INVESTIGATIONS INTO THE NUTRITIONAL REQUIREMENTS OF JUVENILE DUSKY KOB, *ARGYROSOMUS JAPONICUS* (PISCES: SCIAENIDAE), UNDER AMBIENT CULTURE CONDITIONS.

Submitted in fulfilment of the requirements for the degree of

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TABLE OF CONTENTS

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ABSTRACT

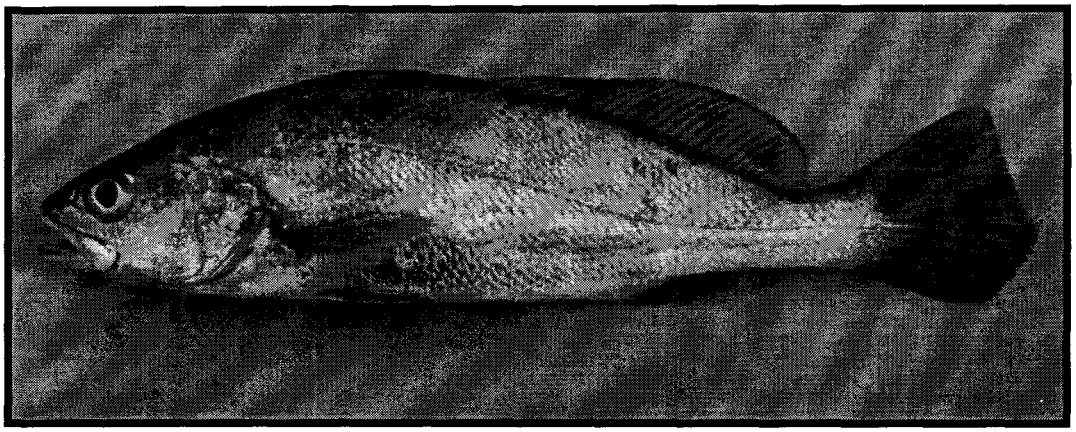
The effect of dietary protein, protein and energy ratios, fish meal replacement by Soya bean meal and feeding frequency was investigated on the growth, feed efficiency and body composition, of juvenile dusky kob, *Argyrosomus japonicus.*

The effect of dietary protein levels was investigated by comparing isocaloric diets containing 35, 40, 45 and 55% protein. Dietary protein inclusion level significantly affected specific growth rates, feed efficiency and body composition (in terms of fat deposition) and results show that a minimum of 45% and maximum of 52.3% dietary protein is optimal.

The protein and energy requirements were investigated by comparing three protein levels (35, 40 and 45%) with three lipid levels (6, 9 and 12%) in a 3x3 factorial design. A diet 45% protein and 9% lipid, with a P: E ratio of 29 mg/kJ and DE of 15.5 kJ/g resulted in an optimal specific growth rate (1.6± 0.2), feed efficiency (FCR= 1.7±0.44; PER= 1.36) and body composition for *A. japonicus.*

To investigate the effect of partial fish meal replacement by Soya bean meal (SBM) in the diet, 20-50% of the protein from fish meal was substituted by protein from SBM, and were compared with a control diet containing only fish meal. No significant differences in the specific growth rates, feed efficiencies and the body composition were recorded for the fish fed the control diet and for fish fed the 20 and 30% SBM treatments. Results show that up to 30% of the protein from fish meal can be replaced with protein from SBM in the diet of this species.

The effect of feeding frequency and gut evacuation time was investigated by comparing four feeding frequencies. Fish were fed to satiation either once, twice, three or four times a day. Feed intake was significantly affected by feeding frequency and fish fed 2, 3 and 4 meals a day ate significantly more food ($p< 0.05$) than the fish fed one meal a day. No significant differences ($p> 0.05$) in the specific growth rates, feed efficiencies and body composition were recorded for fish fed 2, 3 and 4 times a day. Feed intake was used to calculate an optimal daily ration and feeding two meals a day resulted in an optimal daily ration of 4% BW/day. A gut evacuation time of 7.25 hours indicates that fish fed twice a day probably had sufficient time to digest their food and evacuate their guts, resulting in the same daily feed intake as fish given 3 and 4 meals a day. Results conclude that under the present experimental conditions, a minimum daily ration of 4% BW/day, fed twice a day (in the morning and evening) is required for optimal growth (SGR= 1.33± 0.1), feed efficiency (FCR= 1.96± 0.4; PER= 1.44± 0.2) and body composition in juvenile dusky kob.



Frontispiece: Juvenile dusky kob, *Argyrosomus japonicus*

CHAPTER 1

GENERAL INTRODUCTION

The family Sciaenidae includes about 70 genera and 270 species that occur in temperate and tropical regions of the world (Nelson, 1994). Sciaenids are carnivorous fishes usually found in shallow coastal and estuarine water and are well represented in the Indo-Pacific, the Caribbean and in the temperate waters of the Atlantic and Pacific oceans (Nelson, 1994). Sciaenid fishes of the genus *Argyrosomus* occur in the eastern Atlantic and Indo-West Pacific regions and are important food species wherever they are found (Griffiths and Heemstra, 1995).

The dusky kob, *Argyrosomus japonicus* is a sciaenid fish found in both the southern and northern hemispheres (Griffiths and Hecht, 1995). It is found along the entire southern eastern seaboard of Australia and from Hong Kong northwards along the Chinese coast to southern Korea and Japan (Griffiths, 1997a). In Southern Africa (Figure 1.1) it occurs on the east coast from Cape Point to Mozambique, but is especially abundant between Cape Agulhas and Kwazulu Natal (Griffiths, 1996). In 1990, a project by M. H. Griffiths studying the biology of '*Argyrosomus hololepidotus*' revealed that two species *Argyrosomus japonicus* and *Argyrosomus inodorus* were being confused under this name (Griffiths and Heemstra, 1995).

A. japonicus is an important recreational and commercial linefish in South Africa and Australia and attains 50% sexual maturity at a total length (TL) of 107 cm (Griffiths, 1997a). The adults are found in estuaries and the surf zone but are generally restricted to the near shore zone of depths between 10m and 100m (Griffiths, 1997a).

Early juveniles (TL<15 cm) actively recruit into the upper reaches of estuaries, where salinities are low to moderate but later move to the lower reaches and into shallow, coastal waters (Griffiths, 1996). Ter Morshuizen *et al.* (1996) report that juvenile dusky kob in South Africa appear to be more abundant in turbid estuaries such as the Great Fish River than in clear marine dominated ones. The absence of early juvenile dusky kob from the non-turbid Swartvlei and Knysna estuaries (Whitfield and Kok, 1992) suggests the preference of turbid estuaries as nursery areas, as they provide increased protection from predators and adequate supplies of food (Griffiths, 1996). Older juveniles (15-107 cm TL) are found both in estuaries and associated surf zones (Griffiths, 1996).

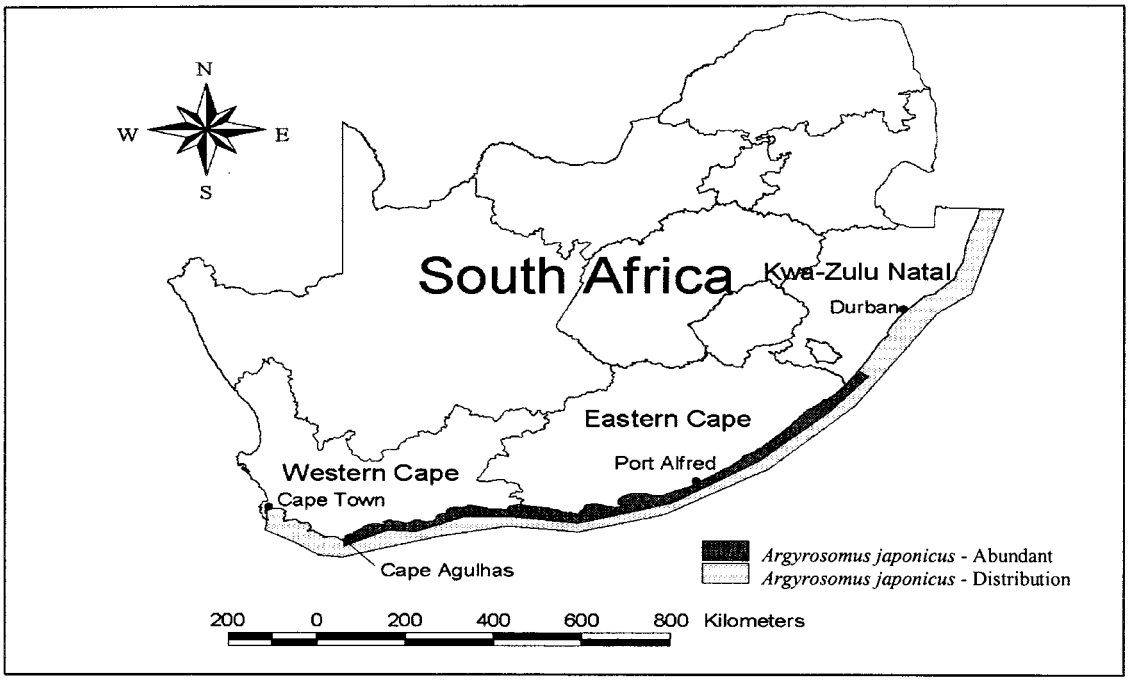


Figure 1.1: Map illustrating the areas of general distribution and abundance for *Argyrosomus japonicus* in South Africa.

There is good evidence to suggest that a large proportion of the adult population in the Cape regions migrate to Kwazulu Natal to spawn (Griffiths, 1996). However, spawning also occurs in the Cape waters in spring and summer and is thought to be induced by changes in water temperature (Griffiths, 1996).

In South Africa, *A. japonicus* lives in excess of 40 years and may attain a maximum length of 1.8m and weight of 75kg (Griffiths, 1997c). Dusky kob has a fast initial growth rate due to the fact that they reach 50% sexual maturity at a reasonably large size (Griffiths, 1996).

Due to its large size, palatability and food value, *A. japonicus* is targeted throughout its distribution (Gray and McDonall, 1993). In South Africa, per-recruit analyses has revealed that *A. japonicus* has been severely over fished and that the spawner biomass has collapsed to about 2.3% of pristine levels (Anon., 2000). In New South Wales, Australia, commercial catches of this species declined from 154 tons in 1992/93 to 88 tons in 1997/98 (Fielder *et al.,* 1999).

Because of declining catches and market demand in Australia, dusky kob (known as mulloway or jewfish) has been identified as a suitable candidate species for aquaculture (O'Sullivan and Ryan, 2001). This has lead to substantial research effort regarding the potential culture of this species. Battaglene *et al.* (1994) successfully induced pond held *A. japonicus* broodstock to spawn by injecting human chorionic gonadotrophin and managed to rear larvae to early juveniles (average weight 21g) in six months. Trials in brackish water ponds at the NSW Fisheries Research Institute in Port Stephens (O'Sullivan and Ryan, 2001) and cage trials in Port Alfred, South Africa (Hecht, personnel communication) have also produced encouraging results regarding the grow out of this species and show that the fish can be grown from 21g to 1.8kg in 16 months.

As in Australia, dusky kob has also been identified as a potential candidate aquaculture species in South Africa. As already mentioned, it is an excellent food fish with a fairly

good market price. In South Africa it currently sells for about 5 US dollar/kg (November 2003), Australia at approximately 6-9 US dollar/kg (November, 2003) and potential for the European and Asian export markets also exists. Dusky kob readily adapts to intensive culture systems and accepts formulated feeds (Hecht, personal communication). They are also euryhaline (Whitfield *et al.*, 1981) and Fielder *et al.* (1999) reported that *A.japonicus* larvae and early juveniles grew well in salinities ranging from 5 to 35 ppt.

Several sciaenid fish form the basis of commercial and recreational fisheries in tropical and temperate regions throughout the world (Rutherford *et al.*, 1989). In recent years several species such as red drum (*Sciaenops ocellatus*), white sea bass (*Atractoscion nobilis*), black drum (*Pogonias chromis*) and spotted sea trout (*Cynoscion nebulosus*) have all been cultured successfully for replenishment of natural populations. Therefore, besides the commercial reasons, the successful culture of this species may also contribute towards the rehabilitation of depleted natural stocks.

The success of a commercial aquaculture operation is dependent on a variety of factors within the fields of biology, engineering and economics (Lazo *et al.*, 1998). One of the most important biological aspects is nutrition or the availability of suitable diets. It is important that available food be efficiently digested and that it provides the required nutrients and supports good growth and overall health (Lazo *et al.*, 1998). Therefore if dusky kob is to be farmed on a commercial basis, it is crucial that the nutrition and feeding requirements be established.

The prime objective in feed formulation is to supply a nutrient density that supports optimal production (Hastings and Dickie, 1972). The first prerequisite of a nutritionally

complete diet is that it contains the optimal quantity and correct ratios of protein, carbohydrates and lipids (Phillips, 1972). These organic macronutrients can be used directly as metabolic fuels, can be stored in the body for use at a later stage or be deposited in the structural materials that represent somatic growth of the animal (Jobling, 2001). Protein, fat and carbohydrates, contribute to production according to their physiological calorific values (Steffens, 1989) and are essential for fish growth, reproduction and health. Any deficiencies or excesses in these substances can lead to reduced growth rates or diseases (NRC, 1993).

Feed represents the largest proportion of the production costs in intensive culture ranging between 30 and 50% of the total operational costs (Kaushik, 1990; Brown *et al.*, 1988). Formulated feeds prepared for intensively reared carnivorous fish typically contain 40-50% protein (Jobling *et al.*, 2001). Given that protein is the most expensive component in fish feed (Chen and Tsai, 1994), it is important that it be used for growth and not energy purposes. Austreng and Refstie (1979) define the optimal protein requirement of a cultured fish as the content of protein in the feed that produces maximum growth, maximum economic profit and maximum protein deposition.

The main contribution of dietary protein is to supply essential amino acids that are required by the fish for replenishment and growth of tissues (Kaushik, 1990). Essential amino acids are those that animals cannot synthesise, or cannot synthesise in sufficient quantity to enable the maintenance of good growth rates, whereas non-essential amino acids can be synthesised (as required) from other compounds (Jobling, 2001).

Dietary protein is also the major source of nitrogenous waste excreted by fish into the water column (Tibbets *et al.*, 2000). Thus, to reduce feed costs and the environmental

impact of fish farming, it is crucial that the supplied protein be used for tissue synthesis and not for maintenance energy.

The proximate composition of any animal provides a general indication of its nutritional status, and aids in defining their actual energy requirements (Marais, 1990). A literature review of the body composition as well as the proximate composition of the natural prey of dusky kob was undertaken. This information together with the protein and lipid requirements of other carnivorous fish species (Table 1.1) was used as the basis for the formulation of the diets in the protein requirement experiment (Chapter 3). Numerous authors have utilised various semi-purified and purified diets to estimate the protein requirements of fish and considerable variation in the results exist (Table 1.1).

Table 1.1: The protein and lipid requirements of various carnivorous marine finfish species.

Species	Protein (%)	Lipid (%)	Reference
Red drum, *Sciaenops ocellatus*	44-45	8-13	Webb & Gatlin (2003), Turano *et al.* (2002), Thoman *et al.* (1999)
European sea bass, *Dicentrarchus labrax*	44	12	Ballestrazzi *et al.* (1994)
Asian sea bass, *Lates calcarifer*	42.5	12	Catacutan & Coloso (1995)
Grouper, *Epinephelus malabaricus*	47.8	8	Chen & Tsai (1994)
Florida pompano, *Trachinotus carolinus*	45	8	Lazo *et al.* (1998)
Gilthead sea bream, *Sparus aurata*	45-50	12-24	Kissil *et al.* (2000)
Striped bass, *Morone saxatilis*	55	16.5	Milliken (1983)
Haddok, *Melanogrammus aeglefinus*	45	12	Kim & Lall (2001)
Spotted sand bass, *Paralabrax maculatofasciatus*	45	8.5	Gonzalez *et al.* (2001)

Stomach content analysis of juvenile dusky kob (< 50mm TL) in some Eastern Cape estuaries (Griffiths (1997a, 1997b; Marais 1984; Smale and Bruton, 1985) reveals that they feed predominantly on mysids (± 99%), but also on small fish (± 1%) such as *Gilchristella aestuarius*, mullet and Gobiidae. *Mesopodopsis slabberi* is the dominant mysid species. The proximate analysis of *M. slabberi* and *A. japonicus* are shown in Table 1.2.

Table 1.2: The proximate composition of the mysid, *Meseopodopsis slabberi* and juvenile dusky kob, *Argyrosomus japonicus* (Irish, 1997; Marais, 1990).

Species	Protein	Lipid	Ash	Energy (kJ/g)
M. slabberi	58.3	1.1	33.9	19.2
A. japonicus	72.7	10.7	16.5	5.5

It is now well established that the contribution of dietary proteins in meeting the energy requirements of fish is relatively high and that the optimisation of protein/energy ratios can considerably improve protein utilisation (Kaushik, 1990). The balance between protein and fat is important in that protein will be used for energy and not for growth if inadequate dietary energy is provided in the diet (Catacutan and Coloso, 1995). In order to minimize the utilization of protein as an energy source, other sources of digestible energy must be supplied (Turano *et al.*, 2002). Carbohydrates and lipids are highly available sources of energy (Turano *et al.*, 2002). However, due to the low digestibility of carbohydrates by most fish species, their use as an energy source is less understood than lipids (Turano *et al.*, 2002). Lipids, specifically marine fish oils are easily digested and provide the n-3 highly unsaturated fatty acids required by marine fish (Sargent *et al.*, 1999).

Increasing the lipid (energy) level in the diet has been shown as a means to spare protein and limit ammonia production in fish species such as carp and Atlantic salmon (McGoogan and Gatlin, 2000). But, if dietary fat levels are excessively high in comparison to protein levels, carcass lipids may be markedly elevated (Page and Andrews, 1973), limiting attractiveness to consumers (McGoogan and Gatlin, 2000). It is thus critical that optimal protein and energy ratios be established so that protein is not wasted, growth is optimal and fat deposition is minimal.

Fish meal has traditionally been the major protein source in fish feeds (Boonyaratpalin *et al.*, 1998). However, because of the recent increase in demand and uncertain availability (Barlow, 1989) the price has increased. Recent debate has also centred on the use of fish meal in aquaculture. Naylor *et al.* (2000) state that many intensive and semi-intensive aquaculture systems use more fish protein (in the form of fish meal) to feed the farmed species, than is supplied from the farmed product. However, according to Tidwell and Allan (2001) fish meal production over the last 15 years has changed very little and that aquaculture has not increased the amount of pelagic fish caught for fish meal manufacture. Nevertheless, manufacturers often over-formulate feeds (in terms of fish meal protein levels), as the information on the dietary requirements for a particular fish species may be inadequate (Naylor, 2001). This emphasises the need to establish nutritionally cost-effective diets. One way of reducing feed costs is to substitute fish meal with a less expensive ingredient such as Soya bean meal (Reigh and Ellis, 1992).

Soya bean meal (SBM) is the most commonly studied replacement of fish meal in both freshwater and marine fish diets (Kaushik, 1990). SBM has a high protein content and favourable amino acid profile (that closely meets the essential amino acid requirements of

fish) is consistently available, relatively cheap and is reported to be palatable to most fish species (Lim and Akiyama, 1992). Many studies have been carried out on various fish species to examine the effects of partial or total replacement of fish meal by Soya bean meal. Results show that generally the incorporation of high levels of SBM in the diets lead to reduced growth performance and feed efficiency (Peres *et al.*, 2003). Tacon (1994) suggests that the poor utilization of high levels of SBM in the diet may be attributed to factors such as improper balance of nutrients (such as amino acids, energy and minerals), lower digestibility, reduced diet palatability and the presence of anti-nutritional factors such as trypsin inhibitors and lectins. However, the common processing techniques of wet and dry heating have been widely and successfully used to reduce the concentration of antinutrients found within plant feeds (Francis *et al.*, 2001). To construct a diet as economically as possible and to reduce the level of fish meal in the diet, the effect of partial fish meal replacement was therefore also investigated.

A basic principle in nutritional theory is that animals eat to satisfy energy requirements (Smith, 1989). Efficient production and growth depend on the fish being fed the best possible diet at levels not exceeding their dietary needs (Charles *et al.*, 1984). Given that feed costs account for a large proportion of the operating costs in intensive culture systems, the size of the daily ration and the frequency of feedings are important management factors that influence growth, feed conversion and the economics of a farming operation (Kaushik and Gomes, 1988). Increased feeding frequency has been shown to improve the growth of numerous fish species (Andrews and Page, 1975; Kayano *et al.*, 1993; Wang *et al.*, 1998) and the variation in reported optimal feeding frequencies can be attributed to fish size (Ruohonen *et al.*, 1998), species (Jobling, 1982),

temperature (Jobling and Davies, 1979), diet properties and the return of appetite (Jobling, 1982).

The amount of food eaten in a meal, the satiation time and the return of appetite are important factors closely related to feeding frequency (Grove *et al.*, 1978). It is desirable to have information on the rate at which ingested food is processed in the digestive tract (Jobling and Davies, 1979), as gastrointestinal evacuation directly influences the amount of food eaten (Storebakken *et al.,* 1999). Studies on numerous fish species suggest that there is a close correlation between the time required to empty the stomach and the return of appetite (Grove *et al.*, 1978). With this in mind, the aim of the final experiment (Chapter 6) was to determine the optimum feeding frequency as well as the gut evacuation rate.

As far as could be ascertained, no data on the nutritional requirements of *A. japonicus* has been published in the primary literature. To achieve optimal growth rates, feed efficiency, body composition and maximum survival, it is vital that these basic nutritional requirements be understood. The overall aim of this study was to establish these basic nutritional requirements, in terms of dietary protein content, protein and energy ratios, fish meal substitution and feeding frequency, and to use this information as a basic "starting point" for the construction of a practical diet for *A. japonicus* for the grow out phase.

The thesis is structured as follows:

Chapter 2 summarises the general methods, fish capture, acclimation and the system used. The dietary protein requirement of juvenile *A. japonicus* is established in Chapter 3.

The results of Chapter 3 are then used as the basis to determine the optimal protein: energy (P: E) requirement in Chapter 4. Based on the optimal P: E results, various isocaloric diets were constructed to examine the effect of partial fish meal replacement by Soya bean meal in the diet (Chapter 5). Chapter 6 investigates the effect of feeding frequency and gut evacuation times using a standard diet containing the established protein and energy levels. Chapter 7 then discusses each experiment separately and examines its contribution to the study as a whole.

CHAPTER 2

GENERAL MATERIALS AND METHODS

Fish capture, weaning and feeding.

Juvenile dusky kob, *Argyrosomus japonicus* were caught in the Great Fish River Estuary (Eastern Cape- 33°30 S, 27°07 E) using a 20m seine net (mesh size <10mm) between the months of January and March in 2002 and 2003. Captured fish were anaesthetized with 2-phenoxyethanol and transported to the Department of Ichthyology and Fisheries Science (DIFS) marine laboratory in Port Alfred. Deacon *et al.* (1997) reported that the routine anaesthesia of 2-phenoxyethanol on spotted grunter had no significant effect on the growth and it was decided that a similar 2-phenoxyethanol concentration of 0.2ml/l would be suitable for use in this study.

