Muresk Institute of Agriculture

Nutrition of marron, *Cherax tenuimanus* (Smith) under different culture environments – a comparative study.

Ravi Fotedar

"This thesis is presented as part of the requirements for

the award of the Degree of Doctor of Philosophy

of the

Curtin University of Technology"

October 1998

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ABSTRACT

Feeding trials were conducted to compare the nutritional requirements of juvenile marron (*Cherax tenuimanus*) under four different cultural environments. The rearing environments included aquaria under controlled laboratory conditions, cages in purpose-built commercial ponds, purpose-built earthen ponds and battery culture called the intensive crayfish culture system (ICCS).

High levels of dietary cod liver oil in the semi-purified diet depressed the specific growth rates of juvenile marron under laboratory conditions. The dietary lipid levels were reflected in the lipid levels of the hepatopancreas of the marron. Dietary fatty acid profiles were also reflected in the fatty acid profiles of the hepatopancreas and subsequently in the tail muscles. Under cage environment in a purpose-built pond, dietary supplementation with cod liver oil (4%) plus sunflower oil (4%) increased the total biomass of juvenile marron. The specific growth rate under these condition had a strong positive correlation with all condition indices measured in the study (dry and wet hepatosomatic indices, dry and wet tail muscles to body weight ratio and moisture content of the hepatopancreas and tail muscles). The lack of protein in formulated practical diets, under commercial farm conditions, did not significantly influence the specific growth of juvenile marron but a diet containing fish protein source and fish oil resulted in a significantly higher survival. Feeding a diet containing fish protein and fish oil resulted in significantly higher wet tail muscles to body weight ratio than was observed with other diets. The inclusion of plant protein in formulated diets had an impact on the pond environment due to significantly higher ammonia levels that resulted in lower survival. Juvenile marron fed with a protein free diet and those fed with a plant protein diet had significantly lower protein levels in their hepatopancreas compared to those fed with diets containing animal protein. Juvenile marron in the battery culture environment tolerated long periods of starvation, utilising energy sources from the hepatopancreas and then from the tail muscles.

Results from one rearing environment could not be directly transferred to another rearing environment. Environmental variables, particularly temperature, nitrogen metabolites and natural productivity of the ecosystem greatly influenced the nutritional requirements of the juvenile marron under culture. Ambient temperature and nitrogen metabolites may exert stronger control on the productivity, and thus profitability, of the commercial marron farms than the inclusion of micronutrients in the formulated diet.

Specific growth rates, biomass, and wet and dry tail muscles to body weight ratios can be used as indicators of nutritional effectiveness of the diet. Further research is required to identify the interaction between the natural productivity of the ecosystem and the need to incorporate essential nutrients in formulated diets for the optimization of the marron production under commercial environment.

To my grandparents

(now deceased)

STATEMENT OF SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from published or unpublished sources has been acknowledged in the text and a list of references is given.

Ravi Fotedar **Date: 14th October 1998**

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ACKNOWLEDGMENTS

There are many people who have directly or indirectly contributed in my endeavour to change my title from Mr to Dr. As this list is quite exhaustive, only a few have been included in this document. I would sincerely like to thank my PhD supervisor Dr Brenton Knott whose continued guidance and support made this thesis happen. His patience and stimulating thoughts are highly acknowledged. I am also grateful to his wife, Kerry (Grope), who did not mind me barging into her house on many occasions and letting me steal her husband.

I wish to thank my main supervisor Assoc. Prof. Louis Evans for the support and guidance she has provided for the last four years. Her ideas and time devoted to this thesis are highly appreciated. Without her help this project would have been traumatic and impossible. I owe my thanks to Prof. Bruce Phillips who, though coming late into the scene, made an important contribution in editing the final version of the thesis. I highly appreciate the help and encouragement provided by Prof. Murray McGregor (the big Boss) during my studentship at Curtin University of Technology. His constant nagging forced me to finish this milestone of my life in time (even though it is late by his standards!).

My thanks go to the Department of Fisheries Western Australia, Marron Growers Association of Western Australia and Eagle Bay Pty Ltd. for their initial contribution towards the Australian Postgraduate Research Award (APRA) scholarship, which partly supported me and my family during my initial years of struggle in Western Australia.

Thanks to John Lang, Hari Cassin and Grahm Dunjey for allowing me to use their facilities for the research. Their support is greatly acknowledged. My thanks also go to some of my favourite farmers turned into friends: Tom Swanell, Frank Major, Hari Cassin, Greg Johnson, Dan Sampi, Roger and Kerri Morgan. I can not ignore your contribution to this research.

I sincerely acknowledge help provided by: Mike Hoxey in preparing test diets and valuable discussions we had while formulating the diets; and Warren Potts timely help in analysis fatty acid profile of cod liver oil and sunflower oil. My thanks also go to Paul Lloyd for supplying water test equipment (even if a bit late, sometimes). My thanks to my colleagues Dr Elena Tsventeko and Yuri Tsventeko for helping me in many ways during my stay here at Aquatic Science. I appreciate greatly the help and guidance provided by Elena in untangling the mysteries of biochemistry. The discussions with Glen Whisson in finalizing the experimental designs have contributed in this research and his valuable comments are appreciated. My thanks to other colleagues Anne, Robyn and Sandra and of course to Agung (Ha! Ha!) for providing an inspiring atmosphere in the office enabling me to concentrate on my research (after regular working hours).

I am thankful to all my students who kept me busy and helped in delaying the submission of my thesis (earlier submission would have been completely premature). I am especially thankful to Jon Arnold, Adrian Hall, David Bloch, Michael Burgess, Shirley (from TAFE), Farah and Theo (from Singapore), Peter Julitz and Rohan Wilson (Scotch College) for helping me from time to time and feeding these creatures called marron when I was busy elsewhere. My thanks also go to Ms. Jane Fewtrell, Ken Gordon, Vicki Lauri, Ned and Adrian Stott, who kept the hatchery and laboratory tidy, so that I could peacefully concentrate on my research. Thanks to Sarah Phipps for data entry and playing with my computer. You did a marvellous job in proof reading and giving final shape to this thesis.

Thanks to Ronlee for keeping me company over a bottle of scotch and lovely lunches during some moments of stress. I also owe appreciation to my Indian friends, Singh Sahib, Pawan, Sam, Anshu and their families, for not insisting that I should reciprocate the parties, which I thoroughly enjoyed with them.

I thank some of the "die-hard supporters" and " ultimate encouragers" of my PhD thesis, including my Masi Sheila and my Uncle Tony Ganju. Masi, I miss your cooking. Other supporters of my thesis are Surender Ji and Mary. Thank you both.

A big thank you to my parents and other relatives in India for not knowing anything about my thesis. Never mind, it is because of you I do exist. I am thankful to my two lovely kids, Aadil and Akash for their understanding and letting me spend my time in writing this thesis and not being very demanding. Now, guys this is the time, pack-up your camping gear and off we go. We have to do lot of catching up. Oops! I almost forgot you Champ. Do join us and I will buy you a new bone for not chewing up my thesis. Thanks to Seema for taking good care of kids while I was busy writing my thesis and occasionally turning up the TV volume which helped me to concentrate on writing my thesis.

*How could I have **fin**SSSS ished this milestone of my life, without your help Moi Japo (my adopted twin brother). Our endless and painful discussions over 1140 mL beer jugs kept my neurons ticking. Moi Japo, we absolutely, maybe, have to write a joint paper in future. Thanks to Tujia, Aku and Ole, Lennu and other friends from Finland where I discovered the missing links in my life. Kippis!*

I am also thankful to the beauty and weirdness of this great country of ours, who inspired me to continue my PhD despite some despairing moments in my life. Last but not least, I want to thank all those people who wanted to help me during my PhD era, but could not because of (I don't know why!). It is the thought that matters and actions are meant for me. I would also like to thank those people who believed or tried to believe that PhD is not my cup of tea. This belief, believe me, kept my belief on.
Kashmiri Aussie

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LIST OF ABBREVIATIONS

0^+	Marron less than one year old
B/m^2	Biomass per unit area in m^2
d	Number of days
DHA	Docosahexaenoic acid
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
fa	Fatty acid
FOC	Free organic carbon
H_{id}	Dry hepatosomatic index (%)
H_{iw}	Wet hepatosomatic index (%)
HM%	Moisture content of hepatopancreas
HUFA	Highly unsaturated fatty acids
LSD	Least square deviation
NFE	Nitrogen free extraction (%)
n_o	Number of marron at the beginning of the trial
n_t	Number of marron at the time t
PUFA	Polyunsaturated fatty acids
r^2	Correlation coefficient
SE	Standard error
SGR	Standard growth rate
T/B_d	Dry tail muscles to wet body weight ratio (%)
T/B_w	Wet tail muscles to wet body weight ratio (%)
TDS	Total dissolved solids
TM%	Moisture content of tail muscles
UV	Ultra violet
W_o	Initial weight
W_t	Weight at the time t

LIST OF SCIENTIFIC AND COMMON NAMES OF FRESHWATER CRAYFISH ONLY USED IN THE THESIS

SCIENTIFIC NAME	COMMON NAME
Astacopsis goudii	Tasmanian freshwater crayfish
Astacus astacus	Noble crayfish
Austropotamobius pallipes	White-clawed crayfish
Cherax albidus	Yabby
Cherax destructor	Yabby
Cherax quadricarinatus	Red claw crayfish
Cherax tenuimanus	Marron
Orconectes limosus	Spiny cheek crayfish
Orconectes rusticus	Rusty crayfish
Orconectes virilis	Virile crayfish
Pacifastacus leniusculus	Signal crayfish
Procambarus acutus acutus	White river crayfish
Procambarus clarkii	Red swamp crayfish

LIST OF PUBLICATIONS *(from the thesis)*

1 Fotedar, R., Evans, L. H. and Knott, B. 1997. The effect of dietary lipid level on the growth and survival of juvenile marron, *Cherax tenuimanus* (Smith). *Freshwater Crayfish* **11**: 417-427.

2 Fotedar, R., Evans, L. H. and Knott, B. 1998 The effect of stocking density on growth and survival of 3-month old juvenile marron, *Cherax tenuimanus* reared in a semi-controlled environment. *Journal of Applied Aquaculture* **8.** (*in press*).

3 The effect of fish and plant oil supplementation growth, Biomass and condition indices of juvenile marron, *Cherax tenuimanus* Smith (1912) cultured in cages. *Freshwater Crayfish 12 (Abstract submitted).*

4 The effect of cod liver oil supplementation on the growth, condition indices and biochemical composition of juvenile marron (Smith). *Journal of World Aquaculture Society (Abstract submitted).*

5 The effect of protein- free, plant and fish protein formulated diets on growth, biomass and condition indices of marron, *Cherax tenuimanus* (Smith 1912) cultured under semi-intensive farming conditions. *Under preparation.*

6 The effect of different periods of starvation and delayed feeding on growth, survival, condition indices and biochemical composition of juvenile marron, *Cherax tenuimanus* (Smith 1912). *Under preparation*

CHAPTER 1

1.0 INTRODUCTION

1.1 PREAMBLE

This thesis is based on an investigation into the impact of different culture environments on the nutritional status of marron (*Cherax tenuimanus*) (Smith 1912), a freshwater crayfish endemic to southwestern Australia (Riek, 1967). It aimed to investigate marron nutrition through studying the role and importance of various nutrients (protein, lipid and fatty acids) and their sources in formulated diets under three different culture systems. Since little information is available specifically on nutrition of freshwater crayfish, except for the reviews of Goddard (1988) and D'Abramo and Robinson (1989), relevant information has been compiled from literature on marine penaeid prawns, lobsters and freshwater prawns. New (1980) compiled a bibliography of prawn nutrition and related research and which serves as a launching pad for crustacean nutritionists. The nutritional status of aquatic species have been assessed in terms of specific growth rates (SGR) in freshwater crayfish (Evans and Jussila, 1997), condition indices in marine fish larvae (Shelbourne, 1957) and biochemical composition of the body tissues (New, 1976; D'Abramo *et al.*, 1980) in decapod crustaceans.

Freshwater crayfish enjoy high consumer acceptability in international markets and consequently their culture has attracted an increasing degree of attention over the last decade (Spittle, 1993; Laud, 1994)). The successful culture of any aquatic species depends on the information acquired in four important areas, namely, biology, culture environment (Ouellet *et al.*, 1995), nutritional requirements and genetic improvement of stock. The nutritional requirements of species are highly dependent on the culture environment and cannot be understood without defining the culture system.

1.2 AQUACULTURE

Most publications on aquaculture refer to the long history of fish farming in Asia, ancient Egypt and in central Europe (Ling, 1977). The Food and Agricultural Organisation (FAO) of the United Nations has defined aquaculture as "the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants Farming implies some form of intervention of the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated" (FAO, 1976). The total world capture fishery has reached a plateau of around 70 to 75 million tonnes. It is estimated that about 100 to 140 million tonnes of edible fish products will be required to meet the demand of the projected world population by the year 2000. There is thus a deficit of approximately 20 to 60 million tons to be made up, and the only major means presently known for this is an accelerated development of aquaculture (Pillay, 1993).

1.2.1 Crayfish Aquaculture

Commercial aquaculture of freshwater crayfish is practiced in North America (Eversole and Pomeroy, 1989; Huner, 1989), Europe (Ackefors, 1993) and Australia (Morrissy, 1992a and b). In North America, red swamp crayfish (*Procambarus clarkii*) accounts for 70-80% of all crayfish harvested from natural fisheries and aquaculture (Huner, 1989). In Europe four species of crayfish are cultured, two indigenous [noble crayfish (*Astacus astacus*) and Turkish crayfish (*Astacus leptodactylus*)] and two introduced [American red swamp crayfish (*P. clarkii*) and signal crayfish (*Pacifastacus leniusculus*)] (Ackefors, 1993).

Commercial aquaculture of crayfish in Australia began in the 1970's (O'Sullivan, 1995) and currently is based on the culture of three species, namely, marron (*C. tenuimanus*) (Smith 1912), in West and South Australia (Evans and Fotedar, 1996; Fotedar *et al.*, 1996a and b), yabby (*Cherax destructor*) Clark 1936 in

Western Australia and eastern states of Australia (Mills and McCloud, 1983) and redclaw (*Cherax quadricarinatus*) (von Martens 1868) in Queensland and Northern Territory (Jones, 1990; Curtis and Jones, 1995a; Jones, 1995b and c). Fast growth, adaptation to different culture systems, low feed costs, high meat yield and ready domestic and overseas markets provide these freshwater crayfish species with the biological, physical and market characteristics necessary for a profitable aquaculture venture (Shipway, 1951; Riek, 1967; Morrissy, 1976). For many years, prior to the development of an aquaculture industry, marron and yabbies were harvested from the wild through recreational fishing.

Table 1.1 **Australian production of crustaceans 1995-96 (Source: O'Sullivan and Kiley, 1996).**

Species	Scientific names	Value ($,000)	Production (tonnes)
Black Tiger prawns	*Penaeus monodon*	18,258.3	1,376.3
Kuruma prawns	*Penaeus japonicus*	15,372.9	228.5
Mud crabs	*Seyla serrata*	2.5	0.3
Brine shrimp	*Artemia sp.*	1,000	3.9
Yabbies	*Cherax destructor*	1,914.4	172.7
Redclaw	*Cherax quadricarinatus*	764.0	55.6
Marron	*Cherax tenuimanus*	713.8	25.5
Freshwater prawn	*Macrobrachium rosenbergii*	1.4	0.1
TOTAL		**38,027.5**	**1,862.9**

As a result of increasing commercial grow-out, particularly in the 1990s, total crayfish production in Australia increased from 50 tonnes in 1988-89 to 172 tonnes in 1991-92. Production further increased to 361 tonnes in 1994-95 but decreased to 253.8 tonnes in 1995-96 (Table 1.1). With demand for crayfish worldwide exceeding supply, there is a keen interest in developing appropriate intensive culture technology for crayfish species. One prerequisite for developing appropriate technology is the availability of nutritional knowledge of the targeted crayfish species.

1.3 NUTRITIONAL REQUIREMENTS

Though research has been directed at nutritional biology in Australian crayfish species (e.g. Morrissy, 1979, 1984; Mills and McCloud, 1983; Geddes *et al.*, 1988, Villarreal, 1988; Jones, 1990, 1995a; Sommer *et al.*, 1991; O'Brien, 1994a and b; Jones *et al.*, 1997), little information is available on the influence of nutrients on freshwater crayfish aquaculture production. The majority of feeding and nutritional information available on freshwater crayfish is based on the American red swamp crayfish (Huner *et al.*, 1975; Huner and Meyers, 1979; Denson and Eversole, 1997). No research has compared the nutritional status of marron fed formulated diets under different cultural environments.

Nutrients are required for the maintenance of life, growth, anabolic synthetic processes (anabolism), reproduction, and other normal physiological functions. Nutrients may be classified as energy and growth nutrients (carbohydrates, fats and proteins) and supporting non-energy nutrients (minerals, vitamins, water, and oxygen). The nutritional value of a diet, different for each species under culture, depends upon the balance between non-energy and energy nutrients, an abundant supply of water and oxygen and the presence of essential nutrients.

1.3.1 Protein and Amino Acids

1.3.1.1 Requirements

Proteins are essential in the diet of crayfish as a source of amino acids, and are the most expensive component (due to its greater bulk) of formulated feeds. Hence, determination of the optimum gross protein level for maximum growth of any crayfish species is of economic significance.

Decapod crustaceans have a wide range of optimal dietary protein requirements in early juvenile stages of development. In a review paper, New (1976), for example, has evaluated the influence of dietary protein on the performance of various

penaeid and palaemonid species. The white shrimp, *Penaeus setiferus*, requires 28 to 32% dietary protein (Andrews *et al.*, 1972) whereas the Japanese kuruma shrimp (*P. japonicus*) requires an optimal dietary protein of 52 to 57% (Deshimaru and Yone, 1978a). Sedgwick (1979) estimated that the optimal dietary protein concentration for the banana prawn (*Penaeus merguiensis*) ranged from 34 to 42% depending upon available energy in the diet.

The dietary protein requirement of the red swamp crayfish ranges between 20 and 30% (Huner and Meyers, 1979), with optimal total animal protein in the range of 15 and 20% of the total diet. Diets with total protein content ranging from 31.7 to 50.5% were fed to white river crayfish (*Procambarus acutus acutus*) (Tarshis, 1978). In these studies, weight gains were highest on those crayfish fed diets containing 50.5% protein. In *A. astacus*, improved condition was noted in crayfish fed a 40% protein diet in comparison to those fed a 30% protein diet (Huner and Lindqvist, 1984). Millikin *et al.* (1980) found a dietary protein requirement of 40% for maximum rates of growth among *M. rosenbergii* juveniles. Hubbard *et al.* (1986) found that a protein level of 30% and an energy level of 0.598 kJ/g (protein to energy ratio 1:120) gave optimum growth and protein deposition in crayfish. Tsvetnenko *et al.* (1995) reported higher growth rates in marron under battery culture system fed 20% or 30% protein diets. Yabby (*C. destructor*) growth was improved when dietary protein level was 30%, a level of 15% resulting in poorer growth and nutritional stress (Jones *et al.*, 1997). However, the exact requirement of protein undoubtedly varies with alterations of the amino acid profile (and thus source of protein), variations in the natural productivity of the water, variation in the dietary supplementation of macro- (required in larger amounts) and micro- (required in trace amounts) nutrients other than protein, and changes in environmental factors. High protein levels in formulated diets may be wasteful, if an excess of protein is utilized for energy and not for growth and other important maintenance functions, for example, the repair of damaged body tissues.

The amino acid profile of a crayfish diet can be predicted from the profile of amino acids from crustacean muscle tissue or from a whole body carcass analysis (Cowey and Tacon, 1983; Wilson and Poe, 1985). However, there is no information on the quantitative amino acid requirement of crayfish that can be applied to diet formulation. Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine are essential in the diets of other animal groups (Claybrook, 1983). ^{14}C studies have confirmed the above requirements, though some possible variations have been reported. The status of arginine as an essential amino acid is in doubt, following studies on *A. astacus* (Zandee, 1966a), whilst asparagine was included as an additional essential amino acid, following studies on *A. leptodactylus* (Willibrordus *et al.*, 1975).

1.3.1.2 Dietary source

There are many protein sources of both plant and animal origin which have been used in the formulation of crayfish diets, including, blends of shrimp meals, fish meals and soybean meals (Meyers *et al.*, 1970; Huner *et al.*, 1975; Tarshis, 1978; Huner and Meyers, 1979; D'Abramo *et al.*, 1985). Reduction in the animal protein content of the diet from 44 to 15% of the total diet had no significant difference on the growth to maturity of male crawfish (Huner and Meyers, 1979). Huner and Meyers (1979) indicated that, for optimal growth of *P. clarkii* in closed systems, more than 50% of the protein should be derived from an animal source. The optimum protein requirements and their sources for selected decapod crustaceans are summarized in Table 1.2.

Balazas and Ross (1976) evaluated selected protein sources and levels on growth, survival and feed conversion of juvenile prawn, *M. rosenbergii*, whereas Clifford and Brick (1978, 1979) measured protein utilization and the physiological response of juvenile prawns fed formulated diets containing different protein concentrations and lipid - to - carbohydrate ratios. They demonstrated that 15-25% dietary protein

Table 1.2 Protein source and optimum protein levels in selected decapod crustaceans.

Species	Protein source	Optimal protein level (%)	Reference
Freshwater crayfish			
Procambarus clarkii		Gross protein: 20-30%; Animal protein: 15-30%	Huner and Meyers (1979)
Cherax tenuimanus	-	30-35	O'Sullivan and Watson (1991)
Marine prawns			
Metapenaeus macleayi	-	27	Macguire and Hume (1982)
M. monoceros	Casein	55	Kanazawa *et al.* (1981)
Penaeus acutusi	-	Up to 50%	Tarshis (1978)
P. aztecus	Fish meal and mixture	< 40	Venkataramiah *et al.* (1975)
P. aztecus	Fish and squid meal	29-31	Shewbart and Miles (1973)
P. brasileinsis	Shrimp meal and casein	45 and 55	Liao *et al.* (1986)
P. californiensis	Mixture	> 44	Colvin and Brand (1977)
P. duorarum	Soybean meal	30	Sick and Andrews (1973)
P. indicus	Shrimp meal and yeast	43	Colvin (1976a)
P. japonicus	Casein and albumin	54	Deshimaru and Kuroki (1974)
P. japonicus	Crab protein	60	Deshimaru and Shigheno (1972)
P. japonicus	Squid meal	-	
P. japonicus	Shrimp meal	> 40	Balazas *et al.* (1973)
P. japonicus	Casein and albumin	> 55	Teshima and Kanazawa (1983)
P. japonicus (zoea)	Casein and albumin	45-55	Teshima and Kanazawa (1984)
P. kerathurus	Mixture	> 40	Fernandez and Puchal (1979)
P. merguiensis	Mussel meal	34-42	Sedgwick (1979)
P. merguiensis	Mixture	50	Aquacop (1978)
P. monodon	–	40	Khannapa (1977)
P. monodon	Casein	40	Aquacop (1978)
P. monodon	Mixture	35	Bages and Sloane (1981)
P. monodon	Mixture	40	Alava and Lim (1983)
P. monodon	Mixture	40-44	Shiau *et al.*(1991)
P. monodon	Casein and fish meal	46	Lee (1971)
P. penicillatus	Fish meal	22-27	Liao *et al.*(1986)
P. setiferus	Fish meal, collagen and squid meal	28-32	Andrews and Sick (1972)
P. stylirostris	Mixture	> 10, 30-35	Colvin and Brand (1977)
P. vannamei	Mixture	30	Cousin *et al.* (1993)
P. vannamei	Mixture	> 30	Colvin and Brand (1977)

level has a strong influence on the magnitude of oxygen uptake and nitrogen production. Measurement of nitrogen excretion and corresponding metabolic

substrate ratios indicated that the protein-sparing effect of non-protein energy sources is maximized at a lipid:carbohydrate ratio of 1:4.

Protein digestibility has been examined in *P. clarkii*, using chromic oxide as an inert reference marker (Nose, 1964). Digestibility figures ranged from 91 to 99% and differences of water temperature in the presence of dextrin (Nose, 1964) in the diet exerted no significance difference on protein digestibility.

1.3.2. Carbohydrates

1.3.2.1. Requirements

Carbohydrate is the most economical dietary energy source in terms of cost / kcal. However, information on the requirements and utilization of carbohydrates by crustaceans is very limited (New, 1976, 1980; Kanazawa, 1984) and carbohydrate requirements of freshwater crayfish are unknown. The type and levels of carbohydrate have been shown to affect the growth of *P. japonicus* (Deshimaru and Yone, 1978b; Abdel-Rahman *et al.*, 1979), *P. aztecus* (Andrews, *et al.*, 1972) and *Penaeus duorarum* (Sick and Andrews, 1973). Wilson (1994) has reviewed comprehensively the utilization of dietary carbohydrate by fish. In general, freshwater and warm water species of fish appear to digest carbohydrates more effectively than marine and cold water fish. D'Abramo and Robinson (1989) reported that crayfish readily utilized diets with high levels of carbohydrates.

Penaeid shrimp species are able to utilize complex sugars more effectively than simple sugars such as glucose (Shiau, 1997); for example, starch and other complex sugars have been used successfully as alternative sources of energy in place of proteins (Hubbard *et al.*, 1986). In contrast, the addition of glucose to the diets of *P. setiferus* resulted in depressed growth whereas supplemental starch did not cause any reduction in growth (Andrews *et al.*, 1972). Growth and survival of *P. duorarum* fed diets containing 40% starch were higher than those fed diets

containing 10% of either glycogen, starch, dextrin, glucose or sucrose (Sick and Andrews, 1973). Deshimaru and Yone (1978b) concluded that feed efficiency of *P. japonicus* was highest for those fed the starch diet, followed by glycogen, sucrose, and dextrin in decreasing order. Growth was also poor in *P. japonicus* fed monosaccharides, glucose, and galactose (Abdel-Rahman *et al.*, 1979). High hepatopancreatic glycogen concentrations were characteristic of shrimp fed diets containing glucose or galactose. Pascual *et al.* (1983) found no correlation between survival and the relative complexity of carbohydrates. They could not draw a confident conclusion because a treatment group fed a glucose containing diet was not included for comparison. Cornstarch is better utilized than glucose by *P. monodon* (Shiau and Peng, 1992). They, however, showed that starch has a better protein-sparing effect than dextrin or glucose. Accordingly, the required dietary protein level for *P. monodon* is lower if starch, instead of glucose or dextrin, is used as the carbohydrate source. A possible explanation for the poor growth performance of shrimps fed glucose containing diets is the inhibition of amino acid absorption in the intestine due to the presence of glucose (Alvarado and Robinson, 1979). An inclusion level of 0.5% of dry weight of chitin in the shrimp feeds has a growth promoting effect as it forms a major structural component of the exoskeleton of shrimp (Akiyama *et al.*, 1992).

1.3.3. Lipids and Fatty Acids

Lipids are a large, varied group of organic compounds (Goldrick, 1971). They function as concentrated energy sources, vitamins, pigments, and essential growth factors for aquatic animals. Lipids are the most energy rich of the nutrient classes, providing approximately 36 kJ/g. compared with 15 kJ/g. for carbohydrates and 19 kJ/g for proteins (De Silva and Anderson, 1995; p 26).

The straight chain may be saturated (containing no double bonds) or unsaturated (containing one or more double bonds). The general formula for saturated fatty acids is $C_nH_{2n+1}COOH$ and the shorthand nomenclature is presented in Table 1.3.

Table 1.3 **Shorthand nomenclature for saturated fatty acids.**

Number of carbons	Common name	Formula
2	Acetic	CH_3COOH
12	Lauric	$C_{11}H_{23}COOH$
14	Myristic	$C_{13}H_{27}COOH$
16	Palmitic	$C_{15}H_{31}COOH$
18	Stearic	$C_{17}H_{35}COOH$
20	Arachidic	$C_{19}H_{39}COOH$

The formula for unsaturated fatty acids is $C_nH_{2n-1}COOH$ and the shorthand nomenclature is presented in Table 1.4. In general, lipids from animal sources contain a higher percentage of saturated fatty acids than lipids from plants, with some notable exceptions.

Table 1.4 **Shorthand nomenclature for unsaturated fatty acids.**

Number of carbon atoms	Number of double bonds	Position of first double bond (series)	Common names	Formula
16	1	ω -9	palmitoleic	$C_{15}H_{29}COOH$
18	1	ω -9	oleic	$C_{17}H_{33}COOH$
18	2	ω -6	linoleic	$C_{17}H_{31}COOH$
20	4	ω -6	arachidonic	$C_{19}H_{31}COOH$
18	3	ω -3	linolenic	$C_{17}H_{29}COOH$
20	5	ω -3	eicosapentaenoic acid	$C_{19}H_{29}COOH$
22	6	ω -3	docosahexaenoic acid	$C_{21}H_{31}COOH$

1.3.3.1. Requirements

Energy metabolism in decapod crustaceans is primarily based on lipids, (Renaud, 1949). The optimum requirements of lipids and sources for some commercially important decapod crustaceans are summarised in Table 1.5.

Table 1.5 **Optimum lipid requirements and lipid sources of the decapod crustaceans listed.**

Species	Lipid sources	Lipid level (%)	Results	Reference
Freshwater crayfish				
Procambarus acutus	Menhaden fish oil	0, 3, 6, 9, 12, 15	0-6% no difference; growth reduction at 9% or more of lipid levels	Davis and Robinson (1986)
Pacifastacus leniusculus	ns	ns	Minimum dietary sterol for survival 0.4% of dry weight. Minimum dietary sterol for optimum growth : 0.5% to 1% of dry weight	D'Abramo *et al.* (1985)
Astacus astacus			Linoleic series fatty acid	Goddard (1988)
Marine lobster				
Homarus americanus	Cod liver oil	1, 5, 10, 15	Optimum weight gain with 5% cod liver oil	Castell and Covey (1976)
Freshwater Prawns				
Macrobrachium rosenbergii	Cod liver oil, corn oil (2:1)	0, 2, 4, 6, 8, 10, 12	Optimum weight gain at 6%	Sheen and D'Abram (1991)
Marine prawns				
Penaeus japonicus	pollack liver oil and soybean oil; 1:1	3, 6, 9, 12	Optimum growth at 6% pollack liver oil: soybean oil (3:1 or 1:1)	Deshimaru *et. al.* (1979)
P. japonicus	Soybean oil, pollack residual oil, short-necked clam oil	8, 12, 16	Optimum growth at 8% short-necked clam oil; growth inhibition at 16%	Kanazawa *et.al.* (1977)

ns – not stated

The nutritional requirements of many commercially important species of crayfish have yet to be assessed; most work conducted primarily concerns sterol requirements (Kanazawa and Teshima, 1971). D'Abramo *et al.* (1985) and Goddard (1988) provided considerable evidence for a low requirement of dietary lipid levels by crustaceans, with indications of growth inhibition when the levels exceed 8% in the diet (Andrews *et al.*, 1972; Forster and Beard; 1973, Davis and Robinson, 1986) with an optimal level ranging from 5 to 8% of the diet (Kanazawa *et al.*, 1977; Davis and Robinson, 1986). Fatty acid lipogenesis in crustaceans,

involves the synthesis of saturated fatty acids from acetate and the conversion of saturated precursors to mono unsaturated products of the palmitoleic (ω-7) and oleic (ω-9) acid series (Castell, 1983). Studies by Colvin (1976b); Kanazawa *et al.* (1979a and b); Bottino *et al.* (1980) and Read (1981) have shown that crustaceans have little or no ability to biosynthesize ω-3 and ω-6, highly unsaturated fatty acids (HUFA) from ω-3 and ω-6, polyunsaturated fatty acids (PUFA) respectively.

Burr and Burr (1930) discovered that animals have an essential dietary requirement for specific types of fatty acids called essential fatty acids (EFA). PUFAs of the linolenic (ω-3) and linoleic (ω-6) families have been recognised as essential nutrients for the growth of crustaceans: *A. astacus*, for example, has an essential requirement of the linoleic series (Zandee, 1966b). In many crustaceans there is a dietary requirement for fatty acids of both the linoleic and linolenic series (Castell and Covey, 1976; Kanazawa *et al.*, 1979a, b and c). In practical ration formulations, therefore, emphasis has been on maintaining a correct dietary ratio and balance between the ω-3 type EFAs (generally found in marine fish oil) and other fatty acids, notably those of the ω-6 type (most abundant in terrestrial plant oils). Depending on the rate at which they are able to desaturate and elongate fatty acids, some marine crustaceans might require eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) to satisfy their EFA requirements. Similarly, cold water crustaceans might be expected, based on differences in fatty acid patterns, to require more linolenic series fatty acids than warm water species.

Experimental diets are generally supplemented with marine or plant oils and with lecithin (Tarshis, 1978; Huner and Lindqvist, 1984; D'Abramo *et al.*, 1985). Lecithin serves as a source of phospholipid, whilst the oils are a source of polyunsaturated fatty acids. Studies on the nutrition of fish and shellfish have shown a general requirement for long chain polyunsaturated fatty acids. These fatty acids have important metabolic significance, being essential for growth,

maintenance and the proper functioning of many physiological processes (Alfin-Slater and Aftergood, 1968).

Lee *et al.* (1967), Castell *et al.* (1972a and b), and Owen *et al.* (1972) have emphasised the role played by the fatty acids of the ω-3 variety in alleviating EFA deficiency symptoms in fish and prawns (Sick and Andrews, 1973). Castell and Covey (1976) used cod liver oil (high in 20:5ω-3 and 22:6ω-3) as a dietary lipid source for adult lobster, *H. americanus* and found 5% lipid level to be optimal for maximum growth. They also reported that cod liver oil produced superior growth and survival of *H. americanus*, compared to lobsters fed corn oil (high in 18:2ω-6). Guary *et al.* (1976), Colvin (1976b), Sandifer and Joseph (1976) and Kanazawa *et al.* (1977) showed that feeding oils rich in either linoleate or linolenate induces high levels of the respective acids in the body tissues.

The qualitative dietary polyunsaturated fatty acid requirements of crustaceans can be identified by a variety of methods: tissue analysis (Alfin-Slater and Aftergood, 1968), lack of biosynthesis, observation of the growth enhancing effects of oils rich in these fatty acids (Read, 1981), and weight gain responses to direct dietary addition of these fatty acids. For example, Alfin-Slater and Aftergood (1968), using penaeid prawns as a test animal, gauged the requirements of an animal fatty acid from the tissue composition. Inspection of penaeid prawn fatty acid patterns (Guary *et al.*, 1974; Gopakumar and Nair, 1975; Bottino *et al.*, 1980; Clarke and Wickins, 1980; Read, 1981) showed a preponderance of short and long chain ω-6 and ω-3 fatty acids characteristic of marine fish lipids (Ackman, 1967). The fatty acid profile varied among different tissues.

1.3.4. Dietary Sterols

Cholesterol is the major sterol in crustaceans and is a precursor of important steroids, nerve cell component, brain and moulting hormones. Crayfish and other arthropods are unable to synthesise sterols (Zandee, 1962, 1966b; Toumanoff, 1967) and require an exogenous source for survival and growth. Dietary sterol levels of 0.3% have been identified as essential for the survival of *P. leniusculus*, whilst levels of 0.4 to 1.0% (dry weight) are required for optimal growth (D'Abramo *et al.*, 1985). A mixture of phytosterols was as effective as an equal amount of cholesterol in the partial satisfaction of the requirement; however, phytosterols could not completely replace the cholesterol requirement (D'Abramo *et al.*, 1985).

1.3.5. Dietary Energy

The understanding of dietary energy values in terms of the actual metabolised energy available to the animal is crucial for diet formulation. Capuzzo (1983) reviewed the general methods of partitioning ingested food energy into various measurable energetic fates in crustaceans. In its simplest form, the energy budget of any growing animal is expressed as:

IE = FE + WE + HE + RE

where the intake of dietary energy (IE) is balanced by the sum of undigested energy lost to the animal through faeces (FE) plus catabolic wastes (WE) and remaining energy available for use by the animal. The available useable energy consists of a combination of total heat production (HE) resulting from both metabolic and behavioural activities, and the net energy gain, which is channelled into growth or recovered energy (RE). The above terminology has been suggested by the U.S. National Research Council (NRC, 1981). The energy and nutrients obtained from the aquatic environment and formulated diets are allocated among basic maintenance (important for survival), growth and reproduction. The amount of resources allocated to growth and reproduction result mainly from the surplus

resources that animals are capable of allocating to these metabolic pathways after the basic maintenance needs are fulfilled. Some characteristics unique to crustaceans appear at several levels of the partitioning (Cuzon and Guillaume, 1997):

- Digestibility and related faecal energy loss (FE) may differ among carnivorous, herbivorous, and scavenger species.
- Waste energy (WE) losses arising from metabolism, primarily through urine and gill excretion, are similar to those determined by calculating for ammoniotelic fish rather than those for higher vertebrates.
- HE includes energy losses associated with moulting (HmE) (Logan and Epifanio, 1978; Capuzzo, 1983).

