INVESTIGATIONS INTO THE DIETARY PROTEIN REQUIREMENTS OF JUVENILE SPOTTED GRUNTER, *Pomadasys commersonnii* (HAEMULIDAE : PISCES)

Submitted in Fulfilment of the

Requirements for the Degree of

MASTER OF SCIENCE

of Rhodes University

by

ABIGAIL IRISH

January 1997

This thesis is dedicated to my family,

...and to the one that got away.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

Firstly, thanks go to my supervisor, Prof. Tom Hecht for his advice both during the experimental stages and also during the writing up of this thesis. Secondly, thanks to the Foundation for Research Development and SANCOR for their much appreciated funding.

The following companies are thanked for their kind donations of feed ingredients; Marine Oil Refineries, Sea Plant Products and Vitamix.

Much appreciation goes to the Department of Animal Science and Poultry Science, University of Pietermaritzburg, and in particular Marianne Hundley, for their assistance with analysis of samples and providing endless support with the protein analysis. In the same way, the Department of Biochemistry, Rhodes University, particularly Rob Van Hille, for his help with the chomic oxide analysis.

I would like to thank my many field assisstents for helping collect the endless fish needed, especially Neil Deacon and Sarah Walmsey. Thanks also to the people who fed and looked after my fish on the occasions that I had to be away.

Horst Kaiser and Sarah Radloff are thanked for their assistance with the statistical work.

I would also like to thank Dr. Tim Andrews for building the greater part of my experimental system and to Tom Shipton for showing me how to use some of the equipment, providing much discussion and for his valuable advice during proof reading of various chapters in this thesis.

Last, but not least, thank you to my parents for supporting me, financially and emotionally. Without them, none of this would have been possible.

ABSTRACT

The proximate composition of juvenile spotted grunter, *Pomadasys commersonnii* and their major prey items were analyzed to test the hypothesis that the dietary protein requirements would approximate the composition of the natural diet. The amino acid profile of juvenile *P. commersonnii* was analyzed to establish the pattern of limiting amino acids for this species. Juvenile *P. commersonnii* feed primarily on the mysid, *Mesopodopsis slabberi*, which has a protein content of 58.27±0.04% and a calorific value of 19.2kJ/g. The first-limiting essential amino acid for this fish was found to be lysine and it was predicted that the optimal dietary protein requirement would be between 50 and 60%.

The optimal dietary protein inclusion level was investigated by feeding semi-purified diets containing graded levels of protein, ranging from 37-62%. Maximum growth was found to occur at dietary protein levels ranging from 40-51%. The best food conversion ratio was achieved at 56% dietary protein and the best protein efficiency ratio was obtained at a dietary protein inclusion level of 37-40%.

The protein to energy ratio was defined by feeding 3 different protein levels at 3 different P:E levels. These semi-purified diets contained 35, 45 or 55% protein and 6, 8 or 12% dietary lipid, resulting in protein to energy ratios ranging from 21.1 to 42.3mg/kJ. The optimal P:E ratio for this species was found to be 26.7mg/kJ. This diet effected a low food conversion ratio (1.72±0.19), a low hepatosomatic index (4.01±0.23) and a high specific growth rate (5.96±0.36).

The essential amino acid requirements of juvenile *P. commersonnii* were investigated. Semi-purified diets were formulated to contain graded levels of crystalline lysine based on the amino acid profile

of the whole body and fed to the fish for a period of 60 days. Fish fed the "ideal protein" diet had significantly higher ($p<0.05$) specific growth rates (2.95±0.18), protein efficiency ratios (0.41±0.05) and significantly lower ($p<0.05$) food conversion ratios (3.02±0.29). Lysine levels in the whole body increased with increasing levels of supplemented lysine. The dietary requirement of lysine was found to be 4.30% of dietary protein.

The crude protein digestibility of six protein sources was investigated. Diets were prepared with 1% chromic oxide as an internal marker. The tested protein sources included low temperature Danish fishmeal, steam dried Chilean fishmeal, blood meal, carcass meal, defatted soyabean meal and *Spirulina* meal. These potential fishmeal substitutes were selected due to their favourable amino acid profiles, and in particular their high levels of lysine, and their high protein content. Both the animal and the plant protein sources were highly digestible. Digestibility co-efficients ranged from 66.09% for *Spirulina* meal to 73.38% for low temperature Danish fishmeal.

The results have shown that juvenile spotted grunter require a minimum of 40% crude protein in their diet, a P:E ratio of 26.7mg/kJ and a lysine concentration of 4.30% of the dietary protein. Long term growth trials are needed to confirm these results. The hypothesis that the proximate composition of the natural diet, and the proximate composition of the animal under investigation, provides a reliable indication of the animals' nutritional requirements was shown to be valid. Differences between the predicted, and the empirically determined optimal protein and protein:energy ratios were slight.

CHAPTER ONE

INTRODUCTION

The spotted grunter, *Pomadasys commersonnii* (Lacepede 1801), belongs to the family Haemulidae (Smith and McKay, 1991). The common name spotted grunter is derived from the rows of distinct small, dark spots which cover the flanks and dorsal surface and the rasping grunt made by the pharyngeal teeth rubbing together when the animal is removed from the water (Smith and McKay, 1991). The spots extend onto the dorsal fins but do not extend onto the head. There is a well-defined dark blotch on the operculum (Van der Elst, 1988). Adults are silver, with a mother-of-pearl sheen on the upper flanks. Juveniles have a purple sheen, no spots and only 2 anal spines (Branch *et al.*, 1994). Adults have 3 anal spines (Smith and McKay, 1991).

The natural distribution of this species is unclear. Blaber (1981) and Van der Elst (1988) describe it as an Indo-Pacific species, inhabiting waters of temperate and tropical coasts, while Day *et al.* (1981), Smith and McKay (1991) and Branch *et al.* (1994) describe it as an Indian ocean species, extending from India to False Bay, but absent from central Indian Ocean islands such as the Seychelles.

Along the east coast of southern Africa spotted grunter are frequently found off sandy beaches or in tidal estuaries (Wallace, 1975; Wallace *et al.*, 1984). Adults spawn at sea during the winter months and the juveniles (<50 mm SL length) recruit into estuaries of the eastern Cape and Natal between July and December (Beckley, 1984; Whitfield, 1990). Juveniles remain in the food-enriched estuarine environment for up to a year where they grow rapidly at 12-15mm SL per month (Day *et al.*, 1981). They return to the open sea (Whitfield, 1990) after a year (at ±200mm SL), where they remain until reaching sexual maturity at approximately 2-3 years of age and ±400mm SL and ±0.8kg (Whitfield, 1990). Following sexual maturity, weight increments range from 0.6 to 0.7kg per annum (Whitfield, 1990). Post-spawning adults may enter the estuaries to feed at the same time as the recruiting juveniles (Whitfield, 1990).

Because of the decline in the South African line fishery there is an emphasis now to develop the mariculture industry in South Africa (Hecht and Britz, 1992). Because of its high price/kilogram,

taste and texture the spotted grunter has been identified as a candidate species for mariculture in South Africa (Deacon and Hecht, 1995a). Under natural conditions these fish attain a market size of approximately 200-300g within the first 12-18 months (Wallace and Schleyer, 1979). Because spotted grunter is an exceptionally fine table fish (Van der Elst, 1988) and has been described as "the premier game fish in many estuaries" (Day *et al.*, 1981), it is a much sought-after angling species (Van der Westhuizen and Marais, 1977; Van der Elst, 1988 and Branch *et al.*, 1994). The spotted grunter is an estuarine dependent fish and tolerates wide salinity and temperature fluctuations (Day *et al.*, 1981). It also readily accepts a moist pellet feed (Deacon, 1997) which facilitates the culture of this species.

The growth and production of all farmed fish is dependent on the intake of essential dietary nutrients. Fish may obtain these nutrients in four different ways, depending on the farming system employed (Tacon, 1995). The first strategy is one which has no exogenous nutrient input. Fish growth, in this example, is entirely dependent on the natural productivity of the water body into which the fish have been placed. This feeding strategy is typical of extensive pond farming systems (Tacon, 1995). The second feeding strategy involves a fertilizer nutrient input. Chemical fertilizers or organic manures are applied as exogenous sources of nutrients to enhance the production of live food organisms in the water body. This strategy is used in extensive or semi-intensive pond farming systems (Tacon, 1995). The third feeding strategy is a supplementary nutrient input where exogenous live or processed feeds are supplied as a supplementary source of dietary nutrients. This strategy is used in semi-intensive pond farming systems (Tacon, 1995). The final feeding strategy involves complete nutrient input, where exogenous live or processed feeds are supplied as the complete source of dietary nutrients. In general, these complete diets take the form of pelleted feeds. These pellets have an overall nutrient profile which approximates as far as is economically viable, the known dietary nutrient requirements of the cultured species. This strategy is used in intensive farming systems (Tacon, 1995).

The primary aim of fish farming is to maximize survival and growth at minimal cost (Knight, 1985). Diet development is a compromise between the ideal situation and practical considerations such as the cost of the ingredients, pelletability, diet acceptability and water stability of the feed (Hardy, 1989). In formulating artificial feeds for carnivorous marine fish, particular attention

must be paid to protein, fatty acid and vitamin content (Tucker, 1992).

The major requirement for growth of juvenile fish is protein (Steffens, 1989). Compared with other vertebrates, fish have a high protein requirement. Chickens, for example, require 20% dietary protein (Leeson and Summers, 1980) and pigs require only 12% dietary protein (Kropf *et al.*, 1959) to achieve maximum growth.

Food represents a large part of the production costs, averaging between 42 and 52% of the total operating costs (Shepherd, 1988). Of all the nutrients, the level and type of protein inclusion is probably the most important as its high price affects the cost of formulated diets (Watanabe, 1988). The protein requirements of fish is closely related to the optimum dietary protein level although these levels are not identical (Watanabe, 1988). The protein requirement is species specific under controlled rearing conditions and is expressed as a value per unit of fish body weight per day. The optimal protein level is determined by the protein requirement, the quality of the protein, the digestible energy levels, the rearing conditions, the manufacturing process for the feed and market costs (Watanabe, 1988). For this reason, the optimal dietary protein requirements must be determined before a possible reduction in dietary protein inclusion level can be investigated. In commercial fish rearing, the primary concern is to find a compromise between the minimum dietary protein content and a high growth rate and satisfactory feed utilization (Steffens, 1989).

Determining the optimal dietary protein inclusion level is of great importance, as is establishing the energetic requirements. Energy is derived from the food and supplies the animals' needs for maintenance and growth (Cho and Bureau, 1995). Energy in the diet is supplied by protein, carbohydrate and lipids. However, fish have a limited ability to digest carbohydrate and therefore utilize proteins and lipids for energy. The energy content of the diet should not be set too low, nor should the protein level be allowed to fall below a certain level if maximal growth is to be achieved (Steffens, 1989). If the energy in the diet is too low, the fish will utilize protein as an energy source, and if there is insufficient protein in the diet then the protein that is available will be used for basal metabolic requirements and not for somatic growth (Cho and Bureau, 1995). For this reason it is important to determine the optimal protein level for growth and survival of

the fish (Tucker, 1992). This level allows the maximum amount of protein to be available for growth by minimizing that used for energy (Tucker, 1992).

As dietary proteins are converted to free amino acids through the actions of various digestive enzymes, the composition of the dietary protein is of particular importance. The released free amino acids are absorbed in the intestinal tract and used by the various tissues for the synthesis of tissue proteins. Excessive amino acids will be used as an energy source which is not cost effective as proteins constitute the most expensive item in fish diets (Benitez, 1989). In the absence of knowledge of the essential amino acid (EAA) requirements, the EAA pattern of the entire body tissue of the fish can provide an approximation of the required amino acid composition of the dietary protein (Cowey and Tacon, 1982; Wilson, 1985). Therefore, this information can be useful in designing test diets for fish when their amino acid requirements have not been established (Wilson, 1989).

Ten amino acids have been isolated as being essential. A diet deficient in any of these amino acids will result in poor growth and a decreased food conversion efficiency, even if the diet has a high protein level (Piper *et al.*, 1982). If the amino acid requirement values are known for a certain species, the nutritional value of a protein can be made directly by comparing the EAA content with the requirement values (Wilson, 1989). This technique is useful in evaluating the limiting amino acid of a potential protein source (Wilson, 1989). There is also the possibility of protein sparing with the use of dispensable amino acids (Cowey, 1993). This can only be investigated once the amino acid requirements of a given species are known. For example, Kim *et al* (1991) re-evaluated the dietary protein requirements of rainbow trout, *Oncorhynchus mykiss*, and found that the protein requirement could be met with a diet containing 25% casein and 10% of a mixture of dispensable amino acids. In other words, the protein requirement of rainbow trout is not more than 25% (previously established to be between 42% and 51%, Austreng and Refstie, 1979) when adequate levels of EAA are supplied. Priority must therefore, be given to the amino acid composition of the protein during the initial feed formulation.

High quality fishmeal is recognized as the best protein source (Pike *et al.*, 1990). It is the most nutritious, digestible and palatable protein but it is also the most expensive, and world supplies

are not expected to meet future demands (Barlow, 1989). Carnivorous fish species represented only 12% of total finfish aquaculture products in 1992 but they consumed about 11% of the total world fishmeal production (Tacon, 1994). Presently fishery products generally make up 70% by weight of the compound aquafeeds for farmed carnivorous fish. These products take the form of high quality fishmeals, fish oil and to a lesser extent fish protein hydrolysates (Tacon, 1993).

Unfortunately, there are no animal or plant proteins that approximate the dietary EAA requirements of farmed fish as well as fishmeal (Tacon and Jackson, 1985). Despite these inherent amino acid imbalances, alternative protein sources, used in conjunction with fishmeal, have been used successfully by researchers (Tacon and Jackson, 1985). Commonly used protein sources include poultry by-product meal (Gallagher and Degani, 1988), meat by-product meal, and cooked soyabean products (Tucker, 1992). The amino acid profiles of various alternative protein sources are outlined in Table 1. Lysine would appear to be the most limiting EAA for juvenile spotted grunter, therefore the most promising alternative protein sources are *Spirulina*, meat and bone meal, soyabean meal, Torula yeast and bloodmeal. The inclusion level of these alternative protein sources is influenced by a number of factors. Animal by-product meals are generally limited by specific amino acid and ash mineral imbalances which necessitate careful formulation to overcome these imbalances (Tacon, 1994). Present inclusion levels in fish diets, of bloodmeal are 4% and meat and bone meal are 10%, with their maximum inclusion levels being 20-35%, respectively (Tacon, 1994). The inclusion levels of single-cell proteins such as *Spirulina* and Torula yeast, *Candida utilis* (a sugar cane by-product), have not been extensively researched but have been included in fish diets at 10%, and in the case of Torula yeast, inclusion levels may be as high as 50% (Tacon, 1994). Uys and Hecht (1985) formulated a successful catfish (*Clarias gariepinus*) larval diet with 69.8% Torula yeast. The inclusion level of oilseeds such as sunflower and soyabean meals is largely dependant on the method of processing as they contain numerous anti-nutritional factors which must be inactivated before these sources can be used (Tacon, 1994). The importance of identifying suitable substitutes for fishmeal is highlighted by artificial diets for channel catfish, *Ictalurus punctatus* which contain only 4-8% fishmeal and are therefore, very cost effective (Lovell, 1992).

Table 1 : Amino acid profiles of various alternative protein sources.

		AMINO ACID (% CRUDE PROTEIN BASIS)										
SOURCE[1]	CRUDE PROTEIN (%)	THR	VAL	MET	ILE	LEU	TYR	PHE	HIS	LYS	ARG	CYS
Menhaden meal (5-02-009)	66.7	2.73	3.52	1.91	3.15	4.89	2.12	2.69	1.58	5.15	4.09	0.61
Solvent extr. Soybean meal (5-04-604)	49.9	1.85	2.25	0.58	2.27	3.65	1.48	2.36	1.19	2.99	3.38	0.83
Mech. extr. Soybean meal (5-04-600)	47.7	1.92	2.53	0.72	2.92	4.02	1.72	2.45	1.26	3.10	3.41	0.63
Bloodmeal (5-00-381)	93.0	3.93	8.13	0.95	0.98	11.86	2.44	6.36	5.59	8.04	3.88	0.78
Mech. extr. Sunflower meal (5-04-738)	41.4	1.37	2.01	0.94	1.76	2.47	1.00	1.80	0.90	1.61	3.45	0.69
Solvent extr. Sunflower meal (5-04-739)	49.8	1.93	2.60	1.16	2.25	3.83	1.39	2.36	1.23	1.92	4.42	0.74

[1] Numbers in parentheses are International Feed Numbers

Table 1 (cont.) : Amino acid profiles of various alternative protein sources.

		AMINO ACID (% CRUDE PROTEIN BASIS)										
SOURCE[1]	CRUDE PROTEIN (%)	THR	VAL	MET	ILE	LEU	TYR	PHE	HIS	LYS	ARG	CYS
Spirulina (5-19-931)	58.6	2.79	3.93	0.85	3.63	4.84	2.42	3.02	1.09	2.79	3.93	0.24
Torula yeast (7-05-534)	49.1	2.64	2.96	0.77	2.85	3.52	0.52	2.85	1.32	3.74	2.64	0.61
Corn gluten (5-28-241)	42.7	1.42	2.26	1.04	2.25	7.22	1.01	2.78	0.97	0.80	1.39	0.67
Poultry by-product meal (5-03-798)	58.7	1.94	4.50	1.06	2.38	4.00	0.94	1.84	1.01	2.89	3.77	0.92
Meat and bone meal (5-00-388)	50.4	1.65	2.45	0.65	1.64	3.06	0.79	1.70	0.96	2.90	3.49	0.50

[1] Numbers in parentheses are International Feed Numbers

The objectives of this study were two-fold. Firstly to determine the dietary protein requirements of juvenile spotted grunter, and secondly to investigate the possibility of reducing the required amount through protein sparing.

The hypothesis that the proximate composition of the animal and its natural diet would provide insight into the dietary requirements of a given species was investigated. This necessitated a literature review regarding the natural feeding habits of spotted grunter. The preferred prey items were identified and the proximate composition of these prey items was investigated. The proximate composition of wild juveniles was also investigated. The juveniles used for proximate analysis were of approximately the same size as the animals used in the experimental growth trials. Based on the proximate composition of the natural diet, and the proximate composition of the juveniles, predictions were made as to the nutritional requirements of the species (Chapter 3).

The predictions resulting from the proximate composition investigations were used to design the optimal dietary protein inclusion experiment (Chapter 4). As dietary energy has a profound effect on the protein requirement, the optimal dietary protein level established in Chapter 4 was used as the basis to define the optimal protein : energy (P:E) ratio (Chapter 5).

The amino acid balance of a protein source determines its' nutritional value (Benitez, 1989). The effect of varying dietary lysine levels was investigated (Chapter 6) using R.P Wilson's (Mississippi State University, pers. comm.) method which gave an indication of the essential amino acids (EAA) requirements. A satisfactory level and balance of amino acids in the diet does not guarantee that ingestion of the diet will satisfy the amino acid requirements of the fish. If the dietary protein is incompletely digested or certain amino acids are not available to the fish then the fish will not grow. Therefore, it is important that the digestibility of various protein used in fish diets be determined (Wilson, 1989). Finally, in order to reduce the level of fishmeal in the diets, as a way to reduce costs, the digestibility of five alternative protein sources was investigated (Chapter 7). The study was concluded with a general discussion on the dietary requirements of the spotted grunter and the theoretical composition of an optimal diet (Chapter 8).

CHAPTER TWO

GENERAL METHODOLOGY

SYSTEM DESIGN AND MANAGEMENT

All experiments were conducted in a 4000 litre recirculating system. The system consisted of twenty seven 40 litre glass aquaria, which were maintained at a capacity of 36l. Each of these tanks was supplied with two water inlet pipes and aerated by means of a single air stone. The overflow from all the tanks drained into a 1000 litre settlement tank. From the settlement tank the water passed through a four-stage (4 x 70 litre) submerged biological filter. Water from the submerged biological filter was then passed through a 2m high trickle filter (250mm diameter) and sand pressure filter before being pumped back to the tanks (Figure 1). The water in the settlement tank was directed through an *in situ* venturi protein skimmer.

Inflow into the tanks was set at 0.6l/min., equivalent to a turnover rate of once every hour. The water was kept at a constant temperature of 24±1.5°C by means of a thermo-controlled 4KW immersion heater in the settlement tank. This temperature has been shown to be optimal for the growth of juvenile spotted grunter (Deacon and Hecht, 1995a). Photoperiod was maintained at "summer" light conditions of 14L:10D (Deacon and Hecht 1995a).Groups of three tanks were luminated with a 1m Biolux fluorescent tube providing a light intensity of 1 500 lux at the water surface. The light intensity in the tanks was measured with a QSL-100 laboratory quantum scalar irradiance meter and was kept constant at 1.95×10^{-3} $\mu E.sec^{-1}.cm^{2}$. Light intensity during the dark period was less than 2 lux. Water quality was monitored weekly and kept at a constant salinity of 33±1ppt with the addition of deionized freshwater. Oxygen saturation was constant at 97±1%. Nitrite levels and total ammonia levels were tested weekly and remained below 0.1mg/l and 0.02mg/l, respectively.

FEED PREPARATION

Unless otherwise stated, the preparation of the diets for all experiments was identical. Two different types of diet were fed to all experimental animals. The first was a moist pellet, acclimation diet which was fed to the fish during the first week of a two week acclimation period. This pellet was fed to the fish to induce them to accept a pelleted feed. This moist pellet diet

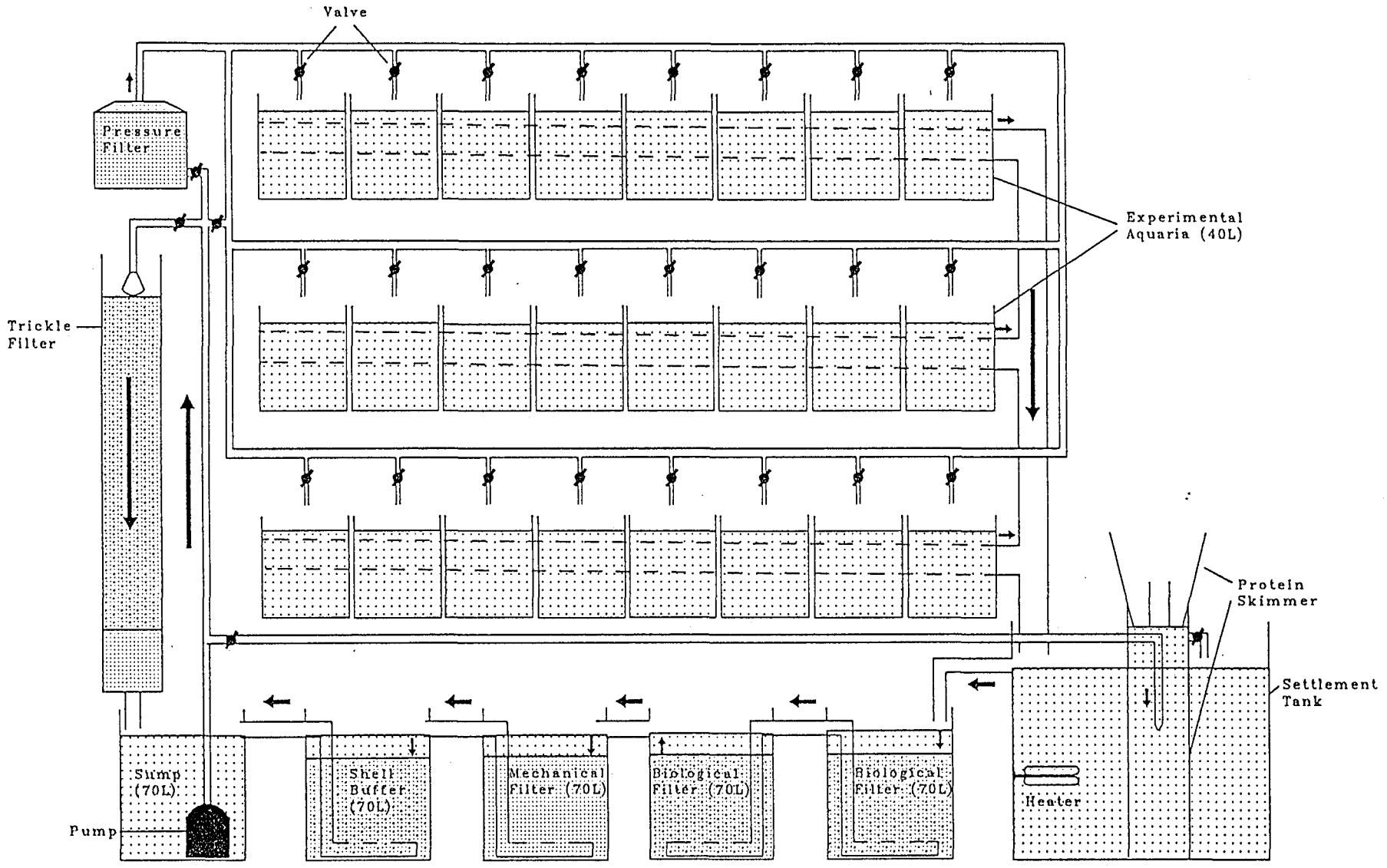

Figure 1 : Schematic plan of the experimental system
(arrows indicate the direction of water flow)

consisted of a 2:1 ratio of minced pilchard (*Sardinops sagax*) and low temperature Danish fishmeal (Fiskernes Fiskeindustri, A.M.B.A). For the last 3 days of the two week acclimation period, the fish were fed their respective semi-purified diets to ensure acceptance of these diets before the growth trial was started.

The protein sources used in all experimental diets were vitamin-free casein and low temperature Danish fishmeal. The diet for the optimal protein determination experiment differed from the other diets in two ways. The first difference was in the use of binding agents. The binding agents for the formulated diets used in the optimal protein inclusion level experiment were, alginate and sodium hexametaphosphate. These binding agents were dissolved in 50ml warm (± 75°C) distilled water, added to the cod liver oil and mixed in with the dry ingredients to form a stiff dough. The reason the binding agents were changed was because the alginate and sodium hexametaphosphate formed a precipitate during the micro-Kjeldhal protein determination. The measured dry ingredients of each of the sequential diets were homogenized. The second difference was in the use of cod liver oil as the sole lipid source (8% of the dry diet). All other diets contained pre-gelatinized starch as the binding agent and the lipid was made up of a 2:1 combination of marine fish oil and soya oil.

The measured dry ingredients were thoroughly mixed to ensure equal distribution of all ingredients. The oil was then evenly dribbled over the dry ingredient. Finally, distilled water was slowly added, while mixing, until a stiff dough was formed. The dough was placed in a commercial food mixer and mechanically kneaded for 10 minutes. It was then extruded through a 2mm diameter die. The strands of the extruded diet were sliced into small pellets (approximately 4mm in length). These pellets were stored in individual containers for each tank at -20°C until used. The containers were weighed before and after each feeding to determine how much food was consumed by the fish in each tank.

FISH AND FEEDING REGIMES

All the fish used in this study were juveniles, with a standard length and weight of between 55-70mm and 3-8g, estimated to be less then 6 months of age (Whitfield, 1990). The fish were caught in the Great Fish River estuary on the south east coast of South Africa (33°30 S 27°07

E) with a 15mm mesh seine net. They were transported to Grahamstown where they were acclimated to tank conditions in the experimental system for a period of two weeks prior to the initiation of the feeding trials.

Before the start of each experiment, the fish were anaethitized and individually marked by means of clipping off the tips of different combinations of dorsal and anal fin spines. These clippings were repeated during the experiments if necessary. Marking of individual fish improved statistical treatment of the data as the growth of individual fish could be monitored (Deacon and Hecht, 1995b). The fish were weighed and measured every 10 days after they had been anaesthetized in a 0.02% solution of 2-phenoxyethanol.l^{-1} which has been shown to have no detrimental effect on the growth of juvenile spotted grunter (Deacon *et al.*, in press). The fish were fed to satiation as restrictive feeding methods have been shown to lower growth responses (Tacon, 1995). They were fed three times daily, which has been shown to be optimal for growth for this species (Deacon and Hecht, 1995b).

