THE DEVELOPMENT OF A PRACTICAL DIET FOR JUVENILE DUSKY KOB, *ARGYROSOMUS JAPONICUS,* FOR THE SOUTH AFRICAN MARICULTURE INDUSTRY

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ABSTRACT

The lack of locally manufactured marine finfish diets motivated the current project, which aimed to develop a practical diet specifically formulated for dusky kob. The first growth trial investigated the effect of pellet texture (i.e. hard versus soft pellets) and the inclusion of an additional binder, test treatments were compared to two reference diets. There was no significant difference in weight gain between fish fed the hard or soft pellets ($p=0.17$), over nine weeks. The fish fed the local trout feed weighed significantly less than those fed the test diets ($p=0.003$). There was no significant difference in leaching rate amongst treatments ($p=0.45$). A hard pelleted diet without gelatin is recommended in the commercial culture of dusky kob. Pellets with different physical shapes were tested to determine the effect of shape on settling speeds. A square shaped pellet had a slower sinking rate compared to a round shaped pellet ($p<0.0001$). Juvenile dusky kob, held in cages, fed the square pellets grew significantly faster than the fish fed the round pellet, with a mean weight gain of 16.81 ± 0.45 g ($p=0.018$).

A growth trial was used to optimize the protein to energy (PE) ratio by adjusting dietary protein and lipid levels. Fish fed the diets with high levels of protein (46 % protein) achieved the better growth rates (125 % weight gain) compared to the lower protein diets (92 % weight gain) ($p<0.0001$). There was no significant difference in total ammonia production (TAN) level for the 46 and 42 % protein diets ($p=0.68$).

The overall performance of the prototype diet was investigated in a closed recirculating system. Growth and FCR of the fish fed the prototype diet was on a par to those fed the commercially available diet currently used in the local farming industry. There was no significant difference in the blood chemistry for fish held at increased stocking densities. The prototype diet is suitable for use in recirculating systems, as there were no negative effects on water quality parameters. This study has laid a foundation for the manufacture of a practical commercial dusky kob diet in South Africa.

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CHAPTER 1

GENERAL INTRODUCTION

As world fish stocks decline rapidly there is an increasing demand for aquaculture to supply consumers with fisheries products (Naylor, *et al*. 2000). Aquaculture is consequently the fastest growing food production sector globally, with an annual production of 51.6 million tons in 2006, compared to the mere 20 million tons produced per year only a decade ago (FAO 2008). South Africa's aquaculture industry has been comparatively slow to develop compared to the international growth of the industry, with the local industry producing about 3500 tons of product per annum, the bulk of which consists of mussels, oyster and abalone, *Haliotis midae*, production (FAO 2008). A number of indigenous linefish species, such as dusky kob, *Argyrosomus japonicus*, yellowtail, *Seriola lalandi*, and white steenbras, *Lithognathus lithognathus,* have been identified as suitable candidates for marine culture (Tyrer 2006, Hecht 2000) and the marine fish farming industry is in its developmental stages in South Africa. Pilot commercial projects are developing both inland recirculating systems, and sea cage grow out facilities (Landman 2006).

Due to its food value, palatability and proximity, the dusky kob, *A. japonicus*, is severely overfished with commercial catches declining rapidly (Fielder *et al*. 1999). The dusky kob belongs to the family Sciaenidae which includes carnivorous fish usually found in temperate regions in shallow coastal and estuarine waters (Nelson 1994). The genus *Argyrosomus* is commonly found in both the Atlantic and Indo-West Pacific regions (Griffiths and Hecht 1995), in the northern and southern hemispheres. They are found from Hong Kong along the Chinese coast, southern Korea and Japan and along the entire south - eastern coast of Australia (Griffiths 1997). In southern Africa dusky kob is found from Cape Point to Mozambique and it is an important food species and recreational and commercial linefish (Griffiths and Heemstra 1995, Griffiths 1997).

Sciaenids are becoming important aquaculture species in Europe, China, Australia and Africa due to their economic importance and high market value (Poli *et al*. 2001, O'Sullivan and Ryan 2001 and El-Shebly *et al*. 2007). The dusky kob grow fast, attaining a market size of one kilogram in 14 to 15 months (Griffiths 1996). The species is also euryhaline, able to withstand a wide range of salinities from 5 to 35 parts per thousand (Whitfield 1998, Fielder *et al*. 1999), and as such is a good candidate for inland recirculating systems and brackish ponds and tanks (O'Sullivan and Ryan 2001). Trials on cultured *Argyrosomus* species were conducted in France and Italy, starting in 1996, when fry production of the meagre, *Argyrosomus regius*, was initiated (Poli *et al*. 2001). In Australia adult *A. japonicus*, known locally as mulloway, were hormonally induced and the larvae were first successfully reared in the early nineties (Battaglene and Talbot 1994). Subsequently, they have been commercially farmed to market size of over 1.0 kg in ponds and sea cages (Fielder and Bardsley 1999). Similarly, in South Africa, dusky kob, *A. japonicus*, and silver kob, *Argyrosomus inodorus*, have been successfully spawned at the Marine and Coastal Management aquarium and the I&J (Pty) Ltd. and Espadon Marine (Pty) Ltd. commercial hatchery facilities (Landman 2006).

Dusky kob is a suitable culture candidate in both inland systems and sea cage environments (Aquaculture SA 2001). In South Africa, dusky kob are successfully grown in pilot, small-scale commercial facilities, in both onshore recirculating systems and sea cages moored in Algoa Bay (Landman 2006). Within inland tank systems, water quality is maintained through recirculation of the water through biological filters, so lowest possible pollution outputs are necessary to maintain good water quality and high levels of dissolved oxygen. Feed is the primary cause of pollutants entering a system (Davis 1990), as uneaten feed causes bacteria to flourish, and digestion of feed by the fish causes levels of nitrogenous excretion to increase. Therefore, one of the research priorities was to develop a diet that reduces ammonia excretion by the fish whilst still maintain high growth rates. Although sea cage systems offer many advantages to inland tanks, the waste outputs from uneaten feed must be properly managed. Whereas fish held in tanks

are able to feed on pellets that are on the tank bottom, fish held in cages are only able to feed whilst the pellets remain within the water column of the cage before they fall through the cage mesh. The proportion of feed that is lost in sea cage culture can be as high as 40 % (Thorpe et al. 1990, Juell 1991). Economically, this increases the expenses of a commercial operation since feed is a primary cost (Halver and Hardy 2002) and, this has negative effects on the surrounding environment and causes benthic substrate pollution (Landman 2006). Thus, any potential diet needs to address the issue of waste outputs for both rearing environments.

The commercial aquaculture of dusky kob is dependent on many factors, including the biological aspects of the species, the conditions and nutrients needed for its survival and optimal growth in cultured environments (Lazo *et al*. 1998). The potential to commercially rear *A. japonicus* has been shown in a number of nutritional, biological and ecological studies (Marais 1984, Ballestrazzi *et al*. 1994, Daniel 2004, Collett *et a*l. 2008). Research shows that dusky kob have high survival rates in both experimental culture conditions and within the farm environment. They tolerate low oxygen levels. They also tolerate relatively high stocking densities of 50 kg m^{-3} (Collett 2007). Their temperature tolerance ranges from 15 to 30 °C (Whitfield 1998), with a preferred range of 24 – 26 °C for growth and aquaculture potential (Collet *et al*. 2008). Similarly, Bernatzeder and Britz (2007) found the selected temperature of juvenile dusky kob held in a horizontal temperature gradient was 25 °C.

Nutrition is regarded as one of the most important biological aspects for successful aquaculture (Halver and Hardy 2002). Feed is the primary operating cost of a commercial finfish farming operation (Andrew *et al*. 2004); with feed constituting as much as 50 % of the total operating expenses of a commercial farm (Davis 1990). This has led to an important area of research within aquaculture; to develop diets that are both cost effective and produce best possible fish growth (Hunter and Roberts, 2000). It is essential that the feed is efficient in delivering the nutrients needed for highest optimal growth and health, as well as being

economically viable. In addition to formulating and manufacturing diets based on the fish nutritional requirements, the feed characteristics, such as size, shape, taste and texture are also important as they influence how and whether a fish will feed on commercial diets (Andrew *et al*. 2004).

The expansion of dusky kob farming will be facilitated by the development of commercial diets. In South Africa there is a lack of a locally produced diet suitable for the growing marine finfish industry. The producers, at the moment, rely either upon a locally produced trout feed or expensive, imported salmonid feeds. It is thus crucial that a nutritionally sound practical diet is established for the expansion of the local industry. There is a need for different types of commercial diets based on the rearing environments of the fish in either recirculating systems or sea cages, as the waste management is different for the specific environments. Thus, a practical diet for the local dusky kob industry must be able to maintain good water quality conditions and remain economical in both systems.

Preliminary experimental nutrition trials demonstrated that juvenile dusky kob feed effectively on artificial pelleted feeds (Daniel 2004, Benjamin *et al*. 2006). Daniel (2004) found captive juvenile dusky kob readily feed on an artificial diet with high protein levels (35 to 55 % crude protein) using fishmeal as a basis for the protein source, and as much as 30 % of the fishmeal could be substituted with soyabean meal (Daniel 2004). Preliminary studies into pellet size have shown different size classes of dusky kob realized no significant difference in growth when fed pellet of varying size from 3 to 8mm (Benjamin *et al*. 2006). In Australia, a practical diet for *A. japonicus* was based on part by published results for the requirements of red drum, *Sciaenops ocellatus*, (Daniel and Robinson, 1986) and the commercial diet for the freshwater silver perch, *Bidyanus bidyanus* (Allan *et al*. 2000). In France, *A. regius*, meagre, are fed diets similar to other Mediterranean marine species, with 45 – 48 % protein (FAO 2004). Previous studies (Daniel 2004) suggest dusky kob may have similar dietary requirements to other marine carnivorous species and growth rates increased significantly with an increase in dietary protein up to 52 %, although their dietary requirements range between 45 and 52 % protein and 9 %

lipid for optimal growth and feed utilization (Daniel 2004). Thus, further studies into the formulation and manufacture of a practical diet will facilitate the growth of the dusky kob industry in South Africa.

The aim of this study was to contribute towards the development of a practical diet for juvenile dusky kob, *A. japonicus*. The objectives of this project were to develop a practical diet that was both nutritionally sound and is suitable to use within recirculating systems and sea cages.

In recirculating systems pellet stability is crucial to deliver nutrients to the fish and limit wastes produced by uneaten feed (Halver and Hardy 2002). The most practical way to increase pellet stability is to introduce a binding agent which keeps the pellet intact when it is submerged in water (Storebakken 1985). Gelatine has been used as a binder in slow-feeding shrimp and abalone diets (Farmanfarmaian *et al*. 1982, Knauer *et al*. 1993 and Britz *et al.* 1994). Therefore, it was evaluated whether the inclusion of a gelatine binder would improve the pellet stability and limit nutrient leaching. Similarly, fish displaying feeding behaviour where pellets are ejected or crushed into particles also increases wastes into a system (Andrew *et al*. 2004, Benjamin *et al*. 2007). The incidence of chewing and ejection of feed was reduced by feeding a softer pellet (Poston 1974, Andrew *et al*. 2004). Thus, the effect pellet hardness had on diet acceptability was evaluated by testing diets with high and low moisture contents.

Aquaculture diets are based on high levels of protein, which supply the necessary amino acids for growth. These amino acids, when in excess, are deaminated for metabolic fuel and this largely increases the ammonia excretion rate of the fish (Shyong *et al*. 1998). By optimizing the protein:energy (P:E) ratio protein retention can be increased in the fish and thus there is a reduction in the amount of amino acids which are deaminated (Lee *et al*. 2002). The growth rates are also increased by the increase in protein retention and thus the feed conversion ratios are more efficient and feed costs are reduced. Ammonia is toxic to fish, above levels of 0.65 mg L^{-1}, and is an important aspect for the maintaining of good water quality within culture systems (Rodrigues *et al*. 2007). Therefore, formulations which reduce

ammonia production whilst maintain high growth rates are highly desirable in intensive recirculating aquaculture. One way of lowering ammonia excretion is to lower the protein levels and increase the energy levels in the diet, where cultured species use the dietary energy for metabolic fuel and the dietary protein solely for growth requirements (Cai *et al*. 1996, Ballestrazzi *et al*. 1994, McGoogan and Gatlin 2000 and Yang *et al*. 2002). Thus, experimental diets with varying protein and energy levels were tested to evaluate the effect on ammonia excretion rates by dusky kob, and to find the optimal P:E ratio for maximal growth and feed efficiency.

The increasing interest in cage culture in South Africa has lead to the necessity to develop a diet which is suitable for cage culture environments. A main disadvantage to cage grow out facilities is the waste outputs from uneaten feed. Unlike fish held in tanks that are able to feed off the tank bottom, fish held in cages only have access to the feed as it falls through the water column of the cage. A possible solution to reducing wastes from uneaten feed is to extend the period of time the pellets are in the cage, by either floating pellets, or by means of slower sinking pellets. Chen (1999) and Elberizon and Kelly (1998) found that pellet with varying sizes had varying setting velocities. The settling velocities of salmonid feed increased from 5 to 12 cm s^{-1} for pellets of 2 mm to 8 mm. The slower the settling velocity of the pellets the longer time period the fish have access to the pellets in the cage (Elberizon and Kelly 1998). Thus, the effect that pellet shape had on the settling velocity of the pellet was tested and the feeding behaviour and growth of dusky kob held in cages when fed pellets of different shapes were evaluated.

A prototype diet was developed and its performance was evaluated by assessing the growth rate, FCR and overall health of the fish and water quality parameters and comparing it with the commercially available feed currently used by local farmers. High stocking densities are utilized in commercial operations increase the economic viability; although the fish may become more susceptible to infection and environmental stressors (Sandnes *et al*. 1988). Collett (2007) found stocking

densities of up to 50 kg m^{-3} did not inhibit the growth of dusky kob. High stocking densities, up to 25 kg m^{-3}, have been successfully used in commercial operations, in the past (Aquaculture SA 2001). The health of fish can be assessed by examining the blood; since haematological characteristics have been shown to be a good indicator of fish health (Blaxhall and Daisley 1973).