Once the fish in the system were actively feeding on finely grated pilchards (*Sardinops sagax*), they were weaned onto dry pellets. Two weeks was decided as an adequate weaning period as Thoman *et al.* (1999) noted that it took about two weeks for red drum to be weaned from a natural minced fish diet to a dry pellet. The fish were weaned onto a trout starter pellet (Table 2.1) by slowly adding a small amount of crumble/pellet to the grated pilchard and then slowly (over the 2 week period) reducing the amount of grated pilchard in the mixture and increasing the crumble/pellet proportion. The fish were considered to be weaned when they readily accepted a diet consisting only of dry pellets.

Table 2.1: Crude proximate composition (%) of the "Aquanutro" trout starter pellet.

Protein	> 45
Fat	> 14
Fibre	< 3
Calcium	< 3
Phosphorus	> 0.7

At the start of each experiment individual fish were measured (Total length to the nearest mm) and weighed (to the nearest gram) and then randomly assigned to a basket (see System design and management below). Each dietary treatment (in all experiments) was replicated three times (3 baskets per treatment) such that 36 fish were used per dietary treatment. All fish were anaesthetized with a 0.2ml/L solution of 2-phenoxyethanol before being weighed and measured.

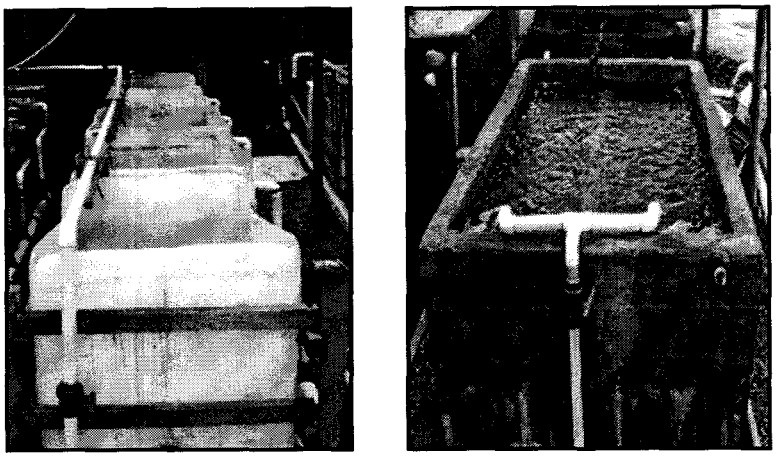
In the nutrition experiments the fish were fed to satiation twice a day at 09h00 and 18h00 hours. Fish in each tank were assumed to be satiated once active foraging at the surface ceased and pellets landed on the tank bottom and were not eaten within 2 minutes.
In similar experiments with red drum, *Sciaenops ocellatus* (Moon and Gatlin, 1994; Turano *et al.,* 2002); European sea bass, *Dicentrarchus labrax* (Peres and Teles, 1999); grouper, *Epinephelus malabaricus* (Chen and Tsai, 1994); Atlantic croaker, *Micropogonias undulatus* (Davis and Arnold, 1997) and Florida pompano, *Trachinotus carolinus* (Lazo *et al.*, 1998), the fish were all hand fed twice a day.

System design and management

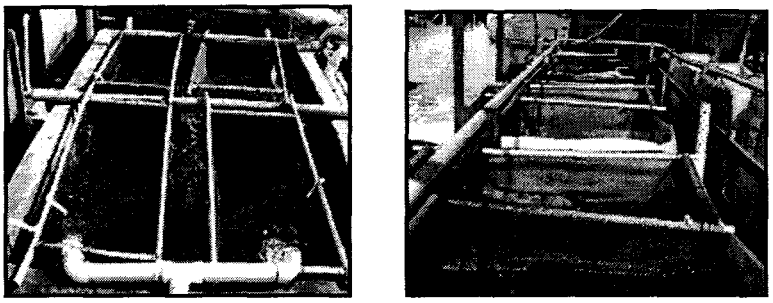
All the experiments were undertaken at the Rhodes University Marine Laboratory in Port Alfred in two semi-recirculating systems (Figure 2.1).

System 1 consisted of ten 1000 litre plastic tubs, linked via a 4.5 kW pump to a biological filter with an operating volume of approximately 4000 litres, while system 2 consisted of four 1500 litre concrete tanks and eight 1000 litre plastic tubs, also linked via a 4.5 kW pump to a 4000 litre biological filter. Both systems were supplied with water from the Kowie River Estuary by a 4.5kW pump at a rate of 40 litres per minute and filtered water for each system was then supplied at a flow rate of approximately 20 litres per minute to each individual tank. The tanks were not aerated due to the high flow rate (and continuous supply of fresh water from the Kowie Estuary) and individual tanks were cleaned as required.

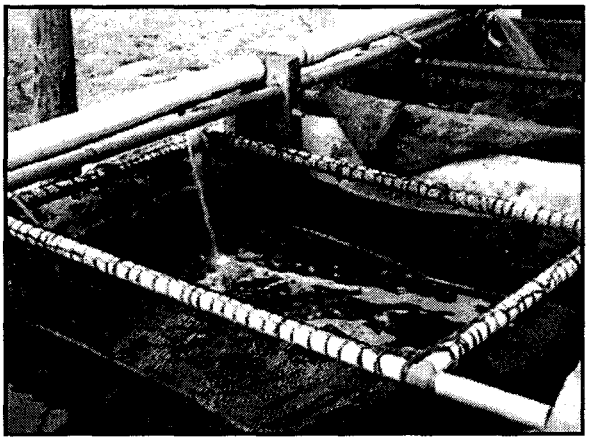
All experiments were undertaken by placing covered plastic mesh baskets ($0.16m^3$ volume, mesh size 5mm) within the tubs or concrete tanks (Picture 2.1, 2.2 and 2.3). Working in baskets allowed for a much quicker handling time when fish were weighed and measured as the baskets (with fish in) were simply removed from the tub/tank and placed in a bath containing 2-phenoxyethanol for anaesthesia. In this way fish only needed to be handled once and the time taken to weigh and measure was decreased, thereby reducing stress.



Picture 2.1: A row of 1000 litre plastic tubs in system 1 and one of the concrete tanks in system 2.



Picture 2.2: Plastic mesh baskets in system 2 (left) and system 1 (right).



Picture 2.3: A close up of a plastic mesh basket in system 1.

Table 2.2: Water quality parameters maintained for the entire duration of the study.

Ammonia (mg/L)	< 0.1
Nitrate (mg/L)	< 20
Nitrite (mg/L)	< 0.1
pH	8.0
Oxygen (%)	80-100

Diet formulation and preparation

The protein sources used in the experimental diets were Casein (80.1% crude protein) and low-temperature Danish fish meal (67.8% crude protein). Soya oil seed cake (47% crude protein) was used in the fish meal replacement experiment. Marine fish oil and soy oil were used as lipid sources and Alpha cellulose was used as a non-nutritive filler, where necessary. Pre-gelatinised starch was used as the carbohydrate source and also doubled up as a binding agent.

The measured dry ingredients of the experimental diets were homogenized and mixed thoroughly. The oil and water were added to the mixed dry ingredients and kneaded by hand to form a consistent dough. The dough was then placed in a commercial food mixer and mechanically kneaded for a further 5-10 minutes before being extruded through a 5mm die. Strands of the extruded diet were then sliced into small pellets approximately 5mm long and sun dried. Diets for each replicate group of fish were kept in individual sealable containers in a freezer until used and containers were weighed prior to each feeding to determine the daily food consumption of the fish in each basket.

Response monitoring

The following growth and nutritional indices were used to evaluate the performance of the fish during the various experiments:

Specific growth rate (SGR): (Ricker, 1979)

SGR = [(Ln (final weight g) – Ln (Initial weight g) / no of days] x 100

Food conversion ratio (FCR): (Cowey, 1992)

FCR = food intake (dry weight g) / body weight gain (wet weight g)

Protein efficiency ratio (PER): (Wilson, 1989)

PER = body weight gain (wet weight g) / protein ingested (grams)

Condition factor (CF): (Bolger and Connolly, 1989)

CF = [body weight (wet weight g) / total length$^{2.84}$] x 10^5

This relationship was determined from a length mass regression for wild fish, where $y = 2.84x - 4.633$ ($r^2 = 0.96$, n = 52) with a fish size range of 8-101g.

Proximate analysis

Prior to the commencement of each experiment 6 randomly selected fish were killed and frozen for proximate analysis and once the experimental period was complete, a further 2 fish (6 per dietary treatment) from each replicate were killed and frozen for analysis.

Proximate analysis was also undertaken on all experimental diets and the following methods were used:

Protein

Protein content analysis was determined using the Micro-Kjeldahl method (Jobling, 2001):

- Approximately 100g of sample were weighed into digestion flasks.
- 2.5g Selenium catalyst and 2.5ml of concentrated sulphuric acid was then added to each flask, including the blanks.
- These flasks were placed on the pre-heated block and washed with 1ml hydrogen peroxide every 10 minutes for 60 minutes.
- Upon completion of the final wash, the digestion flasks 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 and 10ml of 1% Boric acid mixture with indicator was pipetted into each flask.
- Approximately 10ml of distilled water was added to each digestion flask and the contents were then 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 HC*l*

solution to the grey end point and the percentage nitrogen was then calculated from the volume of HCl titrated according to the formula:

N = (M HCl * 14.007 * 100)/ weight of sample (mg) and the percentage protein was calculated as: N x 6.25

Fat

Fat content was determined using the method described by Knauer *et al.* (1994), modified from the chloroform-methanol extraction method (Folch *et al.*, 1957):

- Sacrificed fish were dried to a constant weight at 70°C and then ground with a pestle and mortar.
- Five 0.2g samples of powdered fish were then measured into centrifuge tubes and rehydrated with 3ml distilled water.
- 6.25ml of methanol and 6.25ml of chloroform were then added to each of these solutions and the solutions were homogenized for 2 minutes, after which 6.25ml distilled water was added.
- The solutions were homogenized for another minute and then centrifuged at 3000g for 10 minutes.
- 0.75ml of the bottom layer from each of the solutions was then 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, cooled in a dessicator and then weighed. Percentage fat was then calculated according to the formula:

  % Fat = ((mass of fat (g) * 25/15)/ mass of sample (g))*100

Moisture

- The moisture content was determined by weighing three 1-gram samples before and after drying at 70°C until a constant weight was achieved.

Ash

- Ash content was determined by burning three 0.5g (dried at 70°C) powdered samples in open crucibles in a muffle furnace at 550°C for 7 hours
- The samples were then cooled in a dessicator before weighing (prior to use the crucibles are burnt at 550°C for 8 hrs to prevent contamination).

Energy

The energy content was the calorific value (CV) of the diets and was measured using a CP400 Calorimeter systems apparatus:

- The machine was calibrated at a standard calorific value of 26.454 MJ/kg (using the CV of 0.5g Benzoic acid).
- Samples were dried and weighed and placed individually into the bomb.
- Prior to ignition, pure oxygen at a pressure of 30 bars was pumped into the bomb and the CV of each sample was measured in MJ/kg, read after ignition.

Hepatosomatic index (HSI)

The livers from two fish per tank (6 per dietary treatment) were removed and weighed and used to calculate the HSI. Excessive essential amino acids in the diet cause a decrease in the HSI (Nematipour *et al.*, 1992) and are measured as:

HSI = [liver weight (g)/ total body weight (g)] x 100

Intraperitoneal fat (IPF)

The intraperitoneal fat from two fish (6 per dietary treatment) was removed and weighed. This was used to give an indication of fat deposition (Nematipour *et al.*, 1992) within the body and is calculated as:

IPF ratio = [intraperitoneal fat weight (g)/ total body weight (g)] x 100

Statistical analysis

Following the assumptions for ANOVA tests, the Kolmogorov-Smirnov test was used to check if data was normally distributed and the Levene Test was used to check for homogeneity in variances. A one-way ANOVA was used in all the experiments to test for variation between replicates and if no significant differences were detected then data was pooled. A one-way ANOVA was then used to test for differences between treatments. The Tukey HSD multiple comparison test was then used to evaluate the difference in means between dietary treatments at the 0.05 significance level (Zar, 1984). All statistical analyses were performed using STATISTICA version 6.1 (Statsoft, Tulsa, OK, USA).

CHAPTER 3

THE EFFECT OF DIETARY PROTEIN LEVEL ON GROWTH AND BODY COMPOSITION OF JUVENILE *ARGRYSOMUS JAPONICUS*

Introduction

Proteins are the major organic material in animal tissue, making up approximately 65-75% of the total dry weight (Wilson, 1989). The protein allowances in fish diets are generally higher than those of terrestrial warm-blooded animals because a higher percentage of digestible energy found within proteins, is metabolizable by fish (Lovell, 1989). Fish like other animals, must consume protein to furnish a continual supply of essential amino acids (NRC, 1983).

The dietary protein requirement of a species is one of the most important considerations in aquaculture (NRC, 1983). A continuous supply of amino acids is required to build new tissue (for growth and reproduction), to repair worn tissues, for enzymatic function (Mertz, 1972) and to replace existing proteins used in maintenance (Robinson, 1988). When the requirement for amino acids has been met, excess dietary protein is metabolized for energy (Lochman and Phillips, 1994) as fish have the ability to use protein efficiently as an energy source. It is therefore crucial when formulating diets to minimise the amount of protein used for energy, as protein is the most expensive component in formulated marine fish feeds (Kim and Lall, 2001). The energy requirements should be met by less expensive sources such as lipids and carbohydrates.

Excessive protein, improper protein: energy ratios and imbalanced essential amino acids (within the protein sources), results in excess nitrogenous waste and this can profoundly

affect fish growth and feed costs by decreasing feed performance and overall efficiency (Turano *et al.*, 2002). Optimisation of dietary protein levels, along with increased nutrient retention by the fish, therefore has the ability to reduce nitrogen loading (by reducing the amount of protein that is catabolized into ammonia) and to positively influence production costs by improving feed efficiency (Thoman *et al.*, 1999).

A deficiency of protein in the diet results in a reduction or cessation of growth and a loss of weight (Robinson, 1988) due to the withdrawal of protein from less vital tissues to maintain the functions of more vital bodily functions (Wilson, 1989).

Expressed as percent of dry weight, the concentration of protein in the diet that results in maximum growth is the most frequently used measure of the protein requirement in fish (Bowen, 1987). However, this protein requirement can be influenced by various dietary factors such as dietary protein-energy ratios, protein quality (digestibility, source, availability and balance of amino acids), energy sources (lipids and carbohydrates) as well as factors such as fish size and age, temperature and salinity (Milliken, 1982). It is therefore crucial when determining protein requirements to standardise environmental conditions and dietary ingredients, especially energy sources (Kim *et al.*, 1991).

The optimal dietary protein requirements of several carnivorous fish species have been determined under a variety of conditions and the factors mentioned above may explain the discrepancies in reported values (Table 3.1). Generally, the protein requirements of fish decreases with increasing size and age (Lovell, 1988). In channel catfish, the fry require about 40% protein in the diet, while fingerlings require 30-35% and larger fish about 25-35% (Page and Andrews, 1973). It is therefore important that when evaluating

the protein requirements of various fish, similar size fish be compared. In Table 3.1 the fish referenced were all within a size range comparable in this study.

The protein requirements of the species listed in Table 3.1, together with the information on the stomach content analysis of dusky kob (Marais, 1984; Smale and Bruton, 1985) discussed in Chapter 1 was used to obtain the range of protein inclusion levels (35-55%) compared in this experiment. The standard lipid level of 9% in all diets was based on the literature summarised in Table 1.1 (Chapter 1) and especially the lipid requirements of red drum.

The objective of this study was to investigate the effects of dietary protein level on growth performance, feed efficiency and whole body composition of juvenile *A.japonicus*.

Table 3.1: Protein requirements of various cultured carnivorous marine fish.

Species	Protein requirements (% dry wt)	Average weight (g)	Reference
Red drum, *Sciaenops ocellatus*	45	3.4	Webb and Gatlin (2003)
	44	186	Turano *et al.* (2002)
	40	2	Serrano *et al.* (1992)
	44	50	Thoman *et al.* (1999)
	44	52	Daniels & Robinson (1986)
Juvenile rockfish, *Sebastes schlegeli*	42	22	Lee *et al.* (2002)
Juvenile spotted sand bass, *Paralabrax maculato-fasciatus*	45	9.5	Gonzalez *et al.* (2001)
Juvenile haddok, *Melanogrammus malabaricus*	45	7	Kim & Lall (2001)
European sea bass, *Dicentrarchus labrax*	48	7	Peres & Teles (1999)
	44	76	Ballestrazzi *et al.* (1994)
Juvenile Florida pompano, *Trachinotus carolinus*	45	4.5	Lazo *et al.* (1998)
Asian sea bass, *Lates calcarifer*	42.5	0.9	Catacutan & Coloso (1995)
Malabar grouper, *Epinephelus malabaricus*	48	3.8	Chen & Tsai (1994)
Striped bass, *Morone saxatilisz*	55	1.4	Milliken (1982)
Gilthead sea bream, *Sparus aurata*	40	12-24	Sabaut & Luquet (1973) Kissil (2000)

Materials and Methods

Experimental design

All the fish were captured, acclimated and weaned onto pellets as explained in Chapter 2.

At the start of the 80-day growth trial, 144 juvenile dusky kob with an average (± standard deviation) length of 133.6 ± 17.9mm and weight of 24.8 ± 9.8g, were randomly distributed among 12 baskets. Three replicates of 12 fish (36 per dietary treatment) were fed each diet and individual fish in each basket were weighed (g) and measured (Total length- mm) at the beginning, every 25 days thereafter, and upon completion of the feeding trial.

The fish in each basket were hand fed to satiation twice a day (see Chapter 2) and were assumed to be satiated once active foraging at the surface ceased. The food for each tank was kept in individual containers and these were weighed at the beginning and end of each experimental day.

Several water quality parameters were measured once a week and were maintained within the desired levels outlined in chapter 2. Water temperature and salinity were measured daily. The mean ± standard deviation of temperature and salinity during the 80 day trial were 15.7 ± 2.6°C and 30.2 ± 9.6 ppt, respectively. There was a period where salinity dropped to 5 ppt for about 3 days due to flooding in the Kowie Estuary. However this seemed to have no effect on the fish as no mortalities were recorded and feeding persisted.

At the end of the experiment, two fish from each replicate (6 per dietary treatment) were randomly sampled and killed for proximate analysis. Proximate analysis was undertaken following the methods outlined in chapter 2.

Experimental diets

Four isocaloric (14.5± 0.23 kJ/g) diets were formulated to supply calculated levels of 35, 40, 45 and 55% protein according to the methods outlined in chapter 2. Since digestible energy values of the dietary ingredients have not yet been determined for *A. japonicus*, digestible energy was calculated based on estimated values used in red drum and rainbow trout, of 16.7, 16.7 and 37.7 kJ/g for protein, carbohydrate and lipid respectively (Lee and Putnam, 1973, Serrano *et al.*, 1992). The percent protein in each diet was calculated on a dry matter basis according to the total percentage of crude protein found within casein (80.1% crude protein) and low temperature Danish fish meal (67.8% crude protein). All diets were made isocaloric by adjusting the starch (pre-gelatinised) and cellulose component of the ration in relation to the protein component and by keeping the lipid proportion identical (Table 3.2).

As mentioned, dietary lipid level was fixed at 9% in all the diets as the dietary lipid requirement of cultured carnivorous fish species ranges between 8 and 12% (Table 1.1, Chapter 1).

The composition and proximate analysis of the four test diets are shown in Table 3.2.

Statistical analysis

Specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (CF) were all calculated using the methods outlined in chapter 2.

The Kolmogorov-Smirnov test was used to verify if data was normally distributed and the Levene Test was used to check for homogeneity in variances. Data from the replicates within each dietary treatment were analysed and compared for significant differences

with a one-way ANOVA. If no significant differences were detected between replicates the data were pooled such that comparisons (using ANOVA) could be made between dietary treatments. Tukey's multiple comparison test was used to evaluate the mean difference among individual diets at the 0.05 significance level.

Specific growth rate data was also modelled by polynomial regression analysis so as to calculate at what protein percent SGR was optimal.

Table 3.2: Formulation and proximate composition of the experimental diets (g/100g dry wt).

	Dietary protein level %			
Ingredients	35	40	45	55
Fish meal[a]	31	24	18	10
Casein	18	39	41	60
PGS[b]	36	31	26	15
Marine fish oil	5.9	6.6	7.2	8
Mineral mix[c]	4	4	4	4
Vitamin mix[d]	2	2	2	2
α- Cellulose	3.1	2.4	1.8	1
Calculated %				
Protein	35.5	40.3	45.0	54.8
Lipid	9	9	9	9
Energy (kJ/g)	14.2	14.4	14.6	14.7
Proximate composition				
Crude protein (%)	33.6	39.5	46.8	56.8
Ash (%)	11.2	11.8	12.3	11.7
Lipid (%)	8.7	9.3	9.4	9.1
Gross E (MJ/kg)	18.8	18.6	19.7	19.3

[a] Danish fish meal (low temperature)

[b] Pre-gelatinised starch

[c] Mineral mix (g/kg)- 516g vermiculite, 74g potassium, 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, traces of selenium.

[d] 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

Percentage weight gain, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (CF) responses of *A. japonicus* fed the graded dietary protein levels are presented in Table 3.3.

Table 3.3: The effect of dietary protein levels on weight gain, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (CF) of juvenile *Argyrosomus japonicus* fed for 80 days.

Protein level (%)	35	40	45	55
Initial weight (g)	25.19±1.01	27.65±0.98	23.67±2.22	22.80±0.71
Final weight (g)	35.53±1.92	42.27±1.06	44.13±5.35	41.39±0.65
% Weight gain	41.1±6.0^{a}	53.1±8.8^{a}	86.3±12.5^{b}	81.6±2.8^{b}
Initial length (mm)	133.2±18.5	136.9±20.1	132.9±16.1	131.5±17.4
Final length (mm)	145.9±18.9	148.7±18.6	154.1±16.7	147.4±15.9
SGR	0.44±0.05^{a}	0.54±0.07^{a}	0.79±0.09^{b}	0.75±0.02^{b}
FCR	1.71±0.17^{a}	1.25±0.26^{a}	1.36±0.27^{a}	1.78±0.08^{a}
PER	1.69±0.17^{abc}	2.05±0.39^{b}	1.69±0.33^{abc}	1.02±0.04^{c}
Initial CF	2.73±0.26	2.74±0.40	2.55±0.25	2.59±0.53
Final CF	3.05±0.57^{a}	3.46±0.6^{b}	3.21±0.19^{b}	3.49±0.51^{b}

Data are mean values ± SD of three replicates. Values in each row having the same superscript are not significantly different ($p > 0.05$).

Weight gain in the fish fed dietary protein levels of 45% and 55% was significantly greater ($p<0.05$) than those fed the 35% and 40% protein diets. No significant differences

($p>0.05$) in weight gain were detected between the 45% and 55% dietary protein treatments (Table 3.3).