RESPONSE MONITORING

Growth is the most important criterion for measuring responses in nutritional studies and has been shown to be a sensitive and practical indicator of a particular nutrient or energy level (Lovell, 1989). True growth involves an increase in structural tissue such as muscle and bone. This must be distinguished from an increase in weight due to increased fat deposition (Lovell, 1989). The growth responses measured were; specific growth rate (SGR), protein efficiency ratio(PER), condition factor (CF) and food conversion ratio (FCR).

Specific growth rate (SGR) denotes the average daily growth as a percentage of the initial weight, and is calculated using the formula:

$$SGR = [(LnWt - LnW0)/t] \times 100$$

where Wt = weight of fish at time t

W0 = weight of fish at time 0

t = time in days

(After Ricker, 1979)

Protein efficiency ratio (PER) is the most widely used method for measuring protein utilization in fish (Wilson, 1989) and is calculated by:

PER=(Body weight gain (wet weight g)/Protein ingested(dry weight g)

The condition factor (CF) is a good indicator of the general "well-being or fitness" of the animal (Bolger and Connolly, 1989). The higher the magnitude of the CF the greater is the weight in relation to unit length (Tesch, 1968). Condition factor is calculated from the relationship:

$$CF = [(\text{Body weight (wet weight g)}/\text{Standard length}^{2.71}]X100$$

This relationship was determined from a length/mass regression for wild fish, where y=2.71x-109.52 (r^2=0.97, n=498) with a size range of 2.5-10.0g (N. Deacon, Department of Ichthyology and Fisheries Science, Rhodes University, pers. comm.)

The growth responses of an animal to supplements of essential nutrients are made up of two components (Baker, 1984). Firstly, there is an increase in the food intake and secondly, there is an increase in the metabolic efficiency brought about by the supplementation (Baker, 1984). Measurement of food consumption by fish, especially in experiments with small fish where maximal growth is the aim is therefore, less than exact (Cowey, 1992). Consequently, while the efficiency of food conversion is a normal criterion of response with terrestrial animals, it should be regarded only of a supplementary nature in experiments with fish, hopefully affirming the growth results (Cowey, 1992). It is calculated from:

FCR = [(Food intake (dry weight g)/Body weight gain (wet weight g)]

PROXIMATE COMPOSITION ANALYSIS

Once the experimental period was complete, all the fish were frozen for further analyses. The whole body composition was determined on composite samples consisting of 5 randomly selected fish from each tank (15 fish per dietary treatment). Proximate composition of the diet and of the

fish, both before and after the feeding trial, were determined. The proximate composition of the animals before and after a feeding experiment is important as it elucidates how the absorbed nutrients are utilized (Cowey, 1992).

Protein content was determined using the Micro-Kjeldahl method. Approximately 100mg of sample was weighed into digestion flasks. 2.5g Selenium catalyst and 2.5ml of concentrated sulphuric acid was added to each flask, including the blanks. These flasks were placed on the preheated block and washed with 1ml hydrogen peroxide every 10 minutes for 60 minutes. On completion of the final wash, the digestion tubes were left for a further 20 minutes, then removed from the block and allowed to cool. Whilst the flasks cooled, an equivalent number of 200ml Erhlenmeyer flasks were set up. 10ml of 1% Boric acid mixture with indicator was pipetted into each flask. Approximately 10ml distilled water was added to each digestion flask and the contents transferred to the clean steam-distillation flask. The digestion flask was rinsed with 10ml caustic/hypo mixture and then transferred to the distillation flask. This was then positioned in the Steam-distillation apparatus and distilled for 7 minutes. A prepared 200ml Erhlenmeyer flask was set up in the collecting position so that the tip of the delivery tube was submersed in the boric acid solution. The resultant solution in the Erhlenmeyer flask was titrated with the standard 0.015M HCl solution to the grey end-point. The percentage nitrogen was calculated from the volume of HCl titrated according to the formula:

$$N = (M{\cdot}HCl \times 14.007 \times 100)/\text{weight of sample (mg)}$$

Percentage protein was calculated as N x 6.25 (AOAC, 1984). The procedures for formulating chemical solutions are according to Steyn (1957).

Fat content was determined according to the method used by Knauer *et al.* (1994), modified from Folch *et al.* (1957). Fish were dried to a constant weight at 70°C and then powdered in a mortar. Five 0.2g samples of powdered fish were measured into centrifuge tubes and re-hydrated with 3ml distilled water. 6.25ml methanol and 6.25ml chloroform were added to each of these solutions. The solutions were homogenized for 2 minutes, after which 6.25ml distilled water was added. The solutions were homogenized for another minute and the blender head washed with distilled

water. The solutions were then centrifuged at 3000g for 10 minutes. 0.75ml of the bottom layer from each of the solutions was pipetted into clean, dry crucibles of known weight and evaporated to dryness on a hot plate. The crucibles were then placed in a pre-heated oven at 100°C for 30 minutes. The crucibles were cooled in a desiccator and weighed. Percentage fat was calculated according to the formula:

% fat= [(mass of fat (g) x 25/15)/ mass of sample (g)] x 100

The percentage moisture was determined by weighing 3 x 1.0g samples before and after drying at 70°C until a constant weight was achieved.

Ash was determined by burning 3 x 0.5g dried (at 70°C) powdered sample in open crucibles in a muffle furnace. Prior to use, the crucibles were burnt at 550°C for 8 hours to prevent contamination. The samples were burnt at 550°C for 7 hours (Montgomery and Gerking, 1980) and cooled in a desiccator before weighing.

The calorific value (CV) of the different diets was measured using a CP400 Calorimeter Systems apparatus. The machine was calibrated at a standard calorific value of 26.454MJ/Kg (CV of 0.5g Benzoic acid). Samples were dried and weighed, then placed individually in the bomb. Prior to ignition, pure oxygen at a pressure of 30 bars was pumped into the bomb. This method was shown to be the most accurate when compared to the dichromate wet oxidation and chemical composition methods (Henken *et al.*, 1986).

The livers and intraperitoneal fat from 3 fish per tank (9 fish per dietary treatment) were excised. The hepatosomatic index (HSI) is used an as indicator as excessive essential acids cause a decrease in HSI (Nematipour *et al.*, 1992). The hepatosomatic index is calculated by:

HSI = (liver weight (g) X 100)/body weight (g)

(Nematipour *et al.*, 1992)

Intraperitoneal fat (IPF) ratio gives a good indication of fat deposition and is calculated by:

IPF Ratio = (IPF weight (g) X 100)/body weight (g)

(Nematipour *et al.*, 1992)

STATISTICAL ANALYSIS

A one-way ANOVA was used to check for variation in all responses between replicates. If no significant difference was found, replicates were combined. The growth of individual fish (increase in grammes per week) in the replicates of each treatment were compared by one-way ANOVA. Additional comparison of weight gain was achieved by an analysis of co-variance (one-tailed *F*-test). In this test, the data was linearly transformed using natural logarithms of individual weight. The parameters of FCR, PER and CF were compared using one-way ANOVA and differences among the means were determined by Tukey's Multiple Range Analysis. Bartlett's Test was used to confirm equality of variances among treatments (Zar, 1984). Some of the statistical analysis varied between individual chapters and detail is provided in each chapter.

CHAPTER THREE

NATURAL FEEDING ECOLOGY AND THE CHEMICAL COMPOSITION OF JUVENILE SPOTTED GRUNTER AND PREY ITEMS - IMPLICATIONS FOR DIET FORMULATION STUDIES.

INTRODUCTION

On the basis of dietary physiology, fish can be divided into two main groups; those with and those without a stomach. Stomach less fish are considered to be non-predatory and usually do not possess teeth in the oral cavity. Predatory fish have stomachs and teeth in varying degrees (Steffens, 1989). The relative gut length, gross morphology of the gut and the daily feeding pattern of *Pomadasys commersonnii* conform to the carnivore pattern (Blaber, 1983).

The feeding biology of only two *Pomadasys* species have been extensively studied (Table 1). These are the Piggy, *P. olivaceum* and the spotted grunter, *P. commersonnii*. Most of the published stomach content analyses of these species have been carried out on larger animals then those studied in this investigation. This makes interpretation of results somewhat difficult as findings by Joubert and Hanekom (1980), Lasiak (1982) and Buxton *et al.* (1984) suggest an ontogenetic change in prey selection in *P. olivaceum*, probably resulting from gape limitation. Tentative results of stomach contents of *P. commersonnii* suggest a similar change in diet with increasing size (L. Mahlasela, Department of Ichthyology and Fisheries Science, Rhodes University, unpublished data), although Van der Westhuizen and Marais (1977) found no such difference in diet composition between different size classes. Blaber (1983) suggests that the change in diet from juvenile to adult reflects the increased ability of larger fish to remove larger prey items from the substratum. Juveniles are small enough to select individual zooplankton and small macro benthos while adults are unable to utilize zooplankton but are able to extract larger prey organisms such as pencil bait, *Solen cylindriceus* (Mollusca) and sandprawn, *Callianassa kraussi* (Anomura) from the substratum (Blaber, 1983).

Table 1 : Preferred prey items of 2 *Pomadasys* species.

SPECIES AND PREY ITEM	% FREQUENCY OF OCCURRENCE	REFERENCE
Pomadasys olivaceum (30-70mm SL)		
Copepods	49.6	Lasiak, 1986
Crustacean larvae	28.4	Lasiak, 1986
Mysid *Mesopodopsis slabberi*	55	Buxton *et al.*, 1984
Pomadasys commersonnii (>70mm SL)		
Anomura *Upogebia africana*	13-83 and 41.7	Van der Westhuizen and Marais, 197 Hecht and van der Lingen, 1992
Anomura *Callianassa kraussi*		Whitfield, 1980
Anomura *Macropetasma africanus*	26	Schleyer and Wallace, 1986
Mollusca *Tivela polita*	60	Schleyer and Wallace, 1986
Amphipod *Grandidierella lignorum*	33.3	Hecht and van der Lingen, 1992
Pomadasys commersonnii (30-70mm SL)		
Mysid *Mesopodopsis slabberi*		L. Mahlasela, Rhodes University, Grahamstown, unpublished data

It would appear that *Pomadasys commersonnii* feed mainly on Crustacea (Table 1), although the species of crustacean may differ depending on the area where the fish is found. Spotted grunter, caught from the Swartkops estuary were found to feed primarily on the mudprawn, *Upogebia africana*, (Van Der Westhuizen and Marais, 1977). This was also the case for spotted grunter caught in the Kariega estuary (Hecht and van der Lingen, 1992). Whitfield (1980) found that 65.45% of spotted grunter's total calorific intake in the Mhlanga estuary was provided by the anomuran *Callianassa kraussi*. Schleyer and Wallace (1986) found that spotted grunter caught in the Natal surf zone fed mainly on the sand mussel, *Tivela polita* and the swimming prawn *Macropetasma africanus*. Spotted grunter caught in the Great Fish River were found to prey on mudprawn, mysids and amphipods (Hecht and van der Lingen, 1992). Smaller spotted grunter

preferentially prey on the mysid *Mesopodopsis slabberi* (L. Mahlasela, Department of Ichthyology and Fisheries Science, Rhodes University, unpublished data). Therefore, the description of spotted grunter as an opportunistic macrobenthivore appears to be most fitting, with the diet being dictated by the composition and abundance of the macro benthos in each particular estuarine system (Blaber, 1983).

Proximate composition

The chemical body composition of an animal can serve as a general indication of its nutritional status. Information on the energy content of the whole fish can also help define their energy requirements (Marais, 1990). It has been found that fish may show improved growth when their diets are supplemented with essential amino acids (EAA) to simulate the composition of muscle proteins (Cowey and Tacon, 1982). Essential amino acid requirements have also been shown to correlate well with the EAA profile of whole-body protein. Thus, body protein composition data may provide useful background information which can serve as a basis of the EAA requirement of a given fish species or specific life history stage (Jobling, 1994).

There has only been one study on the body composition of *Pomadasys commersonnii* (Marais, 1990). Unfortunately, this study was carried out on relatively large fish (135± 26g) and size has been shown to have a very definite effect on body composition (Denton and Yousef, 1976; Marais and Erasmus, 1977). As this study is aimed at making a contribution towards formulating an optimal diet for juvenile spotted grunter, as well as a standard growing diet, the published data may have little or no relevance for small juveniles. For this reason, the proximate composition of juveniles (50-70mm SL) caught in the Great Fish River was determined. As no studies have been done on the proximate composition of prey items for this species, the proximate composition of the major prey items was also investigated. The proximate composition of the fish, combined with the proximate composition of the natural diet will serve as the basis upon which to predict the dietary requirements of juvenile spotted grunter.

MATERIALS AND METHODS

Sample collection and preparation

Juvenile fish (50-70mm) were caught by means of a seine netting in the Great Fish River. The fish

were starved for a period of 24 hours to ensure complete purging of the gut. Following purging, 5 animals were frozen at -20 °C for 24 h and set aside for amino acid (AA) analysis. A further 24 fish were killed by over anaesthetizing. These fish were used to analyse the proximate composition according to the methods outlined in Chapter 2. The prey items were caught and brought back to the laboratory in Grahamstown. They were weighed and then dried to a constant weight at 70°C. The proximate composition of the fish and prey items were determined according to the methods outlined in Chapter 2.

The percentage carbohydrate was determined indirectly according to the formula :

% carbohydrate = 100 - (% protein + % fat + % ash)

The AA content of the entire body of the juvenile fish was analysed by the Department of Animal Science and Poultry Science, University of Natal, Pietermaritzburg.

RESULTS

The proximate composition of juvenile spotted grunter is shown in Table 2. There was no significant difference ($p<0.05$) between samples so all results were pooled. There was no intraperitoneal fat deposition in any of the fish examined. There was no difference in moisture or protein levels between the small and larger fish although there was significantly more ($p<0.05$) ash in the juveniles. The juveniles had a lower total body lipid content than the sub-adult fish. The amino acid profile is shown in Table 3. It is evident that the most limiting amino acid was lysine, followed by leucine and arginine.

The proximate composition of the preferred prey items is shown in Table 4. The proximate composition of the 2 preferred prey items of larger spotted grunter is similar, with both *C. kraussi* and *U. africana* containing less protein than *M. slabberi*, the preferred prey item for smaller spotted grunter. The total percent lipid in *C. kraussi* and *U. africana* is higher than the total percent lipid in *M. slabberi*. The softer bodied prey items, *C. kraussi* and *M. slabberi* have less ash than *U. africana*. The calorific content (Table 5) of the prey items is similar, with *U. africana* having a higher calorific content than the other prey items.

Table 2 : Proximate composition of wild juvenile, 50-70mm SL (This study) and of sub-adult spotted grunter (Marais, 1990).

CONSTITUENT	JUVENILE (% ±SD)	SUB-ADULT* (% ±SD)
ASH	4.94±0.92	6.5±0.8
MOISTURE	70.12±1.08	70.1±0.7
PROTEIN	63.25±1.05	63.88±0.12[1]
LIPID	4.71±0.87	5.2±1.3
CARBOHYDRATE	27.10±1.01	N/A
HSI	4.71±0.62	N/A
ENERGY (kJ/g)	5.47±0.66	6.06±0.31

* After Marais (1990)
[1] Calculated from wet weight value

Table 3 : Essential amino acid profile of juvenile (50-70mm) spotted grunter.

AMINO ACID	% SAMPLE
Threonine	2.3764
Valine	2.5556
Methionine	1.7211
Isoleucine	2.2015
Leucine	3.8972
Tyrosine	1.7246
Phenylalanine	2.2273
Histidine	1.1129
Lysine	4.4115
Arginine	3.5307
Cystine	0.895

Table 4 : Proximate composition of three preferred prey items of spotted grunter.

SPECIES	MOISTURE (%±SD)	ASH (%±SD)	PROTEIN (%±SD)	CARBOHYDRATE (%±SD)	LIPID (%±SD)
Callianassa kraussi[1]	82.13 ±0.54	32.12 ±1.79	39.05 ±0.27	27.47 ±0.86	1.36 ±0.32
Upogebia africana[1]	81.67 ±0.81	37.46 ±1.30	41.91 ±0.16	19.3 ±0.62	1.33 ±0.28
Mesopodopsis slabberi[2]	90.92 ±0.27	33.88 ±1.61	58.27 ±0.04	6.75 ±0.11	1.12 ±0.04

[1] Preferred prey item for larger fish (Van der Westhuizen and Marais, 1977; Whitfield, 1980)
[2] Preferred prey item for juvenile fish (L. Mahlasela, Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, unpublished data)

Table 5 : Calorific values of prey items of spotted grunter.

PREY ITEM	CALORIFIC VALUE[1] (kJ/g)	REFERENCE
Upogebia spp	25.4	Thayer *et al.*, 1973
Callianassa kraussi	21.2	Whitfield, 1980
C. stimpsoni	21.5	Thayer *et al.*, 1973
Mesopodopsis spp	19.2	Blaber, 1979

[1] values converted to kJ/g using a conversion factor of 4.184

On the basis that the protein content of the preferred prey item for small juvenile spotted grunter, *M. slabberi,* have a protein content of 58.27%, the optimum dietary protein requirement for small spotted grunter is predicted to fall between 50-60%. It would be likely that the protein requirement for larger animals will be less, possibly falling between 35-45% dietary protein. The optimum protein to energy ratio was calculated from the proximate composition data. The energy content of the natural diet was calculated from the protein, carbohydrate and lipid content of the prey items according to published energy values of 23.8kJ/g, 17.2kJ/g and 39.8kJ/g, respectively, and suggests that the optimum for small animals is approximately 31mg/kJ.

DISCUSSION

Jobling (1995) suggests that the reasons for ontogenic changes in prey selection are twofold. Firstly a small fish would be unable to capture, subdue and swallow a large prey item and secondly, a larger animal would have to expend too much time and energy searching for the large numbers of small prey that would be needed to meet its energetic requirements. The change in diet from juveniles to larger animals in the case of spotted grunter could thus be explained. Juveniles have been found to prefer mysids, particularly *Mesopodopsis slabberi* (L. Mahlasela, Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, unpublished data) which reach approximately 12mm in size (Branch *et al.*, 1994). Larger fish appear to feed mainly on larger prey items such as the mudprawn, *Upogebia africana* and the common sandprawn, *Callianassa kraussi* which reach 40 and 60mm in size respectively (Branch *et al.*, 1994). Not only does the size of these prey items make them unlikely prey candidates for juveniles, but it would be difficult, if not impossible for juvenile spotted grunter to capture these prey items.

Spotted grunter extract prawns from their burrows by using a gill chamber pump action. A jet of water is forced into the burrow system and the prey is blown out and consumed (Day *et al.*, 1981). It would therefore, appear unlikely that juveniles would be able to extract these prey items and that Van der Westhuizen and Marais's (1977) conclusion that there is no difference in diet composition between different size classes is incorrect. The animals sampled by these authors, however, were caught by means of gill-nets and hook-and-line and it is unlikely that any very small (<100mm TL) animals were caught. This aspect of prey selection has, however not been fully investigated.

Marais (1990) found that sub-adult (>100mm TL) spotted grunter can be classified as having intermediate fat and energy levels (5,2± 1,3 % fat and 6.06±0.31 kJ/g energy). Juvenile spotted grunter (<70mm TL), like their sub-adult counterparts can also be described as having intermediate fat and energy levels although they had less fat and a lower energy content than the bigger fish. The ash content of juveniles is lower than in sub-adult fish. Protein and ash have been found to vary according to the age of the fish. Denton and Yousef (1976) found that ash content remained relatively constant during the first 14 months of life in rainbow trout,

Oncorhynchus mykiss. In other words it would appear that the relative proportion of skin, scales and bones does not appear to change with growth during the first year in rainbow trout. On the contrary, Pearse (1925) found that the ash content of brook trout increased during the first year and then remained relatively constant thereafter. This would appear to be the case for spotted grunter.

The tendency for larger (>100mm TL) fish to have a higher energy and fat content, and lower moisture content per gramme body mass than juveniles (Marais and Erasmus, 1977) was partially observed in this study. Juvenile spotted grunter have less energy and fat then larger fish but the moisture content remained relatively constant. The higher lipid content of larger spotted grunter could result from feeding on prey items which have higher lipid content than the prey items selected by smaller spotted grunter.

From the natural diet of this fish, it was possible to predict that the dietary protein requirements will be quite high (50-60%). The energy content, both of the juveniles and the sub-adults, implies that this species may require intermediate values of energy in their diet. The amino acid profile of juvenile spotted grunter reveals a high level of lysine. The level of this amino acid is higher than any of the other amino acids, indicating its' relative importance and suggests that the lysine component of the diet must be evaluated before a balanced diet can be achieved.

CHAPTER FOUR

EFFECTS OF DIETARY CRUDE PROTEIN INCLUSION LEVELS ON GROWTH.

INTRODUCTION

The commercial viability of any intensively cultured fish species depends on its market demand and cost of production. The largest production cost lies in the feed, with protein comprising the most expensive component. It is crucial, therefore, that optimal protein levels are known to ensure the best growth and survival at the lowest cost (Serrano *et al.*, 1992; Chen and Tsai, 1994).

There are a number of factors which influence the growth responses of fish fed varying levels of protein. These include the size of the fish, water temperature, salinity, ration size, the amount of non-protein energy in the feed and the quality of the protein used in the diet. The size of the animal is an important consideration. As the animal grows, its growth potential declines with increasing body size and there is a gradual decrease in specific growth rate (Jobling, 1983a). There is a decrease in metabolic rate with increasing size (Steffens, 1989) and therefore, the relative maintenance rations (food/gram of fish at zero growth rate) also decreases (Wurtsbaugh and Davis, 1977). This means that a diet that provides just enough protein for maximum growth of a young animal will provide excessive protein for an older animal (Bowen, 1987).

Water temperature does not directly influence the amount of protein required but has a definite effect on growth. Brett (1979) describes temperature as being a controlling factor in that it governs the rate of reactions by influencing the state of molecular activation of the metabolites. An increase in temperature will result in an increase in growth rate until an optimum temperature is reached. Above this temperature, conditions will become adverse for the fish and growth rate will decline (Jobling, 1983b). An increase in temperature will also lead to an increase in the rate of digestion (Brett, 1979) and gut evacuation rate (Jobling, 1981; Fauconneau *et al.*, 1983) which in turn results in reduced digestive efficiency (Windell *et al.*, 1976).

The effect of salinity on growth appears to be species specific, governed by the natural environment of the fish and its' tolerance and regulating capacity (Brett, 1979). For example, Jobling (1983b) found that Arctic charr, *Salvelinus alpinus*, showed lower growth rates in saltwater than in freshwater. Red drum, *Sciaenops ocellatus* on the other hand exhibited no difference in specific growth rate between fresh- and saltwater (Wurts and Stickney, 1993).

Feeding regimes adopted in growth trials should permit maximal growth rates. Restricted feeding (a ration of a specific percent of body weight per day) generally prevent maximal growth rates from being achieved. The most satisfactory approach is satiation feeding several times a day (Cowey, 1995). The effects of underfeeding have been shown by Ogino (1980). His work on carp, *Cyprinus carpio* and rainbow trout, *Oncorhynchus mykiss* showed that dietary protein levels increased from 35% to 50% when the feeding ration was lowered from 3.5% to 2.5% of body weight per day. An increase in ration size has been shown to increase growth rates in Atlantic cod, *Gadus morhua* (Houlihan *et al.*, 1988). The effect of non-protein energy and the quality of the protein used are discussed in Chapters 5 and 7 respectively.

Fish, like other animals, do not have a true protein requirement, but have a requirement for a well-balanced mixture of essential and non-essential amino acids (Wilson, 1989). It is, however, the protein in the diet which provides these amino acids. Inadequate protein in the diet will result in a reduction or cessation of growth and a loss of weight due to the withdrawal of protein from less vital tissues to maintain the functions of the more vital tissues (Wilson, 1989).

Dietary protein requirements of carnivorous fish have been well documented (Teng *et al.*, 1978; Millikin, 1982; Lie *et al.*, 1988; Parazo, 1990 and Tucker, 1992) and found to vary greatly. This variation in requirements is probably due to differences in water temperatures, salinity, diet composition, biological value of protein and non-protein energy sources and the size of the fish being studied (NRC, 1983). Protein may be spared by increasing the energy supply from lipids or carbohydrates (Austreng, 1979), however this can only be investigated once an "optimal" dietary protein inclusion level has been established (Fuller, 1988). Once this optimal dietary protein inclusion level has been determined, attempts to reduce it by increasing the dietary lipid can be made (Tibaldi *et al.*, 1996).

This study investigated the optimal dietary protein inclusion level which is necessary for maximum growth of juvenile spotted grunter. This was achieved by assessing the effects of varying levels of dietary protein on growth, feed conversion and protein efficiency ratios. Once this inclusion level had been established, the possible sparing effect by lipid could be investigated in a P:E experiment.

The optimal biotic and abiotic factors of this species have been investigated (Deacon and Hecht, 1995a and b). They found the optimal temperature for growth to be 24°C; the optimal photoperiod to be 14L:10D (1995a) and the optimal feeding frequency to satiation 3X daily (1995b). The use of semi-purified diets in this study made comparisons with findings of other authors possible (Tacon and Cowey, 1985). There is no consensus on a standard reference protein to be used in optimal protein studies, but highly digestible, nutritional proteins are recommended (Tucker, 1992). The most frequently used protein sources are casein and fishmeal (Cowey, 1995). The variation in protein requirements caused by biotic and abiotic factors was thereby minimized.

MATERIALS AND METHODS

The general methods employed are outlined in detail in Chapter 2. The range of dietary protein level were based on the empirically determined optimal protein inclusion levels for other juvenile carnivorous marine finfish outlined in Table 1 and on the predicted protein levels as discussed in Chapter 3. The protein level in the diets ranged from 35-60%.

Preparation of experimental diets

Six semi-purified diets were formulated with protein levels ranging from 35-60% (Table 2) according to the methods outlined in Chapter 2. The protein content of each diet was calculated on a dry matter basis according to the total percentage protein in both the protein sources, fishmeal (71.5% dry matter, Fiskernes Fiskeindustri, A.M.B.A) and casein (92.7% dry matter, International feed number 5-01-162). Diets were based on similar diets used to determine the protein content of juvenile grouper, *Epinephelus malabaricus* (Chen and Tsai, 1994) and red drum, *Sciaenops ocellatus* (Moon and Gatlin, 1994). The diets were kept isocaloric (at 18.23±0.31mg/kJ) with the supplementation of dextrin. The diets were kept isocaloric to avoid

Table 1 : Optimal dietary protein requirements of some juvenile marine carnivores.

FISH SPECIES	FISH SIZE (g)	TEMPERATURE (°C)	SALINITY (ppt)	PROTEIN LEVEL (%)	REFERENCE
Sciaenops ocellatus	46-77	22-26	5-6	35-44	Daniels and Robinson, 1986
Sciaenops ocellatus	2.0	23	6	40-45	Serrano *et al.*, 1992
Dentex dentex	17-20	20	33	44.3-55.8	Tibaldi *et al.*, 1996
Epinephelus malabaricus	3.79± 1.17	26-27	32	47.8	Chen and Tsai, 1994
Scophthalmus maximus		18	35	50-60	Person-Le Ruyet *et al.*, 1991
Dicentrarchus labrax	31-57			50	Hildalgo and Alliot, 1988
Lates calcarifer	1.34± 0.01	28-29	32	42.5	Catacutan and Coloso, 1995
Seriola quinqueradiata				57	Shimeno *et al.*, 1985
Pagrus major	1.6			52	Takeuchi *et al.*, 1991
Pagrus major		16-17.5		50	Foscarini, 1988
Sparus aurata				61	Sabaut and Luquet, 1973
Gadus morhua	173-318			54	Lie *et al.*, 1988

any bias due to energy availability differences

Feeding

Each diet was fed in triplicate to 8 fish per tank. Food for each tank was kept in individual screw top containers which were weighed before and after each feed so as to determine how much food was consumed by the fish in each tank. Feeding rate was to satiation three times daily. Once all tanks had been fed, the process was repeated until the fish were visually assessed as being satiated. This was assumed to occur when the fish moved to the bottom of the tank and ceased feeding and foraging behaviour. Feeding occurred under the optimal conditions of 24.6°C (±0.42) and a salinity of 33±1ppt. Photoperiod was kept constant by means of a time-switch which was checked weekly for any drift. The oxygen saturation was maintained at 97±1% by means of an airstone.

