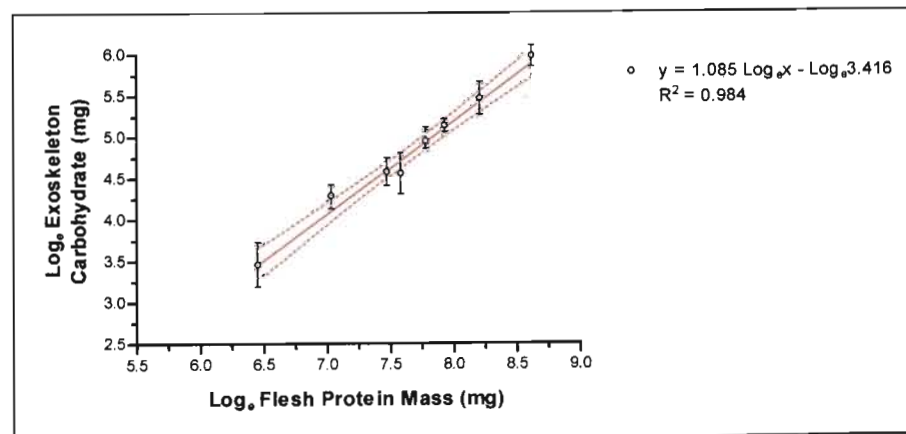
App C Figure 51: The relationship between exoskeleton moisture and flesh protein mass in mixed samples of pond reared prawns.



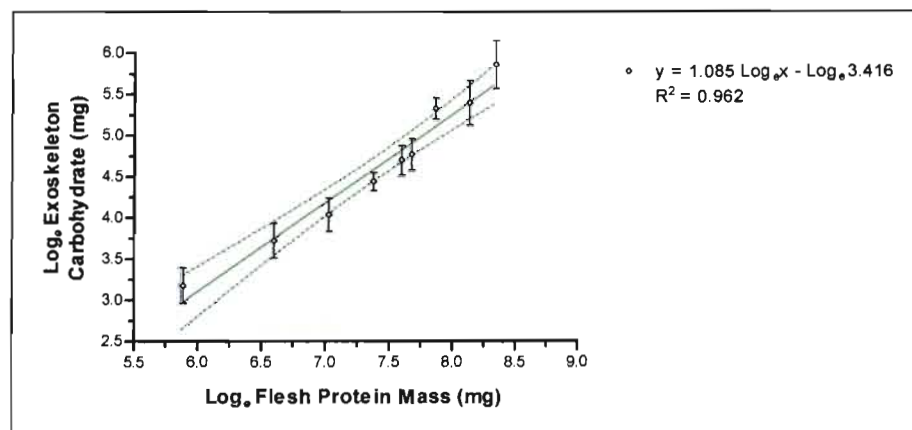
App C Figure 52: The relationship between exoskeleton moisture and flesh protein mass in mixed samples of laboratory reared prawns.



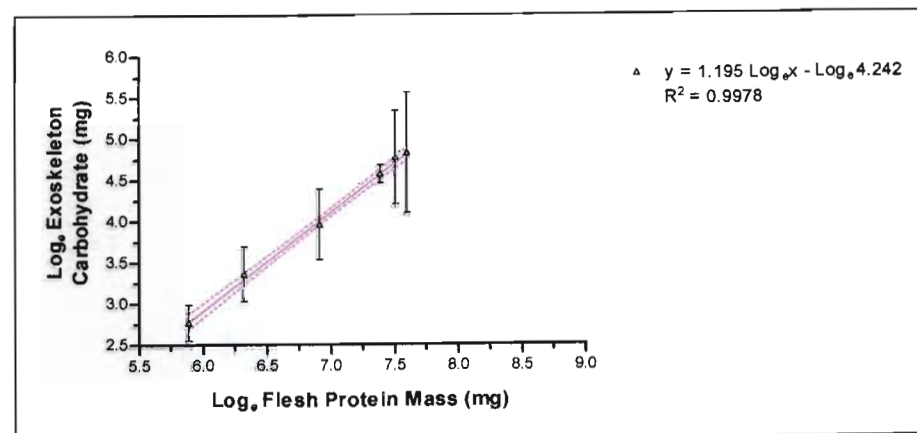
App C Figure 53: The relationship between exoskeleton carbohydrate and flesh protein mass in pond reared male prawns.



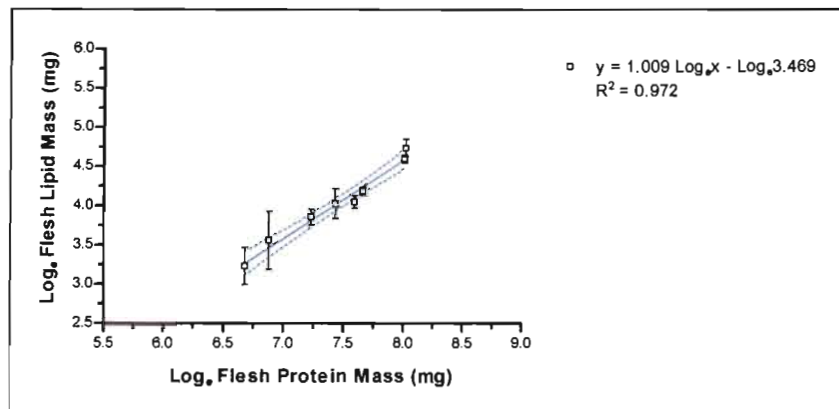
App C Figure 54: The relationship between exoskeleton carbohydrate and flesh protein mass in pond reared female prawns.



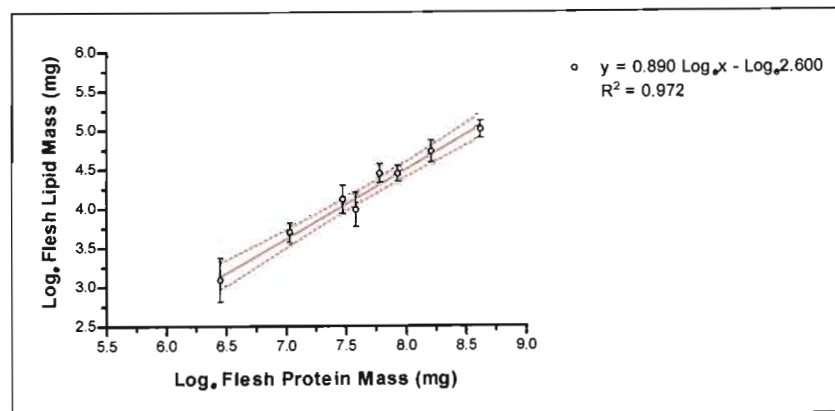
App C Figure 55: The relationship between exoskeleton carbohydrate and flesh protein mass in mixed samples of pond reared prawns.



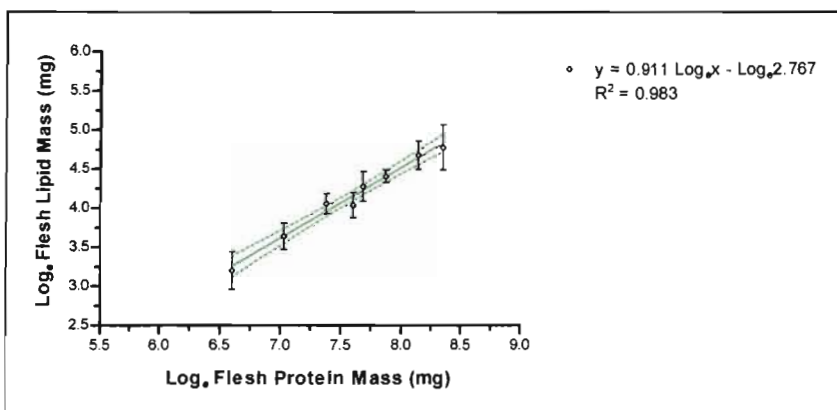
App C Figure 56: The relationship between exoskeleton carbohydrate and flesh protein mass in mixed samples of laboratory reared prawns.



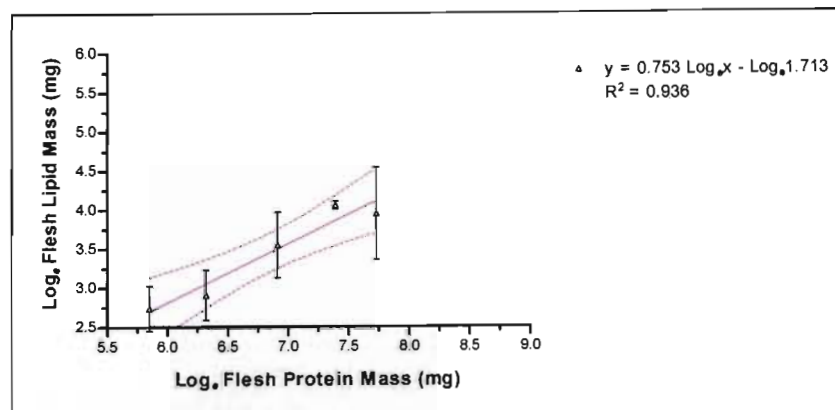
App C Figure 57: The relationship between the Log_{10} of flesh lipid and flesh protein mass in male prawns.



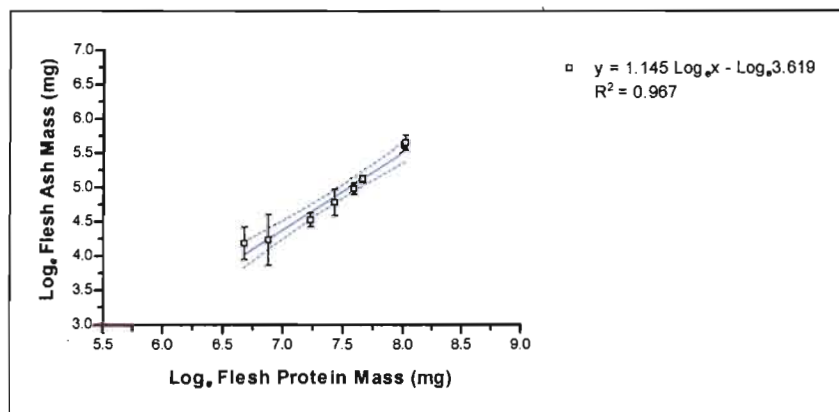
App C Figure 58: The relationship between the Log_{10} of flesh lipid and flesh protein mass in female prawns.



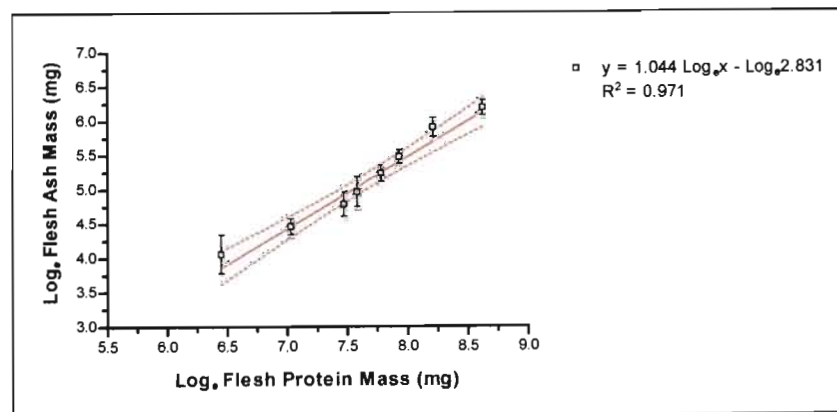
App C Figure 59: The relationship between the Log_{10} of flesh lipid and flesh protein mass in mixed samples of pond reared prawns.



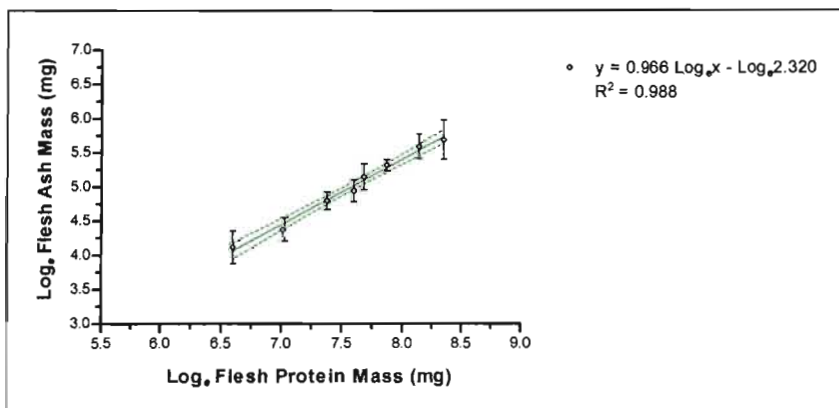
App C Figure 60: The relationship between the Log_{10} of flesh lipid and flesh protein mass in mixed samples of laboratory reared prawns.



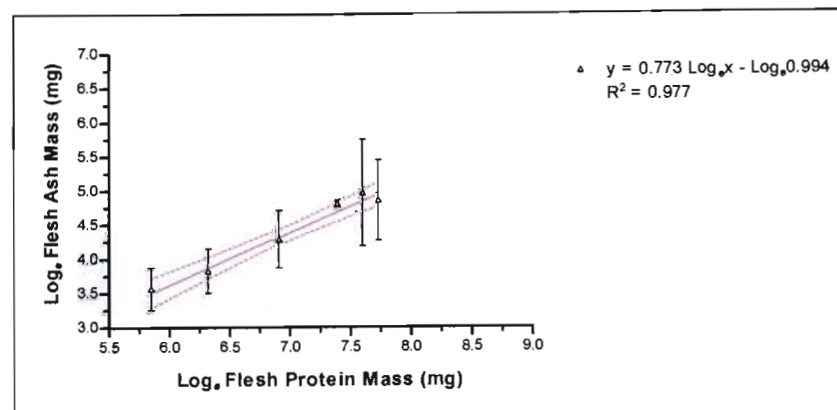
App C Figure 61: The relationship between the Log_{10} of flesh ash and flesh protein mass in male prawns.



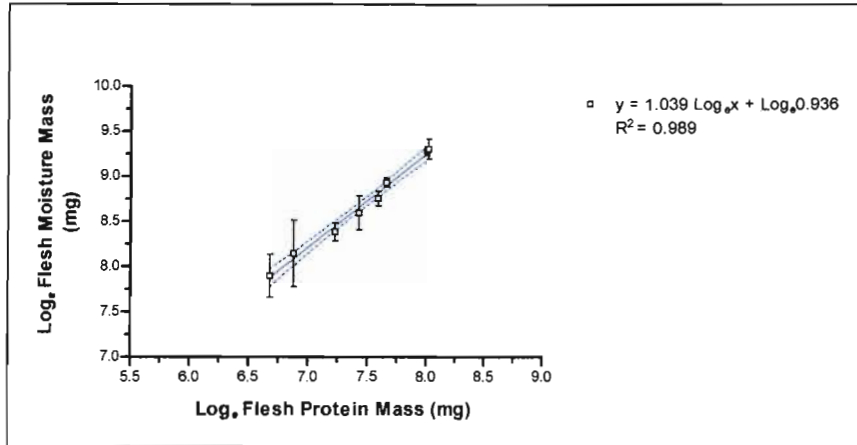
App C Figure 62: The relationship between the Log_{10} of flesh ash and flesh protein mass in female prawns.



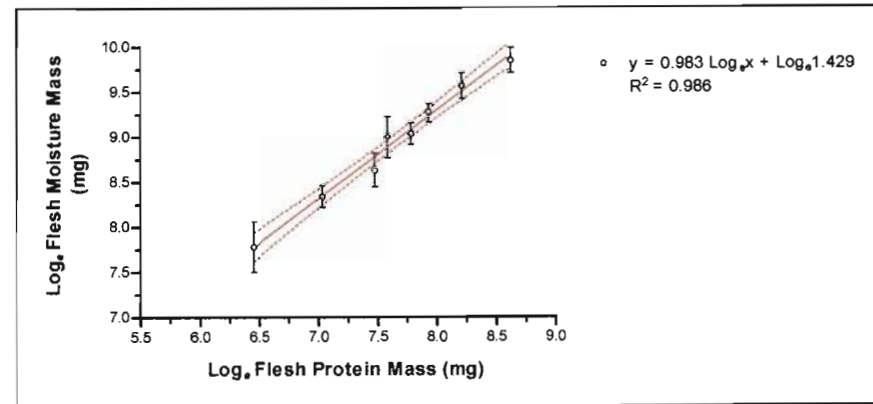
App C Figure 63: The relationship between the Log_{10} of flesh ash and flesh protein mass in mixed samples of pond reared prawns.