The specific growth rate of the fish in the 45% and 55% protein treatments was significantly higher ($p<0.05$) than those in the 35% and 40% protein treatments. The 45% protein diet produced the highest SGR but this did not differ significantly ($p>0.05$) from the 55% protein diet (Table 3.3). The growth response calculated by polynomial regression showed that maximum SGR (x_{max}) was attained at a level of 52.3% dietary protein (Figure 3.1).

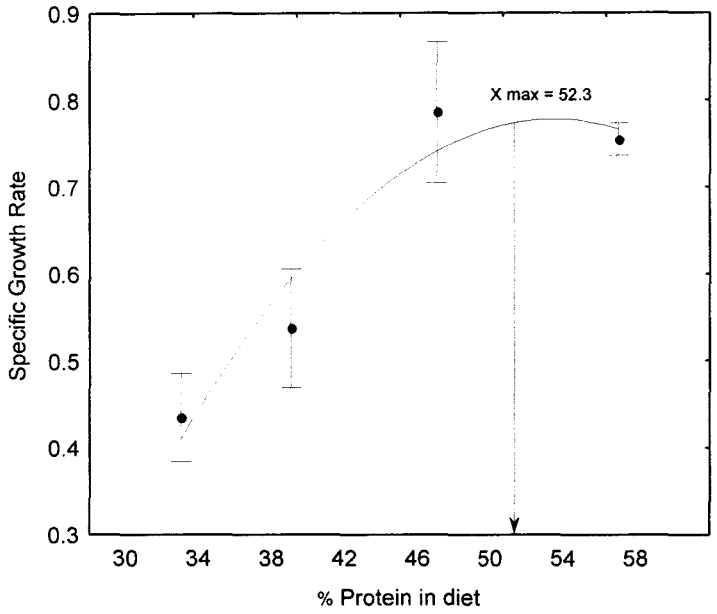


Figure 3.1: A second order polynomial relation between specific growth rate (y) and measured percent protein (x) in the diet for juvenile *Argyrosomus japonicus,* fed for 80 days.

$y = -1.7377 + 0.0942x + 0.0009x^2$, $r^2 = 0.80$. Data points represent the means and vertical bars the standard deviation. X_{max} is the percent protein in the diet required for maximum specific growth rate.

The best food conversion ratios were obtained for the 40% and 45% dietary protein levels (Figure 3.2). However, the FCR values for all dietary treatments did not differ significantly ($p>0.05$).

There was a general decrease in protein efficiency ratio with increasing protein levels (Figure 3.3). The PER of the 40% protein treatment was significantly higher than of the 55% protein treatment.

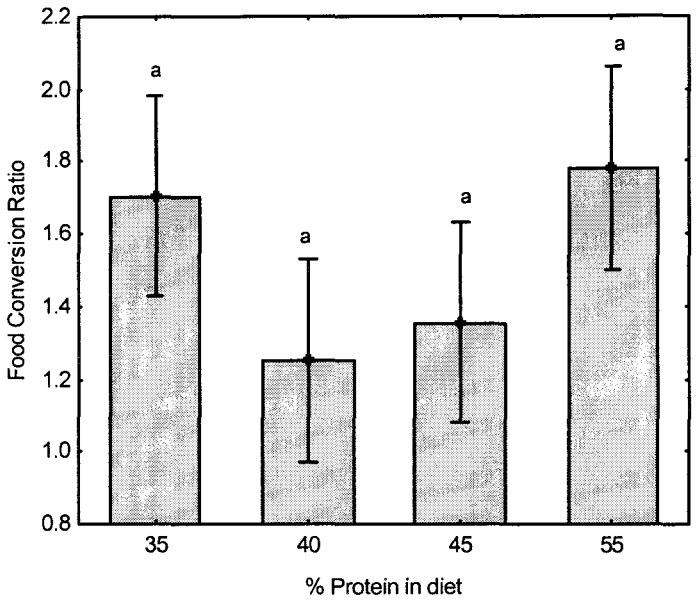


Figure 3.2: Food conversion ratios (FCR) of juvenile *Argyrosomus japonicus* at four protein levels. Error bars denote 95% confidence intervals. Significant differences between means are represented by non-overlapping bars and different superscripts.

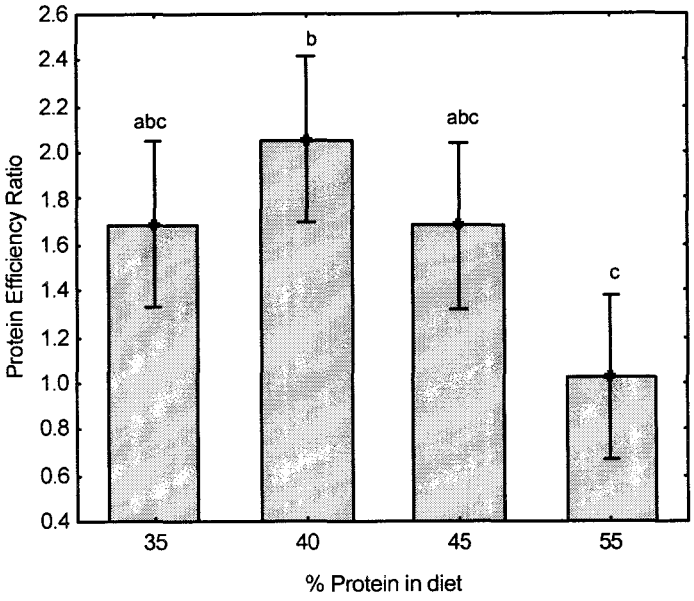


Figure 3.3: Protein efficiency ratios (PER) of juvenile *Argyrosomus japonicus* at four protein levels. Error bars denote 95% confidence intervals. Significant differences between means are represented by non-overlapping bars.

The condition factor of the fish in all treatments at the end of the growth trial was significantly higher than at the start of the experiment ($p<0.05$). At the end of the growth trial the condition factor of the 35% protein treatment was significantly lower ($p<0.05$) than the other diets (Table 3.3).

The proximate composition of the fish at the end of the 80-day feeding trial is summarized in Table 3.4. The various dietary protein levels did not significantly affect the moisture, protein and ash contents of the fish. The fish in the 35% protein treatment had significantly less fat ($p< 0.05$) than the fish fed the other diets.

Table 3.4: Proximate composition of *Argyrosomus japonicus* fed at four protein levels after 80 days.

Protein %	Moisture %	Protein %	Fat %	Ash %
35	72.8±0.4	62.8± 1.69[a]	8.95± 0.29[b]	16.64±0.2[b]
40	71.7±0.5	63.59± 0.95[a]	9.7± 0.18[a]	17.11±0.2[b]
45	71.4±0.7	63.23± 1.33[a]	9.9± 0.53[a]	17.09±0.6[b]
55	72.2±0.6	63.71± 0.8[a]	9.79± 0.39[a]	17.17±0.2[b]

Data are mean values ± SD of three replicates. Mean values in each column having the same superscript are not significantly different ($p > 0.05$).

Discussion

Under the ambient experimental conditions it would appear that juvenile dusky kob require a minimum dietary protein level of 45% for maximum growth in terms of weight gain and specific growth rate. Comparisons of protein requirements amongst different fish species is complicated by differences in fish size, diet formulation and variable culture conditions (Elangovan and Shim, 1997). However, the value reported here for juvenile *A. japonicus* fares within the 40-55% range reported by numerous authors for a variety of cultured carnivorous fish species (Table 3.1).

The specific growth rate of *A.japonicus* increased significantly with increasing dietary protein levels up to 52.3%, where after it decreased. Dietary protein levels above those required for maximum growth have been shown to reduce growth performances in various other species, e.g. juvenile American eel, *Anguilla rostrata* (Tibbets *et al.*, 2000), juvenile grouper, *Epinephelus malabaricus* (Chen and Tsai, 1994) and plaice, *Pleuronectes platessa* (Cowey *et al.*, 1972). Excess protein is not used for normal

metabolic functions, such as tissue growth, but is instead used as a comparatively inefficient energy source (Tibbets *et al.*, 2000).

Decreasing protein efficiency with increasing protein levels in the diet has been reported for other marine fish species (Table 3.5). Similar PER values (Table 3.5) obtained in this experiment with *A.japonicus* indicate that the protein in the diets was used efficiently. Since significant differences were observed in the PER between the 40 and 55% protein treatments it was assumed that a certain proportion of the dietary protein was not assimilated and was used for energy, deposited as fat, or deaminated and excreted as ammonia.

Table 3.5: Protein efficiency ratios (PER) of other marine fish on which similar protein requirement experiments were undertaken.

Species	Protein level (increase)	PER (decrease)	Reference
European sea bass	45-50	1.66-1.18	Ballestrazzi *et al.* (1994)
Gilthead sea beam	42-50	1.5-1.2	Robaina *et al.* (1997)
Spotted sand bass	40-50	1.48-1.34	Gonzalez *et al.* (2001)
Dusky kob	40-55	2.05-1.02	Present results

Since protein is the most expensive component in fish feeds it is important to minimise the protein content in the diet while providing essential amino acids in proportions that promote good growth (Gonzalez *et al.*, 2001). Food conversion ratios are therefore extremely important variables to fish farmers. The FCR values obtained in this experiment (1.25-1.8) are similar or lower than those reported by several authors for other juvenile species of a similar size, such as red drum (FCR = 2.14; Serrano *et al.*, 1992) and estuary grouper (FCR = 1.7-2.85; Teng *et al.*, 1978) and this indicates that the

diets used in this experiment probably and adequately met the protein requirements of juvenile dusky kob.

Varying dietary protein levels in the diet did not affect the body protein, moisture and ash contents of *A.japonicus*. Similarly, body protein was not affected by dietary protein levels in brown trout (Arzel *et al.*, 1995), red drum (Serrano *et al.*, 1992) and channel catfish (Reis *et al.*, 1995) The inverse relationship between whole body fat content and growth rate (increased dietary protein) has been reported in sturgeon (Stuart and Hung, 1989), trout (Austreng and Refstie, 1979) and red drum (Moon and Gatlin, 1994). The lower fat content in the 35% protein treatment may also be explained by the large size variation of the fish at the end of this experimental period.

The results indicate that juvenile dusky kob require a minimum of 45% and no more than 52.3% protein for optimum growth and feed utilization under the present experimental conditions. Since dietary energy has a significant effect on protein utilization in fish and directly affects the quantitative requirements for protein (Wilson, 1989), the next step is to identify the optimal ratio between protein and energy levels, and investigate whether protein sparing can be achieved.

CHAPTER 4

THE EFFECT OF DIETARY PROTEIN AND ENERGY RATIOS ON GROWTH AND BODY COMPOSITION OF JUVENILE *ARGYROSOMUS JAPONICUS*

Introduction

The dietary protein requirement of fish is one of the most important considerations in aquaculture, as protein profoundly affects fish growth and feed costs (Serrano *et al.*, 1992). Due to the fact that food is the principal operating cost in the production of fish, precise information regarding nutritional requirements is essential so that nutritionally complete feeds can be formulated and manufactured economically (Cowey, 1992).

Dietary protein is most often the first nutrient in a diet to be manipulated. As it is the most expensive constituent in the diet and a major source of nitrogen, protein should ideally be used for tissue deposition (Turano *et al.*, 2002). The inclusion of excess dietary protein, incorrect protein and energy (P: E) ratios and unbalanced essential amino acids, can result in excess nitrogenous waste, as well as reduced feed performance and overall cost efficiency (Turano *et al.*, 2002).

Fish are known to utilize protein preferentially to lipid or carbohydrates as an energy source and it is vital to optimise protein utilization for tissue synthesis rather than for energy requirements (Peres *et al.*, 1999). If the protein content of fish diets is too high, the excess is catabolized to provide energy for growth and as a result protein conversion efficiency is reduced (Lee and Putnam, 1973). A way of conserving protein is to replace the excess with an energy rich compound such as lipids. The optimisation of protein and

lipid or protein: energy (P: E) ratios is widely acknowledged as a means of "protein sparing" and in this manner optimal growth rates can be maintained while reducing overall feed costs (Ellis and Reigh, 1991; Serrano *et al.*, 1992).

A protein sparing effect associated with increased dietary energy levels has been reported for numerous fish species (Chou *et al.*, 2001; Samantaray and Mohanty, 1997; Morais *et al.,* 2001; Lee *et al.*, 2002; Lee and Putnam, 1973 and De Silva *et al.*, 1991). Although improved production and protein utilization may be achieved, these high fat diets can lead to elevated lipid levels in the body (Stickney and Andrews, 1972). The benefit of improved protein conversion efficiency due to protein sparing by lipids must therefore be weighed against any disadvantages incurred (Bromley, 1980).

As fish eat to meet an energy need (NRC, 1993) it is important to consider that feeding high-energy diets may result in reduced levels of food consumption. If feed intake is severely reduced, protein intake may be too low to support optimum growth rates. Lowering the protein content of diets may reduce costs, but may also result in reduced weight gain and food conversion efficiency. Reductions in food conversion efficiency can increase feed costs per unit fish produced and this in turn may lead to an increase in the nutrient loading of the culture system, possibly resulting in an increase in overall production costs (Thoman *et al.*, 1999).

There is considerable variation in the reported protein: energy requirements of carnivorous marine finfish species (Table 4.1). This variation may be explained by differences in factors such as water temperature, salinity, diet composition, biological

value of protein sources, non-protein energy sources and size and age of individual fish (NRC, 1983).

Table 4.1: Protein: energy (P: E) ratios of feeds used by other carnivorous marine finfish.

Species	Average size (g)	P: E (mg/kJ)	Reference
Eurasian perch, *Perca fluviatilis*	35- 150	19-25	Mathis *et al.* (2002)
Red drum, *Sciaenops ocellatus*	3.5- 35	24.5- 29.8	McGoogan & Gatlin (2000)
	46- 77	25.6- 28.6	Daniels & Robinson (1986)
	145-183	27.3-29	Turano *et al.* (2002)
Atlantic cod, *Gadus morhua*	233	26.4	Morais *et al.* (2001)
	173-318	33.2	Lie *et al.* (1988)
European sea bass, *Dicentrarchus labrax*	37-57	35	Hidalgo & Alliot (1988)
Asian sea bass, *Lates calcarifer*	15-72	35.1	Tucker *et al.* (1988)
Grouper, *Epinephelus malabaricus*	58- 225	33.8	Teng *et al.* (1978)
Gilthead sea bream, *Sparus aurata*	10	28.5	Lupatsch *et al.* (2001)

Carnivorous marine fish generally have a limited ability to use carbohydrates as an energy source (Chou *et al.*, 2001), but lipids are energy dense nutrients easily metabolised by fish. A balanced diet should include lipids to provide energy and for a constant supply of essential fatty acids. Different carnivorous fish species have been fed dietary lipid levels ranging from 6% to more than 15% with minor differences in growth. However, however higher lipid levels have generally resulted in reduced weight gain and

an increase in the deposition of fat in fish tissues (Robinson, 1988). With this in mind, the balance between protein and lipid in the diet is essential for the future culture of *A. japonicus*, to ensure that growth is optimal and lipid deposition is minimal.

The objective of this experiment was to evaluate the response of juvenile dusky kob to diets containing various protein and energy ratios and to determine the extent to which lipids can spare protein for growth. The minimum protein requirement, as established in the previous chapter, was used as the basis for the protein and energy ratios. Responses were measured in terms of growth, feed efficiency and whole body composition.

Materials and Methods

Experimental diets

Nine experimental diets were formulated to combine three protein levels (35, 40 and 45%) with three lipid levels (12, 9 and 6%) in a 3x3 factorial design.

Since digestible energy values of the dietary ingredients have not yet been determined for *A. japonicus*, digestible energy was calculated based on values used in red drum and rainbow trout, of 16.7, 16.7 and 37.7 kJ/g for protein, carbohydrate and lipid respectively (Lee and Putnam, 1973, Serrano *et al.*, 1992). The resulting protein: energy (P: E) ratios ranging from 21.1 to 30.3 mg/kJ were within an acceptable range (Table 4.2), already established for red drum, *Sciaenops ocellatus,* as well as other cultured carnivorous marine fish.

Low-temperature Danish fish meal (67.8% crude protein) and Casein (80.1% crude protein) were used as protein sources. A combination of marine fish oil and Soya oil supplied the lipid fraction of the diet (in a 2:1 ratio) and pre-gelatinised starch provided the carbohydrates. The procedures for diet preparation are outlined in Chapter 2.

The ingredients and proximate composition of the test diets are presented in Table 4.2

Experimental design

All the fish were captured, acclimated and weaned onto pellets as explained in Chapter 2. Prior to the commencement of this experiment 6 randomly selected fish were killed for proximate analysis.

Juvenile dusky kob with an average weight of 29.2 ± 15.64g and length of 142.6 ± 24.56mm were randomly distributed among 27 baskets. Three replicates of 12 fish (36 per dietary treatment) were fed each diet. Individual fish in each basket were weighed (g) and measured (Total length mm) at the beginning of the 60-day experiment, every 20 days thereafter and upon completion of the feeding trial.

The fish in each basket were hand fed to satiation twice a day and were assumed to be satiated once active foraging at the surface ceased. Individual food from each replicate was kept in individual containers and was weighed at the beginning and end of each day of the experimental period.

At the end of the experiment, two fish from each tank (6 per dietary treatment) were randomly sampled and sacrificed for proximate analysis. Proximate analysis was undertaken following the methods outlined in chapter 2.

Water quality parameters were measured once a week and were maintained within the desired levels outlined in Chapter 2, while temperature and salinity were measured daily. The mean ± standard deviation of temperature and salinity during the 60 day trial were 18.9 ± 3.9°C and 33.1 ± 5.6 ppt, respectively.

Statistical analysis

Percentage weight gain, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF) and body composition data were all calculated using the methods outlined in Chapter 2.

The Kolmogorov-Smirnov test was used to verify if data was normally distributed and the Levene Test was used to check for homogeneity in variances. Data from the replicates within each dietary treatment were analysed and compared for significant differences with a one-way ANOVA. If no significant differences were detected between replicates the data were pooled such that comparisons could be made (by ANOVA) between dietary treatments. Tukey's multiple comparison test was used to evaluate the mean difference among individual diets at the 0.05 significance level.

Table 4.2: Formulation and proximate composition of the 9 experimental diets (g/100g dry weight). P: E ratios increase from left to right.

	Dietary protein: lipid (%)								
Ingredients	35:12	35:9	35:6	40:12	40:9	40:6	45:12	45:9	45:6
Fish meal[a]	26	26	26	35	35	35	45	45	45
Casein	22	22	22	20	20	20	18	18	18
PGS[b]	37.6	40.6	43.6	31.5	34.5	37.5	24.5	27.5	30.5
Marine fish oil	6.3	4.3	2.3	5.7	3.7	1.7	5	3	0
Soya oil	3.1	2.1	1.1	2.8	1.8	0.8	2.5	1.5	1.5
Vitamin mix[c]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mineral mix[d]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Calculated values									
Protein	35.3	35.3	35.3	39.8	39.8	39.8	45	45	45
Lipid	12	9	6	12	9	6	12	9	6
DE (kJ/g)[e]	16.7	16.1	15.4	16.4	15.8	15.2	16.1	15.5	14.9
P: E (mg/kJ)[f]	21.1	22	22.9	24.2	25.2	26.2	27.9	29	30.3
Proximate analysis									
Protein	34.6	37.1	36.1	41.6	41.6	41.4	462	46.7	45.9
Lipid	12.3	8.8	5.7	12.2	9.3	6.4	12.2	9.3	6.1
Ash	6.13	6.3	6.1	7.1	7.2	7.2	8.22	8.5	8.2
Energy (MJ/kg)	18.7	18.3	18.2	18.3	18.1	17.9	18.3	17.9	17.8

[a] Low temperature Danish fish meal.

[b] Pre-gelatinised starch

[c] Vitamins include- 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.

[d] Minerals include- 516g vermiculite, 74g potassium, 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, traces of selenium.

[e] Digestible energy- values are estimated based on 16.7kJ/g for protein and carbohydrate and 37.7kJ/g for lipid.

[f] Protein: Energy ratio = (calculated protein value*10)/ DE value.

Results

Growth responses

Percent weight gain, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and condition factor responses of *A. japonicus* are presented in Table 4.3.

Weight gain in 45% protein and 9% or 12% lipid treatments was significantly greater ($p<0.05$) than at either 40% or 35% protein at all three lipid levels (Table 4.3). The lowest weight gain occurred in the 35% protein and 6% lipid treatment. The greatest weight gain occurred in the fish fed the 45% protein and 12% lipid diet, however this was not significantly different ($p>0.05$) to the 45% protein and 9% lipid diet.

Table 4.3: The effect of protein: lipid ratios on weight gain, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (CF) of juvenile *Argyrosomus japonicus* fed for 60 days.

	Protein: lipid								
	35:12	35:9	35:6	40:12	40:9	40:6	45:12	45:9	45:6
P: E ratio (mg/kJ)	21.13	21.96	22.86	24.22	25.19	26.23	27.9	29.03	30.26
Initial length	163.5 ±28.6	145.2 ±23.6	139.4 ±21.5	146.4 ±21.7	143 ±24.9	146 ±25.9	127.8 ±17.8	134.5 ±16.5	137.3 ±23.6
Final length	196.6 ±25.7	179.6 ±21	161.9 ±16.7	180.5 ±22.7	179 ±21.9	180.5 ±17	181.9 ±14.3	182.4 ±14	173.3 ±16.5
Initial weight	44 ±20.3	32 ±15.4	28.9 ±13.6	29.9 ±13.4	29.6 ±15.9	32.5 ±17.7	20.4 ±8.1	23.2 ±8.0	22.3 ±11.9
Final weight	79.1 ±30.4	58.4 ±21.4	43.4 ±15	59.1 ±19.9	58.4 ±24	60.4 ±24.5	59.9 ±15.6	60.9 ±17.2	51.4 ±18.6
Weight gain (%)	81.1^{ad} ±5.6	82.2^{ad} ±4.5	49.9^{a} ±6.8	98.1^{ad} ±16.3	97.3^{ad} ±1.9	86.8^{ad} ±7.4	195.2^{b} ±44.5	161.3^{bc} ±24.8	120.6^{cd} ±17.7
SGR	0.99^{ab} ±0.05	1.0^{ab} ±0.04	0.67^{b} ±0.08	1.14^{ad} ±0.14	1.13^{ad} ±0.02	1.04^{ad} ±0.07	1.79^{c} ±0.26	1.6^{ce} ±0.16	1.32^{ade} ±0.14
FCR	1.69^{a} ±0.31	1.93^{a} ±0.46	2.86^{b} ±0.12	1.99^{a} ±0.08	1.97^{a} ±0.12	1.67^{a} ±0.29	1.81^{a} ±0.48	1.7^{a} ±0.44	2.10^{a} ±0.41
PER	1.73^{a} ±0.29	1.54^{ab} ±0.34	1.00^{b} ±0.04	1.26^{ab} ±0.05	1.27^{ab} ±0.07	1.52^{ab} ±0.24	1.29^{ab} ±0.34	1.36^{ab} ±0.32	1.09^{ab} ±0.24
Initial CF	2.28 ±0.16	2.24 ±0.18	2.25 ±0.17	2.24 ±0.19	2.25 ±0.17	2.25 ±0.21	2.24 ±0.22	2.24 ±0.18	2.26 ±0.18
Final CF	2.85^{a} ±0.26	2.75^{ab} 0.19	2.75^{ab} ±0.22	2.73^{ab} ±0.26	2.72^{ab} ±0.27	2.76^{ab} ±0.24	2.76^{ab} ±0.18	2.77^{ab} ±0.26	2.65^{b} ±0.33

Data are mean values ± SD of 3 replicates. Mean values in each row having the same superscript are not significantly different ($p>0.05$).