The overall objective of this thesis was thus to develop a practical diet that promotes optimal growth of dusky kob, and to investigate the effects that the diet had on fish health and water quality.

The objectives were to compare:

1. the growth, survival, FCR, specific growth rate (SGR),condition factor (CF), hepatosomatic index (HSI) and visceral index (VSI) of dusky kob fed hard and soft pellets, and to compare the water stability of these pellets (Chapter 2);
2. the growth, survival, FCR, SGR, protein efficiency ratio (PER), CF, HSI, VSI and nitrogen excretion of dusky kob fed diets with varying protein and energy levels (Chapter 3);
3. the growth, survival, FCR, SGR, PER, CF, HSI, VSI of dusky kob kept in cages that were fed different shape pellets that passed through the water column at different sinking velocities (Chapter 4) and
4. the water quality of individual recirculating systems in which the prototype diet was fed to those in which a commercial finfish feed were fed, and compare the growth, survival, FCR, SGR, CF, HSI, VSI, liver glycogen, and haematology and parasite load of dusky kob fed the prototype diet and commercial feed (Chapter 5).

CHAPTER 2

EFFECT OF BINDING AND PELLET TEXTURE ON PELLET STABILITY AND GROWTH OF DUSKY KOB (*ARGYROSOMUS JAPONICUS*)

INTRODUCTION

In commercial finfish aquaculture production, feed is the single most expensive component (Halver and Hardy 2002) and "leaching" may result in considerable financial loss since lost nutrients are not converted into fish growth (Andrew *et al*. 2004). In addition, nutrient wastes have a negative effect on water quality, particularly in recirculating aquaculture systems since they disintegrate into fine particles and suspended and dissolved components that are difficult to filter out of large water systems and result in general fouling and poor water quality (Hilton *et al*. 1981, Farmanfarmaian *et al*. 1982). Pellets must thus remain intact in water until consumed by fish (Halver and Hardy, 2002). Factors such as exposure to water currents, aeration systems and physical mouthing and chewing by the fish can hasten the disintegration of the pellet and result in nutrient losses (Farmanfarmaian *et al*. 1982).

Developing a well bound pellet is essential in pellet formation and contributes to reducing wastage from both dry and moist feeds (Storebakken 1985). Improved pellet stability has been achieved by inclusion of various binders in slow-feeding shrimp (Farmanfarmaian *et al*. 1982), and abalone feeds (Knauer *et al*. 1993 and Britz *et al.* 1994), and similar work has been done on fish feeds (Storebakken 1985 and Storebakken and Austreng 1987). Dusky kob held in tanks tend to be timid when feeding and allow the feed to fall to the tank bottom before ingesting it, thus exposing it to water for a significant period of time and potential leaching losses (Pers. obs.). Therefore, there was a need to ascertain whether the inclusion of a binder in the experimental diets affected nutrient leaching, feed consumption, feed conversion ratio (FCR) and the growth rate of cultured dusky kob.

Pellet hardness and texture may affect the acceptance of the feed by the fish, particularly by marine species. Farmed gilthead sea bream, *Sparus aurata*, have been observed ejecting whole or partially chewed pellets during feeding (Andrew *et al*. 2004). Similar feeding behaviour has also been observed in farmed dusky kob (Benjamin *et al*. 2007). The ejection of whole and crushed pellets may result in increased solid and nitrogenous wastes within a system, as well as a poorer FCR (Andrew *et al*. 2004). The incidence of chewing and ejection of food was however reduced when *S. aurata* were fed a softer pellet (Andrew *et al*. 2004). Furthermore, Poston (1974) found brown trout, *Salmo trutta*, grew slightly faster when fed a moist diet. Thus the effect that pellet hardness had on diet acceptability, feed consumption, FCR and growth rate of cultured dusky kob was evaluated in this study.

The objectives were to: (1) compare the pellet water stability, fish growth, FCR and survival of dusky kob fed the diet formulated for dusky kob requirements to commercial feeds currently available to the finfish aquaculture industry, (2) compare pellet water stability of pellets formulated with and without gelatin and presented as either a hard or soft pellet, and (3) compare pellet water stability, fish growth, FCR and survival of dusky kob fed either a hard or soft pellet, both with and without the additional binder.

MATERIALS AND METHODS

Experimental Animals

Captive bred juvenile *A. japonicus* (approximately 1 g) were acclimated in the same tanks that were subsequently used in the experiment, at a density of 1.5 kg fish m^{-3} at the Rhodes University, Marine Research Laboratory in Port Alfred (South Africa) for 10 weeks. The fish were fed three times a day to apparent satiation on a 1.1 mm crumble trout starter feed (46 % protein, 14 % lipid, Indian Ocean Aquatics (Pty) Ltd, Johannesburg), and then introduced to a 2.2 mm dry pelleted trout feed (45 % protein, 12 % lipid, Indian Ocean Aquatics (Pty) Ltd, Johannesburg) over a period of two weeks.

Experimental System and Stocking Conditions

The experimental system consisted of 21 circular tanks (Figure 1a), (500 L tank^{-1} with a water exchange rate of 200 L tank^{-1} hour^{-1}, Figure 1b), a settling tank (1000 L) and a biological filter (1000 L, with shredded plastic used as the filter media). The water was treated by an ultraviolet light (55W Pro UV, Ultrazap, Johannesburg) and 10 % of the volume of the system was replaced daily with sea water (35 g L^{-1}) from the Kowie River Estuary. The system was heated and temperature was maintained between 20 and 22 °C. Airstones were placed in each tank to aerate the water.

a)  b) 

Figure 1. (a) Experimental system, at the Port Alfred marine research laboratory on the Kowie River estuary, used in dusky kob growth trials, (b) inflow of filtered water into an experimental tank.

One hundred and ten fish of approximately 15.5 g each were randomly assigned to each tank one week before the start of the experiment. The volume of the tank was adjusted to maintain a stocking density of 10 kg m^{-3} every four weeks during the trial; this monthly adjustment was based on the weight of a random sample of 50 fish tank^{-1}.

Dietary Treatments

The four dietary treatments were formulated with total protein and lipid contents of approximately 45 and 11 %, respectively (Table 1), and were manufactured at the

Marifeed (Pty) Ltd factory in Hermanus, South Africa. One formulation included gelatin as an additional binding agent while the second did not, and both formulations were manufactured in either a hard or soft form (Table 1).

Low-temperature, formaldehyde free, fishmeal (66 % crude protein, 8.0 % lipid, Oceana (Pty) Ltd, Cape Town, South Africa) was included as the main protein source. The dietary lipid levels were adjusted to the same level, i.e. 11 %, among treatments with the inclusion of marine fish oil (Oceana (Pty) Ltd, Cape Town, South Africa). Vegetable starch was included as dietary carbohydrate. The additional binding agent, gelatin (Gelatia, Krugersdorp, South Africa), was added at a rate of 3.0 % in experimental diets (Table 1).

The dry ingredients were mixed before the oil, water and additional binding agent were added. The ingredients were subsequently extruded through circular 2.0 mm die and the pellets were cut 3.0 mm long. The pellets used in the soft diets were packaged and immediately frozen at -18 °C. The hard diet pellets were placed on drying trays and dried at 38 °C for 18 hours; the pellets were cooled to room temperature and packaged.

Each diet was fed to the fish in four randomly selected tanks. The two commercially available feeds, a locally manufactured trout feed (protein: 50 %, lipid: 14 %) and an imported marine finfish diet (protein: 52 %, lipid: 10 %) (Table 1), were each fed to two randomly selected tanks of fish.

Table 1: Proximate composition of four experimental diets that included either 10 or 40 % moisture (i.e. hard and soft, respectively), the additional gelatin (i.e. extra binder) and two commercial feeds (i.e. imported feed and trout feed). Each mean in this table reflects a composite of 4 samples.

Composition	Hard	Extra binder hard	Soft	Extra binder soft	Imported Feed	Trout Feed
Protein %	44.04	45.28	43.03	44.87	47.94	41.19
Lipid %	11.27	11.38	11.14	11.38	11.87	13.72
Moisture %	8.70	8.10	39.30	43.20	7.10	7.60
Ash %	11.20	10.73	8.68	9.95	12.49	7.40
Energy (KJ/mg)	20.81	19.30	18.45	18.39	19.66	20.57

Data Collection

At the start and end of the trial fish were individually weighed (0.01 g), and the total length measured (1 mm). All fish were purged for 24 hours prior to handling and subsequently narcotized with 2-phenoxyethanol (ethylene glycol monophenol ether, Merck®, Holland) at 0.2 mL L^{-1} prior to weighing. The specific growth rate (SGR), percentage weight gain, feed conversion ratio (FCR) and condition factors (CF) (Bolger and Connolly, 1989) were calculated using Equations 1 to 4, respectively:

$$SGR = [\ln(\text{final weight(g)}) - \ln(\text{initial weight(g)})] \times 100 / \text{time (days)} \quad (1)$$

$$\% \text{ Weight gain} = [(\text{final weight} - \text{initial weight}) / \text{initial weight}] \times 100 \quad (2)$$

$$FCR = \text{dry feed supplied*(g)} / \text{wet weight (g)} \quad (3)$$

$$CF = \text{as } [\text{weight (g)} / (\text{length (mm)}^{2.84})] \times 10^5 \quad (4)$$

* Air dry (i.e. 10 % moisture).

Two fish per tank were randomly sampled at the end of the experiment; one was immediately frozen for proximate analyses and the second was dissected and the liver and visceral organs were removed and weighed and expressed as a percentage of body weight and used to calculate hepatosomatic (HSI) and visceral (VSI) indices, using Equations 5 and 6:

$$HSI = \text{Liver weight (g)} / \text{Body weight (g)} \times 100 \quad (5)$$

$$VSI = \text{Visceral organs weight (g)} / \text{Body weight (g)} \times 100 \quad (6).$$

Proximate Analysis

Four random samples of each diet were taken from different bags of feed and were combined to form one composite sample for each diet and the randomly sampled fish from each tank were all individually analysed to determine crude protein, lipid, ash, moisture and energy content. Crude protein was determined using the Dumas combustion method in a LECO FP2000 Nitrogen analyser (AOAC, method 990.03). Lipid was extracted from samples by solvent petroleum ether (Soxhlett procedure) using a Buchi 810 Soxhlett fat extractor. The lipid percentage was then calculated by gravimetric analysis (AOAC, method 920.39). Ash content was determined by placing the sample in a furnace for four hours at 550 °C (AOAC, method 942.05). The samples were dried in an air circulated oven at 95 °C for 72 h to determine the moisture content (AOAC, method 934.01). Energy was calculated using a bomb calorimeter (DDS isothermal CP500, Digital Systems, Johannesburg).

Solid Leaching

Solid leaching from the diets was determined by placing approximately 10 g of each diet into a cylindrical container (height: 90 mm X diameter: 70 mm) which was gently lowered into an experimental tank under the same conditions that the fish were held. The water in each container was aerated using an airstone to create water movement in the container. Four replicate samples of each diet were left in the water for either 0, 7.5 or 15 minutes. When removed from the water the samples were oven dried at 100 °C until a constant weight was obtained (i.e. approximately 24 hours). The control samples for each diet, which were not subjected to leaching (i.e. t = 0), were measured and placed in pans and oven dried using the same methods. The amount of feed lost in the stability test was calculated by subtracting the final dry weight from the standard dry weight and expressed as a percentage of solid loss, i.e. leach out rate.

Water Chemistry

The water pH and temperature was measures daily using a pH and temperature probe (Hanna Instruments HI 98128, Rhode Island, USA). A hand-held salinity refractometer (Atago S/Mill-E, Tokyo, Japan) was used to measure salinity weekly. Dissolved oxygen was measured weekly with a dissolved oxygen probe (Hanna Instruments, Dissolved Oxygen Meter HI 1934, Romania). Total ammonia-nitrogen was recorded colourmetrically once a week (NH_4^+ Kit, Merck®, Holland). Water quality parameters were kept within the following limits: The water pH ranged between 7.4 and 7.8 and temperature was maintained between 20 and 22 °C. The oxygen saturation in the tanks was maintained between 65 - 75 % (6.60 – 7.70 mg L^{-1}). The salinity of the water remained 35 g L^{-1} throughout. Total ammonia – nitrogen ranged between 0.00 to 0.25 mg L^{-1} throughout the experiment.

Statistical Analysis

A multi-factor analysis of variance (multi-factor ANOVA) was used to identify interactions between the factors pellet form (i.e. soft and hard pellets) and binding agent (i.e. the presence or absence of the additional binder) at $p<0.05$. If no interaction was found, a one-way analysis of variance (ANOVA) and a Tukeys multiple range analysis or, if the data did not meet the assumptions of an ANOVA, a non-parametric one-way Kruskal Wallis ANOVA was used to compare means between treatments at $p<0.05$. All statistical analyses were calculated using STATISTICA™ 7.0 statistical package (Statsoft, 2004).

RESULTS

Solid Leaching

The pellets in soft form were more stable than the dry test diet pellets, with and without the additional binder (ANOVA: $F_{(1,110)}=9.66$, $p=0.002$; table 2). The additional binder did not significantly improve stability (ANOVA: $F_{(5,18)}=0.84$, $p=0.45$; Table 2). Water stability of the reference diets did not differ significantly from any of the test diets (ANOVA: $F_{(5,18)}=0.84$, $p=0.45$). The mean leach out rate

of the trout diet and the imported marine finfish diet were 0.56 ± 0.05 and 0.36 ± 0.10 %, respectively, after 15 minutes submergence in water.