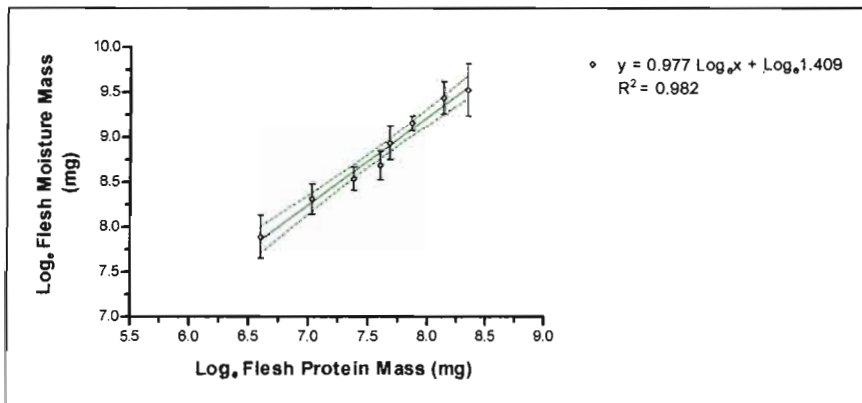
App C Figure 64: The relationship between the Log_{10} of flesh ash and flesh protein mass in mixed samples of laboratory reared prawns.



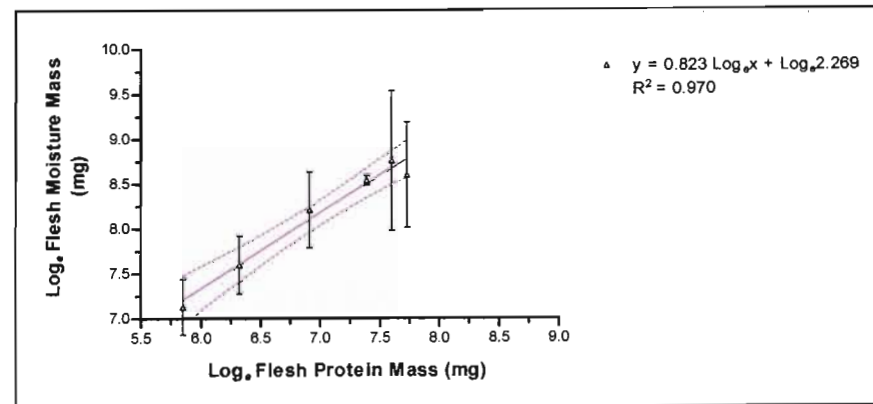
App C Figure 65: The relationship between the Log_{10} of flesh moisture and flesh protein mass in pond reared male prawns.



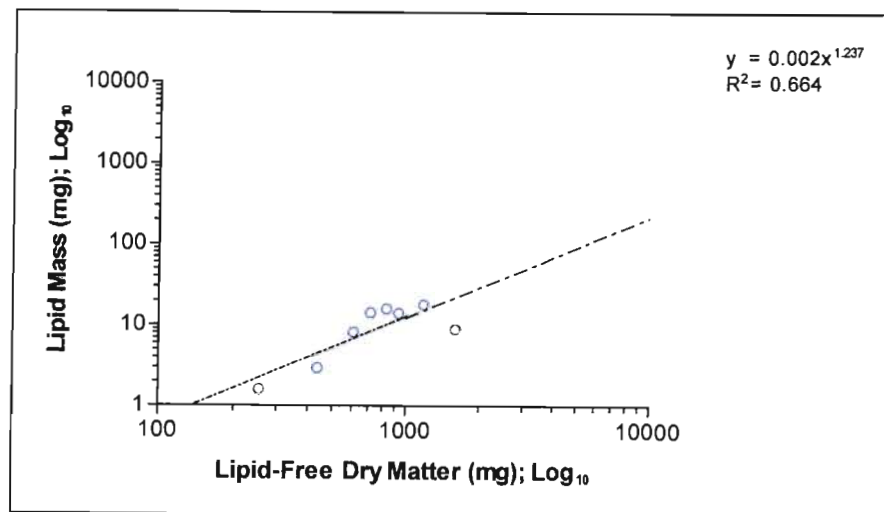
App C Figure 66: The relationship between the Log_{10} of flesh moisture and flesh protein mass in pond reared female prawns.



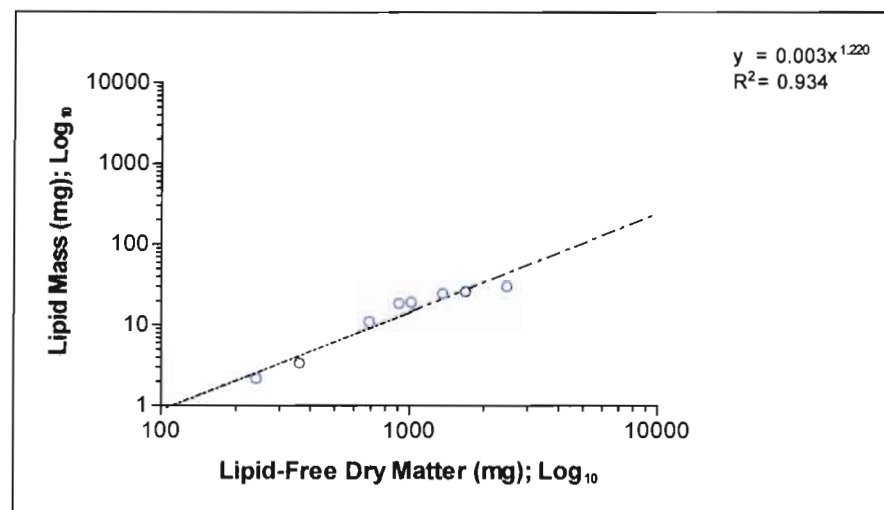
App C Figure 67: The relationship between the Log_{10} of flesh moisture and flesh protein mass in mixed samples of pond reared prawns.



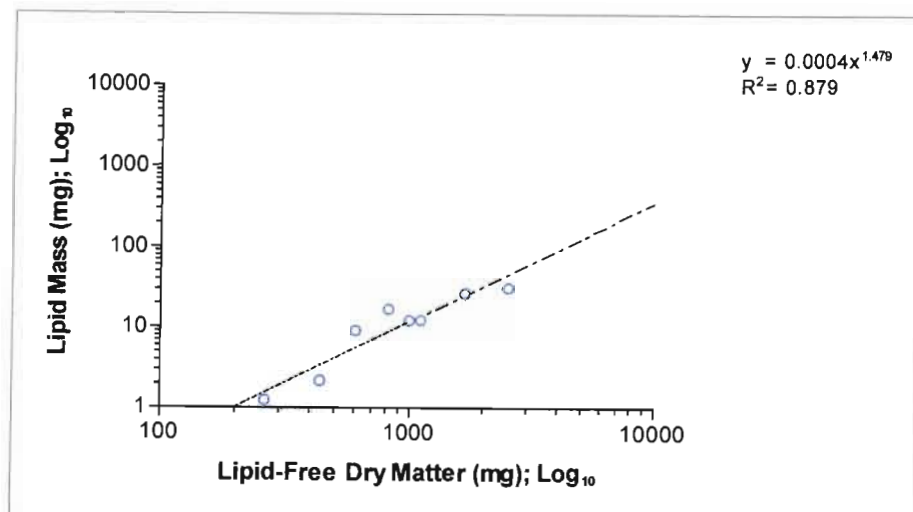
App C Figure 68: The relationship between the Log_{10} of flesh moisture and flesh protein mass in mixed samples of laboratory reared prawns.



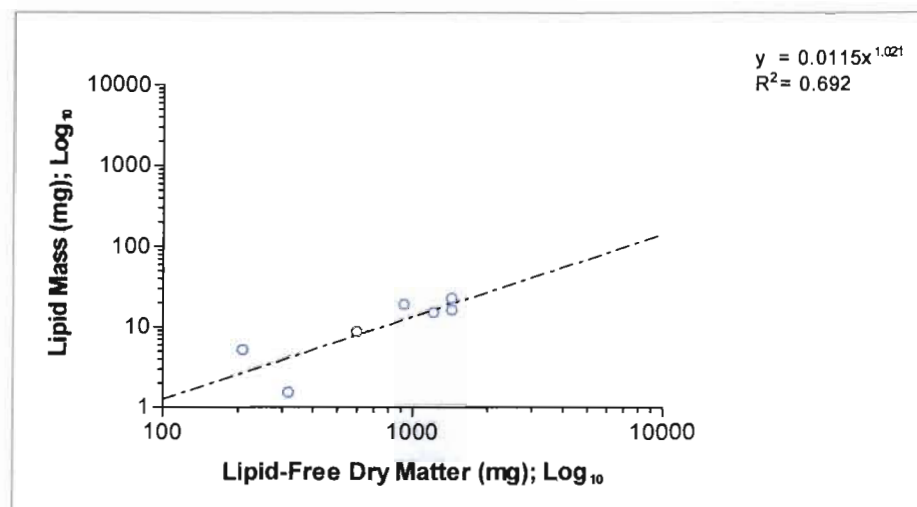
App C Figure 69: Relationship between lipid mass and lipid-free dry matter in the exoskeleton of pond-reared male prawns.



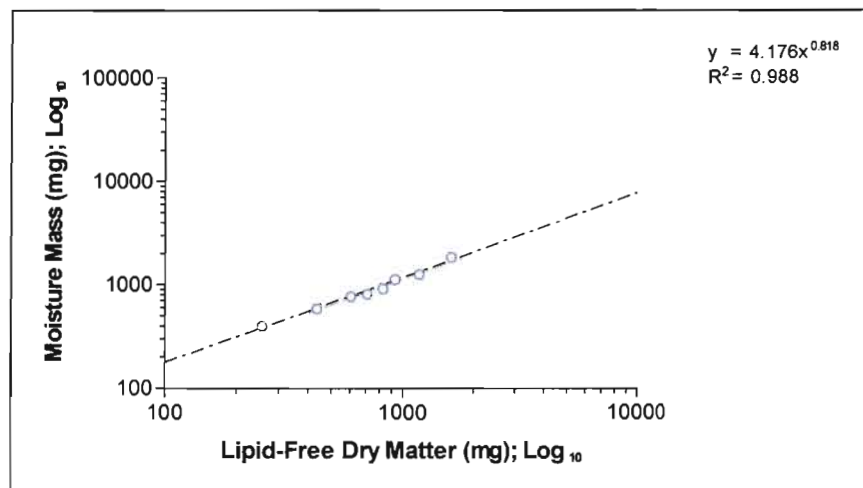
App C Figure 70: Relationship between lipid mass and lipid-free dry matter in the exoskeleton of pond-reared female prawns.



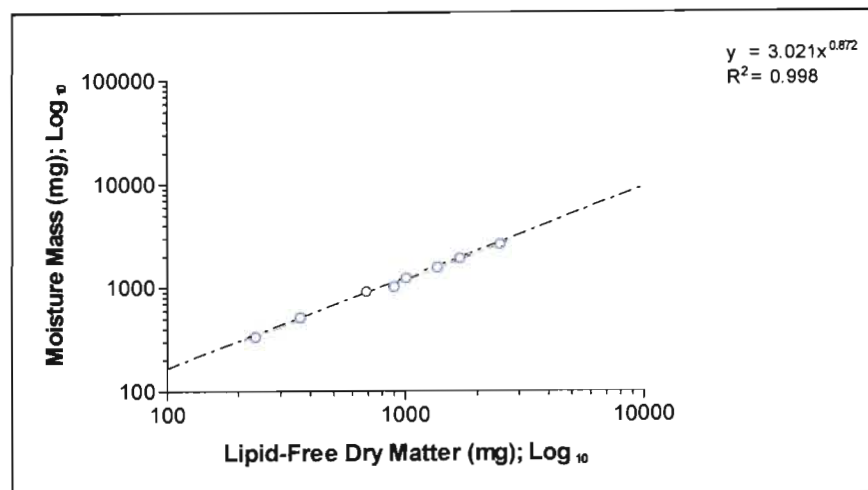
App C Figure 71: Relationship between lipid mass and lipid-free dry matter in the exoskeleton of mixed samples of pond-reared prawns.



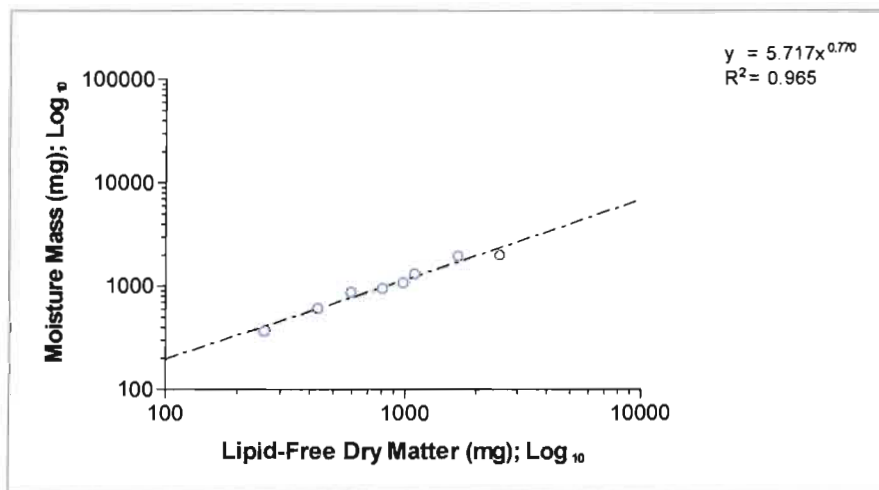
App C Figure 72: Relationship between lipid mass and lipid-free dry matter in the exoskeleton of mixed samples of laboratory-reared prawns.



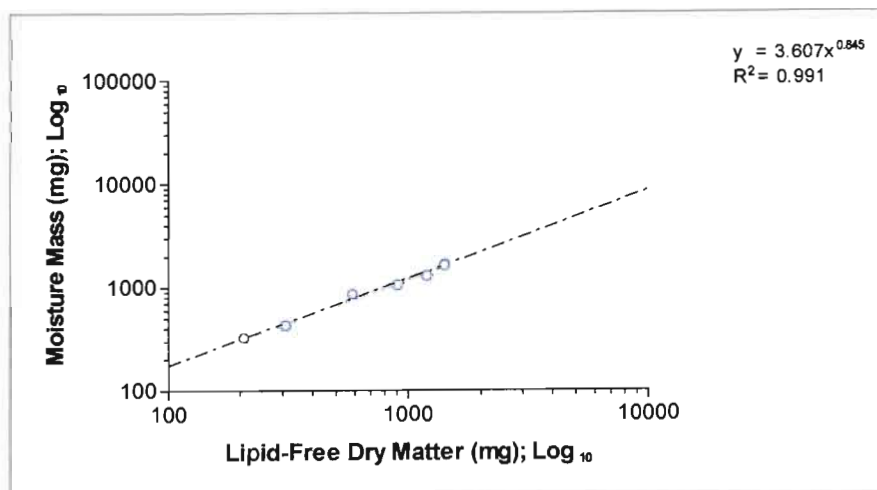
App C Figure 73: Relationship between moisture mass and lipid-free dry matter in the exoskeleton of pond-reared male prawns.



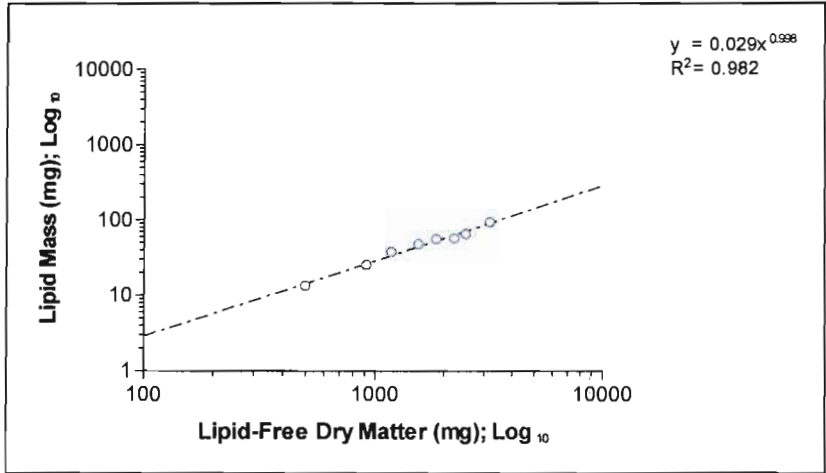
App C Figure 74: Relationship between moisture mass and lipid-free dry matter in the exoskeleton of pond-reared female prawns.