1.3.6. Dietary Protein: Energy ratio

A thorough understanding of dietary protein to energy ratios for crustaceans is presently lacking. Cuzon and Guillaume (1997) suggest that future studies on the dietary protein to energy ratio could be improved by:

- Collection of additional data on digestible energy of usual dietary ingredients.
- Standardization of methodologies including determination of digestibility and the common use of species-specific reference diets providing all essential amino acids for optimum growth and survival.
- Replacement of protein by non-protein sources of energy such as carbohydrates, for example wheat starch.
- Determination of the optimum dietary protein to energy ratio; and
- Precise determination of feed consumption.

Both dietary protein and energy levels influence growth rates when animals are fed *ad libitum* and appetite regulates energy intake. Bautista (1986) and Shiau and Peng (1992) reported that a protein to energy ratio of 0.03 g/kJ is optimal for *P. monodon*, whereas Hajra *et al.* (1988), using isonitrogenous diets, estimated the

optimal dietary protein to energy ratio to be 0.028 g/kJ. This small difference may have been due to the fact that Bautista (1986) and Shiau and Peng (1992) failed to distinguish between digestible energy (DE) and metabolised energy (ME). Both reports concluded that carbohydrate spared the use of protein as an energy source, leading to a decrease in optimal dietary protein levels from 50 to 40% (Bautista, 1986) and 40 to 36% (Shiau and Peng, 1992). In most studies, the actual feed intake is not determined. Rather, the food conversion ratio (FCR) is based upon the amount of feed provided rather than the amount actually consumed. Starch effectively spares protein (Shiau and Peng, 1992) at protein to energy value of 27 and 28 mg/kJ (Alava and Lim, 1983; Alava and Pascual, 1987). Sedgwick (1979) reported that there is a relationship between feed intake and dietary levels of energy and protein in *P. merguiensis.* He evaluated the relationship between energy expenditure and feed intake, energy reserves, and partition of dietary energy in penaeid species. Collectively, these results suggest that an increase in dietary energy tends to increase performance when fed a diet low in protein.

1.4 APPROACHES TO MEASURE NUTRIENT REQUIREMENTS

Techniques which are used to measure the nutritive effectiveness of the diet for crustaceans include measurement of: specific growth rates (Mills and McCloud 1983; Gu *et al.*, 1996; D'Abramo and Castell, 1997); survival rate (Speck and Urich 1969; Gu *et al.*, 1996; D'Abramo and Castell, 1997); total biomass or biomass in various size categories; proximate composition of body tissues (Cowey, 1988); the effect of nutrient deprivation on growth (Mikami and Takashima, 1993; Schultz and Shirley, 1998); and condition indices (Turker and Eversole, 1997), which include wet and dry hepatosomatic indices (McClain, 1995a and b), moisture levels of hepatopancreas (McClain, 1995a and b), wet and dry tail muscles to body weight ratios and moisture levels in tail muscles (Castell and Budson, 1974; Cuzon *et al.*, 1980; Regnault, 1981). All have been followed in this thesis.

1.4.1 Specific Growth Rates

The simplest way to assess growth is to measure absolute weight gains over known time periods and calculate specific growth rates (Ricker, 1979; Busacker *et al.*, 1990). Growth of crustaceans is widely used in the measurement of the nutrient requirements of crustaceans (Gu *et al.*, 1996). Growth is a very complex process with protein synthesis and cellular proliferation during intermoult and a rapid increase in length and weight at moult (Aiken and Waddy, 1992). Growth can be one of the ultimate indications of health and condition because it integrates all of the biotic and abiotic variables acting on the aquatic animal and reflects secondary effects of chronic stress (Le Cren, 1972; Waters, 1977; Larkins, 1978) due to nutrient deprivations. Estimates of marron growth rate, expressed as specific growth rate (SGR) have varied between 0.5 to 1.1 % / day (Morrissy, 1979, 1980, 1990, 1992a; Morrissy *et al.*, 1995a; Jussila, 1996; Evans and Jussila, 1997; Fotedar *et al.*, 1997) in various culture environments, from aquaria to battery culture systems or an intensive crayfish culture system (ICCS).

1.4.2 Biomass and Size Grades

In communal rearing of decapod crustaceans, SGR estimates may not yield a reliable measure of nutritional effectiveness of a test diet as the animals also derive their nutrition by cannibalizing other animals. Further, stocking density of the reared animals may influence the SGR. Therefore, harvested biomass (which incorporates a measure of survival) is a more reliable parameter in the measurement of nutritive effectiveness of a test diet. In a semi-intensive farming situation, categorization of harvested biomass into different size grades makes more commercial sense as different size grades fetch different prices.

1.4.3 Proximate Composition

There has been interest in the proximate composition or the biochemistry of the cultured animals, particularly the effect of diet on animal composition and the

identification of essential dietary requirements (New, 1976). Marine crustaceans tend to have higher levels of linolenic series fatty acids and higher amounts of 20 and 22 carbon polyunsaturated fatty acids (PUFAs) than freshwater crustaceans; freshwater species tend to have higher levels of linoleic series fatty acids. Thus, one might predict that linolenic series fatty acids will have greater essential fatty acids (EFAs) value to marine crustaceans, while fresh water species might require more of the linoleic series of fatty acids or a mixture of both.

Huner *et al.* (1988) compared the proximate analysis of edible tissues of noble crayfish, *A. astacus* and the red swamp crayfish, *P. clarkii* and reported that muscle composition was comparable in both species, having levels of 79-85% moisture, 80-88% protein, 13-50 mg/g lipid, and 19-22 kJ/g energy, respectively. However, waste products remaining after removal of the abdominal muscles and hepatopancreas were: more protein rich (33 *vs.* 24%), had higher energy levels (11.725 *vs.* 10.950 kJ/g), lower ash (33% *vs.* 47%), and lower dry matter (28 *vs.* 37%) levels in *A. astacus* compared with *P. clarkii*, respectively. Anon (1990) reported the proximate composition of tail muscles of *C. tenuimanus*. The proximate analysis of various freshwater crayfish is summerised in Table 1.6.

The cholesterol content in the hepatopancreas of *Procambarus* spp. was reported to be 210 mg per 100 g (Reitz *et al.*, 1990), higher than that of abdominal muscles. Huner *et al.* (1988) reported the proximate analysis of a number of species of freshwater crayfish and their composition as a function of size and maturity.

Table 1.6 Proximate composition (dry weight %) of the listed freshwater crayfish.

Species	Moisture	Ash	Protein	Lipid	Carbohydrate	Reference
Austropotamobius pallipes						
Male	82.54	34.94	42.10	13.97	6.36	Rhodes and
Female	84.23	38.68	43.31	15.54	7.04	Holdich (1984)
Astacus leptodactylus						
Male	83.41	7.05	71.49	3.32	n.s.	Dabrowski *et al.* (1966a)
Female	83.05	7.20	70.80	3.72		
Orconectes limosus						
Male						Dabrowski *et al.*
Female	81.07	n.s.	76.60	n.s.	n.s.	(1966b)
	79.12		54.31			
A. astacus						Dabrowski *et al.*
Male	83.35	7.27	68.11	3.42	n.s.	(1968)
Female	83.11	7.40	67.02	3.79		
Procambarus clarkii	n.s.	n.s.	19 g	1 g	n.s.	Patrick and Moody (1989)
Cherax tenuimanus	75	24.5-26.9	45.8-46.5	5.1-7.3	n.s.	Huner *et al.*, (1997)

n.s. – not stated.

1.4.4 Deprivation of Nutrients

Under intensive crayfish culture systems (ICCSs), the lack of natural productivity, often results in deprivation of certain nutrients available for the cultured crayfish. Further, nutritional deprivation is a natural phase during the life cycle of many crustaceans as a result of onset of extreme temperatures during winter, seasonal elimination of food sources or behavioural patterns during moulting, mating, spawning and rearing of young (Schirf *et al.*, 1987). Marsden *et al.* (1973) Hazlett *et al.* (1975), Cuzon *et al.* (1980) and Regnault (1981) have examined the qualitative and quantitative changes in the body composition of crustaceans in response to starvation. Many species reduce their metabolic rates and start depleting protein, carbohydrate (glycogen) and lipid reserves during nutritional deprivation (Marsden *et al.*, 1973; Hazlett *et al.*, 1975). The relative importance of these reserves and their order of utilization is dependent on species, recent feeding

history and length of starvation (Schirf *et al.*, 1987). In some species carbohydrates, e.g., are used initially, then lipids and finally proteins (Chaisemartin, 1971; Cuzon and Ceccaldi, 1972). In others, for example carbohydrate utilisization is negligible, the reserves used being predominantly lipids (Schafer, 1968) or proteins (Neiland and Scheer, 1953; Marsden *et al.*, 1973). Barclay *et al.* (1983) reported that protein is the major source of energy during 14 days of starvation in *Penaeus esculentus*. Regnault (1981) measuring the oxygen to nitrogen ratio, reported that carbohydrate reserves are quickly exhausted (3-4 days) and that lipids and proteins are the main substrates oxidised to meet the energetic requirements of *Crangon crangon*. It is known that starvation decreases the level of palmitate, hexadecenoate and octadecenoate, and that feeding oils rich in either linoleate or linolenate induces high levels of the respective acids in the body tissues (Colvin, 1976a; Guary *et al.*, 1976; Sandifer and Joseph, 1976; Kanazawa *et al.*, 1977).

Dickson and Giesy (1982) studied the changes in energy metabolism in the hepatopancreas and dorsal tail muscles of the crayfish, *P. clarkii*, in response to short term nutritional deprivation. Schirf *et al.* (1987) showed that both carbohydrate and lipid reserves of the tail muscles decrease significantly after 21 days of starvation in *P. clarkii*, indicating utilization of these energy stores. Hazlett *et al.* (1975) and Speck and Urich (1969) reported similar decreases in carbohydrate and lipid levels in other species of crayfish. Schirf *et al.* (1987) indicated that organs respond differently to nutritional stress. In the muscles, both depletion of carbohydrate and lipid reserve and decrease of ATP (adenosine triphosphate) and the TA pool occurs in response to short-term nutritional deprivation. In contrast, the depletion of energy reserves was not evident in the hepatopancreas, as both carbohydrate and lipid stores remained stable. Starved marron, showed lower muscle carbohydrate levels, hepatosomatic ratios and moisture content than the fed animals (Evans *et al.*, 1992). Abdominal muscles

contributed the most protein (330 mg) and lipid (35 mg) during 14 days of starvation in *P. esculentus*, whereas hepatopancreas accounts for only 18 mg of lipid (Barclay *et al.*, 1983).

Little work has been carried out on the effect of starvation on the structural appearance of midgut gland cells of decapods (Storch and Anger, 1983; Storch *et al.*, 1984). Papathanassiou and King (1984) studied the effect of starvation in the fine structure of the hepatopancreas in the common prawn *Palaemon serratus* (Pennant) and reported that the fine structure of storage lipid cell (R-cells) and of protein producing cells (F-cells) were mainly affected. They also reported that fewer secretory cells (B-cells) were present in the tubules of starved specimens. Evans *et al.* (1992) reported atrophic changes in the hepatopancreas in 27% of starved animals as compared to fed animals.

1.4.5 Conditional Indices

Aquaculturists often need methods that can provide relatively simple and effective indications of how well culture animals are coping with the test diet in their particular environments. Organosomatic indices (ratios of organ weight to body weight) have been widely used in stress-related studies in fish (Goede and Barton, 1990). Shelbourne (1957) was the first person to use the term 'condition' to describe the nutritional status of larval fish, *Pleurocectes platessa*. Condition indices have been reported in molluscs (Lara and Parada, 1991; Karayuecel and Karayuecel, 1997), fish larvae (Setzler-Hamilton and Cowan, 1993) and crustacean larvae (Preston, 1992), for example. Theilacker (1978) first suggested the use of ratios in which the numerator alone was sensitive to the deprivation of nutrients to allow the detection of starving specimens. Weight of hepatopancreas (wet and dry) and weight of tail muscles (wet and dry) is considered, as numerator to determine the ratios (or indices), while wet body weight is a common denominator in all indices.

1.4.5.1 Hepatosomatic indices and/or moisture levels

Animals store extra resources in the hepatopancreas to be utilized later when the environmental conditions favour or trigger either growth or reproduction. Wet and dry hepatosomatic indices and moisture content of hepatopancreas have been used as indicators of nutritional status in crustaceans (Evans *et al.* 1992; Mannonen and Henttonen, 1995; McClain, 1995a and b). Mannonen and Henttonen (1995), while studying the effects of peat mining on a wild population of noble crayfish, concluded that hepatopancreas moisture and energy content could be used to evaluate the condition of crayfish. Jussila and Mannonen (1997) derived an inverse linear relationship between hepatopancreas moisture content and total energy content in marron. Gu *et al.* (1996) have shown that starvation increases the whole body moisture content in red claw (*C. quadricarinatus*) juveniles. The water content of crayfish decreased when the rate of feeding increased and during starvation they catabolised tissue protein to meet their metabolic requirements. Farmed crayfish are more intensively fed than wild marron, resulting in decreased moisture content and increased energy reserves in hepatopancreas (Jarboe and Romaire, 1995; McClain 1995b). McClain (1995a and b) reported that the availability of supplementary feed and population density affect both hepatopancreas moisture and size in red swamp crawfish.

Cockcroft (1997) and Musgrove (1997) have shown that hepatosomatic indices (ratio of the wet and dry weight of hepatopancreas to total wet body weight) and moisture content, when used together, are useful to describe the physiological condition of wild capture lobsters. They also used hepatosomatic indices successfully to estimate the differences between the growth rates among wild captured rock lobster, *Jasus lalandii*, populations and the differences in southern rock lobster (*Jasus edwardsii*) conditions under different feeding treatments. Stewart *et al.* (1967) have shown that 140 days of starvation decreases the

hepatopancreas mean weight of the lobster, *H. americanus,* from 5.2% of wet body weight to 2.6%, while their fed conspecifics hepatopancreas weight was 5%.

Huner *et al.* (1985) reported that, in noble crayfish, energy reserves from the hepatopancreas were depleted during ovarian maturation and this was reflected in an increase in the moisture content of the hepatopancreas and a decline in the abdominal muscles glycogen content. In noble crayfish, Huner *et al.* (1990) observed a high hepatopancreas moisture content, up to 80%, during ovarian maturation, compared to 60-70% of moisture content in reproductively inactive females.

1.4.5.2 Tail muscles to body weight ratios and / or tail moisture levels

If tail muscles are considered as a storage site of the energy reserves, tail muscles to body weight ratio and tail moisture levels can be considered as a condition index. However, there is only limited information available on the use of these indices to measure the nutritional effectiveness of a test diet in freshwater crayfish.

Other parameters that have been used to measure the nutritional effectiveness of test diets include sexual maturity of the animal, viscerosomatic index (Jensen, 1980) and gonadosomatic index in aquatic animals (Mehboob and Sheri, 1997). Turker and Eversole (1997) describe the use of total body electrical conductivity method to estimate lean body mass and total body lipid in live freshwater crayfish and thus 'well being' or condition of the animal.

1.5 Nutritional Value of Freshwater Crayfish

The wide spread interest in eating crayfish and hence the pressure for their culture, has a bonus for humans: crayfish tail meat has a high nutritional value. It provides a source of protein and is rich in certain B vitamins and some minerals such as sodium, potassium, calcium and magnesium. The protein content of crayfish

muscles is less than that of fish muscles, but has increased organoleptic (sensory) properties (Dabrowski *et al.*, 1968). The amino acid profile in different freshwater crayfish species is species specific. Many species of crayfish appear to have high levels of leucine, isoleucine, glutamine and asparagine and a low content of cysteine (Dabrowski *et al.*, 1966a and b, 1968; Suprunovich *et al.*, 1983; Rhodes and Holdich, 1984). Whether this is due to true interspecific variation, or related to the feeding history and regime of the experimental animals prior to analysis is not clear (Rhodes and Holdich, 1984). Ackefors *et al.* (1997) reported that the fatty acid profile of feed provided to crayfish held in captivity is reflected in the fatty acid profile of the hepatopancreas. The crayfish fed marine fish show high proportions of 20:5ω-3, 22:5ω-3 and 22:6ω-3 in their hepatopancreas compared to crayfish fed vegetable or freshwater fish diets.

1.6 Crayfish Culture Systems and Management

In Australia, freshwater crayfish aquaculture is usually undertaken in earthen ponds (Morrissy, 1979, 1992a and b; Geddes *et al.*, 1988; Jones, 1990, 1995b). Tank-based aquaculture is generally employed for breeding, purging and conducting research (Morrissy 1979, Mills and McCloud 1983, Du Boulay *et al.*, 1993). Ackefors *et al.*, (1995) studied growth, frequency and moult intervals of juvenile noble crayfish, *A astacus* in individual experimental compartments. Battery culture or intensive crayfish culture has been described by Morrissy (1984) and is not being used for commercial production of any freshwater crayfish. Three levels of growout in marron farming are being practiced in Western Australia (Morrissy, 1992b). These practices, namely, extensive, semi-intensive and super-intensive are based on three levels of management inputs, capital costs, control over nature and production of marron. In extensive farming existing water bodies (farm dams, lakes, and flooded areas) are utilized. The biomass of marron that can be produced by extensive management style is low: 300-400 kg/ha/year on average. These water bodies can not be drained and there is no water quality

management required. In semi-intensive management style, constructing purpose built, drainable ponds can intensify marron growout. Water quality management, predation control, separate hatchery production of juveniles are in practice in this type of management style. The production of marron is increased to 1000-3000kg/ha/year (Morrissy, 1992b). In super-intensive farming systems, the marron are housed separately in individual compartments in indoor tanks. This system is also called battery culture, intensive crayfish culture system or Nardi system. This higher density of marron (i.e. a large number in a small volume of water) than in ponds permit a higher degree of control. This system is highly capital intensive and has not been proved to be commercially viable but remains subject of a research (Morrissy, 1992b).

1.7 INFLUENCE OF CULTURE ENVIRONMENT ON NUTRITION STATUS

The nutritional requirements of any crustacean species are highly dependent on their cultural environment. The influence of the environment on nutritional requirements poses a complicated research problem in understanding the nutritional requirements of the animal as each environment offers a different set of variables. The nutritional requirement of different species varies due to a combination of factors like biological (genetic, dietary) and environmental (abiotic) factors. Even within single species, one or more of these factors may significantly alter the nutritional status of the animal. Abiotic factors influencing nutritional studies in different culture environments include temperature, nitrogen metabolites, photoperiod, water quality parameters and natural productivity of the ecosystem.

1.7.1 Nature of Artificial Diets

Factors to be considered with respect to the influence of dietary formulations on nutritional requirements include the presentation of the food, feed stability, feeding rates and percentage and source of feed constituents (lipids, carbohydrates and

proteins). Crustacean feeding behaviour involves slow ingestion rates, prolonged handling and long intervals between ingestion of food (Farmanfarmian *et al.*, 1982). Stability of the pellet is one of the crucial parameters affecting both the nutritional status of the animal as well as the natural productivity in the aquatic ecosystem. Further, the prolonged feeding behaviour of crustaceans and specifically of crayfish poses a unique problem in nutritional studies, with the possibility of nutrient-leaching and/or bacterial contamination during experiments (Goddard, 1988). Jussila and Evans (1997) achieved higher specific growth rate (SGR) of marron fed stable pellets than unstable pellets in intensive crayfish culture system (ICCS). However, Bordner *et al.* (1986) stated that pellet stability is not an important factor for the growth and survival of juvenile lobsters.

1.7.2 Environmental Factors

1.7.2.1 Natural productivity of the ecosystem

Detritus, consisting of decomposing plant and animal matter and associated organisms (fungi, bacteria, algae, protozoa and micro crustaceans), constitutes an important food source to bottom feeding crayfish (Mason, 1975; Momot *et al.*, 1978; Reynolds, 1979; Rhodes, 1980). Momot *et al.* (1978) reported that detritus accounts for 13.2 to 21.6% by weight of total food consumed in natural populations of crayfish. In purpose-built and managed crayfish ponds detritus also constitutes an important source of nutrients (Morrissy, 1979; Mills and McCloud, 1983; Huner and Barr, 1984; Avault and Huner, 1985). Avault *et al.* (1983) and Huner and Barr (1984) determined that, in practice, a carbon to nitrogen ratio of 17:1, can be taken as indication that detritus is of satisfactory nutritional value to crayfish.

Plant detritus is the basis of freshwater crayfish nutrition in semi-intensive aquaculture farms (Morrissy, 1992c). Under semi-intensive farming systems, crustaceans can derive up to 50% of their nutrition from natural foods (Apud *et al.*,

1983; Apud, 1985; Lee and Wickins, 1992). Wiernicki (1984) reported an inverse relationship between crayfish size and the assimilation of microorganisms from plant detritus. As mouthparts of small crayfish have two to three times the number of setae as those of large crayfish (Wiernicki, 1984), this differential may be due to the efficiency with which the feeding appendages sweep small particles into the digestive tract. Jones *et al.* (1995) have shown that diets containing mainly zooplankton provided faster growth rates in yabbies, *C. albidus*.

1.7.2.2 Other environmental factors

The culture environment can have a profound affect on proximate analysis of body tissue in general and on the lipid and fatty acids levels in particular. The lipids of shrimps vary in both amount and composition with the various factors like water temperature, food and stage of development, sex and photoperiod (Guary *et al.*, 1975). Each of these factors individually has been shown to affect the lipid composition. An increase in temperature is usually reflected in an enhancement of the concentration of saturated fatty acids and a concomitant decrease in mono and PUFAs (Guary *et al.*, 1975). In planktonic crustaceans, the percentage of C20 to C22 PUFAs increased under high ambient temperatures (Farkas and Herodek, 1964). Further, at high temperatures, when these planktonic crustaceans were fed to freshwater crayfish, the crayfish composition showed preponderance of PUFAs. Cossins (1986) found that there was an increase in PUFA levels by the fatty acid unsaturation of the total phospholipid fraction in the muscles of *A. pallipes* at an acclimation temperature of 4°C, and unsaturation was also influenced by the photoperiod length.

1.8 MARRON BIOLOGY

1.8.1 Taxonomy and Distribution

Crayfish (Crustacea : Malacostraca : Decapoda) occur naturally on all continents except Africa and on a number of coastal and oceanic islands (Hobbs, 1988). Of

the three crayfish families, the Astacidae and Cambaridae (in the superfamily Astacoidea) occur in the Northern Hemisphere, whilst Parastacidae (superfamily Parastacoidea) are confined to the Southern Hemisphere. Australia has a diverse crayfish fauna with about 110 species (Williams 1980; Morgan 1988) representing nine genera. Three of the species, marron (*C. tenuimanus*), yabbies (*C. destructor*) and redclaw (*C. quadricarinatus*) are significant for aquaculture. Species within the genera *Astacopsis* (in Tasmania) and *Euastacus* (eastern Australian mainland states) may also have aquaculture potential but culture conditions for these species have not yet been studied. The Tasmanian freshwater crayfish (*Astacopsis goudii*) is the largest freshwater crayfish in the world (Riek, 1969), reaching a length of 600 mm and biomass of 4.0 kg.

Marron, the third largest crayfish species in Australia, is indigenous to the southwest of Western Australia (Riek, 1967) but has now been translocated by human activities to rivers and drainage systems as far north as Geraldton and northwards (Figure 1.1). Marron are largely detritivores and polytrophic marron are the dominant invertebrate species in their natural environment (Morrissy, 1974) by virtue of their biomass. Marron form the basis of a recreational fishing industry and are important for aquaculture. Commercial marron farming commenced in Western Australia in 1976 and is currently being practiced in Western Australia (Fotedar *et al.*, 1996a and b) and in South Australia. There are 50 licensed farms and a further 77 farms are seeking approvals from the Government agencies (Evans and Fotedar, 1996).

1.8.2 Life Cycle

Most of the growth or development of the eggs occur through summer and autumn. Mating and spawning occur during spring when they are at the age of 1+ years. These processes are dependent on water temperature and photoperiod (Morrissy, 1990). The speed at which the eggs and the hatched young on the tails of females

develop also depends on ambient temperatures and can be prolonged until Christmas in colder regions. The rearing technology of marron has been described by Morrissy (1992b).

Figure 1.1 **Western Australia showing the natural and current distribution of marron and locations of the farm-based experiments (1 - Marron Force and Parkerville; 2- JBMF).**

1.8.3 Digestive System

The digestive system of marron comprises the components of digestive tract and the hepatopancreas (also known as digestive or midgut gland). Knowledge of anatomy and physiology of the digestive system is essential to understand the nutritional status of marron (O'Brien, 1994a).

1.8.3.1 Alimentary tract

In decapod crustaceans the mouth is bordered by the mandibles laterally, the labrum anteriorly and posteriorly by the paragnath lobes of the metasoma (Thomas, 1970, 1986). The alimentary tract is the major organ system suspended in the body cavity of marron and has been described in detail by O'Brien (1990).

1.8.3.2 Hepatopancreas

In crustaceans, the hepatopancreas has the function of both the liver and pancreas of vertebrates (Vonk, 1960). The liver and pancreas of vertebrates are the main sites of both organic and inorganic reserve storage. In addition the liver is the main site for detoxification while the pancreas secretes digestive enzymes. In crayfish, the hepatopancreas occupies most of the cephalothoracic cavity and is in intimate contact with the haemolymph (Holdich and Reeve, 1988). It consists of a complex system of ducts and blind-ending tubules with each duct and tubule lined by a single-layer of epithelial cells (Holdich and Reeve, 1988). The cells comprising the walls of the tubules are of four types (Loizzi, 1971). The E cells at the summit of the tubules develop into R cells, F cells and B cells. R, F, and B cells can be found in the transition and B-cell zones of the tubules while in the proximal zone only R and F cells are present (Vogt *et al.*, 1985).

The main functions of the hepatopancreas are the synthesis and secretion of digestive enzymes, the production of emulsifiers, metabolism of carbohydrates, calcium storage, excretion and storage of heavy metals (Bunt, 1968; Smith *et al.*, 1975). The physiological functions of the hepatopancreas are under neuroendocrine control (Loizzi, 1971; Gibson and Parker, 1979). It serves as a supplementary source of energy during starvation, moulting or during preparation for reproduction (Lindqvist and Louekari, 1975; Haefner and Spaargaren, 1993). The cells of the hepatopancreas pour digestive juices, including proteinases, peptidases, lipases and amylases, into the foregut. Hepatopancreatic cells absorb most of the digested food. Speck and Urich (1970) showed that only 5 percent of

nutrients are absorbed by the midgut in *Orconectes limosus.* In addition, the hepatopancreas of *Austropotamobius pallipes* has been shown to be involved in heavy metal uptake (Lyon and Simkiss, 1984). M. Chambers, who is evaluating the usefulness of marron as a bio-indicator of cadmium toxicity, has found that the rate of cadmium accumulation in the hepatopancreas of marron is related to the cadmium concentration to which animals are exposed (Chambers, 1995).

1.9 Marron Culture

Marron have attracted considerable aquacultural interest in Western Australia, South Australia (Aiken, 1988; Morrissy *et al.*, 1990; Rubino *et al.*, 1990) and in some overseas countries. In Western Australia, aquaculture of marron is being practiced in farm dams, purpose built earthen ponds, plastic lined ponds, and under intensive battery culture, called as intensive crayfish culture system (ICCS). Each of these culture systems provide different biological and physiochemical conditions. These conditions encompass variables such as physical construction of rearing facility, physiochemical properties of water, stocking density, quantity and species diversity of available natural food, and the composition of formulated diet. These variables have a profound impact on management strategies which aquaculturists have to follow to achieve optimum productivity from the culture systems. Optimization of production and minimization of the feeding costs are the two main management objectives of aquaculturists. These objectives can be achieved through cost-effective feed and nutrition management for each particular culture environment.

1.10 AIM

Increased knowledge of marron nutrition is essential for increasing the productivity of existing marron farms and enhancing management methods used for exploiting natural populations. There is no information available on the relationship between the rearing environment and the nutritional requirements of marron. As nutritional

requirements of marron are highly dependent on the set of variables existing within the culture environment, it is essential to study the requirements under a range of typical farm conditions. For example, more nutritious diets will be needed for use in intensive culture systems in an artificial confinement, where little or no wild food is available.

The aim of this research project was to investigate the nutrition of marron under various cultural conditions with special emphasis on lipid nutrition.

1.10.1 Specific Objectives

1.10.1.1 ***To determine the influence of lipid levels in semi-purified diets on growth, survival and lipid levels of marron cultured under laboratory conditions.***

1.10.1.2 ***To study the influence of stocking density on growth, survival and size distribution of marron cultured under conditions similar to those planned for use in cages in farm conditions.***

1.10.1.3 ***To investigate the effect of different lipid sources on growth, survival and nutritional stress in marron, cultured in cages in field trials (using the optimum stocking density from objective 1.10.1.2.).***

1.10.1.4 ***To investigate the influence of protein and lipid sources on growth, survival and body composition of marron cultured in ponds in a semi-intensive commercial marron farm.***

1.10.1.5 ***To investigate the influence of starvation and delayed feeding on growth and survival of marron cultured in an intensive battery culture system.***

1.10.1.6. ***To investigate the utilization of various body nutrients (reserves) during starvation and delayed feeding on marron cultured in an intensive battery culture system.***

1.11 STRUCTURE OF THE THESIS

Chapter 1 is devoted to introducing the topic and reviewing the relevant literature. It highlights the rationale for conducted nutritional research under different rearing conditions. Experiment 1 was performed in glass aquaria in a laboratory environment (Chapter 2) under controlled environmental conditions. Three semi-purified test diets were used in this experiment to investigate the influence of dietary cod liver oil on growth, survival, condition indices, body composition and fatty acid profile of body tissues.

Experiments 2 and 3 were performed in purpose-built cages placed in a hot house (Plate 1; Chapter 3) and marron pond (Plate 2; Chapter 4)) (location in Figure 1.1) respectively. Experiment 2 was conducted to determine the optimum stocking densities of juvenile marron that could be used in future experiments in the cage environment. The experiment was conducted in recirculating water under semi-controlled environmental conditions. Experiment 3 was performed in purpose-built plastic lined, earthen pond (Plate 3; Chapter 4) using the same cages as in experimental 2. The aim of the experiment was to investigate the effect of dietary supplementation of cod liver oil and sunflower oil on growth, survival and condition indices of juvenile marron. The cod liver oil and sunflower oil are easily available in western Australia and are quite consistent in their fatty acid profiles (*Appendix 2, Table 1*). Four practical test diets were evaluated in the experiment.

Experiment 4 was conducted under commercial marron farm situation where 12 purpose built earthen ponds were used (Chapter 5). The main purpose of the experiment was to evaluate the effect of the protein-free formulated diet and two different protein sources on the growth, survival, size grades, total biomass,

proximate composition of body tissues and condition indices of juvenile marron. Four practical test diets were used in this experiment.

Experiment 5 was performed in a battery culture system, referred to as intensive culture system for marron (Plate 4 and 5; Chapter 6). The main aim of the experiment was to investigate the effect of prolonged periods of starvation and delayed re-feeding on growth, survival, condition indices and various energy reserves in hepatopancreas and tail muscles tissues of juvenile marron. Chapter 7 discusses the main results of the thesis and compares the influence of culture environment on nutritional studies of marron. Chapter 7 also highlights the main conclusions from the research and makes recommendations for future nutritional studies in freshwater crayfish in general and marron in particular.

CHAPTER 2

DIETARY LIPID LEVELS

2.1 INTRODUCTION

As marron farming intensifies it has become clear that limited knowledge of the nutritional requirements of marron is impeding growth of the industry. Although considerable nutritional research on feeds and nutrition has been done with penaeid prawns (D'Abramo and Robinson, 1989; Harrison, 1990) and Australian crayfish, namely, *C. destructor*, (Mills and McCloud, 1983; Geddes *et al.*, 1988; Villarreal, 1988; Sommer *et al.*, 1991), *C. tenuimanus*, (Morrissy, 1979, 1984) and *C. quadricarinatus*, (Jones, 1990), little is known of the lipid nutrition of these species. No information is available on the lipid requirements of *C. tenuimanus*.

Knowledge of the exact lipid requirements is essential in order to increase the growth rates, maintain health and to understand the influence of lipid on the nutritional status of other energy sources, such as proteins and carbohydrates. Past nutritional studies with crustaceans, other than crayfish, indicate that optimal lipid levels range from 5 to 8% of the diet (Kanazawa *et al.*, 1977). Experimental diets under laboratory conditions are generally supplemented with fish or vegetable oils as a source of polyunsaturated fatty acids (Tarshis, 1978; Huner and Lindqvist, 1984). O'Brien (1994b) claimed that, in farming conditions, most of the crayfish dietary requirement of lipid comes from natural food rather than supplementary dietary ingredients like fish meal or soybean meal (major protein sources), or through supplementation of pure oils to the diet. But his statement was not supported by experimental evidence.

The quantity of lipid and the fatty acid composition of dietary lipid can influence the growth and survival of decapod crustaceans (Deshimaru *et al.*, 1979; D'Abramo and Sheen, 1993). In dealing with aquacultural situations, natural oils,

rather than artificial oil fractions, are recommended to be included in the diets (Lochmann and Gatlin, 1993). Past research on the dietary use of natural oils has been conducted only on finfish species (Tekeuchi and Watanabe, 1976; Greene and Selivonchick, 1987) and the freshwater prawn, *M. rosenbergii* (Sheen and D'Abramo, 1991). Natural oils contain fatty acids primarily in triglyceride form, and fish usually exhibit better growth and survival performance when fed intact oils rather than isolated or artificial lipid fractions (Greene and Selivonchick, 1987).

The main lipid storage organ in crustaceans is the hepatopancreas. In crayfish hepatopancreas, lipid levels of ≤ 92% of dry weight have been reported (Suprunovich *et al.*, 1983). Sheen and D'Abramo (1991) showed that body lipid levels increase as dietary lipid levels increase in juveniles of *M. rosenbergii*.

The present study investigated the hypothesis that dietary lipids effect growth, survival and body lipid levels of juvenile (0^+) marron reared under laboratory conditions. The exogenous source of dietary lipid was cod liver oil because of its easy availability and known and consistent fatty acid profiles (*Appendix 2, Table I*).

2.2 Materials and Methods

2.2.1 Experimental System and Design

Feeding trials were performed in a temperature-controlled laboratory for 108 days. Twelve glass aquaria (0.91 m x 0.35 m x 0.45 m) containing 120 L of water each, maintained at a constant temperature of 18.5 °C ± 0.1 and fitted with sub-gravel filters, were used in the trial. The tops and the sides of the aquaria were covered with perforated plastic sheet to avoid any stress to the animals due to external disturbances. Small PVC cut-offs and extruded meshes were used as shelters. The

three experimental diets were allocated using a randomized design procedure with four replicates (aquaria) per treatment.

2.2.2 Diet Ingredients, Formulation and Characteristics

Three isoenergetic and isonitrogenous diets (D_1, D_2 and D_3) were prepared using semi-purified ingredients (Table 2.1). The purified cod liver oil (Glen Forrest Stockfeeders, Great Eastern Highway, WA), 6% and 12% (by weight) was added to the diet D_1 to prepare diets D_2 and D_3, respectively. Crude protein content was determined by the Kjeldahl method and a cold extraction procedure using chloroform: methanol was used to determine total lipid content (AOAC, 1990). Nutrient analyses of diets D_1, D_2 and D_3 are described in Table 2.2.

Table 2.1 Ingredients (%) of the three test diets.

Ingredients	Diet D_1	Diet D_2	Diet D_3
Casein	28.10	28.10	28.10
Binder	2.00	2.00	2.00
Limestone	1.20	1.20	1.20
Sugar	33.80	20.30	6.80
Gelatine	4.50	4.50	4.50
Cellulose	30.40	37.90	45.40
Cod liver oil	0.00	6.00	12.00

2.2.3 Experimental Animals

Two hundred juvenile marron (0^+) were harvested from an earthen pond of commercial marron grower T.B.S Pastoral Company, Red Hills, Western Australia. Within two hours of capture they were transported in two polystyrene boxes to the experimental site and placed in two corrugated iron tanks (4 m x 3 m x 0.5 m) of 6,000 L capacity. They were held in the tanks for seven days and on the eighth day they were randomly distributed at a rate of nine animals per aquarium.

Table 2.2 Major nutrient analysis (%) of the three test diets.

Nutrients	Diet D_1	Diet D_2	Diet D_3
Protein	25.57	25.57	25.57
Lipid	0.28	6.28	12.28
Crude Fibre	30.40	37.90	45.40
Calcium [1]	0.46	0.46	0.46
Phosphorus [1]	0.03	0.03	0.03
Salt [1]	0.01	0.01	0.01
Metabolized energy (MJ/Kg) [1]	10.85	10.46	10.07
Lysine [1]	1.82	1.82	1.82
Methionine [1]	0.73	0.73	0.73
Cystine [1]	0.08	0.08	0.08
Threonine [1]	1.00	1.00	1.00
Tryptophane [1]	0.33	0.33	0.33
Leucine [1]	2.25	2.25	2.25
Isoleucine [1]	1.46	1.46	1.46
Arginine [1]	0.92	0.92	0.92
Histidine [1]	0.67	0.67	0.67
Tyrosine [1]	1.41	1.41	1.41
Phenylalanine [1]	1.28	1.28	1.28
Valine [1]	1.64	1.64	1.64
ω-6 [1]	0.06	0.48	0.90
ω-3 [1]	0.00	1.50	3.00

[1] -calculated according to the known nutrient composition of the ingredients used.