At each protein level, the specific growth rate (SGR) improved with increasing lipid fractions (Figure 4.1). The highest SGRs were obtained from feeding the 45% protein and 9 or 12% lipid treatments, with P: E ratios of 29.03 and 27.9mg/kJ, respectively. These were significantly higher than all the 35% and 40% protein dietary treatments. The SGR of the fish fed the 27.9mg/kJ diet was significantly higher ($p<0.05$) than for the fish that received the 30.3mg/kJ diet.

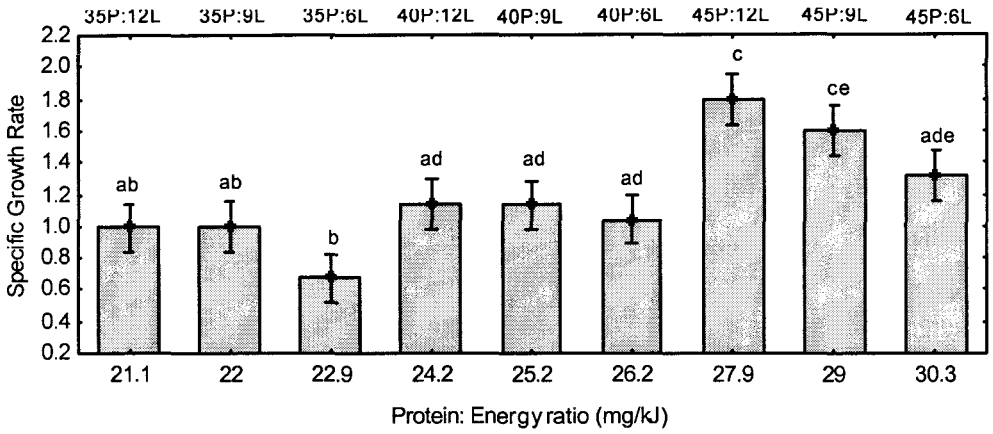


Figure 4.1: The specific growth rate (SGR) of juvenile *Argyrosomus japonicus* fed diets with varying protein and energy ratios. Error bars denote 95% confidence intervals. Significant differences between means are represented by non-overlapping bars and different superscripts.

Besides the 22.9 mg/kJ (35P: 6L) treatment, there was no significant difference in FCR between all other treatments.

The highest protein efficiency ratio (PER) was recorded for the 35% protein and 12% lipid treatment (P: E ratio of 21.1mg/kJ) and this was significantly better than the 35%

protein and 6% lipid treatment. No other significant differences ($p>0.05$) in PER were recorded (Figure 4.3).

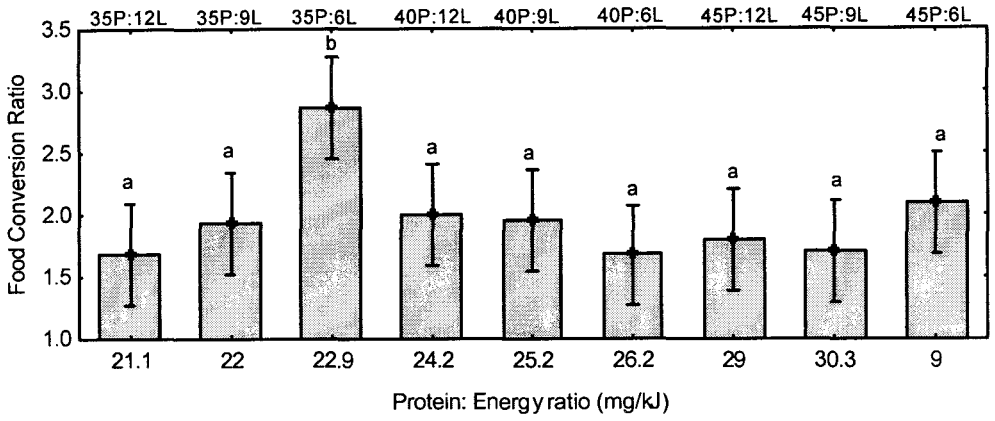


Figure 4.2: The food conversion ratio (FCR) of juvenile *Argyrosomus japonicus* fed diets with different protein and energy ratios. Error bars denote 95% confidence intervals. Significant differences between means are represented by non-overlapping bars and different superscripts.

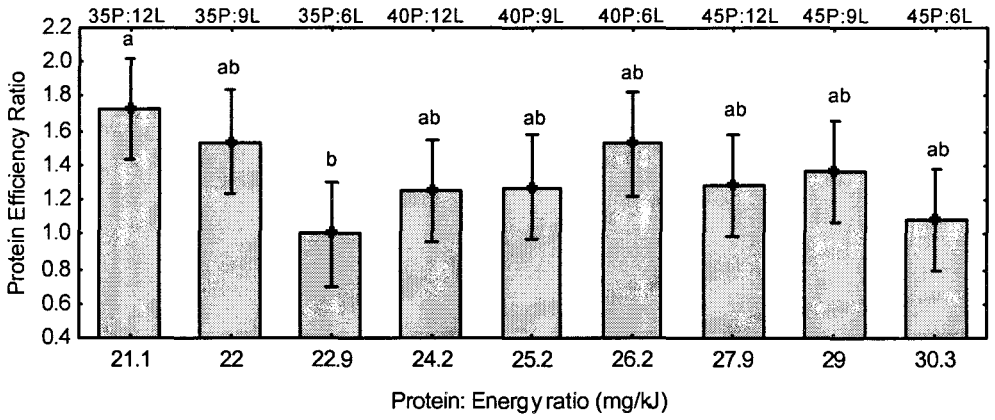


Figure 4.3: The protein efficiency ratio (PER) of juvenile *Argyrosomus japonicus* fed with different protein and energy ratios. Error bars denote 95% confidence intervals. Significant differences between means are represented by non-overlapping bars and different superscripts.

The condition factor (CF) of all the fish at the start of the growth trial was significantly lower ($p<0.05$) than at the end of the experiment. The condition factor of the fish at the start of the growth trial did not differ significantly between treatments ($p>0.05$). At the end of the trial the CF of the fish in the 35% protein and 12% lipid treatment was significantly higher ($p<0.05$) than in the 45% protein and 6% lipid diet (Table 4.3).

Carcass composition

The proximate carcass composition at the start and at the end of the 60-day feeding trial is presented in Table 4.4.

The ash contents of the fish in the 45% protein and 6% or 9% lipid treatments were significantly greater ($p<0.05$) than the fish at the start of the experiment and the fish in all the other treatments. Moisture content of the fish was not significantly affected ($p>0.05$) by the various dietary treatments.

Table 4.4: Initial and final whole body composition of *Argyrosomus japonicus*, expressed as a percent of dry mass.

		Protein: Lipid (%)								
	Initial	35:12	35:9	35:6	40:12	40:9	40:6	45:12	45:9	45:6
Protein	69.1^{a} ±0.5	68.98^{a} ±0.1	68.96^{a} ±0.1	68.9^{a} ±0.4	68.89^{a} ±0.3	68.9^{a} ±0.2	68.9^{a} ±0.04	69.03^{a} ±0.2	69.5^{a} ±0.6	70.2^{b} ±0.2
Lipid	9.8^{ae} ±0.2	10.61^{b} ±0.2	10.55^{b} ±0.1	10.59^{b} ±0.1	10.22^{bc} ±0.4	9.78^{ae} ±0.1	9.69^{ae} ±0.3	10.22^{bc} ±0.2	10.1^{cde} ±0.2	9.73^{ae} ±0.2
Ash	18.1^{a} ±0.1	18.2^{a} ±0.8	18.1^{a} ±0.6	18.3^{a} ±0.1	18.2^{a} ±0.4	18.0^{a} ±0.2	17.9^{a} ±0.3	18.2^{a} ±0.4	18.7^{b} ±0.3	18.9^{b} ±0.4
Moisture	72.1^{a} ±0.7	71.5^{a} ±0.1	71.7^{a} ±0.7	72.4^{a} ±0.5	71.9^{a} ±0.6	72.0^{a} ±0.8	71.9^{a} ±0.4	71.7^{a} ±0.7	71.6^{a} ±0.4	72.0^{a} ±0.1
HSI	1.3^{a} ±0.4	1.8^{a} ±0.02	1.4^{a} ±0.4	1.3^{a} ±0.2	1.9^{a} ±0.7	1.4^{a} ±0.6	1.3^{a} ±0.4	2.1^{a} ±0.4	2.0^{a} ±0.3	1.9^{a} ±0.4
IPF	1.2^{a} ±0.4	2.3^{b} ±0.3	1.3^{a} ±0.5	1.1^{a} ±0.2	1.6^{ab} ±0.4	1.8^{ab} ±0.8	1.25^{a} ±0.4	2.1^{ab} ±0.4	2.0^{ab} ±0.3	1.9^{ab} ±0.4

Data are mean values. Values are mean± SD. Mean values in each column having the same superscript are not significantly different ($p>0.05$). HSI = Hepatosomatic index. IPF = Intraperitoneal fat ratio

A significantly higher ($p<0.05$) percent protein deposition was recorded in the 45% protein and 6% lipid treatment (Figure 4.4). No significant differences in protein content were detected between fish at the start of experiment and all diets excepting the 45% protein and 6% lipid treatment (30.3mg/kJ).

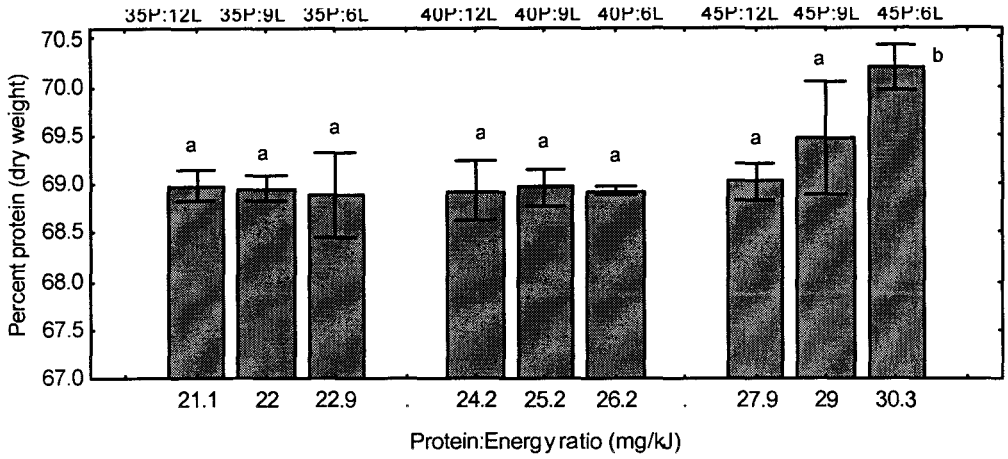


Figure 4.4: The mean protein content of juvenile *Argyrosomus japonicus* fed with different protein and energy ratios. Vertical bars are the standard deviation and significant differences are indicated by different superscripts.

The experimental diets had a significant ($p<0.05$) effect on lipid deposition (Figure 4.5). At each protein level there was a general increase in lipid deposition as dietary lipid level increased. The lowest lipid deposition occurred in fish fed the 6% lipid and 40% or 45% protein diets, and this did not differ significantly ($p>0.05$) from the lipid content of the fish at the start of the experiment. The greatest degree of lipid deposition was recorded in the fish fed the 35% protein diets (Figure 4.5).

No significant differences in the hepatosomatic index (HSI) were recorded in any treatments ($p>0.05$). There was a general increase in intraperitoneal fat (IPF) deposition as dietary lipid increased at each protein level. At the 35% protein level, fish fed a 12% lipid diet had significantly more ($p<0.05$) IPF than those fed 6% and 9% lipid diets (Figure 4.6).

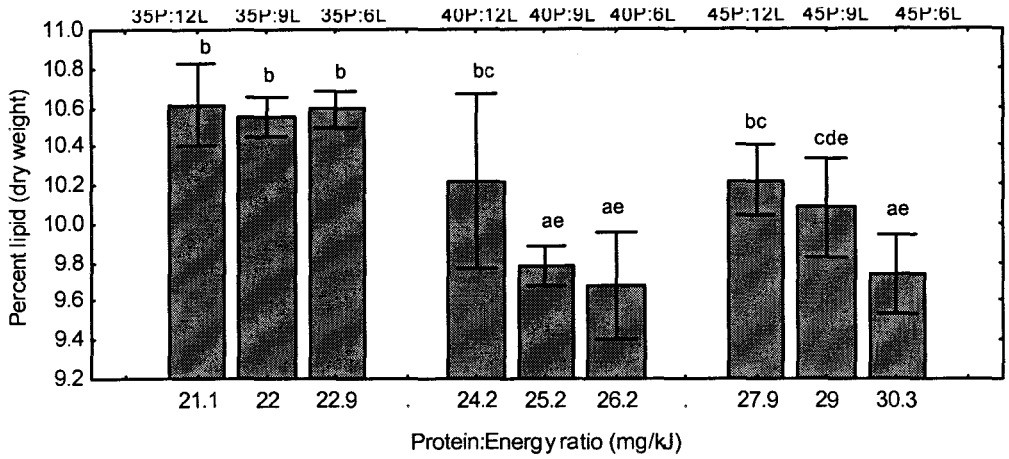


Figure 4.5: The mean lipid content of juvenile *Argyrosomus japonicus* fed with different protein and energy ratios. Vertical bars are the standard deviation and significant differences are indicated by different superscripts.

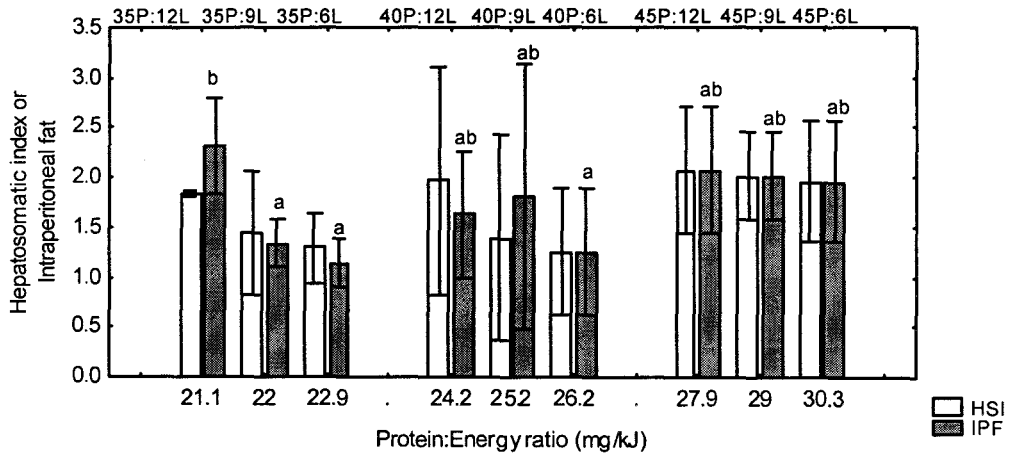


Figure 4.6: The mean hepatosomatic index (HIS) and intraperitoneal fat (IPF) of juvenile *Argyrosomus japonicus* fed with different protein and energy ratios. Vertical bars are the standard deviation and significant differences are indicated by different superscripts.

Discussion

The objective of this study was to investigate whether increasing the lipid level in the diet (while maintaining optimal growth rates, feed efficiency and body composition) could

reduce the minimum dietary protein requirement of 45% established in the previous chapter.

Under the present experimental conditions, the growth of juvenile *A. japonicus* (in weight) appeared to be influenced more by dietary protein than by the dietary lipid levels. Significant reductions in weight gain were observed as the protein content was reduced from 45% to 35%. The best growth rates were attained in the 45% protein and 9 or 12% lipid treatment, at P: E ratios of 29 and 27.9 mg/kJ, respectively (Table 4.3; Figure 4.1). Similar to red drum (Thoman *et al.,* 1999; Daniels and Robinson 1986), juvenile turbot (Bromley, 1980) and numerous other carnivorous fish (Table 4.1), a general decrease in growth was observed (as dietary lipid was reduced) within each protein treatment. In terms of weight and length, dusky kob fared best on a high protein- high-energy diet. The digestibility of carbohydrates in fish diets has been suggested as another possible reason for the reduced growth rates recorded in low protein diets. Higher carbohydrate levels within fish diets have been shown to reduce feed intake and growth rates in other fish species and this may have occurred in this experiment due to the fact that starch levels increased as protein decreased.

The food conversion ratio values (1.7-2.9) obtained in this study were comparable to those recorded for red drum of a similar size (Serrano *et al.*, 1992). Fish fed the 22.9mg/kJ (35P: 6L) diet had a significantly poorer FCR than all the other treatments. This was probably caused by protein being catabolized (due to insufficient dietary lipid) to provide energy for maintenance. This may also explain why the protein efficiency ratio (PER) in the 35% protein and 6% lipid treatment was significantly lower than for the 35% protein and 12% lipid diet.

Decreasing PER values with increased dietary protein levels have been shown for other marine species (Ballestrazzi *et al.*, 1994; Gonzalez *et al.*, 2001). However, no significant differences in PER were recorded between any of the treatments. Using the hypothesis that the 'protein sparing effect' of dietary lipids is usually measured by comparing PER values (Lie *et al.*, 1988), it may be concluded that under the present experimental conditions no protein sparing occurred.

The proximate carcass composition of *A. japonicus* showed that body protein content was inversely related to body fat. Fat levels were reduced and protein deposition improved as dietary P: E ratios increased. The carcass lipid content in *A. japonicus* was greatest in the fish fed a low protein-high fat diet, while carcass protein content was greatest in the fish fed a high protein-low fat diet. Similar proximate analysis results were also reported for Asian sea bass, *L. calcarifer*, and red and blue tilapia, *T. aurea* (Catacutan and Coloso, 1995; Winfree and Stickney, 1981). No significant changes in body fat and protein were found in the 12% lipid level in all three protein treatments. According to Lee and Putnam's (1973) this indicates that the fish are quite capable of converting protein to fat if the diet does not provide adequate amounts of non- protein energy sources.

The hepatosomatic index (HSI) values ranging from 1.3-2.0 were similar to values reported for juvenile *D. labrax* (Peres and Teles, 1999) and juvenile *S. ocellatus* (Daniels and Robinson, 1986; McGoogan and Gatlin, 2000; Turano *et al.*, 2002). Daniels and Robinson (1986) suggest that increases in HSI are directly related to elevated dietary protein and carbohydrate levels. However in this study the HSI values were not significantly different between treatments and appear to be influenced more by dietary lipid level than dietary protein (Figure 4.6). Turano *et al.* (2002) suggest that the lack of

differences in HSI values in their study on red drum were probably due to the larger sized individuals used (145-183g), and that larger fish have a greater ability to tolerate shifts in P: E ratios. However this was not the case in this study as the kob used were smaller than the red drum used by Daniels and Robinson (1986). The absence of any differences in HSI in this study may indicate that kob generally has a good ability to tolerate changes in P: E ratios.

The intraperitoneal fat (IPF) ratios showed a general increase with higher dietary lipid levels. This has also been observed in other marine species such as juvenile red drum (Daniels and Robinson, 1986; Thoman *et al.*, 1999; Serrano *et al.*, 1992) and juvenile turbot (Bromley, 1980) and may indicate that excess energy within a diet can result in increased fat deposition (Turano *et al.*, 2002).

Under the present culture conditions, the best growth and feed efficiencies (as measured by FCR and PER) were observed for the fish fed in the 45% protein and 12 or 9% lipid treatments. However, reducing the dietary lipid from 12% to 9% in this treatment resulted in an increase in protein deposition and a reduction in fat content. Therefore 9% lipid would be preferential in terms of feed costs and kob marketability.

In conclusion, increasing the dietary lipid content did not result in protein sparing and the range of experimental dietary lipid levels used may explain this. Protein sparing by other fish such as turbot (Bromley, 1980) and rainbow trout (Lee and Putnam, 1973) has tended to occur when lipid levels have been greater than the 12% level used in this experiment.

Based on the results of this experiment and under the present experimental conditions, a good quality diet with a minimum of 45% protein, 9% lipid and a P: E ratio of 29mg/kJ appears to be adequate for good growth, feed efficiency and acceptable body composition. This P: E ratio is similar to other carnivorous fish of a similar weight (Table 4.1) and indicates that growth rates are optimised on high protein and moderate lipid level diets.

CHAPTER 5

THE EFFECT OF PARTIAL FISH MEAL REPLACEMENT BY SOYA BEAN MEAL IN THE DIET, ON GROWTH AND BODY COMPOSITION OF JUVENILE *ARGYROSOMUS JAPONICUS*

Introduction

In aquaculture, reducing feed expenditure is essential as these significantly affect production costs. One way of reducing feed costs is to substitute expensive ingredients such as fish meal, with less expensive ingredients, such as soybean meal (Reigh and Ellis, 1992).

Fish meal is a highly sought after protein source (Allan *et al.*, 2000) and is widely used in the formulation and manufacture of aquaculture diets. It contains high levels of essential amino and fatty acids, is low in carbohydrates, contains few anti-nutritional factors and is usually well digested (Allan *et al.*, 2000). However fish meal production already accounts for approximately 35% of the total global fish catch (Allan *et al.*, 2000) and many intensive and semi-intensive aquaculture systems use 2-5 times more fish meal to feed the farmed species, than is supplied by the farmed product (Naylor *et al*, 2000). According to Naylor et al. (2000), for the ten types of commonly farmed fish, an average of 1.9 kg of wild fish is required for every kilogram of fish raised on compound feeds and the growing aquaculture industry cannot continue to rely on finite stocks of wild-caught fish. The use of alternative protein sources for fish meal has therefore become a priority in fish nutrition research, so that commercially stable diets can be produced at a reduced price (Kikuchi, 1999).

Soya bean meal has a high protein content, a favourable amino acid profile (closely resembling the requirements of fish) and a reasonable price, is consistently available and reported to be palatable to most fish species (Elangovan and Shim, 2000). With an annual production of about 30 million MT (Kikuchi, 1999), it is considered to be one of the most suitable and stable supplies of alternative protein sources for replacing fish meal in commercially cultured fish diets.

The value of Soya bean meal (SBM) as a substitute for fish meal in formulated diets has been investigated for a number of fish species (Table 5.1). Results show that considerable variation exists in the ability of different species to efficiently utilize Soya bean protein as an alternative to fish protein in the diet (Reigh and Ellis, 1992). Studies have shown that defatted SBM can substitute between 20% and 50% of the fish meal protein (Table 5.1) without the supplementation of essential amino acids (Kikuchi, 1999). However, the results also show that growth rate and fish meal replacement levels are inversely related (Quartararo *et al.*, 1998). The different levels of fish meal replacement (Table 5.1) by Soya protein reported for the various carnivorous finfish may be attributed to variability in factors such as SBM quality, processing techniques, variation in diet formulations and differences in factors such as fish species and size, culture systems and experimental conditions (Elangovan and Shim, 2000).