Table 2: Mean leach out rate (± standard error) of experimental diets that either included 10 or 40 % moisture (i.e. hard and soft, respectively) and the additional gelatin (i.e. extra binder) after 15 minutes submerged in sea water. Means with a different superscript in the same row are significantly different (ANOVA, $p<0.05$).

	Hard Form	Soft Form	Binder	No Binder
Leaching Rate (%)	0.48 ± 0.06 [a]	0.37 ± 0.14 [b]		
Leaching Rate (%)			0.40 ± 0.13 [a]	0.45 ± 0.07 [a]

Feed Utilization and Fish Growth

There was no interaction between factors: - pellet form (i.e. hard vs. soft diets), and binding agent (i.e. additional binder vs. no additional binder) for mean weight gain (multi-factor ANOVA: $F_{(1,12)}=0.08$, $p=0.79$). There was no significant difference between the growth of fish fed the hard or the soft diet (Kruskal-Wallis: $H_{(1,16)}=1.86$, $p=0.17$), with fish fed both these diets gaining a combined mean of 55.71 ± 7.31 g during the nine week trial (Table 3). Similarly, the difference in growth rate between the diets with and without the additional binder was not significant (Kruskal-Wallis: $H_{(1,16)}=0.28$, $p=0.60$; Table 3).

Table 3: Mean weight and length gain (± standard error) of dusky kob fed (a) soft or hard pellets or (b) a diet with an additional binding agent or no additional binding agent. Means with the different superscript within each row are significantly different (Kruskal-Wallis, $p<0.05$).

	Hard Form	Soft Form	Binder	No Binder
Weight gain (g)	53.85 ± 2.37[a]	57.56 ± 2.57 [a]		
Length gain (mm)	74.72 ± 2.33 [a]	76.60 ± 2.24 [a]		
Weight gain (g)			55.75 ± 2.05 [a]	56.67 ± 2.96 [a]
Length gain (mm)			74.50 ± 3.53 [a]	76.82 ± 8.38 [a]

Combined, the test diets realized an overall mean growth rate (i.e. weight gain) of 356 % over nine weeks (Table 4). There was no significant difference in the growth between the fish fed the test diets and the fish fed the imported finfish feed that resulted in a mean weight gain of 330 %. However, the fish fed the local trout diet gained significantly less weight, realizing a mean weight gain of 230 % over the same period (ANOVA: $F_{(5,14)}$=5.3, p=0.003; Table 4).

The consumption of the dry feeds was similar amongst hard diet treatments, with a combined mean of 5.2 ± 0.2 kg per treatment over the experimental period (ANOVA: $F_{(3,12)}$=2.55, p=0.10). Fish fed the soft diets consumed 25 % more compared to those fed the hard control diet, i.e. 7.1 ± 0.3 vs. 5.3 ± 0.4 kg, however on a dry weight basis, feed intake of the soft and hard feed was not significantly different (ANOVA: $F_{(3,8)}$=3.08, p=0.09) (Table 4).

There was no interaction between factors: - pellet form and binding agent for FCR (multi-factor ANOVA: $F_{(1,12)}$=0.74, p=0.41). The additional binder had no effect on FCR (Kruskal-Wallis: $H_{(1,16)}$=0.40, p=0.53), with fish fed both these diets realizing a combined mean FCR of 1.10 ± 0.05 (Table 4). Fish fed the soft diet had a significantly poorer FCR (air dry ~ 10 % moisture) of 1.18 compared to that of 1.02 for the hard diet (Kruskal- Wallis: $H_{(1,16)}$=9.27, p=0.02; Table 4). The FCR of all test treatments were similar to those of the commercial imported finfish feed (i.e. 0.99 ± 0.03) and outperformed the local trout feed, which realized a mean FCR of 2.02 ± 0.03 (ANOVA: $F_{(5,14)}$=23.44, p<0.001; Table 4).

The mean CF (ANOVA: $F_{(5,14)}$=2.23, p=0.11), HSI (ANOVA: $F_{(5,14)}$=1.49, p=0.25) and VSI (ANOVA: $F_{(5,14)}$=4.98, p=0.80) were similar among all treatments (Table 4). The proximate carcass compositions of composite samples of fish fed each treatment are shown in Table 5.

Table 4: The mean (± standard error) initial size, final size, specific growth rate (SGR)*, percentage weight gain**, feed conversion ratio (FCR)**, condition factor*, hepatosomatic (HSI)* and visceral indices (VSI)* of juvenile dusky kob fed different diets over nine weeks. Means with no superscripts are not significantly different and means with different superscripts in the same row are significantly different (ANOVA* or Kruskal-Wallis ANOVA**, $p<0.05$).

Pellet Form	**Hard**		**Soft**		**Reference Diets**	
Binding Agent	**No Additional**	**Additional**	**No Additional**	**Additional**	**Trout Feed**	**Imported Feed**
Length (mm) Initial	105 ± 0.44	106 ± 0.45	107 ± 0.56	108 ± 0.53	110 ± 0.68	108 ± 0.79
Length (mm) Final	177 ± 4.31 [a]	183 ± 2.59 [a]	184 ± 5.46 [a]	185 ± 2.96 [a]	161 ± 0.99 [b]	184 ± 4.40 [a]
Weight (g) Initial	14.57 ± 0.20	15.34 ± 0.20	15.97 ± 0.25	16.74 ± 0.25	16.74 ± 0.25	15.50 ± 0.37
Weight (g) Final	66.94 ± 4.09 [a]	70.67 ± 3.69 [a]	73.10 ± 5.24 [a]	74.75 ± 3.16 [a]	42.99 ± 1.05 [b]	70.92 ± 4.41 [a]
Weight gain	359 ± 20.22 [a]	361 ± 17.59 [a]	356 ± 21.33 [a]	346 ± 10.81 [a]	155 ± 7.39 [b]	329 ± 9.60 [a]
SGR	2.37 ± 0.07 [a]	2.39 ± 0.06 [a]	2.37 ± 0.07 [a]	2.34 ± 0.04 [a]	1.46 ± 0.05 [b]	2.28 ± 0.03 [a]
Total food intake (kg)	5.3 ± 0.35 [a,c]	5.7 ± 0.12 [a,c]	7.6 ± 0.26 [a,c,d]	7.0 ± 0.17 [a,c,d]	5.1 ± 0.65 [b]	4.4 ± 0.22 [a,d]
FCR	1.03 ± 0.05 [a]	1.01 ± 0.06 [a]	1.23 ± 0.10 [c]	1.12 ± 0.04 [a,c]	2.02 ± 0.03 [b]	0.99 ± 0.03 [a,c]
Condition factor	2.48 ± 0.06	2.47 ± 0.10	2.69 ± 0.07	2.81 ± 0.15	2.37 ± 0.11	2.55 ± 0.13
HSI	2.23 ± 0.31	2.29 ± 0.05	2.64 ± 0.20	1.74 ± 0.32	2.48 ± 0.06	2.47 ± 0.33
VSI	11.27 ± 0.26	11.40 ± 0.60	11.15 ± 0.84	10.54 ± 0.49	14.90 ± 1.52	13.89 ± 0.36

Table 5: Proximate composition of juvenile dusky kob fed experimental diets in either hard or soft form, both with and without gelatin as the additional binder, and commercial diets.

Composition	Hard	Extra binder hard	Soft	Extra binder soft	Imported Feed	Trout Feed	Wild dusky kob*
Protein %	60.72	62.03	61.78	63.86	66.70	59.46	17.10
Lipid %	17.45	19.65	19.73	17.45	19.77	19.06	3.88
Moisture %	72.51	71.99	71.77	72.67	73.39	75.57	74.24
Ash %	18.12	15.00	14.78	16.34	15.64	17.65	2.23
Energy (KJ/mg)	20.56	21.57	21.44	20.36	21.34	19.65	-

*Garb and Gab-Alla (2007)

DISCUSSION

The addition of the binder did not reduce the acceptability of the pellet, nor its utilization by the experimental fish; as food consumption, feed conversion ratio and the growth rate of the fish was similar between treatments. Furthermore, there were no significant differences in visceral and liver weight between the fish fed the test diets and the hepatosomatic index (HSI) values (1.06 – 3.01 %) were similar to levels reported for other Sciaenid species; for instance the brown meagre, *Sciaena umbra*, (1.8 %) (Chatzifotis *et al*. 2006) and red drum, *Sciaenops ocellatus,* (1.75 – 2.28 %) (McGoogan and Gatlin 1999). The relative weight of the hepatopancreas plays an important role in food assimilation (Sureshkumar and Madhusoodana Kurup 1999). The higher the HSI values the higher the rates of food assimilation and growth. Finfish fed diets inadequate in dietary protein and lipids had lower HSI and visceral somatic index (VSI) values (Chaiyapechara et al. 2003).

The soft diets produced similar growth results to the hard diets, with fish fed both diets tripling in weight, thus, in this study dusky kob fed diets containing 10 and 40 % moisture grew at the same rate. A similar result was recorded for brown trout (*Salmo trutta*) which consumed a similar amount of feed (on a dry matter basis) and grew at a similar rate when fed both low- or high-moisture diets (Poston

1974). Pellet texture did not affect the growth or feed intake of gilthead sea bream (*Sparus aurata*) fed a control and soft diet with 8.0 and 12 % moisture contents, respectively (Andrew *et al*. 2004). However, the high moisture diets realized a poorer FCR and require refrigeration and or frequent preparation. The cold temperature storage and short shelf life of the soft diet would increase the costs of feeding. The use of low moisture diets is far more practical to manufacture and store for commercial scale farm operations.

The growth of the fish was not significantly affected by the presence of the gelatin binder in the soft diets and thus it appears unnecessary to use it in practical feeds.

The growth and FCR of dusky kob fed the experimental diets were equivalent to those fed the imported marine finfish feed, formulated for salmonid species. The dusky kob fed the experimental diets grew significantly better than the fish fed the trout feed. The trout feed also realized a significantly poorer FCR than the experimental diets. The lower growth rate and poor FCR of the fish fed the trout feed suggest that the feed ingredients were either less available to the dusky kob, or the essential amino acids or other nutrients did not meet their requirements. The local trout diet, although less costly than the imported diet, is not recommended to marine finfish farmers due to the poor growth rates and FCR.

This study showed that a practical finfish feed can be manufactured using binding technologies that were developed for the abalone feed industry in South Africa. The performance of the experimental diets was on a par with the imported marine finfish diet and outperformed the local trout diet. The addition of the binder did not improve the growth performance of the fish or feeding efficiency (i.e. FCR); therefore, there is no need to include gelatin in the diet. Feeding a soft pellet, containing 40 % moisture, did not increase growth performance of juvenile dusky kob and increased the FCR. A starch- bound hard pellet (10 % moisture)

containing 46 % dietary protein levels is thus recommended as a suitable practical diet for dusky kob in the size range tested here.

CHAPTER 3

EFFECT OF DIETARY PROTEIN AND ENERGY ON GROWTH AND NITROGENOUS WASTE PRODUCTION IN FARMED DUSKY KOB (*ARGYROSOMUS JAPONICUS*)

INTRODUCTION

The lack of a locally manufactured marine finfish feed has motivated research into formulating a diet specifically for dusky kob. Practical diet formulations that promote maximal growth and feed conversion at a competitive cost, and minimize organic wastes are necessary for the growth of the mariculture industry (McGoogan and Gatlin 2000). It is necessary to optimize the dietary protein to energy ratio (P:E) within diets to achieve maximum growth and feed conversion ratios (Lee *et al*. 2002).

Aquaculture waste origins are largely dietary, with as much as 63 % of feed nitrogen ending up as wastes (Cho and Bureau 1997) and within recirculating culture systems it is imperative that pollution is kept to a minimum to ensure good water quality. Based on this, experimental diets were formulated with varying dietary protein and dietary lipid levels to determine their effects on growth and nitrogenous waste production.

Dietary protein is necessary to supply essential amino acids for protein synthesis (McGoogan and Gatlin 1999). However, if amino acids are present in excess they may not be absorbed by the gastrointestinal tract and deaminated for metabolic fuel (Shyong *et al*. 1998). This deamination of excess protein is associated with an increase in ammonia production by the fish which is undesirable (Shyong *et al*. 1998). Ballestrazzi *et al*. (1994), Cai *et al*. (1996) and Yang *et al*. (2002) have investigated the effect of increased levels of dietary protein on ammonia excretion. Thus, protein levels of 42 and 46 % were tested in this experiment to investigate the effect on growth and ammonia production.

One practice employed to limit ammonia excretion in aquaculture is to restrict feeding but this in turn decreases growth. Therefore, formulations which reduce ammonia excretion whilst still maintain rapid growth are desirable in intensive aquaculture. High dietary energy may have a protein-sparing effect, where cultured species use the dietary energy for metabolic fuel rather than the excess dietary protein which results in lower ammonia production (McGoogan and Gatlin 2000). Higher dietary energy generally comes in the form of increasing dietary lipid levels within the diet formulation. Thus, increased levels of lipid where included in the experimental diets to determine if there is a protein-sparing affect which results in lower ammonia production.

The present study was undertaken to investigate the effect of varying the dietary protein and lipid content on the growth and nitrogenous waste, by ammonia production, of juvenile dusky kob reared under conditions simulating commercial mariculture. The objectives were to: (1) compare the growth, feed conversion ratio and survival of dusky kob fed either low or high protein diets, both with increasing levels of lipid energy, (2) compare the nitrogenous wastes produced by dusky kob fed diets of varying dietary protein and lipid levels, and (3) determine the effect of dietary protein and lipid on carcass composition.

METHODS AND MATERIALS

Experimental Animals

Captive bred juvenile dusky kob (*Argyrosomus japonicus*), of approximately 110 g each, were acclimated in the experimental system described in Chapter 2 for one month prior to the trial. They were fed a four mm imported finfish feed (45 % protein, 18 % lipid, Dana Feed A/S # 1845, Denmark) twice daily to apparent satiation.