During the initial acclimatization period of 14 days, the marron were fed diet D_1 on alternate days. The quantity was adjusted as per demand, being approximately 12% of body weight per week. To maintain constant stocking density, a similar sized animal from a reserve tank replaced any animal that died during the acclimatization period. These introduced animals were not considered for any data collection purposes and were made identifiable by punching a small hole in their uropods. At the end of the acclimatization period one marron from each tank was sacrificed for measurement of whole-body proximate composition reducing the final experimental density to eight animals (equivalent to 25 juveniles/m^2).

2.2.4 Biochemical Analysis

Analysis of moisture percentage, crude protein, lipid levels, fibre and ash were performed using standard methods described in AOAC (1990). Fatty acid profiles

were analysed using gas chromatography/mass spectrophotometry (GS/MS) – Hewlett-Packard (Avondale, PA) Model 5971 (after the methods in Tsvetnenko *et al.*, 1996). Chromatography was performed on a DB 23 column (i.d. 0.32 mm and film thickness 0.25 μ; J & W Scientific, Folsom, CA) with helium as the carrier gas. All samples were analysed in triplicate.

2.2.4.1 Moisture percentage

Approximately 5 g of sample was placed in a preweighed (w_0) crucible and the crucible plus sample was then reweighed (w_1). The crucible was then placed in an oven at 105 ^{0}C overnight. Next day the crucible was removed from the oven and placed immediately in a desiccator for approximately 1 hour to cool before reweighing (w_2). Percentage dry matter and moisture were calculated using the following equations:

weight of original sample = $w_1 - w_0$

weight of dry sample = $w_2 - w_0$

percentage dry matter (DM%) = $[(w_2 - w_0) / (w_1 - w_0)] \times 100$

percentage moisture (M%) = 100 - DM

2.2.4.2 Crude protein

The protein nitrogen content of the sample was determined by the Kjeldahl method using a Tecator Digestive System 20 1015 operated by a Tecator Autostep 1012 controller and a Tecator Kjeltec 1030 Auto Analyser. A sample of 0.5 g was transferred into a 250 mL digestion tube and digested with 10 mL of digestion acid (concentrated sulphuric acid: concentrated orthophosphoric acid: 100: 5) in the presence of catalyst for 90 minutes at 420 °C using the Tecator digestor. The tubes were then allowed to cool before adding 75 mL of distilled water. Samples were then analysed by the Kjeltec Auto Analyser. Percentage crude protein was calculated using the following equation:

% crude protein = 14.01 x M x f x 100 (mL titrant – mL blank)] / mg sample

where, 14.01 = atomic weight of nitrogen,
M = Molarity of hydrochloric acid (titrant)
f = Standard Kjeldahl factor (6.25)

2.2.4.3 Crude lipids

The cold extract procedure using methonol: chloroform solvent was used. One gram of marron tissue was homogenised in 10 mL of methanol and 20 mL of chloroform. The suspension was stirred with a magnetic stirrer and then filtered in a Bunnel funnel. The residue was re-extracted using 2:1 methanol: chloroform solvent. Twenty percent of the filtrate was added to approximately 22.5 mL of potassium chloride (0.88%). The mixture was thoroughly shaken and left overnight. The upper aqueous layer (non-lipid) was removed by aspirator and the volume of the lower layer (lipids) was measured. Twenty five percent volume of methanol: potassium chloride (1:1) was added and the upper layer was again removed by aspirator. The lipid layer was then transferred into a pre-weighed round bottom flask (w_1) and the solvent was removed by rotary evaporator at 60 °C. The flask was cooled in a desiccator to a constant weight (w_2). The weight of lipid was determined by subtracting the initial weight of the flask from the final weight ($w_2 - w_1$).

2.2.4.4 Fatty acids

Lipid fractions of approximately 50 mg, dissolved in 1 mL toluene, were subjected to methylation process by adding 2 vol of 1% vol/vol H_2SO_4 in methanol and allowing it to stand for 12 hours at 50 ^{0}C. Five mL of 5% aqueous NaCl solution was added to each sample, followed by 10 mL hexane. The mixture was shaken thoroughly and then allowed to separate. The supernatant from each mixture was removed and stored, and the residue was re-extracted with an additional 10 mL hexane. The supernatant from each sample were pooled and washed with 4 mL of 2% $KHCO_3$ solution. After separation, the supernatant of each sample were dried by passing through a small column of anhydrous Na_2SO_4. These methylated lipid

fractions (FAMES) in hexane were subjected to gas GC/MS spectrophotometry as described by Tsvetnenko *et al.*, (1996) to determine the fatty acid profile.

2.2.4.5 Crude fibre

The lipid-free extract was digested with 200 mL of 1.25% H_2SO_4 by boiling at 100 °C for 30 minutes, then filtered through Whatman No. 541 paper in a Buchner funnel. The residue was further digested with 200 mL of 1.25% NaOH for 30 minutes at 100 °C. After boiling, the digestive samples were filtered through a porous crucible and washed twice with petroleum ether. The crucible plus sample was dried overnight at 105 °C, cooled in a desiccator for an hour and weighed again (w_1). Percentage crude fibre was calculated using the following equation:

$$\% \text{ crude fibre} = [(w_0 - w_1) / \text{weight of sample}] \times 100$$

2.2.4.6 Ash

Approximately 5 g of sample was transferred into a preweighed crucible (w_0) and the weight of the sample and crucible was recorded (w_1). The crucible with the sample was placed in a muffle furnace overnight at 600 °C. The crucible was removed from the furnace, allowed to cool in a desiccator for an hour and reweighed (w_2). Percentage ash was calculated using the following equation:

$$\% \text{ ash} = [(w_2 - w_0) / (w_1 - w_0)] \times 100$$

2.2.5 Water Quality

Ten percent of the water was exchanged twice a week and temperature, pH and dissolved oxygen were recorded twice a week in all aquaria. Total ammonia, nitrite, nitrate, total hardness, and alkalinity were recorded once a month. Sodium, potassium, calcium, magnesium, chloride, (*Appendix 1, Table 1*) were analyzed using an atomic absorption spectrophotometer (Varian/AA-875) before the commencement and at the end of the experiment.

2.2.6 Recording and Data Analysis

Data for growth and survival were recorded every 27 days. Specific growth rate (SGR; as described by Hopkins, 1992) and survival percentage were calculated using the following formulae:

specific growth rate (SGR in $\% d^{-1}$) = $100 (\ln w_t - \ln w_o) / t$

where w_t = weight at time t, namely, 27, 54, 81, and 108 days

w_o = initial weight of the marron

survival (%) = $100 (n_t / n_o)$

n_t = number of animals at the time t, namely, 27, 54, 81, and 108 days

n_o = number of marron at the beginning of the trial

The data were analysed statistically by one-way analysis of variance (ANOVA), analysis of covariance (ANCOVAR; initial weight as covariates) and the LSD multiple-comparison test to identify significant differences among treatment means (Steel and Torrie, 1960, 1980). One way ANOVA analysis, using SPSS ver. 6, was performed to detect any significant differences in water quality among the aquaria with different dietary treatments.

At the end of the experiment, the lipid content and fatty acid profile of one marron from each aquarium was determined. The tail muscles (i.e. the complete mass of muscles in the abdomen of the crayfish) and hepatopancreas (all lobes of the hepatopancreas) of the remaining marron were weighed to determine the wet hepatosomatic index (H_{iw}) and tail muscles to wet body weight ratios (T/B_w) using the following formulae:

$= W H_{wet} \times 100 / W_t$

$T/B_w = W T_{wet} \times 100 / W_t$

where, $W H_{wet}$ = weight of wet hepatopancreas (g)

W_t = total weight of marron (g)

$W T_{wet}$ = weight of wet tail muscles (g)

The whole hepatopancreas and tail muscles were then dried to constant weight at 90 °C for 24 hours. The percentage moisture of hepatopancreas (HM%) and tail

(TM%), dry hepatosomatic index (H_{id}) and dry tail muscles to wet body weight ratios (T/B_d) were calculated using the following formulae:

$$HM\% = (W\,H_{wet} - W\,H_{dry}) \times 100 / W\,H_{wet}$$
$$HT\% = (W\,T_{wet} - W\,T_{dry}) \times 100 / W\,H_{wet}$$
$$H_{id} = W\,H_{dry} \times 100 / W_t$$
$$T/B_d = W\,T_{dry} \times 100 / W_t$$

where, $W\,H_{dry}$ = weight of dry hepatopancreas (g)
$W\,T_{dry}$ = weight of dry tail muscles (g)

2.3 RESULTS

2.3.1 Water Quality

All the water quality variables remained within their optimal range and there were no significant differences ($P > 0.05$) of any water quality variable among any aquaria of the different treatment groups (*Appendix 1, Table 1*). Total ammonia and nitrite were also low but nitrate levels showed a steady increase from 0 to 15 mg/L as the experiment progressed.

2.3.2 Final Weight, Growth and Survival

There were no significant differences in initial average weights of all animals (1.99 ± 0.09 SE g) among any of the treatments (Table 2.3). Up to 27 days, the mean weight did not change significantly ($P > 0.05$) among the three dietary treatments (Table 2.3). Marron fed D_1 achieved a highest final mean weight of 6.34 ± 0.72 g whereas those fed the diet containing 12% cod liver (D_3) weighed only 3.54 ± 0.27 g at the completion of the trial (Table 2.3). Regression analysis between weight and dietary fat levels (Table 2.4) showed a strong negative relation between the weight of marron and the dietary lipid levels from day 54 till the end of the trial. As the observed growth rates were exponential in nature they were converted to the linear form of expression (specific growth rates) using natural log transformations as suggested by Evans and Jussila (1997).

Table 2.3 **The effect of the three dietary lipid levels on weight (mean ± SE g) of 0^+ marron.**

Days	Diet D_1	Diet D_2	Diet D_3
0	$_1$ 1.96 ± 0.09	$_1$ 2.06 ± 0.22	$_1$ 1.85 ± 0.22
27	$_{1,2}$ 2.19 ± 0.09	$_1$ 2.24 ± 0.20	$_1$ 2.17 ± 0.19
54	$_2$ 3.50 ± 0.19 ^{a}	$_{1,2}$ 3.20 ± 0.29 ^{a}	$_1$ 2.43 ± 0.10 ^{b}
81	$_3$ 5.56 ± 0.64 ^{a}	$_{2,3}$ 4.05 ± 0.49 ^{a,b}	$_2$ 3.19 ± 0.30 ^{b}
108	$_3$ 6.34 ± 0.72 ^{a}	$_3$ 5.03 ± 0.55 ^{a,b}	$_2$ 3.54 ± 0.27 ^{b}

a,b - Values in the same row having different superscripts are significantly different ($P < 0.05$)
1,2,3 - Values in the same column having different subscripts are significantly different ($P < 0.05$)

Table 2.4 **Regression analysis of weight as a function of dietary fat level over the experimental period.**

Days	Regression equation	r^2 value
27 days	wt = 2.21 - 0.001 x fat level	0.06
54 days	wt = 3.62 - 0.09 x fat level	0.95
81 days	wt = 5.53 - 0.21 x fat level	0.97
108 days	wt = 6.47 - 0.24 x fat level	0.99

Table 2.5 **The effect of the three dietary lipid levels on SGR (mean ± SE %/day) of 0^+ marron.**

Days	Diet D_1	Diet D_2	Diet D_3
27	$_1$ 0.42 ± 0.07 ^{a,b}	$_1$ 0.32 ± 0.08 ^{a}	$_1$ 0.62 ± 0.10 ^{b}
54	$_2$ 1.07 ± 0.03 ^{a}	$_2$ 0.82 ± 0.05 ^{a,b}	$_1$ 0.53 ± 0.00 ^{b}
81	$_3$ 1.27 ± 0.08 ^{a}	$_2$ 0.83 ± 0.06 ^{b}	$_1$ 0.68 ± 0.08 ^{b}
108	$_2$ 1.07 ± 0.06 ^{a}	$_2$ 0.82 ± 0.07 ^{b}	$_1$ 0.61 ± 0.09 ^{b}

a,b - Values in the same row having different superscripts are significantly different ($P < 0.05$)
1,2,3 - Values in the same column having different subscripts are significantly different ($P < 0.05$).

Different dietary lipid levels (Table 2.5) significantly ($P < 0.05$) affected specific growth rates (SGRs) of marron. The growth rates over different time periods also

showed significant variations. The SGRs for all three dietary levels were low during the first 27 days and then increased. The highest growth rate for any time period (1.27) was achieved using diet D_1. Specific growth rates for this diet declined towards the end of the experiment, the SGR for the period 0-108 days (the entire trial period) was significantly lower than that for the period 0-81 days (Table 2.5). For the first 54 days, the SGR of marron fed diet D_2 increased significantly and thereafter remained static. The marron fed diet D_3 did not show any significant differences ($P > 0.05$) in SGR with time. Regression analysis (Table 2.6) showed a negative relationship between dietary lipid levels and SGR of marron after 54 days of the trial.

Table 2.6 **Regression analysis of SGR as a function of dietary fat level over the experimental period.**

Days	Regression equation	r^2 value
27	sgr = 0.34 + 0. 02 x fat level	0.45
54	sgr = 1.10 - 0.05 x fat level	0.99
81	sgr = 1.24 - 0.05 x fat level	0.91
108	sgr = 1.08 - 0.04 x fat level	0.99

Table 2.7 **The effect of the three dietary lipid levels on survival percentage (mean ± SE %) of 0^+ marron.**

Days	Diet D_1	Diet D_2	Diet D_3
27	$_1$ 85.0 ± 5.0 ^{a}	$_1$ 95.0 ± 5.0 ^{a}	$_1$ 90.0 ± 10.0 ^{a}
54	$_{1,2}$ 75.0 ± 5.0 ^{a}	$_{1,2}$ 85.0 ± 5 ^{a}	$_2$ 60.0 ± 0.0 ^{b}
81	$_{2,3}$ 70.0 ± 5.8 ^{a,b}	$_2$ 80.0 ± 0.0 ^{a}	$_2$ 60.0 ± 0.0 ^{b}
108	$_3$ 60.0 ± 0.0 ^{a}	$_3$ 65.0 ± 5.0 ^{a}	$_2$ 60.0 ± 0.0 ^{a}

a,b - Values in the same row having different superscripts are significantly different ($P < 0.05$)
1,2,3 - Values in the same column having different subscripts are significantly different ($P < 0.05$)

Table 2.8 **Regression analysis of survival percentage as a function of dietary fat level over the experimental period.**

Days	Regression equation	r^2 value
27	survival = 87.44 + 0. 42 x fat level	0.23
54	survival = 79.54 - 0.47 x fat level	0.12
81	survival = 75.59 - 0.91 x fat level	0.27
108	survival = 61.74 - 0.01 x fat level	0.00

Survival at the end of the trial was the same (60%) in all the treatments (Table 2.7). The juvenile marron fed diets D_1 and D_2 exhibited a constant decrease in survival until the end of the trial, whereas marron fed D_3 showed a significantly higher mortality after 54 days and then mortalities ceased. Regression analysis (Table 2.8) showed that survival percentage was not related to dietary lipid levels.

2.3.3 Condition Indices

The moisture content of juvenile marron at the end of the acclimatization period was 80.78 % ± 0.40 (n = 12). At the end of the trial, the percentage moisture levels in the tail muscles (TM%) and hepatopancreas (HM%) were not significantly different among marron (Table 2.9) fed three different experimental diets.

Table 2.9 **Moisture and lipid levels (mean ± SE %) of marron tissue at the end of the experiment.**

Diets	Marron Tissue			
	Tail Muscles		Hepatopancreas	
	Moisture (TM%)	Lipid	Moisture (HM%)	Lipid
D_1	79.95 ± 0.47 [a]	0.92 ± 0.11 [a]	76.45 ± 0.28 [a]	43.95 ± 9.76 [a]
D_2	80.00 ± 0.91 [a]	8.68 ± 1.93 [b]	73.22 ± 3.44 [a]	72.73 ± 10.14 [b]
D_3	82.74 ± 1.17 [a]	10.27 ± 1.99 [b]	76.87 ± 0.58 [a]	71.05 ± 8.99 [b]

a,b - Values in the same column having different superscripts are significantly different ($P < 0.05$).

However, TM% showed an increasing trend as lipid levels in the diets increased from D_1 to D_3. Dry and wet hepatosomatic indices (H_{iw} and H_{id} respectively) of marron with D_2 were significantly higher compared to marron fed D_1 and D_3 (Table 2.10). Wet and dry tail muscles weight to body weight ratios (T/B_w and T/B_d respectively) of marron fed D_2 were also significantly higher than in the other two dietary treatments (Table 2.10).

2.3.4 Proximate Composition

The proximate composition of whole body of marron is shown in Figure 2.1. Lipid levels in the diet and hepatopancreas showed similar trends. Significantly lower levels of lipid levels were found in the tail and hepatopancreas of marron fed D_1 as compared to D_2 and D_3 (Table 2.9).

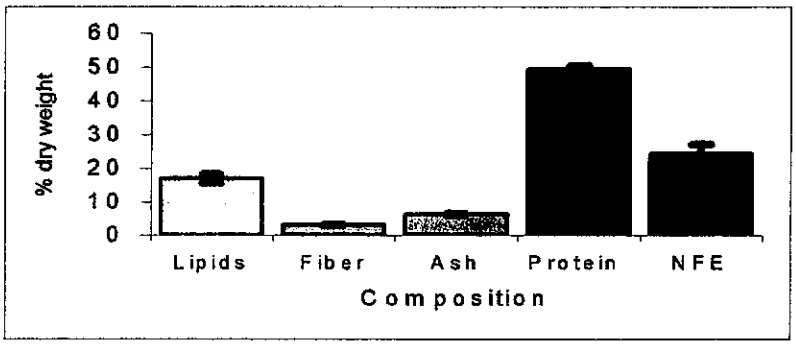


Figure 2.1 **Proximate composition of the whole body of marron at the beginning of the experiment**

Table 2.10 **Wet and dry hepatosomatic indices (H_{iw} and H_{id}; mean ± SE %) and tail muscles to body weight ratio (T/B_w and T/B_d; mean ± SE %) of marron at the end of the experiment.**

Diets	H_{iw}	H_{id}	T/B_w	T/B_d
D_1	6.25 ± 1.33 [a]	1.45 ± 0.32 [a]	16.48 ± 1.58 [a]	3.30 ± 0.35 [a]
D_2	9.11 ± 1.84 [b]	2.92 ± 0.43 [b]	31.51 ± 4.31 [b]	8.37 ± 1.23 [b]
D_3	8.91 ± 0.69 [a,b]	2.06 ± 0.19 [a,b]	24.83 ± 2.7 [a]	4.25 ± 0.47 [a]

a,b - Values in the same column having different superscripts are significantly different ($P < 0.05$)

Table 2.11 **Fatty acid composition (mg/L g of lipid extract) of marron at the beginning and end of the experiment.**

Fatty acids	Initial (whole body) mean ± SE	Hepatopancreas (at the end of the trial)		
		D_1	D_2	D_3
14:00	0.82 ± 0.08	-	8.87	9.12
16:00	15.65 ± 0.81	18.43	25.32	30.67
18:00	7.01 ± 0.31	21.50	4.35	2.2
19:00	1.31 ± 0.13	-	-	-
20:00	1.04 ± 0.07	-	-	-
Total saturated	**25.83 ± 1.24**	**39.93**	**38.54**	**41.99**
16:1 ω-3	-	-	-	-
18:1 ω-9	20.88 ± 0.95	47.55	33.53	22.14
20:1 ω-9	2.31 ± 0.53	-	-	-
22:1 ω-9	1.52 ± 0.03	-	-	-
Total mono-unsaturated	**24.71 ± 1.21**	**47.55**	**33.53**	**22.14**
18:2 ω-6	10.19 ± 0.64	-	4.13	10.82
20:2 ω-6	2.17± 0.15	-	-	-
18:3 ω-3	1.26 ± 0.11	-	-	-
20:4 ω-6	6.71± 0.6	-	-	-
22:4 ω-6	0.82 ± 0.04	-	-	-
PUFA	**21.15 ± 0.60**	-	**4.13**	**10.82**
20:5 ω-3	10.79 ± 0.85	-	16.13	16.5
22.6 ω-3	-	-	6.53	6.0
HUFA	**10.79 ± 0.96**	-	**22.66**	**22.5**
ω-3	12.05 ± 0.77	-	-	-
ω-6	19.89 ± 0.59	-	-	-
ω-9	24.71 ± 1.52	47.55	33.53	22.14
Total	**82.48 ± 1.24**	**87.48**	**98.86**	**97.45**

2.3.5 Fatty acid Profile

Fatty acid levels ≤ 0.7 % were not determined in this study and were considered as trace amounts. Before the trial commenced, HUFAs amounted to approximately 10.8% of the total lipid content, whereas polyunsaturated fatty acids (PUFAs) and mono-unsaturated fatty acids accounted for 21.1 and 24.7% respectively in the

whole body of juvenile marron (Table 2.11). The ω-9 series of fatty acids dominated the fatty acid profile of whole bodies of marron. The increase in dietary lipid levels resulted in an increase in the level of saturated fat in the hepatopancreas. The diet D_3 resulted in lower levels of total mono-unsaturated fatty acids compared to diets D_1 and D_2. Addition of cod liver oil in the diet also resulted in higher levels of unsaturated fatty acids (HUFAs) in the hepatopancreas (22.5 – 22.6%), though cod liver oil had a sizeable percentage (40%) of HUFAs (*Appendix 2, Table I*).

2.4 DISCUSSION

Dietary cod liver oil had an inhibitory effect upon the growth, of *C. tenuimanus* but not survival of juveniles of *C. tenuimanus*. At the end of the trial, dietary lipid levels were reflected in the hepatopancreas and tail muscles of the animal. Further, the fatty acid profile of the diet was also reflected in the fatty acid profile of the hepatopancreas of the animal. Water quality was not considered to be detrimental to the growth of *C. tenuimanus* in the present trial.

2.4.1 Final Weight, Growth and Survival

Multiple regression analysis among weight, lipid level and age (number of days) of marron,

wt. = 2.2191 - 0.6623 x lipid level + 0.7919 x no. of days

ln wt. = 0.7075 - 0.1638 x lipid level + 0.2387 x no. of days

reveals two opposing trends: high dietary lipid levels retard growth; and increasing growth with time. The number of days appeared to be a better predictor of weight than dietary lipid levels. The growth pattern response to three different lipid levels is affected by the age of the animals. Anger (1990) and Ackefors *et al.* (1992) have reported differences in the relative efficiency of lipid utilization with age in crustaceans. In this study the results showed that dietary lipid levels had no influence on the weight during the first 27 days of the trial (Table 2.3). This indicates that it took a considerable time (14 days of acclimatization time plus 27

days of first observation time) for dietary lipids to overcome the effect of lipids stored in the body.

At lower dietary lipid levels (D_1), the SGR started declining after 81 days of trial. At higher dietary lipid levels (D_2 and D_3), the SGR remained static after 54 days of trial indicating that, with increasing age, the animals can respond favourably to higher dietary lipid levels (Ackefors *et al.*, 1992). If the trial had been continued for longer, the SGR of animals fed diet D_1, might have declined below the SGR of marron fed diet D_2. The results also showed that survival was not related to the dietary lipid levels but was a function of time.

Clarke and Wickins (1980) concluded that animal lipid is probably an essential component in the diet of a marine shrimp, *P. merguiensis*, but this study and previous research of Davis and Robinson (1986) and Sheen and D'Abramo (1991), for example, on crayfish and other freshwater crustaceans indicated that there are no optimal dietary animal lipid levels required. Rather, the optimal amount of dietary lipid intake depends upon the fatty acid profile (Reigh and Stickney, 1989), and the amount and quality of dietary protein and energy. Andrews *et al.* (1972), Hajra *et al.* (1988) and Ackefors *et al.* (1992) showed that, not only are protein/energy ratios important in feed formulation of crustacean diets, but lipid/carbohydrate ratios also affect growth and survival. Clifford and Brick (1978, 1979) suggested that the optimum dietary lipid/carbohydrate ratio for *M. rosenbergii* was between 0.25 and 0.33. Fish can derive benefits from increased dietary lipid levels up to 20-30% but in the diets for younger crayfish, the lipid/carbohydrate ratio must be lower than 0.4 (Ackefors *et al.*, 1992). In the present trial, a lipid/carbohydrate ratio of more that 0.2 (diet D_1 and D_2) impaired the growth as juvenile marron seemed unable to utilize the additional dietary lipid levels.

Ackefors *et al.* (1992) showed that lipid levels above 10% in diets depressed growth in juvenile crayfish, *A. astacus.* Davis and Robinson (1986) found that optimal growth occurred at a dietary lipid level of 6% in *P. acutus acutus.* Kanazawa *et al.* (1977), using powdered pollack residual oil as a lipid source for *P. japonicus,* found that optimal growth occurred at an inclusion level of 8% (dry weight). The weight gain was lower when the dietary level was increased to 16%. When the oil content of a diet for *Palaemon serratus* was increased from 7.5% to 15%, the growth rate was significantly reduced (Forster and Beard, 1973). Similarly, Deshimaru *et al.* (1979) demonstrated an optimal dietary level of 6% for *P. japonicus* and found that growth rates were reduced at higher levels.

The mechanism underlying the growth depression observed at high dietary lipid levels is not well understood. The reduced growth response was probably due to insufficient utilization of protein, particularly when other energy sources are available. Under these conditions protein may serve as an energy source. In this trial, consumption was not determined primarily because it was assumed that intake would be more or less equal among treatments since all diets were isoenergetic. However, it appeared that the consumption rate of diet D_3 declined with time, though the small quantities of diet and sub-gravel filtration system in the aquaria made it difficult to quantify reliably and quantitatively the feed consumption rates. The cod liver oil in the diet may have negatively influenced the ingestion rate and digestibility of the diet because of its fatty acid profile.

2.4.2 Condition Indices

Jussila (1997) compared the moisture percentages, wet and dry hepatopancreas indices of noble crayfish (*A. astacus*), signal crayfish (*P. leniusculus*) and marron (*C. tenuimanus*) both of wild capture specimens and also reared in a battery culture environment. He did not observe a consistent relationship between specific growth rates and these indices. Dry weight of hepatopancreas in relation to total wet body weight is considered to be an indicator of physiological state of the animal as well

as a nutritional value of a diet (Speck and Urich, 1969; Marsden *et al.*, 1973; Hazlett *et al.*, 1975; Regnault, 1981), even after a few days (Vogt *et al.*, 1985). In the present trial, D_2-fed marron had the best physiological state based on H_{id} T/B_d indices.

Table 2.12. **Proximate composition (*after converting wet weight into dry weight basis*) of the of freshwater crayfish listed. (*data adapted from Anon (1990) and Huner et al. (1997).***

Proximate composition (g/100 g dry weight)	*C. tenuimanus*	*P. clarkii* (whole animal)	*C. destructor* (whole animal)
Protein	46.5-87.3	85.5-86.3	39.3-47.3
Fat	2.9-5.1	5.9-14	3.0-3.6
Ash	6.4-24.5	5.5-6.4	28.0-33.5
Carbohydrate	5.7	< 4.8	-
Moisture	78.0	79.6-80.0	-

2.4.3 Proximate Composition

Lipids are the main energy reserves in hepatopancreas, often showing an inverse relationship with moisture content (Jussila and Mannonen, 1997). An inverse relationship between moisture and lipid content was not evident in the hepatopancreas of marron in the present trial although a direct relationship between moisture and lipid content was evident in tail muscle tissues. Apparently, nutritional stress caused by the lack of certain nutrients and/or stress caused by the supplementation of cod liver oil in the semi-purified diets was the overriding factor causing increased moisture levels in the hepatopancreas and tail muscles of the juvenile marron. Lipid levels in hepatopancreas of marron fed diets D_2 and D_3 were higher than that reported by Huner *et al.* (1990) in wild caught *A. astacus* in central Finland. These workers reported lipid levels of 28.9 % in July 1987 and 47.5% in August 1988. Lipid levels of tail muscles in crayfish fed D_2 and D_3 were also higher than reported by Huner *et al.* (1990) in the same study but fell within the range reported for *C. tenuimanus* (Anon, 1990) and *P. clarkii* (Huner *et al.*,

1997) (Table 2.12). However, lipid levels in the tail muscles of marron fed D_1 are much lower compared with those values reported by Anon (1990). Huner *et al.* (1997) have compiled the proximate analysis of freshwater crayfish with special emphasis on cambarids.

2.4.4 Fatty acid Profile

At the end of the trial, due to the small sample size of lipids extracted from the tail muscles, it was not possible to determine the fatty acid profile of the tail muscles of the marron fed the experimental diets. However, the fatty acid profile of the hepatopancreas had a close correlation with the fatty acid profile of the pellet, which in turn predominantly followed the cod liver fatty acid profile. In penaeid prawns, qualitative requirements for PUFAs such as linolenic (18:3 ω-3) and linoleic (18:2 ω-6) acids have been documented (Guarry *et al.*, 1976; Kanazawa *et al.*, 1979a and b and c; Xu *et al.*, 1994). However, quantitative and qualitative requirements for any fatty acid have yet to be established for any freshwater crayfish. D'Abramo and Sheen (1993) have reported greater nutritive value of ω-3 series of HUFAs such as eicosapentaenoic acid (EPA; 20:5 ω-3) and docosahexaenoic (DHA; 22;6 ω-3) in *M. rosenbergii*. Guary *et al.* (1976), Kanazawa *et al.*, (1977, 1978; 1979a), Xu *et al.* (1994) have reported the same importance of ω-3 series of HUFAs in penaeid prawns.

Though the body tissues of the marron prior to feeding with the experimental diets contained linolenic, linoleic and eicosapentaenoic fatty acids, only linoleic acid was found in the hepatopancreas of marron fed the high percentage of cod liver oil in the diet (D_3). Inclusion of cod liver oil in the diet decreased the percentage of ω-9 (mainly mono-unsaturated fatty acid) in the hepatopancreas of marron. D'Abramo (1997) recommended that future experiments leading to the precise determination of fatty acid requirements should be directed toward the provision of graded levels of pure fatty acids in a triglyceride or methyl ester form, rather than through the

provision of oil rich in the fatty acids under investigation. As this was not the primary aim of the present trial, only indications concerning the qualitative requirements of these EFAs, can be made here.

In this trial, reduced growth rates under high dietary lipid levels could have been caused by the imbalance in the ratio of ω-3 to ω-6 by the addition of cod liver oil, which is rich in ω-3, highly polyenoic fatty acids (Gruger *et al.*, 1964). There is also evidence that freshwater animals have a requirement of ω-6 fatty acids compared to the ω-3 requirements of marine organisms (D'Abramo and Sheen, 1993). Colvin (1976a) showed that precise dietary levels and a balanced ratio of ω-3 to ω-6 type fatty acids might be necessary for efficient lipid metabolism. Recent studies on the nutrition of fish and shellfish have produced evidence implying a general requirement for long chain polyenoic fatty acids. The role played by the fatty acids of the ω-3 variety in alleviating EFA deficiency symptoms in fish, *Salmo gairdneri*, (Lee *et al.*, 1967, Castell *et al.*, 1972a and b, Owen *et al.*, 1972) and prawns, *Penaeus duorarum*, (Sick and Andrews, 1973) has been stressed. Castell and Covey (1976) used cod liver oil (high in 20:5ω-3 and 22:6ω-3) as a dietary lipid source for adult lobsters, *H. americanus*, and found a 5% lipid level to be optimal. They reported that cod liver oil produced superior growth and survival of lobsters compared to those fed corn oil (high in 18:2ω-6). Merican and Shim (1994) suggested that the fatty acid profile affects the digestibility of individual fatty acids and these effects were dependent on the oil source. Generally, the varying utilization of diets with crude or refined animal and plant lipids suggests that the nutritive value of lipids as dietary lipids is related to the content of free fatty acids and the composition of saturated fatty acids and long-chain unsaturated fatty acids. Marron fed 6% dietary cod liver oil (D_2) has shown higher hepatosomatic indices (both wet and dry) than marron fed with no dietary cod liver oil (D_1) and 12% dietary cod liver oil (D_3) indicates moderate requirements of EFAs supplied through cod liver oil. Sheen and D'Abramo (1991)

indicated that a dietary lipid level ranging from 2% to 12% under a wide range of dietary lipid: carbohydrate ratios was satisfactory for the juvenile freshwater prawn, *M. rosenbergii.* Suggestions for future research are discussed in Chapter 7.

CHAPTER 3

STOCKING DENSITY

3.1 Introduction

A major problem in the commercial culture of marron, also encountered with other crayfish (Lutz and Wolters, 1986), concerns the unpredictable yields resulting from growth at over or under optimal stocking densities. Typically, average weight declines with increasing density (Keller, 1988; Morrissy *et al.*, 1995a) but different culture conditions are likely to influence growth-density relationships.

To date, investigations concerning the effect of density on marron growth have utilized earthen ponds (Morrissy, 1992a, 1995a and b) and outdoor tanks (Whisson, 1995) where factors such as temperature, water quality, predators, food availability, diseases and even density itself may be highly variable and difficult to control. In contrast, a recirculating system can provide relatively stable water temperatures, greater management control and has been used extensively in freshwater crayfish research (Morrissy, 1979; Mills and McCloud, 1983; Kartamulia and Rouse, 1992; Du Boulay *et al.*, 1993).

The aims of the present study were to establish the optimal stocking density giving commercially acceptable growth and harvest rates in a recirculating culture system and to establish the stocking densities for conducting future growth trials in cages under field conditions. This experiment evaluated the effect of stocking density on growth, survival and harvest rates, biomass increment and size frequency distribution under these conditions.

This Chapter has formed the basis of a paper (Fotedar *et al.*, 1998) accepted for the publication in the Journal of Applied Aquaculture and the format of the Chapter reflects the journal format.

3.2 Materials and Methods

3.2.1 Experimental System and Design

The trial was carried out in a greenhouse, at Marron Force Farm, Perth, Western Australia (Figure 1.2). Five cement tanks (5.5 m x 3.5 m x 0.65 m) connected to a recirculating system were used in this trial. Water was distributed separately to each of the tanks through a spray bar from a 40,000 L sump, and was returned to the sump through a biological raceway filter composed of gravel, stones and aquatic plants, ribbon weed (*Vallisneria* spp.) and banana plant (*Nymphoides aquatica*). Each tank was aerated using a mechanical blower.

Plate 3.1 Greenhouse (hot house) where stocking density trial was conducted.

Before the trial commenced, the tanks were dried, scrubbed clean and refilled with ground water supplied *via* a bore. Water was well oxygenated for two days in the main distribution sump prior to the filling of the tanks. Twelve cages (2.2 m x1.5 m x 0.75 m) were fabricated from extruded general purpose nylon, with a mesh size 6 mm^2 wrapped over reinforced PVC pipes. Three cages were placed in each of the four cement tanks. An extra cage with reserve marron was kept in the fifth tank to replace marron that died during acclimatization.

The marron harvested from an earthen pond, (T.B.S. Pastoral Company, Red Hills, Western Australia), were transported in polystyrene boxes to the experimental site and placed directly into the cages within two hours of capture. PVC pipes and onion bags placed inside the cages provided shelter. An initial trial was conducted for one month to evaluate the suitability of the cages for rearing juvenile marron, the effect of the feed on the nutrient load in the system and the efficiency of the biological filter.

At the start of the trial, marron were randomly distributed among twelve cages at three different stocking densities (3, 6, 13 $/m^2$). Each stocking density was replicated four times, each replicate being represented randomly in each cement tank. Test animals were acclimatized for three weeks. At the end of the acclimatization period, survival and initial weights were recorded. To keep the stocking densities constant with each treatment, similar sized animals from those held in reserve in the fifth tank were used to replace the animals that died during the acclimatization period. The marron were 3-month old at the beginning of the trial.

Marron pellets with improved water stability (Table 3.1) were prepared especially for the study. Marron were fed twice weekly at a rate of 10% body weight per week. At the end of the acclimatization period, survival and initial weights were

recorded. The amount of food was increased every ten days by taking samples of marron and adjusted for weight increment. No uneaten food was observed.

Table 3.1 Ingredients (%) and proximate analysis of marron pellets used in the trial.

Ingredients	Inclusion level %	Nutrients	Analysis
Soyabean meal	15.25	Protein	30.73
Lupinseed meal	19.17	Lipid	5.50
Oat Groats	29.81	Crude Fibre	5.90
Fish meal	20.33	Calcium [a]	1.92
Mill Run	10.00	Phosphorus [a]	0.70
Binder	2.00	Salt [a]	0.37
Fish Premix	0.50	Metabolized Energy (MJ/Kg) [a]	10.50
Dicalcium Phosphate	0.29		
Limestone	2.68		
di- Methionine	0.07		

a - Calculated according to the known nutrient composition of the ingredients used

3.2.2 Water Quality

Temperature, pH and dissolved oxygen in all cages were recorded twice weekly at approximately 08.00 hours. Total ammonia, nitrite, nitrate, total hardness, and alkalinity were recorded monthly. Ionic composition was analyzed using an atomic absorption spectrophotometer (Varian /AA-875) before the commencement and at the end of the experiment (*Appendix 1,Table II*). Ten percent of the total volume of fresh water was added once every two weeks to compensate for evaporation losses and to reduce nutrient concentration following episodic rises in nitrite levels. Water was exchanged when required to prevent nitrate levels from exceeding 25 mg/L.