The major limitations in the use of SBM as a replacement for fish meal can be attributed to palatability, the presence of anti-nutritional factors and low levels of some essential amino acids such as methionine (Wilson and Poe, 1985). In some fish, the palatability of diets containing SBM has been improved considerably by adding other protein sources such as corn gluten meal, blood meal, blue mussel meat and Krill (Arndt *et al.*, 1999,

Kikuchi, 1999). Antinutrients have been defined as substances that by themselves, or through their metabolic products, interfere with food utilisation and affect the health and production of animals (Francis *et al.*, 2001). The most commonly studied and known antinutrients found within SBM are the inhibitors of protease enzymes, trypsin and chymotrypsin; some non-digestible carbohydrates such as lectins, saponins and phytates (Elangovan and Shim, 2000); antivitamins and other miscellaneous substances such as mycotoxins (Francis *et al.*, 2001). However, heat treatment applied during the commercial processing of SBM inactivates most of the trypsin inhibitors and other heat sensitive anti-nutritional factors (Peres *et al.*, 2003). However, some antinutrients such as the saponins are heat stable (Francis *et al.*, 2001).

Table 5.1: Levels of Soya bean meal (SBM) protein in the diet of various carnivorous fish species (without the addition of amino acid supplements).

Species	% SBM protein	Reference
Red drum, *Sciaenops ocellatus*	30-50	Gaylord & Gatlin (1996) Reigh & Ellis (1992)
Australian snapper, *Pagrus auratus*	50	Quartararo *et al.* (1998)
Asian sea bass, *Lates calcarifer*	37.5	Boonyaratpalin *et al.* (1998)
European sea bass, *Dicentrarchus labrax*	45	Dias *et al.* (1997)
Gilthead sea bream, *Sparus auratus*	30	Robaina *et al.* (1995)
Japanese flounder, *Paralichthys olivaceus*	45	Kikuchi (1999)
Atlantic salmon, *Salmo salar*	33	Carter & Hauler (2000)
Rainbow trout, *Onchorhynchus mykiss*	37	Refstie *et al.* (2000)

Locally, Soya bean meal (± R3/kg) is substantially cheaper than fish meal (± R5.5/kg). The large difference in price may therefore make a significant difference in feed costs, if a proportion of the fish meal in the diet can be partially substituted by SBM.

The objective of this study was to evaluate the response of juvenile *A. japonicus* to diets in which graded levels of crude protein from fish meal were replaced by Soya bean meal. The protein and energy requirements established in previous chapters were used as the basis for the experimental diets and responses were measured in terms of growth, feed efficiency and body composition.

Materials and Methods

Experimental diets

The ingredient and proximate composition of the test diets are presented in Table 5.2.

Five isocaloric (DE: ±12.6 kJ/g) fish meal based experimental diets were formulated to contain 45% crude protein and 9% lipid. In the control diet, the protein component consisted only of fish meal. In the other diets 20, 30, 40 and 50% of the crude protein from fish meal was replaced by protein from Soya bean meal.

Since digestible energy values of the dietary ingredients have not yet been determined for *A. japonicus*, digestible energy was calculated based on values used in red drum and rainbow trout, of 16.7, 16.7 and 37.7 kJ/g for protein, carbohydrate and lipid respectively (Lee and Putnam, 1973, Serrano *et al.*, 1992). Low-temperature Danish fish meal (67.8% crude protein) and Soya bean meal (47% crude protein) were used as protein sources and marine fish oil supplied the lipid fraction of the diet. Pre-gelatinised starch provided the

carbohydrates and alpha cellulose was used as a non-nutritive filler. The procedures for diet preparation are outlined in the Chapter2.

Table 5.2: Formulation and proximate composition of the experimental diets.

	% Crude protein from Soya bean meal				
Ingredients	Control (0)	20	30	40	50
Fish meal[a]	66	53	46	40	33
Soya bean meal	0	19	29	38	48
PGS [b]	10	10	10	10	10
Marine fish oil	2.4	3.4	4	4.5	5
Mineral mix [c]	2	2	2	2	2
Vitamin mix [d]	2	2	2	2	2
α- Cellulose	17.6	10.6	7	3.5	0
Calculated values					
Protein	44.9	45	44.9	45.1	45
Lipid	9	9	9	9	9
DE (kJ/g) [e]	12.56	12.57	12.58	12.61	12.59
Proximate composition					
Protein	45.2	45.4	45.1	43.5	44.1
Lipid	9.2	9.2	8.9	9.7	9.8
Ash	16.9	14	14.4	13.3	12.1
Gross Energy (MJ/kg)	17.5	17.2	17.8	17.1	17.2

[a] Low-temperature Danish fish meal.

[b] Pre-gelatinised starch.

[c] Vitamins include- 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.

[d] Minerals include- 516g vermiculite, 74g potassium, 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, traces of selenium

[e] Digestible energy- values are based on 16.7kJ/g for protein and carbohydrate and 37.7kJ/g for lipid.

Experimental design

All the fish were captured, acclimated and weaned onto pellets as explained in Chapter 2.

Juvenile dusky kob with an average (± standard deviation) weight of 44.08 ± 11.2g and length of 166.2 ± 15.3mm were randomly distributed among 15 tanks. Three replicates of

12 fish (36 per dietary treatment) were fed each diet. Individual fish in each tank were weighed (g) and measured (Total length- mm) at the beginning of the 60-day experiment, every 20 days thereafter and upon completion of the feeding trial.

The fish in each tank were hand fed to satiation twice a day (see Chapter 2) and were assumed to be satiated once active feeding at the surface ceased. The food for each tank was kept in individual containers. These were weighed at the beginning and end of each day of the experimental period so as to calculate daily feed intake.

At the end of the experiment, two fish from each tank (6 per dietary treatment) were randomly sampled and sacrificed for proximate analysis. Proximate analysis was undertaken following the methods outlined in Chapter 2.

Water quality parameters were measured once a week and were maintained within the desired levels outlined in Chapter 2, while temperature and salinity were measured daily. The mean ± standard deviation of temperature and salinity during the 60 day trial were 17.1 ± 3.9°C and 34.1 ± 3.6 ppt, respectively.

Statistical analysis

Feed intake (FI), percentage weight gain, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF) and whole body composition data were all calculated using the methods outlined in Chapter 2.

The Kolmogorov-Smirnov test was used to verify if data was normally distributed and the Levene Test was used to check for homogeneity in variances. Data from the replicates within each dietary treatment were analysed and compared for significant differences with a one-way ANOVA. If no significant differences were detected between replicates

the data were pooled such that comparisons could be made (by ANOVA) between dietary treatments. Tukey's multiple comparison test was used to evaluate the mean difference among individual diets at the 0.05

Results

Growth responses

Percent weight gain, feed intake (FI), specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (CF) responses of juvenile *A. japonicus* are presented in Table 5.3.

Table 5.3: The effect of fish meal protein replacement by Soya bean meal protein (SBM) on weight gain, feed intake (FI), specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (CF) in juvenile dusky kob, *Argyrosomus japonicus*, fed for 60 days.

	% Crude protein from SBM				
Treatment	Control (0)	20	30	40	50
Initial length	163.8	167.7	165.3	164.9	168.4
	±6.8	±2.0	±3.0	±5.7	±6.4
Final length	204.7	204.9	193.4	185.7	190.2
	±10.0	±4.8	±3.5	±4.2	±2.4
Initial weight	44.7	45	42.4	41	45.4
	±5.1	±2.1	±3.8	±4.2	±5.8
Final weight	91.0	90.8	80.4	68.2	71.1
	±11.1	±4.1	±3.9	±4.4	±3.3
Weight gain	103.61^{a}	101.81^{a}	90.1^{ab}	66.68^{bc}	58.1^{c}
(%)	±2.1	±2.8	±8.4	±9.3	±17.7
FI	3.3^{a}	3.11^{a}	3.04^{a}	2.96^{a}	2.64^{a}
(% Body wt/day)	0.26	0.19	0.11	0.39	0.71
SGR	1.19^{a}	1.17^{a}	1.07^{ab}	0.85^{bc}	0.76^{c}
	±0.02	±0.02	±0.07	±0.09	±0.18
FCR	1.91^{a}	1.83^{a}	2.03^{a}	2.67^{b}	2.75^{b}
	±0.17	±0.12	±0.12	±0.18	±0.32
PER	1.17^{a}	1.22^{a}	1.1a	0.84^{b}	0.81^{b}
	±0.1	±0.1	±0.1	±0.05	±0.3
Initial CF	2.8^{a}	2.6^{b}	2.6^{b}	2.5^{b}	2.6^{b}
	±0.2	±0.2	±0.2	±0.2	±0.2
Final CF	3.06^{ab}	3.05^{ab}	3.17^{b}	3.01^{ac}	2.9^{c}
	±0.2	±0.2	±0.1	±0.2	±0.1

Data values are mean ± SD of 3 replicates. Mean values in each row having the same superscript are not significantly different (ANOVA; $p < 0.05$).

In terms of weight gain and specific growth rate (SGR) there were no significant differences between the control diet and the treatment in which 20% and 30% of the FM protein was replaced by SBM protein (Table 5.3). The 50% SBM protein treatment had a significantly lower ($p<0.05$) weight gain and SGR than the control diet and the 20 and 30% SBM protein treatments. There was a general decrease in SGR as dietary SBM levels increased (Figure 5.1).

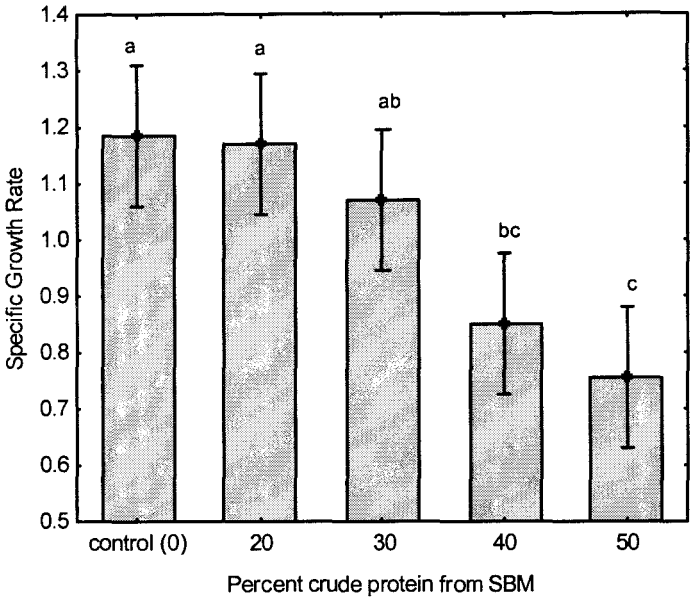


Figure 5.1: The specific growth rate of juvenile *Argyrosomus japonicus* fed diets with graded levels of protein from Soya bean meal. Error bars denote 95% confidence intervals. Significant differences between means are represented by non-overlapping bars and different superscripts.

Feed intake (FI) was not significantly affected ($p>0.05$) by the various experimental diets (Table 5.3) and the food conversion ratio (FCR) ranged from 1.83- 2.75. Fish in the 40% and 50% SBM protein treatments had significantly higher ($p<0.05$) food conversion

ratios than all the other treatments (Figure 5.2). The FCR of fish in the control diet did not differ significantly ($p>0.05$) from the 20% and 30% SBM protein treatments.

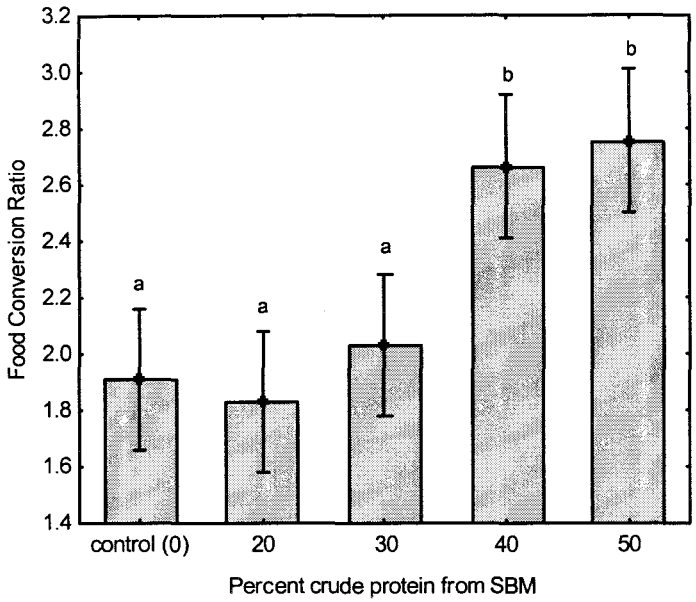


Figure 5.2: The food conversion ratio of juvenile *Argyrosomus japonicus* fed diets with graded levels of protein from Soya bean meal. Error bars denote 95% confidence intervals. Significant differences between means are represented by non-overlapping bars and different superscripts.

There was a general decrease in the protein efficiency ratio (PER) as the amount of SBM protein in the diet increased (Table 5.3, Figure 5.3). The highest PER value was obtained in the 20% SBM protein treatment, however this was not significantly different to the PER values of the fish in the control and 30% SBM treatments. The fish in the treatments in which 40% and 50% of the fish meal protein was replaced with SBM protein, had significantly lower ($p<0.05$) PER values than all other diets (Figure5.3).

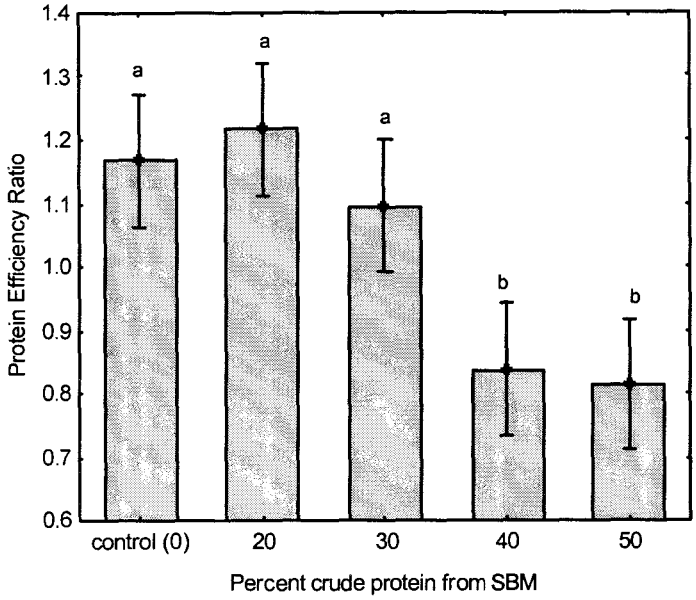


Figure 5.3: The protein efficiency ratio of juvenile *Argyrosomus japonicus* fed diets with graded levels of protein from Soya bean meal. Error bars denote 95% confidence intervals. Significant differences between means are represented by non-overlapping bars and different superscripts.

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At the start of the experiment there were no significant differences ($p>0.05$) in the condition factor of the fish between treatments. However at the end of the study, fish in the 50% SBM protein treatment had a significantly lower CF than those in the control, 20 and 30% SBM treatments (Table 5.3).

Carcass composition

The carcass composition of the fish in different treatments after the 60-day feeding trial is summarised in Table 5.4.

There were no significant differences ($p>0.05$) in the protein, lipid and ash contents between treatments (Table 5.4). However, the moisture content of the fish in the

treatment in which 40% of the fish meal protein was replaced with SBM was significantly higher ($p<0.05$) than of those in the control diet.

Table 5.4: Carcass composition data (% dry weight) of juvenile *Argyrosomus japonicus* fed with graded levels of Soya bean meal (SBM) protein, after 60 days.

	% Crude protein replacement by SBM				
Treatment	Control (0)	20	30	40	50
Protein	65.38 ±1.04	67.63 ±1.96	67.22 ±1.55	65.21 ±1.04	65.55 ±1.13
Lipid	10.15 ±0.47	9.99 ±0.21	9.64 ±0.26	9.60 ±0.22	9.65 ±0.33
Ash	14.77 ±0.15	14.87 ±0.61	14.9 ±0.5	14.66 ±0.23	14.58 ±0.36
Moisture	71.08^{a} ±0.67	72.19^{ab} ±0.53	72.63^{ab} ±0.15	73.54^{b} ±1.17	72.95^{ab} ±1.80
HSI	3.61^{abc} ±0.39	4.01^{b} ±0.55	3.64^{abc} ±0.56	2.36^{c} ±0.87	2.5^{c} ±0.66
IPF	2.03 ±0.38	1.72 ±0.25	1.96 ±0.42	1.05 ±0.15	1.3 ±1.07

Data values are mean ± SD of 3 replicates. Mean values in each row having the same superscript are not significantly different (ANOVA; $p<0.05$). HSI= hepatosomatic index. IPF= Intraperitoneal fat ratio.

The hepatosomatic index (HSI) of the fish in the 40% and 50% SBM treatments was significantly lower ($p<0.05$) than of the fish fed in the 20% SBM protein treatment (Figure 5.4). The highest intraperitoneal fat (IPF) value (2.03) was recorded in the control treatment and the lowest (1.05) was observed in the 40% SBM protein treatment. Although there was a general decrease in IPF as dietary SBM levels increased, no significant differences ($p>0.05$) were detected (Figure 5.4).

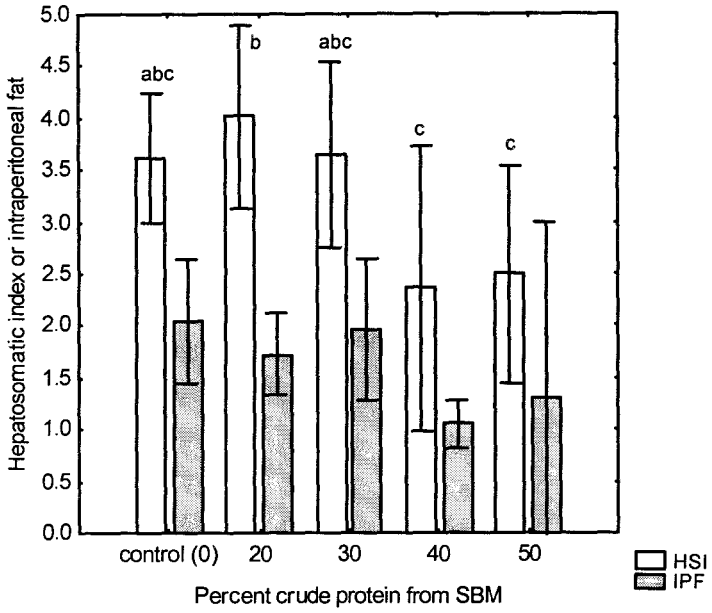

Figure 5.4: The hepatosomatic index (HSI) and intraperitoneal fat (IPF) index of juvenile *Argyrosomus japonicus* fed with graded levels of Soya bean meal (SBM) protein. Columns represent the means and vertical bars the standard deviation. Means with the same superscripts are not significantly different.

Discussion

The inclusion of 29% Soya bean meal in the diet, replacing about 30% of the crude protein supplied by fish meal, did not adversely affect growth, feed efficiency and body composition when compared with a (100% fish meal) control diet. Similar results have been shown for numerous carnivorous species (Table 5.1) and even higher inclusion rates have been demonstrated for red drum with the addition of crystalline amino acids such as methionine (McGoogan and Gatlin, 1997).

In some fish, the palatability of SBM diets significantly reduced feed intake, and in turn growth rates. Kikuchi (1999) reported that about 45% of the protein from fish meal could be replaced with SBM in the diet of Japanese flounder, *Paralichthys olivaceus*, but that

palatability had to be improved with the addition of blood meal, corn gluten meal or blue mussel meal. In this experiment feed intake was not significantly affected (Table 5.3) by the various dietary treatments and this indicates that the palatability of all diets was acceptable.

The specific growth rates for the 20% and 30% SBM diets and the control treatment (1.19-1.07) are consistent with the range of SGR values reported in Chapter 4 as well as for other carnivorous fish such as Atlantic salmon, *Salmo salar* (Refstie *et al.*, 1998) and rainbow trout, *Onchorhynchus mykiss* (Refstie *et al.*, 2000). These results indicate that a certain proportion of the protein from fish meal can be replaced with SBM protein without adverse effects on growth. The lower SGR, percent weight gain and lower PER of the fish in the 40% and 50% SBM protein treatments are also similar to those reported for *S. salar* (Olli *et al.*, 1995) and *P. olivaceus* (Kikuchi, 1999), and may be due to the fact that less essential amino acids are available for growth when fish meal protein is reduced beyond a certain threshold.

The increasing (poorer) FCR recorded in the 40% and 50% SBM protein treatments (Table 5.3) are similar to results obtained for juvenile tinfoil barb, *Barbodes altus* (Elangovan and Shim, 2000), Japanese flounder, *P. olivaceus* (Kikuchi, 1999) and yellowtail, *Seriola quinqueradiata* (Vivyakarn *et al.*, 1992). This indicates that a high level of SBM in the diet of juvenile dusky kob directly affects feed efficiency, which in turn affects growth performance.

Various studies have shown that the growth rate and increased levels of fish meal replacement with Soya bean meal are inversely related (Reigh and Ellis, 1992). Two

explanations have been suggested to explain this. Firstly, that growth is negatively affected by the levels of trypsin inhibitors and antinutrients in SBM diets (Francis *et al.*, 2001), which inhibit protein digestibility (Elangovan and Shim, 2000). During the manufacturing process the SBM used in this experiment was commercially heat-treated. However, the duration and process of heat treatment is unknown and it is assumed that a certain level of trypsin inhibitors and other antinutrients were still present. The reduced PER and higher FCR values observed in the 40% and 50% SBM treatments may therefore have been due to the disruption of digestive processes, caused by elevated levels of anti-nutritional factors in the two high SBM diets.

Secondly, the amino acid balance of SBM, specifically reduced methionine levels, has been suggested as another reason why growth and feed efficiency may be reduced in fish fed high SBM diets. For red drum, *S. ocellatus*, McGoogan and Gatlin (1997) were able to replace up to 90% of the protein from fish meal with SBM, with the addition of essential amino acids. However, in the present experiment no amino acids were added to the higher SBM diets and this may offer another explanation for the reduced growth rates and feed efficiencies observed in the 40% and 50% SBM treatments.

Fat and moisture content in the carcass are usually inversely related, while protein content is reported to be more constant (Belal and Assem, 1995). Although this trend was observed in this experiment, no significant differences in protein, lipid, intraperitoneal fat and ash were detected. In similar fish meal substitution experiments, comparable body composition results have been reported for *P. olivaceus* (Kikuchi, 1999) and *S. salar* (Refstie *et al.*, 2001). The moisture content of fish reared on the control diet was significantly lower than on the diets in which SBM replaced either 30% or 40% of the fish meal protein. Similar increases in moisture content of fish fed comparatively higher

SBM diets were reported in *S. ocellatus*, *S. salar* and *D. labrax* (Reigh and Ellis, 1992; Opstvedt *et al.*, 2003) and according to Jobling (1983) higher body water content suggests that the overall nutritional status of the fish is poorer than in fish with lower body water content.