The experiment ran for a period of 60 days. The fish were weighed (g), measured (SL mm) and individually marked by means of fin spine clipping on the first day of the experiment and every 10 days thereafter. The initial weight and length of the fish was 3.38±0.48g and 55±3mm.

Statistical analysis

A co-efficient of variation in mean weight was performed at the end of the experiment according to the equation :

$$CV\ (\%) = (SD/x)*100$$

where x = mean weight

and CV_1 = CV of sample at time 0

CV_2 = CV of sample at the end of the experiment

so that

$$\Delta CV = CV_2 - CV_1$$

(After Zar, 1984)

Table 2 : Formulation and proximate composition of the test diets.

Ingredient	Diet (g/100g)					
	1	2	3	4	5	6
Fishmeal	24	24	25	25	22	20
Vitamin free Casein	20	25	30	35	43	50
Dextrin	30	25	20	15	10	5
Cod Liver Oil	8	8	8	8	8	8
Mineral mixture[1]	8	8	8	8	8	8
Vitamin mixture[2]	4	4	4	4	4	4
Cellulose	2.50	2.50	1.50	1.50	1.50	1.50
Sodium alginate	2.50	2.50	2.50	2.50	2.50	2.50
Sodium hexamata-phosphate	1	1	1	1	1	1
Calculated protein level	35	40	45	50	55	60
Proximate Composition						
Crude protein (%)	36.67	40.45	46.22	50.86	57.83	61.84
Ash (%)	14.39	14.59	14.27	15.62	14.1	14.44
Gross E (MJ/kg)	17.79	17.82	18.35	18.16	18.93	18.33

[1] Mineral mix (g/kg) : 74g potassium; 516g vermiculite RSU; 14g salt; 0.05g ammonium chloride; 31g choline chloride; 0.31g cobalt; 0.15g copper; 1.5g iron; 0.05g iodine; 0.22g manganese; 41g magnesium; 1g zinc and traces of selenium.

[2] Vitamin mix (IU or g/kg) : 500 000 IU vitamin A; 400 000 IU vitamin D3; 10 000 IU vitamin E; 1g vitamin K3; 0.25g vitamin B1; 1.5g vitamin B2; 0.5g vitamin B6; 25g vitamin C; 2.5g Niacin; 0.09g Folic acid; 0.025g Biotin; 2.5g Calpan; 2.5g Inositol.

RESULTS

The results are summarized in Table 3. Dietary protein content significantly ($p<0.05$) affected weight gain, specific growth rate (SGR) and protein efficiency ratio (PER) but had no effect on food conversion ratio (FCR) of juvenile spotted grunter.

Table 3 : Effects of various dietary protein levels on the growth, feed conversion ratio (FCR) and protein efficiency ratio (PER) of juvenile *Pomadasys commersonnii* fed for 60 days.

DIETARY PROTEIN (%)	WEIGHT GAIN (g)	SGR	FCR	PER
35	5.67 ± 0.51^{a}	2.02 ± 0.15^{a}	1.89 ± 0.21^{a}	1.08 ± 0.09^{ab}
40	7.68 ± 2.87^{b}	2.31 ± 0.11^{b}	1.96 ± 0.25^{a}	1.17 ± 0.12^{a}
45	6.13 ± 2.30^{b}	2.33 ± 0.14^{b}	1.85 ± 0.12^{a}	0.94 ± 0.1^{b}
50	7.15 ± 1.67^{b}	2.42 ± 0.09^{b}	1.67 ± 0.11^{a}	0.88 ± 0.11^{b}
55	5.64 ± 0.80^{a}	2.09 ± 0.13^{a}	1.85 ± 0.17^{a}	0.80 ± 0.15^{bc}
60	5.07 ± 0.45^{a}	1.81 ± 0.15^{a}	1.87 ± 0.24^{a}	0.59 ± 0.14^{c}

Means of 3 replicate groups ± standard deviation with same superscripts are not significantly different ($p<0.05$)

From Figure 1, it can be seen that fish fed the 40-50% protein diets ate significantly less ($p<0.05$) food than those fed the other diets. Fish fed the 60% protein diet ate significantly more ($p<0.05$) food. In terms of nett protein consumption, fish fed the 35 and 40% protein diets consumed significantly less ($p<0.05$) protein than fish fed on the other diets. Fish fed the 60% protein diet consumed nearly twice as much protein than those fed the 35 and 40% protein diets.

Figure 2 shows the effect of varying dietary protein levels on specific growth rates (SGR). At 35% dietary protein SGR is low (2.02 ± 0.15) but increases with increasing dietary protein until an optimum is reached at 50% protein (2.42 ± 0.09). After this optimum SGR decreases until a minimum (1.81 ± 0.15) is reached at 60% dietary protein. There was no significant difference ($p<0.05$) between SGR of diets containing 40-50% protein although the SGR obtained at these

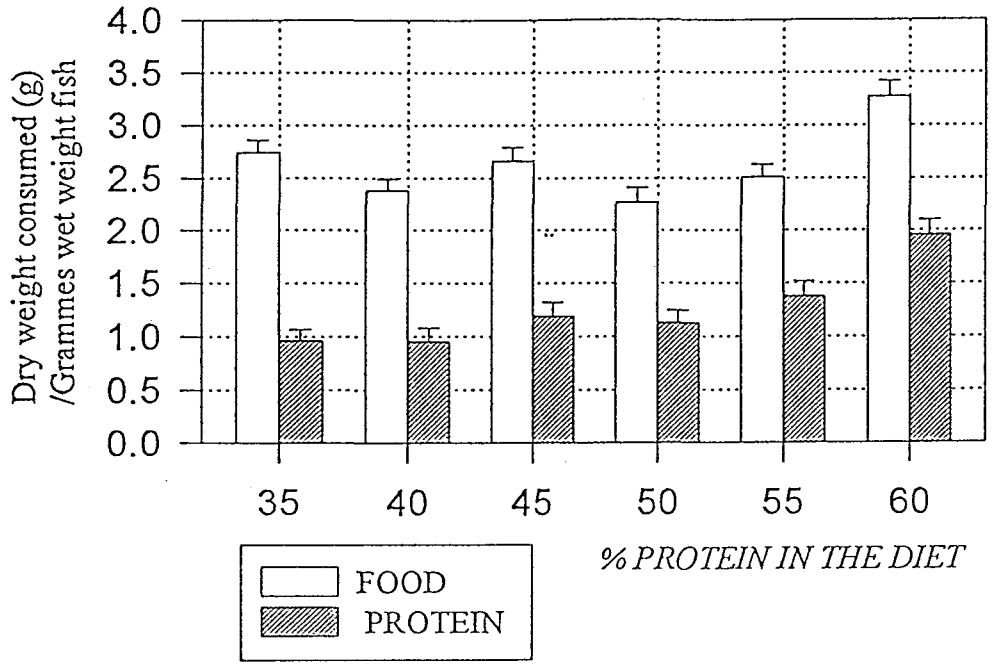

Figure 1 : Food and protein consumption at satiation feeding of diets containing graded levels of protein after a 60 day growth trial (vertical bars = 1/2 standard deviation).

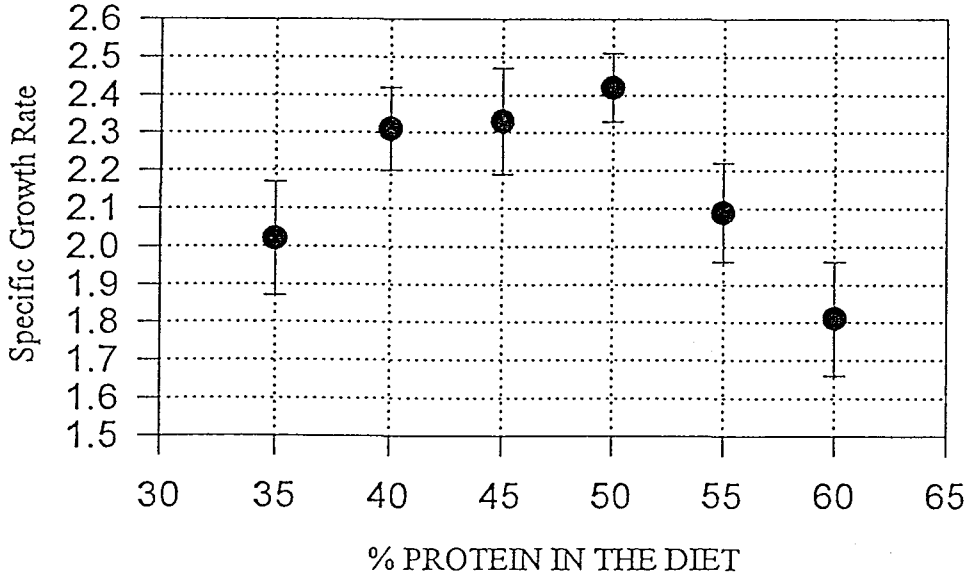

Figure 2 : Effect of varying dietary protein levels at satiation feeding 3x daily on specific growth rate (vertical bars = 1 standard deviation).

levels of protein inclusion were significantly ($p<0.05$) better than SGR obtained at 35, 55 and 60% dietary protein.

There was a decrease in food conversion ratio (FCR) with increasing protein levels (Figure 3) until a low of 1.67±0.11 was obtained at the 50% protein level. After this point, FCR increased with increasing protein levels until a high of 1.87±0.24 was reached at the 60% protein level. There was, however, no significant difference ($p<0.05$) between the FCR obtained from feeding the different diets.

Figure 3 : Effect of varying protein levels at satiation feeding 3x daily on food conversion ratios (vertical bars = 1 standard deviation).

The protein efficiency ratios (PER) are shown in Figure 4. There was a decrease in PER with increasing protein levels. Protein efficiency was best (1.17±0.12) at 40% dietary protein and was significantly higher ($p<0.05$) at this level than at either 50 or 60% dietary protein inclusion levels. The PER at 60% dietary protein was the lowest at 0.59±0.14.

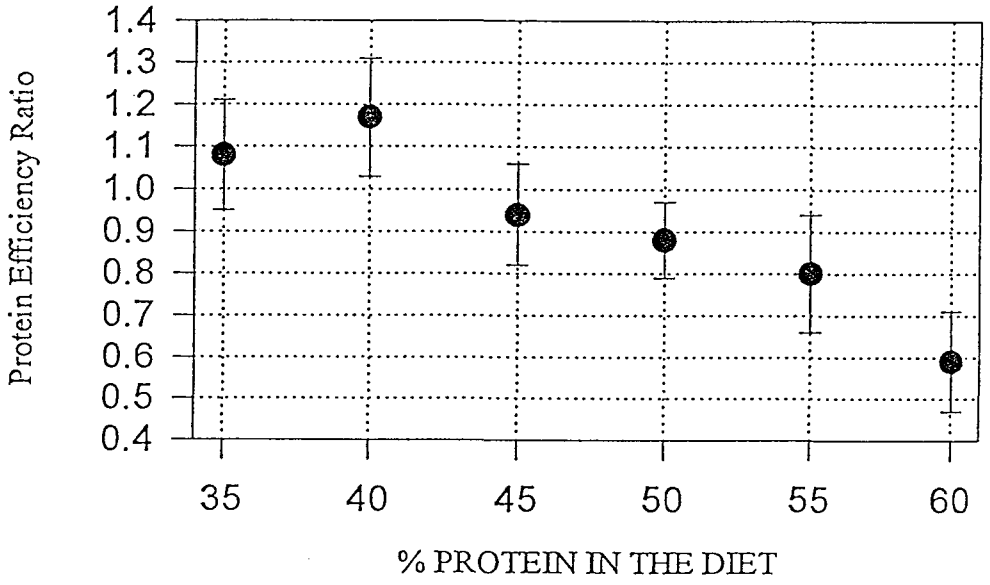

Figure 4 : Effect of varying dietary protein levels at satiation feeding 3x daily on protein efficiency ratios (vertical bars = 1 standard deviation).

The effect of feeding with semi-purified diets with varying protein levels on condition factor (CF) of the fish is shown in Figure 5. The CF of the fish at the beginning of the experiment was significantly ($p<0.05$) lower than at the end of the experiment. The CF of the fish both at the beginning and at the end of the experiment did not differ between treatments.

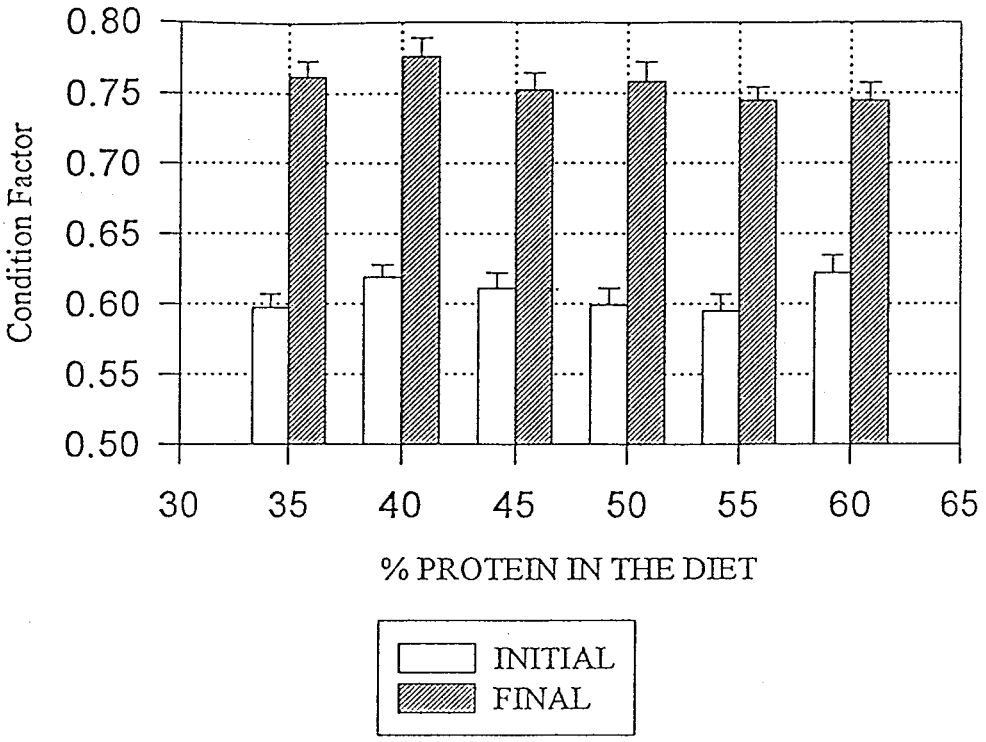

Figure 5 : Effect of feeding varying dietary protein levels at satiation feeding 3x daily on condition factor after a sixty day growth trial (vertical bars = 1/2 standard deviation) .

The co-efficient of variation results are shown in Table 4. It can be seen that feeding the animals with diets containing 40 and 45% dietary protein resulted in a marked decrease in the variation about the mean.

Table 4 : Co-efficient of variation in the mean weight of the fish fed the diets containing graded protein inclusion levels.

% DIETARY PROTEIN	CV_1	CV_2	ΔCV	% IMPROVEMENT
35	32.09	23.72	-8.37	26.08
40	44.72	23.11	-21.61	48.32
45	48.29	23.66	-24.63	51.00
50	37.34	23.64	-13.7	36.69
55	37.13	23.62	-13.51	36.39
60	32.11	23.07	-9.04	28.15

Therefore, it can be seen that the empirically determined optimal dietary protein level for juvenile spotted grunter is 40-50%.

DISCUSSION

Growth is mainly a consequence of protein deposition and this in turn is principally determined by the protein or amino acid supply (Cowey, 1993). The amount of protein required by fish has been shown to be influenced by a number of factors. Fish appear to have a higher protein requirement than terrestrial animals (Tacon and Cowey, 1985). This may be due to their carnivorous/omnivorous feeding habits and/or their apparent use of protein over carbohydrate and lipid as a dietary energy source. Therefore, increasing the amount of non-protein energy in the diet by increasing the amount of lipid or carbohydrate may produce a protein sparing effect (Tacon and Cowey, 1985). Essentially, the energy requirements may be met by the lipid or carbohydrate, allowing the protein to be used more efficiently for somatic growth (Wilson, 1989).

Carnivorous fish generally require high dietary protein levels to ensure fast growth. Salmon and bass require 40-55% dietary protein (Millikin, 1983). The same is true for rainbow trout fingerlings which require a 51% protein inclusion level, (Austreng and Refstie, 1979), juvenile red drum which require a 45% inclusion level (Serrano et al., 1992) and juvenile grouper, *Epinephelus malabaricus* which require a 47.8% protein inclusion level (Chen and Tsai, 1994). Spotted grunter have been described as opportunistic benthivores (Van Der Westhuizen and Marais, 1977). In adults, 75% of their diet in estuaries consists of burrowing anomuran *Upogebia africana* and *Callianassa kraussi* and pencil bait (*Solen cylindraceus*) (Whitfield, 1990). L. Mahlasela (Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, unpublished data) has found that the predominant prey item in juvenile spotted grunter are mysids, particularly *Mesopodopsis slabberi*. The protein content of these prey items is outlined in Chapter 3. The empirically determined optimum dietary protein requirement of 40-50% for juvenile grunter, is therefore not an unexpected result, but is less than the predicted optimum of 50-60% based on the proximate composition of juvenile spotted grunter and their preferred prey items.

Mazid *et al.* (1979) outlined two trends regarding the effect of various levels of dietary protein on growth rate. Firstly, there is an increase in growth rate which is proportional to increased dietary protein up to a threshold level, whereafter growth declines with increasing protein level. This has been found in plaice, *Pleuronectes platessa* (Cowey *et al.*,1972). The second trend shows no decrease in growth rate after the protein threshold has been reached. This has been shown for gilthead bream, *Sparus aurata* (Sabaut and Luquet, 1973). Juvenile spotted grunter clearly responded according to the first trend, with specific growth rate increasing up to a threshold of 50% protein inclusion, thereafter decreasing with increased amounts of dietary protein. Phillips (1979) suggests that this is a consequence of an increase in the requirement of energy to rid the body of excess toxic nitrogenous waste, because of an increase in the rate of amino acid breakdown (Fuller, 1988).

The FCR values achieved in this study were higher than other studies on fish with similar protein requirements (Bowen, 1987). The absolute FCR values achieved in this study serve only as an indication of the optimum protein inclusion rate. As this "grunter" diet becomes better formulated to comply with findings of further work, it is likely to show improvement. The best food conversion ratio was achieved at the upper limit of the optimal protein range (50%), indicating the most efficient utilization of food.

The protein efficiency ratio decreased with increasing dietary protein. This indicates that the maintenance requirement of dietary protein for juvenile spotted grunter, *P. commersonnii*, lies between 35 and 40% protein. Fish are physiologically capable of utilizing a maximum amount of protein for growth, after which the excess protein will either be converted to glycogen for energy or will be deaminated and excreted (Mertz, 1972).

In formulating a diet for large scale grow-out of juvenile spotted grunter, a compromise between maximum growth and minimum production cost must be achieved. Fish fed diets containing 35% and 40% protein ate significantly less ($p<0.05$) protein than fish fed the other diets. Fish fed the 40% protein diet had a significantly higher ($p>0.05$) PER than fish fed the higher protein diets. Therefore,

in terms of protein utilization, a 40% protein diet was optimal. Fish fed the 50% protein diet had the highest SGR but this was not significantly ($p>0.05$) higher than the SGR resulting from feeding diets containing 40 or 45% protein. Fish fed the 50% protein diet ate significantly more ($p>0.05$) protein but exhibited a significantly lower ($p>0.05$) PER. Therefore, in terms of maximum growth at minimum cost, the 40% protein diet was optimal.

CHAPTER FIVE

THE EFFECT OF DIETARY PROTEIN : ENERGY RATIO ON GROWTH AND BODY COMPOSITION.

INTRODUCTION

Fish production can be enhanced by increasing the protein level of the diet to the optimal level (Nematipour *et al.*, 1992). However, this is not cost effective due to the expense of this ingredient. The optimal dietary protein requirement of any fish species is influenced by a number of factors including the protein source, the protein to energy ratio (Sargent *et al.*, 1989), the size and the age of the individual fish, and the ambient temperature (Lovell, 1989).

The absolute protein content of a diet cannot be considered in isolation from the available energy in the diet (Hardy, 1989). The digestible energy available in the diet will determine how much of the incorporated protein can actually be utilized for somatic growth. The digestible energy (DE) of each dietary ingredient must therefore be determined in order to ensure maximum utilization of the diet (Hardy, 1989). The gross energy value of carbohydrates is 17.2 kJ/g and that of proteins slightly higher at 23.4 kJ/g. However, lipids have a much higher energy content at 39.8 kJ/g (Cho *et al.*, 1982). It stands to reason, therefore that variations in the lipid content of the food would have a greater influence on its gross energy value than changes in either protein or carbohydrates (Cho and Bureau, 1995).

Energy metabolism in fish differs from terrestrial animals in two important respects. Firstly, fish are ectotherms and therefore do not have to expend energy in maintaining a constant body temperature. As a result, fish have lower maintenance energy requirements (Cho and Kaushik, 1985). Secondly, fish are able to save 10-20% more energy from the catabolism of protein than their terrestrial counterparts as they do not have to convert ammonia into less toxic substances prior to excretion (Brett and Groves, 1979).

The protein to energy ratio (g protein/kg diet : MJ DE/kg diet) is a measure of the required dietary balance (Steffens, 1989). There is evidence to suggest that fish eat to satisfy their energetic requirements (Lee and Putnam, 1973). In other words, given a food with a satisfactory nutrient balance, fish are able to compensate for a low energy density by eating more of it (feeding conditions and physical capacity of the digestive tract permitting). At maximal physical intake, fish fed high energy diets can ingest more nutrients and will therefore be able to direct protein into somatic growth (Cho and Bureau, 1995). Therefore, the optimal crude protein content of the diet cannot be determined without considering the digestible energy content of the diet.

According to Millikin (1983), optimal feed utilization occurs when fish are fed the lowest dietary protein concentration that supports maximal growth. Protein utilization may be improved by its partial replacement with lipid or carbohydrate in order to produce a protein-sparing effect (Nematipour *et al.*, 1992). Excessive protein in the diet will be used for energy and will result in poor water quality due to an increase in ammonia excretion (Catacutan and Coloso, 1995). If, however, excessive non-protein energy is incorporated into the diet, the protein : energy ratio will be lowered. This may lead to a reduction in growth (Daniels and Robinson, 1986) and an increase in lipid deposition (Watanabe, 1982). However, if a diet is deficient in non-protein energy, protein will be used as an energy source rather than for somatic growth. Similarly, if there is an excess of non-protein energy, the fishes appetite may be satisfied before a sufficient quantity of protein is consumed. This being the case, the requirements for protein synthesis and growth may not be met (Cho and Bureau, 1995).

The true value of a protein in a diet is only fully realised when the protein : energy balance is correct. The energy will then be used for maintenance allowing the animal to move into a positive nitrogen balance in which amino acids are accumulated in body tissue proteins (Fuller, 1988). The use of high-energy/low-protein diets offers the fish farmer a means of reducing protein content in the diets, thereby reducing cost, while maintaining acceptable growth rates (Hillestad and Johnsen, 1994). Due to the limited ability of fish to digest and utilize carbohydrates (Austreng, 1979), energy in formulated fish diets is commonly derived from lipids.

This part of the study was aimed at defining the optimal dietary protein/energy ratios in order to facilitate maximal growth in juvenile spotted grunter, and to determine the extent to which protein can be spared by lipid. This was achieved by assessing the effect of three different P:E ratios at three protein levels on growth, feed conversion, protein efficiency ratios, intraperitoneal fat deposition and hepatosomatic indices.

MATERIALS AND METHODS

Diet preparation

A 3X3 factorial design was employed in which semipurified diets were formulated to contain either 35, 45 or 55% protein and 6, 8 or 12% lipid (Table 1). This resulted in P:E ratios ranging from 21.1mg/kJ to 42.3mg/kJ. Casein and low temperature Danish fishmeal (Fiskernes Fiskeindustri, A.M.B.A) were used as the protein sources. Marine fish oil and soya oil served as the lipid sources. Pregelatinized starch was used as a soluble carbohydrate source. Cellulose was used as the filler.

Published digestible energy values vary greatly. As the values for the individual dietary ingredients have not been determined for spotted grunter, dietary energy was calculated based on the estimated values most commonly used in artificial diet preparation for carnivorous marine fish, of 16.7, 16.7 and 37.7kJ/g for carbohydrate, protein and lipid respectively. This made comparisons with other findings possible (Garling and Wilson, 1977; Serrano *et al.*, 1992).

Fish and feeding trials

The standard length and weight of the fish at the beginning of the growth trial was 66.42±3.16mm and 6.42±0.72g. The fish were acclimated to tank conditions for a period of two weeks prior to the initiation of the feeding trials. During the first week of acclimation the fish were fed pellets consisting of a 2:1 ratio of minced pilchard (*Sardinops sagax*) and low temperature Danish fishmeal (Fiskernes Fiskeindustri, A.M.B.A) to induce acceptance of pellets. During the second week the fish were changed over to their respective semi-purified diets to ensure full acceptance of the diet prior to starting the experiment.

Table 1 : Formulation and proximate composition of the nine test diets.

INGREDIENT	DIET (g/100g)								
	1	2	3	4	5	6	7	8	9
Fishmeal	35	35	35	44	44	44	45	45	45
Vitamin free Casein	11	11	11	15	15	15	25	25	25
PGS[1]	26	34	37	16	24	27	6	14	17
Fish Oil	1.2	2.6	5.2	0.4	1.8	4.5	0.4	1.8	4.4
Soya Oil	0.6	1.3	2.6	0.2	0.9	2.2	0.2	0.9	2.2
Mineral Mix[2]	4	4	4	4	4	4	4	4	4
Vitamin Mix[3]	2	2	2	2	2	2	2	2	2
Cellulose	20.2	10.1	3.2	18.4	8.3	1.3	17.4	7.3	0.4
Proximate Composition									
Crude Protein (%)	35.7	35.5	35.5	44.8	45.3	45.3	54.7	55.4	55.3
Ash (%)	9.4	9.4	10.3	11.7	11.8	10.3	10.4	10.6	11.0
Gross E (MJ/kg)	8.0	8.2	8.5	8.2	8.3	8.5	8.5	8.6	8.8
Calculated Values									
Protein (%)	35	35	35	45	45	45	55	55	55
Carbohydrate (%)	26	34	37	16	24	27	6	14	17
Lipid (%)	6	8	12	6	8	12	6	8	12
DE (kJ/gram)	12.7	14.8	16.8	12.8	14.9	16.9	12.9	15.0	17.0
P:E Ratio (mg/kJ)	28.1	23.9	21.1	35.3	30.3	26.7	42.3	36.8	32.5

[1] Pregelatinized starch

[2] Mineral mix (g/kg) : 74g potassium; 516g vermiculite RSU; 14g salt; 0.05g ammonium chloride; 31g choline chloride; 0.31g cobalt; 0.15g copper; 1.5g iron; 0.05g iodine; 0.22g manganese; 41g magnesium; 1g zinc and traces of selenium.

[3] Vitamin mix (IU or g/kg) : 500 000 IU vitamin A; 400 000 IU vitamin D3; 10 000 IU vitamin E; 1g vitamin K3; 0.25g vitamin B1; 1.5g vitamin B2; 0.5g vitamin B6; 25g vitamin C; 2.5g Niacin; 0.09g Folic acid; 0.025g Biotin; 2.5g Calpan; 2.5g Inositol.

Three replicates of 8 fish (24 fish per treatment) were fed different dietary treatments. The fish were fed to satiation 3x daily. Food for each tank was kept in screw top containers which were weighed before and after each feeding in order to monitor consumption (See Chapter 2 for detail). The fish were weighed and measured every 10 days after they had been anaesthetized in a 0.02% solution of 2-phenoxyethanol.l^{-1} (Deacon *et al.*, in press). The experiment was conducted over a sixty day period in 27 glass aquaria linked to a recirculating system (Chapter 2). The flow rate through the tanks was maintained at 0.6l/min., equivalent to a turnover rate of once every hour. The water temperature was kept at 24±1.5°C by means of an immersion heater. Water quality was closely monitored and kept at a constant salinity of 33±1ppt. Nitrite levels remained below 0.1mg/l and ammonia levels remained below 0.02mg/l.

RESULTS

The results are summarized in Table 2. P:E ratios significantly affected SGR, FCR and PER at $p<0.05$.