3.2.3 Recording and Data Analysis

For each measurement, tanks were drained and all marron were collected for counting and weighing. Individual wet weights (grams to two decimal places) and numbers of marron were measured on days 0, 80 and 160. On these occasions, tanks were drained and all marron counted and weighed. Growth, survival,

biomass, biomass increase per day, harvest rates and size frequency distribution were calculated over two 80 day intervals, i.e. days 0-80 and 80-160 of the trial. Specific growth rate (SGR; % d^{-1}) as described by Hopkins 1992, survival rate (%), harvest rate (g/m^2) and biomass increase per day (g/day) were calculated using the following formulae:

SGR	=	$100 (\ln w_t - \ln w_o) / t$
where w_t	=	weight at time t, calculated after 80 and 160 days
w_o	=	initial weight of the marron at day 0 and 80.
survival rate	=	$100 (n_t / n_o)$
n_t	=	number of marron at time t, calculated after 80 and 160 days
n_0	=	number of marron at day 0 and 80.
harvest rate	=	biomass/area
biomass increase per day	=	$(BM_t - BM_0)/t$
BM_t	=	biomass of total marron at time t, calculated after 80 and 160 days
BM_0	=	total biomass of marron at day 0 and 80.

The data were analyzed statistically using a one-way ANOVA, (initial weight as covariates) and the LSD multiple-comparison test to identify significant differences among treatment means (Steel and Torrie, 1960, 1980). Statistical analysis (One-way ANOVA) was performed to test for significant differences in initial weights of marron among the cages and to detect whether any significant differences in water quality developed among the cages during the trial.

3.3 Results and Discussion

Temperatures in all cages ranged between 19.5 ^{o}C to 26.8 ^{o}C, which covers the optimal temperature of 24 ^{o}C (Morrissy, 1990) for the growth of marron. Dissolved oxygen content of the experiment tanks, near saturation throughout the experiment, averaged between 7.8 and 10.6 mg/L. The lowest dissolved oxygen value recorded was 5.5 mg/l at 23 ^{o}C, well above the incipient lethal oxygen level

(Morrissy *et al.*, 1984). Total ammonia, nitrate, pH and dissolved oxygen (*Appendix 1, Table II*) ranged near the optimal levels for freshwater animals. Water was exchanged to prevent nitrate levels exceeding 25 mg/L, since higher concentrations can generate filamentous algal blooms. Therefore, differences in harvest weight, growth, survival and harvest rates of juvenile marron probably due to density differences ($P < 0.05$).

3.3.1 Harvest Weight

At the conclusion of the trial, the highest mean weight (29.28 ± 2.50 SE g) was achieved at the density 6/m^2, compared with mean weights of 23.01 ± 1.23 SE g and 21.08 ± 1.78 SE g at densities of 3 and 13/m^2, respectively (Table 3.2).

Table 3.2 **Changes in wet weight (mean ± SE g) of juvenile marron with time at three stocking densities.**

Number of days	Stocking density		
	3/m^2	6/m^2	13/m^2
0	3.24 ± 0.15 ^{a}	3.11 ± 0.09 ^{a}	3.06 ± 0.06 ^{a}
80	9.82 ± 0.98 ^{a} (2.76 ± 0.16 ^{a})	11.82 ± 0.86 ^{a} (5.28 ± 0.47 ^{a,b})	7.02 ± 0.47 ^{b} (7.22 ± 1.51 ^{b})
160	23.01 ± 1.23 ^{a} (2.27 ± 0.13 ^{a})	29.28 ± 2.50 ^{b} (3.81 ± 0.36 ^{a})	21.08 ± 1.78 ^{a} (3.00 ± 0.86 ^{a})

a,b - Values in the same row having different superscripts are significantly different ($P < 0.05$). Values in parenthesis are surviving stocking densities in numbers/m^2 (mean number/m^2 ± SE).

These results indicate an optimum harvest weight at the intermediate density of 6/m^2 and thereby differ markedly from previous reports of an inverse relationship between mean harvest weight and stocking density of marron (Morrissy, 1974, 1979; Whisson, 1995) and *Paranephrops planifrons* (Hopkins, 1966). It is perhaps

important to note that the mean weight of 10-month old marron used at the commencement of Whisson's study (6.48-6.81 g) was significantly lower than the final mean weight of 8-month marron in the present study. The mechanisms by which density affects growth rates have to be elucidated, but physical and behavioural factors have been implicated for the density effect on growth in *Orconectes virilis* and *P. clarkii* (Momot and Jones, 1977; McClain, 1995a and b). Schmittou (1970) observed higher growth in channel cat fish (*Ictalurus punctatus*) under high stocking density in floating cages. At lower stocking density, fighting among the fish to establish a hierarchical order resulted in slower growth.

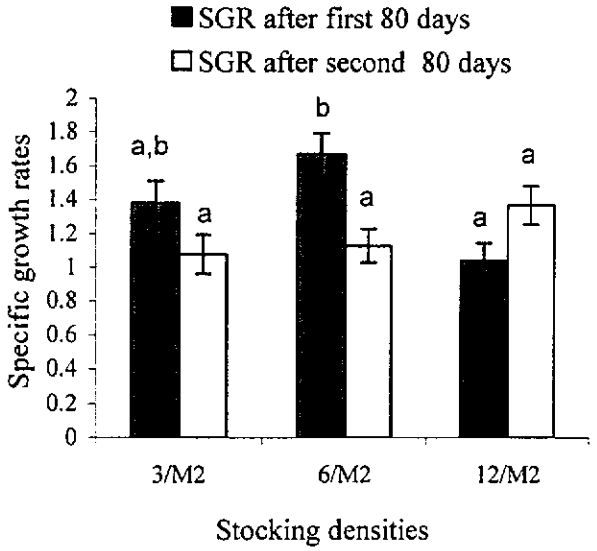


Figure 3.1 **Comparison of specific growth rates (mean ± SE %/day) after first and second 80 day growth periods of juvenile marron reared at three different stocking densities. a,b – different letters indicate significantly different values (P<0.05) across two different lines.**

3.3.2 Growth and Survival

As the growth rate of marron during the trial was exponential, the growth rates were expressed as SGRs, with significant differences ($P < 0.05$) between marron stocked at $6/m^2$ and marron stocked at 3 and $13/m^2$ (Figure 3.1), after the first 80 day trial. However, there were no significant differences in SGR among any stocking densities at the end of the second 80-day trial period or at the end of the

trial (using the160-day period as one continuous growth period). Insignificant differences in SGR at the end of the trial may be attributable to the fact that survival densities of marron had adjusted to equilibrium surviving densities (Table 3.2) as governed by the carrying capacity of the culture system. This suggested that, in a given area, there was a natural regulation of numbers, which was influenced by the carrying capacity of the culture system. Goyert and Avault (1978) indicated that in crawfish, *P. clarkii*, growth was independent of stocking density and was influenced predominantly by different substrates.

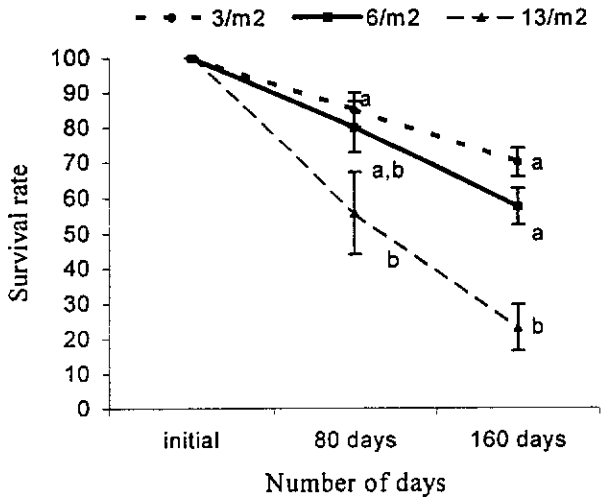


Figure. 3.2 **The survival rate (mean ± SE %) of juvenile marron reared at three stocking densities. a,b – different letters indicate significantly different values (P < 0.05) across different lines.**

Whisson (1995) found no difference in survival of 10-month-old marron among 1, 3, 5, and 7/m^2 densities, whereas in the present trial, the survival rate of marron at lower stocking densities of 3 and 6/m^2 was significantly higher ($P < 0.05$) than at the stocking density of 13/m^2 (Figure 3.2 and Table 3.2). Since no dead marron were found when tanks were drained, it is assumed that animals which died were cannibalized. The higher stocking density may have led to more aggressive encounters and higher than normal mortality rates without influencing SGR of the marron. Mills and McCloud (1983) showed that survival of *C. destructor* was

dependent on stocking densities. However, Morrissy (1992a) indicated that initial stocking density does not closely influence the final yield in *C. tenuimanus*, instead it serves to place the surviving density within a lower or higher range where it influences cohort mean weight.

3.3.3 Harvest Rate and Biomass

The harvest rate at the stocking density of 6/m^2 was 101.32 g/m^2 after 160 days, (equivalent to production estimates of approximately 2.3 tonnes per hectare per year of production), which is significantly higher ($P < 0.05$) than the harvest rates at stocking densities of 3 and 13/m^2 (Table 3.3). It is possible that with further grow-out time higher values could be obtained. The stocking densities of 3 and 13/m^2 gave similar harvest rates of 48.7 and 58.7 g/m^2 respectively (equivalent to production estimates of 1.11 and 1.34 tonnes per hectare per year). The harvest rate of 101.32 g/m^2 at the stocking density of 6 /m^2 is commercially acceptable. The harvest rate at the stocking density of 13/m^2, after 80 days of trial, was not significantly different ($P<0.05$) from stocking densities of 3 and 6/m^2. In fact, harvest rates at stocking densities of 3 and 6/m^2 increased as the trial progressed but the harvest rates at the stocking density of 13/m^2 remained static, indicating the operation of some self-regulatory mechanism in place at higher stocking densities. Self-regulation of the harvest rate may be mediated through various behavioural and physiological factors like vulnerability to cannibalism, nutritional deprivation, and release of growth-inhibitory pheromones, for example. Morrissy *et al.* (1995b), deriving a mathematical equation relating harvest and stock densities using a log-log model, showed their mean weight at harvest was inversely related to surviving density. However, further research is required to investigate the underlying factors controlling the harvest rates.

Biomass increase per day also showed a significant increase ($P < 0.05$) at the

stocking density of 6/m^2 after 160 days of trial (Table 3.3). However, the increase was significantly higher only during the first 80 days of trial and did not change during the second 80 days of the trial (Figure 3.3).

Table 3.3 **S.G.R. (mean ± SE %/day), survival (mean ± SE %), biomass increase (mean ± SE g/day) and harvest rate (mean ± SE g/m^2) of juvenile marron at three stocking densities at the end of the trial.**

Index	Stocking density		
	3/m^2	6/m^2	13/m^2
SGR	1.23 ± 0.06 ^{a}	1.39 ± 0.07^a	1.20 ± 0.06 ^{a}
Survival	70.00 ± 4.08 ^{a}	57.50 ± 5.20 ^{a}	23.13 ± 6.64 ^{b}
Biomass increase/day	0.80 ± 0.076 ^{a}	1.70 ± 0.22 ^{b}	0.45 ± 0.34 ^{a}
Harvest rate	48.68 ± 3.31 ^{a}	101.32 ± 10.55 ^{b}	58.78 ± 16.60 ^{a}

a,b - Values in the same row having different superscripts are significantly different (P < 0.05).

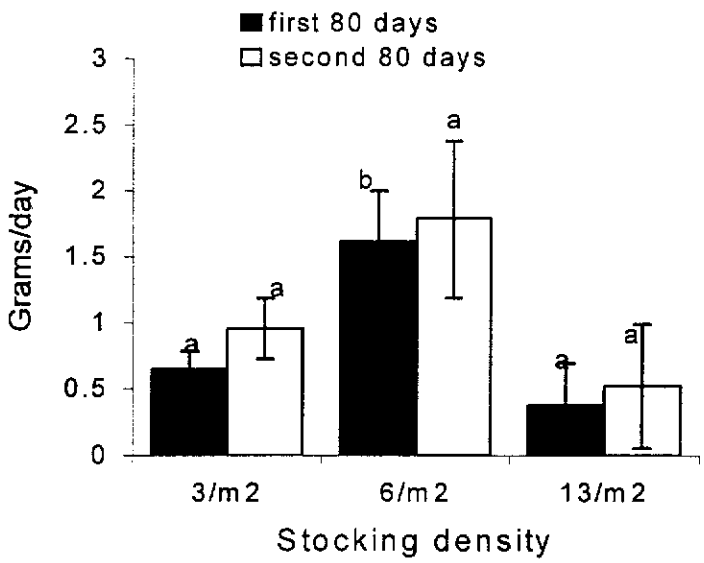


Figure.3.3 **Comparison of biomass increase per day (mean ± SE g/day) after first and second 80 day growth periods of juvenile marron reared at three stocking densities. a,b – different letters indicate significantly different values (P<0.05) across two different time periods.**

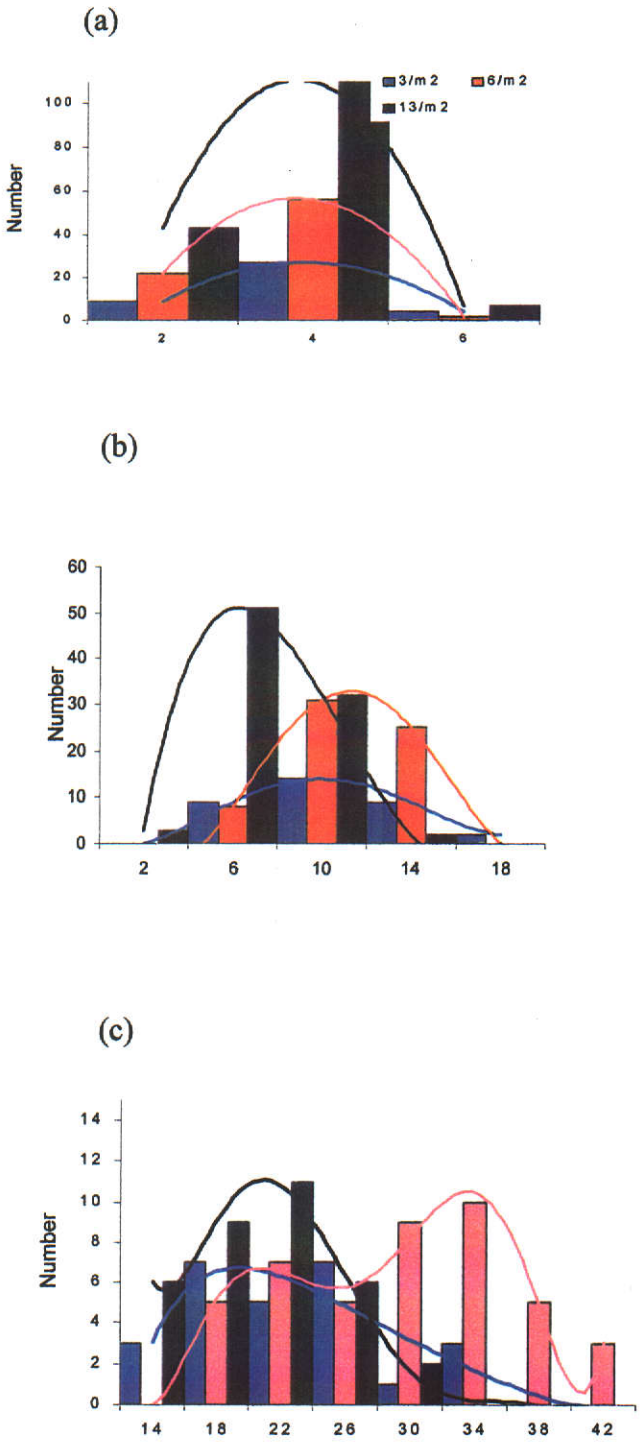


Figure 3.4 **Size-frequency distribution of juvenile marron, stocked at three densities. (a) before the commencement of the trial, (b) after 80 days of trial and (c) after 160 days of trial.**

3.3.4 Size Distribution

Profitable marron farming depends largely on the size distribution of stock under culture because of size-related marron prices. Therefore, it is important to understand the density related size distribution in the present trial. Marron at the beginning of the experiment had a normal weight distribution with most ranging between 3 to 5 g (Figure 3.4). However, at the end of the trial, the weight distribution varied among animals in the three densities. At the density of 6 /m^2, animals had a final weight range of 16-44 g showing two distinct peaks at 20-24 and 32-36 g. The weight distribution was broader at the lowest stocking density of 3/m^2 compared with the other two stocking densities. There were more animals in the size range of 13-25 g at densities of 3 and 13 /m^2. The distribution was negatively skewed at the 6/m^2 stocking density after 80 days, whereas in all other densities it was always positively skewed. At the end of the trial, the distribution was less positively skewed at the stocking density of 6/m^2 indicating more, bigger animals in the distribution. The standard deviation around the mean increased progressively from 80 days to 160 days (Figure 3.4). The trial had two distinct phases, the first phase of 80 days where initial densities were more discrete and more uniform sizes pertained. Less discrete densities but non-uniform size distribution characterized the second 80-day phase. Thus, the response of marron to these two distinct parameters during these phases may be different.

Stocking density combined with higher water temperatures will influence the growth-density relationship in marron (Morrissy *et al.* 1990). A closed recirculating water system operating within a greenhouse environment offers considerable potential for the commercial production of marron. A two-fold increase in yearly crop production over marron raised in earthen ponds may be achieved by maintaining optimum culture conditions on a year-round basis. These results imply that, under semi-controlled culture conditions, rearing marron at a stocking density of 6/m^2 may achieve better harvest rates.

CHAPTER 4

SUPPLEMENTATION OF FISH AND PLANT OIL

4.1 INTRODUCTION

Various oils differ in their fatty acid profiles. Polyunsaturated fatty acids of the ω-3 series (18:3 ω-3; linolenic) and ω-6 series (18:2 ω-6; linoleic), as well as highly unsaturated fatty acids such as, docosahexaenoic acid (22:6 ω-3; DHA) and eicosapentaenoic acid (20:5 ω-3; EPA) have been recognized as important nutrients for the growth of crustaceans (D'Abramo and Sheen, 1993). Read (1981), showed that growth in the Indian white prawn (*P. indicus*) improved when fed purified diets supplemented with both fish oil (rich in ω-3 fatty acids) and sunflower oil (rich in ω-6 fatty acids). However, no information is yet available of a relationship between the qualitative requirements of polyunsaturated fatty acids (PUFAs) and growth and survival in marron.

Rearing conditions can influence the impact of nutrients on the growth performance of freshwater crayfish. The supplementation of certain oils to the semi-purified diet fed to marron reared under controlled laboratory conditions may have different effects on their growth performance compared to marron fed the same diet and reared under farm conditions. Fotedar *et al.* (1997) reported that the supplementation of cod liver oil (containing comparatively high levels of $C \geq 20$ ω-3 PUFAs) to semi-purified diets has an inhibitory effect on the growth performance of marron reared under laboratory conditions. In contrast, Sandifer and Joseph (1976) reported a growth-enhancing response to dietary shrimp head oil (high in ω-3 series of fatty acids) for the freshwater prawn, *M. rosenbergii*, reared under laboratory conditions.

The aim of the experiment was to investigate the hypothesis that dietary supplementation of cod liver oil and sunflower oil to practical diets (diets formulated with locally available ingredients) effect the growth, survival and condition indices of juvenile marron reared in a cage environment under farm conditions.

4.2 MATERIALS AND METHODS

4.2.1 Experimental System and Design

The trial was carried out at a commercial marron farm located at Parkerville, Western Australia, situated approximately 45 km north-east of Perth (Figure 1.2). An existing, purpose-built marron pond (40 m x 25 m x 1-1.5 m) with a gentle slope of 3:1 and drainage pipe fitted with stand-pipe on its deepest end was used in the trial. The pond floor and sides were covered with 2 mm thick UV stable plastic sheet to prevent the seepage of water. Initially, the pond was filled with water for a period of one month to stabilize its banks. All water was then completely drained out and the pond scrubbed clean, dried and refilled with water pumped from an adjacent dam. Sixteen cages, including 13 cages used in the experiment described in Chapter 3, and three new similar cages were employed for this trial. The cages were then placed randomly in the pond and 20 PVC off-cuts and 10 small mats of nylon fibre were used to provide the marron with adequate shelter. Twenty juvenile marron were selected randomly and placed in each cage while the remainder were kept in another circular tank. The marron were then fed the experimental diet (Tables 4.1 and 4.2) in a 4 x 4 experimental design, so that four randomly placed cages received one experimental diet. The marron were fed *ad libitum* which worked out to be approximately 2% of the body weight every alternate day. All cages were allowed to drift freely in the pond to avoid any positioning effect. Further, the cages were randomly reshuffled in the pond every alternate day.

Table 4.1 **Ingredients (%) of the four test diets.**

Ingredients	Diet D_1	Diet D_2	Diet D_3	Diet D_4
Barley	50.6	46.6	46.6	46.6
Binder	2.0	2.0	2.0	2.0
Blood Meal	3.0	3.0	3.0	3.0
Dicalcium Phosphate	1.0	1.0	1.0	1.0
Dried Yeast	5.0	5.0	5.0	5.0
Limestone	1.0	1.0	1.0	1.0
Mill Run	10.0	10.0	10.0	10.0
Molasses	2.0	2.0	2.0	2.0
Salt	0.4	0.4	0.4	0.4
Skim Milk Powder	5.0	5.0	5.0	5.0
Soyabeal Meal	20.0	20.0	20.0	20.0
Cod liver Oil	0.0	4.0	0.0	2.0
Sunflower Oil	0.0	0.0	4.0	2.0

4.2.2 Experimental Animals

Six hundred juvenile marron weighing between 1 and 4 g were collected from a dam in the same farm at Parkerville. Their individual wet weight was recorded. All the marron were fed the diet described in Table 3.1, for a period of 20 days and then starved for another 10 days. On the 30th day, the cages were dragged out of the pond, labeled and all marron were counted and weighed individually. The original stocking density of 20 marron per cage was restored by replacing dead or escaped marron with specimens of similar weight from the reserve circular tank. Escaped marron represented another treatment group (free-range marron) and were not fed formulated feed.

Table 4.2 **Major nutrient analysis (%) of the four test diets.**

Nutrients	Diet D_1	Diet D_2	Diet D_3	Diet D_4
Protein	22.27	21.87	21.87	21.87
Lipid	2.10	6.02	6.02	6.02
Crude Fibre	4.77	4.57	4.57	4.57
Calcium [1]	0.78	0.78	0.78	0.78
Phosphorus [1]	0.64	0.63	0.63	0.63
Salt [1]	0.65	0.64	0.64	0.64
Metabolized energy (kJ/g)	10.64	11.52	11.52	11.52
Lysine [1]	1.43	1.41	1.41	1.41
Methionine [1]	0.37	0.37	0.37	0.37
Cystine [1]	0.27	0.27	0.27	0.27
Threonine [1]	0.88	0.87	0.87	0.87
Tryptophane [1]	0.29	0.29	0.29	0.29
Leucine [1]	1.63	1.60	1.60	1.60
Isoleucine [1]	0.97	0.95	0.95	0.95
Arginine [1]	1.34	1.32	1.32	1.32
Histidine [1]	0.70	0.68	0.68	0.68
Tyrosine [1]	0.67	0.66	0.66	0.66
Phenylalanine [1]	1.63	1.61	1.61	1.61
Valine [1]	1.56	1.14	1.14	1.14
Sodium [1]	0.26	0.25	0.25	0.25
ω-6 [1]	0.93	0.99	2.89	1.94
ω-3 [1]	0.12	1.14	0.23	0.69

[1] – Data calculated according to the known nutrient composition of the ingredients used.

4.2.3 Diet Ingredients, Formulations and Characteristics

Four isoenergetic and isonitrogenous diets (D_1, D_2, D_3, and D_4) were prepared using the practical ingredients described in Table 4.1. Purified cod liver oil (4%) (Glen Forrest Stockfeeders, Great Eastern Highway, WA), was added to diet D_1 to prepare diet D_2, 4% of purified sunflower oil (Glen Forrest Stockfeeders, Great Eastern Highway, WA), was added to D_1 to prepare D_3 and a mixture of 2%

purified cod liver oil and 2% sunflower oil was added to diet D_1 to prepare diet D_4. Crude protein was determined by the Kjeldahl method and a cold extraction procedure using chloroform: methanol extract was used to determine the total lipid content of the diets (Table 4.2). The biochemical analyses of the experimental diets were performed using procedures described in Section 2.2.4.

Plate 4.1 **The plastic-lined pond where supplementation of cod liver oil and sunflower oil trial was conducted.**

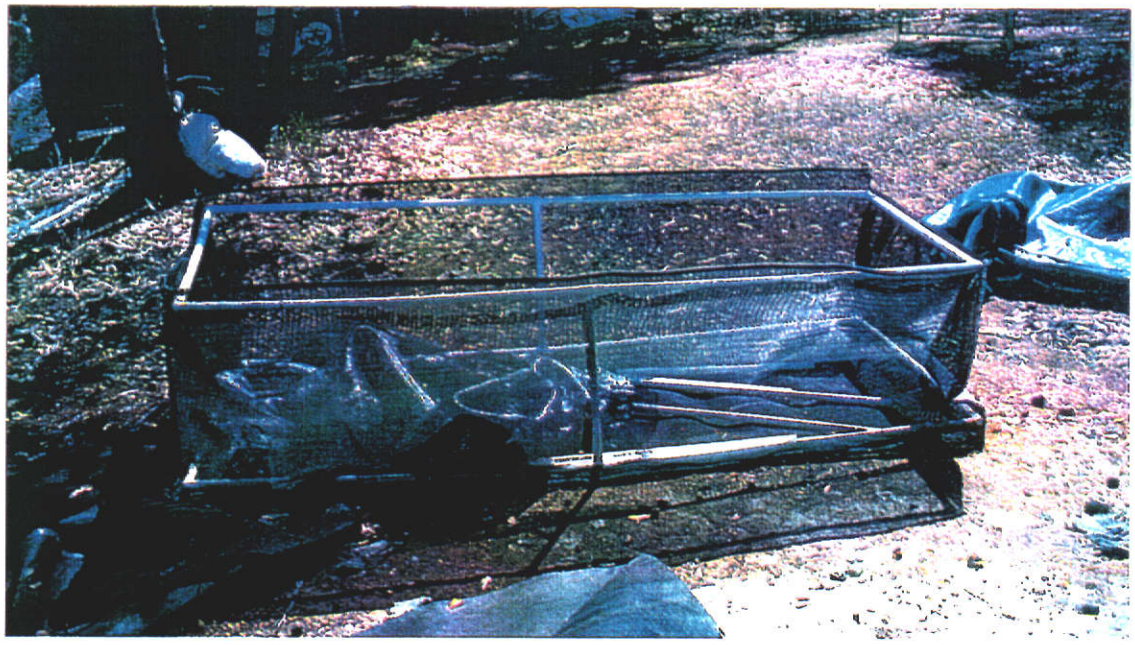


Plate 4.2 **One of the cages used in the trial.**

4.2.4 Water Quality

Temperature, pH and dissolved oxygen levels of the pond water were recorded once weekly. Total ammonia, nitrite, nitrate, total hardness, and alkalinity were recorded monthly. The ionic composition was analyzed using an atomic absorption spectrophotometer (Varian/AA-875) before the commencement and at the end of the experiment (*Appendix 1,Table III*). Twenty percent of the total volume of fresh water was replaced once every month to compensate for evaporation loss and to reduce nutrient concentrations

4.2.5 Recording and Analysis of Data

After 160 days the marron were harvested for counting and weighing to 0.1 gram. Five marron from each cage were immediately placed on ice in an eskee for later moisture and biochemical analyses. The SGR, survival percentage, harvest rate and biomass increase per day were calculated using the formulae described in Chapter 3. The wet and dry hepatosomatic indices, hepatopancreas moisture levels (%), wet and dry tail muscles to wet body weight ratios, and tail muscles moisture (%) were measured as described in Chapter 2.

The data were analyzed statistically using a oneway ANOVA (initial weight as covariates) and the LSD multiple-comparison test was used to identify significant differences among treatment means (Steel and Torrie, 1960,1980). Oneway ANOVA was performed to test for significance of differences in initial weights of marron among the cages.

4.3 RESULTS

4.3.1 Water Quality

Weekly mean water temperature in the pond during the six month period fluctuated between 11 to 19.5 °C, levels which promote no to moderate growth in marron (Morrissy, 1990). Marron cease to grow at temperatures below 12.5 °C and

attain maximum growth rate at 24 °C. Dissolved oxygen levels (6.8 to 10.6 mg/L) remained near 100% saturation at these temperatures. All other water quality parameters including nitrogenous metabolites (ammonia, nitrite and nitrate) were in the range of optimum level for aquatic animals (*Appendix1, Table III*).

4.3.2 Final Weight, Survival and Growth

Marron receiving no supplementary oil in their diet (D_1) grew to a significantly smaller ($P < 0.05$) mean final weight of 7.31 ± 0.46 SE g than for juvenile marron fed diets D_2 (10.6 ± 0.39 SE g), D_3 (9.60 ± 0.49 SE g) and D_4 (11.70 ± 0.62). Further, survival of the marron fed D_1 (51.67 ± 8.44 SE %) was significantly lower than marron receiving diets supplemented with cod live oil (D_2; 77.50 ± 8.32 SE %) and a mixture of cod liver and sunflower oil (D_4; 73.33 ± 2.72 SE %/day) (Table 4.3). Mean harvest weights of marron receiving diets D_2 and D_4, as well as free - range marron outside the cages (which received no direct formulated feed), were not significantly different. Marron receiving dietary supplementation with sunflower oil had a lower mean final weight (9.60 ± 0.49 SE g) (Table 4.3) than marron receiving the mixture of two oils (11.70 ± 0.62 SE g) (Table4.3). After six months, there were no significant differences in the SGR among marron receiving either cod liver oil, sunflower oil or a mixture of these two oils. Marron receiving no oil supplementation in their diet (D_1) showed the lowest SGR of 0.64 ± 0.08 SE %/day (Table 4.3). The difference in the SGR between the marron receiving no oil and those receiving supplementary sunflower oil was not significant ($P > 0.05$). Marron survival at the end of the trial showed the same trend.

4.3.3 *Biomass*

Marron receiving diet D_4 showed the significantly ($P < 0.05$) highest final biomass of 858.83 ± 17.9 SE g (Table 4.3), equivalent to a production level of 4.4 tonnes per hectare per year. At the end of the trial, marron receiving diet D_1 showed the significantly ($P < 0.05$) lowest final biomass of 377 ± 60.97 SE g (Table 4.3).

Marron receiving diet D_4 also yielded significantly highest biomass increase/day of 4.37 ± 0.10 (Table 4.3). The biomass increase/day was the significantly lowest (1.71 ± 0.36 SE g) in marron receiving diet D_1.

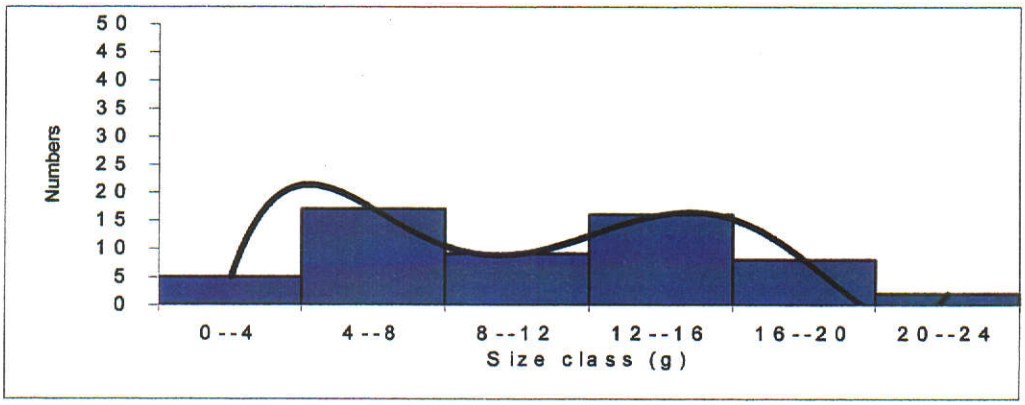
Table 4.3 **Initial weight (mean ± SE g), final weight (mean ± SE g), SGR (mean ± SE % / day), survival (mean ± SE %), biomass (mean ± SE g) and biomass increase per day (mean ± SE g / day) of juvenile marron fed the four test diets.**

Index	D_1	D_2	D_3	D_4	No formulated feed (free-range)
Initial weight	2.30 ± 0.13	2.40 ± 0.11	2.30 ± 0.04	2.40 ± 0.03	2.40 ± 0.10
Final weight	7.31 ± 0.46 ^{a}	10.6 ± 0.39 ^{b,c}	9.60 ± 0.49 ^{b}	11.70 ± 0.62 ^{c}	11.00 ± 0.70 ^{c}
SGR	0.64 ± 0.08 ^{a}	0.83 ± 0.02 ^{b}	0.75 ± 0.04 ^{a,b}	0.88 ± 0.03 ^{b}	0.85
Survival %	51.67 ± 8.44 ^{a}	77.50 ± 8.32 ^{b}	66.67 ± 1.36 ^{a,b}	73.33 ± 2.72 ^{b}	
Biomass	377.50 ± 60.97 ^{a}	823.8 ± 67.50 ^{c}	588.67 ± 28.29 ^{b}	858.83 ± 17.90 ^{c}	
Biomass increase/day	1.71 ± 0.36 ^{a}	4.18 ± 0.39 ^{c}	2.89 ± 0.14 ^{b}	4.37 ± 0.10 ^{c}	

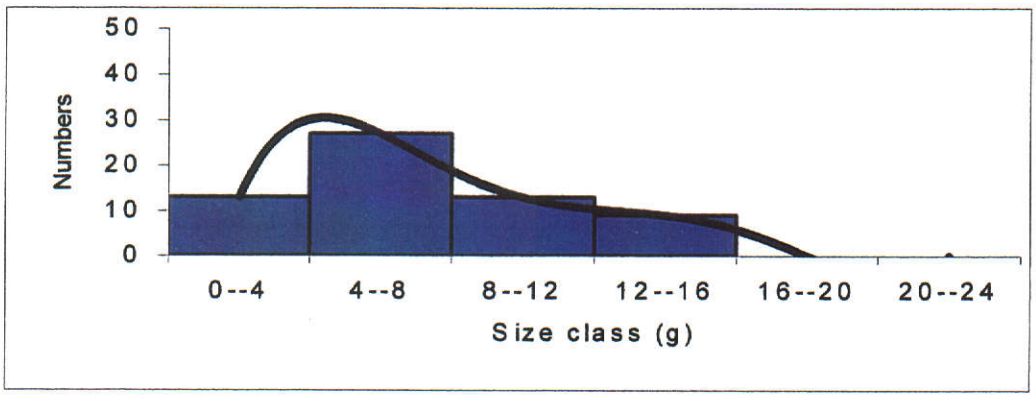
a, b, c values in the same row having different superscripts are significantly different ($P < 0.05$).

4.3.4 Size Distribution

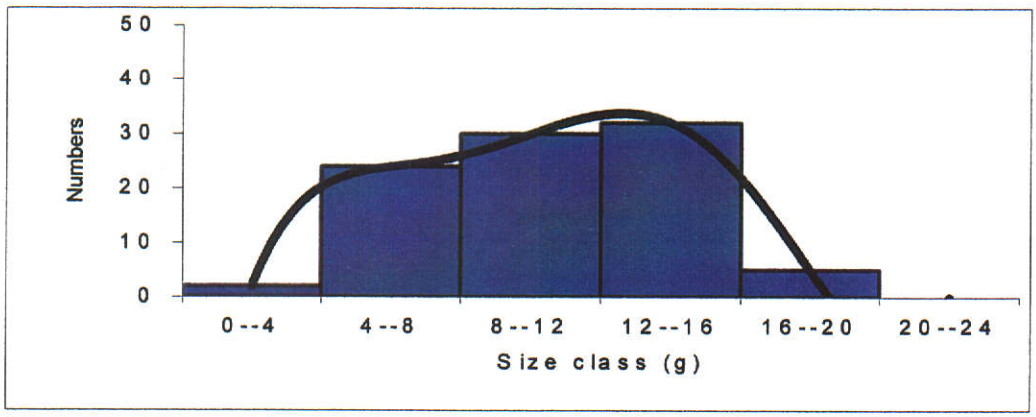
At the end of the trial, marron receiving diets D_1 and D_3 had a positively skewed size distribution, whereas marron receiving diets D_2 and D_4 had flatter and bimodal size distributions.