Experimental System and Stocking Conditions

The same experimental system described in Chapter 2 was used here, (500 L $tank^{-1}$), as in Chapter 2. Water flow rate into each tank was set at 200 L h^{-1}. Each tank was aerated and water temperature was controlled by thermostatically controlled heaters set at 24 °C.

Twenty eight fish of approximately 114 g each were randomly assigned to each tank, at a stocking density of 10 kg m^{-3}, one week before the start of the experiment. As the fish grew throughout the trial the volume of water in the tanks was adjusted every four weeks during the trial to maintain a density of 10 kg m^{-3}.

Dietary Treatments

Six diets were formulated, each with either 42 % (i.e. low protein) or 46 % (i.e. high protein) protein and each of these were formulated with one of three lipid levels: 6 % (LF), 12 % (MF) or 18 % (HF) crude lipid (Table 1). These diets were manufactured at the Marifeed (Pty) Ltd. factory in Hermanus, South Africa, using low-temperature, formaldehyde free, fishmeal (66 % crude protein, 8.0 % lipid, Oceana (Pty) Ltd, Cape Town, South Africa) as the main protein source. The dietary lipid was maintained among treatments using marine fish oil (Oceana (Pty) Ltd, Cape Town, South Africa). Vegetable starch was added as a carbohydrate and gelatin (Gelatia, Krugersdorp, South Africa) was added as an additional binding agent (Table 1).

The feed was extruded through a circular 4.0 mm die, using proprietary technologies developed for the abalone-farming feed industry, and the pellets were cut 3.0 mm long. They were dried at 38 °C for 18 hours after which they were cooled to room temperature and packaged.

Each of the experimental diets (Table 1) and a commercial marine finfish feed (45 % protein; 18 % lipid; Dana Feed, Denmark), which was included as a reference

diet, were each fed to three randomly selected tanks of fish for the duration of the 12 week trial.

Table 1: Proximate composition and protein to energy ratios (P:E ratio) (g protein/ MJ.kg^{-1} energy) of six experimental diets that were formulated with either high or low protein (HP or LP) each with either low, medium or high lipid (LF, MF or HF), respectively and one commercial finfish feed (REF) fed to juvenile dusky kob, *Argyrosomus japonicus*.

Formulated Diet:	**HPLF**	**HPMF**	**HPHF**	**LPLF**	**LPMF**	**LPHF**	**REF**
Protein (%)	**46**	**46**	**46**	**42**	**42**	**42**	**45**
Lipid (%)	**6**	**12**	**18**	**6**	**12**	**18**	**18**
Protein (%)	44.35	47.45	44.98	40.61	40.65	42.45	42.51
Lipid (%)	6.89	13.21	16.80	4.42	10.82	17.19	17.79
Moisture (%)	10.23	12.91	10.97	9.00	9.80	7.60	7.80
Ash (%)	7.84	12.87	8.41	12.24	10.79	10.70	7.10
Energy (kJ/mg)	18.94	17.83	20.53	17.48	18.61	20.70	21.54
P:E ratio	2.52	2.38	2.26	2.32	2.19	2.08	2.20

Data collection and proximate analysis were measured as in Chapter 2.

Ammonia Excretion

At the end of the growth trial, feed was withheld from the fish for 24 h. Immediately before feeding, one fish from each tank (i.e. three replicates per treatment) was randomly selected and placed into an isolated chamber (25 L) filled with seawater from the fish culture system. These fish were used as the control of unfed fish- the ammonia concentration (TAN mg L^{-1}), temperature (°C) and pH in each chamber were recorded at the time that the fish were placed into them, and again every two hours for the next 10 h. The rest of the fish were subsequently fed the respective diets (Table 1). After reaching apparent satiation, another fish from each tank was randomly selected and placed into similar chambers, at which time the same water quality parameters were recorded over a similar time period. Water quality parameters were also taken from three additional chambers that contained seawater and no fish (i.e. a second control).

All the chambers were kept in a constant temperature environment of 22 °C for the duration of the experiment.

Total ammonia nitrogen (TAN mg L^{-1}) was recorded from sampled water using an ammonium test kit (Merck®, Holland) and reading the absorbance of each solution on a double-beam spectrophotometer (Shimadzu UV-150-02, Kyoto, Japan). A standard curve was determined by testing NH_4Cl solutions with known ammonia concentrations. Ammonia production was calculated as mg TAN kg^{-1} of fish h^{-1}.

Water Chemistry in the Culture tanks during the Trial

The water chemistry was measured using the same method as described in Chapter 2. Water quality parameters were kept within the following limits: The water pH ranged between 7.3 and 7.9 and temperature was maintained between 22 and 24 °C. The oxygen saturation in the tanks was maintained between 65 - 75 % (6.60 – 7.70 mg L^{-1}). The salinity of the water remained 35 g L^{-1} throughout. TAN ranged between 0.00 to 0.25 mg L^{-1} and free ammonium nitrogen (FAN) ranged between 0.000 and 0.012 mg L^{-1} throughout the experiment.

Statistical Analysis

A multi-factor analysis of variance (multi-factor ANOVA) was used to identify interactions between dietary protein and dietary lipid at $p<0.05$. If no interactions were found, a one-way analysis of variance (ANOVA) was carried out. If a significant difference was found, a Tukey's multiple range analysis was used to compare means between treatments at $p<0.05$. If the data did not meet the assumptions of an ANOVA, a non-parametric one-way Kruskal-Wallis ANOVA was used to compare means at $p<0.05$. All test treatment means were combined and compared to the mean generated from the reference diet (i.e. the imported commercial feed) using a Student's *t*-test at $p<0.05$. All statistical analyses were calculated using STATISTICA™ 7.0 (Statsoft, 2004).

RESULTS

Fish Growth and Feed Utilization

There was a significant interaction between factors: - dietary protein and energy level on weight gain (multi-factor ANOVA: $F_{(2,12)}$=17.42, p=0.0003; Figure 1a) and specific growth rate (SGR) (multi-factor ANOVA: $F_{(2,12)}$=20.50, p=0.00013; Figure 1b). Weight gain and SGR gain were highest for the fish fed the high protein, high energy diet with a mean weight gain of 156 ± 5.80 g (136 %) (Table 2). SGR decreased as energy, supplied as additional lipid, increased at the low protein level, and increased as energy increased at the high protein level. The fish fed the low protein, high (18 %) lipid diet realised the poorest mean weight gain of 73 ± 7.04 g (65 %). The fish fed the imported finfish feed gained significantly less weight, realizing a mean weight gain of 87 ± 5.80 g or 77 % compared with an overall mean of 124 ± 7.34 g or 109 % for the test treatments combined (Student's *t*-test: t=2.12, p=0.047; Table 2).

There was a significant interaction between: - dietary protein and energy levels for feed intake (multi-factor ANOVA: $F_{(2,12)}$=22.765, p=0.00008). Feed intake increased as the dietary lipid level increased for the fish fed the high protein diets. In contrast, the feed intake by the fish fed the low protein diets decreased as the dietary lipid levels increased (Figure 2a). The feed intake of the experimental fish fed the test treatments and imported feed were similar, with a mean intake of 4.05 ± 0.17 kg per treatment over the experimental period (Student's *t*-test: t=1.16, p=0.232).

Similarly, there was a significant interaction between: - dietary protein and energy levels on FCR (multi-factor ANOVA: $F_{(2,12)}$=9.58, p=0.003; Figure 2b), with a particularly poor FCR (i.e. 1.93 ± 0.19) recorded for the fish fed the low protein, high energy diet, whereas there was no change in FCR with increasing dietary lipid level for fish fed the high protein diets. There was no significant difference in FCR for fish fed the test treatments combined and those fed the imported feed (Student's *t*-test: t=-1.46, p=0.16), with an overall FCR of 1.32 ± 0.07 (Table 2).

The protein efficiency ratio (PER) was significantly affected by the test diet formulations (multi-factor ANOVA: $F_{(2,12)}$=13.17, p=0.0009; Figure 3). The efficiency of the protein decomposition was negatively affected by increasing lipid levels for the low (42 %) protein diets, and there was no evident trend for protein deposition for the high (46 %) protein diets. PER was similar for fish the test treatments and the imported feed (Student's *t*-test: t=1.77, p=0.09; Table 2).

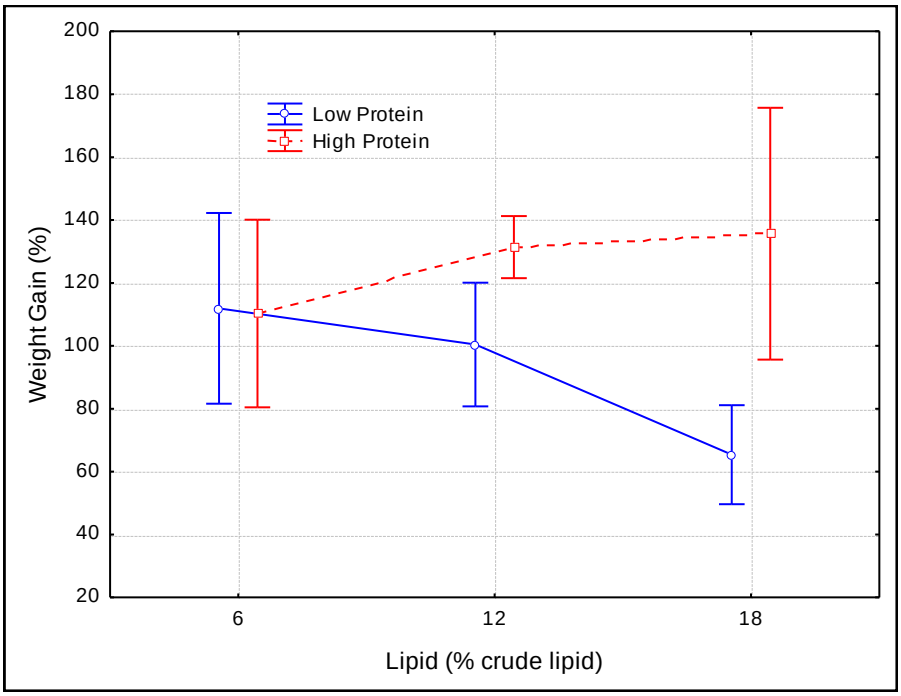
Survival was high for fish fed all treatments, i.e. 90 ± 5 % (Table 2). There was no interaction between factors: - dietary protein and energy levels in the test treatments (multi-factor ANOVA: $F_{(2,12)}$=0.15, p=0.859; Table 2). Survival was similar for fish fed the test treatments and the imported feed (Student's *t*-test: t=1.77, p=0.09; Table 2).

There was no significant interaction for factors:- dietary protein and energy levels for CF (multi-factor ANOVA: $F_{(2,12)}$=18.43, p=0.20) and VSI (multi-factor ANOVA: $F_{(2,12)}$=1.00, p=0.39; Table 2). There was a significant difference in CF for fish fed the test treatments and the imported feed (Student's t-test: t=2.15, p=0.044), with a mean CF of 2.87 ± 0.02 and 2.76 ± 0.03, respectively (Table 2). There was a significant interaction between factors for HSI (multi-factor ANOVA: $F_{(2,12)}$=4.188, p=0.042; Table 2), with a drop in the HSI of fish fed a low lipid, high protein diet. Fish carcass composition appeared to be similar between treatments (Table 3). The lipid composition (% total body weight^{-1}) of the fish carcasses increased as dietary lipid increased in the experimental diets ($y = 13.534 \ln(x) - 19.584$; $R^2 = 0.98$; Figure 4).

Table 2: Mean (± Standard error) initial and final length and weight, weight gain, feed intake and conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), survival, condition factor (CF), hepatosomatic (HSI) and visceral indices (VSI) of dusky kob fed diets with different protein and lipid levels, and a commercial feed (REF). The symbol † marks a significant interaction between dietary protein and dietary lipid in the row (multi-factor ANOVA, $p < 0.05$). The symbol ‡ indicates a significant difference between the reference diet and the combined mean of all test treatments in the row (Student t-test, $p < 0.05$).