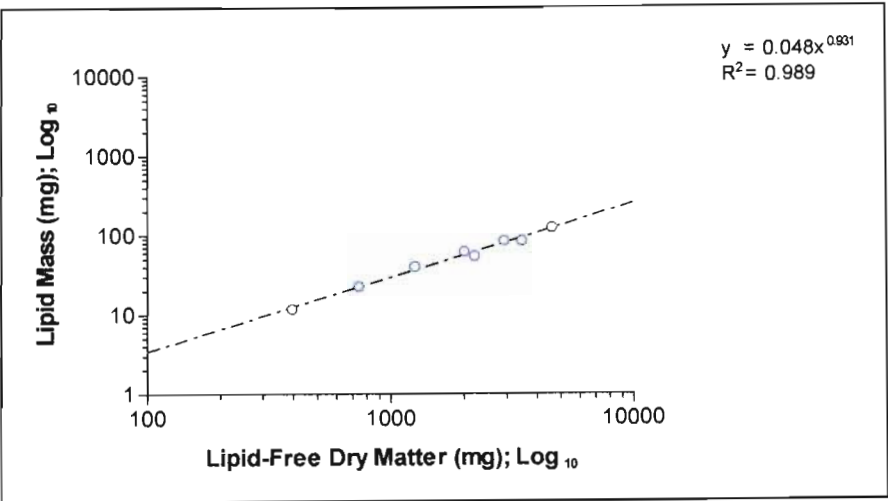
App C Figure 75: Relationship between moisture mass and lipid-free dry matter in the exoskeleton of mixed samples of pond-reared prawns.



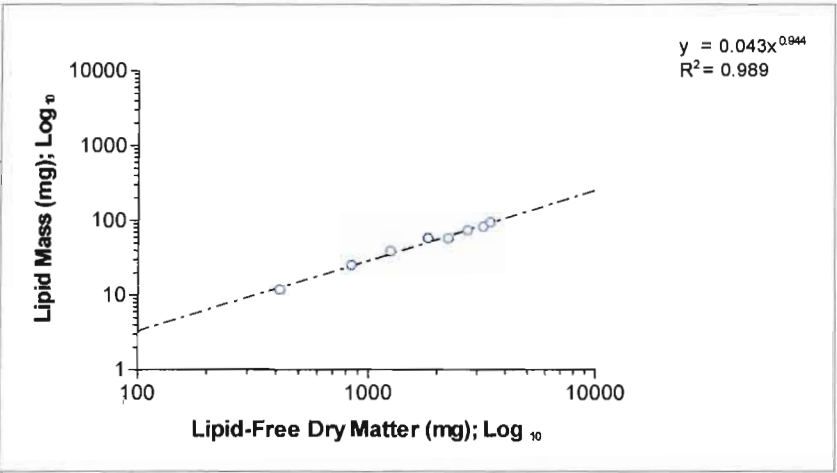
App C Figure 76: Relationship between moisture mass and lipid-free dry matter in the exoskeleton of mixed samples of laboratory-reared prawns.



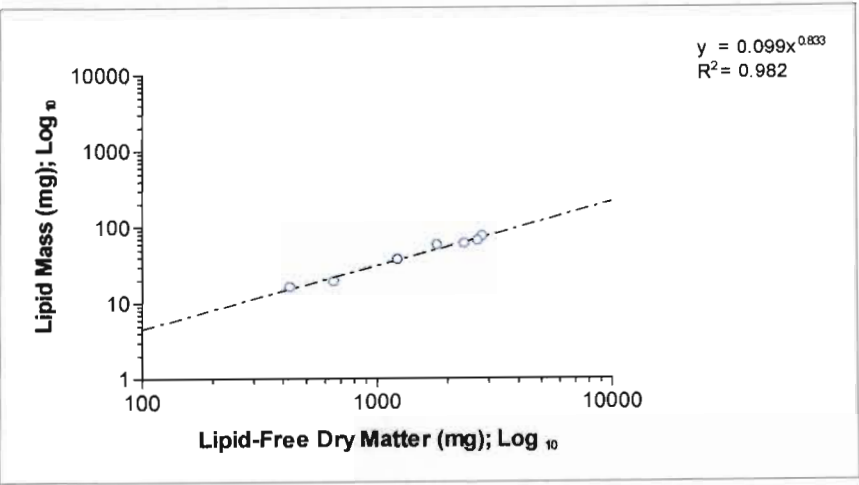
App C Figure 77: Relationship between lipid mass and lipid-free dry matter in the flesh of pond-reared male prawns.



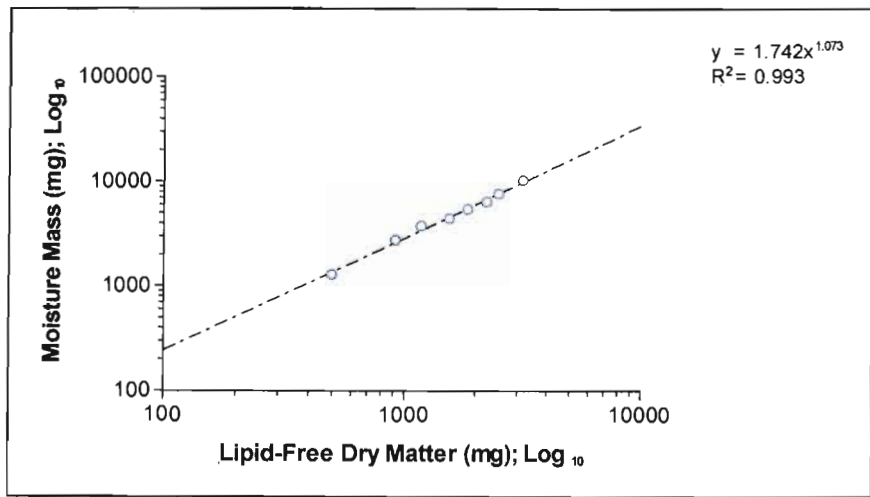
App C Figure 78: Relationship between lipid mass and lipid-free dry matter in the flesh of pond-reared female prawns.



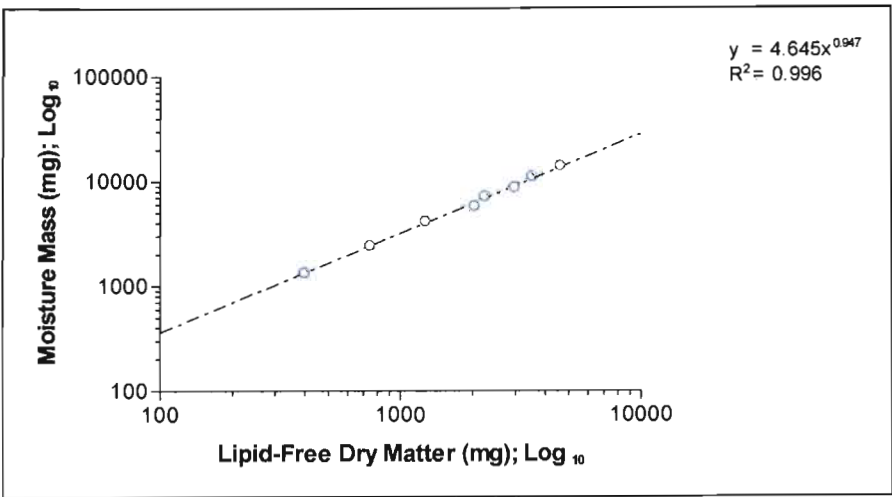
App C Figure 79: Relationship between lipid mass and lipid-free dry matter in the flesh of mixed samples of pond-reared prawns.



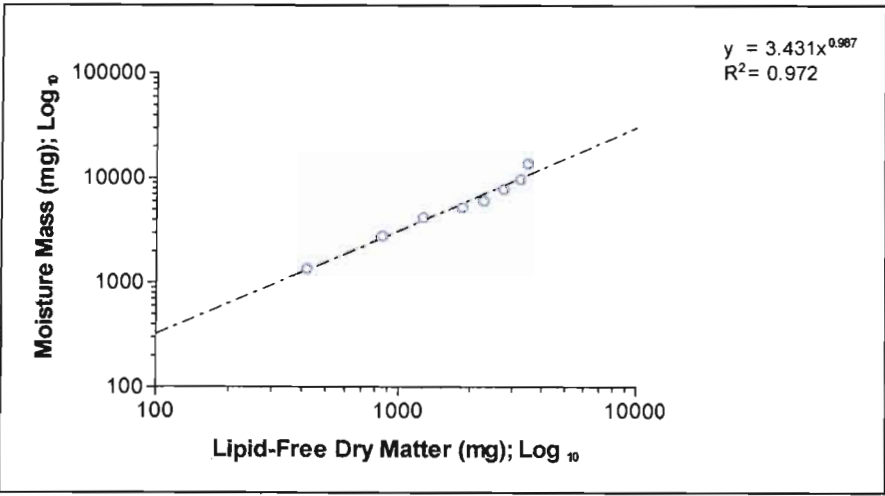
App C Figure 80: Relationship between lipid mass and lipid-free dry matter in the flesh of mixed samples of laboratory-reared prawns.



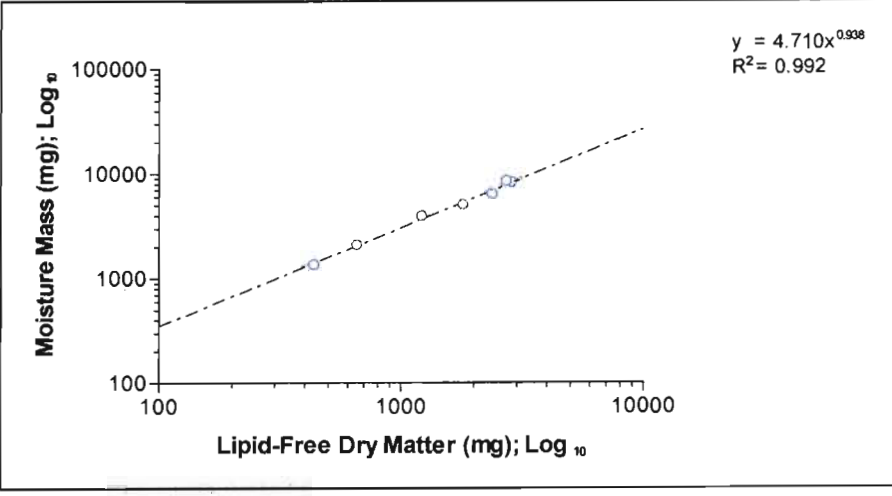
App C Figure 81: Relationship between moisture mass and lipid-free dry matter in the flesh of pond-reared male prawns.



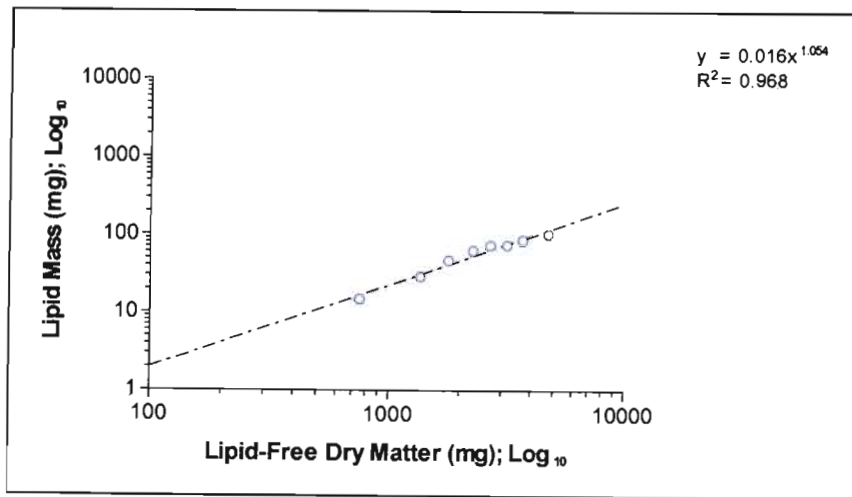
App C Figure 82: Relationship between moisture mass and lipid-free dry matter in the flesh of pond-reared female prawns.



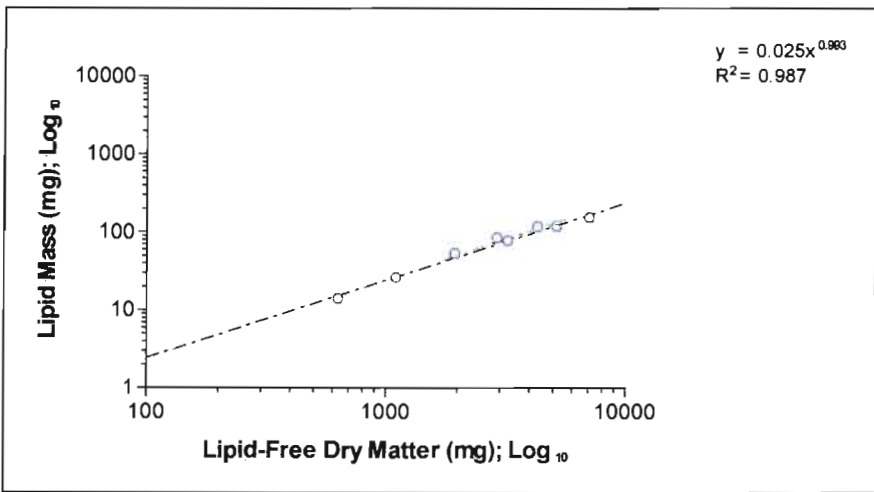
App C Figure 83: Relationship between moisture mass and lipid-free dry matter in the flesh of mixed samples of pond-reared prawns.



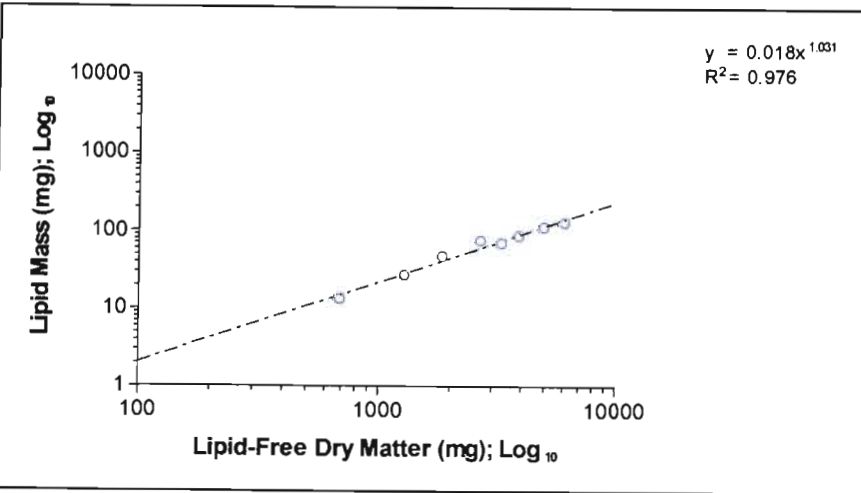
App C Figure 84: Relationship between moisture mass and lipid-free dry matter in the flesh of mixed samples of laboratory-reared prawns.



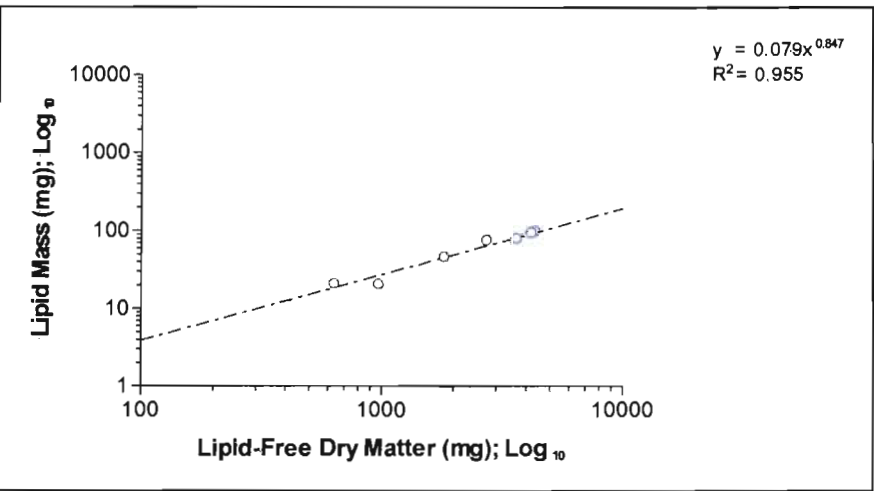
App C Figure 85: Relationship between lipid mass and lipid-free dry matter in the empty body of pond-reared male prawns.