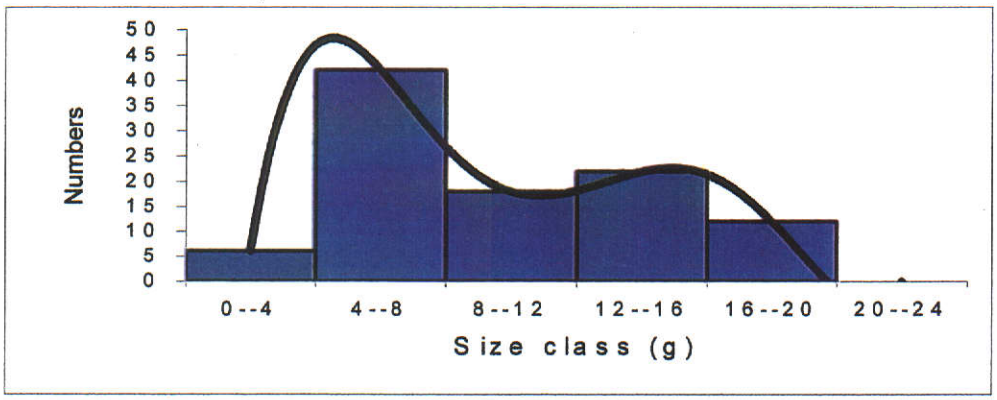
a) size distribution of marron receiving no formulated diet.



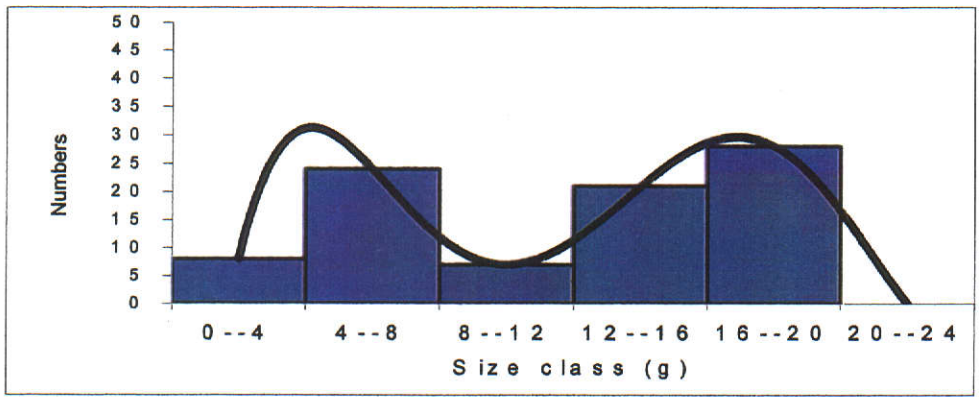
b) size distribution of marron fed without dietary oil supplementation.



c) size distribution of marron fed dietary supplementation of cod liver oil.



d) size distribution of marron fed dietary supplementation of sunflower oil.



e) size distribution of marron fed dietary supplementation of a mixture of cod liver and sunflower oils.

Figure 4.1 Size distribution of juvenile marron fed one of four diets and those receiving no supplementary food.

4.3.5 Condition Indices

Marron outside the cages receiving no formulated feed had significantly lower ($P < 0.05$) wet and dry hepatosomatic indices (H_{iw} and H_{id}), as well as low wet and dry tail to body weight ratios (T/B_w and T/B_d), compared to marron fed formulated feed inside the cages (Table 4.4). These free-ranging marron also had the highest moisture levels in tail muscles (TM%) and in hepatopancreas (HM%). There were significant differences in all condition indices (H_{iw}, H_{id} T/B_w, T/B_d, TM% and

HM%) between marron receiving no supplementary oil in their diets (D_1) and marron receiving some kind of dietary oil supplementation (D_2, D_3 and D_4) (Table 4.4). There was no significant difference ($P > 0.05$) in wet tail muscles to body weight ratio (T/B_w) among any marron groups receiving any supplementary oil.

The marron fed on diet D_2 had a significantly higher ($P < 0.05$) H_{id} (Table 4.4) compared to marron receiving diet D_3. Oil supplementation had an opposite influence on hepatopancreatic moisture percentage (HM%) and H_{iw}, so that marron receiving a mixture of sunflower and cod liver oil had a lower HM% (56.82 ± 0.63) (Table 4.4). Dry tail muscles to body weight ratio (T/B_d) showed no significant difference ($P > 0.05$) between marron fed supplementation of either cod liver oil or sunflower oil, however, a mixture of these two oils resulted in significantly higher T/B_d. There was no significant difference in T/B_w between marron fed either cod liver oil or sunflower oil (Table 4.4). The trend in these indices are analysed by regression analysis and are explained in Chapter 7.

Table 4.4 **Condition indices (H_{iw}, H_{id}, HM%, T/B_w, T/B_d and TM%) of marron fed the four test diets**

Index	D_1	D_2	D_3	D_4	No formulated feed (free-range)
H_{iw}	3.20 ± 0.05^{b}	$4.92 \pm 0.08^{c,d}$	4.73 ± 0.07^{c}	5.14 ± 0.09^{d}	2.54 ± 0.07^{a}
H_{id}	1.04 ± 0.02^{b}	2.05 ± 0.06^{d}	1.91 ± 0.05^{c}	2.22 ± 0.05^{e}	0.61 ± 0.02^{a}
HM %	67.34 ± 0.30^{c}	$58.46 \pm 0.73^{a,b}$	59.74 ± 0.48^{b}	56.82 ± 0.63^{a}	76.08 ± 0.50^{d}
T/B_w	25.25 ± 0.49^{b}	32.92 ± 0.54^{c}	32.81 ± 0.48^{c}	34.56 ± 0.74^{c}	23.25 ± 0.74^{a}
T/B_d	4.46 ± 0.17^{b}	6.76 ± 0.14^{c}	6.53 ± 0.19^{c}	7.71 ± 0.24^{d}	3.65 ± 0.23^{a}
TM%	83.02 ± 0.34^{c}	79.82 ± 0.22^{b}	79.76 ± 0.41^{b}	77.74 ± 0.38^{a}	84.38 ± 0.59^{d}

a, b, c values in the same row having different superscripts are significantly different ($P < 0.05$).

4.4 DISCUSSION

Cod liver oil is a rich source of ω –3 fatty acids whereas sunflower oil is a source of ω – 6 fatty acids (Gunstone and Padley, 1965, *Appendix 2, Table I*). Cod liver oil supplementation increased the level of ω-3 fatty acids in the diet D_2 and supplementation by sunflower oil increased the percentage of ω-6 fatty acid in diet D_3. Oil supplementation of practical diets has a positive effect on final weight, growth, survival and biomass of marron. Supplementation with any dietary oil (D_2, D_3 or D_4) resulted in less nutritional / physiological stress in marron as indicated by higher H_{iw} and lower HM% (Table 4.4) .

4.4.1 Final Weight, Growth

The importance of ω-3 HUFAs in the nutrition of aquatic animals (Guary *et al.*, 1976; Kanazawa *et al.*, 1977, 1979a; D'Abramo and Sheen, 1993; Xu *et al.*, 1994) is consistent with results of the present trial where supplementation of cod liver oil alone or in combination with sunflower oil resulted in significantly ($P < 0.05$) higher final mean weight than was achieved by supplementation of sunflower oil alone. Cod liver oil in the diet of American lobster (*H. americanus*) resulted in greater percent weight gain and feed conversions due to an essential fatty acid requirement by the lobster for the linolenic series ω-3 or other fatty acids present in the cod liver oil (Castell and Covey, 1976). However, supplementation with only cod liver oil (6%) in a semi-purified diet resulted in the depression in SGR (Fotedar *et al.*, 1997), indicating that experimental conditions, ingredients of the diet and age of experimental animals may influence the effect of oil supplementation on the growth of marron.

Kanazawa *et al.* (1977) demonstrated that a combination of fish oil rich in ω-3 fatty acids and plant oil rich in ω-6 fatty acids is more effective for growth of prawns than the individual oils alone. Growth was increased when juveniles of red

drum, (*Sciaenops ocellatus*) were fed diets supplemented with linseed and menhaden oil (Lochmann and Gatlin, 1993). The latter oil contains substantial amounts of ω-3 HUFAs that are necessary to satisfy the EFA requirement of fish of that species.

4.4.2 Survival

It is assumed that most of the mortalities during communal rearing of crayfish are associated with cannibalism (Mason, 1978; Pursiainen *et al.*, 1983; Ackefors *et al.*, 1989; Gydemo and Westin, 1989) and the rate of cannibalism is increased by the lack of adequate nutrition in the diet (Celada *et al.*, 1989; D'Abramo and Robinson, 1989). Significantly higher survival was observed in marron receiving diets D_2 and D_4, both of which contained cod liver oil. Supplementation of the basic D_1 diet with sunflower oil (D_3) did not alter the survival rate of marron, although addition of cod liver oil (diets D_2 and D_4) did significantly increase survival, indicating that cod liver oil contributed some essential nutrients in the form of fatty acids to the practical diets.

Supplementation of diets with cod liver oil resulted in higher survival. Mortalities in cages were due to predation and/or cannibalism and not for physiological reasons. Marron fed diets without cod liver oil may have been subjected to increased levels of cannibalism as a result of nutrient inadequacies of the diets. In the experiment described in Chapter 2, supplementation of cod liver oil to a semi-purified diet under laboratory conditions did not alter the survival of younger marron of 1.99 ± 0.09 SE g mean weight (Fotedar *et al.*, 1997). The survival percentage after 108 days was 60 to 65% lower than found in the current study. This may be due to environmental factors, mainly temperature. Morrissy (1990) reported that under low temperatures there is a requirement by marron of ω-3 fatty acids. Consequently the semi-purified diets used under controlled and sterile conditions (Chapter 2) may have been nutritionally inadequate compared with the practical diets used in the presence of natural food in commercial settings (Chapter 4).

Marron outside the cages did not receive any formulated feed and their only source of nutrition was derived from the natural primary productivity of the pond. These marron had a similar final weight to that of marron fed cod liver oil and a mixture of cod liver and sunflower oil. The leaching of nutrients from all practical diets from the cages presumably contributed to the natural productivity in the pond water. In fact, freshwater crayfish are reported to obtain 50% of their nutritional requirements from natural productivity of the surrounding waters (Apud *et al.*, 1983; Apud, 1985; Lee and Wickins, 1992). The comparable final mean weight of free-range marron can also be attributed to their lower stocking density compared with crayfish inside the cages. The survival rate of free-range marron could not be determined as it was not possible to drain completely the pond immediately upon concluding the trial.

4.4.3 Biomass

Marron fed diets supplemented with cod liver oil (D_2 and D_4) reached higher biomass than those without cod liver oil. Both of these diets contained the ω-3 HUFA known to meet the EFA requirements in a majority of crustaceans (D'Abramo and Sheen, 1993). Biomass gain of marron fed diets containing cod liver oil suggests their fatty acids are important for both growth and survival. Results of this study suggest that dietary levels of cod liver oil as low as 2% are sufficient to satisfy the EFA requirements.

The outdoor farm conditions combined with the enclosed cage design of the experiment system precluded any assessment of ingestion rates; the rate of ingestion among all treatment groups was assumed to be equal considering all experimental diets were isoenergetic.

High moisture levels in both hepatopancreas and tail tissue are clear indications that the free-range marron without supplementary feed were nutritionally/ physiologically stressed compared to the marron inside the cages and receiving

supplementary feed but without any oil augmentations (Table 4.4). This is due to the fact that all pellets without oil supplementation do contain some natural oil (2%; Table 4.2) through other ingredients. This suggests that the requirement of total oil is more than 2% for marron.

4.4.4 Size Distribution

At the end of the trial, marron receiving diets without oil supplementation (D_1) and with sunflower oil (D_3) had a positively skewed size distributions indicating that a few specimen were growing more quickly than others in the experiment - probably by deriving more nutrition through cannibalising weaker members of the group or competing more successfully for food. Free-range marron receiving no formulated diet, marron receiving diets supplemented with cod liver oil and marron receiving diets D_2 and D_4 showed flatter size-distributions (Figure 4.1), but the reason(s) for this increased scatter size is (are) not clear.

4.4.5 Condition Indices

Supplementation of diets with cod liver oil resulted in nutritionally less stressed marron as indicated by the higher wet hepatopancreas index (H_{iw}) and lower hepatosomatic moisture (HM%) levels. Dry hepatosomatic index (H_{id}) of marron receiving the mixture of cod liver and sunflower oil was significantly higher than H_{iw} of marron receiving other dietary treatments, indicating that marron have an energy reserve (mainly, lipids, proteins and carbohydrates) in the hepatopancreas (Jusilla and Mannonen, 1997).

The difference between tail moisture levels (TM%) and dry tail muscles to body weight ratio (T/B_d) was evident only in marron receiving a combination of dietary oil and / or individual oils. These findings are consistent with previously reported results (D'Abramo and Sheen, 1991) of the requirements for both ω-3 and ω-6 series fatty acids in the diets of omnivore crustaceans.

CHAPTER 5

DIETARY PROTEIN AND LIPID SOURCE

5.1 INTRODUCTION

Feed accounts for 40-60% of the production costs in aquaculture (D'Abramo and Sheen, 1991; Akiyama *et al.*, 1992; Sarac *et al.*, 1993), with protein sources accounting for a significant proportion of this cost (New, 1976; Akiyama and Dominy, 1991). The nutritional and energy requirements of aquatic animals can be provided from protein, carbohydrate and lipid sources; should one source be in short supply in the diet, the animal can adjust to some extent to obtain its requirements from the others. This apparently simple physiological shunt, however, has implications for aquaculture, including a change in the fatty acid profile of food and making it more expensive. Further, the use of protein as an energy source can lead to deterioration in environmental conditions caused by deteriorating water quality.

The majority of artificial diets for decapod crustaceans in Australia, including those of freshwater crayfish, are presently produced using imported fishmeal as the protein source. Recent nutrition research has focused on the evaluation of alternative cheap and cost-effective protein sources based on plant products (Kanazawa, 1991; Agung *et al.*, 1995). However, plant sources also provide some dietary oil components and this influences the fatty acid profile of the formulated diet. Thus, alteration of the protein source in the diet can significantly affect the dietary fatty acid profile. The effects of the replacement of fishmeal with plant-based protein sources on the lipid composition of aquaculture diets have not been studied in detail. Furthermore, no research has yet investigated the effect of the interaction between lipids and proteins on freshwater crayfish growth under semi-intensive farming conditions, where pond flora and fauna provide a significant source of nutrition (Moore, 1986; D'Abramo and Sheen, 1991).

Worldwide, over 95% of freshwater crayfish aquaculture is conducted under extensive or semi-intensive earthen pond-based farming systems wherein their nutrients are supplied by a combination of formulated feeds and natural productivity within the pond ecosystem. Thus, most of the nutritional research on freshwater crayfish has little commercial farm applicability (Tacon, 1996) if the research is conducted under artificially controlled, laboratory conditions which do not reflect, accurately, natural pond productivity (Lim and Persyn, 1989).

The aim of this experiment was to

1. evaluate the effect of the absence of supplementary dietary protein and
2. evaluate the influence of fish and plant protein and lipid source on the growth, survival, condition indices and body composition of marron cultured under commercial, semi-intensive farm conditions.
3. investigate the effect of interaction of dietary proteins and lipids on the growth of marron.

The study investigated two hypotheses, namely,

1. the quantity and source of dietary protein effects the growth, survival, biomass and condition indices of the marron cultured under commercial farm conditions.
2. the source of oil and protein have no interactive influence on marron.

5.2 Materials and Methods

5.2.1 Experimental System

Twelve purpose-built, drainable, earthen ponds located at Jurien Bay Marron Farm (JBMF), Jurien, Western Australia (Figure 1.2), were used to evaluate growth and survival of marron fed four test diets. All ponds were 70 x 15 m, varying in depth from 1 to 1.5 m deep. The slope of all pond embankments was 3:1. Ponds were randomly selected out of 38 ponds available at JBMF. All ponds were completely drainable *via* a PVC pipe (100 mm diameter). By lifting the standpipe from the outlet at the deepest end of the pond, the majority of marron could be drained with the water flow into a cement

sump (3m x 3m x 2m) constructed inside a shed, 50 to 100 m away from the experimental ponds, depending on the location of the pond. Ponds where feeding trials were performed are shown in Plate 5.1 and Figure 5.1.

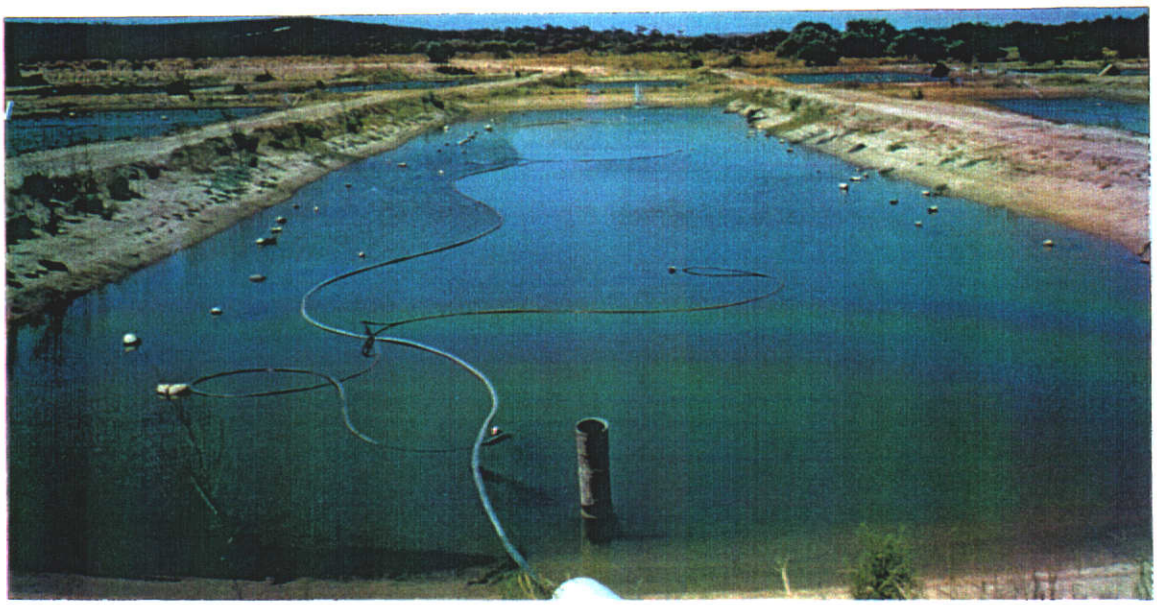


Plate 5.1 **Ponds at the JBMF where feeding trial was conducted.**

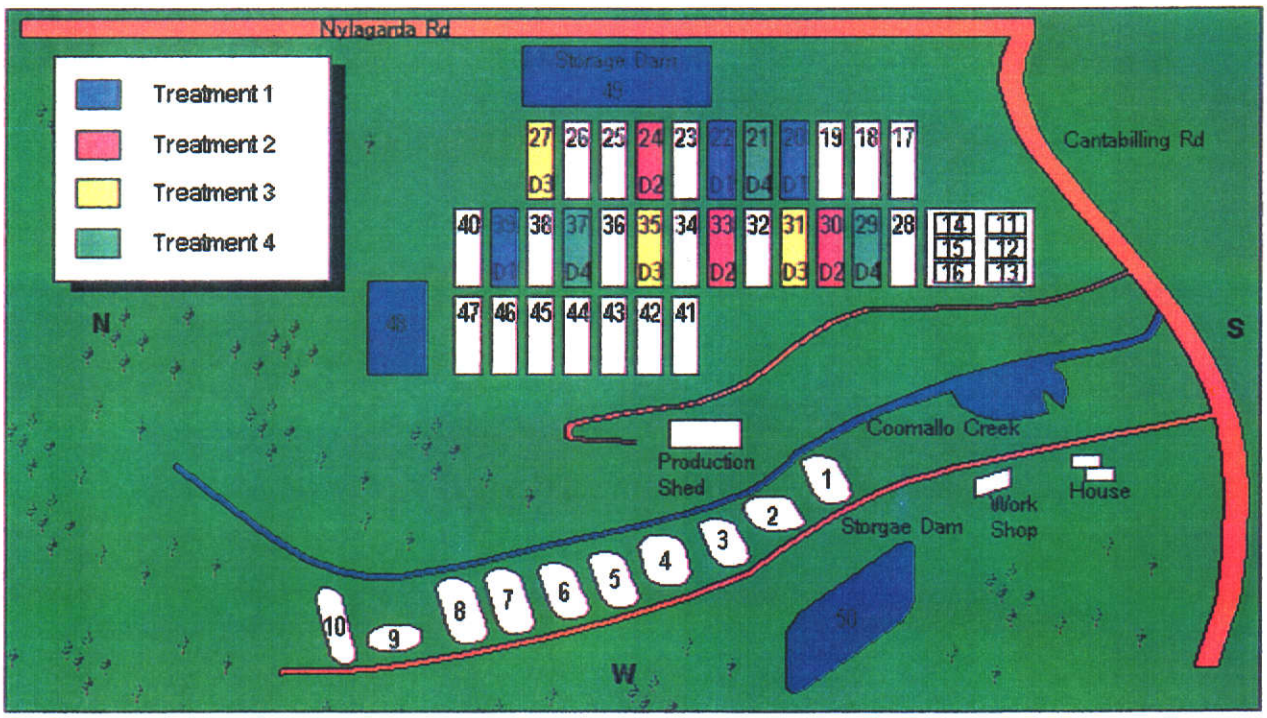


Figure 5.1 **Diagrammatic view of the outlay of the farm and the experimental ponds.**

Before the experiment commenced, the ponds were sun-dried, raked to rid the sediments of any entrapped noxious gases and then compacted. Pond preparation was completed by applying 2.5 kg of lime and 10 kg of inorganic fertiliser mix (nitrogen: phosphorus: potassium in the ratio of 5:5:1) per pond. The ponds were then filled with water from a header dam (20,000 m^3) in which water was supplied from a subterranean supply through a bore. After a week, when the pond water had turned a light green colour due to the production of unicellular green algae, the ponds were stocked with 3 juvenile marron/ m^2, (mean weight 1.3 ± 0.28 SE g) equivalent to 4.01 ± 0.28 SE g/m^2, representing 300 marron per pond. The marron were obtained from JBMF. Each pond was aerated through six air-lift pumps connected to an air blower. The blower was linked to a timer and automatically turned on from 04.00 to 10.00 hours daily. Sixty marron shelters, made from prawn nets, were placed in each pond. The bottom end of each shelter was attached to a brick and the other end to a float, effectively suspending each shelter at a fixed position in the water column.

Table 5.1 Ingredients (%) of the four test diets.

Ingredients	Diet D_1	Diet D_2	Diet D_3	Diet D_4
Lupin	71.50	-	-	-
Fish Premix	0.30	0.30	0.30	0.30
Limestone	2.00	-	-	1.50
Salt	0.50	-	-	0.50
Maize Starch	19.00	53.60	53.60	82.60
Dicalcium Phosphate	3.20	-	-	4.00
Cod Liver Oil	3.50		4.90	3.50
Cellulose	-	2.70	2.70	3.00
Sunflower Oil	-	4.90		4.60
Fish Meal 65	-	38.50	38.50	-
Total	100.00	100.00	100.00	100.00

5.2.2 Diet Preparation and Experiment design

Four isonitrogenous and isoenergetic semi-purified test diets were prepared using ingredients as described in Table 5.1. Fish meal and dehulled white lupin, *Lupinus albus,* were the sources of protein and lipid and cod liver oil and sunflower oil provided the additional sources of lipid to the diet.

Table 5.2 Major nutrient analysis (%) of the four test diets.

Nutrients	Diet D_1	Diet D_2	Diet D_3	Diet D_4
Protein	25.03	25.03	25.03	0.00
Lipid	8.36	8.36	8.36	8.10
Crude Fibre	3.07	3.08	3.08	3.00
Salt [1]	0.57	0.58	0.58	0.58
Metabolized Energy (kJ/g) [1]	10.86	13.79	13.79	14.32
Lysine [1]	1.17	1.89	1.89	0.00
Methionine [1]	0.17	0.73	0.73	0.00
Cystine [1]	0.34	0.23	0.23	0.00
Threonine [1]	0.74	1.04	1.03	0.00
Tryptophane [1]	0.29	0.29	0.29	0.00
Leucine [1]	1.58	1.92	1.92	0.00
Isoleucine [1]	0.89	1.15	1.15	0.00
Arginine [1]	2.55	1.30	1.30	0.00
Histidine [1]	0.75	0.58	0.58	0.00
Tyrosine [1]	1.14	0.95	0.95	0.00
Phenylalanine [1]	1.66	0.92	0.92	0.00
Valine [1]	0.85	1.31	1.31	0.00
ω-6 [1]	1.59	2.50	0.18	2.39
ω-3 [1]	1.07	0.84	1.92	1.01

[1] - Data calculated according to the known nutrient composition of the dietary ingredients.

Nutrient analyses of the four diets are described in Table 5.2. Marron in the ponds were fed one of these four test diets. The four test diets were randomly assigned to three replicate ponds. The dietary treatments are summerised in Table 5.3. The marron were fed

by placing feed at four fixed positions in each pond, at 17.00 hours at two day intervals. They were fed to satiation, which was subsequently estimated to be about 2 - 6% of their body weight.

Table 5.3 Test diets and their protein and lipid content and sources.

	Diet D_1	**Diet D_2**	**Diet D_3**	**Diet D_4**
Protein source	Plant	Fish	Fish	Negligible (< 0.4%)
Lipid (% diet)	3.5 %	3.5%	3.5%	Negligible
Supplemented oils (% diet; exogenous lipid sources)	Fish oil (3.5%)	Sunflower oil (3.5%)	Fish oil (3.5%)	Fish oil (3.5%) & Plant oil (3.5 %)

5.2.3 Water Quality

Of the several water quality parameters measured, those which are known to be sensitive to general environmental conditions (temperature, pH and dissolved oxygen) were measured twice daily in all ponds, at approximately 08.00 and 15.00 hours. Those which vary but at a slower rate (nutrient concentration such as, total ammonia, nitrite, nitrate) were measured at frequency commensurate with their rate of change (bimonthly). Those which generally reflect geochemical influences (the ionic composition of pond water) were also measured bimonthly using atomic absorption spectrophotometery (Varian/AA-875) and is shown in *Appendix 1, Table IV.* About 10% of the original water volume was added once every two weeks to compensate for evaporation losses.

5.2.4 Recording and data analysis

At the end of the trial all ponds were drained completely. Most of the marron were automatically harvested into the sump inside the shed; those remaining in the ponds were picked-up manually. All marron from each pond were graded according to commercial grades or size categories: 20 – 40 g (grade 1), 41 – 70 g (grade 2), 71 – 100 g (grade 3), 101 – 120 g (grade 4), 121 – 160 g (grade 5), 161 – 200 g (grade 6) and a sub-standard category comprising marron of all sizes with broken or missing claws. The total biomass in each size grade was also calculated by weighing the marron in that particular size

grade. Specific growth rates, survival, biomass, harvest rates and size frequencies were calculated over the period of the complete trial as described in Chapter 3. The tail muscles and hepatopancreas from 10 marron from each pond were weighed to calculate the wet and dry hepatosomatic indices, and wet and dry tail to body ratios as described in Chapter 2. Dry hepatopancreas and tail muscles tissue was analysed for proximate biochemical composition of crude protein, lipids, carbohydrates, ash and fatty acid profiles.

The data were analysed statistically by oneway ANOVA, (initial weight as covariate) and the LSD multiple-comparison test to identify significant differences among treatment means (Steel and Torrie, 1960, 1980). Statistical analyses were also performed to test for significant differences in temperature, pH and dissolved oxygen among treatment groups over time.

5.3 RESULTS

5.3.1 Water Quality

Mean monthly temperature, pH and dissolved oxygen levels are given in Figures 5.2, 5.3 and 5.4 respectively. Ionic composition of pond water (*Appendix 1, Table IV*) showed a general trend of increasing salt content with time but not of a magnitude to influence significantly the results. Nitrite and nitrate levels ranged from 0.015 to 0.5 and 0.5 to 5.0 mg/L (Table 5.5) respectively. Total ammonia levels were high (0.05 to 0.5 mg/L) during the last four months of the trial (Table 5. 5). At the end of the trial, ponds receiving diet D_4 had significantly lower ($P < 0.05$) levels of ammonia compared to other ponds (Figure 5.5). There were significantly higher levels of ammonia in ponds receiving diet D_1 than in ponds receiving diets D_2 and D_3.

There were no significant differences in water temperatures in any of the experimental ponds. However, mean pH and dissolved oxygen levels of water in the ponds where marron received diet D_1 were significantly higher ($P < 0.05$) than in the remainder of the ponds.

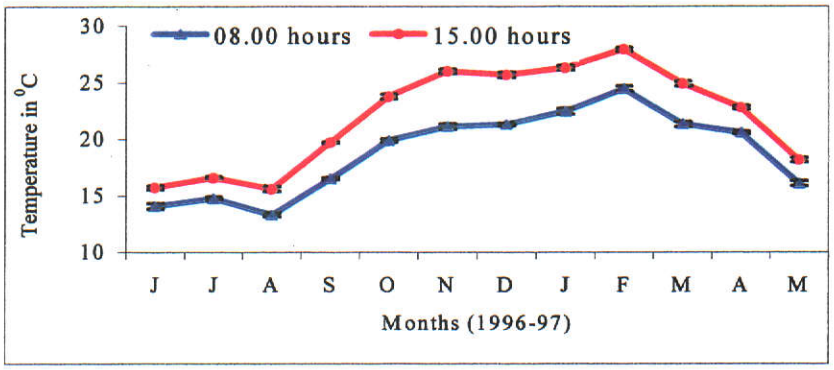


Figure 5.2 **Mean monthly temperature (°C ± SE) fluctuations at 08.00 and 15.00 hours.**

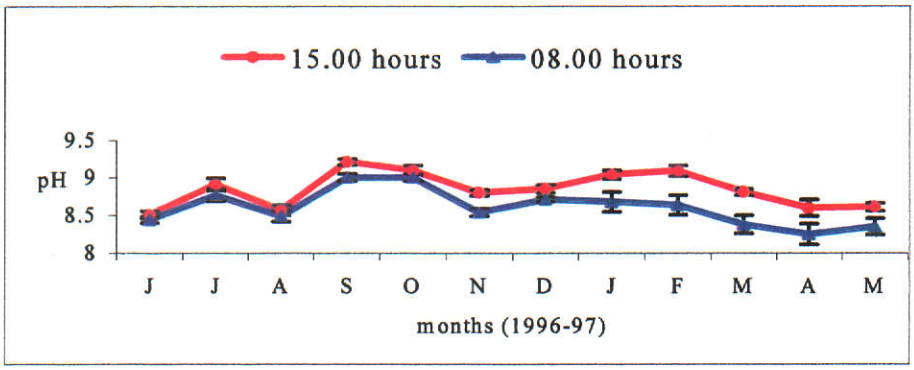


Figure 5.3 **Mean monthly pH (± SE) fluctuations at 08.00 and 15.00 hours during the trial.**

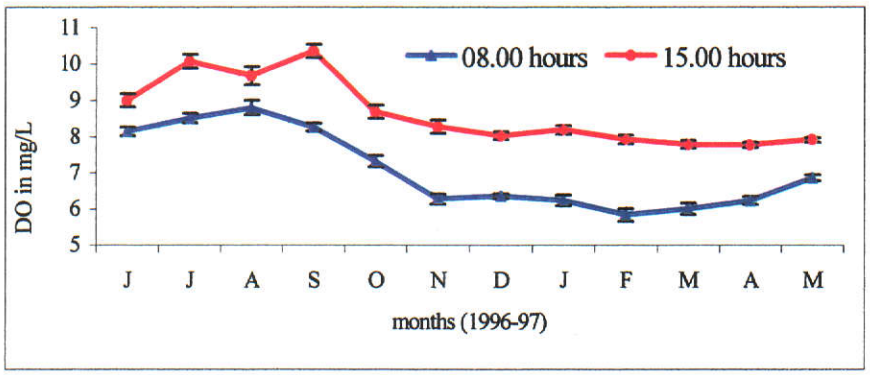


Figure 5.4 **Mean monthly dissolved oxygen (DO) (mg/L ± SE) fluctuations at 08.00 hours and 15.00 hours.**

There were no significant differences in water temperatures between: September, 1996 and May, 1997; December, 1996 and January, 1997; November, 1996 and March, 1997 (Table 5.4). However, between November 1996, to March, 1997 water temperatures were consistently higher than 21° C. During the entire trial the daily minimum water temperature ranged from 11.1 to 26.9 ^{0}C and daily maximum water temperature ranged from 13.6 to 29.7 ^{0}C.

Table 5.4 **Mean monthly minimum - maximum temperature (^{0}C), pH and dissolved oxygen (mg /L) in all ponds during the experiment.**

Months	Temp		pH		Dissolved Oxygen	
	Min	Max	Min	Max	Min	Max
June 96	14.1 ± 0.3^{b}	15.7 ± 0.2^{a}	$8.4 \pm 0.1^{a,b,c,d}$	8.5 ± 0.1^{a}	8.1 ± 0.1^{e}	9.0 ± 0.2^{d}
July 96	14.8 ± 0.1^{c}	16.6 ± 0.1^{b}	$8.7 \pm 0.1^{e,f}$	$8.9 \pm 0.1^{c,d,e}$	$8.51 \pm 0.1^{e,f}$	$10.1 \pm 0.2^{e,f}$
Aug 96	13.3 ± 0.1^{a}	15.6 ± 0.2^{a}	$8.5 \pm 0.1^{a,b,c,d}$	8.6 ± 0.1^{a}	8.8 ± 0.2^{f}	9.7 ± 0.3^{e}
Sept 96	16.5 ± 0.1^{d}	19.7 ± 0.1^{d}	9.0 ± 0.1^{f}	9.2 ± 0.0^{f}	8.3 ± 0.1^{e}	10.4 ± 0.2^{f}
Oct 96	19.9 ± 0.2^{e}	23.8 ± 0.2^{f}	$9.0 \pm 0.1^{d,f}$	$9.1 \pm 0.1^{e,f}$	7.3 ± 0.2^{d}	$8.7 \pm 0.2^{c,d,f}$
Nov 96	21.2 ± 0.3^{f}	$26.0 \pm 0.2^{h,i}$	$8.6 \pm 0.1^{c,e}$	$8.8 \pm 0.0^{b,c}$	6.3 ± 0.2^{b}	8.3 ± 0.2^{c}
Dec 96	22.3 ± 0.1^{g}	25.7 ± 0.2^{h}	$8.7 \pm 0.0^{c,d,e}$	$8.9 \pm 0.0^{c,d}$	6.4 ± 0.1^{b}	$8.0 \pm 0.1^{a,b}$
Jan 97	22.5 ± 0.3^{g}	26.3 ± 0.2^{i}	$8.6 \pm 0.1^{d,e}$	$9.0 \pm 0.1^{d,e,f}$	6.3 ± 0.2^{b}	8.2 ± 0.1^{b}
Feb 97	24.5 ± 0.2^{h}	27.9 ± 0.2^{j}	$8.6 \pm 0.1^{c,d,e}$	$9.1 \pm 0.1^{e,f}$	5.8 ± 0.2^{a}	$7.9 \pm 0.1^{a,b}$
March 97	21.3 ± 0.2^{f}	24.9 ± 0.2^{g}	$8.4 \pm 0.1^{a,b,c}$	$8.8 \pm 0.0^{b,c}$	$7.0 \pm 0.2^{a,b}$	7.8 ± 0.1^{a}
April 97	20.6 ± 0.1^{i}	22.8 ± 0.1^{e}	8.2 ± 0.1^{a}	8.6 ± 0.1^{a}	6.2 ± 0.1^{b}	7.8 ± 0.1^{a}
May 97	16.5 ± 0.1^{d}	18.2 ± 0.2^{c}	$8.4 \pm 0.1^{a,b}$	$8.6 \pm 0.1^{a,b}$	6.9 ± 0.1^{c}	$7.9 \pm 0.1^{a,b}$

a,b,c,d,e,f,g,h Values in the same column having different superscripts are significantly different ($P < 0.05$)

Table 5.5 **Range of nitrogenous metabolites (mg/L) in all experimental ponds measured during the experiment.**

Metabolites	June 96 - Sept 96	Oct 96 - Jan 97	Feb 97 - May 97
Total ammonia	0.02 - 0.05	0.05 - 0.25	0.05 - 0.50
Nitrites	0.03 - 0.50	0.02 - 0.30	0.01 - 0.02
Nitrates	0.50 - 5.00	0.50 - 2.00	0.50 - 5.00

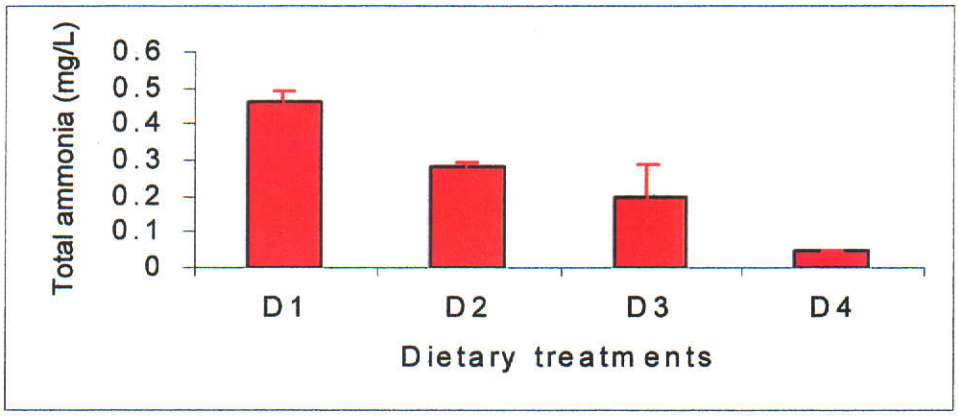


Figure 5.5 **Mean level (mean + SE mg/L) of total ammonia at the end of the experiment.**

Table 5.6 **Initial weight (mean ± SE g) MIW, final weight (mean ± SE g) MFW, specific growth rate (mean ± SE g/day %), survival (mean ± SE %) and biomass (mean ± SE g) of juvenile marron fed the four test diets.**

	Diet D_1	Diet D_2	Diet D_3	Diet D_4
MIW	4.01 ± 0.65 [a]	4.80 ± 0.59 [a]	3.76 ± 0.43 [a]	3.48 ± 0.10 [a]
MFW	67.35 ± 15.18 [a]	47.45 ±12.4 [a]	62.94 ± 6.72 [a]	50.94 ± 4.83 [a]
SGR	1.12 ± 0.15 [a]	0.89 ± 0.14 [a]	1.13 ± 0.05 [a]	1.07 ± 0.05 [a]
Survival %	13.82 ± 8.60 [a]	17.93 ± 4.23 [a]	26.78 ± 8.82 [a,b]	34.66 ± 9.04 [b]
Final Biomass	24.83 ± 15.40 [a]	27.97 ±10.80 [a]	48.7 ±12.85 [a]	53.87 ± 11.63 [a]

a,b Values in the same row having different superscripts are significantly different ($P < 0.05$).