The HSI values (above 2) observed in this experiment are similar to values reported for *D. labrax,* in which hepatic fat deposition is high when fed plant protein based diets (Ballestrazzi *et al.*, 1998; Kaushik *et al.*, 2003). It has been hypothesised that the replacement of fish meal by plant protein sources such as soy protein concentrates may variably affect the hepatic lipogenic enzyme activities (Kaushik *et al.*, 2003). This may have occurred in this experiment and offers an explanation for the high HSI values recorded.

This study has shown that Soya bean meal may be used as a source of protein in combination with fish meal in the diets of juvenile *A. japonicus*, and that up to 30% of the fish meal protein can be replaced by protein from Soya bean meal, without adverse effects on growth performance, feed efficiency and body composition. Due to the fact that SBM is a much cheaper protein source than fish meal, future research needs to focus on the supplementation of essential amino acids and the role of anti-nutrition factors found in SBM, so that higher levels of Soya bean meal may be included in the diet of juvenile dusky kob.

CHAPTER 6

THE EFFECT OF FEEDING FREQUENCY AND GUT EVACUATION TIME ON THE GROWTH AND BODY COMPOSITION OF JUVENILE *ARGYROSOMUS JAPONICUS.*

Introduction

A major objective in the commercial culture of fish is to produce a high quality product at minimal cost. It is thus essential that fish are fed in an effective way so that feed utilization is optimal (Sveier and Lied, 1998).

Feed costs account for between 40 and 60% of the operating costs in intensive culture systems. The size of the daily ration and the frequency and timing of feedings are therefore key factors of feed management that influence growth, feed conversion and the economics of a farming operation (Bascinar *et al.*, 2001). Food is considered to be the most important biotic factor affecting metabolism and growth in fish (Charles *et al.*, 1984) and studies on the ration and frequency of feeding have aimed at achieving optimum levels of both. To ensure that growth rates remain optimal and costs as low as possible (by minimizing food wastage), fish depend on being fed the best possible diets at levels that do not exceed their needs (Charles *et al.*, 1984).

The relationship between food consumption and growth is an important ecological variable and an important base in the culture of a fish species (Klaoudatos and Apostolopoulos, 1986). The manipulation of food consumption, by the control of timing and frequency of feed delivery, has the ability to influence commercially important traits such as biomass gain, feed: gain ratio, organ and tissue composition and relative size and

size variation (Sveier and Lied, 1998). Feeding frequency directly affects the consumption and food intake of fish. Food intake increases as feeding frequency increases and the lowest frequency at which optimal growth is achieved, is termed the "optimum" feeding frequency (Grayton and Beamish, 1977). Considerable differences in the "optimum" feeding frequency for various fish species exist (Table 6.1). These differences may be due to physical attributes (such as size, age or species of fish), abiotic factors (such as temperature, light, pH, salinity and water quality) and biotic factors such as stocking density and social structure (Grayton and Beamish, 1977; Kestemont and Baras, 2001).

Table 6.1: The "optimum" feeding frequencies of various cultured fish species.

Species	Feeding frequency (Meals per day)	Reference
Yellowtail flounder, *Limanda ferruginea*	2	Dwyer *et al.* (2002)
Hybrid sunfish, *Lepomis cyanellus*	3	Wang *et al.* (1998)
Red-spotted grouper, *Epinephelus akaara*	6	Kayano *et al.* (1993)
Channel catfish, *Ictalurus punctatus*	2	Andrews & Page (1975)
Croaker, *Micropogonias furnieri*	1	Abud (1990)
Rainbow trout, *Oncorhynchus mykiss*	3	Ruohonen *et al.* (1998)
Korean rockfish, *Sebastes schlegeli*	1	Lee *et al.* (2000)
Flounder, *Paralichthys olivaceus*	2-3	Lee *et al.* (2000)
Sunshine bass, *Morone chrysops* & striped bass, *M. saxatilis*	2	Thompson *et al.* (2000)
Milkfish, *Chanos chanos*	2	Teshima *et al.* 1984
European seabass, *Dicentrarchus labrax*	2	Tsevis *et al.* (1992)

Food intake is governed by hunger level or satiation level, which in turn depends on the amount of food remaining in the stomach (Brett, 1971). The rate at which ingested food is processed in the digestive tract is desirable information to the fish farmer as gastric digestion and evacuation is frequently used in the estimation of daily food consumption (Jobling and Davies, 1979). It is therefore necessary to have a general idea of gut evacuation or gastric digestion (Charles *et al.*, 1984) rates. Gastric/gut evacuation time (GET) is defined by Smith (1989) as the time taken for ingested food to move entirely through the gastrointestinal tract. It has also been established that the return of appetite and the frequency of feeding are directly related to the gastric evacuation time (Grove *et al.*, 1978). Knowledge of gastric evacuation times together with results from feeding frequency experiments therefore provide the necessary information such that growth rates may be optimized, feed efficiency improved and overall feed costs reduced by limiting waste from overfeeding.

The objectives of this experiment were to examine the response of juvenile dusky kob to varying feeding frequencies, establish gut evacuation times, determine a daily ration amount (as a percentage of body weight per day) and establish the "optimum" feeding frequency. Responses were measured in terms of growth performance, feed efficiency and whole body composition.

Materials and methods

All the fish were captured, acclimated and weaned onto pellets as explained in Chapter 2.

Feeding frequency

Juvenile dusky kob with an average (± standard deviation) weight of 34.6 ± 17.3g and length of 146.2 ± 25.5mm were randomly distributed among 12 tanks (see Chapter 2). Three replicates of 12 fish (36 per dietary treatment) were fed at one of four feeding frequencies: once daily at 0900 h, twice daily at 0900 h and 1800 h, three times daily at 0900 h, 1400 h and 1800 h, and four times daily at 0900 h, 1200 h, 1400 h and 1800 h. Individual fish in each tank were weighed (g) and measured (Total length- mm) at the beginning of the 60-day experiment, every 20 days thereafter and upon completion of the feeding trial.

The fish in each replicate were hand fed to satiation at the desired feeding frequency and were assumed to be satiated (see Chapter 2) once active foraging at the surface ceased. The food (Table 6.2) for each tank was kept in individual containers and was weighed at the beginning and end of each day of the experimental period so as to calculate daily food intake.

The diet used in this experiment was constructed using the optimal protein and energy requirements established in Chapter 4. The proximate composition of the diet is shown in Table 6.2 and the procedures for diet preparation are outlined in Chapter 2.

Table 6.2: Ingredients and proximate composition of the standard diet fed at all feeding frequencies.

Ingredient	(g/100g)
Fish meal	45
Casein	18
Fish oil	3
Soya oil	1.5
PGS	27.5
Vitamin/mineral mix	5
Calculated values	
Digestible energy (kJ/g)	15.5
Protein: energy (mg/kJ)	29.0
Proximate analysis	
Protein	46.7
Lipid	9.3
Ash	8.5
Energy (MJ/kg)	17.9

PGS – Pre-gelatinised starch

At the end of the experiment, two fish from each tank (6 per dietary treatment) were randomly sampled and sacrificed for proximate analysis. Proximate analysis was undertaken following the methods outlined in Chapter 2.

Water quality parameters were measured once a week and were maintained within the desired levels outlined in the materials and methods chapter, while temperature and salinity were measured daily. The mean ± standard deviation of temperature and salinity during the 60 day trial were 18.9 ± 3.9°C and 33.1 ± 5.6 ppt, respectively.

Gut evacuation time

For this experiment, food marked with a 1% chromic oxide dye was substituted for the standard diet in the feeding frequency experiment on day 30 and day 50. The aim was to determine the time it took for food to be digested and reach the rectum/anus for evacuation. Besides the chromic oxide marker, the pellet used for this experiment was identical in its proximate composition to the diet used in the feeding frequency experiment. The use of chromic oxide as a reference marker in this experiment was based on the assumption that the compound moves uniformly with the food as it passes through the digestive tract and that it is inert and not absorbed by the fish (Zarate, 1999).

To asses the presence of faecal matter in the rectum, fish were anaesthetised in a 0.2ml/l solution of 2-phenoxyethanol for 1 minute or until they could be handled properly. Once anaesthetised, fish were dried on the underside and assessed for the presence of faeces by applying gentle pressure around the anal area. Every hour from the first feeding at 0900 h, five fish from each replicate were removed, anaesthetised and assessed for the presence of faecal material in the rectum. At the first appearance of green dye in the faeces, a further 10 fish from each treatment were removed and examined. The total gut evacuation time was determined as the time it took 75% of the fish in each treatment to evacuate dyed faeces.

Ration

To calculate the optimal daily ration, the feed intake in the feeding frequency that resulted in optimal growth, feed efficiency and body composition was used. The daily

ration (FI) expressed as a percentage wet body weight per day was calculated using the equation:

FI (% body wt/ day)=[average daily food consumed(g)] / [initial biomass of fish(g)]*100

Statistical analysis

Percent weight gain, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF) and whole body composition data were all calculated using the methods outlined in Chapter 2.

The Kolmogorov-Smirnov test was used to verify if data was normally distributed and the Levene Test was used to check for homogeneity in variances. Data from the replicates within each dietary treatment were analysed and compared for significant differences with a one-way ANOVA. If no significant differences were detected between replicates the data were pooled such that comparisons could be made (by ANOVA) between dietary treatments. Tukey's multiple comparison test was used to evaluate the mean difference among individual diets at the 0.05

The recorded percent of fish evacuating their guts between the 2^{nd} and 8^{th} hour after feeding were subject to linear regression analysis and differences in the rates between treatments were discerned by an analysis of covariance (ANCOVA; $p<0.05$).

Results

Growth responses

Percent weight gain, feed intake (FI), specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (CF) responses of juvenile *A. japonicus* are presented in Table 6.3.

Table 6.3: The effect of feeding frequencies on the percent weight gain, feed intake (FI), specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and condition factor of juvenile *Argyrosomus japonicus* fed for 60 days.

	Feeding frequency (meals per day)			
	1	2	3	4
Initial length	148.3 ±25.4	132.5 ±27.3	155.5 ±22.1	148.4 ±22.1
Final length	182.3 ±22.1	178.1 ±19.9	208.5 ±17.3	202.9 ±18.9
Initial weight	40.9 ±21.7	27.4 ±16.0	37.4 ±14.1	32.7 ±14.1
Final weight	61.2 ±23.0	60.3 ±18.6	90.4 ±22.6	83.3 ±23.9
Weight gain (%)	52.9^{a} ±24.8	122.2^{b} ±18.3	142.5^{b} ±9.1	155.9^{b} ±7.4
FI (% Body wt/day)	1.4^{a} ±0.8	4.0^{b} ±1.0	4.6^{b} ±0.4	4.7^{b} ±0.4
SGR	0.69^{a} ±0.27	1.33^{b} ±0.14	1.48^{b} ±0.06	1.57^{b} ±0.05
FCR	1.56^{a} ±0.19	1.96^{a} ±0.43	1.95^{a} ±0.21	1.80^{a} ±0.06
PER	1.44^{a} ±0.19	1.17^{a} ±0.24	1.15^{a} ±0.12	1.23^{a} ±0.04
Initial CF	3.18 ±0.37	2.94 ±1.04	2.6 ±0.3	2.58 ±0.27
Final CF	2.75^{a} ±0.15	2.93^{b} ±0.18	2.84^{ab} ±0.16	2.82^{a} ±0.2

Data values are mean ± SD of 3 replicates. Mean values in each row having the same superscript are not significantly different (ANOVA, $p < 0.05$).

In terms of weight gain, the lowest specific growth rate and percent weight gain was observed for the fish that were fed once a day and these values were significantly lower ($p < 0.05$) than for all other treatments. There were no significant differences ($p > 0.05$) in the SGR or percent weight gain for fish fed two, three and four times a day (Figure 6.1).

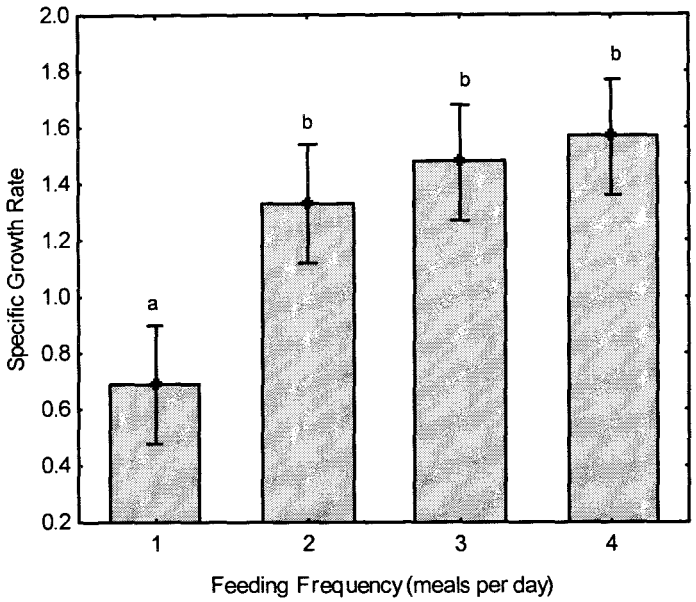

Figure 6.1: The specific growth rate of juvenile *Argyrosomus japonicus* fed at various feeding frequencies for 60 days. Error bars denote 95% confidence intervals. Significant differences between means are represented by non-overlapping bars and different superscripts.

In terms of feed intake, the fish fed one meal per day consumed a ration equivalent to 1.4% of their body weight per day. This was significantly lower ($p < 0.05$) than the fish in the remaining three feeding frequencies (Table 6.3; Figure 6.2), in which the fish consumed a ration equivalent to 4, 4.6 and 4.7% of their body weight per day,

respectively. There was no significant difference (p> 0.05) in feed intake (FI) between the two, three and four times daily feeding treatments (Figure 6.2).

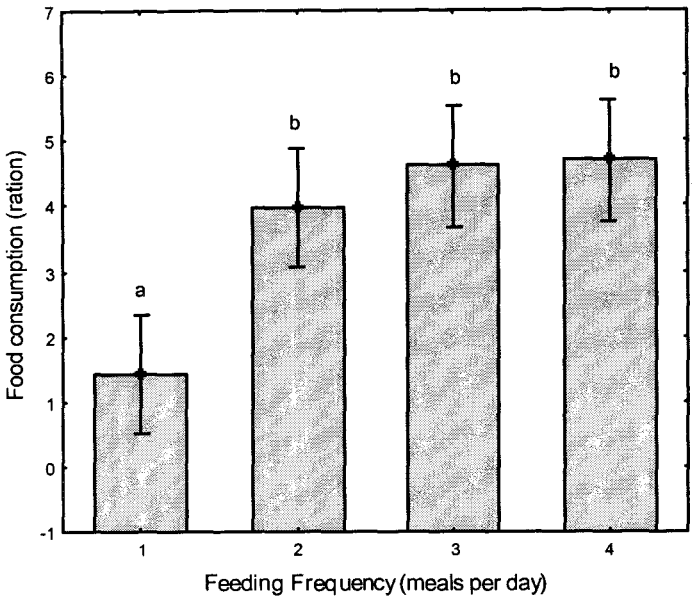

Figure 6.2: The food consumption (% wet body wt/day) of juvenile dusky kob, *Argyrosomus japonicus*, fed at various feeding frequencies for 60 days. Error bars denote 95% confidence intervals. Significant differences between means are represented by non-overlapping bars and different superscripts.

The lowest FCR value (1.56) was observed in the fish that were fed once a day but this was not significantly different (p> 0,05) to those fed two, three or four times a day (Figure 6.3).

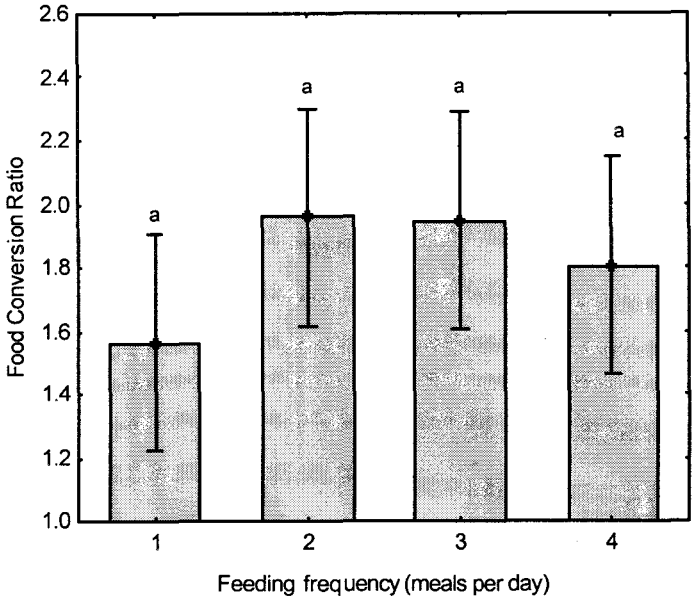

Figure 6.3: The food conversion ratio of juvenile *Argyrosomus japonicus* fed at various feeding frequencies for 60 days. Error bars denote 95% confidence intervals. Significant differences between means are represented by non-overlapping bars and different superscripts.

Although, the best protein efficiency ratio (1.44) was observed in the fish fed once per day there were no significant differences ($p > 0.05$) in PER between any of the treatments (Figure 6.4).

At the end of the experiment, the condition factor (CF) of the fish fed twice a day was significantly higher ($p > 0.05$) than those fed once and four times daily (Table 6.3).

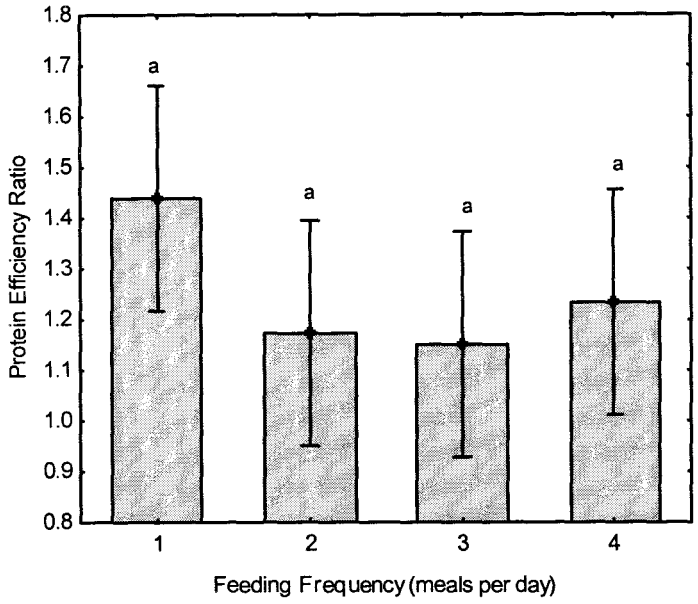

Figure 6.4: The protein efficiency ratio of juvenile *Argyrosomus japonicus* fed at various feeding frequencies for 60 days. Error bars denote 95% confidence intervals. Significant differences between means are represented by non-overlapping bars and different superscripts.

Carcass composition

The proximate body composition at the end of the 60-day feeding trial is summarised in Table 6.4.

The moisture content was not affected by any of the feeding frequencies and no significant differences ($p > 0.05$) were detected between treatments (Table 6.4).

Fish fed once per day had significantly less ($p < 0.05$) protein and lipid deposition, but significantly greater ($p < 0.05$) ash contents than the fish fed at the other three feeding

frequencies (Table 6.4). No significant differences ($p > 0.05$) in protein, lipid and ash contents were observed between the fish fed two, three and four times a day.

Table 6.4: Whole body composition data (% dry weight) of juvenile *Argyrosomus japonicus* fed at various feeding frequencies for 60 days.

	Feeding frequency (meals per day)			
	1	2	3	4
Protein	64.71^{a}	69.31^{b}	67.79^{b}	68.90^{b}
	0.8	0.75	0.95	0.50
Lipid	11.05^{a}	12.06^{b}	12.47^{b}	12.06^{b}
	0.15	0.27	0.39	0.13
Ash	20.43^{a}	17.48^{b}	16.58^{b}	16.51^{b}
	0.71	0.69	0.75	0.69
Moisture	70.78^{a}	72.01^{a}	71.14^{a}	70.85^{a}
	1.13	0.74	0.81	0.96
HSI	1.05^{a}	1.29^{ab}	1.83^{b}	1.95^{b}
	0.11	0.46	0.44	0.28
IPF	0.98^{a}	1.28^{a}	2.06^{b}	2.09^{b}
	0.16	0.15	0.54	0.45

Data values are mean values of 3 replicates. Mean values in each row having the same superscript are not significantly different ($p < 0.05$). HSI = hepatosomatic index. IPF = intraperitoneal fat ratio.

The hepatosomatic index (HSI) of the fish fed three and four times a day was significantly higher ($p < 0.05$) than the fish that received one meal per day (Table 6.4; Figure 6.5). The HSI value of fish fed twice a day was not significantly ($p > 0.05$) different to any of the other feeding frequencies.

The fish fed once and twice a day had significantly lower ($p < 0.05$) intraperitoneal fat (IPF) ratios than the fish fed three and four times a day (Table 6.4; Figure 6.5).

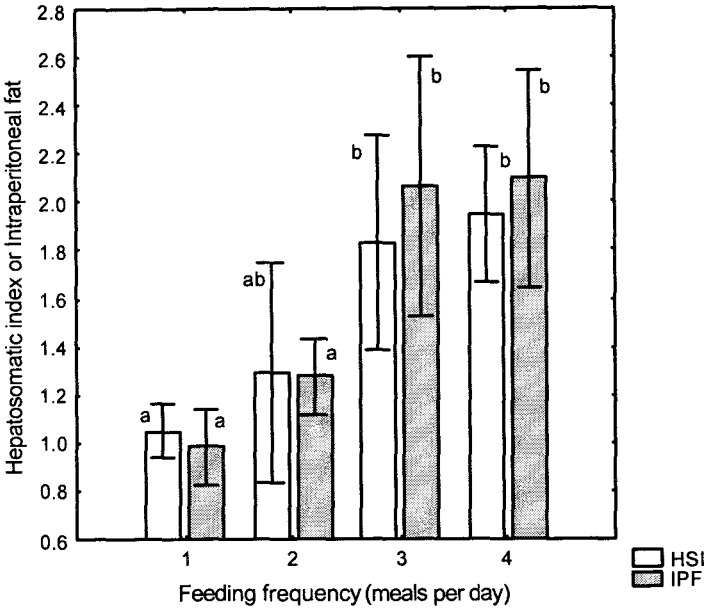

Figure 6.5: The hepatosomatic index (HSI) and intraperitoneal fat (IPF) index of juvenile *Argyrosomus japonicus* fed at various feeding frequencies for 60 days. Columns represent the means and vertical bars the standard deviation. Means with the same superscripts are not significantly different.

Gut evacuation time

The gut evacuation rates between the 2nd and 8th hour after feeding, expressed as linear equations are presented in Table 6.5

Fish fed one meal a day had a significantly slower (ANCOVA, $p<0.05$) rate of gut evacuation than the fish fed at all the other feeding frequencies. There were no significant differences ($p>0.05$) in the gut evacuation rates between fish fed two, three and four times a day (Table 6.5).