Food and protein consumption

The amount of food or protein consumed per gramme of fish is shown in Figure 1. There was no significant difference ($p<0.05$) in the amount of food or protein consumed per gramme of fish between dietary treatments.

Proximate composition

The proximate composition of the fish before and after completion of the feeding trial are summarized in Table 3. The dietary treatments had no significant ($p<0.05$) effect on the moisture content of the fish (Figure 2). The ash content of the fish upon completion of the feeding trial was significantly lower ($p<0.05$) than that of fish at the start of the trial (Table 3). Fish fed diets containing 45% protein and 8% and 12% lipid had significantly less ($p<0.05$) ash than fish fed the other diets.

Table 2 : Growth responses of fish fed diets containing varying P:E ratios for 60 days.

	DIET NUMBER								
PARAMETER	1-35P:6L	2-35P:8L	3-35P:12L	4-45P:6L	5-45P:8L	6-45P:12L	7-55P:6L	8-55P:8L	9-55P:12L
Initial weight	4.17 ±2.28	6.37 ±1.92	7.78 ±2.93	6.41 ±2.16	6.67 ±2.58	7.49 ±2.57	6.75 ±2.38	5.37 ±1.91	6.44 ±1.11
Final weight	9.65 ±4.77	13.59 ±3.61	16.25 ±4.61	14.63 ±3.67	16.56 ±5.32	17.83 ±5.19	15.59 ±4.93	14.05 ±4.57	18.35 ±4.25
Initial CF°	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Final CF°	0.08	0.08	0.09	0.08	0.09	0.09	0.08	0.08	0.08
Feed consumed[1]	12.27 ±2.99	15.38 ±1.28	17.62 ±1.04	14.71 ±1.46	17.36 ±1.30	18.23 ±1.49	14.68 ±1.77	14.61 ±1.24	18.11 ±2.32
Protein consumed[1]	4.38^{a} ±1.07	5.46^{b} ±0.45	6.26^{c} ±0.37	6.59^{c} ±0.66	7.87^{d} ±0.59	8.26^{d} ±0.68	8.03^{d} ±0.97	8.09^{d} ±0.69	9.96^{e} ±1.78
FCR	2.43^{a} ±0.40	2.27^{b} ±0.29	2.43^{a} ±0.35	1.86^{d} ±0.20	2.08^{c} ±0.24	1.72^{d} ±0.19	1.88^{d} ±0.12	1.91^{c} ±0.25	2.23^{b} ±0.31
SGR	4.81^{a} ±0.43	5.08^{b} ±0.51	5.43^{c} ±0.33	5.51^{cd} ±0.32	5.58^{cd} ±0.31	5.96^{e} ±0.36	5.35^{bc} ±0.37	5.41^{c} ±0.32	5.38^{bc} ±0.36
PER	1.05^{a} ±0.09	1.06^{ab} ±0.09	1.11^{b} ±0.12	1.21^{bc} ±0.11	1.29^{cd} ±0.17	1.25^{cd} ±0.13	1.38^{d} ±0.16	1.37^{d} ±0.15	1.30^{cd} ±0.14

^{abcde} Means of 3 replicate ± standard deviation groups with the same superscripts are not significantly different ($p<0.05$)
[1] dry weight consumed per wet weight gramme fish
° standard deviation was negligible (<0.001)

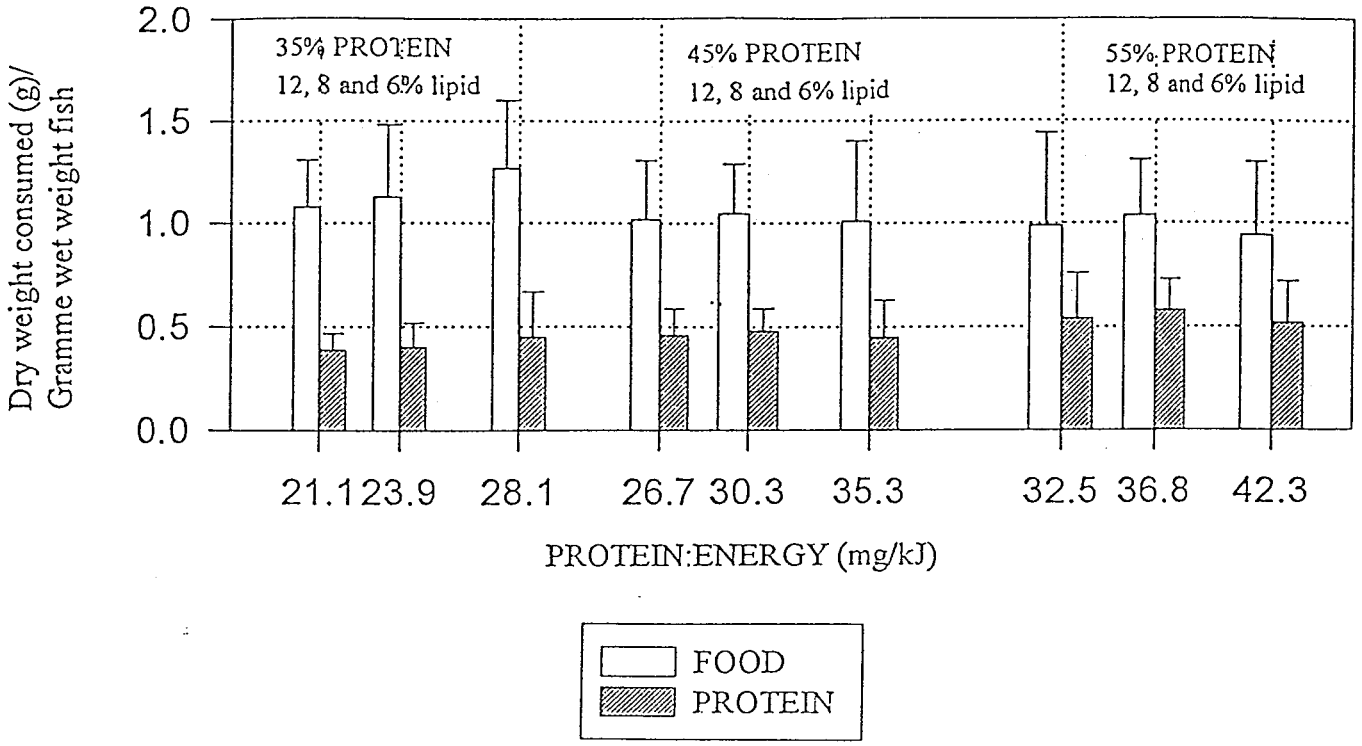

Figure 1 : Food and protein consumption by the fish at satiation feeding 3x daily of different P:E diets (vertical bars = 1/2 standard deviation).

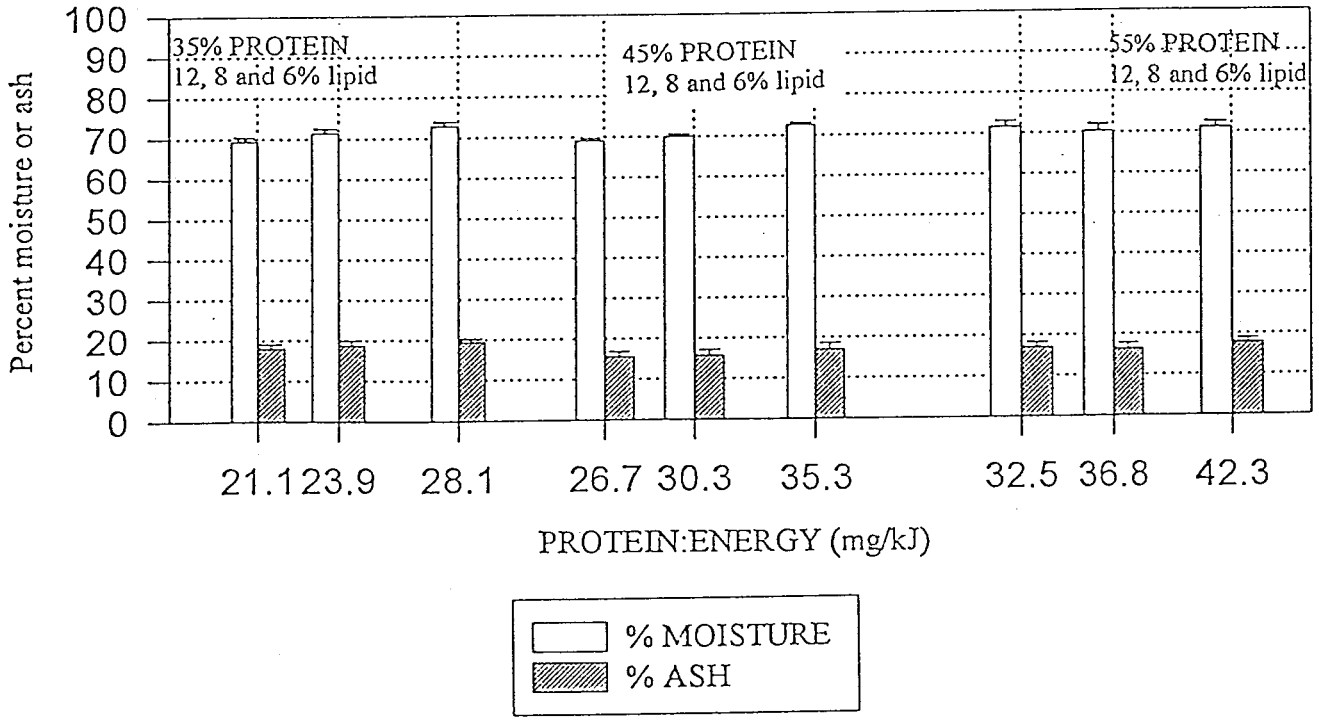

Figure 2 : Effect on moisture and ash content of varying P:E ratios at different protein levels at satiation feeding 3x daily (vertical bars = 1/2 standard deviation).

Table 3 : Body composition of fish before and after feeding with the test diets for 60 days.

FISH	% MOISTURE	% ASH	% PROTEIN	% LIPID	HSI[1]	IPF[2]
Wild	70.12±1.08	24.94±1.12^{a}	63.25±1.05^{a}	4.70±0.87^{a}	4.71±0.62^{c}	N/A
D1-35P:6L	72.79±1.15	19.31±1.04^{b}	50.27±0.05^{b}	16.96±0.84^{b}	4.77±0.46^{c}	2.55±0.75^{a}
D2-35P:8L	71.42±1.12	18.79±1.14^{b}	49.83±0.1^{bc}	20.62±0.92^{c}	4.89±0.56^{c}	4.27±0.89^{bc}
D3-35P:12L	69.30±1.06	17.9±3.09^{b}	48.31±0.35^{c}	21.66±0.10^{c}	4.07±0.19^{a}	7.23±1.23^{e}
D4-45P:6L	72.51±0.63	16.98±2.56^{b}	51.67±0.97^{b}	17.84±0.76^{b}	4.29±0.18^{ab}	5.24±1.02^{bcd}
D5-45P:8L	69.93±1.67	15.7±1.43^{c}	47.68±0.62^{c}	25.45±0.89^{d}	4.16±0.13^{ab}	5.79±1.09^{cde}
D6-45P:12L	68.98±0.61	15.54±2.26^{c}	43.98±0.96^{e}	25.86±0.95^{d}	4.01±0.23^{a}	6.52±0.52^{de}
D7-55P:6L	71.06±1.55	17.67±1.21^{b}	52.57±0.43^{b}	20.00±0.76^{c}	4.11±0.11^{ab}	2.26±0.46^{a}
D8-55P:8L	70.37±1.69	16.29±2.53^{b}	48.55±0.47^{c}	21.12±1.05^{c}	4.09±0.2^{a}	3.86±1.03^{ab}
D9-55P:12L	71.54±1.48	17.01±2.23^{b}	45.72±0.28^{d}	22.26±0.94^{cd}	3.89±0.08^{a}	4.42±1.68^{c}

^{abcde} Means of 3 replicate groups ± standard deviation with the same superscript are not significantly different ($p<0.05$)

[1] HSI = Hepatosomatic index

[2] IPF = Intraperitoneal fat index

The protein content of the fish fed the test diets was significantly lower ($p<0.05$) than at the start of the experiment (Table 3). Within each protein level, there was a decrease in protein deposition with increasing dietary lipid levels (Figure 3). Fish fed diets containing 12% lipid, at all protein levels, had significantly less protein deposition than was observed in fish fed diets containing 6%. Fish fed on the 45% and 55% protein diets with 6% dietary lipid showed a slight increase in protein deposition, although this was not significant ($p>0.05$).

The various test diets had a significant effect ($p<0.05$) on gross lipid deposition (Figure 3). Fish fed the formulated diets had significantly ($p>0.05$) more lipid than wild fish. Fish fed 45% protein and 6% lipid (35.3mg/kJ), and fish fed 35% protein and 6% lipid (28.1mg/kJ) had the lowest total lipid content. There was an increase in lipid deposition with increasing dietary lipid. The increase in the percentage body lipid did not appear to be detrimental to the fish as no pathological disorders or ill-effects were observed during this 60 day growth trial.

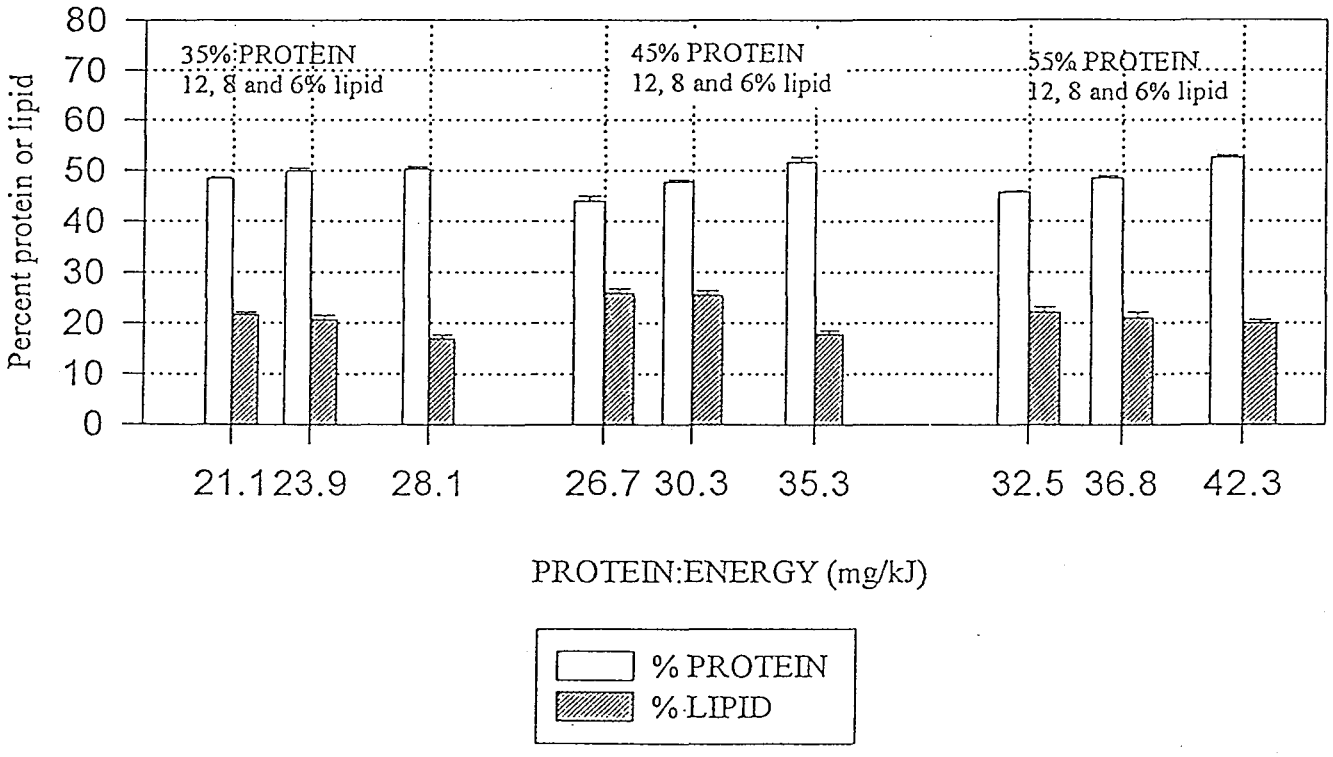

Figure 3 : Effect on protein and lipid content of varying P:E ratios at different protein levels at satiation feeding 3x daily (vertical bars = 1/2 standard deviation).

Hepatosomatic indices (HSI) of the fish at the start of the experiment and those fed diets with 35% protein with either 6% or 8% lipid were not significantly different ($p<0.05$). A significant difference ($p<0.05$) in HSI was found between fish at the start of the experiment and those fed the other dietary treatments (Figure 4). There was an increase in the deposition of intraperitoneal fat with an increase in dietary lipid (Figure 4). There was no significant difference ($p>0.05$) in the deposition of intraperitoneal fat in fish fed the 45% protein diets. Fish fed diet containing 35% and 55% protein and 6% lipid had significantly less deposited intraperitoneal fat than fish fed the other diets (Figure 4). As the total carcass lipid concentration increased, there were corresponding increases in percent moisture and decreases in percent ash.

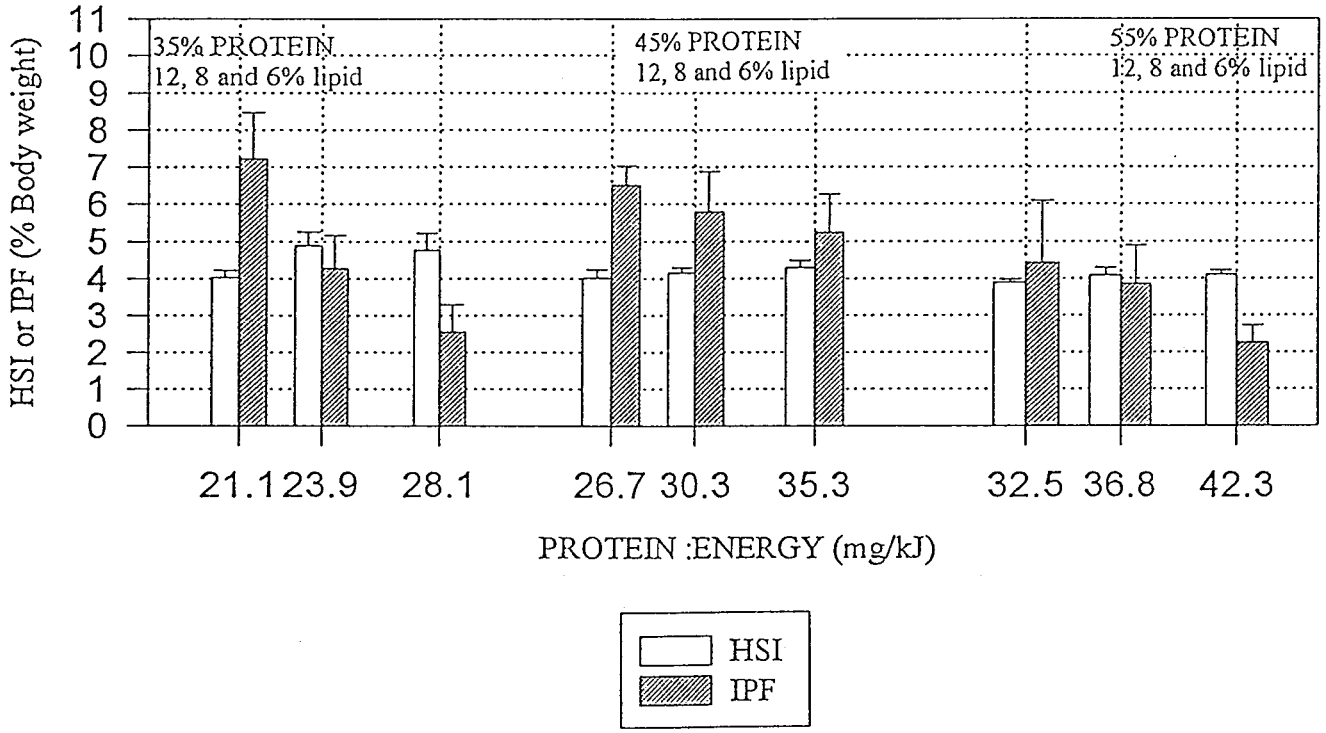

Figure 4 : Effect of varying P:E ratios at different protein levels at satiation feeding 3x daily on hepatosomatic index and intraperitoneal fat (vertical bars = 1/2 standard deviation).

Growth parameters

The effects of varying P:E ratios at the different protein levels on food conversion ratios (FCR) are shown in Figure 5. A decrease in FCR was observed with increasing P:E ratios until a FCR of 1.72 was observed in the 45% protein: 12% lipid diet whereafter FCR gradually started to increase. There was no significant difference ($p<0.05$) in the FCR's of the fish fed the 35% protein diets. Fish fed the 45% protein diet with P:E ratios of 26.7mg/kJ and 35.3mg/kJ, and fish fed the 55% protein diet with a P:E ratio of 42.3mg/kJ had significantly lower ($p<0.05$) FCR's than fish fed the other diets.

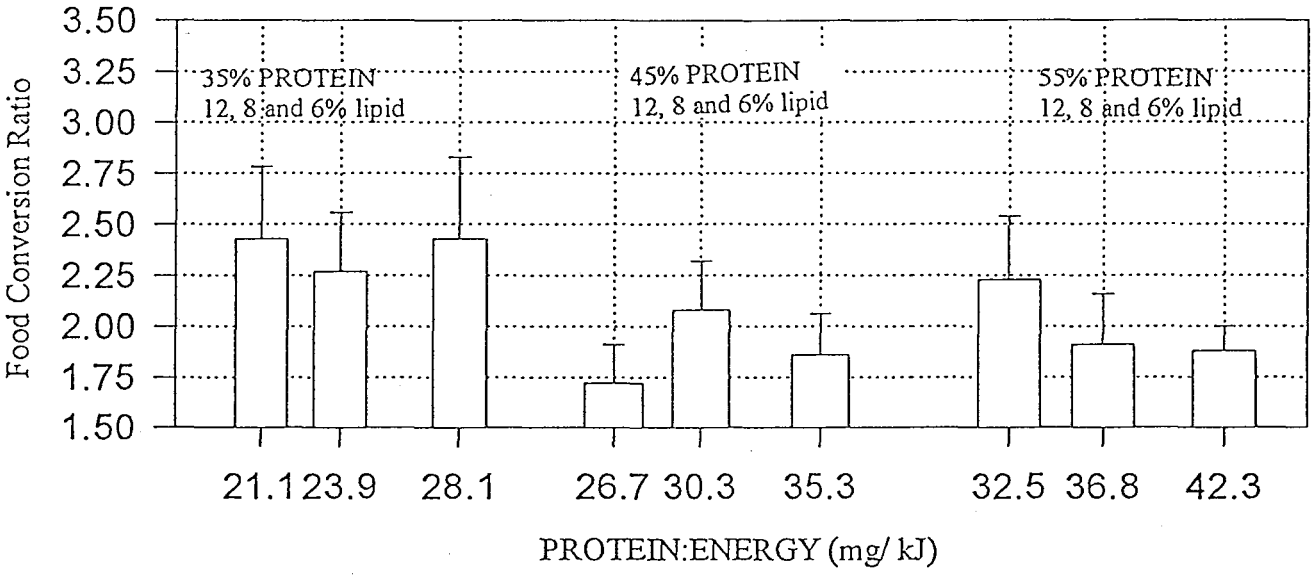

Figure 5 :Effect on food conversion ratio of varying P:E ratios at different protein levels at satiation feeding 3x daily (vertical bars = 1/2 standard deviation).

Specific growth rate is shown in Figure 6. The best SGR resulted from feeding a 45% protein diet with 12% lipid, and a P:E ratio of 26.7mg/kJ. Fish fed diets containing 35 and 45% protein showed increasing SGR with increasing lipid (decreasing P:E ratios) levels. The SGR at both these protein levels and 12% lipid were significantly higher ($p>0.05$) than at the lower lipid levels. There was no significant difference ($p>0.05$) between the SGR of fish fed the 55% protein diets at the three different lipid levels.

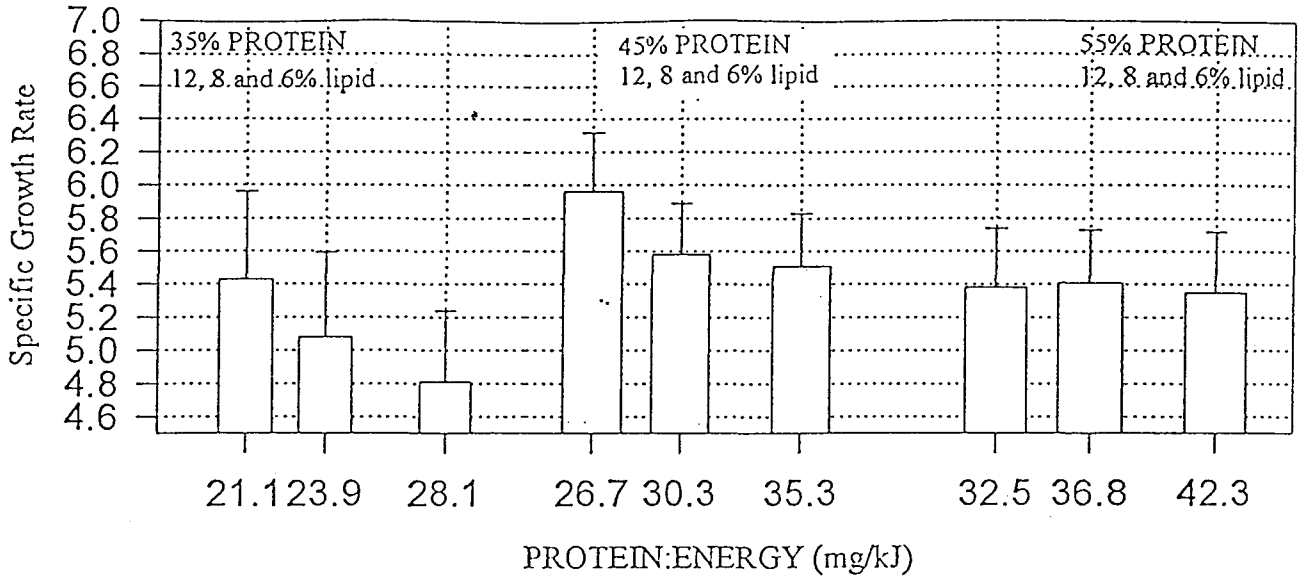

Figure 6 : Effect on specific growth rate of varying P:E ratios at different protein levels at satiation feeding 3x daily (vertical bars = 1/2 standard deviation).

The protein efficiency ratios are shown in Figure 7. There was an increase in PER corresponding to increases in lipid levels in fish fed the 35% protein diets. There was no significant difference in the PER of fish fed the 45% protein diets at the three different lipid levels. The same pattern was observed in fish fed the 55% protein diets, although a slight increase in PER was observed with increasing P:E ratios. This increase was not significant ($p>0.05$). The best PER were observed in fish fed the 35% protein diets.

Fish fed diets containing 12% lipid at each of these dietary protein levels had a significantly ($p>0.05$) higher SGR (Figure 6) than fish fed corresponding protein levels and 6% dietary lipid. The SGR at a P:E ratio of 21.1mg/kJ approached that recorded at 26.7mg/kJ (Figure 6), although this did not result in protein sparing.