5.3.2 Final Weights, Growth and Survival

There were no significant differences in final weights and SGRs among different test diets (Table 5.6). Survival was low in all ponds (13.82 to 34.66 %), although significantly higher ($P < 0.05$) for marron fed diet D_4 compared to marron fed diets D_1 and D_2. However, there were no significant differences among number of marron surviving in different size grades fed four test diets (Table 5.7). Significantly ($P < 0.05$) fewer marron survived in the initial two cumulative size grades (20 -70 g), which received diet D_1 than

marron receiving diet D_2, D_3 and D_4 (Table 5.8). There was no significant ($P > 0.05$) difference in survival of 20 – 70 g marron fed diets D_1, D_2 and D_3.

Table 5.7 **Number of surviving marron (mean ± SE %) of juvenile marron in each size-grade fed the four test diets.**

Size- grade (g)	Diet D_1	Diet D_2	Diet D_3	Diet D_4
20-40	138.66 ± 80.54 [a]	204 ± 52.7 [a]	264.33 ± 145.34 [a]	428.67 ± 52.83 [a]
41-70	148.00 ±114.18 [a]	172.33 ± 78.19 [a]	282.00 ± 65.09 [a]	398.33 ± 105.84 [a]
71-100	56.00 ± 41.20 [a]	56.67 ± 33.95 [a]	135.33 ±15.96 [a]	126.33 ± 39.26 [a]
101-120	17.00 ± 11.53 [a]	15.67 ± 9.39 [a]	35.33 ± 17.48 [a]	33.00 ± 14.36 [a]
121-160	18.00 ± 15.50 [a]	20.33 ± 9.87 [a]	53.00 ± 24.06 [a]	31.33 ± 11.57 [a]
161-200	3.67 ± 2.73 [a]	7.00 ± 5.57 [a]	21.66 ± 9.39 [a]	10.67 ± 5.70 [a]
Broken clawed	33.33 ± 13.57 [a]	43.00 ± 22.50 [a]	11.67 ± 6.93 [a]	11.33 ± 1.20 [a]

Values in the same row having different superscripts are significantly different ($P < 0.05$).

Table 5.8 **Cumulative surviving numbers of marron at the end of the trial.**

Cumulative size grades (g)	Diet D_1	Diet D_2	Diet D_3	Diet D_4
20 – 40	138.66 ± 80.54 [a]	204.00 ± 52.70 [a]	264.33 ± 145.34 [a]	428.67 ± 52.83 [a]
20 – 70	286.67 ± 190.88 [a]	376.33 ± 103.66 [a,b]	546.33 ± 210.35 [a,b]	827.00 ± 130.66 [b]
20 – 100	342.67 ± 231.54 [a]	433.00 ±116.92 [a]	681.67 ± 226.17 [a]	953.33 ± 224.02 [a]
20 – 120	359.67 ± 242.92 [a]	448.67 ± 117.73 [a]	717.00 ± 240.22 [a]	986.33 ± 221.44 [a]
20 – 160	377.67 ± 257.95 [a]	469.00 ± 120.61 [a]	770.00 ± 255.31 [a]	1017.67 ± 179.86 [a]
20 – 200	381.33 ± 260.68 [a]	476.00 ± 123.75 [a]	791.66 ± 258.21 [a]	1028.33 ± 179.44 [a]
Total	414.67 ± 257.88 [a]	519.00 ± 145.72 [a]	803.33 ± 264.8 [a]	1039.67 ± 178.61 [a]

a, b Values in the same row having different superscripts are significantly different ($P < 0.05$)

5.3.3 Total Biomass

At the end of the trial, total biomass as well as biomass in each size grade [except in size grade 1 (20 – 40 g)] was similar among all dietary treatments (Table 5.9). Marron receiving diet D_4 of the size grade 1 had a significantly higher ($P < 0.05$) biomass than marron receiving diet D_1. There were no significant differences in biomass of marron between marron fed diets D_1, D_2 and D_3 on one hand and D_2, D_3 and D_4 on the other.

Table 5.9 Total biomass (mean ± SE kg) of juvenile marron in each size-grade fed the four test diets.

Size-grades (g)	Diet D_1	Diet D_2	Diet D_3	Diet D_4
20-40	3.33 ± 1.93 [a]	4.90 ± 1.3.0 [a,b]	5.60 ± 2.7 [a,b]	10.23 ± 2.10 [b]
41-70	8.13 ± 6.27 [a]	9.50 ± 4.30 [a]	15.70 ± 3.47 [a]	21.90 ± 5.83 [a]
71-100	4.77 ± 3.48 [a]	4.83 ± 2.89 [a]	11.60 ± 1.15 [a]	10.63 ± 3.26 [a]
101-120	1.90 ± 1.25	1.77 ± 0.99	3.87 ± 1.85	3.67 ± 1.58
121-160	2.50 ± 2.20	2.43 ± 1.60	7.07 ± 3.25	4.37 ± 1.65
161-200	0.67 ± 0.52	1.20 ± 1.00	3.80 ± 1.80	1.90 ± 1.00
Broken clawed animals	3.53 ± 1.39	3.33 ± 1.67	1.00 ± 0.64	1.17 ± 0.22

Values in the same row having different superscripts are significantly different ($P < 0.05$).

5.3.4 Condition Indices

Wet and dry hepatosomatic indices (H_{iw} and H_{id}) between marron at the beginning of the trial and again at the end of the trial were similar ($P > 0.05$). Further, H_{iw} and H_{id} did not show any significant differences among the four test diets (Table 5.11). There were no significant differences ($P > 0.05$) in H_{iw} and H_{id} between the male and female components in any of the dietary treatments (Table 5.12 and 5.13). However, at the end of the trial, the mean wet tail muscles to body weight ratio (T/B_w) for the entire female component (33.26 ± 1.55 SE %) of test animals was significantly higher ($P < 0.05$) than for the

corresponding value for the males (31.40 ± 0.75 SE %). Marron fed diet D_3 had significantly higher T/B_w than marron fed diets D_2 and D_4. The four dietary treatments did not have any significant influence on the T/B_d of marron.

Table 5.10 Cumulative biomass of marron fed the four test diets.

Cumulative grades (g)	Diet D_1	Diet D_2	Diet D_3	Diet D_4
20 – 40	3.33 ± 1.93 [a]	4.90 ± 1.27 [a,b]	5.63 ± 2.74 [a,b]	10.23 ± 1.21 [b]
20 – 70	11.47 ± 8.09 [a]	14.40 ± 4.75 [a]	21.33 ± 6.20 [a]	32.13 ± 6.24 [a]
20 – 100	16.23 ± 11.56 [a]	19.23 ± 6.65 [a]	32.93 ± 7.34 [a]	42.77 ± 9.51 [a]
20 – 120	18.13 ± 12.8 [a]	21.00 ± 7.09 [a]	36.79 ± 8.79 [a]	46.43 ± 10.63 [a]
20 – 160	20.63 ± 14.97 [a]	23.43 ± 8.42 [a]	43.86 ± 11.18 [a]	50.80 ± 11.3 [a]
20 – 200	21.30 ± 15.49 [a]	24.63 ± 9.27 [a]	47.63 ± 12.19 [a]	52.70 ± 11.57 [a]
All surviving animals	**24.83 ± 15.4** [a]	**27.97 ± 10.8** [a]	**48.73 ± 12.83** [a]	**53.86 ± 11.63** [a]

a, b Values in the same row having different superscripts are significantly different ($P < 0.05$).

Table 5.11 Condition indices (H_{iw}, H_{id}, T/B_w and T/B_d) of juvenile marron fed the four test diets.

	Initial	Diet D_1	Diet D_2	Diet D_3	Diet D_4
H_{iw}	4.63 ± 0.50 [a]	4.56 ± 0.30 [a]	5.11 ± 0.23 [a]	4.83 ± 0.23 [a]	4.37 ± 0.18 [a]
T/B_w	26.62 ± 2.70 [a]	32.96 ± 0.83 [a,b]	30.87 ± 0.82 [a]	34.45 ± 0.87 [b]	30.89 ± 1.19 [a]
H_{id}	1.78 ± 0.10 [a]	1.27 ± 0.07 [a]	1.67 ± 0.23 [a]	1.12 ± 0.05 [a]	1.17 ± 0.10 [a]
T/B_d	4.74 ± 0.50 [a]	6.65 ± 0.78 [b]	6.33 ± 0.17 [b]	6.92 ± 0.30 [b]	6.91 ± 0.25 [b]

a,b - Values in the same row having different superscripts are significantly different ($P < 0.05$).

Table 5.12 **Wet hepatosomatic index and wet tail muscles to body weight ratio of the male population of marron fed the four test diets.**

Condition indices	Initial	Diet D_1	Diet D_2	Diet D_3	Diet D_4
H_{iw}	4.85 ± 0.83^{a}	4.42 ± 0.50^{a}	5.12 ± 0.24^{a}	5.31 ± 0.51^{a}	4.58 ± 0.19^{a}
T/B_w	27.91 ± 4.32^{a}	33.42 ± 1.13^{a}	31.00 ± 1.09^{a}	32.50 ± 0.58^{a}	29.79 ± 0.19^{a}

a,b - Values in the same row having different superscripts are significantly different ($P < 0.05$).

Table 5.13 **Wet hepatosomatic index and wet tail muscles to body weight ratio of the female population of marron fed the four test diets.**

Condition indices	Initial	Diet D_1	Diet D_2	Diet D_3	Diet D_4
H_{iw}	4.30 ± 0.38^{a}	4.70 ± 0.94^{a}	5.10 ± 0.40^{a}	4.59 ± 0.21^{a}	4.16 ± 0.30^{a}
T/B_w	24.67 ± 2.13^{b}	32.49 ± 1.28^{a}	30.74 ± 1.30^{a}	35.42 ± 1.19^{a}	31.98 ± 2.41^{a}

a,b - Values in the same row having different superscripts are significantly different ($P < 0.05$).

Table 5.14 **Proximate composition (%) of the hepatopancreas of juvenile marron fed the four test diets.**

Composition	Initial	Diet D_1	Diet D_2	Diet D_3	Diet D_4
Moisture	57.65 ± 1.47^{a}	$63.60 \pm 3.59^{a,b}$	66.40 ± 2.02^{b}	$69.95 \pm 2.75^{b,c}$	71.25 ± 0.75^{c}
Fat	55.50 ± 0.50^{b}	$48.80 \pm 5.58^{a,b}$	$46.72 \pm 2.18^{a,b}$	44.07 ± 2.47^{a}	42.65 ± 0.45^{a}
Protein	20.82 ± 1.94^{c}	27.83 ± 1.70^{a}	31.56 ± 3.07^{b}	30.89 ± 3.12^{b}	$28.44 \pm 2.17^{a,b}$
Ash	0.83 ± 0.28^{b}	3.60 ± 0.69^{a}	3.93 ± 0.36^{a}	4.15 ± 0.77^{a}	5.47 ± 0.84^{a}

a,b,c - Values in the same row having different superscripts are significantly different ($P < 0.05$).

5.3.5 Proximate Composition

Hepatopancreatic moisture (HM%) was significantly lower ($P < 0.05$) in marron receiving diets D_1 and D_2 than for marron receiving diet D_4 (Table 5.14). However, there were no significant differences ($P > 0.05$) in tail muscles moisture (TM%) for marron receiving

diets D_1, D_2 and D_3. Marron fed diet D_4 had significantly higher ($P < 0.05$) TM% (Table 5.15). There was a progressive increase in HM% for marron receiving diets D_1 to D_4. There was no significant difference ($P > 0.05$) in TM% between marron receiving diet D_1 at the beginning and end of the trial.

Table 5.15 Proximate composition (%) of the tail muscles of juvenile marron fed the four test diets.

Composition	Initial	Diet D_1	Diet D_2	Diet D_3	Diet D_4
Moisture	$80.58 \pm 0.33^{a,b}$	78.40 ± 0.20^{a}	$79.33 \pm 0.71^{a,b}$	$80.60 \pm 0.71^{a,b}$	82.05 ± 1.25^{b}
Fat	0.50 ± 0.00^{a}	0.71 ± 0.02^{a}	0.83 ± 0.07^{a}	0.84 ± 0.25^{a}	0.85 ± 0.25^{a}
Protein	91.24 ± 4.23^{b}	76.25 ± 3.13^{a}	79.38 ± 5.46^{a}	73.75 ± 1.30^{a}	76.25 ± 3.31^{a}
Ash	4.07 ± 2.48^{b}	6.70 ± 0.00^{a}	6.50 ± 0.10^{a}	7.10 ± 0.38^{a}	6.70 ± 0.74^{a}

a,b - Values in the same row having different superscripts are significantly different ($P < 0.05$).

Hepatopancreatic lipid levels decreased significantly ($P < 0.05$) over the trial for marron receiving diets D_3 and D_4 (Table 5.14). However, there were no significant differences in hepatopancreatic lipid levels for marron receiving the four diets. Fat content of the tail muscles ranged from 0.5 % in the beginning of the trial to 0.85 % at the end of the trial for the marron receiving diet D_4. There was no significant difference ($P > 0.05$) in tail muscles lipid levels among marron receiving diets (Table 5.15). All hepatopancreatic protein levels increased but not significantly ($P > 0.05$) over the duration of the trial (Table 5.14). Tail muscles protein level of all marron was significantly lower ($P < 0.05$) at the end of the trial than at the beginning (Table 5.15). Hepatopancreatic ash content (Table 5.14) and ash content of the tail muscles (Table 5.15) were significantly lower ($P < 0.05$) in the beginning of the trial than at the end. However, there were no significant differences in the ash content of hepatopancreas (Table 5.14) and tail muscles (Table 5.15) at the end of the trial.

5.3.6 Fatty Acid Profile

Fatty acid profiles showed a preponderance of mono-unsaturated fatty acids in hepatopancreas (Table 5.16) and polyunsaturated fatty acids in tail muscles (Table 5.17).

Diet had a clear effect on the percentage of EPA and DHA in both hepatopancreas and tail muscles of marron. The main polyunsaturated fatty acids were predominately represented by linolenic (18:3 ω-3) and linoleic acids (18:2 ω-6) in both tail muscles and hepatopancreas. The total percentage of mono-unsaturated fatty acids was comparatively less in the tail muscles of marron fed diets D_3 and D_4 compared to levels in those fed diets D_2 and D_3. The concentrations of 20:5 ω-3; EPA and 22:6 ω-3; DHA were higher in marron tail muscles for diets D_3 and D_4 (Table 5.17).

Table 5.16 Fatty acid profile (% of total extracted lipids) of hepatopancreas of marron fed the four test diets and before trial commenced.

		initial	Diet D_1	Diet D_2	Diet D_3	Diet D_4
Myristic acid	C14:0	1.2	1.7	2.2	3.2	1.0
Palmictic acid	C16:0	4.4	6.3	10.9	13.6	4.5
Stearic acid	C18:0	1.2	1.3	2.1	2.6	0.85
Archidic Acid	C20:0	0.0	0.2	0.3	0.3	0.1
Behenic Acid	C22:0	0.0	1.8	3.1	4.8	1.3
Lignoceric Acid	C24:0	0.0	0.1	0.1	0.9	0.3
Total saturated		**6.8**	**11.4**	**18.8**	**25.4**	**8.1**
Palmitoleic acid	C16:1	19.4	13.4	6.2	8.1	14.0
Oleic acid	C18:1	17.8	18.4	31.3	24.8	19.4
Gadoleic Acid	C20:1	6.5	0.42	2.4	4.9	2.8
Total mono-unsaturated	**43.7**		**32.2**	**39.9**	**37.8**	**36.2**
Linoleic Acid	C18:2	13.0	15.2	7.8	7.7	4.9
Linolenic acid	C18:3	7.5	10.9	2.6	2.5	2.7
PUFA		**20.5**	**26.13**	**10.4**	**10.2**	**7.64**
Eicosapentaenoic acid	C20:5 ω-3	2.5	12.5	11.4	13.5	19.5
Docosahexaenoic acid	C22:6 ω-3	0.5	9.5	8.7	8.8	13.5
HUFA		**3.0**	**22.0**	**20.0**	**22.3**	**33.0**

Table 5.17 Fatty acid profile (% of total lipids) of tail muscles of marron fed the four test diets and before trial commenced.

Fatty acids		initial	Diet D_1	Diet D_2	Diet D_3	Diet D_4
Myristic acid	C14:0	6.2	2.5	3.5	4.8	5.1
Palmictic acid	C16:0	4.8	0.5	0.5	4.5	2.5
Stearic acid	C18:0	4.6	14	12.2	6.9	3.3
Archidic Acid	C20:0	1.3	0.5	0.5	6.0	3.5
Behenic Acid	C22:0	2.1	0.5	0.5	0.5	0.5
Lignoceric Acid	C24:0	1.8	0.5	0.5	0.5	0.5
Total saturated		**20.8**	**18.5**	**17.7**	**23.2**	**15.4**
Palmitoleic acid	C16:1 ω-3	0.2	1.4	2.1	0.2	0.1
Oleic acid	C18:1 ω-9	10.9	16.0	15.6	1.8	0.6
Gadoleic Acid	C20:1 ω-9	0.0	0.5	1.5	0.5	0.5
	C22:1 ω-9	0.0	0.0	2.6	2.5	1.5
Total mono-unsaturated	**11.1**		**17.9**	**21.8**	**5**	**2.7**
Linoleic Acid	C18:2 ω-6	29.2	22.7	23.0	20.2	24.1
Linolenic acid	C18:3 ω-3	20.2	10.3	10.5	10.1	10.1
PUFA		**49.4**	**33.0**	**33.5**	**30.3**	**34.2**
Eicosapentaenoic acid	C20:5 ω-3	0.5	6.5	6.0	16.5	14.5
Docosahexaenoic acid	C22:6 ω-3	0.5	0.5	1.0	7.5	6.5
HUFA		**1.0**	**7.0**	**7.0**	**24.0**	**21.0**

5.4 DISCUSSION

The final weight and SGR of marron were not influenced by dietary protein level or by the dietary protein and lipid source. However, survival was influenced by both the source and level of dietary protein. The deterioration of environmental quality through increasing ammonia levels in the ponds probably contributed to the low survival of

marron under high ambient temperatures. The size distribution of marron was also influenced by the diet. Of the condition indices measured, only HM% was influenced by diet.

5.4.1 Survival and Water Quality

The entire trial was marked by the low percentage survival (13.82 to 34.66 %) of marron in all of the experimental ponds. The average survival of the entire JBMF (excluding experimental ponds used in this trial) was 16% for marron fed commercial diet having protein levels of 25% over the same period. The diet contained both plant and animal sources of protein. The ingredient and biochemical composition of the diet is given in *Appendix 2, Table II* and *Table III.* Warm air temperatures prevailed between November, 1996 to March, 1997 with mean monthly water temperatures rising above the optimum level of 24 ^{0}C (Table 5.4.).

Although the daily readings may not have been precisely at the extreme range of the parameter values on each day, they would have been close to the time, when the extremes are generally observed. Given this logic, it is reasonable to assess that that observed parameters are close to the true values. Nowadays, the small error of uncertainty can be readily solved with the use of data loggers.

The survival of marron receiving diet D_4 was significantly higher ($P < 0.05$) than marron receiving diets D_1 and D_2, indicating that inclusion of protein in the diet decreased survival. This can be attributed directly to the decline in water quality (in terms of total and un-ionized ammonia) under high ambient temperatures at times when any protein was present in the diet. Seasonal variation in yield is well correlated with pond water temperature (Teichert-Coddington *et al.*, 1994). Ammonia is the major nitrogenous waste product for Crustacea (Claybrook, 1983). Total ammonia levels in pond water where protein-enriched diets (D_1, D_2 and D_3) were applied were significantly higher than the pond water where negligible protein was present in the diet (D_4). Further, the ponds

receiving plant protein (D_1) had significantly higher levels of ammonia in the water compared to ponds with marron fed animal proteins (D_2 and D3).

Since the ultimate consequence of protein sparing is a shift in protein metabolism, Clifford and Brick, (1978) used the level of nitrogen excretion to quantify the magnitude of sparing in juveniles of the fresh water prawn, *M. rosenbergii*, and suggested that those diets which do not provide sufficient lipid or carbohydrate to satisfy the immediate basal energy requirements of the prawn therefore necessitates mobilization of protein for catabolic processes, thus increasing levels of total ammonia in the water. As diet D_4 had a very low protein to energy ratio, the marron receiving this diet must have used energy from non-protein energy sources (mainly carbohydrates and lipids) and thus had a minimum contribution to increasing the ammonia load in the pond water. It is also evident that marron could either deaminate plant protein in diet D_1 for energy uses or could not digest this protein, either way, increasing the ammonia content of the water.

For crustaceans, the reported 96-hr LC_{50} values of un-ionized ammonia ranges from 0.40 to 2.65 mg/L (Wickins, 1976; Armstrong *et al.*, 1976; Hymel, 1985). Lourey and Mitchell (1995) recommended a "safe" level of 0.05 mg/L of un-ionized ammonia for Australian yabby (*C. destructor*) culture. Levels above 0.096 mg/L resulted in reduced growth rates of the animals. If the tolerance range is similar for marron, and using the conversions outlined by Trussell (1972) and Emerson *et al.*, (1975), safe total ammonia concentrations under various pH and temperature conditions can be calculated. The total ammonia levels in the ponds receiving diets D_1 were well above the safe level and in ponds receiving diets D_2 and D_3 fluctuated around the safe level. In contrast to earlier reports, Liu *et al.* (1995) reported that another Australian freshwater crayfish, redclaw (*C. quadricarinatus*) is relatively tolerant to ammonia, but somewhat sensitive to nitrites when compared with other crustaceans.

At the end of the trial, test pellets were analysed to identify any changes in the biochemical composition which occurred during storage on the farm (*Appendix 2, Table IV*). No significant changes were detected.

5.4.2 Final Weight and Growth

5.4.2.1 Protein levels

Results indicate that provision of protein and its sources are not important for marron growth in a commercial, semi-intensive environment. D'Abramo and Conklin (1996) reported that crustaceans do not have a specific requirement of protein from their formulated diet as long as the required levels of the essential amino acids are supplied through natural productivity of the ecosystem. Huner and Meyers (1979) reported that amino acid profiles in freshwater crayfish should be considered since the natural diet includes animal matter which may provide essential amino acids not normally available in vegetable protein substrates.

Freshwater crayfish are able to utilize different types of food items occurring naturally in the pond ecosystem. Brown *et al.*, (1992) reported weight gains of 272% in small unfed crayfish, *P. clarkii*, and attributed this, in part, to grazing on bacteria. The most rapid growth in *Cherax albidus* has been recorded in animals fed a diet consisting entirely of zooplankton (Mitchell and Collins, 1989; Jones *et al.*, 1995)

Post-larvae of the freshwater prawn, *M. rosenbergii,* grew 180% larger on natural productivity of the water than on the formulated feed alone (Stahl, 1979). Lower protein levels are suspected to be adequate in semi-intensive production of shrimps (Akiyama, 1992) because the contribution from the natural production to growth (Hunter *et al.*, 1987; Anderson *et al.*, 1987) is adequate. Tidwell *et al.* (1997) reported that formulated feeds low in some of the essential amino acids need not closely match the nutritional requirements of prawn, *M. rosenbergii,* as long as these amino acids are supplied through natural productivity. These amino acids may be available through the natural zooplankton and microorganisms occurring due to natural productivity of the pond ecosystem.

Further, the reduction of dietary protein from 23% to negligible levels and the consequent increase in non-protein nutrient, mainly carbohydrates, (as lipid content was the same in

all dietary treatments) did not reduce the SGR, indicating energy from the non-protein sources spared the protein utilization. The requirements of essential amino acids possibly would have been met from the nutrient available in the natural productivity of the pond ecosystems. Sedgwick (1979) reported that the growth of *P. merguiensis* did not change when fed diets in which the protein level was reduced from 51 to 34%, while maintaining a constant dietary energy content from non-protein nutrients. Further, Teichert-Coddington and Rodriguez (1995) reported that dietary protein level had no significant effect on the yield of shrimp, *P. vannamei*. The dietary protein content for American lobster was reduced to 40% from the established requirement of 60% when fed an isocaloric diet with high carbohydrate content (Capuzzo and Lancaster, 1979; D'Abramo *et al.*, 1981). Andrews *et al.* (1972) demonstrated that growth rate and minimum protein requirements of penaeid shrimps are directly dependent upon the presence of other dietary components from which carbon chains can be catabolised for basal energy requirements.

5.4.2.2 Protein source

Reigh and Ellis (1994) demonstrated that commonly used plant protein and animal protein supplements differ in nutritional value for red swamp crayfish, *P. clarkii*. In the present experiment, the absence of an affect of protein source on SGR of marron can be attributed to the fact that, under semi-intensive farming conditions, natural flora and fauna of the pond negates the effect of protein source in the formulated diets. Feeding trials conducted under laboratory conditions have shown that growth rates in *P. indicus* (Colvin, 1976a), *P. japonicus* (Deshimaru and Shigheno, 1972; Teshima *et al.*, 1986), *P. setiferus* (Andrews *et al.*, 1972; Lee and Lawrence, 1985), and *P. vannamei* (Smith *et al.*, 1985; Dominy and Ako, 1988) are influenced by the source of protein in the diet.

5.4.2.3 Lipid source

The final mean weight and SGR of marron receiving only fish lipids, for example in diet D_3 did not significantly differ ($P > 0.05$) from those values of marron receiving diets D_1, D_2 and D_4, all containing plant lipids supplemented exogenously (D_2 and D_4) or as supplied directly from the plant source (D_1). The results showed that the lipid source did

not affect marron growth in semi-intensive marron ponds. It appears that the source of lipids is not important in commercial farm ponds as essential fatty acids can be provided from the natural pond ecosystem.

Hubbard *et al.* (1986) showed that when red-swamp crayfish (*P. clarkii*) were fed a high protein diet (40%) – low energy diet (0.6 kJ/g), their growth was depressed compared to being fed other low energy diets. Apparently there was insufficient non-protein energy in the low-energy diet, thus protein was deaminated and utilized as an energy source. In the present study, the protein to energy ratio ranged from 23.03g/MJ in diet D_1 to negligible in diet D_4. Hubbard *et al.* (1986) estimated the protein requirement and optimum protein / energy ratio to be 30% and 0.05 g/kJ, respectively in red-swamp crayfish and these estimates were quite similar to *A. astacus* requirements (Ackefors *et al.*, 1992). However, there is no consistency in the optimum dietary ratio of protein to energy in crustaceans as evident by past research. Sarac (1994) has reviewed the literature available on dietary protein to energy ratio for penaeid prawns.

5.4.3 Size Distribution

Different size (weight) grades command different prices in the international and domestic markets so it is necessary to evaluate the influence of dietary treatments on the numbers surviving in each size grade. Marron fed diet D_4 had a significantly higher survival in the size grade of 20 to 70 g than marron fed diet D_1 (Table 5.8), indicates that inclusion of lupin based protein in the diets of marron increased the mortality of marron.

5.4.4 Condition Indices

There were no significant differences ($P > 0.05$) in wet and dry hepatosomatic indices (H_{iw} and H_{id}) of marron among any dietary treatments. Further, the test diets did not influence these indices, as there was no changes throughout the experiment. This indicates that under these farming conditions natural productivity of the ecosystem is an overriding factor, providing all the essential nutrients.

There were no significant differences in HM% values of marron receiving either source of dietary protein in the presence of plant oil. However, the HM% of marron receiving the diet with negligible protein level (D_4) was significantly higher than that of marron receiving diets D_1 and D_2, indicating that marron fed diet D_4 were nutritionally stressed compared with marron fed diets D_1 and D_2. Jones *et al.* (1997) also reported that low dietary protein levels cause nutritional stress in yabbies. Marron receiving plant protein did not show significant changes in HM% throughout the experiment, indicating that the nutritional status of these had not altered. As marron for this experiment came initially from a pond abundant in aquatic vegetation and phytoplankton, it may be assumed that the protein requirements of the marron at the beginning of the trial were being supplied from plant sources, both natural vegetation and phytoplankton within the pond ecosystem.

Wet tail muscles to body weight ratio (T/B_w) of marron receiving no nutrients from plant sources (D_3) was significantly higher ($P < 0.05$) than T/B_w of marron fed an exogenous source of plant oil (D_2 and D_4) but was not significantly different from that of marron fed an exogenous source of fish oil (D_1). As the T/B_w and T/B_d values were significantly higher at the conclusion compared to the commencement of trial, and since there was no significant differences in the moisture contents between the dietary treatments, the ratio increases were due to an increase in ash and NFE contents (Table 5.15). Fat content was not significantly altered, whereas protein content of the tail muscles significantly decreased ($P < 0.05$) after receiving test diets. Huner and Lindqvist (1984) showed that high dietary protein levels lead to increased levels of glycogen and lipids in the hepatopancreas of the freshwater crayfish, *A. astacus*. Similarly high dietary protein levels produced higher concentrations of hemolymph protein which may be indication of better physiological condition and better growth rate, as found in lobsters (Castell and Budson, 1974).

5.4.5 Body Composition

There were no significant differences in proximate composition of various nutrients (proteins, fat) in hepatopancreatic tissue among marron receiving four different diets. This contrasts with the findings of Jones *et al.* (1997), who found increased levels of protein

and ash in the carcass of yabbies and a decrease in lipid levels and energy when fed higher dietary proteins. When the initial and final proximate composition of various nutrients in the hepatopancreas are compared, some of the test diets had a significant impact. For example, the fat content of the hepatopancreas significantly decreased following feeding with protein-free or plant oil-free diets. However, the protein content of the hepatopancreas of marron did not change significantly when fed protein-free diet or plant sourced protein diet. The ash content of the hepatopancreas increased significantly after being fed the test diets. This increase cannot be explained by dietary treatments but can be viewed as a function of the physiological age of the animals.

5.4.6 Fatty Acid Profile

Though the fatty acid profile of all the test diets is expected to be the similar, there was a difference in the fatty acid profiles of tail muscles of marron fed diets D_1 and D_2 on the one hand and D_3 and D_4 on the other hand. This difference is more pronounced in terms of mono-unsaturated fatty acids and HUFAs. Marron fed diets D_3 and D_4 had healthy fatty acid profiles if humans nutrition is a valid comparison. The fatty acid profile of hepatopancreas was less diverse among marron fed different test diets as compared to their tail muscles.

As dietary treatments did not influence SGR of marron but the protein free diet (D_4) and the plant oil–free diet (D_3) increased overall survival percentage of marron as well as number of marron in size grade of 20 to 40 g, it is recommended that under semi-intensive farm conditions the minimal protein should be used while formulating the diets and this should preferably come from an animal source supplemented with fish oil.

CHAPTER 6

STARVATION AND DELAYED FEEDING

6.1 INTRODUCTION

Crustaceans, like many other aquatic organisms, are subjected to scarcity of food and prolonged starvation in their natural environment (Gu *et al.*, 1996). They are also subjected to periodic fasting during, immediately before and immediately after, moulting. As a consequence, they undergo some alterations in their normal physiological and biochemical processes and their nutritional status is altered during these non-feeding periods (Hochachka and Somero, 1984). Depletion of protein, carbohydrate, glycogen and lipid reserves and reduction in metabolic rates have been associated with nutritional deprivation in crayfish and other crustaceans (Speck and Urich, 1969; Marsden *et al.*, 1973; Hazlett *et al.*, 1975; Regnault, 1981 and Edsman *et al.*, 1993). Schirf *et al.* (1987) studied the qualitative and quantitative changes in proximate composition of the tail muscles and hepatopancreas in red swamp crayfish (*P. clarkii*). Hepatosomatic index and tail muscles carbohydrate levels have been used previously as indicators of nutritional deprivation in *C. tenuimanus* (Evans *et al.*, 1992).

After certain periods of starvation, it takes some time to regain the normal nutritional or physiological state in juvenile redclaw crayfish (*C. quadricarinatus*) (Gu *et al.*, 1996). Protein synthesis in juvenile signal crayfish (*P. leniusculus*) was reported to increase again after the first day of re-feeding and return to normal concentrations after four days of feeding (Edsman *et al.*, 1993).

The aim of the present study was to investigate the hypothesis that different starvation periods effect the growth, survival, and proximate composition of body tissues and conditional indices in marron. The study also investigated the hypothesis that refeeding marron after various periods of starvation influences

the proximate composition of body tissues and conditional indices. The experiment consisted of two trials of 120 and 150 days, respectively. Trial 1 was carried out to investigate the nutritional contribution of exuvia of marron by allowing the marron to feed on their own exuvia. It is a common practice in intensive crayfish culture system (ICCS) to allow marron to feed on their exuvia. Trial 1 aimed to investigate the impact of feeding on exuvia on growth and condition indices in marron. The contribution of exuvia to marron growth and condition indices needed to be determined in order to clarify one protocol of the starvation experiment (trial 2): did the exuvia need to be removed?

6.2 MATERIALS AND METHODS

6.2.1 TRIAL 1

6.2.1.1 Experimental System

The trial was conducted for 120 days in a battery culture system called a 'Nardi' system or intensive crayfish culture system (ICCS; O'Sullivan, 1990). The system consisted of four stainless steel culture tanks in the form of raceways (360 cm (l) x 45.5 cm (w) x 21 cm (h)) connected to a round, fiberglass sump tank of 2800 L capacity. Four culture tanks were placed at two different heights occupying two tiers. Each tank was divided into 20 compartments (43 cm (L) x 16 cm (W) x 21 cm (H)) with 3 mm thick polypropylene dividers which had 6 mm diameter holes to allow water flow. Each compartment was covered with a black perforated polypropylene lid. Each culture tank had a water inlet from both ends and an outlet in the middle, connected to the sump tank. Water was circulated continuously from the sump tank by a pump to the culture tanks. Water flowed by gravity back into the sump tank through four independent PVC pipes at a total flow rate of 50 L per minute.

6.2.1.2 Experimental Design

One hundred marron were harvested from two cement tanks of a commercial marron farm, Cassin Farm, Parkerville, Western Australia (Plate 1.2). They were transported in two polystyrene boxes to the experimental site and placed in a corrugated iron tank (4m x 3m x 0.5 m) of 6,000 L capacity. They were held for five days in the tank and were not fed prior to placement in the ICCS.

Eighty marron, mean weight of 16.25 ± 0.60 SE g, were used in the trial and one marron was placed into each compartment. Each compartment was marked from 1 to 80. A PVC pipe 15 cm long and 50 mm diameter was placed inside each compartment to provide a shelter for individual marron. All marron were acclimatized for a period of two weeks during which they were fed a reference diet (Table 3.1 of Chapter 3) of 22 % protein, 8% lipid, 6% fibre on alternate days, for a period of two weeks. Similar-sized marron were used to replace mortalities during the acclimatization period. Individual weight was recorded for each marron at the end of the acclimatization period. Marron in compartments numbered 1 to 20 were fed a marron reference diet alone and exuvia were removed immediately after moulting (treatment D_1). Marron in compartments 21 to 40 were not fed and the exuvia were not removed (treatment D_2), marron being allowed to feed on their own exuvia. Marron in compartments 41 to 60 were fed the reference diet and exuvia were not removed (treatment D_3). Marron in compartments 61 to 80 were not fed and exuvia were removed immediately after moulting (D_4). At the end of the trial, all surviving marron were dissected and analyzed for hepatosomatic indices and tail muscles to wet body weight ratios.

6.2.1.3 Water Quality

Temperature, pH, and dissolved oxygen were recorded on alternate days before feeding at 15.00 hrs. Total ammonia, nitrite and nitrate were recorded once a month. Total ionic composition (*Appendix I, Table VI*) of water was recorded before and immediately after completion of the trial.

6.2.1.4 Recording and Data Analysis

Data for growth and survival were recorded at the end of the trial. Specific growth rate and survival percentages were also calculated using the equations described in Chapter 2. The data were analyzed statistically using one-way analysis of variance (ANOVA) and the LSD multiple–comparison test to identify significant differences among treatment groups (Steel and Torrie, 1960, 1980) for SGR and condition indices.