Diet	**HPLF**	**HPMF**	**HPHF**	**LPLF**	**LPMF**	**LPHF**	**REF**
Protein %	**46**	**46**	**46**	**42**	**42**	**42**	**45**
Lipid %	**6**	**12**	**18**	**6**	**12**	**18**	**18**
Initial Weight (g)	108.64 ± 2.18	110.77 ± 7.15	115.51 ± 4.09	113.73 ± 9.97	123.6 ± 3.10	111.5 ± 4.76	112.69 ± 3.30
Initial Length (mm)	213 ± 0.73	214 ± 4.61	217 ± 2.47	215 ± 5.48	221 ± 0.90	213 ± 3.13	215 ± 1.13
Weight Gain (g) †	119.55 ± 6.00	145.23 ± 7.50	155.93 ± 5.80	127.31 ± 13.45	123.9 ± 4.27	73.26 ± 7.04	87.28 ± 5.80 ‡
Weight gain (%) †	110 ± 6.93	131 ± 2.29	136 ± 9.31	112 ± 7.05	100 ± 4.57	65 ± 3.65	77 ± 3.27 ‡
Feed Intake (kg) †	3.9 ± 0.03	4.5 ± 0.16	4.6 ± 0.11	4.6 ± 0.18	4.3 ± 0.17	3.5 ± 0.12	3.9 ± 0.40
FCR	1.05 ± 0.02	1.11 ± 0.02	1.05 ± 0.05	1.31 ± 0.10	1.24 ± 0.03	1.93 ± 0.19	1.58 ± 0.07
SGR †	0.95 ± 0.04	1.08 ± 0.01	1.10 ± 0.05	0.96 ± 0.04	0.89 ± 0.03	0.64 ± 0.03	0.73 ± 0.02 ‡
PER	1.95 ± 0.06	1.66 ± 0.02	1.89 ± 0.05	1.68 ± 0.11	1.63 ± 0.05	1.12 ± 0.11	1.35 ± 0.04
Survival (%)	92 ± 1.19	85 ± 3.15	89 ± 2.06	94 ± 2.38	84 ± 2.38	92 ± 3.15	95 ± 2.38
CF	2.91 ± 0.09	2.87 ± 0.02	2.87 ± 0.02	2.76 ± 0.03	2.93 ± 0.01	2.9 ± 0.03	2.76 ± 0.03 ‡
HSI (%) †	1.85 ± 0.05	2.25 ± 0.04	2.35 ± 0.18	2.46 ± 0.09	2.22 ± 0.14	2.4 ± 0.15	2.38 ± 0.17
VSI (%)	11.68 ± 0.13	12.17 ± 0.26	11.66 ± 0.67	12.75 ± 0.51	12.01 ± 0.41	11.83 ± 0.5	12.23 ± 0.53

a)



b)

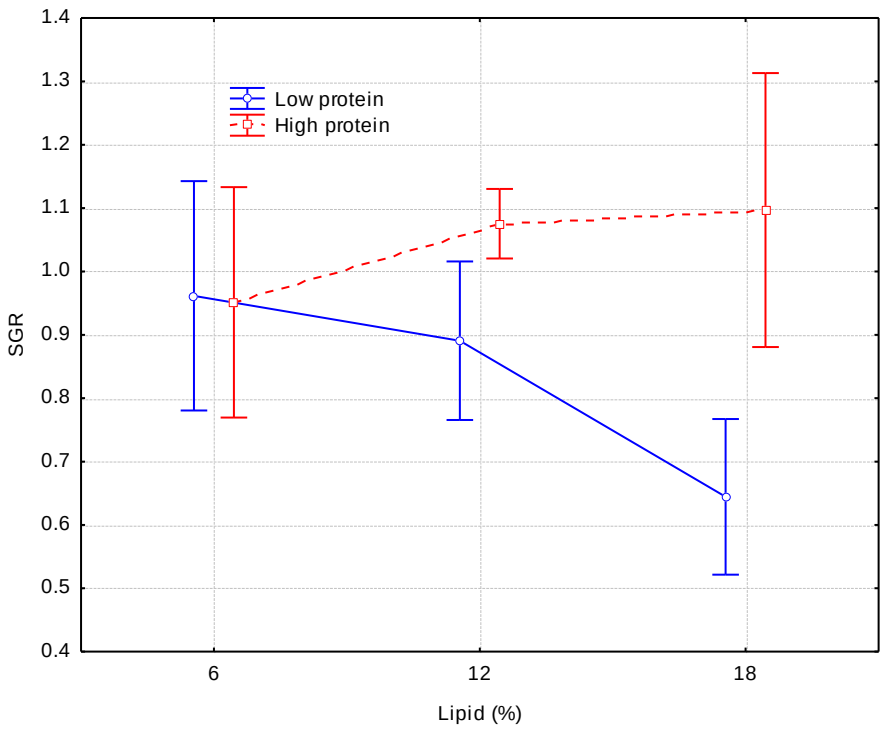
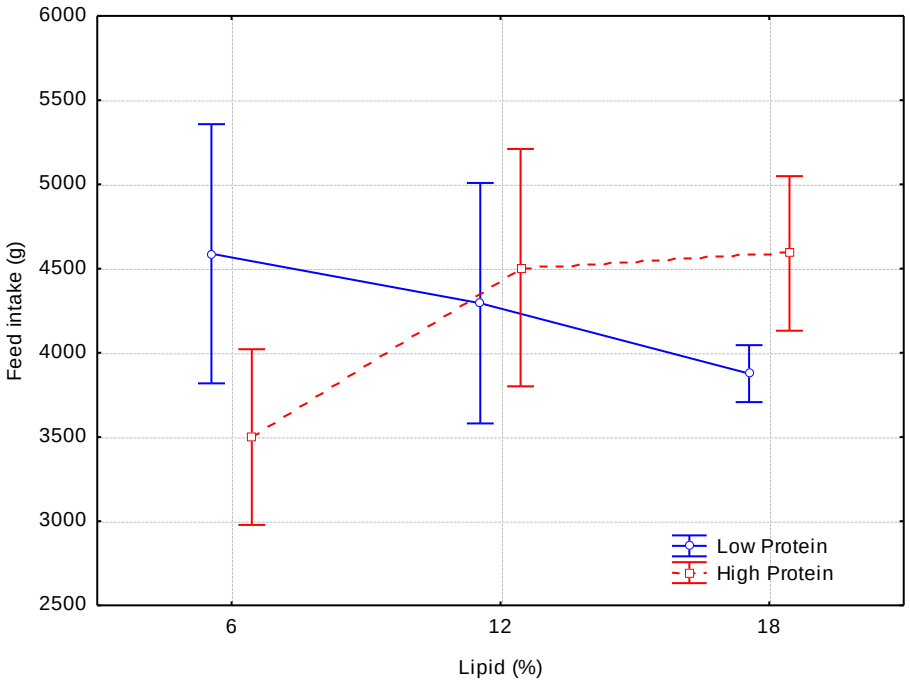


Figure 1. Changes in (a) weight gain (%) and (b) specific growth rate (SGR) (N=5) of dusky kob fed either a low (42 %) or high (46 %) protein diets with increasing levels of dietary lipid (multi-factor ANOVA: $F_{(2,12)}$=17.42, p=0.0003) and (multi-factor ANOVA: $F_{(2,12)}$=20.50, p<0.001), respectively. Error bars represent a 95 % confidence interval.

a)



b)

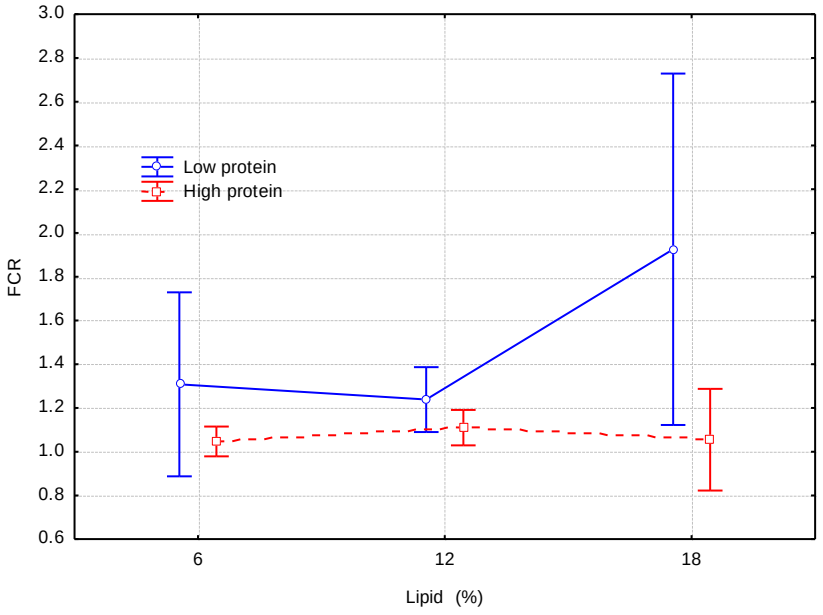


Figure 2. (a) Feed intake (multi-factor ANOVA: $F_{(2,12)}$=22.77, p<0.0001) and (b) feed conversion ratio (FCR) (multi-factor ANOVA: $F_{(2,12)}$=9.58, p=0.003) (N=5) of dusky kob fed low (42 %) and high (46 %) protein diets with increasing dietary lipid levels over 11 weeks. Error bars represent a 95 % confidence interval.

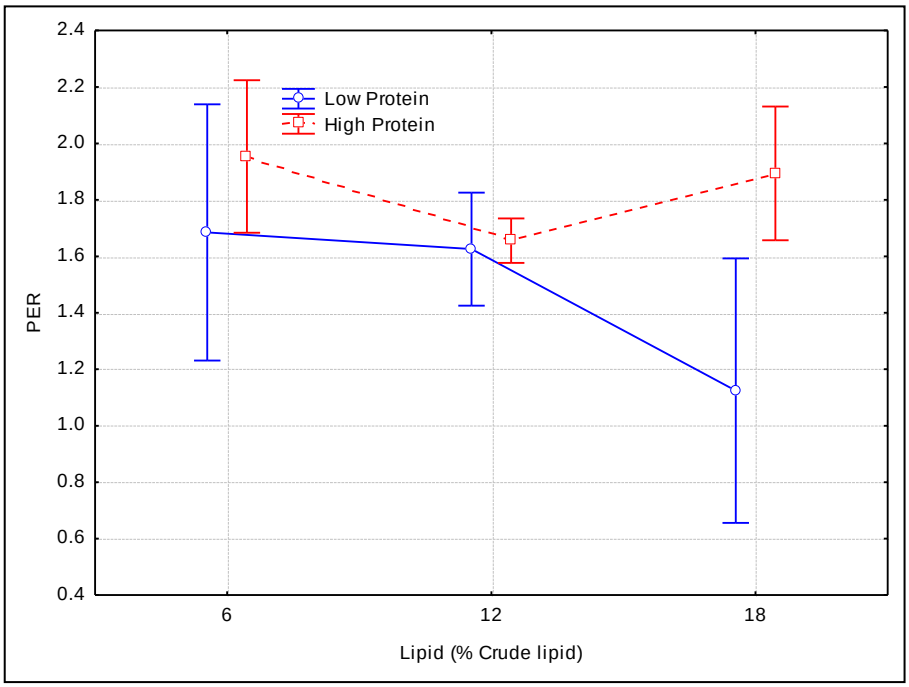


Figure 3. Protein efficiency ratio (PER) of dusky kob fed either low or high protein diets with increasing crude dietary lipid (multi-factor ANOVA: $F_{(2,12)}$=13.17, p=0.0009) over 11 weeks. Error bars represent a 95 % confidence interval.

Table 3: Proximate composition dusky kob fed six experimental diets, with varying dietary protein and lipid levels, and one commercial finfish feed (REF).

Diet	HPLF	HPMF	HPHF	LPLF	LPMF	LPHF	REF
Protein %	**46**	**46**	**46**	**42**	**42**	**42**	**45**
Lipid %	**6**	**12**	**18**	**6**	**12**	**18**	**18**
Protein (%)	69.30	60.72	67.20	68.18	62.79	60.86	61.53
Moisture (%)	74.37	81.63	82.67	76.14	80.39	73.37	77.40
Ash (%)	20.67	18.12	21.04	21.22	16.74	20.77	16.00
Energy (kJ/mg)	17.52	18.03	18.18	17.15	20.31	18.73	21.17

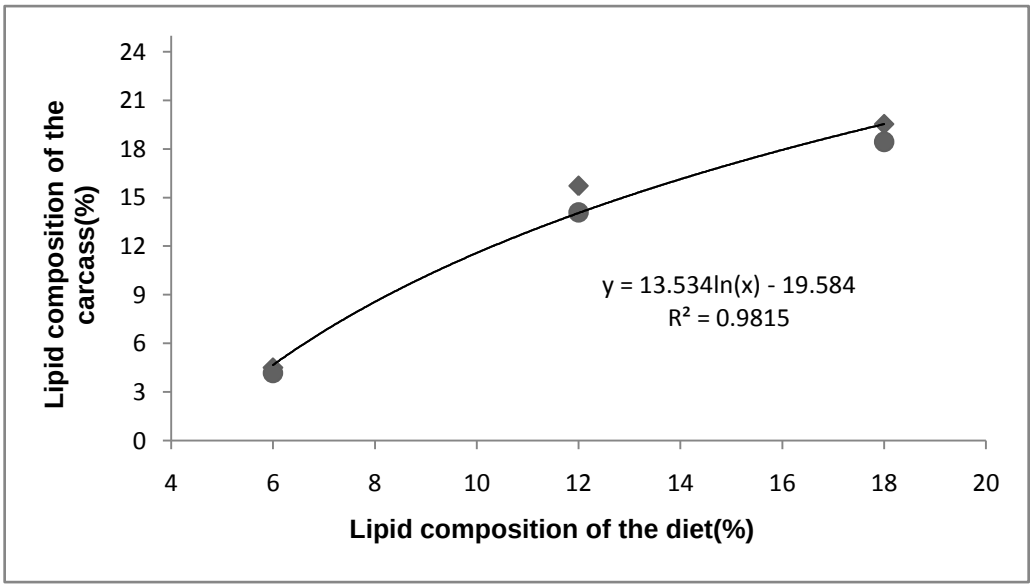


Figure 4. The carcass lipid composition (% total body weight^{-1}) of dusky kob, *Argyrosomus japonicus*, compared to the increasing dietary lipid content in high (46%) and low (42 %) protein diets.

There was no significant interaction between: - dietary protein and lipid levels on ammonia production over a 10 hour period (multi-factor ANOVA: $F_{(2,98)}$=0.39, p=0.68; Table 4). TAN production was not significantly affected by dietary protein levels (ANOVA: $F_{(1,88)}$=1.97, p=0.16) with fish fed the low protein diets producing 1.74 ± 0.29 mg kg^{-1} L^{-1} h^{-1} compared to the fish fed the high protein diets which produced 2.65 ± 0.38 mg L^{-1} kg^{-1} h^{-1} 10 hours after feeding. Similarly, dietary energy did not significantly reduce ammonia

Table 4: Mean (± standard error) rate of ammonia production of dusky kob 10 h after feeding different experimental diets with a combination of either 42 or 46 % protein (i.e. LP and HP, respectively) and either 6, 12 and 18 % fat (i.e. LF, MF and HF, respectively) or a commercial feed (i.e. REFERENCE). Means with the different superscripts in the same line are significantly different (ANOVA, p=0.37).