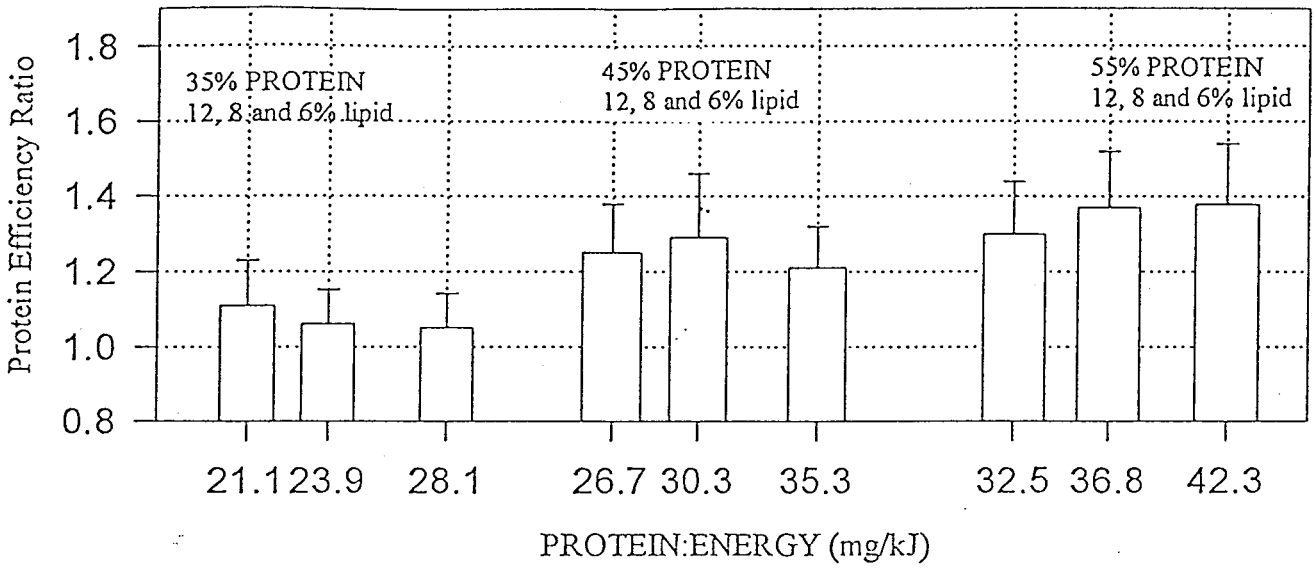

Figure 7 : Effect on protein efficiency ratios of varying P:E ratios at different protein levels at satiation feeding 3x daily (vertical bars = 1/2 standard deviation).

The diet containing 45% protein and 12% lipid (P:E = 26.7mg/kJ) resulted in the best SGR and FCR. There was also more protein and less fat deposited in fish fed this diet than in any of the 35% protein diets. The HSI of fish fed diets containing 12% lipid was significantly lower ($p<0.05$) than fish fed diets containing 6 or 8% lipid. Therefore, on the basis of growth performance and body composition, the optimal P:E ratio for this species was estimated to be 26.7mg/kJ.

DISCUSSION

The range of dietary lipids used in this study were based on research on juvenile red drum, *Sciaenops ocellatus* (lipid levels ranging from 6-10%; Ellis and Reigh, 1991), juvenile gilthead seabream, *Sparus aurata* (lipid levels ranging from 8-10% ; Ibeas *et al.*, 1994) and juvenile Asian seabass, *Lates calcarifer* where dietary lipid levels of 7% and 14% (Lochmann and Gatlin, 1993) and 5-15% were used (Catacutan and Coloso, 1995).

Fish oils are rich in highly unsaturated fatty acids (HUFA) (Lochmann and Gatlin, 1993) and polyunsaturated fatty acids (PUFA), particularly of the ώ3 series. Good growth and feed conversion have been associated with diets containing these lipid configurations (Watanabe, 1982; Steffens, 1989). Significantly higher FCR's have been recorded in juvenile gilthead seabream fed diets containing in excess of 6% cod liver oil than those fed diets containing less cod liver oil (Kalogeropoulos *et al.*, 1992). It was also found that elevated levels of cod liver oil in the diet reduced the HSI's and liver lipid deposition. Deshimaru *et al.* (1982) reported higher FCR's and growth in yellowtail, *Seriola quinqueradiata*, fed diets rich in HUFA's. Polyunsaturated fats are good energy sources resulting in sparing action on dietary protein (Watanabe, 1982). Marine fish have a higher demand for ώ3 fatty acids then freshwater fish (Steffens, 1989). Fish cannot synthesize fatty acids containing the ώ3 or ώ6 configuration. These must, therefore be supplied in the diet (Lee and Sinnhuber, 1989). A lack of linolenic acid (18:2ώ6) can cause stunted growth, a poor FCR and fin erosion. Soya bean oil is a good source of this fatty acid (Steffens, 1989) and was included in the present test diets in conjunction with fish oil.

A review of the literature revealed three trends for protein deposition associated with increasing levels dietary lipid. The first pattern involves an increase in protein deposition associated with increasing dietary lipid levels coupled with an increase in fat deposition. This increase in protein deposition was noted by Catacutan and Coloso (1995) in their work on juvenile Asian seabass. Cho and Bureau (1995) reported that as excess energy intake increases in salmonids, the total amount of protein deposited increases but the proportion of the energy retained as fat increases at an even faster rate, so that increased energy intake leads to increased fat deposition.

The second pattern involves a decrease in protein deposition with increasing dietary lipid. This pattern was observed in juvenile turbot (Bromley, 1980), juvenile red drum (Daniels and Robinson, 1986) and rainbow trout (Reinitez *et al.*, 1979). Results from this study indicate that juvenile spotted grunter follow this pattern of a decline in the amount of protein deposited associated with increased dietary lipid levels.

Finally, Parazo (1990) in his work on rabbitfish, found that body protein content remained constant and essentially independent of dietary energy levels but that feeding diets with high

energy content resulted in fatty fish. It would therefore appear that the relationship between dietary lipid levels and body composition, in particular protein deposition, is species specific and that dietary lipid levels have a greater effect on protein deposition then dietary protein levels.

The pattern of decreasing hepatosomatic indices with increasing dietary lipid or P:E ratios found in juvenile spotted grunter was also evident in hybrid striped bass (Nematipour *et al.*, 1992). In a study on juvenile dentex, *Dentex dentex* Tibaldi *et al.* (1996) found that different dietary treatments with elevated lipid levels (12% and 17.3%) did not affect the liver or viscerosomatic indices. In contrast, a number of authors (Daniels and Robinson, 1986; De Silva *et al.*, 1991 and Shimeno *et al.*, 1980) found that hepatosomatic indices increased as a function of increasing dietary lipid levels.

The higher intraperitoneal fat indices resulting from high dietary energy or lipid levels as exhibited by juvenile spotted grunter has also been reported for other species such as juvenile red drum (Daniels and Robinson, 1986), channel catfish fry (Reis *et al.*, 1989), juvenile turbot (Bromley, 1980) and carp fingerlings (Zeitler *et al.*, 1984).

The use of higher dietary lipid levels as a means of reducing the protein requirement has been well documented and protein sparing has been noted for a number of species such as turbot (Bromley, 1980), red tilapia (De Silva *et al.*, 1991), red drum (Ellis and Reigh, 1991), Atlantic salmon smolt (Hillestad and Johnsen, 1994) and juvenile dentex (Tibaldi *et al.*, 1996). Increasing the amount of dietary lipid in this study did not result in protein sparing. A number of reasons could be put forward to explain this. The lack of protein sparing could be as a result of not supplying a broad enough range of dietary lipid levels as the protein sparing capabilities of red tilapia were maximized at 18% dietary lipid (De Silva *et al.*, 1991), at 17.4% for dentex (Tibaldi *et al.*, 1996) and 28% for Atlantic salmon (Hillestad and Johnsen, 1994), although protein sparing was observed in turbot on a diet supplemented with 6% lipid (Bromley, 1980) and at 10% lipid in red drum (Ellis and Reigh, 1991). The juvenile spotted grunter in this study were fed to satiation three times daily, which has been shown to be optimal for growth (Deacon and Hecht, 1995b). However, Bromley (1980) found that protein sparing in turbot was marginal at satiation feeding rates.

The pattern of increasing specific growth rate to a maximum with increasing P:E ratios observed in this study, has been reported by other authors (Lee and Putnam, 1973; Zeitler *et al.*, 1984 and Lie *et al.*, 1988).

The optimal P:E ratio of 26.7mg/kJ resulting from a 45% protein : 12% lipid diet resulted in optimal growth in juvenile spotted grunter. This P:E ratio is lower than the P:E ratios of feeds used successfully for raising other marine fish (Table 4), although these levels may not necessarily be optimal (Tucker, 1992). The size of the fish fed these diets was larger (15-318g) than the size of the fish in this study (4.17-7.78g), and this may affect the requirement. Feeding juvenile spotted grunter high levels of lipid led to increased fat deposition and lower protein deposition.

Table 4 : Protein : energy ratios of feeds used successfully for raising marine fish (After Tucker, 1992).

SPECIES	WEIGHT (g)	P:E (mg/kJ)	REFERENCE
Sciaenops ocellatus	46-77	28.7	Daniels and Robinson, 1986
Seriola quinqueradiata	88-214	35.3	Shimeno *et al.*, 1985
Gadus morhua	173-318	33.2	Lie *et al.*, 1988
Epinephelus malabaricus	58-225	33.8	Teng *et al.*, 1978
Dicentrarchus labrax	37-57	35	Hidalgo and Alliot, 1988
Lates calcarifer	15-72	35.1	Tucker *et al.*, 1988

CHAPTER SIX

ESSENTIAL AMINO ACID REQUIREMENTS.

INTRODUCTION

It has been suggested that all fish, common with other vertebrates, require the same ten indispensable or essential amino acids (Ketola, 1982). These amino acids are : arginine (arg), histidine (his), isoleucine (ile), leucine (leu), lysine (lys), methionine (met), threonine (thr), phenylalanine (phe), tryptophan (trp) and valine (val), although cystine and tyrosine are sometimes considered to be semi-essential amino acids (Steffens, 1989). Cystine spares part of the methionine requirement in some fish, and although not proven, it is thought that tyrosine probably has a similar sparing effect on phenylalanine in fish diets (Ketola, 1982).

These essential amino acids (EAA's) are provided in the diet by the protein, but the nutritive value of the dietary protein is dependent on the extent to which the composition of its EAA's fulfil the requirement of the organism (Benitez, 1989). The quantitative requirement for each individual amino acid varies considerably between different species, as does the ratio between the amino acids (Wilson, 1985). There are a number of different methods that can be employed to quantify the EAA requirements. These include the use of test diets utilizing crystalline amino acids (Mertz, 1972), and the use of radioactive labelled amino acids (Walton, 1985). Test diets can take one of many forms. The protein source in the test diet can be made up of only crystalline amino acids with the amino acid being tested being supplied in varying concentrations. Another method involves using semi-purified and practical type diets. The semi-purified diet method involves the use of an unbalanced protein such as corn gluten as the major source of intact protein which is deficient in certain essential amino acids (Wilson, 1985). This type of diet requires supplementation with small amounts of a limited number of crystalline amino acids in the test diets. These diets are then formulated with the crystalline amino acids according to the requirements of the fish under study (Wilson, 1985). The practical type diet involves using normal feedstuffs to provide the majority of amino acids. These may be formulated to either make up a fixed amount of the desired protein level with the remaining amount being made up of crystalline amino acids or to be deficient in only the amino acid being studied (Wilson, 1985).

Quantitative EAA requirements have been calculated for a number of fish species, including various salmonids, channel catfish, Japanese eel, gilthead bream and common carp (Wilson, 1985). Quantitative determination of EAA requirements is very time consuming as the effect of each individual amino acid is assessed in a lengthy growth trial (Tacon and Cowey, 1985). Therefore, another approach has been developed. This qualitative method involves using ion-exchange chromatography and spectrophotometry to obtain an accurate analysis of amino acids in whole fish tissue (Benitez, 1989). It is now widely accepted that the amino acid profile of a fish provides a reliable indication of the balance of amino acid levels required in the diet of that species (Wilson, 1985; Benitez, 1989; Moon and Gatlin, 1991). This approach has been used in the development of artificial diets for prawns, *Peneaus japonicus* (Deshimaru and Shigeno, 1972) and has been shown to be successful. The overriding advantage of the qualitative approach is that all ten EAA's can be determined in a single analysis as the requirements of the other nine EAA are "pegged" to the empirically determined requirement of the most limiting amino acid..

In formulating fish feeds to meet the amino acid requirements, the total amino acid content of the feed ingredients must be corrected for availability to allow the optimum amounts of amino acid in the diet (Lovell, 1989). Most proteins do not have an ideal balance of amino acids. One amino acid is usually the most deficient relative to the animals' needs and the others are to some degree in excess and are degraded. The "ideal" protein is one that is completely balanced and which is therefore potentially fully utilized (Fuller, 1988).

The purpose of this study was to quantify the essential amino acid requirements of juvenile spotted grunter. This was achieved by obtaining the amino acid profile of the fish and identifying the most limiting EAA. The amino acid composition of juvenile spotted grunter showed lysine to be the first limiting amino acid. This information provided the foundation upon which an "ideal" protein (R.P. Wilson, Mississippi State University, pers. comm.) could be formulated. The test diets were formulated to contain graded levels of lysine.

MATERIALS AND METHODS

Using amino acid profiles of the two protein sources and the amino acid profile of juvenile spotted grunter, the theoretical essential amino acid ratio (Ogata *et al.*, 1983; Moon and Gatlin, 1991) was calculated (Table 1). This ratio, as defined by Ogata *et al.*(1983) is expressed as follows :

$$A/E \text{ ratio} = (EAA/\text{Total EAA})X\ 1000$$

where EAA = essential amino acid value in g/100g dry matter and total EAA includes tyrosine and cystine (combined with phenylalanine and methionine respectively). These ratios provide additional information concerning the amino acid requirements of the fish under study as they can be used to predict the requirements of the other essential amino acids (Moon and Gatlin, 1991).

Table 1 : Essential amino acid ratio of juvenile *Pomadasys commersonnii* , low temperature Danish fishmeal and vitamin-free casein.

AMINO ACID	*P.commersonnii*	FISHMEAL	VITAMIN- FREE CASEIN
Arginine	149	127*	83*
Histidine	47	55	54
Isoleucine	94	102	137
Leucine	165	165	211
Lysine	187	174*	171*
Phe + Tyr	84	90	115
Cys + Met	76	69*	65*
Threonine	100	95*	93*
Valine	109	123	160

* denotes limiting amino acids relative to the EAA ratio of juvenile spotted grunter

Therefore, using a combination of low temperature Danish fishmeal and casein as the protein sources, a diet with a 45% protein content was developed. The combination of these two protein sources resulted in a diet with an A/E ratio which, when compared to that of the juvenile spotted grunter (Table 2), would appear to be primarily limiting in arginine and lysine, with threonine and

methionine limiting to a lesser extent. Using the A/E ratio values, six semipurified test diets were supplemented with graded levels of crystalline lysine (Table 3), with diet 4 being the "ideal protein" diet and diet 1 being the control diet with no supplemental lysine.

Table 2 : Essential amino acid ratio of juvenile *Pomadasys commersonnii* and the combined resultant crude protein source.

AMINO ACID	*P.commersonnii*	CRUDE DIETARY PROTEIN SOURCE
Arginine	149	123*
Histidine	47	55
Isoleucine	94	110
Leucine	165	176
Lysine	187	173*
Phe + Tyr	84	96
Cys + Met	76	68*
Threonine	100	95*
Valine	109	132

* denotes limiting amino acids relative to the EAA ratio of juvenile spotted grunter

The crystalline L-lysine was mixed with the oil prior to mixing it with the dry ingredients so as to retard digestion and leaching as much as possible. This allowed digestion of the crystalline amino acid to be delayed until the amino acids present in the intact protein source had been liberated by the digestion process.

The level of lysine in the diet which resulted in the best growth and food conversion was then assumed to be "ideal". The requirements of the other 9 EAA were then calculated using Moon and Gatlin's (1991) method based on the A/E ratios. Once the optimal level of lysine had been empirically determined, the EAA requirements of the other nine essential amino acids were calculated according to the formula of Moon and Gatlin (1991):

$$Req_x = (\%\ \text{lysine} \times A/E_x)/(A/E_{lys})$$

where x is the unknown amino acid.

Table 3 : Test diet formulation and composition.

	DIET (g/100g)					
INGREDIENT	1 Control	2	3	4 "Ideal"	5	6
Fishmeal	44	44	44	44	44	44
Casein	14	14	14	14	14	14
Pregelatinized Starch	27	27	27	27	27	27
Fish Oil	4.5	4.5	4.5	4.5	4.5	4.5
Soya Oil	2.2	2.2	2.2	2.2	2.2	2.2
Mineral Mix[1]	4	4	4	4	4	4
Vitamin Mix[2]	2	2	2	2	2	2
Crystalline L-Lysine	0	0.05	0.1	0.2	0.3	0.35
Cellulose	2.3	2.25	2.2	2.1	2.0	1.95
Proximate Composition						
Crude Protein (%)	44.2	44.8	45.0	45.3	44.3	44.5
Ash (%)	14.52 ±0.24	14.28 ±0.35	14.15±0 .19	13.96 ±0.73	14.2 ±0.54	14.1 ±0.76
Gross E (MJ/kg)	19.19 ±0.31	19.14 ±0.62	19.14 ±0.37	18.95 ±0.27	19.05 ±0.32	19.35 ±0.12

[1] Mineral mix (g/kg) : 74g potassium; 516g vermiculite RSU; 14g salt; 0.05g ammonium chloride; 31g choline chloride; 0.31g cobalt; 0.15g copper; 1.5g iron; 0.05g iodine; 0.22g manganese; 41g magnesium; 1g zinc and traces of selenium.

[2] Vitamin mix (IU or g/kg) : 500 000 IU vitamin A; 400 000 IU vitamin D3; 10 000 IU vitamin E; 1g vitamin K3; 0.25g vitamin B1; 1.5g vitamin B2; 0.5g vitamin B6; 25g vitamin C; 2.5g Niacin; 0.09g Folic acid; 0.025g Biotin; 2.5g Calpan; 2.5g Inositol.

RESULTS

The amount of food and protein consumed per gramme of fish is shown in Figure 1. There was no significant difference ($p<0.05$) in consumption between any of the diets.

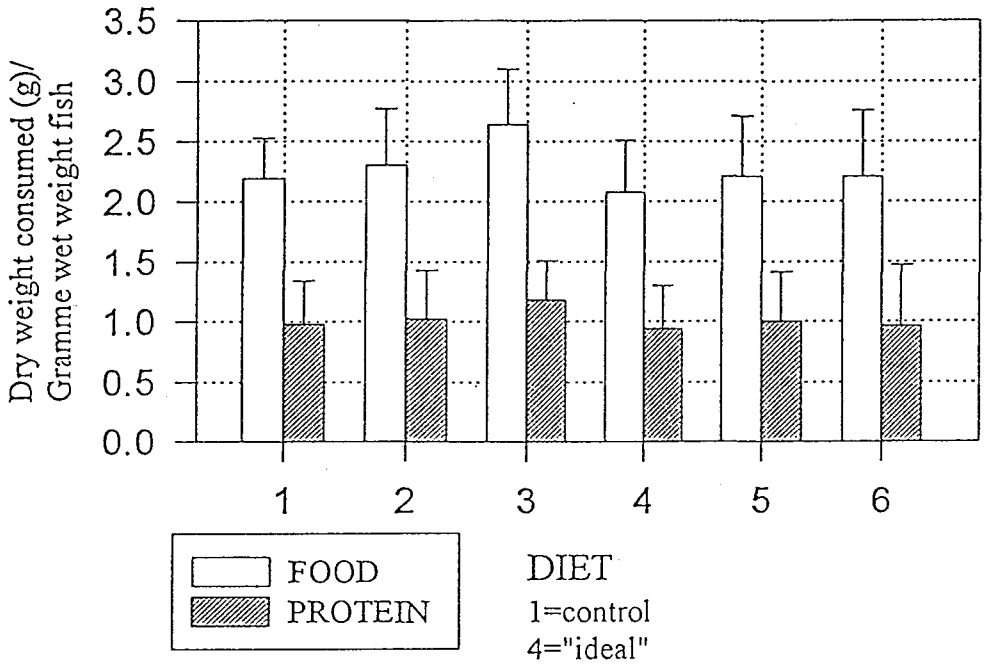

Figure 1 : Food and protein consumption at satiation feeding 3x daily of diets containing graded levels of lysine (vertical bars = 1/2 standard deviation).

Proximate composition

The proximate composition of the fish before and after the feeding trial are shown in Table 4. The percentage moisture (Figure 2) of wild caught juveniles was significantly less ($p<0.05$) than the percentage moisture of fish fed the supplemented diets (diets 2-6). There was no significant difference ($p<0.05$) in moisture between the wild caught fish and those fed the control (unsupplemented) diet. Fish fed the "ideal protein" diet (diet 4) had significantly less ($p<0.05$) moisture than fish fed the other supplemented diets (diets 2, 3, 5 and 6). Fish fed diets with limiting and "ideal" lysine levels (diets 2, 3 and 4) had significantly less ($p<0.05$) ash (Figure 3) than wild caught juveniles, fish fed the control diet and diets with excessive lysine (diets 5 and 6).

Table 4 : Body composition of fish prior to (wild) and after (D1-D6) completion of the essential amino acid supplementation feeding trial.

FISH	% MOISTURE	% ASH	% PROTEIN	HSI	IPF	% LIPID
Wild	70.12^{a} ±1.08	24.94^{a} ±1.12	63.24^{a} ±1.05	4.71^{a} ±0.62	N/A	4.70^{c} ±0.87
D1- 0g[1] Control	71.59^{a} ±1.05	19.06^{bc} ±1.11	51.73^{d} ±1.02	4.66^{a} ±0.28	3.33^{c} ±0.86	5.46^{a} ±0.62
D2 - 0.05g	75.71^{c} ±1.06	18.55^{c} ±1.15	56.66^{c} ±1.13	4.26^{c} ±0.26	3.17^{b} ±0.21	4.91^{b} ±0.59
D3 - 0.1g	75.67^{c} ±1.11	18.39^{c} ±1.20	57.62^{c} ±1.21	4.01^{d} ±0.53	3.08^{a} ±0.12	4.71^{c} ±0.68
D4 - 0.2g Ideal	73.72^{b} ±1.09	18.86^{c} ±1.14	59.72^{b} ±1.11	4.19^{c} ±0.61	3.45^{c} ±0.29	4.45^{d} ±0.59
D5 - 0.3g	75.21^{c} ±1.21	20.17^{b} ±1.19	56.13^{c} ±1.16	4.23^{c} ±0.22	3.04^{a} ±0.36	3.11^{c} ±0.73
D6 - 0.35g	76.05^{c} ±1.18	19.69^{b} ±1.21	57.26^{c} ±1.19	4.42^{b} ±0.51	3.19^{b} ±0.67	3.38^{c} ±0.81

[1] g crystalline lysine per 100g dry diet
[abcde] Means of three replicate groups ± standard deviation with the same superscripts are not significantly different ($p<0.05$)

Wild caught fish had a significantly higher ($p<0.05$) protein content than fish fed the formulated diets (Figure 3). There was a gradual increase in gross protein content with increasing levels of lysine supplementation until a peak, corresponding with the "ideal protein" diet, after which gross protein content decreased. There was a decrease in the lipid content (Figure 3) with increasing levels of lysine supplementation. The HSI and IPF are shown in Figure 4. Wild caught fish and fish fed the control diet had significantly higher ($p<0.05$) HSI's than fish fed the supplemented diets. Fish fed the diet with the highest level of lysine supplementation (diet 6 - 0.35g/100g dry diet) also had relatively high HSI's. The HSI recorded in diet 3 was significantly lower ($p<0.05$) than the HSI recorded in the other diets. Fish fed the "ideal protein" diet had significantly higher ($p<0.05$) levels of protein and fat deposition than fish fed any other diets. Therefore, in terms of proximate composition, the "ideal" diet with a lysine level of 4.30% of the dietary protein was optimal.

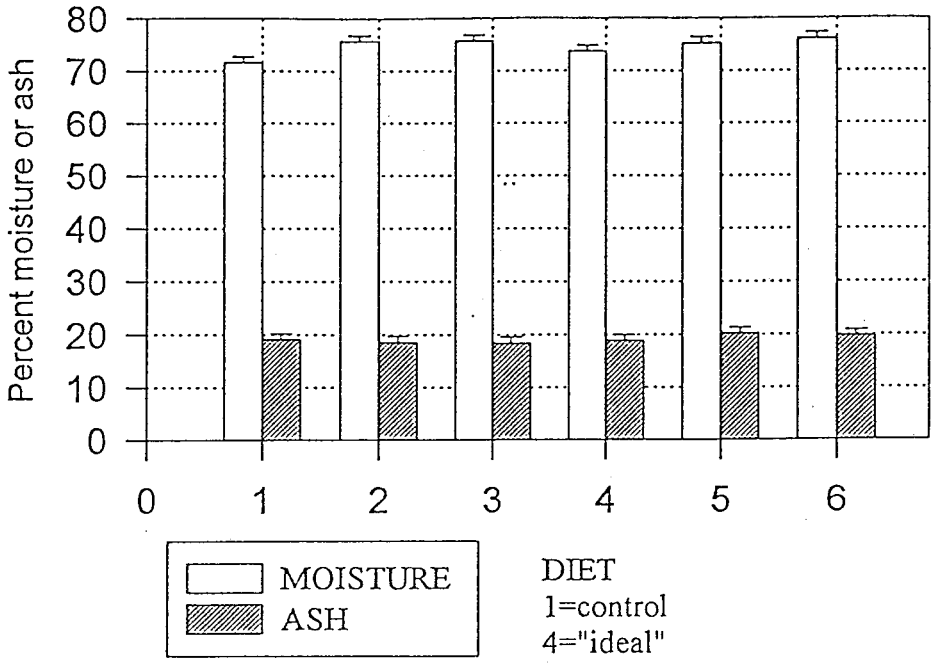

Figure 2 : Effect of feeding graded levels of lysine on the ash and moisture content of the fish (vertical bars = 1/2 standard deviation).

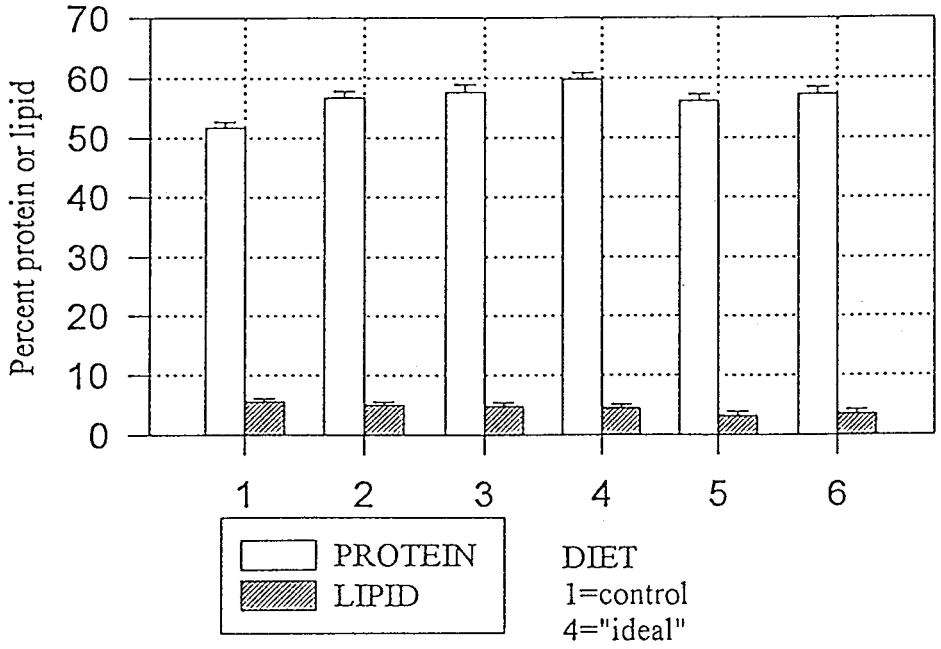

Figure 3 : Effect of feeding graded levels of lysine on the protein and lipid content of the fish (vertical bars = 1/2 standard deviation).