Table 6.5: The gut evacuation rate between the 2nd and 8th hour after feeding, expressed by linear regression equations for juvenile dusky kob, *Argyrosomus japonicus* fed at four feeding frequencies.

Feeding frequency (meals per day)	Linear regression equation	r^2
One	$y = -34.1 + 13.31x$ [a]	0.94
Two	$y = -43.9 + 17.78x$ [b]	0.95
Three	$y = -35.6 + 19.67x$ [b]	0.97
Four	$y = -32.62 + 19.3x$ [b]	0.94

Equations in the column having the same superscript are not significantly different (ANCOVA, $p<0.05$)

The fish fed one meal per day took an average of 7.25 hours before 75% of the fish had evacuated dyed faeces (Figure 6.6), while those fed twice a day took an average of 6.2 hours. The quickest evacuation times were observed in the fish fed three and four times a day, with an average gut evacuation time of 5.5 hours after feeding (Figure 6.6).

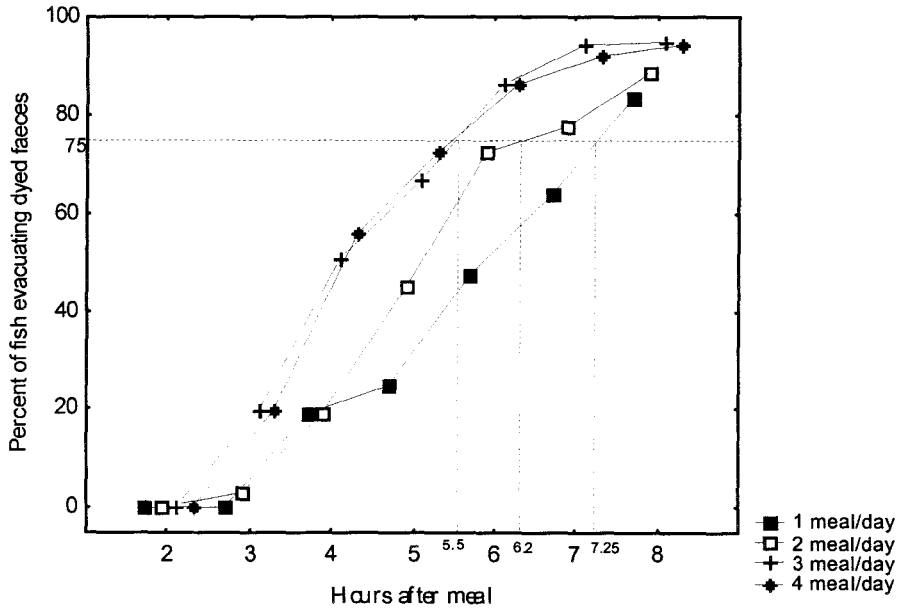

Figure 6.6: The gut evacuation times for juvenile *Argyrosomus japonicus* fed at various feeding frequencies (time taken to evacuate dyed faeces).

Discussion

Feeding frequency had a significant effect on food consumption, growth rate and body composition of juvenile dusky kob. Both feed intake and growth rates increased from one meal a day to two meals a day. However, further increases in feeding frequency from two to four meals a day did not result in better growth, improved feed efficiency or carcass quality. Similarly, feeding juvenile channel catfish, Atlantic cod and European sea bass twice a day was found to be optimal in terms of growth rates and feed efficiency (Andrews and Page, 1975; Folkvord and Ottera, 1993; Tsevis *et al.*, 1992).

The lack of significant differences in food conversion ratios supports the argument that the effect of feeding frequency on food conversion is usually small (Wang *et al.*, 1998). Fish that are fed more frequently and consume more food utilise that food as efficiently as fish fed less frequently, and that food consumption and not conversion is the growth-limiting factor (Andrews and Page, 1975). In terms of PER, the lack of significant differences between treatments provides further evidence that food consumption is the limiting factor in terms of growth, as the protein that was provided to fish fed once a day was utilised just as efficiently as by those that were fed more meals per day.

As ration is considered to an important factor in the growth of cultured fish, any restriction to it results in lower metabolic rates (Fiogbe and Kestemont, 2003). In this experiment fish fed one meal consumed a significantly smaller ration (1.4% BW/day) than those in the other feeding frequency treatments. Resultant inferior metabolic rates may have caused the slower growth rate observed in the one meal per day treatment. Brett and Grove (1979) state that any ration between the amount required for body maintenance and that required for maximum growth causes weight increase. Furthermore,

the authors state that the greatest weight gain per unit of given ration is obtained before the maximum feeding level and that this level is considered optimal in terms of biological conversion.

Studies on other fish species have shown that food consumption and growth generally increase with increased feeding frequency up to a certain point (Andrews and Page, 1975; Grayton and Beamish, 1977). However this was not the case in this experiment as food consumption was the same for the fish fed two, three and four times a day (Figure 6.2). Grove *et al.* (1978) suggest that stomach fullness is a major factor controlling appetite and feeding motivation in cultured fish and the size of the meal at each feeding in this study seems to have been dictated by stomach fullness. According to the gut evacuation times (Figure 6.6), fish fed three and four times a day took an average of 5.5 hours to evacuate their stomachs. This gut evacuation time (5.5 hours) together with the known feeding intervals and calculated daily ration, suggests that the feed intake per meal in the fish fed three and four times a day was reduced because of incomplete digestion and the presence of food in the stomach. Grove *et al.* (1978) have shown that there is a close correlation between the time required to evacuate the stomach and the return of appetite. Jobling (1982) suggests that fish may also adapt to more restricted feeding regimes by increasing food intake at each meal. Although the feed deprivation time of approximately 8.5 hours in fish fed twice a day is not as long as the times investigated by Jobling (1982), it may still tender another possible explanation for the food intake results obtained. Unfortunately it is not possible to use gut evacuation rates of other fish species as references for comparison because gut evacuation times are highly variable and are influenced by factors such as gut length, diet, water temperature and meal size (Smith, 1989).

Protein and lipid accumulation in juvenile dusky kob was significantly affected by increasing feeding frequency. The fish fed once a day had significantly less protein and fat deposition than those in the other treatments. Kayano *et al.* (1993) postulate that low feeding frequencies may cause metabolic changes that depress protein assimilation and accumulation of lipid in the muscle. This has been found in channel catfish (Noeske-Hallin *et al.*, 1985) and rainbow trout (Grayton and Beamish, 1977), in which increased lipid levels were reported when feeding frequency was increased from one meal to two meals a day. As already hypothesized, the resultant smaller fish size and decreased protein and fat accumulation, are the result of the fish being offered and eating less food than the fish in the other treatments.

The fish fed three and four times a day had more IPF than those at the two lower feeding frequencies. Kayano *et al.* (1993) suggested that the low accumulation of intraperitoneal fat, caused by infrequent feedings, could be attributed to a low conversion of dietary protein. Fish fed three and four times daily would have received more dietary protein than the other fish and the surplus energy derived from the extra food may have accumulated and deposited as fat within certain tissues and the gut cavity.

The significantly higher HSI values of the fish fed three and four times a day are similar to those in juvenile sunshine bass (*Morone chrysops)*, in which greater values were reported for fish fed at the highest feeding frequency (Thompson *et al.*, 2000). According to Tyler and Dunn (1976), the liver weight should alter appreciably with increased ration or feeding frequency and they found that liver weight was positively correlated with calories consumed. In this experiment, fish fed three and four times a day would have

received more calories (due to a higher daily feed intake) than the fish fed once a day, and this may explain the larger HSI values.

The results of this experiment raise the question of the effect of feeding time on the growth and feed efficiency in juvenile kob. Fish fed twice a day consumed as much food as those fed three and four times a day and perhaps this indicates that dusky kob prefer to feed in the morning and early evening. Traditionally, the quantities of feed delivered to cultured fish have usually been regulated according to temperature and fish size (Bolliet *et al.*, 2001). Feed has often been distributed in normal working hours, regardless of any natural feeding rhythm of the species. Thus the timing of feed provision may not match the peak of appetite, leading to poor growth, feed utilisation and feed wastage (Bolliet *et al.*, 2001). To investigate this hypothesis further one would need to ascertain at what time of day juvenile dusky kob, under culture conditions and in their natural habitat, are predominantly feeding, and compare similar feeding frequency responses under light and dark regimes.

Under the present experimental conditions the "optimum" feeding frequency is assumed to be twice a day. Feeding fish to satiation more than twice a day did not result in superior growth, feed efficiency or body composition. Feeding fish above the "optimum" frequency is likely to increase production costs because more time and labour are required and more feed is likely to be wasted (Lee *et al.*, 2000).

Feeding fish two, three and four times day to satiation, resulted in a daily feed intake of 4.0, 4.6 and 4.7% of wet body weight per day (BW/day), respectively. As no significant differences were detected in growth rates, feed efficiency and body composition between

the fish in these feeding frequencies it is recommended that a minimum ration of 4.0% BW/ day^{-1} be fed twice a day to fish with an average weight of 35g. In general smaller fish consume more feed when expressed as a percent of body weight than do larger fish (Gatlin, 2002). Red drum weighing less than 5g consume over 7% BW/day, while 50g red drum consume approximately 5% BW/day (Gatlin, 2002). Although the optimal daily ration in this experiment (4% BW/day) is lower than the recommended ration for red drum of a similar size, it is still comparable and the difference may be attributed to species differences or water temperature.

Due to the fact that food consumption changes as fish size changes, future research should focus on the relationship between feeding frequency and ration and fish size, feeding frequency and temperature, as well as feeding frequency and time of feeding.

CHAPTER 7

GENERAL DISCUSSION

This final discussion will examine each experiment within the study individually and discuss their relevant contribution towards the understanding of the basic nutritional requirements of a grow out diet for juvenile dusky kob, *A. japonicus*.

As discussed in the general introduction, dusky kob have been severely over fished in South Africa (Anon, 2000) and Australia (O'Sullivan and Ryan, 2001). The dramatic decline in the natural stocks, together with the popularity of this species have lead to studies on the suitability of this species for aquaculture. As far as could be ascertained, published studies thus far have examined the screening of *A. japonicus* as a candidate for aquaculture (Hecht, personal communication), hormone induction and larval rearing (Battaglene and Talbot, 1994), the effects of salinity on growth and survival (Fielder and Bardsley, 1999) and the enhancement of mulloway in intermittently opening lagoons (Fielder *et al.*, 1999). The taxonomy, life history, growth and feeding of dusky kob is also fairly well understood (Griffiths, 1996; Griffiths, 1997a; Griffiths and Hecht, 1995; Griffiths and Heemstra, 1995) and available information suggests that dusky kob have various attributes (euryhaline, fast initial growth, good palatability) required for successful culture. Some unpublished information and "grey" literature also exists regarding the experimental and the potential for culture of this species within South Africa and Australia, however no information is available regarding the dietary requirements of this species under culture conditions.

Due to the high running costs involved in a commercial scale fish farming operation (and with our knowledge of the life history of this species), it is assumed that the grow out phase of this species would probably occur under natural (as opposed to controlled) conditions. This study was therefore conducted under ambient conditions as it was decided that this method would make the results more relevant to any future commercial grow out of this species. The location of the DIFS Marine Laboratory in Port Alfred is such that any temperature and salinities recorded in this study would fall within the natural range of known conditions for this species.

A complete diet should meet an animals needs for essential nutrients and supply energy for maintenance, growth and reproduction (Jobling *et al.*, 2001). It is vital that these factors be addressed when feeds are being formulated. The aim of this study was therefore to establish the basic nutritional requirements of juvenile dusky kob, by investigating the effects of protein inclusion levels, protein and energy ratios, fish meal substitution by Soya bean meal and feeding frequency, under ambient conditions. Due to the fact that the experiments in this study were conducted at different times of the year (under varying ambient conditions), the results of each experiment cannot be compared with one another. Therefore each experiments results will be discussed separately.

Fish like all other animals, do not have a true protein requirement but rather a requirement for a well-balanced mixture of essential and non-essential amino acids (Wilson, 1989). In the protein requirement experiment (Chapter 3), a minimum protein inclusion level of 45% and maximum of 52.3% (obtained from a polynomial regression model) was found to be optimal in terms of growth, feed efficiency and body composition. This is similar to the protein requirement of European sea bass,

Dicentrarchus labrax (Kaushik, 2002), gilthead sea bream, *Sparus aurata* (Koven, 2002) and red drum, *Sciaenops ocellatus* (Serrano *et al.*, 1992), all of which are carnivorous marine fish species.

In addition to supplying amino acids for protein synthesis, dietary protein may also be catabolized for energy (Gatlin, 2002). Chapter 4 investigated the optimal protein: energy (P: E) ratio and examined if the optimal protein inclusion level of 45% (obtained in Chapter3) could be reduced by increasing dietary lipid. A digestible energy (DE) level of 15.5 kJ/g with a protein: energy (P: E) ratio of 29 mg/kJ was found to be optimal for juvenile dusky kob. This result is also very similar to red drum where optimal growth and body composition was observed in fish fed a diet containing 15 kJ/g DE and a P: E ratio of 28.6 mg/kJ (Daniels and Robinson, 1986). Kob appears to be highly proficient at using dietary protein for energy (as other carnivorous fish are), and this may be due to the fact that ammonia from deaminated protein is excreted via the gills with limited energy expenditure (NRC, 1993). Protein sparing did not occur in this experiment and in future investigations it is recommended that higher lipid levels be compared

Costs associated with diets and feeding generally constitute the largest expense in intensive fish production (Gatlin, 2002). From a nutritional point of view, it has been established that in all fish diets, protein is the most important nutrient and that fish meal remains the major source of dietary protein (Kaushik, 1990). Globally, efforts are being made to reduce the amount of fish meal in aquaculture diets (Kaushik, 1990). One way is to substitute fish meal with a less expensive plant protein source such as Soya bean meal (SBM). In Chapter 5, varying levels of fish meal were replaced by SBM in isocaloric diets containing 45% protein and 9% lipid. Results showed that up to 30% of the protein

from fish meal could be replaced with SBM protein, without any adverse effects on growth, feed efficiency and body composition. Similar to red drum, *S. ocellatus* (Gaylord & Gatlin, 1996); gilthead sea bream, *S. aurata* (Robaina *et al.*, 1995) and Atlantic salmon, *S. salar* (Carter & Hauler, 2000); dusky kob showed a good tolerance to changes in diet composition. As fish meal prices continue to increase, and knowing that dusky kob respond well to dietary changes, it is suggested that future nutritional studies investigate a) other less expensive alternative protein sources, and b) higher levels of fish meal substitution by plant proteins with the addition of amino acid supplements, such as methionine.

A number of experiments with various fish species have been conducted in order to determine the minimum number of feedings required to produce maximum growth rates (Jobling, 1982). In the feeding frequency experiment (Chapter 6), feeding to satiation twice per day produced optimal growth, feed efficiency and body composition. Feeding twice a day to satiation resulted in an equivalent daily ration of 4% BW/day. The relationship between feeding frequency and gut evacuation was also investigated as there is a recognised link with feeding frequency, stomach fullness and the return of appetite (Grove *et al.*, 1978). The rate at which ingested food is processed in the digestive tract is desirable information to the fish farmer due to the fact that food consumed can be measured and used as a tool to determine productivity (Jobling and Davies, 1979).

Fish fed twice a day would have had approximately 9 hours before the next feeding and with a gut evacuation rate of 7.25 hours it may have enabled them to ingest more food per meal than fish fed three and four meals a day.

In their natural habitat it is known that dusky kob (200-300mm TL) feed predominantly on mysids, while larger fish (220-870mm TL) feed mainly on fish (Marais, 1984; Coetzee and Pool, 1991; Smale and Bruton, 1985). It is assumed that kob in the wild would spend more energy catching fish prey than they would for mysids, which occur in large swarms (Coetzee and Pool, 1991). This suggests that there feeding frequency within their natural environment may be reduced at a certain size or age, because food may be harder to come by. Therefore, a future experiment is recommended to investigate the relationship between fish size, ration size and feeding frequency. Furthermore, it is suggested that the relationship between ration, feeding frequency, temperature and time of feeding (light or dark) be investigated as many fish display rhythmic patterns of feeding and are generally classified as being diurnal, nocturnal or crepuscular feeders (Bolliet *et al.*, 2001).

The basic nutritional requirements of juvenile dusky kob for optimal growth under the present ambient study conditions (average temperature- 19°C, salinity- 33 ppt, pH- 8; Oxygen- 80-100%) are presented in Table 7.1.

It is suggested that the established diet be fed at a ration of 4% BW/day twice a day (in the morning and evening). Comparative diets for other carnivorous fish are also presented in Table 7.1 and the variation in nutritional requirements may be attributed to differences in study methods, conditions and the size and age of fish compared (Wilson, 1989).

Table 7.1: The minimum dietary requirements of juvenile dusky kob, *A. japonicus*, compared with the requirements of other cultured carnivorous fish species.

	Species			
Ingredient	*Argyrosomus japonicus*	*Sciaenops ocellatus*	*Dicentrarchus labrax*	*Sparus aurata*
Protein	45	40-45	45	55
Lipid	9	8	12	12-24
Feed frequency	2		2-3	
P: E (mg/kJ)	29	28.6	19-35	28.5
DE (kJ/g)	15.5	15	21	20

P: E – Protein: Energy ratio
DE – Digestible energy

Juvenile dusky kob was easily weaned and readily accepted a dry pellet. Generally, this species displayed good growth rates and feed efficiencies (FCR and PER) that were comparable, if not better than some presently farmed species. Dusky kob showed a remarkable tolerance to a range of environmental conditions and experimental diets throughout the entire study period. Its suitability to culture systems, excellent body composition attributes and popularity as a food fish throughout its natural distribution, suggests that *A. japonicus* could compete locally and in export markets with other fish species.

CHAPTER 8

REFERENCES

Allan. G. L., Parkinson. S., Booth. M. A., Stone. D. A. J., Rowland. S. J., Frances. J. and R. Warner-Smith 2000. Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. *Aquaculture,* 186: 293-310

Andrews, J. W. and Page, J. W. 1975. The effects of frequency of feeding on culture of catfish. *Transactions of the American Fisheries Society*, 2: 317- 321

Anonymous, 2000. Breakthrough in Fish Breeding. Http://www.environment.gov.za

Aristizabal Abud, E. O. 1990. Effect of feeding frequency in juvenile croaker, *Micrpogonias furnieri* (Pisces: Sciaenidae) *Journal of Fish Biology*, 37: 987-988

Arndt, R. E., Hardy, R. W., Sugiura, S. H. and Dong, F. M. 1999. Effects of heat treatment and substitution level on palatability and nutritional value of soy defatted flour in feeds for coho salmon, *Onchorhynchus kisutch. Aquaculture*, 180: 129-145

Arzel, J., Metailler, R., Kerleguer, C., Delliou, H. H. and Guillaume, J. 1995. The protein requirement of brown trout (*Salmo trutta*) fry. *Aquaculture,* 130: 67-68

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

Ballestrazzi, R., Lanari, D., Agaro, A. and Mion, A. 1994. The effect of dietary protein level and source on growth, body composition, total ammonia and reactive phosphate excretion of growing sea bass (*Dicentrarchus labrax*). *Aquaculture*, 127: 197-206

Ballestrazzi, R., Lanari, D., D'Agaro, E., 1998. Performance, nutrient retention efficiency, total ammonia and reactive phosphorus excretion of growing European sea-bass (*Dicentrarchus labrax,* L.) as affected by diet processing and feeding level. *Aquaculture*, 161, 55–65.

Barlow, S. 1989. Fish meal world outlook to the year 2000. *Fish Farmer.* Sept/Oct., pp 40-43

Battaglene, S. C. and Talbot, R. B. 1994. Hormone induction and larval rearing of mulloway, *Argyrosomus hololepidotus* (Pisces: Sciaenidae). *Aquaculture*, 126: 73-81

Bascinar, N., Okumus, I., Bascinar, N. S. and Saglam, H. E. 2001. The influence of daily feeding frequency on growth and feed consumption of rainbow trout fingerlings (*Oncorhynchus mykiss*) reared at 18.5-22.5°C. *The Israeli Journal of Aquaculture*, 53(2): 80-83

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

Bolliet, V., Azzaydi, M. and Boujard, T. 2001. Effects of feeding time on feed intake and growth. In: *Food Intake in Fish.* (Eds) Houlihan, D., Boujard, T. and Jobling, M. Blackwell Science Ltd., Oxford, London, pp. 233

Boonyaratpalin. M., Suraneiranat. P. and Tunpibal, T. 1998. Replacement of fish meal with various types of soybean products in diets for the Asian seabass, *Lates calcarifer. Aquaculture*, 161: 67-78

Brett, J. R. 1971. Satiation time, appetite and maximum food intake of sockeye salmon (*Oncorhynchus mykiss*). *Journal of the Fisheries Research Board of Canada*, 28: 409-415

Bromley, P. J. 1980. Effect of dietary protein, lipid and energy content on the growth of

turbot (*Scopthalmus maximus* L.). *Aquaculture*, 19: 359-369

Brown, P. B., Allen Davis, D. and Robinson, E. H. 1988. An estimate of the lysine requirement of juvenile red drum, *Sciaenops ocellatus*. *Journal of the World Aquaculture Society,* 19 (3): 109-112

Carter. C. G. and R. C. Hauler. 2000. Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. *Aquaculture*, 185: 299- 311

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

Charles, P. M., Sebastion, S. M., Raj, M. C. and Marian, M. P. 1984. Effect of feeding frequency on growth and food conversion of *Cyprinus carpio* fry. *Aquaculture*, 40: 293-300

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

Chou, R., Su, M. and Chen, H. 2001. Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron candum*). *Aquaculture,* 193: 81-89

Coetzee, D. J. and Pool, R. C. 1991. Diet of the larger fish species in the Breede River estuary, with emphasis on the prey species *Palaemon capensis*. Bontebok, 7: 22-26

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

Cowey, T. B., Pope, J. A., Adron, J. W. and Blair, A. 1972 Studies on the nutrition of marine flat fish. The protein requirement of plaice, *Pleuronectes platessa*. *British Journal of Nutrition*, 28: 447-456

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. and Arnold, C. R. 1997. Response of Atlantic croaker fingerlings to practical diet formulations with varying protein and energy contents. *Journal of the World Aquaculture Society,* 28(3): 241-248

Deacon, N., White, H. and Hecht, T. 1997. Isolation of the effective concentration of 2-phenoxyethanol for anaesthesia in the spotted grunter, *Pomadasys commersonii*, and its effect on growth. *Aquarium Sciences and Conservation,* **1**: 19-27

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

Dias, J., Gomes, E. F. and Kaushik, S. J. 1997. Improvement of feed intake through supplementation with an attractant mix in European sea bass fed plant-protein rich diets. *Aquatic Living Resources*, 10: 385-389

Dwyer, K. S., Brown, J. A., Parrish, C. and Lall, S. P. 2002 Feeding frequency affects food consumption, feeding pattern and growth of juvenile yellowtail flounder (*Limanda ferruginea*). *Aquaculture*, 213: 279-292

Elangovan A. and Shim, K. F. 1997. Growth response of juvenile *Barbodes altus* fed isocaloric diets with variable protein levels. *Aquaculture*, 158: 321-329