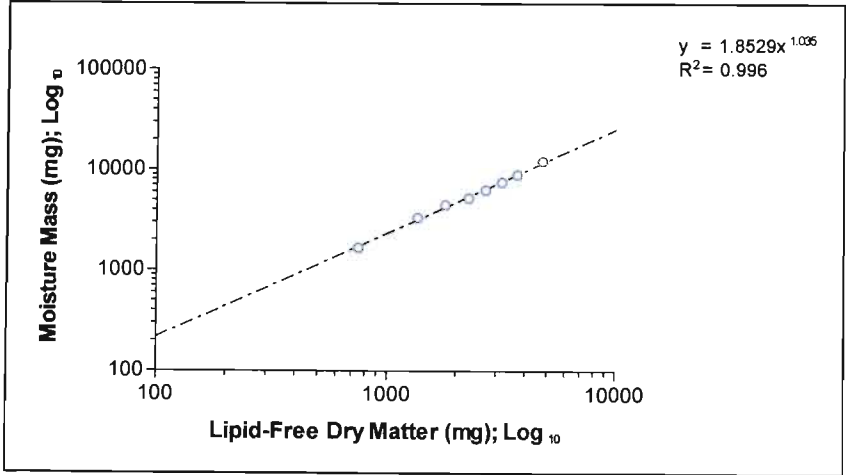
App C Figure 86: Relationship between lipid mass and lipid-free dry matter in the empty body of pond-reared female prawns.



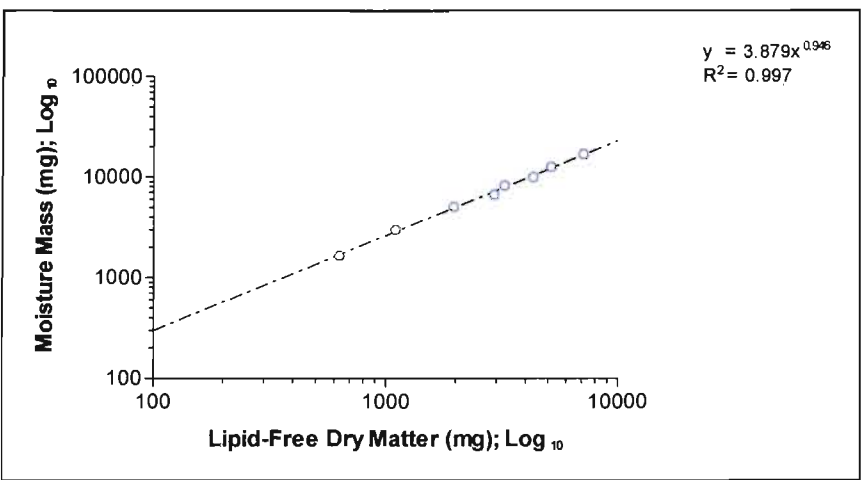
App C Figure 87: Relationship between lipid mass and lipid-free dry matter in the empty body of mixed samples of pond-reared prawns.



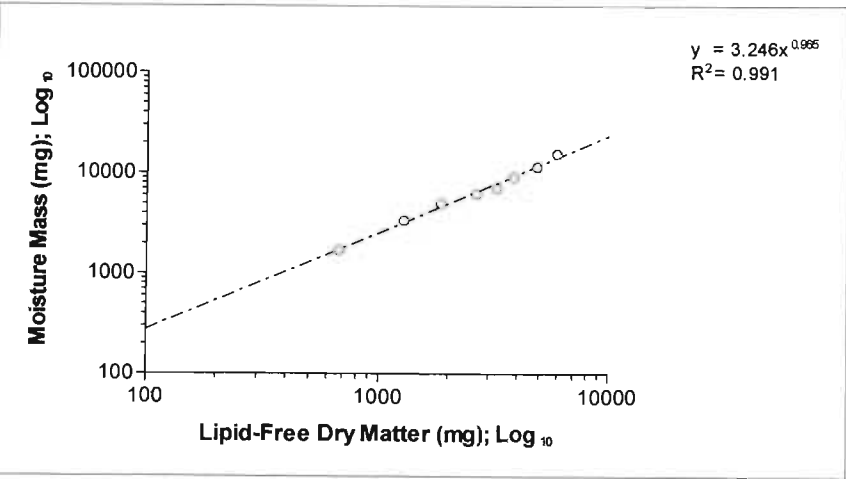
App C Figure 88: Relationship between lipid mass and lipid-free dry matter in the empty-body of mixed samples of laboratory-reared prawns.



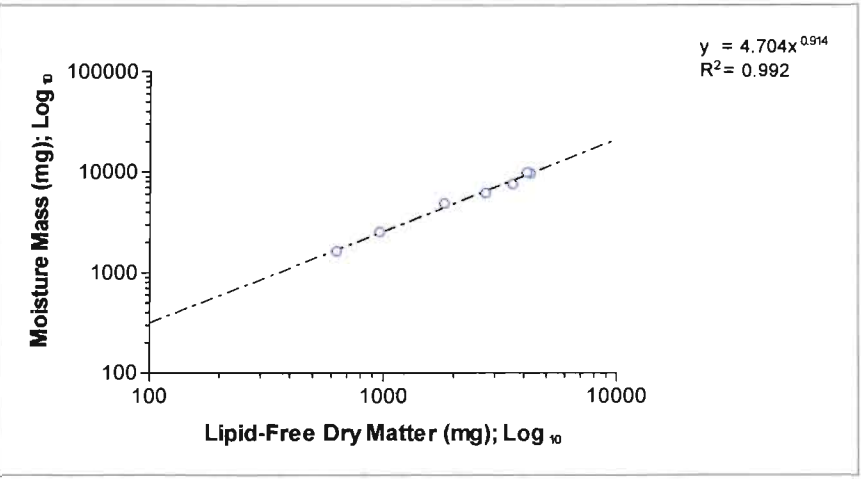
App C Figure 89: Relationship between moisture mass and lipid-free dry matter in the empty body of pond-reared male prawns.



App C Figure 90: Relationship between moisture mass and lipid-free dry matter in the empty body of pond-reared female prawns.

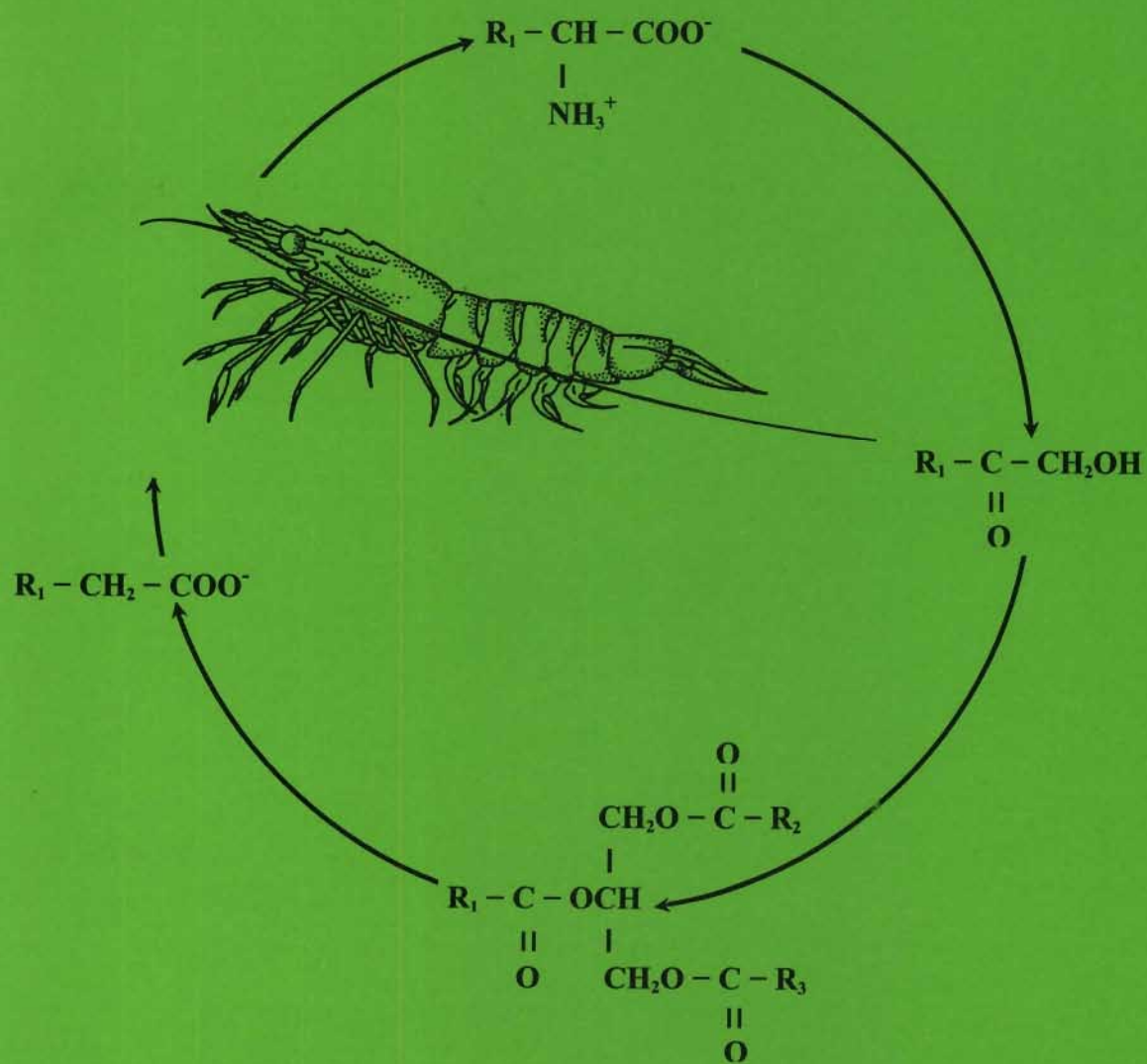


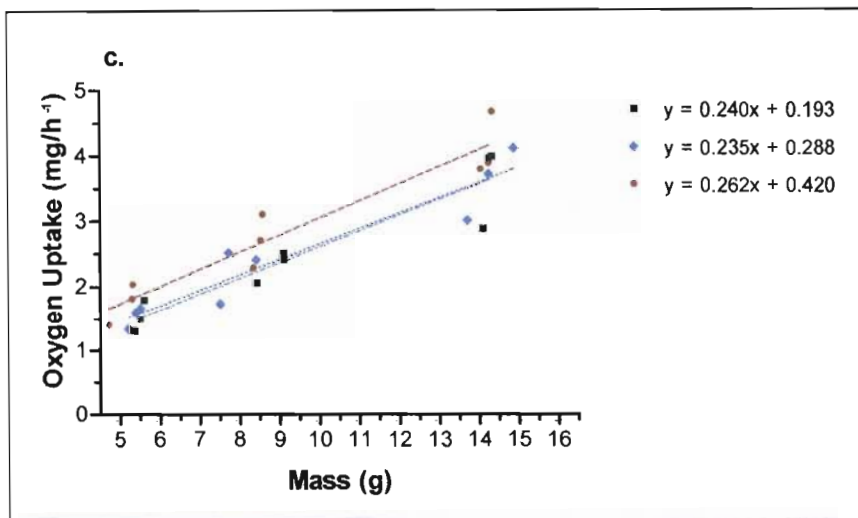
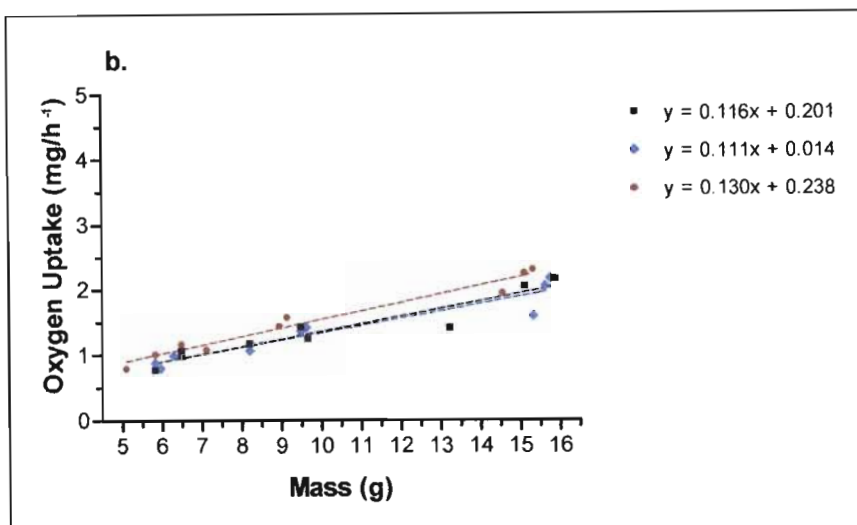
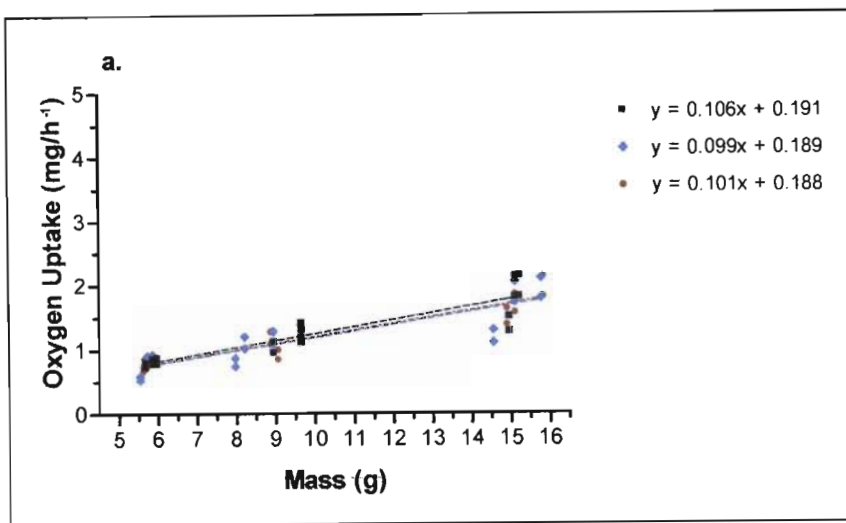
App C Figure 91: Relationship between moisture mass and lipid-free dry matter in the empty body of mixed samples of pond-reared prawns.



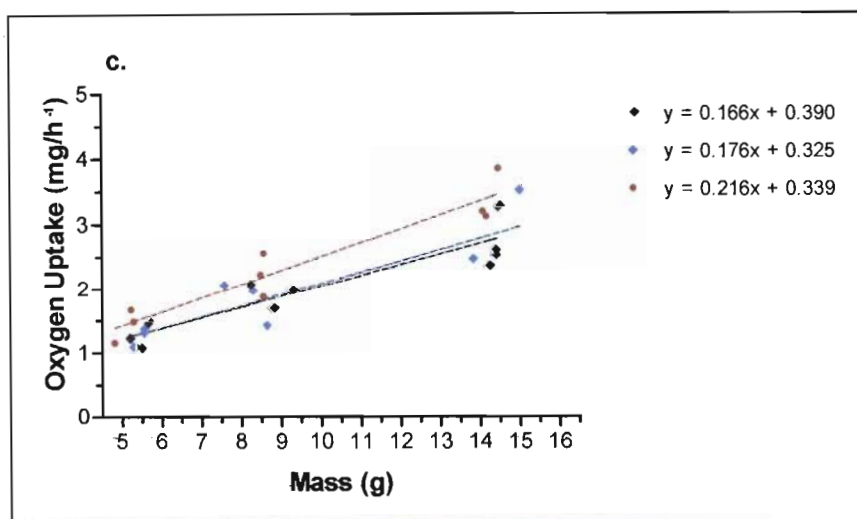
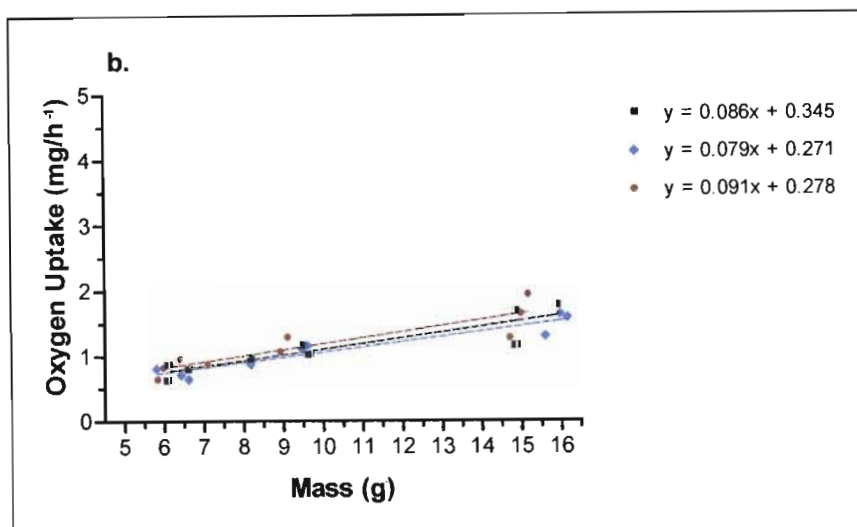
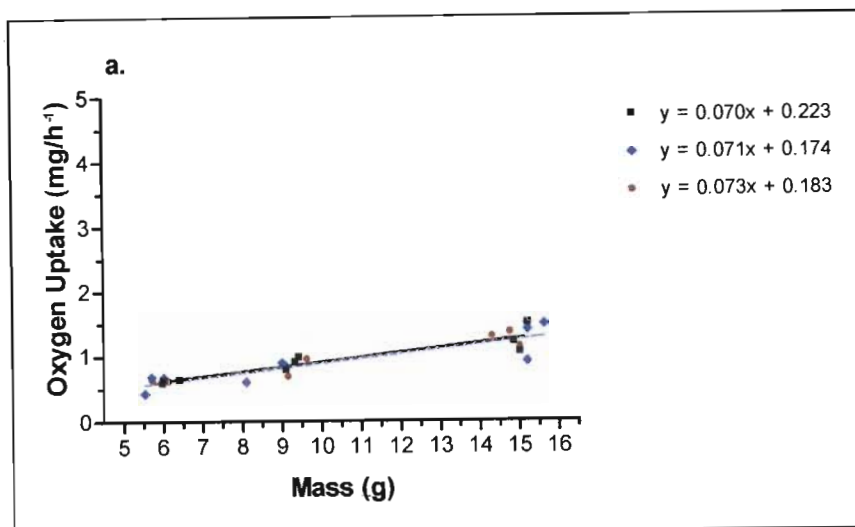
App C Figure 92: Relationship between moisture mass and lipid-free dry matter in the empty body of mixed samples of laboratory-reared prawns.