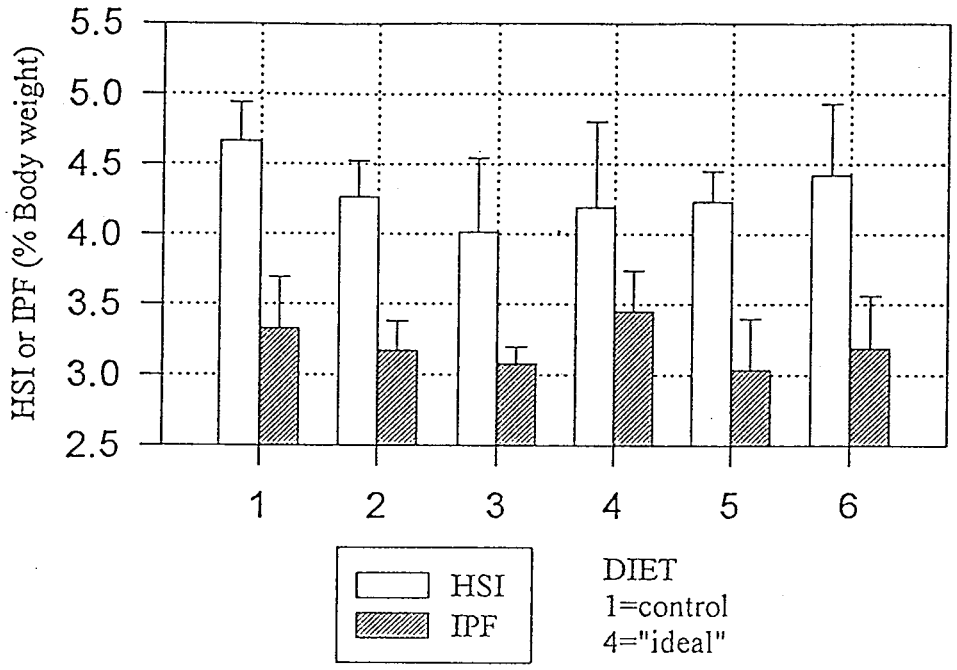

Figure 4 : Effect of feeding graded levels of lysine on HSI and IPF of the fish on completion of the growth trial (vertical bars = 1/2 standard deviation).

Amino acid profiles

The requirements of the remaining 9 EAA's were calculated according to Moon and Gatlin (1991) and are outlined in Table 5.

Table 5 : Calculated requirements of 8 essential amino acids based on the lysine requirement (4.30% dietary protein) after Moon and Gatlin, 1991.

AMINO ACID	DIETARY REQUIREMENT (% dietary protein)
Arginine	3.44
Histidine	1.08
Isoleucine	2.11
Leucine	3.78
Phenylaline	2.19
Methionine	1.68
Threonine	2.31
Valine	2.49

Supplementing diets with crystalline amino acids had a definite effect on the amino acid profile of the fish (Table 6). It is clear from this table that juvenile spotted grunter are able to assimilate crystalline forms of amino acids. This can be seen with the increasing lysine level resulting from feeding with increased levels of lysine. It would also appear that feeding with graded levels of crystalline lysine affected the assimilation of the other amino acids as the profiles of fish fed different levels of lysine show differences in the levels of the other amino acids.

Growth responses

The growth responses are outlined in Table 7. The "ideal protein" diet resulted in a significantly lower ($p<0.05$) FCR than those achieved from feeding any of the other diets (Figure 5).

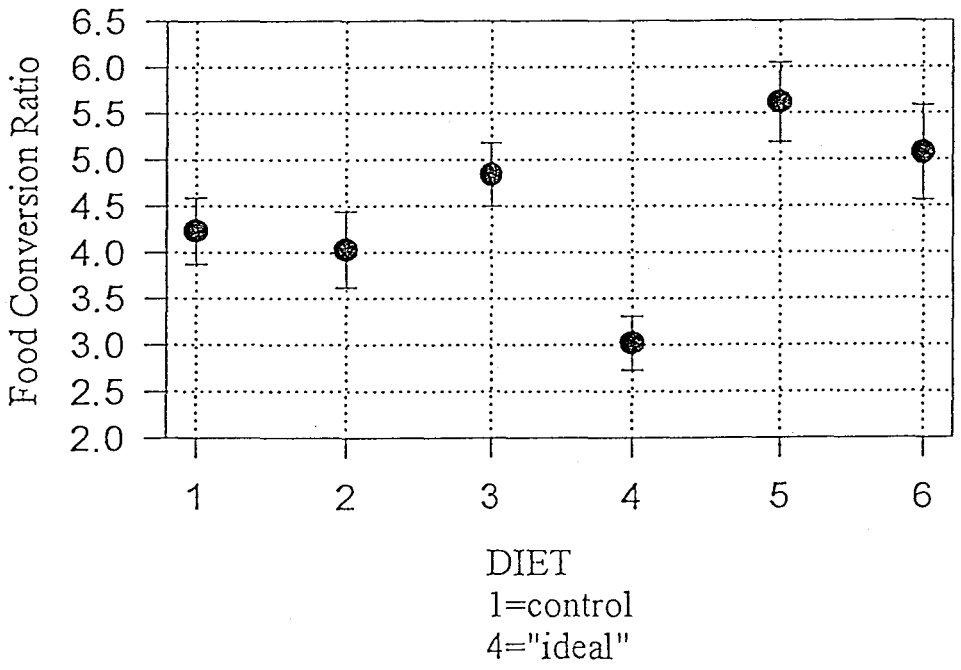

Figure 5 : Effect of feeding graded levels of lysine on food conversion ratios (vertical bars = 1 standard deviation).

Table 6 : Comparison of amino acid profiles of whole fish tissue of wild caught juveniles and fish on completion of the feeding trial.

AMINO ACID	WILD FISH	FISH 1[a] Control	FISH 2	FISH 3	FISH 4 Ideal	FISH 5	FISH 6
Threonine	2.37	2.54	2.49	2.38	2.35	2.47	2.49
Valine	2.56	2.83	2.73	2.68	2.66	2.68	2.72
Methionine	1.72	1.70	1.59	1.57	1.62	1.64	1.68
Isoleucine	2.20	2.37	2.32	2.26	2.33	2.26	2.25
Leucine	3.89	4.15	4.15	3.94	3.94	4.07	4.06
Tyrosine	1.72	1.65	1.65	1.63	1.68	1.66	1.74
Phenylaline	2.23	2.32	2.33	2.25	2.22	2.28	2.31
Histidine	1.11	1.17	1.15	1.09	1.13	1.15	1.15
Lysine	4.41	4.23	4.13	4.02	4.30	4.33	4.37
Arginine	3.53	3.39	3.28	3.25	3.32	3.37	3.44

[a] numbers represent the number of the diet fed to the fish

Table 7 : Performance of juvenile spotted grunter fed diets with graded levels of lysine for 60 days.

	DIET NUMBER					
PARAMETER	1- 0g[2] Control	2- 0.05g	3-0.1g	4-0.2g Ideal	5-0.3g	6-0.35g
Initial weight	6.11 ±0.23	5.19 ±0.36	4.73 ±0.38	5.18 ±0.32	5.54 ±0.26	4.89 ±0.65
Final weight	8.68 ±0.31	7.16 ±0.26	6.49 ±0.33	8.32 ±0.24	6.9 ±0.27	6.21 ±0.30
Initial CF	0.71 ±0.16	0.68 ±0.21	0.69 ±0.23	0.70 ±0.12	0.68 ±0.22	0.67 ±0.25
Final CF	0.79 ±0.11	0.79 ±0.09	0.79 ±0.09	0.79 ±0.10	0.77 ±0.08	0.74 ±0.11
Feed consumed[1]	19.03 ±0.56	16.47 ±0.62	17.11 ±0.58	17.32 ±0.69	15.23 ±0.72	13.70 ±1.02
Protein consumed[1]	8.47 ±0.41	7.30 ±0.48	7.67 ±0.43	7.80 ±0.52	6.90 ±0.54	5.99 ±0.75
FCR	4.23^{b} ±0.36	4.03^{b} ±0.41	4.84^{b} ±0.34	3.02^{c} ±0.29	5.62^{a} ±0.43	5.07^{a} ±0.51
SGR	2.55^{b} ±0.19	2.29^{b} ±0.19	1.89^{c} ±0.26	2.95^{a} ±0.18	1.88^{c} ±0.20	2.38^{b} ±0.22
PER	0.33^{b} ±0.06	0.24^{c} ±0.07	0.22^{c} ±0.09	0.41^{a} ±0.05	0.21^{c} ±0.08	0.22^{c} ±0.09

[1] consumption per fish g dry weight
[2] g crystalline lysine per 100g dry diet
[abc] Means of three replicates ± standard deviation with the same superscripts are not significantly different ($p<0.05$)

Fish fed the control diet and those diets with limiting levels of lysine (diets 2 and 3) showed no difference in their respective FCR's. The fish fed diets with excessive amounts of lysine (diets 5 and 6) had significantly higher ($p<0.05$) FCR's than any of the other diets. The low FCR exhibited by fish fed the "ideal protein" diet was enhanced by a corresponding significantly higher ($p<0.05$) SGR (Figure 6) than was achieved by any of the other diets. There was little variation in the PER's (Figure 7) of the fish fed the supplemented diets with the exception of the fish fed the "ideal protein" diet

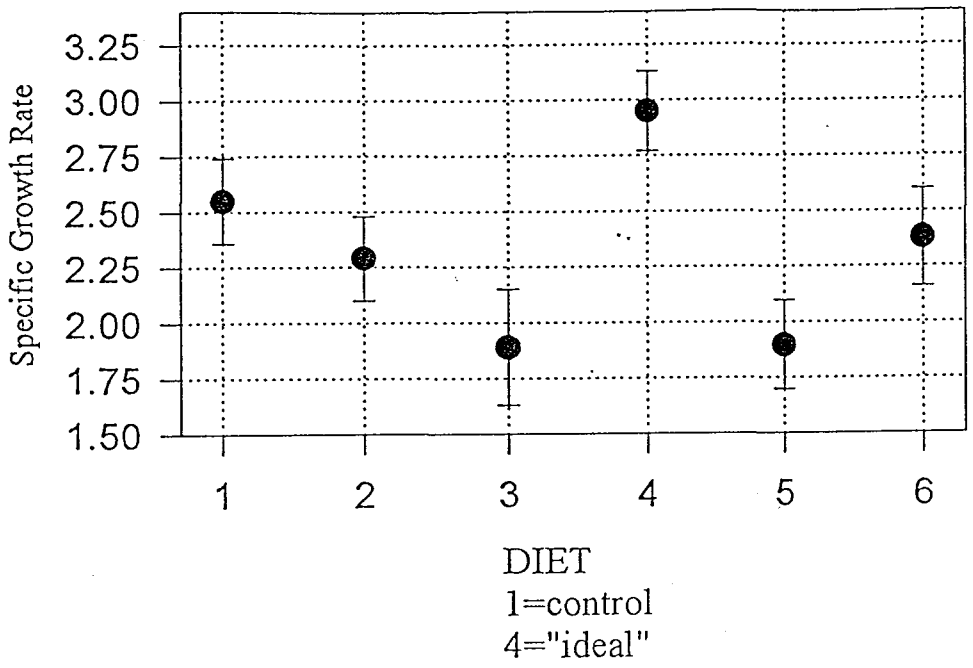

Figure 6 : Effect of feeding graded levels of lysine on specific growth rate (vertical bars = 1 standard deviation).

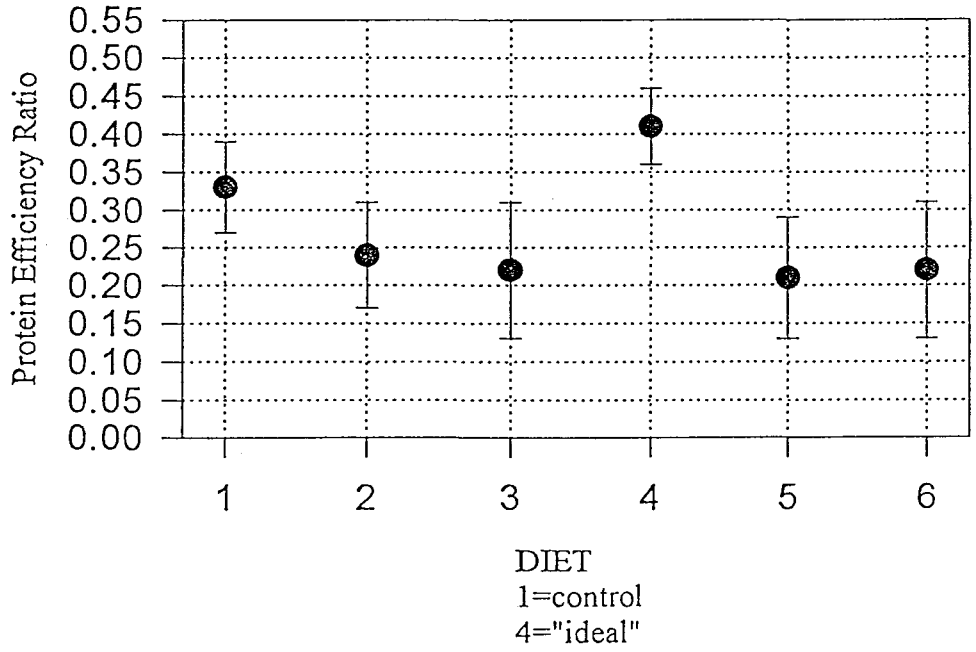

Figure 7 : Effect of feeding graded levels of lysine on protein efficiency ratios (vertical bars = 1 standard deviation).

which exhibited significantly higher ($p<0.05$) PER's than any of the other fish. The PER of the fish fed the control diet was also significantly higher ($p<0.05$) than that resulting from feeding the supplemented diets although it was not as high as the "ideal" diet.

DISCUSSION

The nutritive value of a dietary protein is dependent on the extent to which the composition of its essential amino acids fulfil the requirements of the organism (Benitez, 1989). There are various ways to establish the EAA requirements of any particular species. The most frequently used method is to base the EAA requirements on the profiles of either the whole body tissue, muscle tissue or the eggs of the species under study (Moon and Gatlin, 1991).

Ogata *et al.* (1983) found that cherry salmon, *Oncorhynchus masou* and amago salmon, *O. rhoduru* fed diets with crystalline amino acids to simulate the essential amino acid (A/E) ratio of the whole body had significantly higher growth rates, feed efficiencies and protein efficiencies than those fed diets with no crystalline amino acid supplementation. Ketola (1982) found that rainbow trout, *O. mykiss* fed on diets based on the amino acid composition of trout eggs, showed improved growth, fin condition and survival. Moon and Gatlin (1991) used the A/E ratio of muscle tissue as the basis of their test diets to predict the requirements of the other indispensable amino acids and found that this method resulted in strong correlations when compared with quantitative requirements of amino acids. Similar correlations have been observed with coho salmon, *O. kisutch* (Arai, 1981), cherry salmon (Ogata *et al.*, 1983) and channel catfish, *Ictalurus punctatus* (Wilson and Poe, 1985). The justification for using this method in this study is therefore valid.

Cowey (1992) found that diets containing large amounts of free AA gave rise to lower growth rates than those obtained when all the protein in the diet was supplied as high quality intact protein. This is substantiated by Jackson and Capper (1982) who found that while supplementation with crystalline AA could be beneficial, growth on a complete free AA diet was considerably less than growth on a casein control diet and that growth rates deteriorate with increasing levels of free AA. This is indicative of an inhibitory effect of excess AA. Cowey (1995) found a similar trend and suggested

that the depressed growth rates may be due to the crystalline AA being assimilated more rapidly then the AA present in the diet as a component of the whole protein. Cho *et al.* (1992) overcame this problem by blending the AA supplement with agar before mixing with other ingredients in order to retard absorption of the crystalline AA while digestion of the intact dietary protein occurred. Similarly, in this study, the crystalline lysine was mixed with the oil prior to mixing with the other ingredients.

The pattern of improved growth with increasing levels of supplemented lysine up to a point (1.02% of the total dry diet) and a subsequent decrease in SGR with further additions of lysine observed with juvenile spotted grunter has also been noted for a number of other aquaculture species. Channel catfish fingerlings exhibited a depression of growth at lysine levels higher then 1.5% of the dry diet (Robinson *et al.*, 1980). This may be due to a lysine/arginine antagonism, as it has been widely documented that excessive dietary lysine increases the arginine requirement (Tibaldi *et al.*, 1994). The adverse effect of disproportionate amounts of lysine has been explained through a reduction in food intake, poor utilization of amino acids through competition by the amino acids for absorptive sites in the intestine and renal tubes, and by an increase in amino acid degradation through interference of their natural metabolism (Harper *et al.*, 1970). The reason for the decline in SGR just prior to and just following the "ideal" lysine level remains unclear, but it is possibly as a result of antagonism between the amino acids.

Another response to lysine supplementation is an increase in growth up to a point with little variation after that. This is the case with red drum, *Sciaenops ocellatus* which, when fed diets containing graded levels of lysine, ranging from 1.2% to 2.4% of the dry diet, showed significantly lower weight gains and feed efficiencies at lysine levels of 1.2% when compared with fish fed higher lysine levels. There was no difference between growth or feed efficiencies of fish fed 1.6,2.0 and 2.4% dietary lysine (Brown *et al.*, 1988). The lysine requirement of red drum is therefore considered to be not greater then 1.6% of the dry diet. This was also evident in fingerling seabass, *Dicentrarchus labrax*, where fish fed moderate excesses of lysine (3.25% dietary protein) did not exhibit impaired growth rates, protein retention or HSI (Tibaldi *et al.*, 1994). Keembiyehetty and Gatlin (1992) found a

similar trend with juvenile hybrid striped bass (*Morone chrysops* x *M. saxatilis*) where increasing levels of dietary lysine above 1.44% did not have an influence on weight gain. These authors also found overt signs of lysine deficiency such as suppressed growth and feed efficiency in fish fed diets containing 1.14% lysine. As with spotted grunter, these fish did not show any evidence of pathological symptoms such as fin erosion reported by Ketola (1979, 1982) for fingerling rainbow trout.

There are conflicting reports with regard to tilapia. Jackson and Capper (1982) found that *Oreochromis mossambicus* were able to utilize free AA for growth and like spotted grunter and channel catfish exhibited slightly depressed growth rates after a 1.62% lysine level was reached. On the contrary, Viola *et al.* (1994a) found that increasing lysine levels in diets for hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) did not yield higher weight gains or significant changes in carcass composition and in fact caused a decrease in protein retention. In a similar study Viola *et al.* (1994b) suggested that hybrid tilapia appeared to be indifferent to lysine supplementation.

It would seem therefore, that the required levels of lysine are highly species specific, and that the responses of fish which can utilize free AA are very variable. The lysine requirement of juvenile spotted grunter expressed as dry weight of the diet appears to be quite low but when the requirements of lysine are expressed as a percentage of dietary protein, the estimated value for spotted grunter (4.30%) falls within the same range as those for other fish, namely 4.1-5.3% of the dietary protein (Tibaldi and Lanari, 1991). The unexpected patterns in the FCR and SGR resulting from feeding with graded levels of crystalline lysine remain unclear. Optimal lysine levels for juvenile spotted grunter are 1.02% dry diet or 4.30% of the dietary protein.

CHAPTER SEVEN

IN VIVO ASSESSMENT OF THE APPARENT CRUDE PROTEIN DIGESTIBILITY OF ALTERNATIVE PROTEIN SOURCES.

INTRODUCTION

At present fishmeal is the single most important protein source in the aquaculture industry. High quality fishmeal is well recognized as the best protein source (Pike *et al.*, 1990). It is also very expensive. Not only is the cost of fishmeal a problem, but future supplies are not expected to meet future requirements (Barlow, 1989). This will lead to further increases in the price. Primary targets for cost reduction in formulated diets are the source and level of protein in the diet (Davis *et al.*, 1995). If intensive fish farming systems are to be sustainable in the long term, it is essential that alternative and more sustainable protein sources be found and their potential as fishmeal substitutes be assessed (Dong *et al.*, 1993). Partial or total replacement of fishmeal as the primary protein source could be of considerable economic advantage, even if a moderate reduction in feed efficiency occurs (Hajen *et al.*, 1993). The supply of fishmeal is stagnating while the aquaculture industry is growing and until suitable alternative protein sources are identified, the cost of raising fish can be expected to increase significantly (Rumsey, 1993). During the past 20 years, world fishmeal supplies have increased by only 27% and world fishmeal production is projected to decline by 5% between 1990 and 2000 (Rumsey, 1993).

While a wide variety of different fishmeal substitutes have been evaluated for carnivorous fish species, only a few show real potential. These include single-cell proteins such as yeast; plant oilseed cake and legumes such as soyabean and sunflower seed; terrestrial animal by-product meals such as carcass meal, bone meal and poultry by-product meal and finally miscellaneous plant protein sources such as corn gluten (Tacon, 1994).

Long term feeding trials are the most accurate means of assessing the nutritive value of fishmeal substitutes. These are slow and often expensive trials. A more rapid method of assessing the nutritive

value of an ingredient is to measure its digestibility. Digestibility is important as an indicator of potentially available energy and nutrients for growth, maintenance and reproduction of the animal and has become a major focus in aquaculture nutrition for assessing ingredient or diet quality (Sadiku and Jauncey, 1995).

There are two ways in which digestibility can be determined. The first method is directly, which involves measuring all the nutrient consumed and excreted. Quantitative recovery of the fed nutrients voided in the faeces is difficult due to their aqueous environment which leads to leaching of the nutrients. This problem is further exacerbated in the case of spotted grunter whose feeding behaviour often involves ingestion, regurgitation and re-ingestion. They are also very active feeders which causes further break up of the feed and faecal material.

The second method, and the method used in this study, is the indirect method. This involves the measurement of the ratio's of nutrient to some indigestible component (indicator) in the feed and in the faeces. The indicator must be indigestible, unaltered chemically, non-toxic to the fish, able to pass through the gut and be conveniently analysed (Lovell, 1989). The advantage of this method is that it circumvents the need to collect all the faeces produced from a meal (Wee *et al.*, 1992). The method used to collect faeces in digestibility trials has been shown to have an effect on the co-efficients obtained (Sullivan and Reigh, 1995). Methods which involve faecal collection from the water typically produce higher digestibility co-efficients than methods in which the faeces are collected from the fish itself (Henken *et al.*, 1985).

Environmental conditions in the production system, feeding practises and diet manufacturing techniques can affect digestibility under practical conditions. Digestibility co-efficients are also known to increase as a function of body size. This may be due to an increase in the length of the intestine and the time required for digestion to be completed (Ferraris *et al.*, 1986). Therefore, digestibility co-efficients are not constants (McGoogan and Reigh, 1996) and should be used as guidelines rather then as absolutes.

As already mentioned (Chapter 6), the essential amino acid balance of the feed plays a vital role in the efficient utilization of any dietary protein source. Therefore, when considering the incorporation of an alternative protein source into an artificial diet, the amino acid balance of that source must also be considered alongside digestibility. Apart from fishmeal, there are no animal or plant proteins available to feed producers with an amino acid profile approximating the dietary EAA requirements of farmed fish. Consequently it is not surprising that attempts to totally replace the fishmeal component of practical fish feeds with alternative protein sources has not been successful. When compared with fishmeal, the majority of alternative protein sources are deficient in a specific amino acid or suffer from an imbalance of amino acids. Despite these inherent amino acid imbalances, some researchers have had success in utilizing non-conventional protein sources in conjunction with fishmeal (Tacon and Jackson, 1985).

The aim of this part of the study was to assess the apparent digestibilities of various alternative protein sources as well as the more conventional sources for artificial feeds for juvenile spotted grunter.

MATERIALS AND METHODS

Six test diets were formulated with six different protein sources to contain approximately 40% protein (Table 1). The method used was based on the method used by Ferraris *et al.* (1986) in their study with milkfish, *Chanos chanos*. Prior to the diet formulation, samples of the potential fishmeal substitutes were obtained from the manufacturers and tested for crude protein content using the micro-Kjeldhal method. The formulations were based on these values. The variation in the protein content may result from differences in the protein content of the different batches of supplied protein source. The results may be somewhat misleading, but nevertheless provide some guidelines for substitution.

The test diets were made up with the same dietary components as used in previous diets, with the exception that chromic oxide was included at 1% as the internal marker. The concentrations of these ingredients was the same as used previously, with the concentration of the filler, cellulose, varying.

This variation in the concentration of cellulose was as a result of different quantities of the protein source being required to result in approximately 40% of the diet.

Table 1: Composition of the six test diets.

	DIET (g/100g)					
INGREDIENT	BLOOD MEAL	CARCASS MEAL	DANISH FISH-MEAL	CHILEAN FISH-MEAL	*SPIRULINA*	DEFATTED SOYAMEAL
PROTEIN SOURCE	34	56	42	49	59	66
PGS[1]	15	15	15	15	15	15
FISH OIL	5.2	5.2	5.2	5.2	5.2	5.2
SOYA OIL	2.6	2.6	2.6	2.6	2.6	2.6
MINERAL MIX[2]	4	4	4	4	4	4
VITAMIN MIX[3]	2	2	2	2	2	2
CELLULOSE	36.2	14.2	28.2	21.2	11.2	4.2
Cr_2O_3	1	1	1	1	1	1
PROTEIN CONTENT (%)	34.64	36.04	37.75	38.99	38.69	35.43

[1] Pregelatinized starch

[2] Mineral mix (g/kg) : 74g potassium; 516g vermiculite RSU; 14g salt; 0.05g ammonium chloride; 31g choline chloride; 0.31g cobalt; 0.15g copper; 1.5g iron; 0.05g iodine; 0.22g manganese; 41g magnesium; 1g zinc and traces of selenium.

[3] Vitamin mix (IU or g/kg) : 500 000 IU vitamin A; 400 000 IU vitamin D3; 10 000 IU vitamin E; 1g vitamin K3; 0.25g vitamin B1; 1.5g vitamin B2; 0.5g vitamin B6; 25g vitamin C; 2.5g Niacin; 0.09g Folic acid; 0.025g Biotin; 2.5g Calpan; 2.5g Inositol.

All diets were fed in triplicate to 24 fish for a period of four days prior to stripping to ensure the absence of any other food remnants in the faeces. Prior to first feeding, the tanks containing the fish were completely scrubbed down to remove any algal growth and residual feed and faecal material.

The tanks were cleaned daily thereafter. The fish were fed daily at 08H30 and again at 12H00 and manually stripped of faecal material at 15H30, after anaesthetizing them in a solution of 0,2ml 2-phenoxyethanol.l^{-1}. Manual stripping, if possible, has been found to produce the most reliable results (Austreng, 1978; Hajen *et al.*, 1993). After approximately 1 minute in the anaesthetic, the fish were removed, blotted dry on the underside and stripped of faeces by applying gentle pressure to the abdomen between the anal fin and the anus. Faeces were collected into sterile glass vials and dried in a convection oven at 70°C. Before any samples were placed in the drying oven, a 10% solution of formalin in a glass petri dish was left to evaporate over night to prevent fungal and bacterial contamination (Rouhani, 1993).

Protein content of the food and the faeces was determined using the micro-Kjeldhal method outlined earlier (Chapter 2). Following standard micro-Kjeldhal digestion, chromic oxide concentration was determined using a GBC-909 Atomic Absorption spectrophotometer. A calibration curve was constructed using a standard chromium solution. Absorption was measured at 357.9μm using nitrous oxide acetylene (R. Van Hille, Department of Biochemistry, Rhodes University, Grahamstown, pers. comm.). Atomic absorption spectrophotometry has been shown to be the most efficient method to determine chromic oxide concentration (Lied *et al.*, 1982).

Apparent digestibility (AD) was calculated using the Maynard and Loosli (1956) equation :

$$AD\ (\%) = \frac{\dfrac{(\%\ \text{protein in diet}) - (\%\ \text{protein in faeces})}{(\%\ \text{indicator in diet}) \quad (\%\ \text{indicator in faeces})}}{\dfrac{\%\ \text{protein in diet}}{\%\ \text{indicator in diet}}} \times 100$$

It was not possible to determine true digestibility as the fish would not accept a no-protein diet and were too small to force feed. As digestibility changes as a fish grows and smaller fish have the tendency to lose more protein from endogenous sources (Ferraris *et al.*, 1986), using larger fish to determine the amount of endogenous protein in the faeces would not have been valid. Marine fish, however, exhibit higher rates of food movement which causes dilution of endogenous protein

production (Ferraris *et al.*, 1986).

RESULTS

The apparent digestibilities of the six protein sources are shown in Table 2. The digestibility of these alternative protein sources was found to be quite high, ranging from 66% to 73%. The digestibility of both of the fishmeals tested were higher than the other protein sources, with the low temperature Danish fishmeal being more digestible than the Chilean fishmeal.

Table 2 : Apparent crude protein digestibilities of the tested alternative protein sources.

PROTEIN SOURCE	APPARENT DIGESTIBILITY (%)
Animal source	
Danish fishmeal	73.38
Chilean fishmeal	71.94
Blood meal	69.60
Carcass meal	70.42
Plant source	
Defatted soyabean meal	71.44
Spirulina	66.09

The difference in digestibility between the animal and plant protein sources was slight, with defatted soyabean meal digestibility being comparable to the Chilean fishmeal. The least digestible protein was *Spirulina*.