6.2.2 TRIAL 2

6.2.2.1 Experimental System

The trial was performed for 150 days in a modified ICCS or 'Nardi' system having different dimensions from those used in trial 1. The four stainless steel culture tanks had the dimensions of 624 cm x 47.5 cm x 21 cm connected to a round, fibreglass sump tank of 3100 L capacity. Each tank was divided into 38 compartments (44 cm (l) x 15.6 cm (w) x 20 cm (h)) with 3 mm thick polypropylene dividers which had 6 mm diameter holes to allow water flow. Each compartment was covered with a black, perforated polypropylene lid. Each culture tank had a water inlet from both ends and an outlet in the middle, connected to the sump tank. Water was continuously circulated from the sump tank by a pump to the culture tanks *via* a polystyrene pipe so that each compartment received independent water through a small irrigation polystyrene pipe. Water fed by gravity back into the sump tank through four independent PVC pipes at a total flow rate of 50 L per minute. The Nardi system was modified for this trial by installing a biological filter inside the sump. Eighty percent of the flow was directed into the bottom of the biological filter containing off-cuts of PVC and polystyrene pipes. The biological filter medium also contained 50 L of plastic bio balls. The water dropped back into the sump tank from the top end of the biological filter and was automatically aerated.

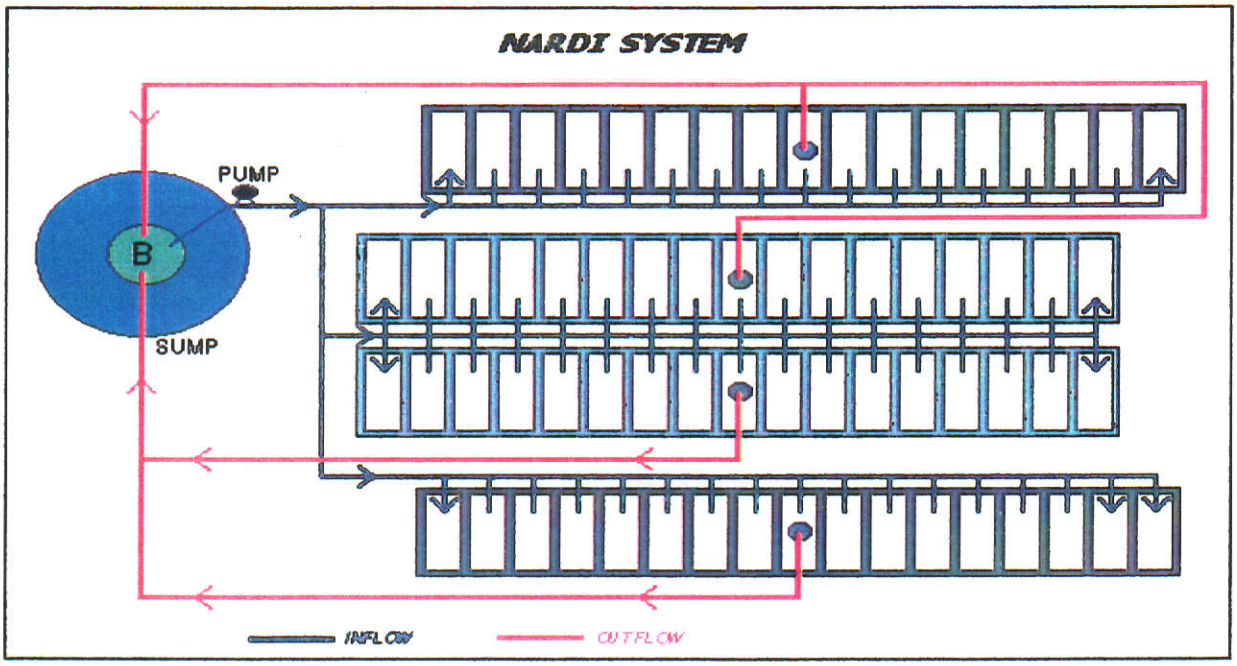


Figure 6.1 **A diagrammatic sketch of the ICCS (S = sump; B = biological filter; C= compartments; p = pump and arrow shows the direction of flow of water.**

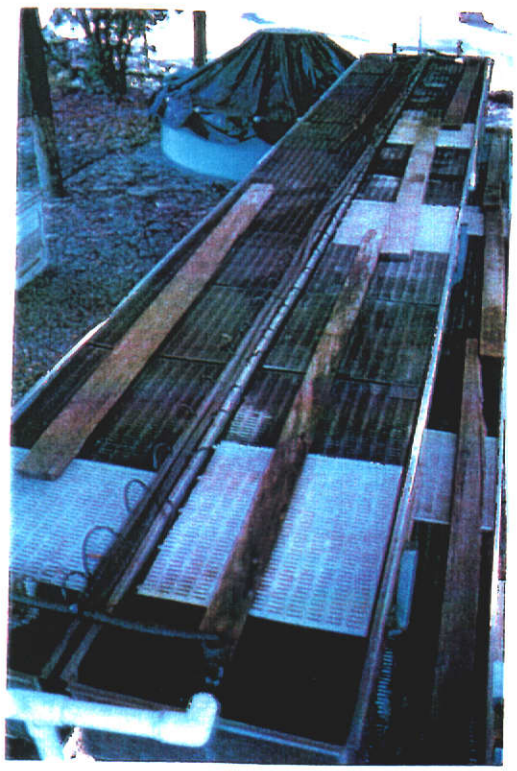


Plate 6.1 **Battery culture system, ICCS where trial 2 was conducted.**

Plate 6.2 **Two marron in compartments of the ICCS**

6.2.2.2 Experimental Design

Two hundred marron were harvested from two cement tanks of commercial marron farm, Cassin Farm, Parkerville, Western Australia. They were transported in two polystyrene boxes to the experimental site and placed in a corrugated iron tank (4m x 3m x 0.5 m) of 6,000 L capacity. They were held for five days in the tank and were not fed.

One hundred and fifty compartments were used in the trial and one marron was placed into each compartment. Each compartment was given a number from 1 to 150. A piece of PVC pipe of 15 cm long and 50 mm diameter was placed inside each compartment to provide shelter for individual marron. All marron were acclimatized for a period of four weeks during which they were fed alternate days with a reference diet of 26 % protein, 8% lipid and 4.7 % fibre. A similar-sized marron replaced any dead marron during the acclimatization period. Individual weight of marron was recorded at the end of the acclimatization period. On day 1

of the trial, feeding was stopped to all marron except all those in compartments numbered 1 to 30.

The animals were selected at random and partitioned according to the treatment in Table 6.1. Following sacrifice, specimens were weighed individually and dissected. Their tail muscles and hepatopancreas were weighed to determine the wet hepatosomatic index and tail to body ratios using the equations described in Chapter 2. The dry hepatosomatic index, hepatosomatic moisture percentages, dry tail to body ratio and tail moisture percentage was also determined by the procedures described in Chapter 2. The dry samples of hepatopancreas and tail muscles were refrigerated for proximate analysis by the methods described in Chapter 2.

Table 6.1 Summary of the dietary treatments in the ICCS.

Dietary treatment symbol	Dietary treatment	Number of specimen	Day of sacrifice
D_1	Marron continuously starved for 30 days	5	30
D_2	Marron continuously starved for 60 days	5	60
D_3	Marron continuous starved for 120 days	5	120
D_4	Marron continuously starved for 150 days	5	150
D_5	Marron continuously fed for 30 days	5	30
D_6	Marron continuously fed for 60 days	5	60
D_7	Marron continuously fed for 120 days	5	120
D_8	Marron continuously fed for 150 day	5	150
D_9	Marron fed for 30 days after 30 days of starvation	5	60
D_{10}	Marron fed for 90 days after 30 days of starvation	5	120
D_{11}	Marron fed for 120 days after 30 days of starvation	5	150
D_{12}	Marron fed for 60 days after 60 days of starvation	5	120
D_{13}	Marron fed for 90 days after 60 days of starvation	5	150
D_{14}	Marron fed for 30 days after 120 days of starvation	5	150

6.2.2.3 Water Quality

Temperature, pH, and dissolved oxygen were recorded on alternate days before feeding at 15.00 hours. Total ammonia, nitrite and nitrate were recorded once a month. Total ionic composition of water was recorded before and after the commencement of the trial.

6.2.2.4 Recording and Data Analysis

Data for growth and survival were recorded on days 30, 60, 120 and 150. Specific growth rate and survival were also calculated using the equations described in Chapter 2. When a marron moulted it was weighed the next day to calculate the moult-increment (W_m) using the following equation:

$W_m = (W_a - W_b) \times 100 / W_b$
where, W_a = weight after the moult, g
W_b = Weight before moult, g.

The data were analyzed statistically by one-way analysis of variance (ANOVA) and LSD multiple–comparison test to identify significant differences among treatment groups (Steel and Torrie, 1960) for SGR, hepatosomatic indices (wet and dry) tail to body ratio (dry and wet) and proximate analysis of hepatopancreas and tail muscles.

6.3 RESULTS

6.3.1 Water Quality

Monthly maximum/minimum temperature range is shown in *Appendix I, Figure I* and pH, dissolved oxygen, nitrogen metabolites and ionic composition of water is shown in *Appendix I, Table VI.*

6.3.2 Weight

In trial 1, there were no significant differences ($P > 0.05$) among the mean final weights of marron for the four dietary treatments (Table 6.2). Completely starved marron (D_4) grew to a mean final weight of 18.46 ± 1.43 SE g, which was not

significantly different than the mean final weight of marron fed the other three diets.

Table 6.2 Effect of starvation and feed exuvia on final weight (mean ± SE g) of marron.

Dietary Treatments	Initial wet weight (s)	Final wet weight (g)
D_1	17.44 ± 1.42 [a]	19.12 ± 1.69 [a]
D_2	15.92 ± 0.92 [a]	16.31 ± 1.21 [a]
D_3	14.71 ± 0.85 [a]	16.50 ± 0.95 [a]
D_4	16.94 ± 1.02 [a]	18.46 ± 1.43 [a]

In trial 2, feeding marron for 150 days did not significantly increase ($P > 0.05$) their final weight (Table 6.3). However, starvation of marron for 60, 120 and 150 days (D_2, D_3 and D_4 respectively) resulted in significantly lower mean final weight of marron than in the treatments involving continuous feeding for the same periods (D_6, D_7 and D_8). There was no significant difference ($P > 0.05$) in mean final weight between marron starved and continuously fed for 30 days. Feeding marron for 30 and 90 days after 30 days of initial starvation (D_9, D_{10}) did not influence their mean final weight, whereas 30-day starved marron, when fed for 120 days (D_{11}), reached a mean final weight (22.89 ± 5.88 SE g) which was a biomass similar to that of fed marron (D_5, D_6, D_7 and D_8). Marron fed for 30 days after being subjected to 120 days of starvation (D_{11}) did not exhibit any significant increase in mean final weight compared to marron when starved for four different periods (D_1, D_2, D_3 and D_4).

6.3.3 Growth and Survival

In trial 1, marron receiving reference diet plus their exuvia (D_3) had a significantly higher ($P < 0.05$) SGR compared to the completely starved marron (D_4) and marron receiving only their exuvia as a sole source of nutrition (D_2) (Table 6.4). There was no significant difference in SGR among marron fed: only reference

diet (D_1); those feeding on their exuvia only (D_2); and those completely starved (D_4). Marron receiving the reference diet showed high survival (88.9 and 100%) during the trial, whereas, in totally starved marron survival was lowest (72.2%). All marron showed similar intermoult intervals (Table 6.4).

Table 6.3 **Effect of feeding and starvation on final weight (mean ± SE g) and SGR (mean ± SE % / day) of marron. S – starvation and F – feeding. Numericals followed after S and F are number of days.**

Treatments	Final weight	SGR
D_1 (S 30)	20.60 ± 2.43^{b}	0.41 ± 0.18^{a}
D_2 (S 60)	18.10 ± 0.76^{b}	$0.18 \pm 0.06^{b,c}$
D_3 (S 120)	18.80 ± 1.98^{b}	$0.11 \pm 0.06^{c,d}$
D_4 (S 150)	20.50 ± 1.51^{b}	0.03 ± 0.01^{d}
D_5 (F 30)	$23.60 \pm 3.96^{a,b}$	$0.33 \pm 0.13^{a,b}$
D_6 (F 60)	29.40 ± 4.16^{a}	$0.20 \pm 0.04^{b,c}$
D_7(F 120)	27.50 ± 4.35^{a}	$0.17 \pm 0.04^{b,c}$
D_8 (F 150)	29.75 ± 1.82^{a}	$0.22 \pm 0.02^{b,c}$
D_9 (S 30: F 30)	18.80 ± 3.54^{b}	$0.17 \pm 0.03^{b,c}$
D_{10} (S 30: F 60)	17.70 ± 1.55^{b}	0.13 ± 0.04^{c}
D_{11} (S 30: F 120)	22.89 ± 2.98^{a}	$0.11 \pm 0.02^{c,d}$
D_{12} (S 60: F 60)	22.30 ± 5.88^{a}	0.11 ± 0.03^{c}
D_{13} (S 60: F 90)	22.90 ± 1.95^{a}	$0.10 \pm 0.02^{c,d}$
D_{14} (S 120: F 30)	$21.53 \pm 2.45^{a,b}$	$0.07 \pm 0.04^{c,d}$

Table 6.4 **Effect of starvation and feeding exuvia on survival (mean ± SE %) , SGR (mean ± SE %/day) and inter-moult period (mean ± SE days) of juvenile marron.**

Dietary Treatments	SGR	Survival %	Inter-moult period (days)
D_1	$0.17 \pm 0.02^{a,b}$	100.00	16.46 ± 2.83^{a}
D_2	0.11 ± 0.03^{b}	76.47	21.82 ± 4.54^{a}
D_3	0.24 ± 0.04^{a}	88.89	21.25 ± 3.33^{a}
D_4	0.13 ± 0.03^{b}	72.22	16.21 ± 3.38^{a}

Values in the same column having different superscript are significantly different ($P < 0.05$).

In trial 2, the SGR of starved marron declined steadily over the 150 days of starvation as shown regression coefficient ($R^2 = 0.86$) between SGR and period of starvation (Table 6.3 and Figure 6.2). Up to day 120, the SGR of starved marron (D_1, D_2 and D_3) did not differ significantly ($P > 0.05$) from the SGR of marron fed for the same time period (D_5, D_6 and D_7). However, by day 150, the SGR of continuously starved marron (0.03 ± 0.01 g ; D_4) was significantly lower ($P < 0.05$) than the SGR of continuously fed marron (0.22 ± 0.02 SE g ; D_8). Continuously fed marron did not show any correlation to SGR ($R^2 = 0.43$) in ICCS.

There were no significant differences in SGR among 30-day starved marron fed for 30, 60 and 120 days (D_9, D_{10} and D_{11} ; Figure 6.3), although SGR declined steadily ($R^2 = 0.76$). Further, there was no significant difference in SGR between marron fed for 60 and 90 days after being starved for 60 days (D_{12} and D_{13} ; Table 6.3). Feeding marron for 30 days after being subjected to 120 days of starvation (D_{14}) did not significantly improve their SGR than of marron which were continuously starved for 150 days (D_4).

In trial 2, marron starved for 150 days had the lowest survival of 60% whereas marron continuously fed had a survival rate of 97% (Figure 6.4). The survival of marron dropped steadily as the starvation period increased from 30 to 120 days. (Figure 6.4).

6.3.4 Condition Indices

In trial 1, there were no significant differences between hepatosomatic indices (wet and dry; H_{iw} and H_{id}) and moisture content of hepatopancreas (HM%) of marron fed reference diet (D_1) alone and marron fed the reference diet plus their exuvia (D_3) (Table 6.5). Similarly, there were no significant differences in H_{iw}, H_{id} and HM% of completely starved marron (D_4) and marron fed exuvia only (D_2).

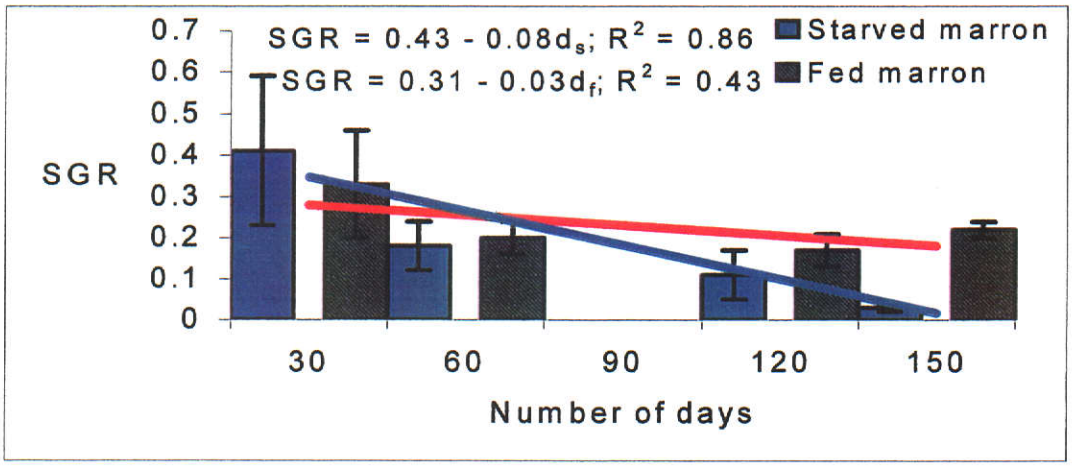


Figure 6.2 **Influence of prolonged starvation periods on SGR (mean ± SE %/day) of marron.**

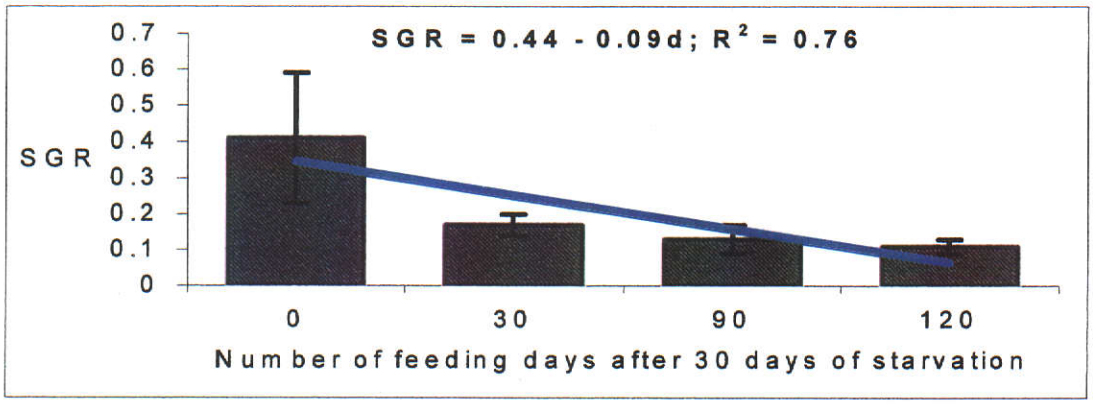


Figure 6.3 **Specific growth rate (mean ± SE %/day) of marron initially starved for 30 days.**

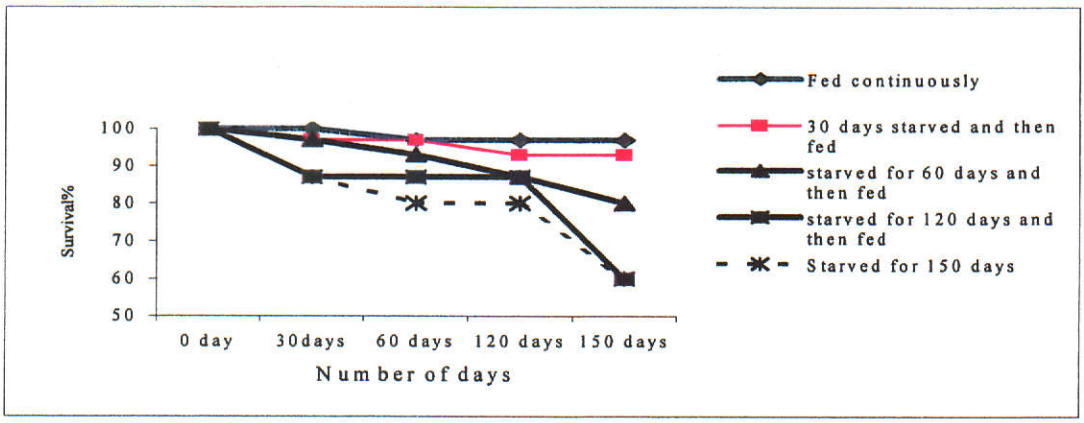


Figure 6.4 **Effect of starvation and delayed feeding on survival percentage of marron.**

However, H_{iw} and H_{id} were significantly higher and the HM% was significantly lower ($P < 0.05$) in marron fed reference diets (D_1 and D_3). There were no significant differences in wet tail muscles to body weight ratio (T/B_w) of marron among any dietary treatments. However, dry tail muscles to body weight ratio (T/B_d) in starved marron (D_4) was significantly lower ($P < 0.05$) than T/B_d of marron fed reference diet plus their exuvia (D_3). Percentage moisture levels in tail muscles (TM%) were not significantly different among marron receiving four different dietary treatments.

Table 6.5 **Effect of starvation and feeding exuvia on the conditional indices of juvenile marron.**

Condition Indices	D_1	D_2	D_3	D_4
H_{iw}	5.43 ± 0.30^{a}	1.80 ± 0.32^{b}	4.79 ± 0.31^{a}	1.92 ± 0.16^{b}
H_{id}	1.16 ± 0.08^{a}	0.27 ± 0.11^{b}	1.25 ± 0.12^{a}	0.24 ± 0.02^{b}
HM%	78.58 ± 1.19^{a}	85.22 ± 2.30^{b}	73.39 ± 2.23^{a}	87.34 ± 0.04^{b}
T/B_w	18.07 ± 0.44^{a}	18.21 ± 0.14^{a}	22.90 ± 2.44^{a}	23.31 ± 2.32^{a}
T/B_d	$3.37 \pm 0.08^{a,b}$	$3.05 \pm 0.10^{a,b}$	3.56 ± 0.29^{a}	3.00 ± 0.07^{b}
TM%	81.37 ± 0.09^{a}	83.24 ± 0.47^{a}	83.00 ± 2.17^{a}	85.91 ± 0.42^{a}

Values in the same row having different superscripts are significantly different ($P < 0.05$).

In trial 2, starvation till 150 days did not influence the wet hepatosomatic index (H_{iw}) and moisture percentage in hepatopancreas (HM%) (Table 6.6). However, marron starved initially for 30 days had a significantly higher dry hepatosomatic index (H_{id}) than marron which were starved for more than 30 days. Starvation periods of 60 days and more did not influence significantly ($P > 0.05$) H_{iw}, H_{id} and HM% marron (Table 6.6). There were no significant differences in H_{iw}, H_{id} and HM% between 30-day fed marron and 30-day starved marron. However, H_{iw} and H_{id} were significantly lower in marron starved for 60, 120 and 150 days compared to marron which were continuously fed for similar periods. Starvation for 60, 120 and 150 days resulted in higher HM% of marron compared to HM% of fed

marron. Feeding marron for 60 days after being subjected 30 days of starvation initially resulted in significantly higher H_{iw}, H_{id} and HM% values compared to marron which were fed for 30 days after being subjected to the same initial period of starvation. Feeding to 60 - days starved marron did not influenced H_{iw}, H_{id} and HM%. There was no significant difference in H_{iw}, H_{id} and HM% between marron fed for 30 days after being subjected to 120 days of initial starvation and marron continuously starved for 150 days.

Table 6.6 **Effect of feeding and starvation on the mean wet and dry hepatosomatic indices (H_{iw} and H_{id}) and moisture percentage in the hepatopancreas (HM%) of juvenile marron.**

Treatments	H_{iw}	H_{id}	HM%
D_1	$2.53 \pm 0.35^{a,b}$	$0.48 \pm 0.03^{b,c}$	$84.56 \pm 8.59^{a,b,c,d,e,f}$
D_2	$2.50 \pm 0.33^{a,b}$	0.21 ± 0.05^{a}	91.80 ± 1.23^{f}
D_3	1.84 ± 0.19^{a}	0.16 ± 0.04^{a}	$90.68 \pm 2.32^{e,f}$
D_4	$2.59 \pm 0.28^{a,b}$	0.28 ± 0.03^{a}	$89.08 \pm 0.80^{d,e,f}$
D_5	$3.24 \pm 0.41^{b,c}$	$0.47 \pm 0.17^{b,c}$	$86.86 \pm 3.51^{b,d,e,f}$
D_6	$4.34 \pm 0.61^{c,d,e}$	$0.83 \pm 0.18^{c,d,e}$	$81.42 \pm 1.85^{a,b,c,d}$
D_7	$4.53 \pm 0.32^{d,e}$	$0.95 \pm 0.07^{d,e}$	78.75 ± 2.03^{a}
D_8	$4.95 \pm 0.02^{e,f}$	$0.68 \pm 0.01^{b,c,d}$	76.17 ± 0.09^{a}
D_9	$3.50 \pm 0.14^{b,c,d}$	$0.41 \pm 0.07^{a,b}$	$88.24 \pm 1.85^{d,e,f}$
D_{10}	5.90 ± 0.71^{f}	1.07 ± 0.07^{e}	$81.17 \pm 1.98^{a,b,c}$
D_{11}	$5.08 \pm 0.33^{e,f}$	$0.82 \pm 0.09^{c,d,e}$	$84.13 \pm 1.07^{a,b,c,d,e,f}$
D_{12}	$4.90 \pm 0.27^{e,f}$	$1.00 \pm 0.08^{d,e}$	$79.56 \pm 1.31^{a,b}$
D_{13}	$4.87 \pm 0.33^{e,f}$	$0.81 \pm 0.07^{c,d,e}$	$83.44 \pm 0.93^{a,b,c,d,e}$
D_{14}	$2.68 \pm 0.25^{a,b}$	$0.40 \pm 0.18^{a,b}$	$85.06 \pm 0.91^{a,b,d,e,f}$

There were significant differences in wet and dry tail muscles to body weight ratios (T/B_w and T/B_d, respectively) between marron starved for 60 days and marron starved for 120 days (Table 6.6). After 60 days of starvation, the percentage moisture content of the tail muscles (TM%; 87.42 ± 0.64) decreased significantly compared to that (84.32 ± 01.22) of marron starved for 30 days.

There were no significant differences in TM% among marron starved for 60, 120 and 150 days. After 120 and 150 days of starvation T/B_w and T/B_d of marron significantly ($P < 0.05$) decreased compared to marron fed for the same time periods, whereas, TM% of the marron increased significantly only after 60 days of starvation (Table 6.6). After 120 days of experimentation starved marron had significantly lower T/B_w and T/B_d compared to marron fed for 120 days. After 150 days of starvation, T/B_w and T/B_d values decreased significantly compared to marron fed for the same time period. The percentage moisture content in the tail muscles (TM%) of marron were significantly higher in marron starved for 60, 120 and 150 days compared to marron fed for similar periods. Feeding for 60 days following 30 days of starvation significantly increased their T/B_w and T/B_d values but did not influence TM%. Feeding for 120 days following 30 days of starvation resulted in T/B_w and T/B_d levels similar to those of marron fed for only 30 days. Feeding 30–days starved marron for 30, 60 and 120 days did not significantly influence the TM%. However, marron that were initially starved for 60 days, then fed for 120 days increased their TM% compared to marron fed only for 60 days after being subjected to the same period of starvation. Thirty days of feeding following 120 days of starvation did not influence T/B_w and T/B_d and TM% compared to marron continuously starved for 150 days.

6.3.5 Body Composition

Starvation resulted in a steady decline in lipid and protein content (as per dry weight basis) in the hepatopancreas (Table 6.8). Starvation for 150 days resulted in a decline in hepatopancreatic lipid level from 51.1 to 10.5 mg/100 mg of dry weight. Similarly, the protein content in the same marron decreased steadily from 30.4 mg/ 100 mg to 21.1 mg/100 mg of dry hepatopancreas. Apparently, starvation of marron till 120 days did not influence the protein content in the tail muscles (Table 6.9). Continuous feeding for 150 days did not increase the lipid and protein contents of hepatopancreas (Figures 6.7 and 6.9). Ash content of hepatopancreas and tail muscles remained unaffected by the dietary treatments. The influence of

various dietary treatments on the proximate analysis of hepatopancreas and tail muscles is summarized in Tables 6.8 and 6.9.

Table 6.7 **Effect of feeding and starvation on the mean wet and dry tail muscles to body weight (T/B_w and T/B_d)) and moisture percentage in the tail muscles (TM%) of juvenile marron.**

Treatments	T/B_w	T/B_d	TM %
D_1	$25.79 \pm 0.51^{c,d}$	$4.05 \pm 0.35^{d,e,f,g}$	$84.32 \pm 1.22^{a,b,c,d}$
D_2	$27.00 \pm 0.63^{c,d}$	$3.41 \pm 0.24^{b,c,d,e}$	87.42 ± 0.64^{e}
D_3	19.32 ± 0.09^{a}	$2.49 \pm 0.25^{a,b}$	87.16 ± 0.81^{e}
D_4	15.06 ± 1.22^{a}	2.04 ± 0.18^{a}	$86.38 \pm 0.59^{d,e}$
D_5	$27.35 \pm 1.21^{c,d}$	$4.44 \pm 0.30^{e,f,g}$	83.80 ± 0.66^{a}
D_6	$26.15 \pm 0.78^{c,d}$	$4.35 \pm 0.19^{e,f,g}$	83.35 ± 0.53^{a}
D_7	$24.75 \pm 2.05^{b,c}$	$4.12 \pm 0.45^{d,e,f,g}$	$83.48 \pm 0.38^{a,b}$
D_8	$27.95 \pm 0.04^{b,c}$	4.05 ± 0.01^{e}	82.99 ± 0.09^{a}
D_9	$25.08 \pm 0.78^{b,c}$	$3.80 \pm 0.20^{c,d,e,f}$	$84.89 \pm 0.49^{a,b,c,d}$
D_{10}	30.44 ± 4.80^{d}	4.94 ± 0.82^{g}	$83.84 \pm 0.44^{a,b,c}$
D_{11}	$20.37 \pm 1.24^{a,b}$	$2.89 \pm 0.19^{b,c,d}$	$85.81 \pm 0.41^{a,b,c,d}$
D_{12}	$20.27 \pm 1.85^{a,b}$	$3.17 \pm 0.38^{a,b}$	84.50 ± 0.61^{a}
D_{13}	19.11 ± 0.85^{a}	$2.76 \pm 0.18^{a,b}$	$85.65 \pm 0.54^{b,c,d,e}$
D_{14}	15.59 ± 0.94^{a}	2.00 ± 0.14^{a}	87.15 ± 0.47^{e}

6.4 DISCUSSION

Trial 1 showed that availability of exuvia as a food source did not significantly influence the mean final weight, SGR or any of the condition indices of marron, however, it did influence survival of marron. Though exuvia of marron are a rich source of calcium (54 – 78% and 0.32 – 0.75% respectively) (Jussila et al., 1995), no energy value can be associated with this, but it was observed that uneaten exuvia can become the substrate for some benthic organisms and fungi, from where marron could perhaps derive some nutrition. As feeding on exuvia had influenced the survival of the marron, exuvia were removed from the compartments in trial 2.

Table 6.8 **Proximate composition in mg / 100 mg of dry hepatopancreas of juvenile marron.**

Treatments	Lipid	Protein	Ash	Moisture %
D_1	51.1	30.4	2.8	84.56
D_2	49.3	24.8	4.5	90.68
D_3	18.5	22.2	4.1	90.7
D_4	10.5	21.1	3.9	89.08
D_5	54.9	24.7	4.1	86.86
D_6	31.1	25.2	4.3	84.4
D_7	32.9	26.5	4.4	78.75
D_8	41.1	25.3	3.3	76.17
D_{10}	46.2	22.7	4.5	81.17
D_{11}	47.1	23.6	5.0	84.13
D_{12}	14.8	21.4	3.6	79.56
D_{13}	23.7	21.3	4.6	83.44
D_{14}	17.0	22.0	4.4	85.06

Table 6.8 **Proximate composition in mg / 100 mg of dry tail muscles of juvenile marron.**

Treatment	Lipid	Protein	Ash	Fibre	Moisture %
D_1	3.1	90.6	4.9	0.01	84.32
D_2	3.1	90.7	3.5	0.02	85.70
D_3	2.4	90.8	1.3	0.04	87.42
D_4	0.7	87.8	2.1	0.01	86.38
D_5	3	89.9	6.6	0.06	83.80
D_6	3.4	88.8	4.5	0.01	83.50
D_7	0.7	89.4	1.2	0.01	83.48
D_8	5.2	92.6	3.9	0.01	82.99
D_{10}	0.6	86.4	1.3	0.01	83.84
D_{11}	2.4	88.0	3.8	0.01	85.81
D_{12}	0.7	88.6	1.3	0.01	84.50
D_{13}	2.4	86.3	2.6	0.01	85.65
D_{14}	3.1	87.3	2.3	0.01	87.15

In trial 2, dietary treatments D_5, D_6, D_7 and D_8 can be considered as controlled treatments where juvenile marron were fed continuously for a period of 30, 60, 120 and 150 days respectively. Four different starvation periods had influenced the mean final weight, SGR and survival of the marron. Re-feeding marron after

various periods of starvation also had an influence on the SGR. Four starvation periods did influence the H_{id} and T/B_w, T/B_d and TM% and body composition of the marron.

6.4.1 Weight

The final weight and SGR of marron did not increase during continuous feeding, indicating that the intensive crayfish culture system (ICCS) environment does not provide suitable conditions for marron growth. This information is supported by the work of Jussila (1997). After 60 days of starvation, the mean final weight of marron started to show a significant decline. It took 120 days of re-feeding to 30-day starved marron to recover and show significant increase in mean final weight. Gu *et al.* (1996) demonstrated that starvation caused a marked decrease in mean final weight of juveniles of redclaw crayfish (*C. quadricarinatus*). But as soon as starved juveniles were provided with food they began to increase in weight, but at a slower rate than the controls.

6.4.2 Growth and Survival

The SGR of continuously fed marron in ICCS ranged from 0.17 to 0.33 %/ day which is lower than SGR of marron observed in aquaria under laboratory conditions (SGR of 0.32 to 1.27%/ day) (Fotedar *et al.*, 1997) and marron reared in cages (SGR of 1.20 to 1.39 %/day) (Fotedar *et al.*, 1998) but comparable to other studies of marron reared in ICCS (SGR of 0.4 to 0.8 %/ day) (Jussila, 1997).

Without supplemental feeding, ICCS is unable to sustain similar growth of marron in a way that a pond environment can. For 120 days, the SGR showed no difference between fed and starved marron, indicating that it took 150 days of starvation before the SGR was affected.

Meade and Watts (1997) demonstrated that short-term nutrient deprivation did not have long term consequences in terms of weight gain in juvenile red claw crayfish

(*C. quadricarinatus*). Crayfish that exhibited minimum weight gain when cultured initially using poor-quality feeds exhibited improved weight gain when fed high quality feed. Further, Bosworth and Wolters (1995) demonstrated compensatory growth in juvenile red swap crayfish (*P. clarkii*) starved for 30 days. Compensatory growth is a phase of higher growth than normal after a period of starvation.

In the present study, survival was negatively correlated with starvation periods (R^2 = 0.86). Mortalities continued to occur after feeding was resumed following periods of starvation of 60 days or greater, indicating that juvenile marron starved for this length of time may suffer a significant irreversible decline in body reserves (or health) only able to benefit from subsequent 120 days of continuous feeding. It was not until 120 days of feeding after a period of starvation that mortalities ceased. This is in contrast to a study of redclaw crayfish (*C. quadricarinatus*) juveniles reported by by Gu *et al.* (1996). The two species may have different mechanism of energy storage which reflect their natural environments.

Mikami and Takashima (1993) demonstrated that starvation decreased the first moulting interval of newly hatched phyllosoma larvae of the spiny lobster, *P. japonicus*. Longer periods of time-to-first-feeding lengthened the intermoult period of the first instar of *P. japonicus* and *Thenus* sp. larvae without affecting the duration of later intermoult periods or growth rates (Mikami *et al.*, 1995).

6.4.3 Condition Indices

Sixty days of starvation resulted in an increase in TM% but it was only after 120 days of starvation that wet and dry tail muscles to body weight ratio (T/B_w and T/B_d) decreased significantly. The finding that starvation caused an increase in percentage water content agrees with the previous studies on other crayfish species (Claybrook, 1983; Gu *et. al.*, 1996). The reason why TM% did not change after

starving beyond 60 days may be because there is a threshold limit for percentage water content in the tail muscles which was reached after 60 days of starvation.

As expected, TM%, and H_{iw}, did not increase during the length of the starvation, perhaps because the threshold for water content in the hepatopancreas was reached well before the 30 days of starvation. However, it took 60 days of continuous starvation for a marron to show a decline in H_{id}. The HM% value in marron continuously fed for 30 days did not differ from the HM% of marron starved for the same length of time, indicating that marron in the ICCS system were already nutritionally/physiologically stressed. It took 60 days of continuous feeding for them to recover from the stress. If percentage moisture content of the hepatopancreas and tail muscles can give any indication of nutritional/ physiological stress in marron, then marron never recovered after 30 days of starvation. However, the H_{iw} recovered after 60 days of re-feeding to marron initially starved for 30 days and the T/B_w increased after 120 days of re-feeding to 30-day starved marron. Re-feeding to marron subjected to 120 days of starvation did not lead to any recovery in terms of HM%, TM% or hepatosomatic indices (wet and dry) and tail muscles to body ratio (wet and dry).