Appendix D

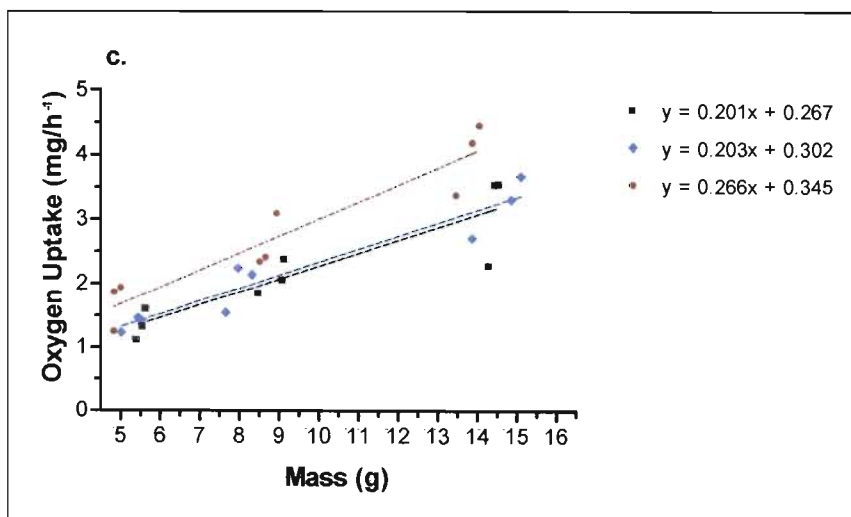
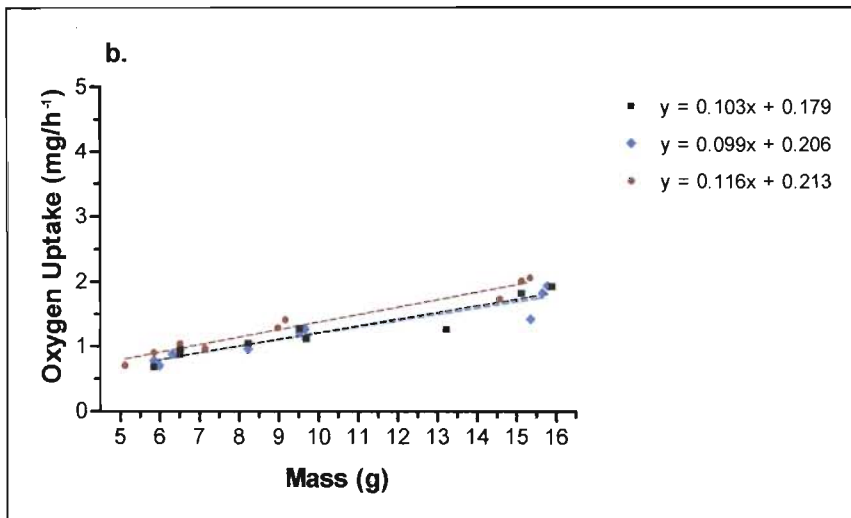
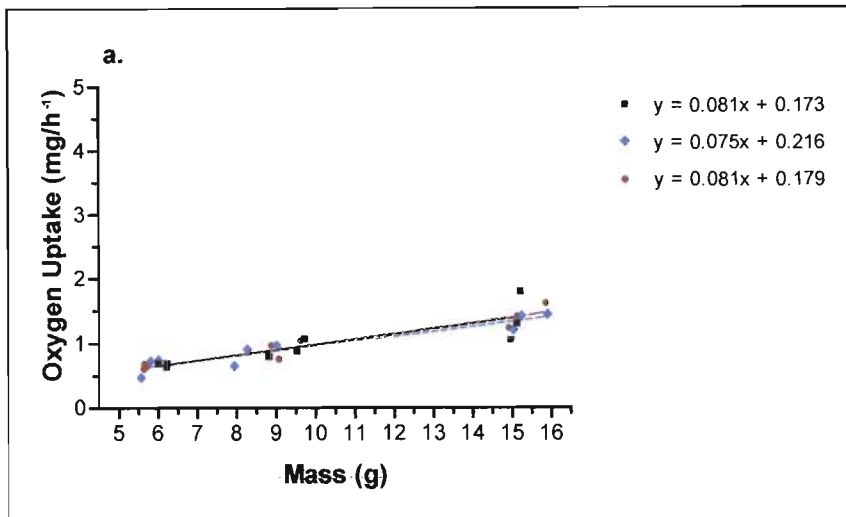




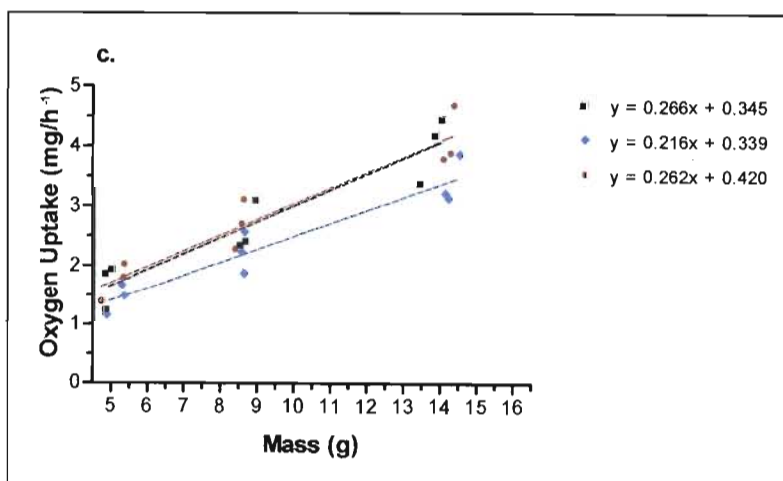
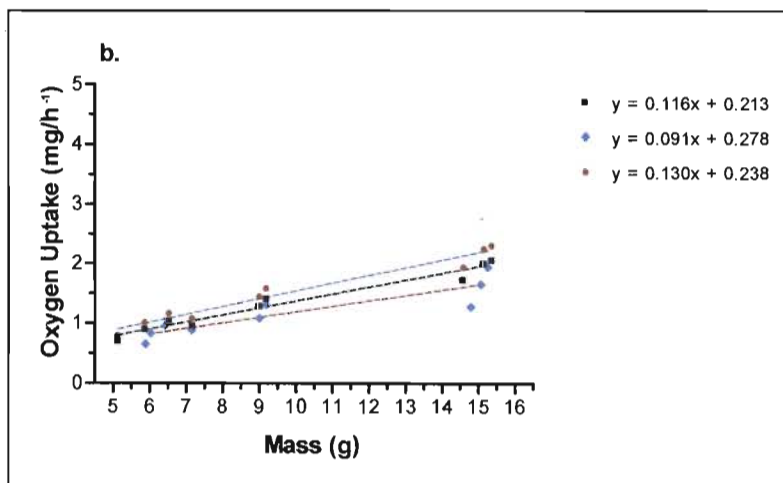
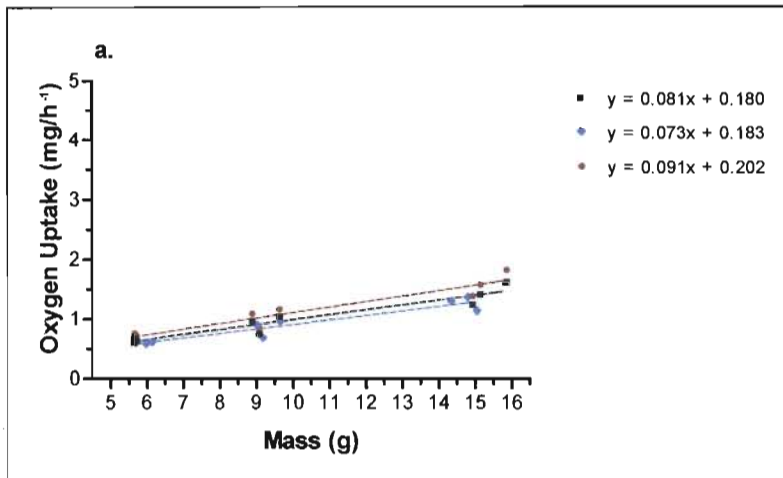
App D figure 1: The mean oxygen consumption of fasting *Fenneropenaeus indicus* reared in 15 ppt and fed diets containing ● 20, ◆ 30 and ■ 40% crude protein (CP) and at temperatures 20°C (a.), 25°C (b.) and 30°C (c.).



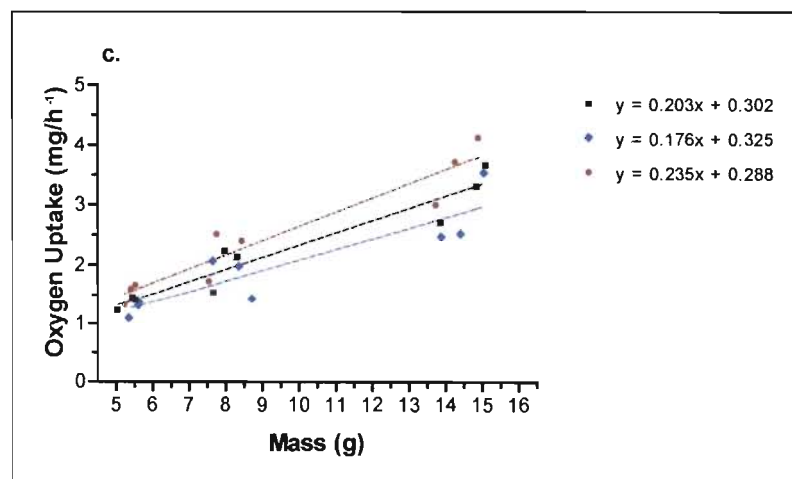
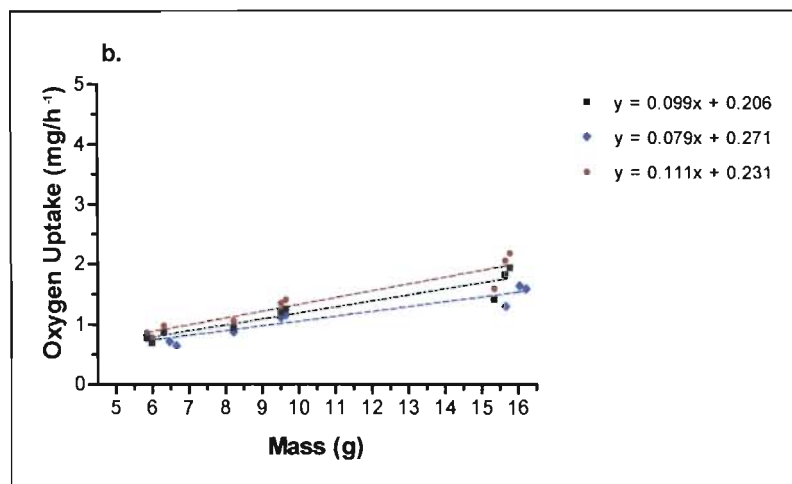
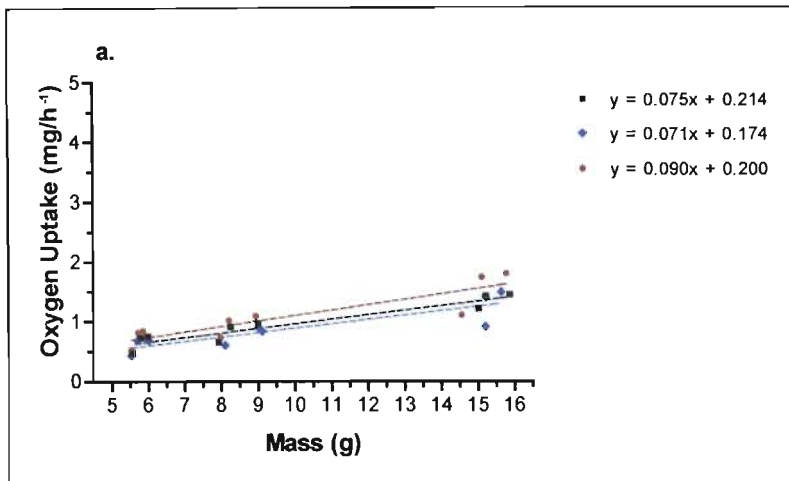
App D figure 2: The mean oxygen consumption of fasting *Fenneropenaeus indicus* reared in 25 ppt and fed diets containing 20, 30 and 40% crude protein (CP) and at temperatures 20°C (a.), 25°C (b.) and 30°C (c.).



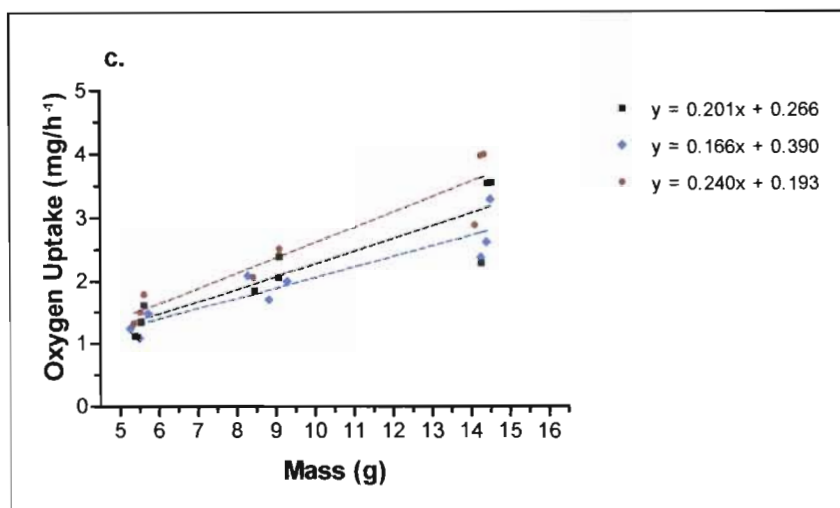
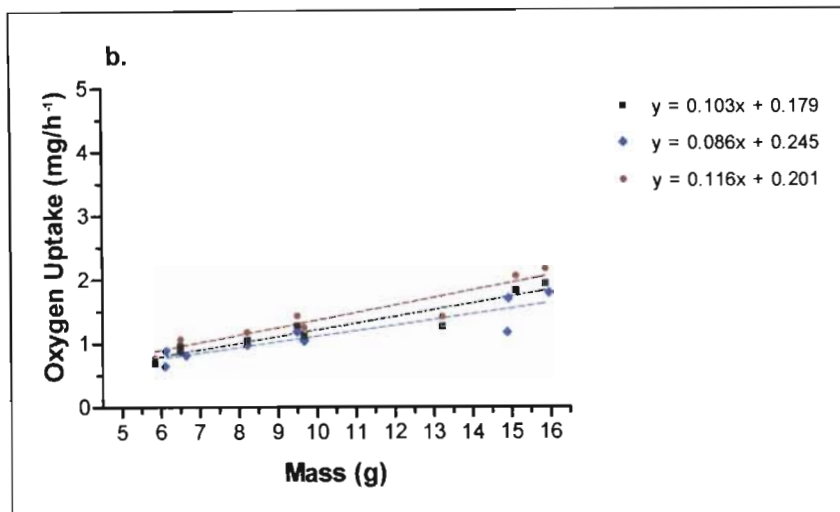
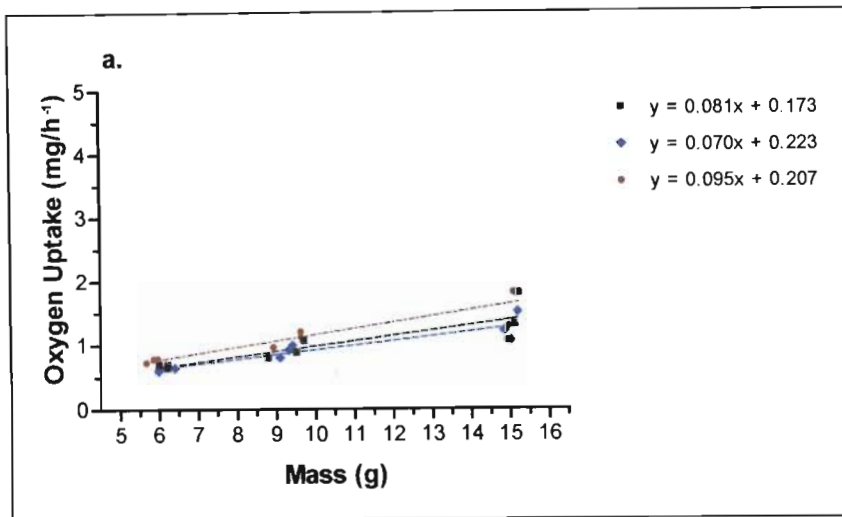
App D figure 3: The mean oxygen consumption of fasting *Fenneropenaeus indicus* reared in 35 ppt and fed diets containing 20, 30 and 40% crude protein (CP) and at temperatures 20°C (a.), 25°C (b.) and 30°C (c.).