DISCUSSION

There have been numerous studies on fishmeal alternatives. Plant proteins appear to be digested as efficiently by carnivorous and omnivorous fish as animal proteins (Cho and Cowey, 1991; Wilson, 1991 and Sullivan and Reigh, 1995). The factor which prohibits the inclusion of alternative plant proteins at high concentrations does not appear to be digestibility but rather palatability (McGoogan and Reigh, 1996). For example, Davis *et al.* (1995) found that increasing the amount of soyabean protein in diets for red drum, *Sciaenops ocellatus* led to reduced palatability, and a reduction in food intake and feed efficiency. However, these authors found that soya protein was a suitable fishmeal substitute as long as chemical attractants were included to increase palatability.

The most widely used plant protein in fish diets is soyabean meal. The two main reasons for this are its favourable amino acid profile and the fact that it is consistently available (Watanabe *et al.*, 1992). It is also cost effective as soyabean products are almost half the price of fishmeal (Dabrowski *et al.*, 1989). Soyabean production has grown by 176% during the past 20 years and is expected to increase by a further 40% by the year 2000 (Rumsey, 1993).

The digestibility of soyabean meal appears to be species specific with the digestibility of soyabean meal for seabream, *Sparus aurata* is 92.12% (Nengas *et al.*, 1996); for rainbow trout, *Oncorhynchus mykiss* is 91.54% (Watanabe and Pongmaneerat, 1993); for red drum is 86% (Gaylord and Gatlin, 1996) and for channel catfish, *Ictalurus punctatus* is 79.95% (Sullivan and Reigh, 1995). The digestibility of soyabean meal for juvenile spotted grunter was found to be lower (71.44%) but this value may be unreliable due to the fishes poor appetite when fed this protein source. This reduction in appetite when fed diets with high inclusion levels of soyabean is not uncommon and has been reported for chinook salmon, *O. tshawytscha* (Hajen *et al.*, 1993) and red drum (Reigh and Ellis, 1992; Davis *et al.*, 1995), although Watanabe *et al.* (1992) found no such reduction in appetite in yellowtail, *Seriola quinqueradiata* fed high soyabean meal inclusion diets.

The most commonly used single-cell protein (SCP) in aquafeeds is yeast, either in the form of brewers/bakers yeast or commercially produced candida/torula yeast preparations (Tacon, 1994).

In most monogastric animals however, high dietary inclusion levels of SCP cause toxicological effects due to the high content of nucleic acid nitrogen (Rumsey, 1993). However, some fish have high levels of the liver enzyme uricase, which catalyses the degeneration of the toxic end-product of nucleic acid metabolism (uric acid) to carbon dioxide and urea (Rumsey, 1993). Therefore, the potential of this alternative protein source, in certain species of fish, remains valid. Lake trout, *Salvelinus namaycush* for example, have been successfully reared on diets containing up to 50% yeast (Rumsey *et al.*, 1990). This is substantially higher than the inclusion level of 16% of the bacterial SCP *Brevibacterium lactofermentum* in rainbow trout diets (Kiessling and Askbrandt, 1993). The apparent crude protein digestibility of this bacterial SCP is estimated to be 75% for rainbow trout (Kiessling and Askbrandt, 1993).

Another promising potential SCP is unicellular algae meal such as *Spirulina* (Hepher *et al.*, 1979). In his work on silver seabream, *Rhabdosargus sarba,* El-Sayed (1994) found that fingerling seabream utilized *Spirulina* meal more efficiently than soyabean meal or chicken offal meal, and inclusion levels up to 50% caused no significant difference in growth. Chow and Woo (1990) also found that *Spirulina,* supplemented with methionine, successfully replaced fishmeal as a protein source for Nile tilapia. Unfortunately, the only reported crude protein digestibility value for *Spirulina* is 87.1% for mirror carp, *Cyprinus carpio* (Atack *et al.*, 1979). The crude protein digestibility of *Spirulina* for juvenile spotted grunter was low (66.09%) and further investigations are required before this can be established as a true potential fishmeal alternative.

Published results on the apparent digestibility of crude protein of bloodmeal are extremely variable (Cho *et al.*, 1982; Asgard and Austreng, 1986; Hajen *et al.*, 1993). This may be due to processing effects as overheating will result in reduced digestibility. This is hypothesized to be the reason for the low crude protein digestibility of bloodmeal (29.4%) for chinook salmon (Hajen *et al.*, 1993). Apart from this example, crude protein digestibility of bloodmeal is relatively high with digestibility co-efficients ranging from 100% for red drum, *Sciaenops ocellatus* (McGoogan and Reigh, 1996), 86% for hybrid striped bass (Sullivan and Reigh, 1995) to 74% for channel catfish (Tucker and Robinson, 1990). The crude protein digestibility co-efficient of bloodmeal for spotted grunter of

69.60% is low by comparison. A possible explanation for this may be excessive heating during processing as indicated by the dark purple colour and the presence of charred material.

Like bloodmeal, the reported crude protein digestibility of carcass or meat and bone meal is highly variable. The crude protein digestibility of bloodmeal for rainbow trout ranges between 74.06% (McGoogan and Reigh, 1996) and 85% (Cho and Cowey, 1991), 78.9% for red drum (Gaylord and Gatlin, 1996), 73% for hybrid striped bass (Sullivan and Reigh, 1993) and 61% for channel catfish (Tucker and Robinson, 1990). There is the extreme case where animal by-product meal has been shown to be able to be used as a sole protein source in commercial diet for Nile tilapia, *Oreochromis niloticus*, without affecting growth or food utilization (Rodriguez-Serna *et al.*, 1996). This allows for improved economics of feeding in comparison with fishmeal and bears further investigation in the case of spotted grunter. The crude protein digestibility of carcass meal for spotted grunter was 70.42%. Although the reported crude protein digestibilities of carcass meal are generally quite high, this alternative protein source is characterized by ash/mineral imbalances which may limit inclusion levels (Tacon, 1994).

The crude protein digestibility of fishmeal is very variable and is determined largely by the method of processing (McCallum and Higgs, 1989). Freeze-drying produces the highest quality fishmeal, followed by low temperature drying, with high temperature dried fishmeal having a drastically reduced protein quality (McCallum and Higgs, 1989). Generally speaking, fishmeal is highly digestible, with co-efficients of Menhaden fishmeal ranging from 96% for red drum (McGoogan and Reigh, 1996) to 85% for channel catfish (Tucker and Robinson, 1990). The crude protein digestibility co-efficient for both fishmeals tested was low (73.38% for Danish fishmeal and 71.94% for Chilean fishmeal) by comparison. The crude protein digestibility of fishmeal for red drum has however, been reported to be as low as 77% (Gaylord and Gatlin, 1996), again indicating the variation in crude protein digestibility of fishmeal. Notwithstanding this variability, the incorporation of high nutritive value marine protein sources, such as fishmeal, into diets has been shown to improve protein conversion in salmon (Higgs, 1986). Therefore, although it is expensive, fishmeal does contribute to the minimization of cost per kilogram weight gain (Higgs, 1986).

It is unlikely that fishmeal will be totally replaced by alternative protein sources in the diets for carnivorous marine fish, but with the use of chemical attractants and/or crystalline amino acid supplementation, alternative protein sources may reduce the fishmeal component in artificial diets (Tacon, 1994). It is clear from the apparent protein digestibilities of the alternative protein sources investigated in this study that defatted soyabean meal, carcass meal and blood meal are potential substitutes for fishmeal in diets for juvenile spotted grunter. *Spirulina* does not appear to be a potential substitute due to its' low digestibility and its' lack of availability. Commercially grown *Spirulina* is also very expensive (El-Sayed, 1994) which further negates its' value as a fishmeal substitute. Defatted soyabean meal, carcass meal and blood meal need to be further investigated by means of growth trials. These growth trial should be designed to assess both the effect on growth of long term feeding with substitute protein sources and also the optimal inclusion levels of these sources.

CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSION

In this final discussion the results from the various feeding trials of the various feeding trials from the previous chapters are considered in their entirety in terms of their contribution towards the development of an optimal diet for juvenile spotted grunter. The results obtained in this study are also compared to those obtained for other carnivorous marine fish. Their applicability to similar species is discussed and future studies, before an optimal diet can be formulated for spotted grunter, are identified.

In order for marine fish farming to be feasible, food formulation should result in adequate growth and survival at the least possible cost (Knight, 1985). With few exceptions, such as mullet, *Mugil cephalus* and milkfish, *Chanos chanos*, most aquaculture candidates are either pelagic carnivores, demersal carnivores or demersal omnivores (Tucker, 1992). In formulating feeds for carnivorous and omnivorous marine fish, particular attention must be paid to the protein, fatty acid and vitamin content of the feeds (Tucker, 1992). This study was aimed at investigating aspects of the protein requirements of juvenile spotted grunter, *Pomadasys commersonnii*.

The specific growth rate (SGR), protein efficiency ratios (PER) and the food conversion ratios (FCR) from the three growth trials are summarized in Table 1.

As each experiment was conducted at different times and with different fish, albeit under similar conditions, the results of the individual experiments cannot be compared directly with one another. For this reason, the results of each experiment will be discussed separately.

The proximate composition of the natural diet was found to contain 50-60% protein. This was used as a guide for quantitative studies on protein requirements. However, it was found that fishmeal and casein diets with 40-50% protein produced optimal growth, and that the minimum dietary protein

requirement for juvenile spotted grunter should not be less than 40% (Table 1). The predicted inclusion level of crude protein of 50-60%, was therefore higher than required by the fish but nevertheless provided the information upon which to develop the necessary range of dietary protein levels to be investigated.

Table 1 : Growth parameters from the sequential growth trials.

EXPERIMENT	OPTIMAL CONDITION	SGR	PER	FCR
Crude protein	40% dietary protein	2.31 ±0.11	1.17 ±0.12	1.96 ±0.25
Protein:Energy	26.7mg/kJ	5.96 ±0.36	1.25 ±0.13	1.72 ±0.19
EAA - "ideal"	lysine = 4.30% of dietary protein	2.95 ±0.18	0.41 ±0.05	3.02 ±0.29
EAA - control	N/A	2.55 ±0.19	0.33 ±0.06	4.23 ±0.36

The optimal protein/energy requirement for juvenile spotted grunter was found to be 26.7mg/kJ (Table 1). Feeding spotted grunter a diet with this protein:energy ratio was found to be optimal in terms of SGR, PER and FCR. The empirically determined P:E ratio was also higher than the predicted level of 31mg/kJ, although this was not much higher than the experimentally determined level. There is a possibility that feeding a 35% crude protein diet with lipid levels greater than 12% may result in protein sparing, however further investigation is necessary.

Optimal growth was obtained with a diet containing a P:E ratio of 26.7mg/kJ. This diet was used as the control diet for the lysine evaluation experiment (Chapter 6). From Table 1 it is clear that supplementing with crystalline lysine improved SGR, PER and FCR. Supplementing with crystalline lysine also had a significant effect ($p<0.05$) on the proximate composition of the fish (Table 2). Supplementation with crystalline lysine resulted in a significant ($p<0.05$) increase in protein deposition by almost 8% and a significant ($p<0.05$) decrease in gross lipid content by approximately 0.5%. It is also clear that juvenile spotted grunter are capable of utilizing crystalline amino acids to enhance growth. The optimal lysine level was found to be 4.30% of dietary protein.

Table 2 : Proximate composition of fish fed the optimal P:E and the "ideal" and control lysine diets.

EXPERIMENT	% PROTEIN	% LIPID	HSI	IPF
EAA - "ideal" (Chapter 6)	59.72±1.11	4.45±0.59	4.19±0.61	3.45±0.29
EAA - control	51.73±1.02	5.06±0.62	4.66±0.28	3.33±0.86

HSI = Hepatosomatic index
IPF = Intraperitoneal fat ratio

Therefore, the optimal diet in terms of protein, for juvenile spotted grunter based on these studies should contain not less than 40% crude dietary protein, a P:E of 26.7mg/kJ and a lysine level of 4.30% of the dietary protein.

Fishmeal is an expensive protein source (Barlow, 1989) Projections of future world production and supply suggest that the price will increase over time. This will have adverse effects on feed prices. In order to reduce feed cost, there is a movement within the industry to find less expensive alternative protein sources. With this in mind, crude protein digestibility of various alternative protein sources as well as the crude protein digestibility of low temperature Danish and Chilean fishmeals was investigated. Low temperature fishmeal has been found to be the most digestible and palatable protein source in artificial feed formulations for fish (McCallum and Higgs, 1989; Pike *et al.*, 1990). Juvenile spotted grunter are able to utilize both animal and plant protein sources. Digestibility co-

efficients appear to be species specific (Chapter 7). Future studies in this area should concentrate on formulating test diets containing various inclusion levels of the suitable alternative protein sources. These diets should be evaluated by running extended growth trials. The results from these trials would allow for least costing of a feed.

The combined optimal dietary protein and P:E requirements of five potential mariculture species are outlined in Table 3. It is clear that the optimal dietary protein requirements for juvenile carnivorous marine fish range from 40% for red drum, *Sciaenops ocellatus* (Serrano *et al.*, 1992) to 57% for yellowtail, *Seriola quinqueradiata* (Shimeno *et al.*, 1985). Optimal P:E ratios ranged from 28.7mg/kJ to 35.3mg/kJ for these fish.

The dietary protein and protein:energy requirements (40% dietary protein and 26.7mg/kJ) of juvenile spotted grunter are very similar to the requirements of juvenile red drum. It is interesting to note that the natural diet of juvenile red drum is very similar to that of juvenile spotted grunter. In both cases juveniles feed primarily on mysids (L. Mahlasela, Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, unpublished data; Hildebrand and Schroeder, 1928). As they grow, there is a shift in both cases towards larger crustaceans such as prawns and shrimp (Van Der Westhuizen and Marias, 1977; Whitfield, 1980; Hildebrand and Schroeder, 1928).

It is clear that the proximate composition of the natural diet is a valid starting point in nutritional studies. The similarity of the crude protein and P:E requirements of juvenile red drum and juvenile spotted grunter suggests that if the nutritional requirements are not known for a certain species, then feed formulations for that species should be based on the known requirements of a fish having a similar natural diet to the unknown species.

Research on the dietary nutrient requirements of potential mariculture species should be designed and conducted in such a manner that the results can be applied under practical farming conditions (Tacon, 1995). At present, pelleted feed for juvenile spotted grunter is in the form of a moist pellet as they will not accept a dry pellet (N. Deacon, Department of Ichthyology and Fisheries Science, Rhodes

Table 3 : Optimal dietary crude protein and P:E requirements of five mariculture species.

FISH	NATURAL DIET	% CRUDE PROTEIN	P:E (mg/kJ)
Sciaenops ocellatus Red drum	Crustaceans, with juveniles preferring *Mysis* and larger fish preferring shrimp (Hildebrand and Schroeder, 1928)	40-45 (Serrano *et al.*, 1992)	28.7 (Daniels and Robinson, 1986)
Epinephelus malabaricus Grouper	Mainly fish, but also crayfish and crabs (Van Der Elst, 1988)	47.8 (Chen and Tsai, 1994)	33.8 (Teng *et al.*, 1978)
Seriola quinqueradiata Yellowtail	Fish (Steffens, 1989)	57 (Shimeno *et al.*, 1985)	35.3 (Shimeno *et al.*, 1985)
Pagrus major Red sea bream	Invertebrates (Steffens, 1989)	55 (Takeuchi *et al.*, 1991)	34.7 (Takeuchi *et al.*, 1991)
Dicentrarchus labrax Sea bass	Crustaceans and fish (Steffens, 1989)	47-50 Alliot *et al.*, 1974)	35.0 (Hildalgo and Alliot, 1988)

University, Grahamstown, pers. comm. and this study). Moist pellets are not ideal as they cannot be made in bulk and stored. While many commercially grown marine fish, such as red sea bream, *Pagrus major*, flounder, *Platichthys flesus* and striped jack , *Pseudocaranx dentex* , accept a hard-dry pellet, this is not the case for other species (Watanabe et al., 1991a). Yellowtail, *Seriola quinqueradiata*, will initially accept a hard-dry pellet but will frequently regurgitate the pellet due to its' texture (Watanabe et al., 1991a). Recently Watanabe *et al.* (1991a) developed a soft-dry pellet which was not only accepted by yellowtail but also enhanced growth. The possibility of juvenile spotted grunter accepting this type of pellet should be investigated as it would help simplify the feeding and manufacturing process.

In conclusion, the objectives of this study have been met in that the optimal crude protein requirements, the optimal P:E ratio and the optimal lysine content of the diet have been determined. Some alternative protein sources have been identified and the crude protein digestibility of these sources have been determined. It has also been shown that the hypothesis that the proximate composition of the natural diet and the proximate composition of wild juveniles provide valuable information regarding the animals' nutritional requirements is valid, and should be used as a starting point in other nutritional studies.

REFERENCES

Alliot, E.; Febvre, A.; Metailler, R. and Pastoureaud, A. 1974. Besoins nutritifs du bar *Dicentrarchus labrax*. Etude du taux de proteines et du taux de lipides dans le regime. *Actes Colloq. CNEXO*, 1 : 215-228.

Asgard, T. and Austreng, E. 1986. Blood, ensiled or frozen, as feed for salmonids. *Aquaculture*, 55 : 263-284.

Association of Official Analytical Chemists (AOAC). 1984. Official Methods of Analysis, 14th ed. AOAC, Washington, DC.

Atack, T.H.; Jauncey, K. and Matty, A.J. 1979. The utilization of some single-cell proteins by fingerling mirror carp (*Cyprinus carpio*). *Aquaculture*, 18 : 337-348.

Austreng, E. 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. *Aquaculture*, 13 : 265-272.

Austreng, E. 1979. Fat levels and fat sources in dry diets for salmonid fish. In : *Finfish Nutrition and Fishfeed Technology*. (Eds). Halver and Tiews, pp. 313-326.

Austreng, E. and Refstie, T. 1979. Effects of varying dietary protein level in different families of rainbow trout. *Aquaculture*, 18 : 145-156.

Arai, S. 1981. Amino acid ratios of whole bodied coho salmon and the formulation of test diets. *Bull. Jap. Soc. Sci. Fish.*, 47 : 547-550.

Baker, D.H. 1984. Equalized versus *ad libitum* feeding. *Nutr. Rev.*, 42 : 269-273.

Barlow, S. 1989. Fishmeal - World outlook to the year 2000. *Fish Farmer* : Sept/Oct 1989 pp. 12-15.

Beckley, L.E. 1984. The ichthyofauna of the Sundays Estuary, South Africa, with particular reference to the juvenile marine component. *Estuaries*, 7 : 248-258.

Benitez, L.V. 1989. Amino acid and fatty acid profiles in aquaculture nutrition studies. In : *Fish Nutrition Research in Asia. Proc. of the 3rd Asian Fish Nutrition Network Meeting. Asian Fish. Soc. Spec. Publ.*, 4 166p. Asian Fisheries Society, Manila, Philippines.

Blaber, S.J.M. 1979. The biology of filter feeding teleost in Lake St Lucia, Zululand. *J. Fish Biol.*, 15 : 37-59.

Blaber, S.J.M. 1981. The zoogeographical affinities of estuarine fishes in south-east Africa. *S. Afr. J. Sci.*, 77 : 305-307.

Blaber, S.J.M. 1983. The feeding ecology of *Pomadasys commersonnii* and *Rhabdosargus sarba* in Natal estuaries. SANCOR Report FRD Pretoria 1983 : 228-232.

Bolger, T. and Connolly, P.L. 1989. The selection of suitable indices for the measurement and analysis of fish condition. *J. Fish Biol.*, 34 : 171-182.

Bowen, S.H. 1987. Dietary Protein Requirements of Fishes - A Reassessment. *Can. J. Fish. Aquat. Sci.*, 44 : 1995-2001.

Branch, G.M.; Griffiths, C.L.; Branch, M.L. and Beckley, L.E. 1994. *Two Oceans. A Guide to the Marine Life of Southern Africa.* David Philip Publishers, Cape Town.

Brett, 1979. Environmental factors and growth. In : *Fish Physiology*, Vol. 8. (Eds.) Hoar, W.S.; Randall, J.R. and Brett, J.R. Academic Press, London, pp. 599-675.

Brett, J.R. and Groves, T.D.D. 1979. Physiological energetics. In : *Fish Physiology*. (Eds.) Hoar, W.S.; Randall, D.J. and Brett, J.R. New York, Academic Press, Vol. 8. pp. 279-352.

Bromley, P.J. 1980. Effects of dietary protein, lipid and energy content on the growth of turbot (*Scophthalmus maximus* L.). *Aquaculture*, 19 : 359-365.

Brown, P.B; Davis, D.A. and Robinson, E.H. 1988. An estimate of the dietary lysine requirement of juvenile red drum *Sciaenops ocellatus*. *J. World Aquac. Soc.*, 19(3) : 109-112.

Buxton, C.D.; Smale, M.J.; Wallace, J.H. and Cockcroft, V.G. 1984. Inshore small-mesh trawling survey of the Cape south coast. Part 4. Contributions to the biology of some Teleostei and Chondrichthyes. *S. Afr. J. Zool.*, 19 : 180-188.

Catacutan, M.R and Coloso, R.M. 1995. Effect of dietary protein to energy ratios on growth, survival, and body composition of juvenile Asian seabass, *Lates calcarifer*. *Aquaculture*, 131 : 125-133.

Chen, H-Y and Tsai, J-C. 1994. Optimal dietary protein level for the growth of juvenile grouper, *Epinephelus malabaricus*, fed semipurified diets. *Aquaculture*, 119 : 265-271.

Cho, C.Y; Bayley, H.S and Slinger, S.J. 1974. Partial replacement of herring meal with soybean meal and other changes in a diet for rainbow trout (*Salmon gairdneri*). *J. Fish Res. Bd Can.*, 31 : 1523-1528.

Cho, C.Y.; Slinger, S.J and Bayley, H.S. 1982. Bioenergetics of salmonid fishes : energy intake, expenditure and productivity. *Comp. Biochem. Physiol.*, 73B : 25-41.

Cho, C.Y. and Kaushik, S.J. 1985. Effects of protein intake on metabolizable and net energy values of fish diets. In : *Nutrition and Feeding in Fish.* (Eds.) Cowey, C.B.; Mackie, A.M and Bell, J.G. Academic Press, London, pp. 95-117.

Cho, C.Y. and Cowey, C.B. 1991. Rainbow trout, *Oncorhynchus mykiss.* In: *Handbook of Nutrient Requirements of Finfish.* (Ed.) Wilson, R.P. CRC Press, Boca Raton, pp. 131-143.

Cho, C.Y.; Kaushik, S.J. and Woodward, B. 1992. Dietary arginine requirements of young rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol.*, 102A : 211-216.

Cho, C.Y. and Bureau, D.P. 1995. Determination of the energy requirements of fish with particular reference to salmonids. *J. Appl. Ichthyol.*, 11 : 141-163.

Chow, C.Y. and Woo, N.Y.S. 1990. Bioenergetic studies on an omnivorous fish *Oreochromis mossambicus* : Evaluation of the utilization of *Spirulina* algae in feeds. In: *Proc. 2nd Asian Fish. Forum, Tokyo, Japan.* (Eds.) Hiranu, R. and Hanyu, I. pp. 291-294.

Cowey, C.B. 1992. Nutrition : Estimation requirements for rainbow trout. *Aquaculture*, 100 : 177-189.

Cowey, C.B. 1993. Protein Metabolism in Fish. In : *Coastal and Estuarine Studies. Aquaculture : Fundamental and Applied Research.* (Eds.) Lahlou, B. and Vitiello, P. American Geophysical Union, Washington D.C, pp. 125-137.

Cowey, C.B. 1995. Protein and amino acid requirements : A critique of methods. *J. Appl. Ichthyol.*, 11 : 199-204.

Cowey, C.B and Tacon, A.G.J. 1982. Fish Nutrition - Relevance to Invertebrates. In : *Proceedings of the second International Conference on Aquaculture Nutrition. Rehoboth Beach, Delaware.* (Eds.) G.D Pruder, C. Langdon and D. Conklin, pp 13-30. World Mariculture Society, Special Publication number 2, Louisiana State University, Louisiana.

Cowey, C.B.; Pope, J.A., Adron, J.W. and Blair, A. 1972. Studies on the nutrition of marine flatfish. The protein requirement of plaice *Pleuronectes platessa. Br. J. Nutr.*, 28 : 447-456.

Dabrowski, K.; Poczycznski, P.; Kock, G. and Berger, B. 1989. Effects of partially or totally replacing fishmeal protein on growth, food utilization and proteolytic enzyme activities in rainbow trout (*Salmon gairdneri*) : New *in vivo* test for exocrine pancreatic secretion. *Aquaculture*, 77 ; 29-49.

Daniels, W.H and Robinson, E.H. 1986. Protein and energy requirements of juvenile red drum *(Sciaenops ocellatus). Aquaculture*, 53 : 243-252.

Davis, D.A.; Jirsa, D. and Arnold, C.R. 1995. Evaluation of soybean proteins as replacements for Menhaden fish meal in practical diets for the red drum, *Sciaenops ocellatus. J. World Aqua. Soc.*, 26(1) : 48-58.

Day, J.H.; Blaber, S.J.M. and Wallace, J.H. 1981. Estuarine fishes. In : *Estuarine Ecology with Particular Reference to southern Africa.* Cape Town, A.A Balkema, pp. 197-221.

Deacon, N.R. 1997. The environmental requirements for hatchery rearing juvenile spotted grunter, *Pomadasys commersonnii*, (Haemulidae : Pisces). PhD. Thesis, Rhodes University, Grahamstown, South Africa.

Deacon, N. and Hecht, T. 1995a. The effect of temperature and photoperiod on the growth of juvenile spotted grunter, *Pomadasys commersonnii*, (Haemulidae : Pisces). *S.A J. Mar. Sci.*,(In Press).

Deacon, N. and Hecht, T. 1995b. The effect of feeding frequency on gut evacuation and its implications for food intake and growth of juvenile spotted grunter, *Pomadasys commersonnii*, (Haemulidae : Pisces). *Aquaculture Nutrition* (In Press).

Deacon, N.; White, H. and Hecht, T. (In Press). Isolation of the effective concentration of 2-phenoxyethanol for anaesthesia in fish and its effect on growth. *Aquarium Sci. Conserv.*

Denton, J.E and Yousef, M.K. 1976. Body composition and organ weights of rainbow trout, *Salmon gairdneri. J. Fish Biol.*, 8(6) : 489-499.

Deshimaru, D. and Shigeno, K. 1972. Introduction to the artificial diet for prawn (*Penaeus japonicus*). *Aquaculture*, 1 : 115-133.

Deshimaru, O; Kuroki, K and Yone, Y. 1982. Nutritive Values of Various Oils for Yellowtail. *Jap. Soc. Sci. Fish.*, 48(8) : 1155-1157.

De Silva, S.S; Gunasekera, R.M and Shim, K.F. 1991. Interactions of varying dietary protein and lipid levels in young red tilapia : evidence of protein sparing. *Aquaculture*, 95 : 305-318.

Dong, F.M.; Hardy, W.H.; Haard, N.F.; Barrows, F.T; Rasco, B.A.; Fairgrieve, W.T. and Forster, I.P. 1993. Chemical composition and protein digestibility of protein by-product meals for salmonid diets. *Aquaculture*, 116 : 149-158.

Ellis, S.C and Reigh, R.C. 1991. Effects of dietary lipid and carbohydrate levels on growth and body composition of juvenile red drum, *Sciaenops ocellatus*. *Aquaculture*, 97 : 383-394.

El-Sayed, A-F. M. 1994. Evaluation of soybean meal, *Spirulina* meal and chicken offal meal as protein sources for silver seabream (*Rhabdosargus sarba*) fingerlings. *Aquaculture*, 127: 169-176.

Fauconneau, B.; Choubert, G.; Blanc, D.; Breque, J. and Luquet, P. 1983. Influence of environmental temperature on flow rate of foodstuffs through the gastrointestinal tract of rainbow trout. *Aquaculture*, 34 : 27-39.