Time (h)	Diet	Ammonia Production (mg TAN L^{-1} kg^{-1} h^{-1})																							
	HPLF				HPMF				HPHF				LPLF				LPMF				LPHF				REFERENCE			
2	0.39	±	0.14	a	0.46	±	0.16	a	0.18	±	0.11	a	0.22	±	0.07	a	0.44	±	0.06	a	0.26	±	0.06	a	0.45	±	0.09	a
4	0.33	±	0.10	a	0.45	±	0.11	a	0.28	±	0.12	a	0.41	±	0.09	a	0.39	±	0.17	a	0.19	±	0.09	a	0.38	±	0.09	a
6	0.33	±	0.09	a	0.65	±	0.17	a	0.46	±	0.09	a	0.38	±	0.04	a	0.46	±	0.13	a	0.19	±	0.09	a	0.37	±	0.07	a
8	0.53	±	0.11	a	0.75	±	0.27	a	0.46	±	0.16	a	0.42	±	0.11	a	0.38	±	0.09	a	0.20	±	0.04	a	0.58	±	0.02	a
10	2.36	±	0.33	a	3.33	±	0.26	a	2.25	±	0.55	a	2.24	±	0.08	a	1.62	±	0.34	a	1.35	±	0.44	a	2.10	±	0.29	a

production (ANOVA: $F_{(2,87)}$=0.51, p=0.45), although there was a trend towards lower ammonia production, after 8 and 10 hours postprandial, in the low protein diets as the energy level increased (Table 4).

DISCUSSION

Growth performance and feed utilization by dusky kob was significantly better in diets containing higher, 46 %, dietary protein compared to the low (42 %) protein diets. An increase in dietary protein results in an increase in essential amino acids which are the building blocks for protein synthesis and hence overall growth (McGoogan and Gatlin 1999). Dusky kob may have similar optimum dietary protein requirements to other Sciaenidae species. Red drum, *Sciaenops ocellatus*, requires a 44 % dietary protein as dry weight (Daniels and Robinson 1986), whereas Serrano *et al*. (1992) recommended 40 – 45 % protein and 10 % lipid for the same species.

Weight gain of dusky kob fed the 18 % lipid diets was significantly higher at the high protein (46 %) diet than that of the fish fed the low protein (42 %) diet. With a high protein (46 %) diet, the fish were able to utilize the additional dietary protein, as evidenced by the better feed intake, growth, FCR and PER values; whereas with the lower protein (42 %) diet, increasing dietary lipid suppressed feed intake, and growth and feed conversion was poorer.

The decreased feed intake with increasing dietary lipid levels in dusky kob fed the low protein diets indicates a protein: energy (P:E) imbalance. Although, it is suggested that feed intake is solely governed by dietary energy levels in several species (NRC 1993, Booth et al. 2005), the dusky kob fed the 42 % protein, high lipid diets consumed less total energy indicating some form of inhibition as a result of a low P:E ratio. It has been suggested that feed intake in certain species is regulated through a lipostatic negative feedback mechanism, where the amount of lipid stored in the body may regulate the animal's appetite, through a feedback inhibition regulating energy balance (Jobling and Miglavs 1993). This lipostatic

mechanism of feed intake control and energy regulation has been postulated for Arctic charr, *Salvelinus alpinus* (Jobling and Miglavs 1993). Similarly, feed intake by Malabar grouper (*Epinephelus malabaricus)* was inversely affected by increasing dietary lipid (Tuan and Williams 2007).

The superior growth rates, PER and FCR of dusky kob fed the high protein diet indicate that an increase in dietary energy supplied by lipid promotes improved protein utilization at a higher dietary protein level. The fish fed the high protein, high energy diet, had a P:E ratio of 2.26 which is the suggested optimal. Little other work has addressed the digestible protein and energy requirements of dusky kob (Pirozzi et al. 2008). For example, the optimal P:E ratio for growth of Australian snapper, *Pagrus auratus*, is 2.8 (Booth *et al*. 2005).

Dusky kob fed diets with various dietary protein to energy (P:E) ratios did not influence the condition factor (CF), hepatosomatic index (HSI) and visceral index (VSI). Although, fish fed the high protein, low fat (HPLF) diet did show slightly lower hepatosomatic index value. The observed HSI values of brown meagre, *Sciaena umbra,* were found to be lower when fish were fed a HPLF diet (Chatzifotis *et al*. 2006). The HSI values of dusky kob were approximately 2.3 % of body weight, which is similar to cultured brown meagre, *S. umbra*, (1.8 %) (Chatzifotis *et al*. 2006), red drum, *Sciaenops ocellatus,* (1.75 – 2.28 %) (McGoogan and Gatlin 1999), and red tilapia, (*Oreochromis mossambicus* and *Oreochromis niloticus* hybrid), (1.67 – 5.41 %) (De Silva *et al.* 1991). Daniels and Robinson (1986) suggested HSI increases with an increase in dietary protein, however, in this study there were no significant differences in HSI values between treatments.

The total carcass lipid levels increased as the dietary lipid levels increased in both the low and high protein diets. Piccolo *et al*. (2008) found meagre, *Argyrosomus regius*, fillets increased in fat content when fed a diet with a lower P:E ratio of 2.26 compared to a diet with a P:E ratio of 3.36. In this study, dusky kob were fed diets

with P:E ratios as low as 2.08, which would account for the increase in carcass lipid. Recently, consumer demand for healthier food products have resulted in studies to manipulate healthy fats, such as the 20-carbon omega-3 fatty acids, found in marine sources (Hargis and Van Elswyk 1993). This result indicates that fish farmers can manipulate the level of fat in the fillet of dusky kob by adjusting the diet to suit the consumer market.

There was no significant difference in ammonia production between treatments. An increase in dietary protein is associated with increased ammonia excretion by the fish; as excess amino acids, supplied by dietary protein, are deaminated for metabolic fuel which increases ammonia excretion (Shyong *et al*. 1998). However, in the marine herbivorous fish, *Cebidichthys violaceus*, nitrogen excretion did not increase with an increase in dietary protein, but may have been due to the decrease in feed consumption rates with increase in dietary protein levels (Horn, *et al*. 1995). In the present study, dusky kob feed intake increased when the dietary protein level was increased, yet ammonia excretion did not increase proportionally. This is explained by the higher PER values for the high (46 %) protein diets indicating that a greater proportion of dietary nitrogen was retained in the flesh as protein deposition. The deamination of protein was thus reduced, stabilizing ammonia production (Webb and Gatlin 2003).

Ammonia is known to be toxic to fish, and special attention must be paid to its toxicity in intensive aquaculture systems, where high concentrations can easily be reached (Rodrigues *et al*. 2007). Feeding ceased in juvenile cobia, *Rachycentron canadum*, at ammonia concentrations above 0.65 mg L^{-1} kg^{-1} (Rodrigues *et al*. 2007) and red drum (*S. ocellatus*) ammonia excretion peaked to 2.97 mg L^{-1} kg^{-1} six hours postprandial when fed a 45 % protein diet (Webb and Gatlin 2003). Ammonia production in this study was below 0.55 mg L^{-1} kg^{-1} eight hours postprandial and below 2.36 mg L^{-1} kg^{-1} 10 hours postprandial, with the exception of the high protein, medium lipid diet. Thus, the high protein diets did not cause an increase in ammonia production compared to the low protein diets. The high

protein diets produced improved growth rates and are thus recommended for use intensive aquaculture feeds for juvenile dusky kob.

In conclusion, there was an increase in weight gain, SGR and PER with increasing lipid levels at the 46 % protein level, and a decrease in weight gain, SGR and PER at the 42 % protein level. It is suggested that a fishmeal based diet containing 46 % dietary protein, 18 % lipid with a P:E ratio of 2.26 (g protein/MJ.kg^{-1} energy) is close to optimal for cultured dusky kob.

CHAPTER 4

EFFECT OF PELLET SHAPE ON FEEDING RESPONSE OF CAGED JUVENILE DUSKY KOB (*ARGYROSOMUS JAPONICUS*)

INTRODUCTION

Cage culture is the predominant method of farming marine finfish species globally (Mortensen *et al*. 2007), but the development of open sea cage culture in South Africa is quite recent. Open sea-cage culture of *Argyrosomus japonicus*, dusky kob, and *Argyrosomus inodorus*, silver kob are currently in a pilot commercial stage (Landman 2006). An experiment to test the performance of dusky kob in cages (150 – 1200 m^3) moored in the mariculture concession off Algoa Bay close to the Port Elizabeth harbour took place in 2007/8, and a further site for cages has been proposed to for Mossel Bay (Schoonbee and Bok 2006). In South Australia and New South Wales *A. japonicus* is currently cultured commercially utilizing sea cages (Fielder *et al*. 1999).

The growing interest in cage culture of dusky kob necessitates the development of a diet that is practical to use in cage conditions. While cage farming offers many advantages over inland tank or raceway systems:- such as lower capital and operating costs, and water supply that never fails; the waste output from uneaten feed must be properly managed (Mortensen *et al*. 2007). Whereas fish held in tanks are able to feed on pellets that are on the tank bottom, fish held in cages are only able to feed whilst the pellets remain within the water column of the cage before they fall through the cage mesh. Once the pellets fall through the cage they are completely lost to the fish. The proportion of feed that is lost in sea cage culture has been reported to range from 1 to 40 % (Thorpe *et al*. 1990, Juell 1991). Feed consumption is thus determined by the amount of feed that can be consumed by the fish in the time the pellets sink in the cage water column. As dusky kob tend to be shy fish which wait for pellets to sink below the surface (Per. Obs.), feed loss is a particular concern in cages. It was hypothesized that reducing

pellet settling velocity, by manipulation of the pellet shape, would increase the availability of the pellet to the fish and reduce feed waste.

The objectives of this study were to (1) compare the pellet settling velocity of an experimental diet presented as either a round, cylindrical pellet or a flat, square pellet, and (2) determine the feeding response, survival, feed conversion ratio and growth rate of juvenile dusky kob fed an experimental diet presented in these two pellet shapes.

MATERIALS AND METHODS

Experimental Animals

Captive bred juvenile *A. japonicus* (approximately 1.0 g) were acclimated in four circular floating cages at the small boat harbour in Port Alfred (South Africa) for 12 weeks prior to the trial. The fish were fed twice a day to apparent satiation on a 1.0 mm imported finfish feed (52 % protein, 12 % lipid, Dana Feed A/S # 1845, Denmark).

Experimental System and Stocking Conditions

The experimental system consisted of four circular plastic cages (diameter: 1.5 m, volume: 3.3 m^2). Each cage consisted of a cylinder and base of semi-rigid polyethylene mesh netting (9 mm diameter mesh holes) attached to a 60 mm (internal diameter) polyethylene pipe hoop (Figure 1a). The cage tops were covered with shade cloth material. The cylindrical plastic cages, floated on the surface of the water to a depth of 0.9 m, and were moored to the sides of a harbour jetty (Figure 1b).

One hundred and seventy five fish of approximately 15 g each were randomly assigned to each cage at a stocking density of 1.0 kg m^{-3}. Fish were fed to apparent satiation twice a day for eight weeks.

a)  b) 

Figure 1. (a) View of constructed experimental cage and (b) cages moored at the Port Alfred harbour.

Dietary Treatments

Two pellet shapes were produced: - one treatment included a 2 mm round, cylindrical pellet and the other a 2 mm square, flat pellet, both of similar weight of 0.06 ± 0.17 g. The two dietary treatments were manufactured at the Marifeed (Pty) Ltd factory in Hermanus, South Africa, using a proprietary formulation that resulted in a diet with 460 g kg^{-1} crude protein, 150 g kg^{-1} crude lipid, 110 g kg^{-1} crude ash, 80 g kg^{-1} moisture and 20.8 MJ kg^{-1} energy. Low-temperature, formaldehyde free, fishmeal was included as the main protein source (66 % crude protein, 8.0 % lipid, Oceana (Pty) Ltd, Cape Town, South Africa). The dietary lipid levels were adjusted to the same level, i.e. 15 %, between treatments with the inclusion of marine fish oil (Oceana (Pty) Ltd, Cape Town, South Africa). Vegetable starch and gelatin (Gelatia, Krugersdorp, South Africa) were used as the primary binding agents.

The ingredients were extruded through either a circular 2.0 mm die and the pellets were cut 2.0 mm in length for the cylindrical pellet shape, or through a square 2.0 mm die and cut 2.0 mm in length for the square pellet shape. The pellets were

placed on drying trays and dried at 38 °C for 18 hours, after which they were cooled to room temperature and packaged.

Each diet was fed to the fish in two randomly selected cages. Data collection took place and proximate analyses were recorded as described in Chapter 2.

Pellet Settling Velocity

A 90 cm length of 25 cm diameter glass tube was used for assessing pellet sinking velocity in sea water (35 g L^{-1}) at 22 °C. Individual pellets were introduced into the tube just below the water surface and the settling velocities determined by timing the fall between two points that were 70 cm apart. The first point was 5 cm below the water surface and the second point was 10 cm from the bottom of the tube. Fifty randomly selected pellets of each shape were tested. The water in the tube was changed prior to testing each pellet shape. Pellets which came into contact with the tube wall or those interfered by air bubbles were excluded from the analysis.

Feeding Behaviour

Feeding behaviour in the cages was recorded by determining the time the fish spent feeding in the water column within in a single feed. The feeding time was measured from the time the fish began to feed at the surface of the water and followed the pellets to the cage bottom until the pellets fell through the cage bottom and the fish stopped feeding. Feeding behaviour was observed on 12 randomly selected days.

Water Chemistry

The water pH and temperature was measured in the cages weekly using a pH and temperature meter (Hanna Instruments HI 98128, Rhode Island, USA). A hand-held salinity refractometer (Atago S/Mill-E, Tokyo, Japan) was used to measure salinity weekly. Dissolved oxygen was measured weekly with a dissolved oxygen probe (Hanna Instruments, Dissolved Oxygen Meter HI 1934, Romania). Total

ammonia nitrogen was recorded using a colourmetric titration kit once a week (Merck®, Holland). Water quality parameters were within the following limits: The pH ranged between 7.7 and 8.10 and temperature ranged between 19 and 22 °C, with an overall mean of 20.20 ± 0.74 °C. The oxygen saturation in the water ranged between 70 - 85 % (7.50 – 8.20 mg L^{-1}). The salinity of the water remained 35 g L^{-1} throughout. Total ammonia nitrogen remained undetectable throughout the experiment.