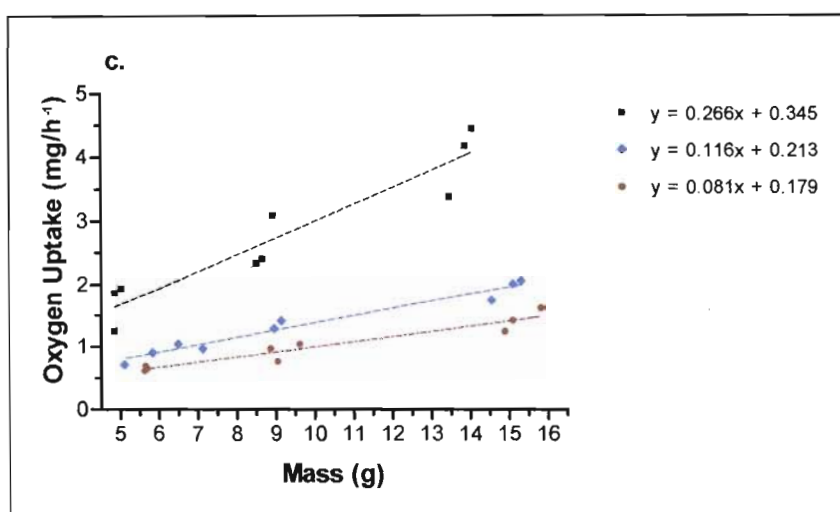
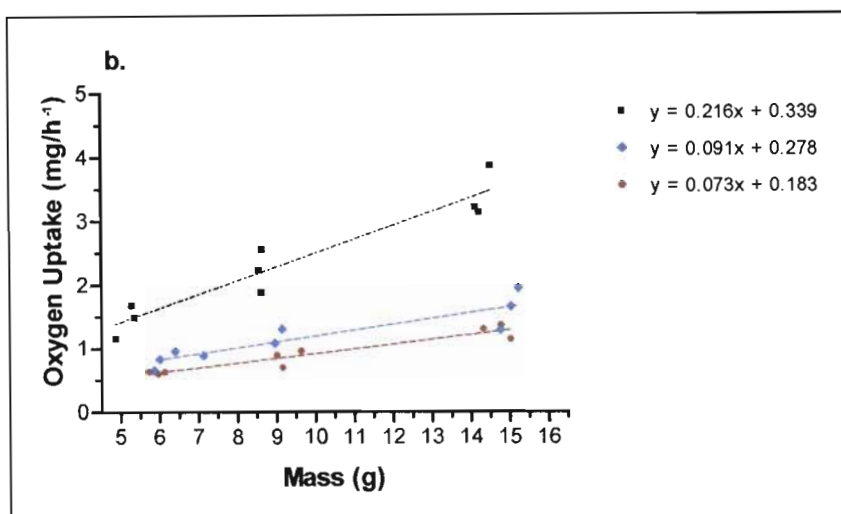
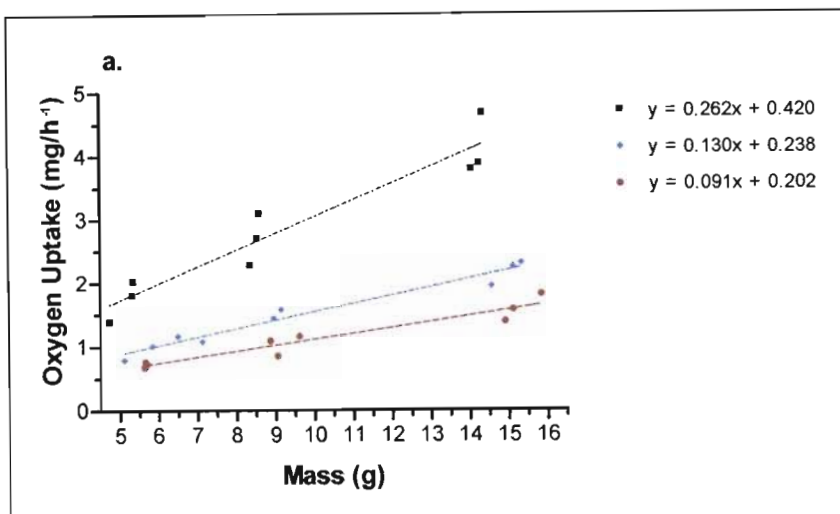
App D figure 4: The mean oxygen consumption of fasting *Fenneropenaeus indicus* reared on a diet containing 20% crude protein (CP) in salinities ● 15ppt, ◆ 25ppt and ■ 35ppt and at temperatures 20°C (a.), 25°C (b.) and 30°C (c.)



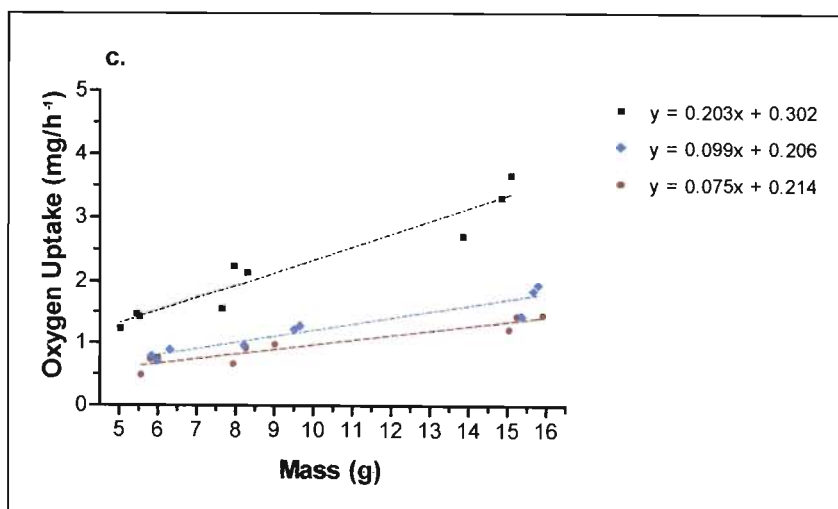
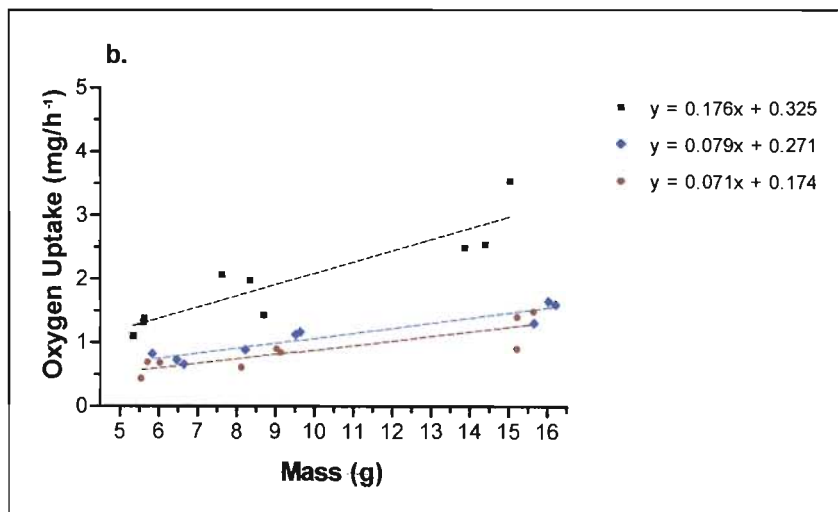
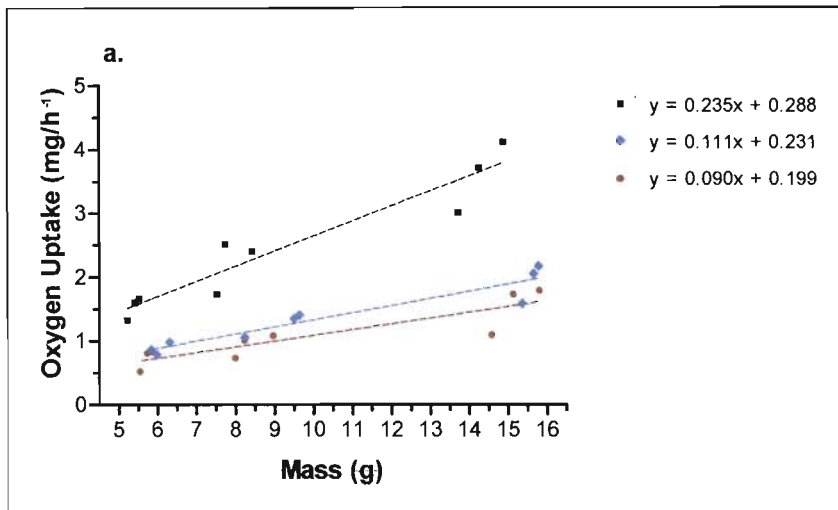
App D figure 5: The mean oxygen consumption of fasting *Fenneropenaeus indicus* reared on a diet containing 30% crude protein (CP) in salinities ● 15ppt, ◆ 25ppt and ■ 35ppt and at temperatures 20°C (a.), 25°C (b.) and 30°C (c.).



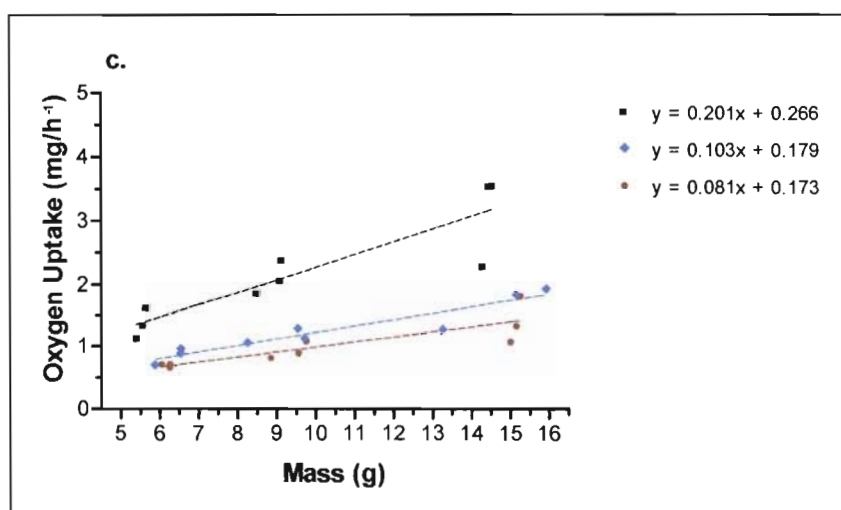
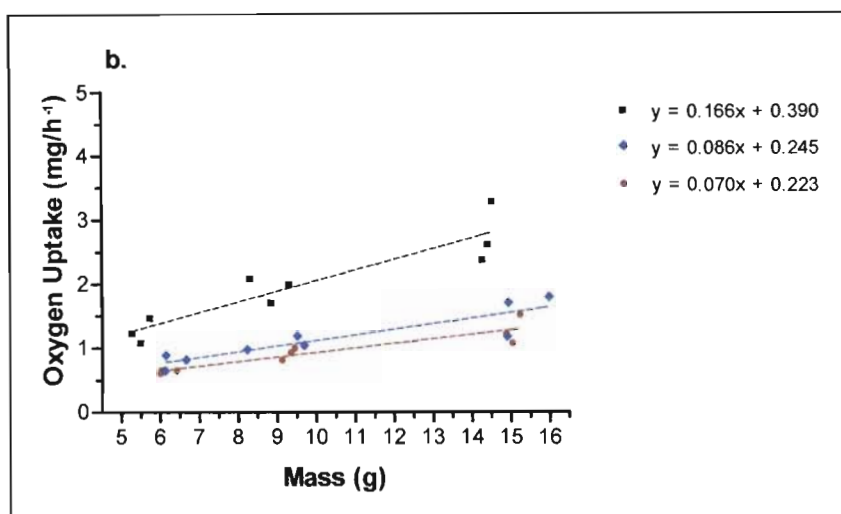
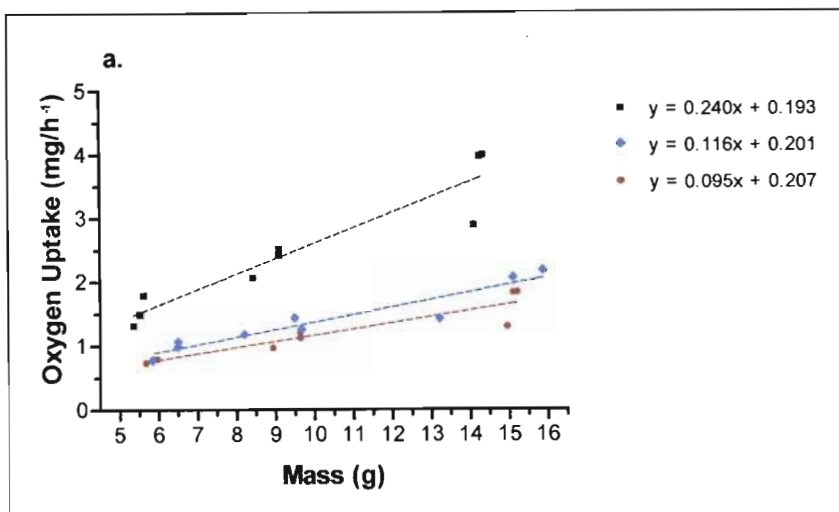
App D figure 6: The mean oxygen consumption of fasting *Fenneropenaeus indicus* reared on a diet containing 40% crude protein (CP) in salinities ● 15ppt, ◆ 25ppt and ■ 35ppt and at temperatures 20°C (a.), 25°C (b.) and 30°C (c.).



App D figure 7: The mean oxygen consumption of fasting *Fenneropenaeus indicus* reared on a diet containing 20% crude protein (CP) at temperatures \bullet 20°C, \blacklozenge 25°C and \blacksquare 30°C and in salinities 15ppt (a.), 25ppt (b.) and 35ppt (c.).