Ferraris, R.P.; Catacutan, M.R.; Mabelin, R.L. and Jazul, A.P. 1986. Digestibility in milkfish, *Chanos chanos* (Forsskal) : Effects of protein source, fish size and salinity. *Aquaculture*, 59 :93-105.

Folch, J.; Lees, M. and Sloane Stanley, G.Y. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226 : 497-509.

Foscarini, R. 1988. A review : Intensive farming procedure for Red Seabream (*Pagrus major*) in Japan. *Aquaculture*, 72 : 191-246.

Fuller M.F. 1988. Methods of protein evaluation for non-ruminants. In : *Feed Science* (Ed). Orskov. Elsevier Science Publishers, New York, pp. 81-101.

Gallager, M.L and Degani, G. 1988. Poultry meal and poultry oil as sources of protein and lipid in the diet of European eels (*Anguilla anguilla*). *Aquaculture,* 73 : 177-187.

Gaylord, T.G. and Gatlin III, D.M. 1996. Determination of digestibility co-efficients of various feedstuffs for red drum (*Sciaenops ocellatus*). *Aquaculture*, 139 : 303-314.

Hajen, W.E.; Higgs, D.A.; Beames, R.M. and Dosanjh, B.S. 1993. Digestibility of various feedstuffs by post-juvenile chinook salmon (*Oncorhynchus tshawytscha*) in sea water. 2. Measurement of digestibility. *Aquaculture*, 112 : 333-348.

Hardy, R.W. 1989. Diet preparation. In : *Fish Nutrition* 2nd Edition. (Ed.) J.E. Halver, pp. 476-548, Academic Press, San Diego.

Harper, A.E.; Benvenga, N.J. and Wolhueter, R.M. 1970. Effects of ingestion of disproportionate amounts of amino acids. *Physiol. Rev.*, 50 : 428-543.

Hecht T. and Britz, P.J. 1992. The current status, future prospects and environmental implications of mariculture in South Africa. *S. Afr.J. Sci.*, 88(6) : 335-342.

Hecht, T. and van der Lingen, C.D. 1992. Turbidity-induced changes in feeding strategies of fish in estuaries. *S. Afr. J. Zool.*, 27(3) : 95-107.

Henken, A.M.; Kleingeld, D.W. and Tijssen, P.A.T. 1985. The effect of feeding level on apparent digestibility of dietary dry matter, crude protein and gross energy in the African catfish (*Clarius gariepinus*). *Aquaculture*, 51 : 1-11.

Henken, A.M.; Lucas, H.; Tijssen, P.A.T and Machiels, M.A.M. 1986. A comparison between methods used to determine the energy content of feed, fish and faeces samples. *Aquaculture,* 58 : 195-201.

Hepher, B.; Sandbank, E. and Shelef, G. 1979. Alternative protein sources for warmwater fish diets. In: *Finfish Nutrition and Fishfeed Technology*. (Eds.) Halver, J.E. and Tiews, K. Schriften der Bundesforschungsanstalt fur Fischerei, Berlin, pp. 327-341.

Hidalgo, F. and Alliot, E. 1988. Influence of water temperature on protein requirement and protein utilization in juvenile sea bass, *Dicentrarchus labrax*. *Aquaculture*, 72 : 115-129.

Higgs, D.A. 1986. Feeding fish efficiently. *Can. Aqua.*, 2(2) : 15-26.

Hildebrand, S.F. and Schroeder, W.C. 1928. Fishes of Chesapeake Bay. *Bulletin of the United States Bureau of Fisheries.* Vol. : XLIII(1) : 276-277.

Hillestad, M. and Johnsen, F. 1994. High-energy/low-protein diets for Atlantic Salmon : effects on growth, nutrient retention and slaughter quality. *Aquaculture*, 124 : 109-116.

Houlihan, D.F.; Hall, S.J.; Gray, C. and Noble, B.S. 1988. Growth rates and protein turnover in Atlantic cod, *Gadus morhua*. *Can. J. Fish. Aquat. Sci.*, 45 : 951-964.

Ibeas, C.; Izquierdo, M.S. and Lorenzo, A. 1994. Effect of different levels of *n*-3 highly unsaturated fatty acids on growth and fatty acid composition of juvenile gilthead seabream (*Sparus aurata*). *Aquaculture*, 127 : 177-188.

Jackson, A.J. and Capper, B.S. 1982. Investigations into the requirements of the tilapia *Sarotherodon mossambicus* for dietary methionine, lysine and arginine in semi-synthetic diets. *Aquaculture*, 29 : 289-297.

Jobling, M. 1981. Mathematical models of gastric emptying and the estimation of daily rates of food consumption for fish. *J. Fish Biol.*, 19 : 245-257.

Jobling, M. 1983a. Growth studies with fish - overcoming the problems of size variation. *J. Fish Biol.*, 22 : 153-157.

Jobling, M. 1983b. Influence of body weight and temperature on growth rates of Arctic charr, *Salvelinus alpinus* (L.). *J. Fish Biol.*, 22 : 471-475.

Jobling, M. 1994. Nutritional Requirements. In: *Fish Bioenergetics. Fish and Fisheries Series 13*. Chapman and Hall, London. pp. 31-76.

Jobling, M. 1995. Foodstuffs and Feeding. In: *Environmental Biology of Fishes. Fish and Fisheries Series 16*. Chapman and Hall, London. pp. 47-92.

Joubert, C.S.W. and Hanekom, P.B. 1980. A study of feeding in some inshore reef fish of the Natal Coast, South Africa. *S. Afr. J. Zool.*, 15 : 262-309.

Kalogeropoulos, N.; Alexis, M.N. and Henderson, R.J. 1992. Effects of dietary soybean and cod-liver oil levels on growth and body composition of gilthead bream (*Sparus aurata*). *Aquaculture*, 104 : 293-308.

Kaushik, S.J. and Fauconneau, B. 1984. Effects of lysine administration on plasma arginine and on some nitrogenous catabolites in rainbow trout. *Comp. Biochem. Physiol.*, 79A : 459-462.

Keembiyehetty, C.N. and Gatlin III, D.M. 1992. Dietary lysine requirement of juvenile hybrid striped bass (*Morone chrysops* x *M. saxatilus*). *Aquaculture*, 104 : 271-277.

Ketola, H.G. 1979. Influence of dietary lysine on "fin rot", survival, and growth of fry of rainbow trout. *Fish Health News*, 8(2) : 6.

Ketola, H.G. 1982. Amino acid nutrition of fishes : requirements and supplementation of diets. *Comp. Biochem. Physiol.*, 73B(1) : 17-24.

Kim, K-I.; Kayes, T.B. and Amundsen, C.H. 1991. Purified diet development and re-evaluation of the dietary protein requirement of fingerling rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 96 : 57-67.

Kiessling, A. and Askbrandt, S. 1993. Nutritive value of two bacterial strains of single-cell protein for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 109: 119-130.

Knauer, J.; Hecht, T. and Duncan, J.R. 1994. Proximate composition of the South African abalone, *Haliotis midae* (Haliotidae:Gastropoda). *Aquac. Fish. Man.*, 25 : 351-354.

Knight, B. 1985. Energetics and Fish Farming. In : *Fish Energetics : New Perspectives.* (Eds.) Tytler, P. and Calow, P. John Hopkins University, Baltimore, Maryland. pp. 309-340.

Kropf, D.H.; Bray, P.H.; Phillips, P.H. and Grummer, R.H. 1959. Effect of protein level and quality in swine rations upon growth and carcass development. *J. Anim. Sci.*, 18 : 755-762.

Lasiak, T.A. 1982. Structural and functional aspects of the surf-zone fish community in the Eastern Cape. Ph.D. thesis, University of Port Elizabeth, South Africa.

Lasiak, T.A. 1986. Juveniles, food and the surf zone habitat : implications for teleost nursery areas. *S. Afr. J.Zool.*, 21 : 51-56.

Lee, D.J and Putnam, G.B. 1973. The response of rainbow trout to varying protein/energy ratios in a test diet. *J. Nutr.*, 103 : 916-922.

Lee, D.J. and Sinnhuber, R.O. 1989. Lipids. In: *Fish Nutrition.* 2nd Edition. (Ed.) J.E. Halver, pp , Academic Press, San Diego.

Leeson, S. and Summers, J.D. 1980. Production and carcass characteristics of the broiler chicken. *Poult. Sci.*, 59 : 786-798.

Lie, O.; Lied, E. and Lambertsen, G. 1988. Feed optimization in Atlantic cod (*Gadus morhua*) : Fat versus protein content in the feed. *Aquaculture*, 69 : 333-341.

Lied, E.; Julshamn, K. and Braekkan, O.R. 1982. Determination of protein digestibility in Atlantic cod (*Gadus morhua*) with internal and external markers. *Can. J. Fish. Aquat. Sci.*, 39 : 854-861.

Lochmann, R.T and Gatlin III, D.M. 1993. Evaluation of different types and levels of triglycerides, singly and in combination with different levels of ω-3 highly unsaturated fatty acid ethyl esters in diets of juvenile red drum, *Sciaenops ocellatus. Aquaculture*, 114 : 113-130.

Lovell, T. 1989. *Nutrition and Feeding of Fish.* Van Nostrand Reinhold, New York, pp. 2-71.

Lovell, R.T. 1992. Replacing fishmeal in channel catfish diets. In: *Proceedings of Aquaculture Nutrition Workshop, Salamander Bay.* (Eds.) G.L Allan and W. Dall, pp. 118-121.

Marais, J.F.K and Erasmus, T. 1977. Body composition of *Mugil cephalus, Liza dumerili, Liza richardsoni* and *Liza tricuspidens* (Teleostei : Mugilidae) caught in the Swartkops estuary. *Aquaculture*, 10 : 75-86.

Marais, J.F.K. 1984. Feeding ecology of major carnivores from four eastern Cape estuaries. *S. Afr. J. Zool.*, 19 : 210-223.

Marais, J.F.K. 1990. Body composition of ten marine migrants and one freshwater fish species caught in estuaries of the eastern Cape, South Africa. *S. Afr. J. Mar. Sci.*, 9 : 135-140.

Maynard, L.A. and Loosli, J.K. 1956. *Animal Nutrition.* McGraw Hill, New York, 4th Edition, 484pp.

Mazid, M.A; Tanaka, Y; Katayama, T; Rahman, M.A; Simpson, K.L and Chichester, C.O. 1979. Growth response of *Tilapia zillii* fingerlings fed iso-caloric diets with variable protein levels. *Aquaculture*, **115** : 115-122.

McCallum, I.M. and Higgs, D.A. 1989. An assessment of processing effects on the nutritive value of marine protein sources for juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture*, 77 : 181-200.

McGoogan, B.B. and Reigh, R.C. 1996. Apparent digestibility of selected ingredients in red drum (*Sciaenops ocellatus*) diets. *Aquaculture*, **141** : 233-244.

Mertz, E.T. 1972. Lipid Requirements. In : *Fish Nutrition.* (Ed.) Halver, J.E. Academic Press, Inc., New York, pp. 106-143.

Millikin, M.R. 1982. Effects of dietary protein concentration on growth, feed efficiency, and body composition of age-0 striped bass. *Trans. Amer. Fish. Soc.*, **111** : 373-378.

Millikin, M.R. 1983. Interactive effects of dietary protein and lipid on growth and protein utilization of age-0 striped bass. *Trans. Am. Fish. Soc.*, **112** : 185-193.

Montgomery, W.L and Gerking, S.D. 1980. Marine macroalgae as foods for fishes : an evaluation of potential food quality. *Environ. Biol. Fish*, 5 : 143-153.

Moon, H.Y. and Gatlin III, D.M. 1991. Total sulphur amino acid requirement of juvenile red drum, *Sciaenops ocellatus*. *Aquaculture*, 95 : 97-106.

Moon, H.Y. and Gatlin III, D.M. 1994. Effects of dietary animal proteins on growth and body composition of the red drum (*Sciaenops ocellatus*). *Aquaculture*, 120 : 327-340.

Nematipour, G.R; Brown, M.L and Gatlin III, D.M. 1992. Effects of dietary energy : protein ratio on growth characteristics and body composition of hybrid striped bass, *Morone chrysops* ♀ X *M. saxatilis* ♂. *Aquaculture*, 107 : 359-368.

Nengas, I.; Alexis, M.N. and Davies, S.J. 1996. Partial substitution of fishmeal with soybean meal products and derivatives in diets for the gilthead seabream *Sparus aurata* (L.). *Aquac. Res.*, 27 : 147-156.

NRC (National Research Council). 1983. Nutrient requirements of Warmwater Fishes and Shellfishes. National Academy Press, Washington, 102 pp.

Ogata, H.; Arai, S. and Nose, T. 1983. Growth responses of cherry salmon *Oncorhynchus masou* and amago salmon *O. rhodurus* fry fed purified casein diets supplemented with amino acids. *Bull. Jap. Soc. Sci. Fish.*, 49(9) : 1381-1385.

Ogino, C. 1980. Protein requirement of carp and rainbow trout. *Bull. Jap. Soc. Sci. Fish.*, 46 : 385-388.

Parazo, M.M. 1990. Effect of dietary protein and energy level on growth, protein utilization and carcass composition of rabbitfish, *Siganus guttatus*. *Aquaculture*, 86 : 41-49.

Pearse, A.S. 1925. The chemical composition of certain freshwater fishes. *Ecology*, 6 : 7-16.

Person-Le Ruyet, J.; Baudin-Laurencin, F.; Devauchelle, N.; Mètailler, R.; Nicolas, J.L.; Robin, J. and Guillaume, J. 1991. Culture of turbot (*Scophthalmus maximus*). In : *Handbook of Mariculture Volume II : Finfish Aquaculture.* (Ed.) McVey, J.P. CRC Press, Florida, pp. 21-41.

Phillips, A.M. 1979. Calorie and Energy Requirement. In : *Fish Nutrition.* (Ed.) Halver, J.E. Academic Press, Inc., New York, pp. 1-28.

Pike, I.H.; Andorsdottir, G. and Mundheim, H. 1990. The role of fish meal in diets for salmonids. *Internat. Assoc. Fish Meal Manufacturers, Hertfordshire, England* 24 : 35pp.

Piper, G; McElwain, I.B; Orme, L.E; McRaren, J.P; Fowler, L.G and Leonard, J.R. 1982. Fish hatchery management. United States Department of Interior Fish and Wildlife Service, Washington.

Reigh, R.C. and Ellis, S.C. 1992. Effects of dietary soybean and fish-protein ratios on growth and body composition of red drum (*Sciaenops ocellatus*) fed isonitrogenous diets. *Aquaculture*, 104 : 279-292.

Reinitz, G.L; Orme, L.E and Hitzel, F.N. 1979. Variation of body composition and growth among strains of rainbow trout. *Trans. Amer. Fish. Soc.*, 108 : 204-207.

Reis, L.M; Reutebuch, E.M and Lovell, R.T. 1989. Protein-to-energy ratios in production diets and growth, feed conversion and body composition of channel catfish, *Ictalurus punctatus. Aquaculture*, 77 : 21-27.

Ricker, W.E. 1979. Growth rates and models. In: *Fish Physiology, Volume VIII.* (Eds.) Hoar, D.J.; Randall, D.J. and Brett, J.R. Academic Press, New York, pp. 677-743.

Robinson, E.H.; Wilson, R.P. and Poe, W.E. 1980. Re-evaluation of the lysine requirement and lysine utilization by fingerling channel catfish. *J. Nutr.*, **110** : 2313-2316.

Rodriguez-Serna, M.; Olivera-Novoa, M.A. and Carmona-Osalde, C. 1996. Nutritional value of animal by-product meal in practical diets for Nile tilapia *Oreochromis niloticus* (L.) fry. *Aqua. Res.*, 27 : 67-73.

Rouhani, Q.A. 1993. Digestible energy as a criterion for the development of diets for the African sharptooth catfish, *Clarias gariepinus* (Pisces : Clariidae). MSc thesis, Rhodes University, Grahamstown, South Africa, pp. 4-29.

Rumsey, G.L.; Hughes, S.G. and Kinsella, J.E. 1990. Use of dietary yeast (*Saccharomyces cervisa*) nitrogen by lake trout. *J. World Aqua. Soc.*, 21 : 205-209.

Rumsey, G.L. 1993. Fishmeal and alternative sources of protein in fish feeds. Update 1993. *Fisheries*, 18(7) : 14-19.

Sabaut, J.J. and Luquet, P. 1973. Nutritional requirements of the gilthead bream *Chrysophyrys aurata.* Quantitative protein requirements. *Mar. Biol.*, 18 :50-54.

Sadiku, S.O.E. and Jauncey, K. 1995. Digestibility, apparent amino acid availability and waste generation potential of soybean flour : Poultry meat meal blend based diets for tilapia, *Oreochromis niloticus* (L.), fingerlings. *Aqua. Res.*, 26 : 651-657.

Sargent, J.J; Henderson, R.J and Tochar, D.R. 1989. The Lipids. In: *Fish Nutrition.* 2nd Edition. (Ed.) J.E. Halver, pp , Academic Press, San Diego.

Schleyer, M.H and Wallace, J.H. 1986. Stomach contents of some shore-caught teleosts of Natal, South Africa. *S. Afr. J. Zool.*, 21(3) : 272-274.

Serrano, J.A; Nematipour, G.R and Gatlin III, D.M. 1992. Dietary protein requirements of the red drum (*Sciaenops ocellatus*) and relative use of dietary carbohydrates and lipid. *Aquaculture*, 101 : 283-291.

Shepherd, J. 1988. Commercial development and future prospects. In : *Intensive Fish Framing*. (Eds.) Shepherd, J. and Bromage, N. BSP Professional Books, London, pp. 333-364.

Shimeno, S; Hosokawa, H; Takeda, M. and Kajiyama, H. 1980. Effects of calorie to protein ratios in formulated diets on the growth, feed conversion and body composition of young yellowtail, *Seriola quinqueradiata*. *Bull. Jpn. Soc. Sci. Fish.*, 46 : 1083-1088.

Shimeno, S.; Hosokawa, H.; Takeda, M.; Kajiyama, H. and Kaisho, T. 1985. Effect of dietary lipid and carbohydrate on growth, feed conversion and body composition in young yellowtail. *Bull. Jap. Soc. Sci. Fish.*, 51 : 1893-1898.

Smith, M.M. and McKay, R.J. 1991. Haemulidae. In: *Smith's Sea Fishes*. (Eds.) Smith, M.M. and Heemstra, P.C. Southern Book Publishers, Halfway House. pp. 564-571.

Steffens, W. 1989. *Principles of Fish Nutrition*. Halsted Press, New York.

Steyn, W.J.A. 1957. A statistical study of the errors involved in the sampling and chemical analysis of soils and plants with particular reference to citrus and pineapples. Ph.D. thesis, Rhodes University, Grahamstown, South Africa, pp. 65-66.

Sullivan, J.A. and Reigh, R.C. 1995. Apparent digestibility of selected feedstuffs in diets for hybrid striped bass (*Morone saxatilus* ♀ x *M.chrysops* ♂). *Aquaculture*, **138** : 313-322.

Tacon, A.G.J. and Jackson, A.J. 1985. Utilization of conventional and unconventional protein sources in practical feeds. In : *Nutrition and Feeding in Fish*, (Eds.) Cowey, C.B.; Mackie, A.M. and Bell, J.G. Academic Press, London, pp. 119-145.

Tacon, A.G.J. and Cowey, C.B. 1985. Protein and amino acid requirements. In: *Fish Energetics : New Perspectives*. (Eds.) Tytler, P. and Calow, P. John Hopkins University, Baltimore, Maryland. pp. 155-183.

Tacon, A.G.J. 1993. Feed ingredients for warmwater fish : Fish meal and other processed feedstuffs. *FAO Fisheries Circular*, 866 : 64p.

Tacon, A.G.J. 1994. Feed ingredients for carnivorous fish species : Alternatives to fishmeal and other fishery resources. *FAO Fisheries Circular*, 881 : 35p.

Tacon, A.G.J. 1995. Application of nutrient requirement data under practical conditions : special problems of intensive and semi-intensive fish farming systems. *J. Appl. Ichthyol.* **11** : 205-214.

Takeuchi, T.; Shiina, Y. and Watanabe, T. 1991. Suitable protein and lipid levels in diet for fingerlings of red seabream, *Pagrus major*. *Nippon Suisan Gakkaishi*, 57(2) : 293-299.

Tesch, F.W. 1968. Age and Growth. In : Ricker, W.E. *Methods for Assessment of Fish Production in Freshwater*. IBP Handbook Number 3, Blackwell Scientific Publication, Oxford, Edinburgh, pp. 93-123.

Teng, S-K.; Chua, T-E and Lim, P-E. 1978. Preliminary observation on the dietary protein requirement of estuary grouper, *Epinephelus salmoides* Maxwell, cultured in floating net-cages. *Aquaculture*, 15 : 257-271.

Thayer, G.W.; Schaaf, W.E.; Angelovic, J.W. and LaCroix, M.W. 1973. Caloric measurements of some estuarine organisms. *Fish. Bull.*, 71(1) : 289-296.

Tibaldi, E. and Lanari, D. 1991. Optimal dietary lysine levels for growth and protein utilization of fingerling sea bass (*Dicentrarchus labrax* L.) fed semi-purified diets. *Aquaculture*, 95 : 297-304.

Tibaldi, E.; Tulli, F. and Lanari, D. 1994. Arginine requirement and effect of different arginine and lysine levels for fingerling sea bass (*Dicentrarchus labrax*). *Aquaculture*, 127 : 207-218.

Tibaldi, E.; Beraldo, P; Volpelli, L.A and Pinosa, M. 1996. Growth response of juvenile dentex (*Dentex dentex* L.) to varying protein level and protein to lipid ratio in practical diets. *Aquaculture*, 139 :91-99.

Tucker, J.W., Jr., Mackinnon, M.R., Russell, D.J., O'Brien, J.J. and Cazzola, E. 1988. Growth of juvenile barramundi (*Lates calcarifer*) on dry feeds. *Prog. Fish-Cult.*, 50 : 81-85.

Tucker, C.S. and Robinson, E.H. 1990. *Channel Catfish Farming Handbook.* Van Nostrand Reinhold, New York, NY, 454pp.

Tucker, J.W. 1992. Marine fish nutrition. In : *Proceedings of Aquaculture Nutrition Workshop, Salamander Bay.* (Eds.) G.L Allan and W. Dall, pp. 25-40.

Uys, W. and Hecht, T. 1985. Evaluation and preparation of an optimal dry feed for the primary nursing of *Clarius gariepinus* larvae (Pisces:Clariidae). *Aquaculture*, 47 : 173-183.

Van Der Elst, R. 1988. *A Guide to the Common Sea Fishes of Southern Africa Second Edition.* Struik, Cape Town. pp. 209.

Van Der Westhuizen, H.C and Marais, J.F.K. 1977. Stomach content analysis of *Pomadasys commersonnii* from the Swartkops estuary (Pisces : Pomadasidae). *Zool. Afr.*, 12 : 500-504.

Viola, S.; Angeoni, H.; Gur, N. and Lahav, E. 1994a. Growth performance of hybrid tilapia fed two levels of lysine at three levels of protein. *Israeli J. Aqua.*, 46(4) : 212-222.

Viola, S.; Angeoni, H. and Lahav, E. 1994b. Present limits of protein sparing by amino acid supplementation of practical carp and tilapia feeds. *Israeli J. Aqua.*, 46(4) : 203-211.

Wallace, J.H. 1975. The estuarine fishes of the east coast of South Africa. I. Species composition and length distribution in the estuarine and marine environment. II. Seasonal abundance and migration. *Invest. Rep. Oceanogr. Res. Inst.*, 40 : 1-72.

Wallace, J.H. and Schleyer, M.H. 1979. Age determination in two important species of South African angling fishes, the kob (*Argyrosomus hololeidotus*), and the spotted grunter (*Pomadasys commersonnii*). *Trans. Roy. Soc. S. Afr.*, 44 : 15-26.

Wallace, J.H; Kok, H.M and Beckley, L.E. 1984. Inshore small-mesh trawling survey of the Cape south coast. Part 2. Occurrence of estuarine associated fishes. *S. Afr. J. Zool.*, 19 : 165-169.

Walton, M.J. 1985. Aspect of amino acid metabolism in teleost fish. In: *Nutrition and Feeding in Fish.* (Eds.) Cowey, C.B.; Mackie, A.M. and Bell, J.G. Academic Press, London. pp. 47-67.

Watanabe, T. 1982. Lipid Nutrition in Fish. *Comp. Biochem. Physiol.*, 73 : 3-15.

Watanabe, T. 1988. Nutrition and Growth. In : *Intensive Fish Farming.* (Eds.) Shepherd, J. and Bromage, N. BSP Professional Books, London, pp. 154-195.

Watanabe, T.; Sakamoto, H.; Abiru, M. and Yamashita, J. 1991a. Development of a New Type of Dry Pellet for Yellowtail. *Nippon Suisan Gakkaishi*, 57(5) : 891-897.

Watanabe, T.; Lee, M-J.; Mizutani,J.; Yamanda, T.; Satoh, S.; Takeuchi, T.; Yoshida, N.; Kitada, T. and Arakawa, T. 1991b. Effective components in cuttlefish meal and raw krill for improvement of quality of red seabream *Pagrus major* eggs. *Bull. Jap. Soc. Sci. Fish.*, 57 : 681-694.

Watanabe, T.; Viyakarn, V.; Kimura, H.; Ogawa, K.; Okamoto, N. and Iso, N. 1992. Utilization of soybean meal as a protein source in a newly developed soft-dry pellet for yellowtail. *Nippon Suisan Gakkaishi*, 58(9) : 1761-1773.

Watanabe, T. and Pongmaneerat, J. 1993. Potential of soybean meal as a protein source in extruded pellets for rainbow trout. *Nippon Suisan Gakkaishi*, 59(8) : 1415-1423.

Wee, K.L; Maguire, G.B and Hindrum, S.M. 1992. Methodology for digestibility studies with abalone. I. Preliminary studies on the feeding and defaecatory behaviour of blacklip abalone, *Haliotis rubra*, fed natural and artificial diets. In : G.L Allan and W. Dall (Eds.), *Proc. Aquaculture Nutrition Workshop.* pp. 192-196.

Whitfield, A.K. 1980. A quantitative study of the trophic relationships within the fish community of the Mhlanga estuary. *Est. Coast. Mar. Sci.*, **10** : 417-435.

Whitfield, A.K. 1990. Estuarine fishes, illustrated by the spotted grunter. *S. Afr. Nat. Sci. Rep.*, **167** : 73-75.

Wilson, R.P. 1985. Amino acid and protein requirements of fish. *Nutrition and Feeding in Fish*, Academic Press, London, pp. 1-16.

Wilson, R.P. 1989. Amino Acids and Proteins. In: *Fish Nutrition*. 2nd Edition. (Ed.) Halver, J.E, Academic Press, San Diego, pp. 111-151.

Wilson, R.P. 1991. Channel catfish, *Ictalurus punctatus*. In: *Handbook of Nutrient Requirements of Finfish*. (Ed.) Wilson, R.P., CRC Press, Boca Raton, pp. 35-53.

Wilson, R.P. and Poe, W.E. 1985. Relationship of whole body and egg essential amino acid patterns to amino acid requirement patterns in channel catfish, *Ictalurus punctatus*. *Comp. Biochem. Physiol.*, 80B : 385-388.

Windell, J.T.; Kitchell, J.E.; Norris, D.O.; Norris, J.S. and Foltz, J.W. 1976. Temperature and rate of gastric evacuation by rainbow trout. *Trans. Amer. Fish. Soc.*, **106** : 712-717.

Wurtsbaugh, W.A. and Davis, G.E. 1977. Effects of temperature and ration level on the growth and food conversion efficiency of *Salmo gairdneri* Richardson. *J. Fish Biol.*, **11** : 87-98.

Wurts, W.A. and Stickney, R.A. 1993. Growth rates of juvenile red drum *Sciaenops ocellatus* reared on commercial salmon feed in fresh and saltwater. *J. World Aqua. Soc.*, 24(3) : 422-424.

Zar, J.H. 1984. *Biostatistical Analysis.* Second Edition, Prentice-Hall, Inc., New Jersey, pp. 32.

Zeitler, M.H; Kirchgessner, M and Schwarz, F.J. 1984. Effects of different protein and energy supplies on carcass composition of carp (*Cyprinus carpio* L.). *Aquaculture*, 36 : 37-48.