Statistical Analysis

A Student's *t*-test or, if the data did not meet the assumptions, of a parametric analysis, a non-parametric Mann-Whitley test was used to compare means between treatments at $p<0.05$. All statistical analyses were calculated using the STATISTICA™ 7.0 statistical package (Statsoft, 2004).

RESULTS

Feed Utilization and Fish Growth

The fish readily accepted the test diets and the feed intake was similar between treatments, with the fish fed the square pellet and those fed the cylindrical pellet consuming 3.9 ± 0.13 and 4.1 ± 0.19 kg over the experimental period, respectively (Student's *t*-test: t=-0.72, p=0.54).

Pellet form affected growth, with fish fed the cylindrical pellet gaining an overall mean of 13.36 ± 0.38 g, compared to those fed the square pellet gaining 16.71 ± 0.11 g during the eight week trial (Student's *t*-test: t=8.67, p=0.01). Similarly, the square pellet realized significantly higher SGR (Student's *t*-test: t=4.50, p=0.04; Table 1).

The FCR recorded for the square pellet (1.2 ± 0.08) was slightly lower than of the cylindrical shape pellet (1.4 ± 0.02), but the difference was not statistically significant (Student's *t*-test: t=2.37, p=0.14).

Table 1: The mean (± standard error) initial weight and length, the length and weight gain*, percentage weight gain (weight gain %)**, specific growth rate (SGR)*, feed conversion ratio (FCR)*, survival*, condition factor*, hepatosomatic (HSI)* and visceral indices (VSI)* of juvenile dusky kob grown in cages for eight weeks and fed different pellet shapes. Means with different superscripts in the same line are significantly different (Student's *t*-test* or Mann-Whitley**, p<0.05).

	Cylindrical	**Square**
Initial Length (mm)	104 ± 2.70[a]	102 ± 1.26[a]
Initial Weight (g)	14.9 ± 0.54[a]	14.7 ± 0.20[a]
Length gain (mm)	32 ± 1.57[a]	30 ± 0.94[a]
Weight gain (g)	13.4 ± 0.48[a]	16.8 ± 0.11[b]
Weight gain %	91 ± 1.32[a]	113 ± 4.82[b]
SGR	1.06 ± 0.04[a]	1.24 ± 0.01[b]
Total food intake (kg)	4.1 ± 0.19[a]	3.9 ± 0.13[a]
FCR	1.4 ± 0.02[a]	1.2 ± 0.08[a]
Survival %	92 ± 4.24[a]	91 ± 1.21[a]
Condition factor	2.59 ± 0.03[a]	2.76 ± 0.01[b]
HSI %	2.03 ± 0.03[a]	2.11 ± 0.02[a]
VSI %	8.07 ± 0.27[a]	9.00 ± 0.17[a]

There was no significant difference in survival amongst treatments, with a combined mean of 91 ± 2.85 % (Student's *t*-test: t=-0.32, p=0.78; Table 1). The HSI and the VSI were similar among treatments, with the cylindrical and square pellet mean HSI values of 2.03 ± 0.03 and 2.11 ± 0.02 (Student's *t*-test: t=1.92, p=0.20) and mean VSI values of 8.07 ± 0.27 and 9.00 ± 0.17 (Student's *t*-test: t=2.94, p=0.10), respectively. There was a significant difference in CF between treatments with the fish fed the square pellet realizing a mean CF value of 2.76 ± 0.01 compared to those fed the cylindrical pellet realizing a mean of 2.59 ± 0.03 (Student's *t*-test: t=4.70, p=0.04; Table 1). Fish carcass composition appeared to be similar between treatments (Table 2).

Table 2: Initial and final proximate composition of composite samples of *Argyrosomus japonicus* fed experimental diets for eight weeks.

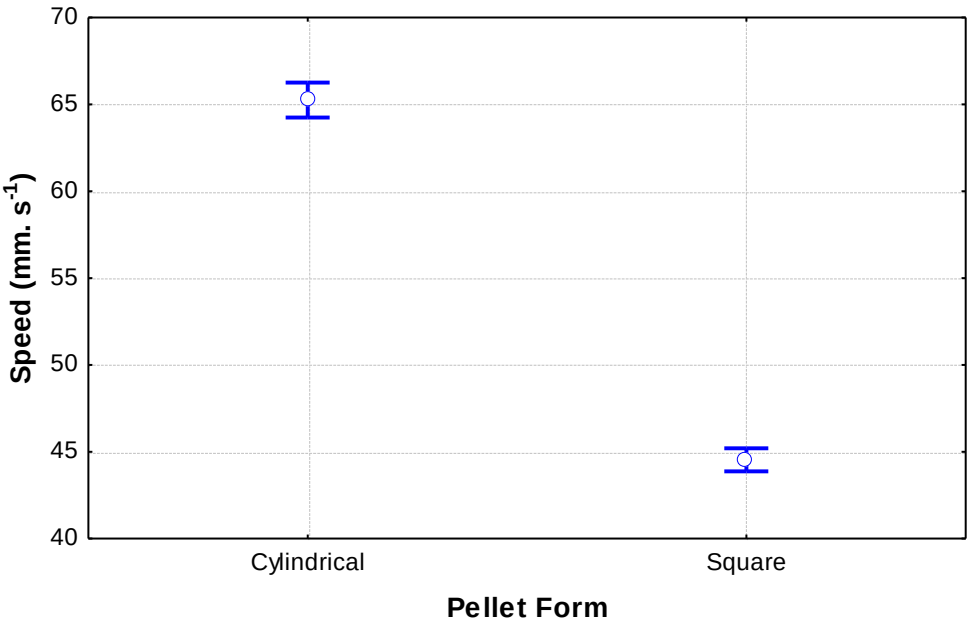
Composition	Initial	Cylindrical	Square
Protein %	62.04	60.33	60.81
Lipid %	10.48	14.71	13.59
Moisture %	74.40	73.80	73.38
Ash %	19.58	17.83	17.82
Energy (MJ kg^{-1})	17.94	19.67	20.03

Settling Speed and Feeding Period

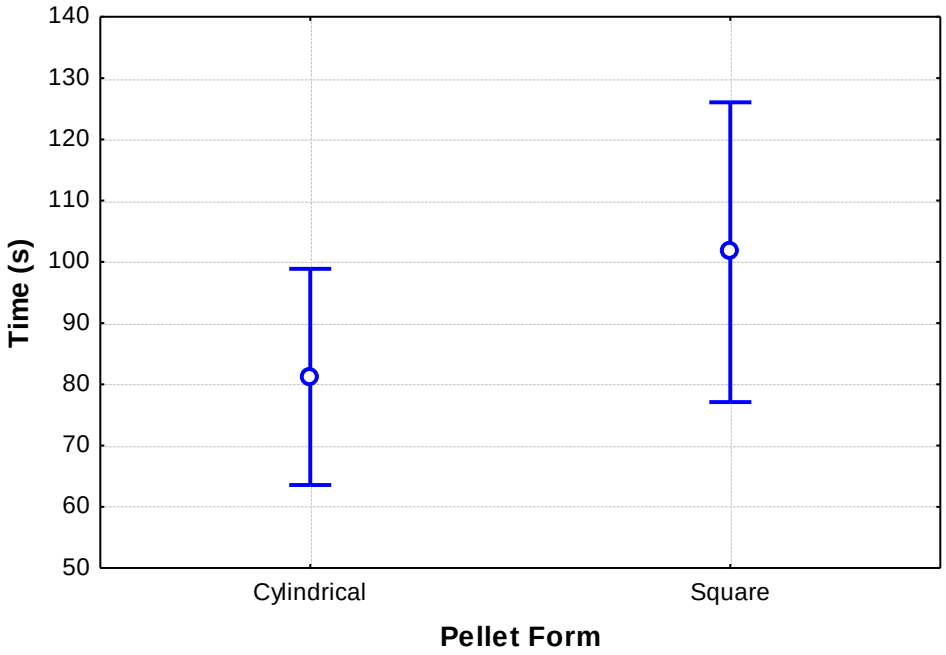
There was a significant difference in settling speed between pellet shapes (Mann-Whitley: $Z_{(1,98)}$=8.62, p<0.0001; Figure 2a), with the cylindrical pellets settling at 65 ± 0.5 mm s^{-1} compared to the square pellets at a slower rate of 45 ± 0.3 mm s^{-1}. The feeding period of the fish fed the square pellet was longer (i.e. 101 ± 11 s) compared to those fed the cylindrical pellet (i.e. 81 ± 8 s), but did not significantly differ (Student's *t*-test: t=1.46, p=0.16; Figure 2b).

DISCUSSION

The results of this study are similar to settling rates found for marine aquaculture feeds of similar sizes. Published data on settling velocity for artificial aquaculture feeds is sparse. Chen (1999) found velocity increased with pellet size from 5.6 cm s^{-1} (2.0 mm pellet) to 13.9 cm s^{-1} (10 mm pellet) and settling velocities increased from 5.0 to 12 cm s^{-1} for freshwater salmonid pellet diets (2.0 mm and 8.0 mm pellet sizes) (Elberizon and Kelly 1998). In this study the use of a large number of sampled pellets (50 replicates per treatment) increased the reliability of the settling velocity data (variation of 5 %). Furthermore, the experimental water column was as deep as those used in feed settlement studies by Chen et al. (1999) (i.e. 50 cm), and deeper than those used by Elberizon and Kelly (1998) (i.e. 30 cm), the deeper water column reduced any influence of drag from the tube wall or resistance from the tube bottom.



a)



b)

Figure 2. (a) Settling velocity (Mann-Whitley: $Z_{(1,98)}$=8.62, p<0.0001) and (b) feeding period (Student's *t*-test: t=1.46, p=0.16) of juvenile dusky kob fed two pellet shapes, cylindrical and round, in a cage culture system. The bars represent 95 % confidence intervals.

The settling rates differed between different shaped pellets, with mean values of 4.5 cm s^{-1} for the square shape pellet and 6.5 cm s^{-1} for the cylindrical shape pellet. The time taken for pellets to fall through a 15 m cage was estimated to be approximately 5 min 33 s for the square pellet and 4 min 11 s for the cylindrical pellet. Dusky kob held in captivity tend to be timid when feeding and allow the feed to fall through the water column before ingesting it (pers. obs.) Thus, the longer time that the square pellet would be suspended within the cage would provide the fish with longer access to feed which is supported by the better growth and FCR results produced by the fish fed the square pellet over the eight week experimental period. The feed conversion ratio (FCR) was slightly better for the fish fed the slower sinking square pellet than those fed the faster sinking cylindrical pellet.

In this study, manipulating the physical shape of artificial aquaculture feeds resulted in pellets with different settling velocities. The fish fed the slower sinking pellets realized better growth and FCR, since the slower sinking pellet spent a significantly longer period in the cage. Thus, the square pellet shape is recommended for cage culture for juvenile dusky kob.

CHAPTER 5
EFFECT OF THE TEST DIET ON GROWTH AND HEALTH OF THE DUKY KOB IN RECIRCULATING SYSTEMS

INTRODUCTION

Feed formulations are based on published nutritional requirements and digestibility of nutrients for specific species. The results of the present study and those of Daniel (2004), indicate dusky kob may have similar dietary protein requirements to other marine carnivorous species (Marais 1984, Daniel 2004, Ballestrazzi *et al*. 1994). Once a prototype diet is developed, it is necessary to test it in commercial scale conditions. One of the aims of the project was to develop a diet for use in recirculating systems that would produce competitive growth performance and FCR without compromising the system water quality or fish health. Thus, the best performing formulation in Chapter 3 (46 %, 18 % lipid) was used to manufacture a commercial prototype diet. The prototype diet was compared with the growth of fish fed a commercially available imported feed in recirculating systems stocked at a commercial density.

The maintenance of water quality parameters, such as nitrogen and oxygen, are imperative for recirculating systems (Masser *et al*. 1999). Waste build up from uneaten feed and feces and ammonia excretion by the fish leads to high levels of nitrogen, which may become toxic to fish, and bacteria flourishes causing levels of oxygen to decrease (Davis 1990). Dissolved oxygen is an important factor for metabolic requirements and survival of fish (Wedemeyer 2000) and thus needs to be kept within acceptable levels in recirculating systems. The performance of the prototype diet and the effect on water quality parameters in recirculating systems needs to be determined. Thus, the effects of both the prototype diet and imported diet on water quality parameters were investigated.

Modern intensive aquaculture utilizes high stocking densities, which may often increase susceptibility to infection and environmental stresses (Sandnes *et al*.

1988). In Australia, dusky kob is farmed at stocking densities of up to 25 kg m^{-3} (O'Sullivan and Ryan 2001) and in South Africa stocking densities of up to 11 kg m^{-3} are used in cage culture production (Schoonbee and Bok 2006). Furthermore, dusky kob was successfully cultured in experimental trials at densities of 50 kg m^{-3} without inhibiting growth (Collett 2008). An increase in stocking density, defined as fish biomass per unit water volume (Wedemeyer 2000), is an important factor influencing growth, survival and health of cultured fish species (Rowland *et al*. 2006). The overall health of the fish can be assessed by changes in haematology, as haematological characteristics are sensitive to fish health (Blaxhall and Daisley 1973). Thus, the effect of increasing stocking density on fish health was assessed for the fish fed the prototype diet and compared to those fed the commercial feed.

The objectives were to: (1) compare fish growth, feed conversion ratio and survival of dusky kob fed the prototype diet, formulated for dusky kob, to the commercial salmonid feed currently available to the finfish aquaculture industry, (2) compare water quality parameters between the recirculating systems of fish fed the two treatments and (3) compare the overall health of fish fed the two treatments.