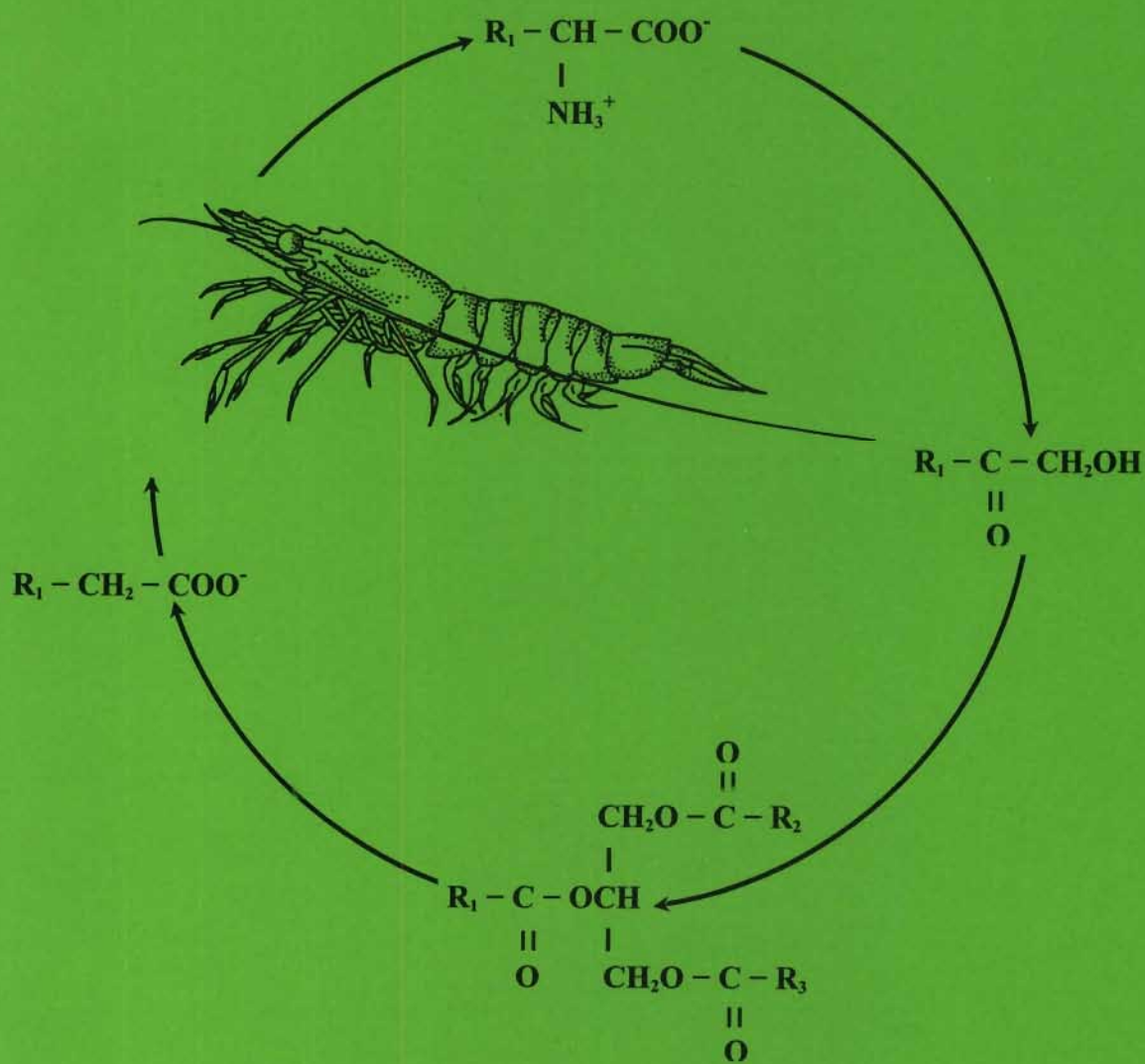


App D figure 8: The mean oxygen consumption of fasting *Fenneropenaeus indicus* reared on a diet containing 30% crude protein (CP) at temperatures \bullet 20°C, \blacklozenge 25°C and \blacksquare 30°C and in salinities 15ppt (a.), 25ppt (b.) and 35ppt (c.).



App D figure 9: The mean oxygen consumption of fasting *Fenneropenaeus indicus* reared on a diet containing 40% crude protein (CP) at temperatures \bullet 20°C, \blacklozenge 25°C and \blacksquare 30°C in salinities 15ppt (a.), 25ppt (b.) and 35ppt (c.).

Appendix E



App E. 1. Determination of Q_{10} values.

The Q_{10} value was calculated using the following formula (Schmidt-Nielsen 1997).

$$R_2 = R_1 \times Q_{10}^{\frac{T_2 - T_1}{10}}$$

where R_1 and R_2 are oxygen rates ($\mu\text{g atoms O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) at temperatures T_1 and T_2 .

App E. 2. Effect of temperature on Q_{10} values.

The thermal coefficient (Q_{10}) is a function of body mass with estimates thereof for different temperature increments and salinities shown in Table E. In this study Q_{10} values for *F. indicus* varied between 0.41 and 2.59 and were found to decrease with an increase in temperature. The highest value of 2.59 was recorded for 15 g prawns reared on 21 % CP, at 35 ‰ and between 20° – 30 °C. In contrast the lowest value was reported to be 0.65 for 5 g prawns reared on a 31 % CP at 25 ‰.

A temperature increment of 20° – 25 °C had a greater influence on respiration than 25° – 30 °C since Q_{10} estimates were higher amongst the former interval as opposed to the latter (Table 7.5). In all cases Q_{10} values increased with an increase in body mass for each of the intervals with the 20° – 30 °C interval displaying expectantly higher values than those recorded for the 5 °C intervals. Results indicate the sensitivity of species metabolic rate to a 5° – 10 °C change in temperature.

App E. 2. The influence of temperature on Q_{10} values.

By comparison with other crustaceans, Q_{10} values presented in Table E were lower than those reported for *Callinassa kraussi* (Hanekom & Baird, 1987) and *P. monodon* (Liao & Mural, 1986) for similar temperatures intervals. Higher Q_{10} values for *P. monodon* are indicative of a higher growth metabolism whereas with regards to *C. kraussi* differences were attributed to a difference in approaches and the sensitivity of the organism to a change in temperature.

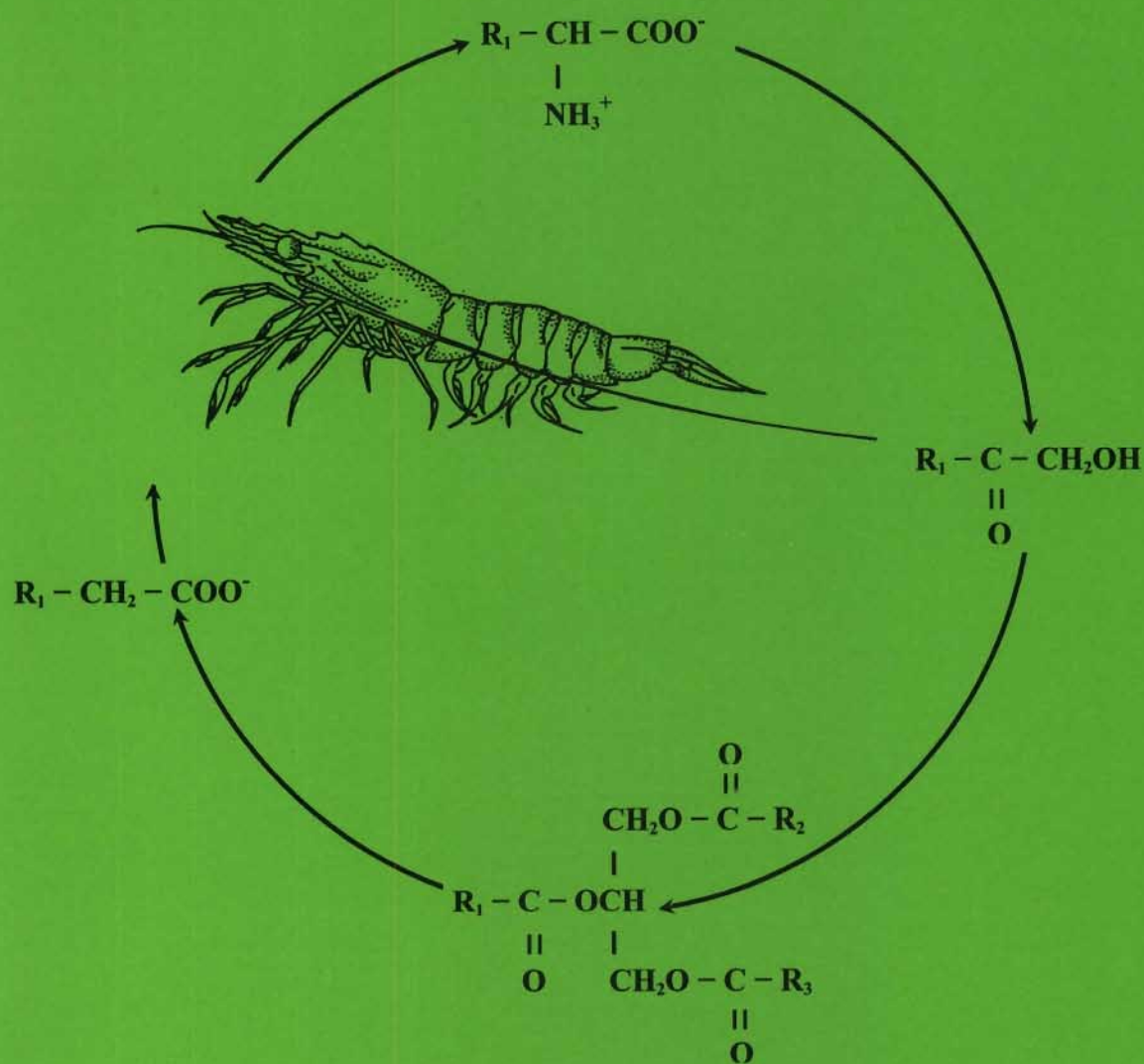
An almost threefold increase in Q_{10} values for 14 prawns between a 20° – 30 °C indicates that metabolic rate of older animals were influenced by a 10 °C change as opposed to 6 and 9 g prawns. A 2.6 increase does not seem excessive especially when compared to a Q_{10} value of 2 obtained for 0.1 g *F. indicus* for the interval of 25° – 35 °C (Kutty *et al.*, 1971). Q_{10} values in this study are considered to be low when compared to invertebrates that inhabit intertidal zones

whereby they experience large variations and are exposed to extreme temperatures with a change in tides.

Table E: Summary of Q_{10} values of juvenile *F. indicus* established at different temperatures intervals and at 15, 25 and 35 ‰ fed test diets containing 21, 31 and 41 % crude protein.

Diet Protein (%)	Salinity (‰)	Mean Live Mass (g)	Temperature Interval (°C)		
			20 – 25	25 - 30	20 - 30
21	15	6	0.82	0.59	1.02
		9	0.94	0.88	1.66
		14	1.21	1.03	2.53
	25	6	0.73	0.54	0.82
		9	0.83	0.83	1.37
		14	1.01	1.05	2.13
	35	6	0.77	0.67	1.04
		9	0.89	0.95	1.69
		14	1.14	1.13	2.59
31	15	6	0.72	0.54	0.80
		9	0.92	0.64	1.26
		14	1.09	0.94	2.06
	25	6	0.65	0.49	0.66
		9	0.84	0.58	1.04
		14	0.93	0.85	1.58
	35	6	0.68	0.51	0.72
		9	0.87	0.61	1.13
		14	1.04	0.89	1.88
41	15	6	0.75	0.45	0.76
		9	0.74	0.72	1.23
		14	1.00	0.98	1.97
	25	6	0.68	0.41	0.63
		9	0.77	0.66	1.02
		14	0.96	0.76	1.50
	35	6	0.70	0.42	0.67
		9	0.85	0.69	1.18
		14	0.98	0.88	1.73

Appendix F



App F.1. The model proposed by Bureau et al. (2000) for L. vannamei.

In order to determine the daily feed ration Bureau *et al.* (2000) highlighted six simple steps, which were:

Step 1.: DIET selection:

The first step requires that the feed composition be defined. The practical diet used had the following:

crude protein	= 40 %,
lipid	= 8 %
carbohydrate	= 30 %
digestible energy	= 17 MJ/kg

Step 2.: GROWTH prediction:

The second step requires that the growth be predicted and for this Bureau *et al.* (2000) estimated a gain of 2 g. prawn⁻¹. week⁻¹.

where	Initial mass was:	10 g
	Water temperature:	26 °C
	Over a period of:	7 days

Step 3.: ENERGY GAIN (RE) predicted:

This step requires that the energy retained in the body be known or measured.

Gross energy content of prawn carcass	= 5.2 kJ/g
Mass gain	= 2 g
∴ Energy retained (RE)	= 5.2 × 2 = 10.4 kJ prawn ⁻¹ week ⁻¹

Step 4.: WASTE estimation:

Since maintenance of life processes (integrity of the tissues of the animal, osmoregulation, respiration, circulation, swimming *etc*) and the deposition of body components have costs in terms of nutrients and feed energy, step four requires that these losses be estimated. Energy losses were considered to be due to maintenance, feeding, moulting, non-faecal and exuviae costs. Thus, based on data from Gauguelin (1996) *cited* in Bureau *et al.* (2000) the costs needed for maintenance were calculated as:

$$\begin{aligned} \text{HE}_e &= 45 (0.010)^{-1} \cdot \text{d}^{-1} &= 0.45 \text{ kJ prawn}^{-1} \cdot \text{d}^{-1} \\ \text{HE}_e &= 0.45 \text{ kJ prawn}^{-1} \cdot \text{d}^{-1} \times 7 \text{ days} &= 3.20 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1} \end{aligned}$$

Predicting heat increment losses due to feeding (HE_i):

Warukamkul *et al.* (2000) cited in Bureau *et al.* (2000) suggested that the partial efficiency of ME utilised to promote a defined increment in live mass (k_{pl}) of *P. mondon* was estimated to be about 0.5; therefore, for every 0.5 kJ of RE or HE_e , 0.5 kJ is expended as HE_i . HE_i was estimated to be $0.5/0.5 = 1.0$. Hence it follows that:

$$HE_i = (HE_e + RE) = (3.2 + 10.4) = 13.6 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1}$$

Determination of losses caused due to moulting:

Characteristics unique to crustaceans include periodic energy losses associated with moulting, HE_x . In *L. stylirostris* and *L. vannamei* 25 % of the RE accumulated during the intermoult phase was considered to be expended due the metabolic cost of moulting (Read & Caulton, 1980). Thus, moulting energy losses (HE_x) were calculated using the following:

$$HE_x = 0.25 \times (RE) = 0.25 \times (10.4) = 2.6 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1}$$

Predicting non-faecal energy losses (UE + ZE):

Bureau *et al.* (2000) estimated non-faecal energy losses to be 8 % of the total energy required for retention, maintenance, feeding and moulting. Thus, non-faecal losses were considered to be:

$$\begin{aligned} UE + ZE &= 0.08 \times (RE + HE_e + HE_i + HE_x) \\ &= 0.08 \times (10.4 + 3.2 + 13.6 + 2.6) = 2.4 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1} \end{aligned}$$

Predicting surface losses, exuviae (SE):

Data from Cousin *et al.* (1996) suggest that losses associated with exuviae were less than 3 % of the gross energy intake for *L. stylirostris* and *L. vannamei* (Cousin, 1995):

$$SE = 0.03 \times (RE + HE_e + HE_i + HE_x) = 0.9$$

Step 5.: RATION allowance:

Daily or weekly rations, are calculated by adding all losses incurred due to the assimilation and deposition of nutrients, waste formation and excretion, the heat lost due to maintenance and dividing by digestible energy (DE) of a well-balanced feed. The amount calculated generally represents the minimal feed required to achieve the predicted growth of the prawn (Bureau *et al.*, 2000). Thus, food allowance requires that the total digestible energy requirement is calculated first.

$$\begin{aligned} \text{DE requirement} &= (RE + HE_e + HE_i + HE_x + (UE + ZE) + SE) \\ &= (10.4 + 3.2 + 13.6 + 2.6 + 2.4 + 0.9) = 33.1 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1} \end{aligned}$$

$$\text{DE content of feed:} = 17 \text{ MJ/kg or kJ/g}$$

$$\begin{aligned} \text{Ration allowance} &= (33.1 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1}) \div 17 \text{ MJ.kg}^{-1} \\ &= 1.95 \text{ g feed per prawn}^{-1} \cdot \text{week}^{-1} \end{aligned}$$

$$= 0.28 \text{ g prawn}^{-1} \cdot \text{d}^{-1}$$

$$\text{Expected feed efficiency} = \text{live mass gained/feed consumed} = 2.00 \div 1.95 = 1.03$$

$$\text{Expected FCR} = \text{feed consumed/live mass gained} = 1.95 \div 2.00 = 0.98$$

Note: The amount depends on the composition of the feed since this affects the amount required. In general if fed two diets with similar protein and energy content, a higher intake for a feed with a lower nutrient density will be required in order for the animal to achieve the same performance level (Bureau *et al.*, 2000). For example if DE content of feed is only 14 MJ/kg instead of 17 MJ/kg then:

$$\begin{aligned} \text{DE requirement} &= (\text{RE} + \text{HE}_f + \text{HE}_i + \text{HE}_x + (\text{UE} + \text{ZE}) + \text{SE}) \\ &= (10.4 + 3.2 + 13.6 + 2.6 + 2.4 + 0.9) = 33.1 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1} \end{aligned}$$

$$\begin{aligned} \text{Ration allowance} &= 32.2 \text{ kJ prawn}^{-1} \cdot \text{week}^{-1} / 14 \text{ MJ.kg}^{-1} \\ &= 2.36 \text{ g feed per prawn over 7 days} \\ &= 0.34 \text{ g prawn}^{-1} \cdot \text{d}^{-1} \end{aligned}$$

$$\text{Expected feed efficiency} = \text{live mass gained/feed consumed} = 2.0 \div 2.36 = 0.85$$

$$\text{Expected FCR} = \text{feed consumed/live mass gained} = 2.36 \div 2.0 = 1.18.$$

Step 6.: FEEDING strategies

Feed rations should be given in a manner that allows adequate opportunity for the prawn to achieve its growth potential while minimizing waste (Bureau *et al.*, 2000).