Elangovan. A. and Shim. K. F. 2000. The influence of replacing fish meal partially in the diet with soybean meal on growth and body composition of juvenile tin foil barb (*Barbodes altus). Aquaculture*, 189: 133-144

Elliot, J. M. 1976. Body composition of brown trout (*Salmo trutta*) in relation to temperature and ration size. *Journal of Animal Ecology*, 45: 273-289

Ellis, S. C. and Reigh, R. C. 1991. Effects of dietary lipid and carbohydrate levels on

growth and body composition of juvenile red drum, *Scaienops ocellatus*. *Aquaculture*, 97: 383-394

Fielder, D. S., and Bardsley, W. 1999. A preliminary study on the effects of salinity on growth and survival of mulloway *Argyrosomus japonicus* larvae and juveniles. *Journal of the World Aquaculture Society*, 30(3): 381-387

Fielder, S., Bardsley, W. and Allen, G. L. 1999. Enhancement of mulloway in intermittently opening lagoons. *NSW Fisheries Final Report Series,* No. 14. FRDC project No. 95/148

Fiogbe, E. D. and Kestemont, P. 2003. Optimum daily ration for Eurasian perch *Perca fluviatilis* L. reared at its optimum growing temperature. *Aquaculture*, 216: 243-252

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

Folvord, A. and Ottera, H. 1993. Effects of initial size ditribution, day length and feeding frequency on growth, survival and cannibalism in juvenile Atlantic cod (*Gadus morhua* L.). *Aquaculture*, 114: 243-260

Francis, G., Harinder, M. P. S. and Becker, K. 2001. Antinutritional factors present in plant derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, 199: 197-227

Gatlin III, D. M. 2002. Red drum, *Scaienops ocellatus*. In: *Nutrient requirements and feeding of finfish for aquaculture.* (Eds.) Webster, C. D. and Lim, C. E. CABI Publishing, New York, USA. pp. 147

Gaylord, T. G. and Gatlin III, D. M. 1996. Determination of digestibility coefficients of various feed stuffs for red drum (*Scaienops ocellatus*). *Aquaculture*, 139: 303-314

Gonzalez, C. A., Civera-Cerecedo, R., Ortiz-Galindo, J. L., Dumas, S., Moreno-Legoretta, M. and Grayeb-Del Alamo, T. 2001. Effect of dietary protein level on growth and body composition of juvenile spotted sand bass, *Paralabrax maculatofasciatus*, fed practical diets. *Aquaculture*, 194: 151-159

Gray, C. A. and McDonall, V. C. 1993. Distribution and growth of juvenile mulloway, *Argyrosomus hololepidotus* (Pisces: Sciaenidae), in the Hawkesbury River, South-eastern Australia. *Australian Journal of Marine and Freshwater Research*, 44: 401-409

Grayton, B. D. and Beamish, F. W. H. 1977. Effects of feeding frequency on food intake, growth and body composition of rainbow trout (*Salmo gairdneri*). *Aquaculture*, 11: 159-172

Griffiths, M. H. 1996. Life history of the dusky kob *Argyrosomus japonicus* (Sciaenidae) off the east coast of South Africa. *South African Journal of Marine Science*, 17: 135-154

Griffiths, M. H. 1997a. Feeding ecology of South African *Argyrosomus japonicus* (Pisces: Sciaenidae), with emphasis on the eastern Cape surf zone. *South African Journal of Marine Science*, 18: 249-264

Griffiths, M. H. 1997b. Influence of prey availability on the distribution of dusky kob, *Argyrosomus japonicus* (Sciaenidae) in the great fish river estuary, with notes on the diet of early juveniles from three other estuarine systems. *South African Journal of Marine Science*, 18: 137-145

Griffiths, M. H 1997c. Management of South African dusky kob, Argyrosomus japonicus (Sciaenidae) based on per recruit models. *South African Journal of Marine Science*, 18: 213-228

Griffiths, M. and Hecht, T. 1995. Age and growth of South African dusky kob, *Argyrosomus japonicus* (Sciaenidae) based on otoliths. *South African Journal of Marine Science*, 16: 119-128

Griffiths, M. H. and Heemstra, P. H. 1995. A contribution to the taxonomy of the marine fish genus *Argyrosomus* (Perciformes: Sciaenidae), with descriptions of two new species from southern Africa. *J. L. B. Smith Institute of Ichthyology: Ichthyological Bulletin*, 45: 1-40

Grove, D. J., Loizides, L. G. and Nott, J. 1978. Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. *Journal of Fish Biology*, 12: 507-516

Hastings, W. H. and Dickie, L.M. 1972. Feed formulation and Evaluation. In: *Fish Nutrition. 1st Edition*. (Ed.) Halver, J. Academic Press, London (Ltd), pp. 327

Hepher, B. 1988. *Nutrition of Pond Fishes*. Cambridge University Press, Cambridge, UK.

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

Irish, A. 1997. Investigations into the dietary protein requirements of juvenile spotted grunter, *Pomadasys commersonnii* (Haemulidae: Pisces). *MSc Thesis*, Rhodes University, Grahamstown, South Africa

Jobling, M. 1982. Some observations on the effects of feeding frequency on the food intake and growth of plaice, *Pleuronectes platessa* L. *Journal of Fish Biology*, 20: 431-444

Jobling, 1983. Effect of feeding frequency on food intake and growth of Arctic charr, *Salvelinus alpinus* L. *Journal of Fish Biology*, 23: 177-185

Jobling, M. 2001. Feed Composition and Analysis. In: *Food Intake in Fish*. (Eds) Houlihan, D., Boujard, T. and Jobling, M. Blackwell Science Ltd, Oxford, England. pp. 1-24

Jobling, M. and Davies, S. 1979. Gastric evacuation in Plaice, *Pleuronectes platessa* L.: effects of temperature and meal size. *Journal of Fish Biology*, 14: 539-546

Jobling, M., Gomes, E. and Dias, J. 2001. Feed types, Manufacture and Ingredients. In: *Food Intake in Fish*. (Eds) Houlihan, D., Boujard, T. and Jobling, M. Blackwell Science Ltd, Oxford, England. pp. 25-48

Kaushik, S. 1990. Use of alternative protein sources for the rearing of carnivorous fish. In: *Mediterranean Aquaculture*. (Eds) Flos, R., Tort, L. and Torres, P. Ellis Horwood Limited, West Sussex, England. pp. 125-142

Kaushik, S. J. 2002. European sea bass, *Dicentrarchus labrax*. In: *Nutrient Requirements and Feeding of Finfish for Aquaculture*. (Eds.) Webster, C. D. and Lim, C. E. CABI Publishing, New York, USA. pp 28-39

Kaushik, S. J. and Gomes, E. F. 1988. Effect of frequency of feeding on nitrogen and energy balance in rainbow trout under maintenance conditions. *Aquaculture*, 73: 207-216

Kayano, Y., Yao, S., Yamamoto, S. and Nakagawa, H. 1993. Effects of feeding frequency on the growth and body constituents of young red spotted grouper, *Epinephelus akaara*. *Aquaculture*, 110: 271-278

Kestemont, P. and Baras, E. 2001. Environmental factors and feed intake: Mechanisms and Interactions. In: *Food Intake in Fish*. (Eds) Houlihan, D., Boujard, T. and Jobling, M. 2001. Iowa State University Press, Blackwell Science Ltd. pp. 131-156

Kikuchi, K. 1999. Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (*Paralichthys olivaceus*). *Aquaculture*, 179: 3-11

Kim J. and Lall, S. P. 2001. Effects of dietary protein level on growth and utilization of protein and energy by juvenile haddock (*Melanogrammus aeglefinus*). *Aquaculture*, 195: 311-319

Kim. K, Terrence. B. K. and Amundson, C. H. 1991. Purified diet development and re-evaluation of the dietary protein requirement of fingerling rainbow trout (*Onchorhynchus mykiss*). *Aquaculture*, 96: 57-67

Kissil, G. W., Lupatsch, I., Higgs, D. A. and Hardy, R. W. 2000. Dietary substitution of soy and rapeseed concentrates for fish meal and their effects on growth and nutrient utilization in gilthead sea bream, *Sparus aurata. Aquaculture Research,* 31: 595-602

Klaoudatos, S and Apostolopoulos, J. 1986. Food intake, growth maintenance and food conversion efficiency in the gilthead sea bream (*Sparus auratus*). *Aquaculture*, 17: 221-229

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

Koven, W. 2002. Gilt-head sea bream. In: *Nutrient requirements and feeding of finfish for aquaculture.* (Eds.) Webster, C. D. and Lim, C. E. CABI Publishing, New York, USA. pp. 64-78

Lazo J. P., Davis D. A. and Arnold, C. R. 1998. The effects of dietary protein level on growth, feed efficiency and survival of juvenile Florida pompano (*Trachinotus carolinus*). *Aquaculture*, 169: 225-232

Lee, S-M., Cho, S. H. and Kim, D-J. 2000. Effects of feeding frequency and dietary energy level on growth and body composition of juvenile flounder, *Paralichthys olivaceus. Aquaculture Research,* 31: 917-921

Lee, S., Hwang, U. and Cho, S. H. 2000. Effects of feeding frequency and dietary moisture content on growth, body composition and gastric evacuation of juvenile Korean rockfish (*Sebastes schlegeli*). *Aquaculture*, 187: 399- 409

Lee, S., Jeon, I. G. and Lee, J. Y. 2002. Effects of digestible protein and lipid levels in

practical diets on growth, protein utilization and body composition of juvenile rock fish (*Sebastes schlegeli*). *Aquaculture*, 211: 227-239

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

Lim, C. and Akiyama, D. M. 1992. Full-fat soybean meal utilisation by fish. *Asian Fisheries Science*, 5: 181-197

Lochman, R. T. and Phillips, H. 1996. Dietary protein requirement of juvenile golden shiners (*Notemigonus crysoleucas*) and gold fish (*Carassius auratus*) in aquaria. *Aquaculture*, 128: 277-285

Lovell, T. 1989. The Nutrients. In: *Nutrition and Feeding of Fish*. Van Nostrand Reinhold, New York.

Lupatsch, I., Kissil, G. W., Sklan, D. and Pfeffer, E. 1998. Energy and protein requirements for maintenance and growth in gilthead sea bream (*Sparus aurata* L). *Aquaculture Nutrition*, 7: 71-80

Marais, J. F. K. 1984. Feeding ecology of major carnivorous fish from four eastern Cape estuaries. *South African Journal of Zoology,* 19(3): 210-223

Marais, J. F. K. 1990. Body composition of ten marine migrants and one freshwater species caught in estuaries in the eastern Cape, South Africa. *South African Journal of Marine Science*, 9: 135-140

Mathis, N., Fiedt, C. and Brun-Bellut, J. 2002. Influence of protein/energy ratio on carcass quality during the grow out period of Eurasian perch (*Perca fluviatilis*). *Aquaculture*, 217: 453-464

McGoogan, B. B. and Gatlin, D. M. 1997. Effects of replacing fish meal with soybean meal in diets for red drum, *Scaienops ocellatus*, and potential for palatability enhancement. *Journal of the World Aquaculture Society,* 28: 374-385

McGoogan, B. B. and Gatlin, D. M. 2000. Dietary manipulations affecting growth and nitrogenous waste production of red drum, *Sciaenops ocellatus*. II. Effects of energy level and nutrient density at various feeding rates. *Aquaculture,* 182: 271-285

Mertz, E. T. 1972. Protein and Amino acid needs. In: *Fish Nutrition. 1st Edition.* (Ed) Halver, J. Academic Press, London, pp. 106-144

Millikin, M. R. 1982. Effects of dietary protein concentration on growth, feed efficiency and body composition of age- 0 striped bass. *Transactions of the American Fisheries Society,* 111: 373-378

Milliken, M. R. 1983. Interactive effects of dietary protein and lipid on growth and protein utilization of age-0 striped bass. *Transactions of the American Fisheries Society*, 112: 183-193

Moon, H. Y. L and Gatlin D. M. 1994. Effects of dietary animal proteins on growth and body composition of the red drum (*Sciaenops ocellatus*). *Aquaculture*, 20: 327-340

Morais, S., Bell, J. G., Robertson, D. A., Roy, W. J. and Morris, P. C. 2001. Protein/lipid ratios in extruded diets for Atlantic cod (*Gadus morhua*): effects on growth, feed utilization, muscle composition and liver histology. *Aquaculture*, 203: 101-119

National Research Council (NRC). 1983. *Nutrient requirements of Warm water fishes and Shellfishes*. National Academy Press, Washington, DC.

National Research Council (NRC). 1993. *Nutrient Requirements of Fishes*. National Academy Press, Washington, DC.

Naylor, R. 2001. Effects of aquaculture on world fish supplies. *Issues in Ecology*, 8: 1-14

Naylor, R., Goldburg, R., Primavera, J. H., Kautsky, N., Beveridge, M. C., Clay, J.,

Folke, C., Lubchenco, J., Mooney, H. and Troell, M. 2000. Effects of aquaculture on world fish supplies. *Nature*, 405: 1017-1024

Nelson, J. S. 1994. *Fishes of the World. 3rd Edition*. John Wiley and Sons, New York, NY.

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

Noeske, T. A., Spieler, R. E., Parker, N. C. and Suttle, M. A. 1985. Feeding time differentially affects fattening and growth of channel catfish. *Journal of Nutrition*, 115:1228-1232

Olli, J. J., Krogdahl, A. and Vabeno, A. 1995. Dehulled solvent extracted soybean meal as protein source in diets for Atlantic salmon, *Salmo salar*. *Aquaculture Research*, 26: 167-174

Opstvedt. J., Aksnes. A., Hope. B. and Pike, I. H. 2003. Efficiency of feed utilization in Atlantic salmon (*Salmo salar L.*) fed diets with increasing substitution of fish meal with vegetable proteins. *Aquaculture*, 221: 365-379

O'Sullivan, D. and Ryan, M. 2001. Mulloway trials suggest opportunities for brackish water ponds. *Austasia Aquaculture*, April/May: 22-26

Page, J. W. and Andrews, J. W. 1973. Interactions of dietary levels of protein and energy on channel catfish (*Ictalurus punctatus*). *Journal of Nutrition*, 103: 1339-1346

Peres. H., Chlorn. L. and P. H. Klesius. 2003. Nutritional value of heat-treated soybean meal for channel catfish (*Ictalurus punctatus*). *Aquaculture,* 225: 67-82

Peres, H. and Oliva-Teles, A. 1999. Influence of temperature on protein utilization in juvenile European sea bass (*Dicentrarchus labrax*). *Aquaculture*, 170: 337-348

Peres, H. and Olivia-Teles, A. 1999. Effect of dietary lipid level on growth performance and feed utilization by European sea bass juveniles (*Dicentrarchus labrax*). *Aquaculture,* 179: 325-334

Phillips, M. 1972. Calorie and Energy Requirement. In: *Fish Nutrition. 1st Edition.* (Ed.) Halver, J. E. Academic Press, London (Ltd), pp. 1-28

Quartararo. N., Allan. G. L. and Bell, J. D. 1998. Replacement of fish meal in diets for Australian snapper, *Pagras auratus. Aquaculture*, 166: 279-295

Refstie. S., Korsoen. O. J., Storebakken. T., Baeverfjord. G., Lein. I. and Roem, A. J. 2000. Differing nutritional responses to dietary soybean meal in rainbow trout (*Onchorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Aquaculture*, 190: 49-63

Refstie. S., Sorebakken. T., Baeverfjord. G. and. Roem, A. J. 2001. Long-term protein and lipid growth of Atlantic salmon (*Salmo salar*) fed diets with partial replacement of fish meal by soy protein products at medium or high lipid level. *Aquaculture*, 193: 91-106.

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

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

Robaina, L., Moyano, F. J., Izquierdo, M. S., Socorro, J., Vergara, J. M, Montero, D. and

Fernandez-Palacios, H. 1995. Soya bean and lupin seed meals as protein sources in diets for gilthead sea bream (Sparus aurata). Nutritional and histological implications. *Aquaculture*, 157: 347-359

Robinson, E. H. 1988. Nutritional requirements of red drum: A review. *Contributions in Marine Science*, 30: 11-20

Ruohonen, K., Vielma, J. and Grove, D. J. 1998. Effects of feeding frequency on growth and food utilization of rainbow trout (*Onchorhynchus mykiss*) fed low fat herring on fry pellets. *Aquaculture*, 165: 111-121

Rutherford, A. S., Tilmant, J. T., Thue, E. B. and Schmidt, T. W. 1989. Fishery harvest and population dynamics of spotted sea trout, *Cynoscion nebulosus*, in Florida Bay and adjacent waters. *Bulletin of Marine Science*, 44: 108-125

Rychly, J. 1980. Nitrogen balance in trout. II. Nitrogen excretion and retention after feeding diets with varying protein and carbohydrate levels. *Aquaculture,* 20: 343-350

Sabaut, J.J. and P. Luquet. 1973. Nutritional requirements of gilthead bream *Chrysophrys aurata.* Quantitative protein requirements. *Marine Biology*, 18: 50-54.

Samantaray, K. and Mohanty, S. S. 1997. Interactions of dietary levels of protein and energy on fingerling snakehead, *Channa striata. Aquaculture*, 156: 241-249

Sargent, J., McEvoy, L., Estevez, E., Bell, G., Bell, M., Henderson, J. and Tocher, D. 1999. Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture*, 179: 217-229

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

Smale, M. J. and Bruton, M. N. 1985. Predation and prey selectivity by *Argyrosomus*

hololepidotus (Sciaenidae) in south-eastern Cape waters of South Africa. *South African Journal of Zoology*, 20(3): 97-108

Smith, R. R. 1989. Nutritional Energetics. In*: Fish Nutrition. 2nd Edition.* (Ed) Halver, J. Academic Press. Pp. 1-29

Steffens, W. 1989. Principles of Metabolism. In: *Principles of Fish Nutrition*. Ellis Horwood Limited, West Sussex, England.

Stickney, R. R. and Andrews, J. W. 1972. Effects of dietary lipids on growth, food conversion, lipid and fatty acid composition of channel catfish. *Journal of Nutrition*, 102: 249-258

Storebakken, T., Kvien, I. S., Shearer, K. D., Grisdale-Holland, B.and Helland, S. J. 1999. Estimation of gastrointestinal rate in Atlantic salmon (*Salmo salar*) using inert markers and collection of faeces by sieving: evacuation of diets with fish meal, soy bean meal or bacterial meal. *Aquaculture*, 172: 291-299

Stuart, J. S. and Hung, S. S. O. 1989. Growth of juvenile white sturgeon (*Acipenser transmontanus*) fed different proteins. *Aquaculture*, 76: 303-316

Sveier, H and Lied, E. 1998. The effect of feeding regime on growth, feed utilization and weight dispersion in large Atlantic salmon (*Salmo salar*) reared in seawater. *Aquaculture*, 165: 333-345

Tacon, A. G. J. 1994. Feed ingredients for carnivorous fish species: alternatives to fish meal and other dietary resources. *FAO Fisheries Circular,* 881: 35

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

Ter Morshuizen, L. D., Whitfield, A. K. and Paterson. A. W. 1996. Distribution patterns of fishes in an eastern Cape estuary and river with particular emphasis on the ebb

and flow region. *Transactions of the Royal Society of South Africa*, 51: 257-280

Teshima, S-I., Kanazawa, A. and Kawamura, G. 1984. Effects of several factors on growth of milkfish (*Chanos chanos*) fingerlings reared with artificial diets in aquaria. *Aquaculture*, 37: 39-50

Thoman, E. S., Davis, D. A. and Arnold, C. R. 1999. Evaluation of grow out diets with varying protein and energy levels for red drum (*Scaienops ocellatus*). *Aquaculture*, 176: 343-353

Thompson, K. R., Webster, C. D., Morgan, A. M. and Grisby, E. J. 2000. Effects of different feeding frequencies on growth, body composition and fillet composition of juvenile sunshine bass, *Morone chrysops & M. saxatitlis*, grown indoors. *Journal of Applied Aquaculture*, 10(2): 55-65

Tibbets, S. M., Lall, S. P. and Anderson, D. M. 2000. Dietary protein requirement of juvenile American eel (*Anguilla rostrata*) fed practical diets. *Aquaculture*, 186: 145-155

Tidwell, J. H. and Allan, G. L. 2001. Fish as food: aquacultures contribution. Ecological and economic impacts and contributions of fish farming and capture fisheries. *EMBO Reports*, 2(11): 958-963

Tsevis, N. S., Klaoudato, S. and Conides, A. 1992. Food conversion budget in sea bass, *Dicentrarchus labrax*, fingerlings under two different feeding frequency patterns. *Aquaculture*, 101: 293-304

Tucker, J. W., Mackinnon, M. R., Russel, D. J., O'Brien, J. J. and Cazzola, E. 1988. Growth of juvenile barramundi on dry feeds. *Progressive Fish-Culture*, 50: 81-85

Turano, M. J., Davis, D. A. and Arnold, C. R. 2002. Optimization of grow out diets for red drum, *Sciaenops ocellatus*. *Aquaculture Nutrition,* 8: 95-101

Tyler, A. V. and Dunn, R. S. 1976. Ration, growth and measures of somatic and organ

condition in relation to meal frequency in winter flounder, *Pseudopleuronectes americanus*, with hypotheses regarding population homeostasis. *Journal of the Fisheries Research Board of Canada*, 33(1): 63-75

Vivyakarn, V., Watanabe, T., Aoki, H., Tsuda, H., Sakamoto, H., Akamoto, N., Iso, N., Satoh, S. and Takeuchi, T. 1992. Use of Soybean meal as a substitute for fish meal in a newly developed soft-dry pellet for yellowtail. *Nippon Suisan Gakkaishi*, 58: 1761-1773

Wang, N., Hayward, R. S. and Noltie, D. B. 1998. Effect of feeding frequency on food consumption, growth, size variation and feeding pattern of age-0 hybrid sunfish. *Aquaculture*, 165: 261- 267

Webb, K. A. and Gatlin, D. M. 2003. Effects of dietary protein level and form on production characteristics and ammonia excretion of red drum, *Sciaenops ocellatus*. *Aquaculture*, 225: 17-26.

Whitfield, A. K., Blaber, S. J. M. and Cyrus, D. P. 1981. Salinity ranges of some South African fish species occurring in estuaries. *South African Journal of Zoology*, 16: 151-155

Whitfield, A. K. and Kok, H. M. 1992. Recruitment of juvenile marine fishes into permanently and seasonally open estuarine systems on the southern coast of South Africa. *Ichthyological Bulletin of J. L. B. Smith Institute of Ichthyology*, 57: 1-39

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. and Poe, W. E. 1985. Effects of feeding soybean meal with varying trypsin inhibitor activities on growth of fingerling channel catfish. *Aquaculture*, 46: 19-25

Winfree, R. A. and Stickney, R. R. 1981. Effects of dietary protein and energy on growth,

feed conversion efficiency, and body composition of *T. aurea*. *Journal of Nutrition*, 111: 1001-1012

Zarate, D. D. and Lovell, R. T. 1999. Effects of feeding frequency and rate of stomach evacuation on utilization of dietary free and protein bound lysine for growth by channel catfish, *Ictalurus punctatus*. *Aquaculture Nutrition*, 5: 17-22

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