Whyte *et al.* (1986) used the ratio of wet weight of hepatopancreas of whole body as an index of physiological condition in the prawn, *Pandalus platyceros,* and demonstrated that the ratio decreased on starvation and increased on re-feeding. Twelve day starved redclaw crayfish (*C. quadricarinatus*) juveniles can regain their protein and water content and return to their original physiological status after being re- fed for 6 days (Gu *et al.*, 1996).

Armitage and Wall (1982) suggested that changes in moisture content in the body tissue reflect physiological changes during starvation. There was a marked decrease in the average thickness of the hepatopancreas in starved juvenile American lobster (*H. americanus*) and disintegration of at least two (F and R) of

the cell types (Niles *et al.*, 1993). The finding that starvation caused an increase in moisture content agrees with previous studies in other crayfish species (Claybrook, 1983). Armitage *et al.* (1973) suggested that in the freshwater crayfish, *Orconectes nailis*, fluctuations in moisture content occurred during the moult and reproductive cycle.

In the present study, marron analysed for condition indices were from the same reproductive state (immature) and marron at an immediate pre- or post-moult stage were not considered for the analysis as described by Schafer (1968) in other crustaceans. A decline in condition indices is usually interpreted as being due to depletion of energy reserves such as stored energy glycogen or body fat (Geode and Barton, 1990). This decline may not be due entirely to starvation but reflect a change in feeding patterns, which could be behavioural response to certain stressors (Brown et al., 1987), or an increase in metabolic rate in response to stress (Schreck, 1981, 1982; Barton and Schreck, 1987).

6.4.4 Body Composition

The regression analyses in Table 6.10 provide evidence for the catabolization of lipids in hepatopancreas and tail muscles in juvenile marron for metabolic requirements during starvation, as indicated by the decreasing percentage dry weight of lipids. Table 6.10 also indicates the utilization of protein levels in the hepatopancreas during continuous starvation.

Protein reserves in the hepatopancreas were depleted at a faster rate than protein reserves in the tail muscles. Starvation did not result in any trend in the utilization of protein in the tail muscles, indicating that the hepatopancreas of juvenile marron is the main site for energy reserves. There is conflicting evidence in the literature on the order of utilization of major energy reserves in decapod crustaceans. Lipid was considered to be utilized first in the crayfish, *Orconectes nair* (Armitage *et al.*, 1972), protein was metabolized primarily in *O. virilis*

(Hazlett *et al.*, 1975) and both lipid and protein were used during progressive starvation of *O. limosus* (Speck and Urich, 1969). Four-week starvation of the shrimp, *Crangon crangon* resulted in the utilization of carbohydrate, then lipid and finally protein reserves (Cuzon and Ceccaldi, 1972). Major depletion of lipid reserves in the starved shore crab, *Carcinus maenas,* (Heath and Barnes, 1970) contrasts with the predominant use of protein reserves in *Hemigrapsus nudus* (Neiland and Scheer 1953) and in the lobsters, *Panulirus longipes,* and *Nephrops norvegicus* (Dall 1974, 1981). Biochemical composition of the hepatopancreas of the pink shrimp, *P. duoranum,* starved for 12 days suggested the initial use of lipid, then protein and finally carbohydrate reserves (Schafer, 1968). Biochemical changes in tissue sections of the prawn, *P. esculentus*, showed that protein from the tail muscles to be the major source of energy and lipid of secondary importance (Barclay *et al.*, 1983). Biochemical changes in starved kuruma prawns (*P. japonicus*) suggested a reseve utilization of carbohydrates, lipid and then protein (Cuzon *et al.*, 1980).

Table 6.10 **Regression analysis of lipid and protein levels in the hepatopancreas and tail muscles as a function of starvation and feeding periods (d_s and d_f respectively) in marron.**

Hepatopancreas		Tail muscles	
Regression equation	**R^2**	**Regression equation**	**R^2**
Lipid level = 70.5 – 15.26 d_s	0.89	Lipid level = 4.3– 0.79 d_s	0.81
Protein level = 32.25 – 3.05 d_s	0.90	Protein level = 92.05 – 0.83 d_s	0.54
Lipid level = 49.9 – 3.96 d_f	0.20	Lipid level = 2.1 + 0.39 d_f	0.07
Protein level = 24.65 + 0.31 d_f	0.28	Protein level = 88.0 + 0.87 d_f	0.45

This study has demonstrated that juveniles marron can tolerate relatively long periods of starvation or low food availability by catabolizing stored energy sources such as lipid and protein in the hepatopancreas and by moisture uptake into the hepatopancreas. However, in community rearing, starvation may lead to an increase in the rate of cannibalism (Gydemo and Westin, 1992). Starving freshwater crayfish, *O. limosus*, for 15 days caused a 2% decrease in total body

protein and starvation for 41 days caused a decline of 11% in total body proteins. The prawn, *Pandalus platyceros*, has a level of metabolic reserves sufficient for live-holding in a fasting condition for at least 40 days with minimal mortalities (Whyte *et al.*, 1986).

Apparently, water content of hepatopancreas is the most sensitive indicator of nutritional/physiological stress to the juvenile marron as compared to water content in the tail muscles. Whyte *et al.* (1986) demonstrated that moisture uptake occurred in all segments of the prawn, *P. platyceros,* but most significantly in the hepatopancreas after 22 days of starvation. Starvation starts influencing wet and dry tail to body weight ratios only after 120 days of continuous starvation in marron. The energy reserves in the form of proteins in the tail muscles are the last option for catabolism.

CHAPTER 7

DISCUSSION AND SUMMARY

7.1 INTRODUCTION

Production of marron through aquaculture has not improved as expected for in Western Australia, for example, and, indeed, has failed to keep pace with the culture of other crustaceans. One of the main reasons for limited growth in aquacultural production of marron may be the lack of knowledge of the nutrient requirements, and the role that rearing environments play in influencing the nutrient requirements of the animal. Indeed, there is a very little information available on the dietary nutrient requirements of many decapod crustaceans under different rearing conditions including the most common semi-intensive pond conditions (Tacon, 1996). Much of the early nutritional research on crustaceans focused on the kuruma shrimp (*P. japonicus*) (Kanazawa, 1985), but more recently the nutritional requirements of *P. monodon* and *M. rosenbergii* (D'Abramo, 1997) have been comprehensively investigated. However, no specific information is available on the nutrient requirements of marron (*C. tenuimanus*) under different culture conditions and the influence of culture environments on their nutritional requirements.

Nutritional trials under controlled laboratory conditions using purified or semi-purified diets provide scope to conduct basic research to determine the specific nutrient requirement in isolation and without the confounding complexities of highly variable natural environments and diets. Therefore, the use of purified and semi-purified diets under controlled laboratory conditions is highly recommended in order to develop and understand the requirements of each of the nutrient components of the diet. However, nutritional trials in the past have had highly variable results because most laboratory-based crayfish feeding experiments were performed with crude practical diets or using feed formulated primarily for other aquatic species (Celada *et*

al., 1989, 1993). Further, the results obtained from these conditions cannot be translated directly to farm situations, necessary for the optimization of feeding and management strategies and prediction of production under farm conditions.

Nutritional trials conducted under farm conditions may fail to take into account the importance of a particular nutrient in the diet of the crayfish due to the complexity and interplay of different environmental variables, and the presence of varied natural productivity unique to each farm site. Also, under field conditions it is difficult to identify the separate inputs of nutrition and ecology in quantifying the contribution of natural productivity in the overall nutritional budget of pond-reared animals (Tacon, 1996). Further, complex field conditions combined with the natural productivity of the ecosystem and nutrient leaching from the formulated feed may mask quantification of one of the important nutritional parameters, namely, ingestion rate.

This thesis investigated the nutritional requirements of marron under different culture conditions, ranging from laboratory aquaria to experiments carried out using a semi-enclosed recirculating system, purpose-built cages in outdoor ponds, purpose-built commercial earthen ponds and battery culture systems where individual marron were housed in separate recirculating environments. The thesis researched the role of protein and lipid nutrition in marron in different culture environments, highlighting the importance of taking into account the cultural environment for the nutritional studies. The thesis also evaluated how the nutritional effectiveness of a particular test diet can be quantified for comparisons between different culture methods.

7.2 Main findings and recommendations for future research

- **Measurement of nutritional effectiveness**

The SGR of marron cannot be considered as a sole indicator of nutritional effectiveness of a test diet because it is influenced by the natural productivity of the

ecosystem under farm conditions and the rate of cannibalism. An alternative approach to the assessment of the nutritional effectiveness of a test diet is to consider the energy reserves (lipids, proteins and carbohydrates) of the hepatopancreas and tail muscles. As energy reserves (dry weight) in the hepatopancreas and tail muscles have shown a strong positive correlation with the SGR and the biomass per unit area in all rearing environments, the hepatopancreas and the tail muscles seem to be the storage sites for energy reserves in marron. Moisture content and dry weight of the hepatopancreas are relatively more sensitive indices of nutritive effectiveness of the formulated diets and/or environmental conditions of the rearing system than the moisture content and tail muscles weight to body weight ratios (wet and dry). Furthermore, a weight change associated with a reduction in energy reserves may be offset by increase in uptake of water into the body tissues (Niami, 1972; Adams *et al.*, 1985, Cunjak and Power, 1986). An increase in body tissue weight may be a long-term phenomenon or a short-term response to a stressor such as handling (Stevens, 1972).

The condition indices, and in particular hepatosomatic indices, may vary with season, with input and distribution of energy available to the aquatic animals, and with sex and life history (White and Fletcher, 1985). In aquatic animals hepatosomatic indices may increase following stress or exposure to pollutants or toxicants (Buckley, *et al.*, 1985). The increase in liver weight is due to hyperplasia (increase in cell numbers) or hypertrophy (increase in cell size) as an adaptive response to increase the capacity of the liver to detoxify foreign compounds via the mixed- function oxidase system (Addison, 1984; Heath, 1987).

Table 7.1 **Regression analysis of the various indices as a function of SGR of juvenile marron cultured in three different environments.**

Culture environment	Regression equations	R^2 values
Cages at commercial farm	SGR = 0.27 + 0.11 H_{iw}	0.88
	SGR = 0.43 + 0.19 H_{id}	0.91
	SGR = 2.08 – 0.02 HM%	0.92
	SGR = 4.41 – 0.05 TM%	0.90
	SGR = 0.05 + 0.02 T/B_w	0.85
	SGR = 0.30 + 0.07 T/B_d	0.94
Earthen ponds at commerci farm	SGR = 6.32 – 1.52 H_{iw}	0.29
	SGR = 3.41 – 2.01 H_{id}	0.80
	SGR = 65.89 + 1.83 HM%	0.01
	SGR = 79.40 + 0.67 TM%	0.01
	SGR = 19.24 + 12.49 T/B_w	0.63
	SGR = 4.68 + 1.93 T/B_d	0.63
Battery culture system (trial 1)	SGR = - 0.71 + 25.83 H_{iw}	0.61
	SGR = - 58.0 + 6.18 H_{id}	0.58
	SGR = 98.1 – 104.86 HM%	0.90
	SGR = 85.34 – 12.09 TM%	0.14
	SGR = 17.39 + 19.92 T/B_w	0.16
	SGR = 2.52 + 4.41 T/B_d	0.90
Battery culture system (trial 2)	SGR = 0.4 –1.73 H_{iw}	0.03
	SGR = 0.59 - 0.02 H_{id}	0.01
	SGR = 85.67– 5.80 HM%	0.03
	SGR = 86.24 – 6.64 TM%	0.28
	SGR = 17.94 + 28.06 T/B_w	0.45
	SGR = 2.47 + 5.42 T/B_d	0.46

Condition indices of marron reared in the cage environment had a strong correlations with the SGR (Table 7.1). However, these relationships were generally weaker in

earthen ponds under commercial conditions and in the ICCS environment. The relationship between SGR and H_{id} was strongly negative in marron reared on the commercial marron farm. It seems that under high temperature and high accumulations of nitrogen metabolites in the rearing environment, energy reserves in the hepatopancreas are channeled for reproduction. Morrissy (1992b) suggested that marron reproduce normally at the 1^+ stage, whereas, in the trial conducted at the commercial marron farm, JBMF, there were indications that, under environmentally stressed conditions, marron may alter their normal strategy and can be reproductively active at an earlier physiological age (Chapter 5). Until the nutritional requirements of essential and trace nutrients for marron are fully known, emphasis has to be given to the quantity and source of energy in marron reared under commercial farms. Consequently the impact of suboptimal environmental conditions like high temperatures and poor water quality on the reproductive strategies of marron need to be elucidated and their geographical implications determined. In addition, research needs to be performed on anti-nutritional factors of certain grains and impact of their dietary inclusions on the digestibility of marron. Marron samples collected for biochemical analysis and assessment of condition indices in experiment 4 (Chapter 5) at JBMF revealed that the majority of specimens had fully mature gonads and majority of females carried eggs under the abdomen suggesting that energy was being directed towards reproduction.

Under ICCS conditions, there was a strong positive correlation between the dry tail muscles to body weight ratio and the SGR but a strong negative relation between HM% and SGR. There was a negative correlation between the dry tail muscles to body weight ratio and the biomass per unit area of marron both in cages and in free-range situations (Chapter 4).

Table 7.2 also suggests that when the biomass of the marron per unit area in cages increases, the moisture content of the tail muscles and hepatopancreas decrease. Both of these indices were positively correlated with biomass per unit area in the free-

range marron at the commercial farm (Chapter 5). An increase in the moisture content in the marron hepatopancreas and tail muscles are indicative of stress in marron. The results show that stress in marron increases as the biomass per unit area increases.

Table 7.2 **Regression analysis of the various indices as a function of density (biomass/m^2) of juvenile marron cultured in two different environments.**

Culture environment	Regression equations	R^2 values
Cages at commercial farm	$B / M^2 = 2.34 + 0.66\ H_{iw}$	0.85
	$B / M^2 = 0.50 + 0.40\ H_{id}$	0.88
	$B / M^2 = 72.20 - 3.53$ HM%	0.88
	$B / M^2 = 85.27 - 1.58$ TM%	0.80
	$B / M^2 = 21.47 + 3.02\ T/B_w$	0.81
	$B / M^2 = 2.98 + 1.03\ T/B_d$	0.87
Earthen ponds at commercial farm	$B / M^2 = 4.98 - 0.01\ H_{iw}$	0.24
	$B / M^2 = 1.61 - 0.01\ H_{id}$	0.51
	$B / M^2 = 62.25 + 0.21$ HM%	0.90
	$B / M^2 = 77.57 + 0.09$ TM%	0.90
	$B / M^2 = 31.97 + 0.01\ T/B_w$	0.01
	$B / M^2 = 6.30 + 0.02\ T/B_d$	0.74

The regression analyses between all six condition indices (H_{iw}, H_{id}, HM%, T/B_w, T/B_d and TM%) pooled from all rearing environments investigated in this study are shown in Table 7.3. As there is a different degree of correlation between SGR and these indices under different culture conditions, pooling the data reduces the strength of correlation. It is evident from Table 7.3 that the moisture content of the tail muscles (TM%) followed by dry tail muscles to body weight ratio (T/B_d) bear strong correlations with the SGR of marron. The dry weight of tail muscles comprises proteins, carbohydrates, lipids (energy reserves) fiber and ash; as energy reserves in tail muscles are utilized, through growth for example, their supplies are replenished

through feeding. The correlation indicates that energy reserves are possibly, stored in the tail muscles for long-term utilisation.

Table 7.3 **Regression analysis of the various indices as a function of SGR (%/day) of juvenile marron cultured in all four culture environments (data pooled from all four rearing environments).**

Regression equation	R^2 value
SGR = 0.02 + 0.09 H_{iw}	0.20
SGR = 0.05 + 0.37 H_{id}	0.45
SGR = 2.73 – 0.03 HM%	0.61
SGR = - 0.06 + 0.04 T/B_w	0.44
SGR = - 0.30 + 0.17 T/B_d	0.66
SGR = 10.0 – 0.12 TM%	0.70

- High levels (12%) of dietary fish lipid (cod liver oil) in the semi-purified diet inhibited the growth of juvenile marron cultured under controlled laboratory conditions (Chapter 2). These results conform to the often-observed relationship of comparatively high dietary lipid levels and reduced growth rates in certain species of aquatic animals, such as *P. japonicus* (Kanazawa *et al.*, 1977; Deshimaru *et al.*, 1979), *P. acutus acutus* (Davis and Robinson, 1986) and *A. astacus* (Ackefors *et al.*, 1992). Dietary lipid levels influence growth by altering the lipid/carbohydrate and ω3/ω6 ratios and possibly by effecting the ingestion rate of marron (Chapter 2). Nevertheless, supplementation of fish oil into the practical diets for marron cultured under farm conditions is recommended (Chapter 4). There is a requirement of both fish and plant oil in the diet of marron. The augmentation of a mixture of animal and plant oil to practical diets provided maximal growth (SGR of 0.88) and biomass (858.83 g) in the cage environment in commercial farm conditions (Chapter 4). The minimum

requirement of fish oil appears to be 2%. Total requirement of oil in the practical formulated feed for marron is therefore more than 2% (Chapter 4).

The study also suggests that both ω-3 and ω-6 fatty acids are important in the nutrition of marron and increasing the relative proportion of ω-3 and ω-6 fatty acids in their composition increases the efficiency of a practical marron diet. In a commercial aquaculture farm setting, intact oils in practical diets, rather than in semi-purified diets have more commercial applications. A positive application of the response to the practical diets was evident by incorporating natural oils containing linolenic and linoleic PUFAs in diets consisting of ingredients that would routinely be used in formulating commercial diets for marron. Natural oils contain fatty acids primarily in triglyceride form and experimental animals usually exhibit better performance when fed intact oils in practical diets rather than isolated or artificial lipid fraction in purified or semi-purified diets (Greene and Selivonchick, 1987). For example, similar responses have been observed in plaice, (Cowey and Sargent, 1972), red sea bream, (Yone and Fujii, 1975) and turbot, (Bell *et al.*, 1985) fed natural oils.

The results indicate that there is a need to conduct nutritional research using semi purified or purified diets under controlled laboratory conditions to elucidate the underlying factors which are responsible for depression in growth from the use of high dietary levels of cod liver oil. The research needs to be conducted for a longer duration than the present study of 108 days to compensate for the slow growth of the animals under these conditions. Alternative sources of fish oil, such as menhaden oil or other locally available oils, should be investigated under the same cultural conditions.

- The nature and extent of the changes in dietary lipid levels are first reflected in the hepatopancreas and then in the tail muscles of cultured marron (Chapter 2). This has an important implication on human health and marketability of marron.

There is a need for future investigations on the influence of different levels and sources of dietary lipids and fatty acid profiles on the enhancement of marron tail quality in terms of nutritional composition so that it provides additional health benefits to human nutrition (Fotedar, 1995). The trial under commercial farm conditions (Chapter 5) did not show any direct correlation between the fatty acid profile of the diet and the fatty acid profile of the body tissue of the marron. However, there is a trend evident in data that the fatty acid profile of the diet is immediately reflected in the hepatopancreas and then in the tail muscles. Future research needs to be directed towards investigating the relationship between the biochemical composition of the formulated diet and nutritional composition of the tail of marron.

- In a commercial marron farm where water exchange is minimal, a diet low in protein, particularly animal-sourced protein, is recommended (Chapter 5). As dietary protein increases, there is an increase in the proportion of non-protein energy requirements (Hubbard *et al.*, 1986). Further, freshwater crayfish deaminate and utilize the excess dietary protein for energy requirements. In the absence of a non-protein energy source in the diet, deamination of protein leads to an increase in the ammonia loading of water, thereby creating water quality problems. Therefore, use of excessive protein in feeds not only reduces profitability, but also increases nitrogenous wastes and generates poor feed conversions. Minimization of wastes in ponds is desirable for long-term, sustainable marron production. It is essential that the protein in commercially manufactured marron diets be provided at the required level, and from a source that is a cheap and easily digestible to achieve both an economically acceptable growth and optimum water quality.

  Lipid levels and sources are more crucial than protein levels in commercial marron farming. Differences in food habits, size and rearing conditions can have a greater influence on protein requirements of the studied animal. For example,

dietary protein requirements of penaeid prawns ranges from 23 to 62% (Kanazawa, 1981; Dall *et al.*, 1990). If a diet is deficient in energy from lipids and carbohydrates, the protein will be metabolized for energy resulting in inadequate protein available for growth and a waste of dollars for the aquaculturist. Diets which contained high levels of digestible carbohydrates in the form of dextrin and relatively low levels of lipids were well utilized in white crayfish (*P. acutus acutus*) (Davis and Robinson, 1986). This indicates that the dietary carbohydrate/lipid ratio is of importance and that carbohydrates are better utilized as an energy source in freshwater crayfish. Deshimaru and Kuroki (1974) demonstrated that growth of the prawn, *P. japonicus*, increased when carbohydrate (glycogen) was added to the diets containing 0, 6, 12% lipid.

Although a protein-low diet may be best for maintaining optimum water quality, there are practical difficulties in preparing such a diet. Assuming that the moisture content of the hepatopancreas (HM%) and tail muscles (HM%) are indicators of nutritional stress, then a diet containing the minimum amount of fish protein and supplemented with fish oil is the most preferable commercial diet.

- Biomass in aquaculture is highly dependent on growth and density relationships. As density exceeds a certain threshold, growth rates decrease. The conflicting nature of these two factors prevent biomass and growth rates being simultaneously maximised. As stocking densities can have greater influence on the test results as mortalities may adjust the densities, future studies where mortalities are replaced with marked animals to maintain the stocking densities through out the study period, should be attempted. Further, rearing conditions can influence this relationship. A stocking density of $6/m^2$ of marron in cages yielded maximal SGR (1.39) and harvest rates (101.32 g/m^2) in the semi-controlled recirculating conditions (Chapter 3). The effect of stocking density on these factors has a significant impact on the profit of a marron farm and knowledge of optimum stocking density may help improve management under semi-controlled

recirculating environments. Therefore, stocking density need to be considered from situation to situation.

Table 7.4 **The summary of optimum survival, specific growth rate (SGR), condition indices (H_{iw}, H_{id}, HM%, T/B_w, T/B_d and TM%) of juvenile marron cultured in four different environments.**

	Laboratory	Cages	Semi-intensive farm	ICCS
Survival	65.00 ± 5.00	70.00 ± 4.08 [1]	34.66 ± 9.04	100.00 [1]
	-	77.50 ± 8.32 [2]	-	100.00 [2]
SGR	1.07 ± 0.06	1.39 ± 0.07 [1]	1.13 ± 0.05	0.24 ± 0.04 [1]
	-	0.88 ± 0.03 [2]	-	0.41 ± 0.18 [2]
H_{iw}		-	5.11 ± 0.23	5.43 ± 0.30 [1]
	9.11 ± 1.84	5.14 ± 0.09 [2]	-	5.90 ± 0.71 [2]
H_{id}		-	1.78 ± 0.10	1.25 ± 0.12 [1]
	2.92 ± 0.43	2.22 ± 0.05 [2]	-	1.07 ± 0.07 [2]
HM%		-	57.65 ± 1.47	73.39 ± 2.23 [1]
	73.22 ± 3.44	56.82 ± 0.63 [2]	-	79.56 ± 1.31 [2]
T/B_d		-	6.92 ± 0.30	3.56 ± 0.29 [1]
	8.37± 1.23	7.71 ± 0.24 [2]	-	4.94 ± 0.82 [2]
T/B_w		-	34.45 ± 0.87	23.31 ± 2.32 [1]
	31.51 ± 4.31	34.56 ± 0.74 [2]	-	30.44 ± 4.80 [2]
TM%	79.95 ± 0.47	-	78.40 ± 0.20	-
	-	83.02 ± 0.34 [2]	-	82.99 ± 0.09 [2]

[1, 2] – showing results obtained from trial 1 and trial 2 respectively.

- Results obtained under different culture conditions are summarized in Table 7.4. Growth rates (SGR of 1.39) are maximal in cages where optimum temperature was provided by the continuously circulating water in a semi-controlled environment. Therefore, optimal temperature of recirculating water plays a significant contribution in the growth of marron. Seals *et al.* (1997) demonstrated

that in white river crayfish (*P. acutus acutus*) feed consumption rates increased as ambient temperature increased. Growth rates were minimal in intensive crayfish culture system (ICCS) environment, indicating that a confined environment and a lack of natural productivity is not suitable for achieving optimal marron growth. However, under ICCS survival was 100%, suggesting that cannibalism is the main cause of mortality in communal rearing systems. Therefore, marron can obtain their nutrients from cannibalising other, weaker animals and thus some manage to grow at the expense of the overall survival rates in the culture system.

- Comparing different condition indices (H_{iw}, H_{id}, HM%, T/B_w, T/B_d and TM%) across the various culture systems (Table 7.3), it is evident that marron are nutritionally/physiologically stressed under laboratory and intensive crayfish culture system (ICCS) environments. This is probably due to the fact that these two systems do not provide ideal culture conditions due to the lack of certain nutrients, plankton and healthy detritus from where some of the essential and trace nutrients may be obtained. The supply of a formulated pelleted diet can be considered as the sole source of nutrition by maintaining clean battery system (Morrissy, 1984, 1992c). Although, ICCS provides a controlled experiment system which is ideal for studying aggressive and cannibalistic species of freshwater crayfish, its commercial applicability has yet to be proven (Jussila, 1997).

ICCS environments can be used to gain insights into ways to improve the growth rates of marron. The flow-through system of ICCS can be used to study the influence of natural productivity of the ecosystem on marron growth. Investigations need to be carried out on the nutritional contribution to marron by the primary productivity of the natural ecosystem under various rearing conditions. The relationship between different organosomatic indices with the physiological age of the marron need also be investigated.

- Juvenile marron can tolerate relatively long periods of starvation or low food availability, utilizing energy sources from the hepatopancreas first and then from the tail muscles and by absorbing water into the hepatopancreas and into the tail muscles. During starvation, lipids are catabolized at a faster rate than the proteins from the hepatopancreas. The best indicator to determine the stress caused by nutritional deprivation needs to be further investigated using purified diets under controlled conditions in marron.

  The formulated feeds used in the study were able to provide an adequate energy source in terms of protein, lipids and carbohydrates but failed to provide other non-energy essential nutrients. O'Brien (1994b, 1995) described marron as detritivores based on an analysis of their gut content; Momot (1995), however, suggested that freshwater crayfish should be termed obligate carnivores. Both of these studies were conducted on natural populations of crayfish and did not consider the role of formulated diets in the feeding biology of the animal. Further, there is need to consider that different crayfish species may have entirely different feeding behaviour and nutritional requirements.

- Nutritional research conducted in one culture environment may not be directly applicable to another culture environment. To achieve realistic and commercially applicable conclusions from feeding and nutritional trials, it is essential that experimental animals be reared under conditions resembling those of the intended commercial farm. Further, growth performance of the experimental animals in nutritional trials should be comparable to the growth performance observations in animals reared under farming conditions (Tacon, 1996). Researchers need to specify cultural conditions when describing the nutritional requirements of cultured crayfish. Further research needs to be carried out to develop approaches to conduct nutritional research on farmed crayfish to ensure that results can be applied under commercial farming conditions.

Chambers (*pers. comm. September, 1998*) collected different marron samples from six different geographical regions of West Australia and derived a model [Ln (weight) = constant + geographic location (orbital carapace length)] to signify the relationship between length-weight and location. Comparison of marron of the same orbital-carapace length from the northern part of their geographical range in Western Australia with those from the southern part of the range indicated that northern marron were less heavy. Higher temperature regimes in the northern region causes an increase in the frequency of moult rates and SGRs, but the tail muscle production can not keep pace (Chambers, *pers. commun. September, 1998*). In stress-related studies in fish species, the most common condition indices are ratios between morphological and anatomical features expressed as weight / length^3 (Le Cren, 1951; Carlander, 1969; Everhart and Youngs, 1981). Thus, further research is required to investigate the nutritional requirements and condition indices at different geographic regions.

The results obtained from the above research will be helpful in establishing standard and commercially viable formulated diets complete in basic nutrients appropriate for a given culture environment. This research has provided sound knowledge on the influence of culture environment on the nutritional requirements of the juvenile marron and has paved the way for the development of commercial diet for marron on a particular culture system. The research has also paved the way for conducting research in the commercial marron farm environment. The research has clearly suggested that some of the conditions can be used as conditional factors to predict the growth rates or nutritional status of the marron.

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Table I. **Water quality variables in glass aquaria (Chapter 2).**

Variables	Mean ± SE	Variables	Mean ± SE
Temperature [1]	18.5 ± 1.0 ^{0}C	Magnesium [2]	11 mg/l
Dissolved oxygen [1]	5.9±0.6 mg/l	Soluble Iron [2]	<.05
pH [1]	8.1±0.2	Chloride [2]	100 mg/l
Total Ammonia [1]	0.4±0.1 mg/l	Carbonate [2]	Nil
Nitrite [1]	0.025-0.15 mg/l	Bicarbonate [2]	120-125 mg/l
Nitrate [1]	0-15 mg/l	Sulphate [2]	40 mg/l
Total Hardness [1]	7.2-12 mg/l	Manganese [2]	<.05
Carbonate Hardness [2]	5.2-8 mg/l	Ortho Phosphate [2]	0.25-0.8
Sodium [2]	54.5-55.5 mg/l	Cobalt [2]	<0.05
Potassium [2]	10 mg/l	Zinc [2]	<0.05
Calcium [2]	31-31.5 mg/l	Copper [2]	<0.05

[1] - water samples analysed from all the aquaria
[2] -water samples analysed only twice during the whole experiment by mixing the water from all aquaria.

Table II **Water quality variables in cages under semi-controlled recirculating system. (Chapter 3).**

Variables	Range	Variables	Range
Temperature [1]	19.5-26.8 ^{0}C	Magnesium [2]	17.70 mg/L
Dissolved oxygen [1]	7.8-10.6 mg/L:	Soluble Iron [2]	0.06 mg/L
pH [1]	7.2-8.4	Chloride [2]	100 mg/L
Total ammonia [1]	0.2-0.4 mg/L	Carbonate [2]	0.0
Nitrite [1]	0.15-0.25 mg/L	Bicarbonate [2]	120-125 mg/L
Nitrate [1]	2-25 mg/L	Sulphate [2]	40 mg/L
Total dissolved solids [1]	613 –825 mg/L	Manganese [2]	<.01 mg/L
Sodium [2]	128 –142 mg/L	Ortho Phosphate [2]	0.25-0.80 mg/L
Potassium [2]	5.15-6.50 mg/L	Carbon [2]	7.07 mg/L
Calcium [2]	80.0-95.2 mg/L	Zinc [2]	<0.05 mg/L

[1] - water samples analyzed from all the cages
[2] - water samples analyzed by mixing the water from all cages

Table III **Water quality variables in cages at commercial marron farm (Chapter 4).**

Variables	Range	Variables	Range
Temperature	11.0 - 19.5 ^{0}C	Magnesium	17.7 mg/L
Dissolved oxygen	6.8 - 10.6 mg/L	Soluble Iron	0.1 mg/L
pH	7.2 - 8.4	Chloride	100.0 mg/L
Total Ammonia	0.2 - 0.4 mg/L	Carbonate	0.0 mg/L
Nitrite	0.2 - 0.3 mg/L	Bicarbonate	120.0 – 125.0 mg/L
Nitrate	2.0 – 25.0 mg/L	Sulphate	40.0 mg/L
Total dissolved solids	613.0 – 825.0 mg/L	Manganese	< 0.01 mg/L
Sodium	128.0 – 142.0 mg/L	Ortho Phosphate	0.3 - 0.8 mg/L
Potassium	5.2 - 6.5 mg/L	Carbon	7.1 mg/L
Calcium	80.0 - 95.2 mg/L	Zinc	< 0.05 mg/L

Table IV **Water quality variables in experimental ponds during the experiment (Chapter 5).**

Parameters	June 96 - Sept 96	Oct 96 - Jan 97	Feb 97 - May 97
Conductivity	256 - 301	294 - 447	336 -391
TDS	1020 - 1050 mg / L	2000 - 3040 mg / L	NA
Na	321 - 390 mg / L	453 - 690 mg / L	614 - 692 mg / L
K	25.5 - 31.1 mg / L	30.1 - 46.2 mg / L	36 - 48.7 mg / L
Ca	36.6 - 49.9 mg / L	14.2 - 38.9 mg / L	20.5 - 43 mg / L
Mg	37.0 - 43.9 mg/L	47.0 - 82.5 mg/L	70.1 - 84.9 mg/L
Fe unfiltered	0.03 - 0.70 mg / L	0.03 - 0.48 mg / L	0.10 - 0.49 mg / L
Mn unfiltered	0.04 - 0.08 mg / L	0.06 - 0.10 mg / L	0.09 - 0.33 mg / L
Al	0.15 - 0. 28 mg / L	0.03 - 0.26 mg / L	0.13 -0.05 mg / L
S	22.3 - 27.0 mg / L	26.5 - 37.7 mg / L	27.5 - 40.5 mg / L
Si	11.8 - 13.6 mg / L	10.4 - 14.3 mg / L	10.8 - 16 mg / L
C	2.22 - 3.12 mg / L	4.28 - 7.08 mg / L	3.74 - 4.55 mg / L

NA – Not available

Table V **Water quality variables in ICCS during trial 1 (Chapter 6).**

Variables	Range	Variables	Range
Temperature	17.6-27.5 ^{0}C	Magnesium	77.5-91.2 mg/L
Dissolved oxygen	6.9-7.4 mg/L	Soluble Iron	0.06 mg/L
pH	7.8-8.9	Chloride	165.00 mg/L
Total Ammonia	0.2-0.5 mg/L	Carbonate	0.00 mg/L
Nitrite	0.25-0.75 mg/L	Bicarbonate	110.00 mg/L
Nitrate	1.5-24.5 mg/L	Sulphate	35.00mg/L 0
Total dissolved solids	879-988 mg/L	Manganese	<0.05 mg/L
Sodium	75.00 mg/L	Copper	<0.05 mg/L
Potassium	6.00 mg/L	Soluble Iron	<0.05 mg/L
Calcium	22.0 mg/L	Zinc	<0.05 mg/L

Table VI **Water quality variables in ICCS during trial 2 (Chapter 6).**

Variables	Range	Variables	Range
Temperature	19.9-28.5 ^{0}C	Magnesium	52.1-54.5 mg/L
Dissolved oxygen	6.9-7.7 mg/L	Soluble Iron	< 0.05 mg/L
pH	7.1-8.1	Chloride	110.00 mg/L
Total Ammonia	0.05 mg/L	Carbonate	0.00 mg/L
Nitrite	0.15 mg/L	Bicarbonate	105.00 mg/L
Nitrate	1-5 mg/L	Sulphate	30.00mg/L 0
Total dissolved solids	203-208 mg/L	Manganese	<0.05 mg/L
Sodium	100.0 mg/L	Copper	<0.05 mg/L
Potassium	4.50 mg/L	Soluble Iron	<0.05 mg/L
Calcium	24.0 mg/L	Zinc	<0.05 mg/L

Appendix 2

Table 1 Fatty acid profile of sunflower oil and cod liver oil.

nomenclature	Traditional nomenclature	Sunflower oil	Cod liver oil
14:0	Myristic		5.8
16:0	Palmitic	7.0	8.4
16:1	Palmitoleic		20.0
18:0	Stearic	5.0	0.6
18:1	Oleic	19.0	14.1
18:2 - ω6	Linoleic	68.0	2.1
18:3 - ω3	Linolenic	1.0	1.2
18: 4 - ω3	-		2.7
20:1	Gadoleic		2.3
20:4 - ω6	-		2.1
20:5 - ω3	EPA		25.4
22:6 - ω3 (+22:5 ω - 3)	DHA		9.6
	ω-3 HUFA	1.0	38.9
	EPA:DHA		2.65

Table II Ingredients (%) of the diet.

Ingredients	Inclusion level (%)
Barley	56.60
Binder	2.00
Cod liver oil	3.00
Blood meal	3.20
Dicalcium phosphate	1.80
Dried Yeast	5.50
Limestone	1.40
Mill run	10.00
Molasses	2.00
Salt	0.40
Skim milk powder	5.00
Soyabean meal	20.00

Table III **Nutrient analysis (%; dry weight basis)**

Nutrients	Diet D_1
Protein	22.27
Lipid	8.11
Crude Fibre	4.77
Calcium [1]	0.78
Phosphorus [1]	0.03
Salt [1]	0.64
Metabolized energy (MJ/Kg)	10.64
ω-6 [1]	0.93
ω-3 [1]	2.12

Table IV **Proximate composition of pellet trial in mg per 100 mg (dry weight), at the end of the trial (Chapter 5)**

Composition	Diet D_1	Diet D_2	Diet D_3	Diet D_4
Crude protein	25.5	24.6	23.6	0.44
Fibre	4.4	4.5	3.5	4.5
Ash	3.7	9.1	7.7	9.0
Fat	6.1	6.4	6.3	6.2

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