Appendix G

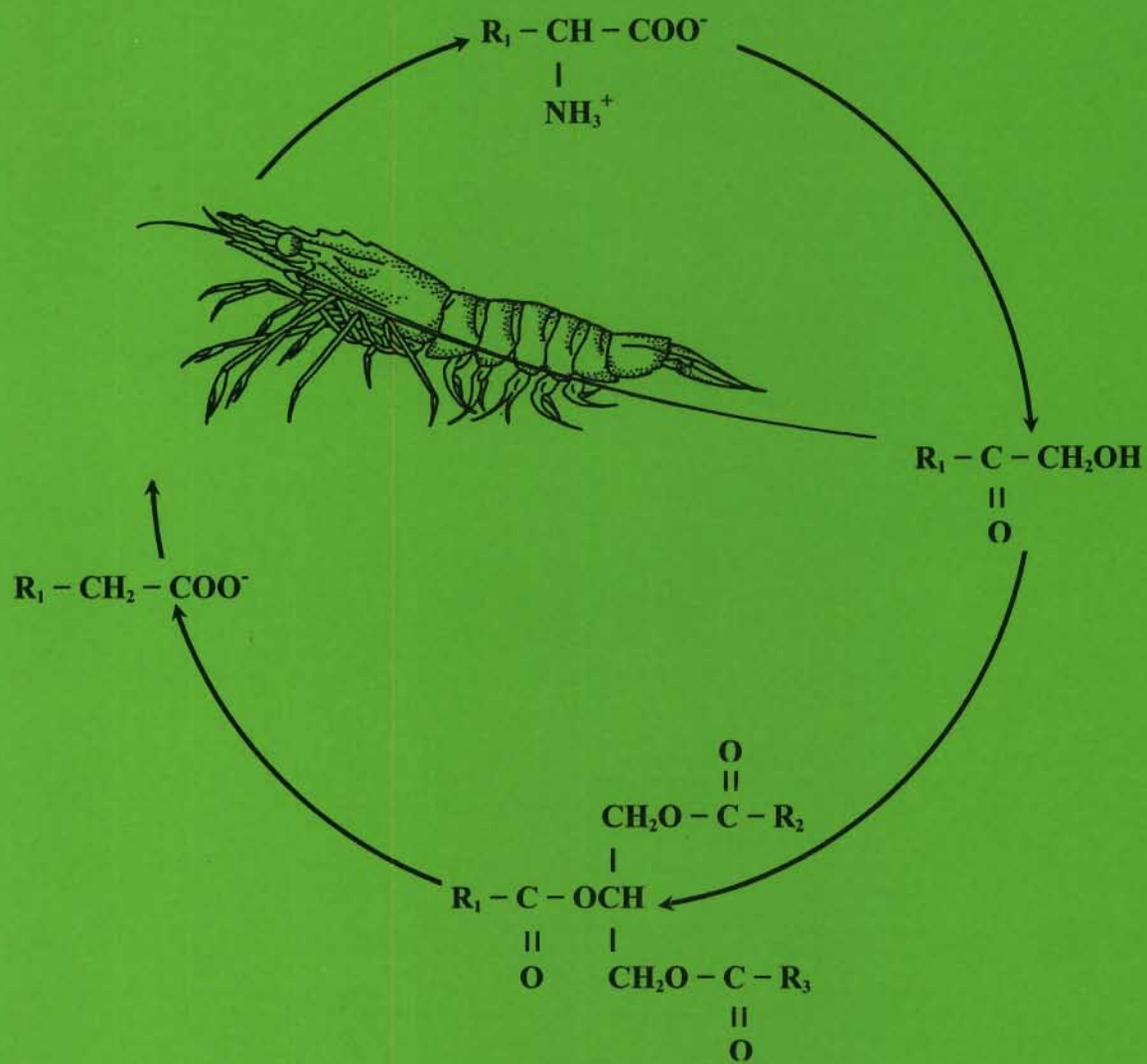


Table App G: Ingredients (mg) and dietary composition used to formulate[†] dietary levels of protein that will satisfy the amino acid requirements in growing *F. indicus*.

Ingredients	Live Mass (g)								
	3	6	9	12	15	20	25	32	35
Wheat meal	47.25	51.26	53.04	54.07	54.65	56.81	59.05	59.36	59.60
Soybean meal 44	24.44	7.74	0.15	-	-	-	-	-	-
Soybean meal 46	2.90	12.29	16.18	13.3	10.94	4.42	-	-	-
Soybean meal 50	0.76	-	-	-	-	-	-	-	-
Sunflower meal 34	11.42	15.46	17.38	19.38	20.78	25.09	28.07	27.97	27.90
Sunflower meal 37	-	-	-	-	0.39	0.45	-	-	-
L-Lysine	0.57	0.62	0.64	0.65	0.65	0.68	0.37	0.16	-
DL-Methionine	0.14	0.11	0.09	0.08	0.06	0.03	-	-	-
L-Threonine	0.02	0.02	0.02	0.02	0.02	0.02	-	-	-
Wheat gluten	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Brewers yeast	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mono calcium phosphate	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Soylecithin	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Fish oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin & Mineral mix	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cholestrol	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
On a dry mass basis (g/100g)									
Protein	27.31	22.94	22.28	21.69	21.40	20.15	18.82	18.67	18.56
Fat	6.51	6.52	6.53	6.54	6.56	6.57	6.58	6.58	6.59
Ash	3.77	3.58	3.49	3.44	3.44	3.34	3.22	3.21	3.21
Fibre	6.54	6.76	6.88	7.24	6.56	8.27	8.84	8.82	8.81

[†] Based on EERQ and an EEC of 10.96 kJ/g.

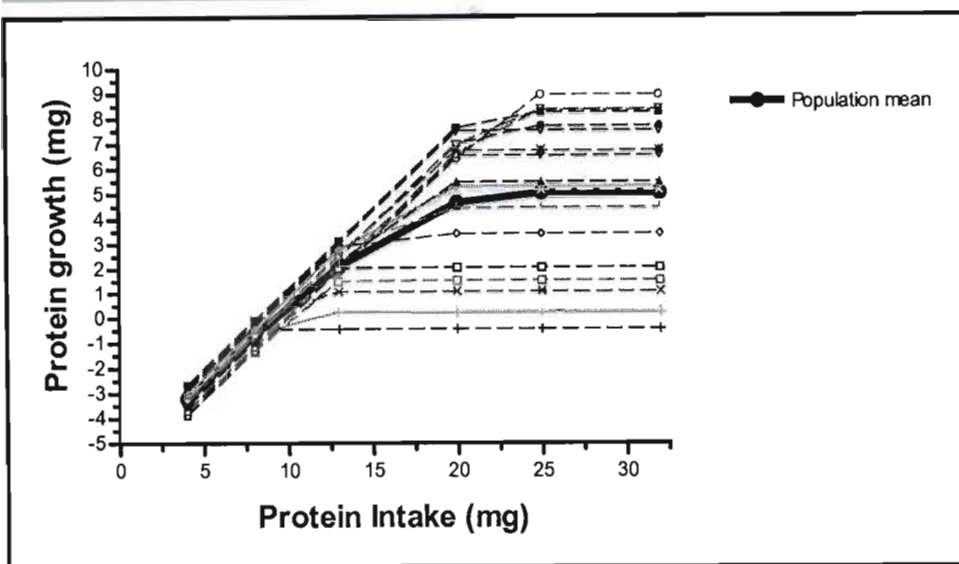


Figure App G 1: The mean (---) and population (—) responses of 2 g *Fenneropenaeus indicus* to protein intake.

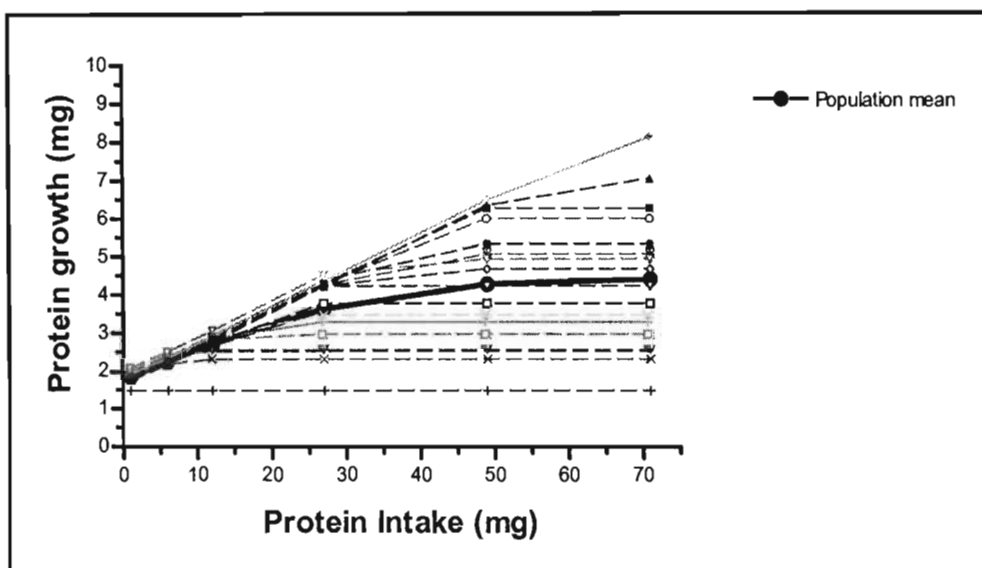


Figure App G 2: The mean (---) and population (—) responses of 7 g *Fenneropenaeus indicus* to protein intake.

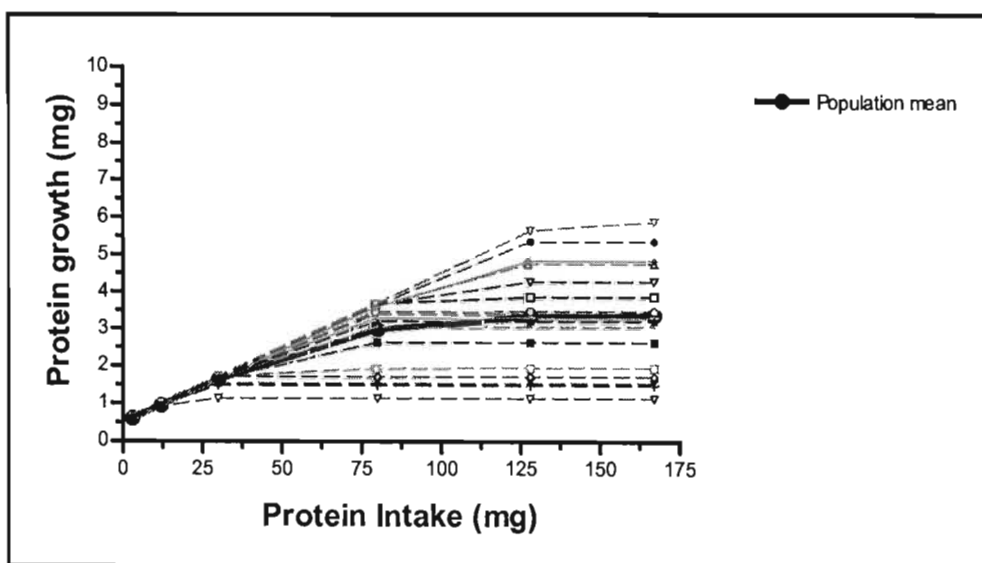


Figure App G 3: The mean (---) and population (—) responses of 14 g *Fenneropenaeus indicus* to protein intake.

Appendix H

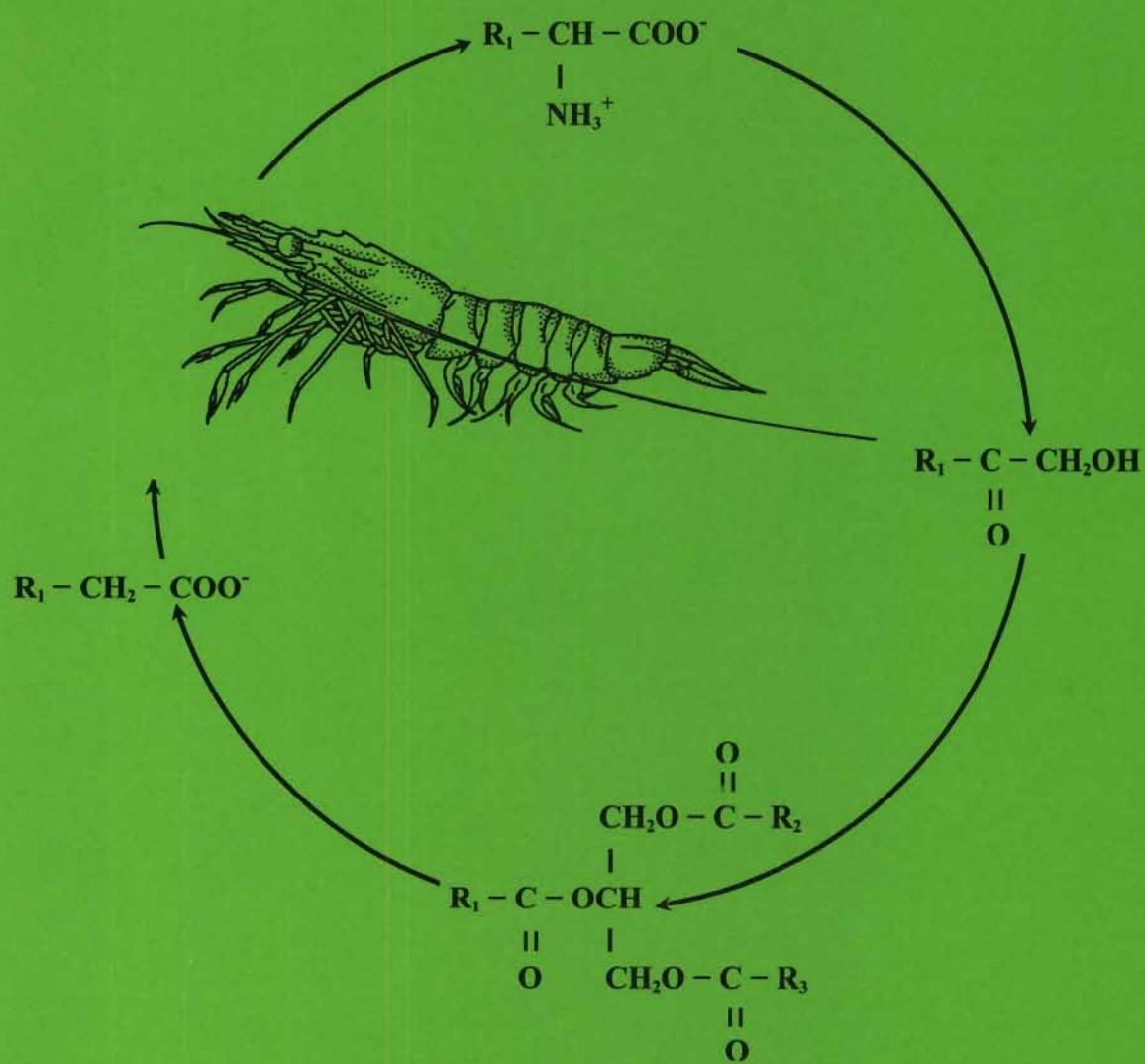


Table App H: The amino acids and nutrient composition in feedstuff used for substitution trials.

Amino Acids	Soya meal	Wheat gluten	Brewers yeast	Wheat flour	Fish meal	Casein
Lysine	3.165	1.076	2.981	0.221	4.604	7.846
Arginine	4.504	3.710	2.248	0.348	3.303	3.835
Methionine	0.528	1.057	0.477	0.111	1.791	3.088
Threonine	1.886	1.746	1.752	0.251	2.510	4.310
Isoleucine	2.485	3.129	2.002	0.367	3.153	6.291
Leucine	4.076	5.816	2.841	0.654	4.774	9.670
Histidine	1.164	1.389	0.843	0.228	1.330	2.846
Phenylalanine	2.035	3.396	1.369	0.410	2.100	5.282
Valine	2.848	3.780	2.633	0.470	4.061	7.373
Proximate composition of the feed ingredients.						
Moisture	10.000	8.400	12.100	12.100	8.300	8.891
Crude Protein	49.520	80.920	41.390	12.320	66.570	92.311
Lipid	2.640	1.530	0.390	1.400	9.430	0.659
Ash	6.860	0.860	6.490	0.700	15.880	2.417
Crude Fibre	4.320	0.410	0.640	0.670	-	0.217
GE (MJ/kg)	17.766	21.396	16.925	15.933	19.023	21.69

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