MATERIALS AND METHODS

Experimental System

The experimental system consisted of eight circular tanks, (with a volume of 500 L $tank^{-1}$ when full) that were isolated from each other. Each tank was connected to its own biological filter (500 L, with oyster shells used as the filter media) and water was exchanged between each tank and filter using a 12 W submersible pump (AquaH2O, ASP-100, Johannesburg, South Africa) at a rate of 200 L h^{-1}. Ten percent of the volume of water in each system was replaced daily with sea water (35 g L^{-1}) from the Kowie River Estuary. Each system was heated using two 300 W aquarium glass heaters (AquaH2O, AT-380, Johannesburg, South Africa) and temperature was maintained between 22 and 24 °C. Airstones were placed in

each tank and biological filter to aerate the water. The filters were conditioned for three weeks prior to the start of the trial.

Experimental Animals

Thirty six captive bred juvenile dusky kob (*Argyrosomus japonicus*), approximately 365 g each, were randomly assigned to each of the eight tanks and acclimated in the experimental system for one week. They were fed a 4.0 mm imported finfish feed (45 % protein; 18 % lipid; Dana Feed A/S # 1845, Denmark) twice daily to apparent satiation.

Dietary Treatments

The dietary treatment was manufactured at the Marifeed (Pty) Ltd factory in Hermanus, South Africa, using a proprietary formulation that resulted in a diet with 460 g kg^{-1} crude protein and 170 g kg^{-1} crude lipid, 85 g kg^{-1} crude ash, 80 g kg^{-1} moisture and 20.5 KJ mg^{-1} energy. Low-temperature, formaldehyde free maasbanker fishmeal was included as the main protein source (71 % crude protein; 9.0 % lipid, Oceana (Pty) Ltd., Cape Town, South Africa), and the dietary lipid level was adjusted to 16 % with the inclusion of marine fish oil (Oceana (Pty) Ltd., Cape Town, South Africa). Vegetable starch and gelatin (Gelatia, Krugersdorp, South Africa) were used as the primary binding agents.

The ingredients were extruded through circular 4.0 mm die and the pellets were cut 3.0 mm long. The pellets were placed on drying trays and dried at 38 °C for 18 hours, after which they were cooled to room temperature and packaged. The experimental diet and the commercially feed (45 % crude protein; 18 % crude lipid), were each fed to four randomly selected tanks of fish.

Fish Growth and Nutritional Indices

At the start and end of the trial fish were purged for 24 hours prior to handling and subsequently narcotized with 2-phenoxyethanol (ethylene glycol monophenol ether, Merck®, Holland) at 0.2 mL L^{-1}. All fish were individually weighed (0.01 g),

and the total length measured (1.0 mm). The specific growth rate (SGR), percentage weight gain, feed conversion ratios (FCR) and condition factors (CF) (Bolger and Connolly 1989) were calculated using Equations 1 to 4(Chapter 2)

Haematology and Liver Glycogen

Two fish per tank were randomly sampled at the end of the experiment. Blood and a section of gill filament from the gill arch were taken from the first fish, which was then frozen at - 10 °C for later proximate analyses. Blood was sampled by cardiac puncture using a 22 gauge, 1.5 inch needle and 3.0 cc syringes. The needle and syringe were heparinized with sodium heparin (5 000 i.u. mL^{-1} Bodene (Pty) Ltd, Intramed). Blood was stored in dipotassium ethylenediamine tetra acetic acid (EDTA) vacuum tubes and kept at 4°C until analyzed. A drop of blood was placed into an Accu-Check® (Roche (Pty) Ltd., Mannheim, Germany) glucose test strip. The strip was then placed into the Accu-Check® sensor. Blood was analyzed for red blood cell (RBC) count, heamoglobin and heamatocrit levels. Analysis was run in a hematology analyser (Beckman Coulter, AC.T5 DIFF, Florida, USA). A sample of blood and gill filaments was taken from the second fish, before the liver and visceral organs were removed and weighed. Hepatosomatic index (HSI) and visceral index (VSI), were calculated using Equations 5 and 6 (Chapter 2)

The liver was subsequently frozen in liquid nitrogen (- 210 °C) for later glycogen analysis, which was carried out using methods described by Woodcock and Bekendorff (2008): - approximately 0.250 g of liver sample was homogenized, 0.6 M perchloric acid (PCA) was added at a ratio of 1:5 and samples were incubated at room temperature for 30 minutes. Samples were centrifuged for 10 minutes at 10 000 rpm. Six hundred µL of supernatant was centrifuged for five minutes at 10 000 rpm. Seventy seven µL of the supernatant was added to 500 µL iodine (I_2KI) reagent and 77 µL of the supernatant was added to 500 µL triple distilled water, to use as the blank sample. Samples were incubated at room temperature for 20 minutes then 200 µL of each sample was added to a microplate. Absorbance of the solution was recorded using a 96 microtitre spectrophotometer (PowerWave

X, Biotek Instruments, USA) at a wave length of 460 nm. The absorbance readings were converted to glycogen concentrations using a standard curve prepared with oyster glycogen (Sigma Chemicals, USA).

Proximate analysis of fish and feed samples were measured as in Chapter 2.

Disease Monitoring and Control

A single fish was randomly sampled from two randomly selected tanks two weeks into the trial, skin scrapings were taken and a piece of gill filament was cut from the gill arch. The gill filaments and skin scrapping samples were mounted onto slides and viewed under a standard light microscope, and examined for ectoparasites, viz. gill flukes.

Due to the fluke infection found on the fish gills, all the fish in all the tanks were treated with praziquantel, at a dosage of 150 mg kg^{-1}, (K. Christison, *pers comm*.). The medication was given orally by coating the pelleted feed in the praziquantel; these pellets were also covered in cod liver oil. The fish were fed the medicated pellets on day 26, 33 and 40 of the trial. Excess pellets were removed from the tanks two hours after the fish were fed.

Haematology Comparison at a Higher Stocking Density

After the 11 week growth trial the stocking density of six tanks: - three tanks of fish fed the prototype diet and three tanks of fish fed the commercial feed, were increased to 50 kg m^{-3} by lowering the water level in each tank. The fish were fed to apparent satiation twice a day with the respective diets. After 12 days three fish per tank were randomly sampled and blood samples were drawn from each fish. Blood was analysed for glucose, red blood cell (RBC) count, white blood cell (WBC) count, heamoglobin and haematocrit using methods described above.

Water Chemistry

The water chemistry was measured using the same method as described in Chapter 2. Water quality parameters were kept within the following limits: - the water pH ranged between 7.5 and 7.9 and temperature was maintained between 19 and 22 °C. The oxygen saturation in the tanks was maintained between 70 - 85 % (7.20 – 8.10 mg L^{-1}). The salinity of the water remained 35 g L^{-1} throughout. Total ammonia nitrogen ranged between 0.00 to 0.25 mg L^{-1} throughout the experiment.

Water Chemistry over Eight Hour Post Feeding

Water quality parameters were tested in six of the individual treatment systems after the feeding trial. The pH, temperature and dissolved oxygen and the total dissolved solids (TDS probe 98312, Hanna Instruments, South Africa) were recorded every two hours over an eight hour period, after the fish had been fed their respective diets, in triplicate (i.e. three tanks of fish per treatment). Water samples were collected over the same time period and were frozen at – 10 °C for later total ammonia nitrogen analysis. Total ammonia nitrogen was tested using an ammonium test kit (Merck®, Holland). The samples were read using a double-beam spectrophotometer at 640 nm (Shimadzu UV-150-02, Kyoto, Japan). The absorbancy readings were converted to ammonia concentrations using a standard curve prepared with known concentrations of ammonium chloride (NH_4Cl).

Biological Oxygen Demand Test

Water samples were collected after feeding for a five-day biological oxygen demand (BOD) test (Delzer and McKenzie 2003). BOD bottles, 250 mL, with ground glass stoppers were thoroughly wash with iodine tincture solution and rinsed with deionised water. Bottles were completely filled with water samples from six treatment tanks in duplicate. Two bottles were filled with distilled water, and this acted as a control. The dissolved oxygen in the water of each bottle was recorded at the start of the trial (Dissolved Oxygen Meter, Hanna Instruments HI 1934, Romania). A magnetic stirrer was placed into each bottle and the glass

stoppers were securely fitting ensuring no air bubbles formed in the bottles. The bottles were covered in foil to protect the samples from light and stored at approximately 22 °C for five days. After five days the water samples were mixed using a magnetic plate stirrer and the dissolved oxygen was recorded. The BOD was calculated using the Equation 7:

$$DOB_5 \text{ (mg } L^{-1}) = D_1 - D_2, \quad (7)$$

where D_1 is the initial sample dissolved oxygen concentration and D_2 is the sample dissolved oxygen after five days.

Statistical Analysis

A Student's *t*-test or, if the data did not meet the assumptions of a parametric analysis, a non-parametric Mann-Whitley test was used to compare means between treatments at $p<0.05$. All statistical analyses were calculated using STATISTICA™ 7.0 statistical package (Statsoft, 2004).

RESULTS

Fish Growth and Feed Utilization

The fish growth was similar for both treatments with the fish fed the prototype diet gaining an overall mean (± standard error) of 204.9 ± 18.2 g compared to the fish fed the commercial feed gaining an overall mean of 231.5 ± 14.5 g (Mann-Whitley Z=-0.86, p=0.39). The fish fed the two treatments had similar SGR, with a combined mean of 1.83 ± 0.03 (Student's *t*-test: t=-1.03, p=0.34; Table 1).

The feed intake of the prototype diet and the commercial feed were similar, with a mean intake of 5.63 ± 0.33 kg per treatment over the experimental period (Student's *t*-test: t=-1.62, p=0.16; Table 1). Similarly, feed conversion ratios (FCR) between the treatments were also the same (Table 1), with the prototype diet and commercial feed realizing a combined mean of 1.06 ± 0.06 (Student's *t*-test: t=0.19, p=0.56).

Table 1: Mean (± standard error) (N=4) initial and final weight and length*, growth performance**, specific growth rate* (SGR) and feed intake*, feed conversion ratio* (FCR) and survival** of dusky kob fed an experimental prototype diet and a commercial feed. Means with no superscript in the same line are not significantly different (Student's *t*-test* or non-parametric Mann-Whitley test**, p<0.05).

	Prototype Diet	Commercial Feed
Length (mm) Initial	306 ± 5.64	310 ± 6.19
Length (mm) Final	354 ± 4.67	361 ± 7.81
Weight (g) Initial	357.5 ± 19.14	373.7 ± 23.10
Weight (g) Final	562.4 ± 24.72	605.2 ± 36.37
Weight gain %	58 ± 5.95	62 ± 1.91
Survival %	68 ± 2.56	70 ± 5.40
SGR	1.81 ± 0.03	1.84 ± 0.02
Total food intake (kg)	5.25 ± 0.35	6.01 ± 0.31
FCR	1.07 ± 0.07	1.05 ± 0.05

The mean CF (Student's *t*-test: t=-1.99, p=0.09), HSI (Student's *t*-test: t=-0.48, p=0.65) and VSI (Student's *t*-test: t=0.27, p=0.80) were similar among treatments (Table 2).

Liver Glycogen

There was a significant difference in the hepatosomatic glycogen between the treatments, with the fish fed the prototype diet storing an overall mean of 19.56 ± 0.88 mg glycogen mg liver^{-1} compared to the fish fed the commercial feed storing a mean of 24.62 ± 0.69 mg glycogen g liver^{-1} (Student's *t*-test: t=-3.32, p<0.0001; Table 2).

Disease Monitoring and Survival

The mean prevalence of the parasite infection was 67 % for both treatments combined. There was no significant difference in intensity between the fish fed the two treatments (Student's *t*-test: t=0.40, p=0.70; Table 2). Survival was affected by the parasitic infection within the first two weeks of the trial and ranged between

60 – 78 %. However, there was no significant difference in survival between treatments (Mann-Whitley Z=-0.14, p=0.89; Table 1).

Table 2: Mean (± standard error) (N=4) condition factor (CF), hepatosomatic index (HSI), visceral index (VSI), hepatosomatic glycogen, and incidence of *Diplectanum oliveri* in dusky kob. Means with different superscripts in the same line are significantly different and means with no superscript in the same line are not significantly different (Student's *t*-test: p<0.05).

	Prototype Diet			Commercial Feed		
CF	3.06	±	0.02	3.13	±	0.02
HSI %	1.94	±	0.12	2.05	±	0.19
VSI %	7.24	±	0.61	7.05	±	0.32
Glycogen (mg glycogen g liver^{-1})	19.56	±	0.88 [a]	24.62	±	0.69 [b]
Parasite Incidence	3	±	1.1	2	±	0.7

Haematology Analysis

The red blood cell (RBC) count, haemaglobin and haematocrit levels were similar for the fish fed the prototype diet and the imported feed under low density conditions (i.e. density 25 kg m^{-3}) (Student's *t*-test: p=0.77, p=0.77 and p=0.71, respectively; Table 3). Blood glucose differed significantly between the two treatments (Student's *t*-test: t=11.92, p<0.0001). The fish fed the prototype diet had a combined mean glucose level of 9.5 ± 0.5 mmol L^{-1} compared to those fed the commercial feed which had a far lower mean glucose level of 3.2 ± 0.2 mmol L^{-1}.

Table 3: Mean (± standard error) of blood variables for dusky kob kept at a low density (i.e. 25 kg m^{-3}). Means with different superscripts in the same line are significantly different and means with no superscripts in the same line are not significantly different (Student's *t*-test: p<0.05).

	Prototype Diet	Commercial Feed
Haematocrit %	43 ± 0.93	48 ± 4.83
Haemoglobin g 100 mL^{-1}	7.70 ± 0.15	8.03 ± 0.78
RBC $x10^{12}$ L^{-1}	2.19 ± 0.05[a]	2.85 ± 0.21[b]
Glucose mmol L^{-1}	10.02 ± 0.18[a]	5.08 ± 0.39[b]

Haematology Comparison at a Higher Stocking density

The haemaglobin and haematocrit levels did not increase significantly at the higher stocking density (i.e. 50 kg m^{-3}; Table 4). The red blood cell (RBC) count and blood glucose levels of the fish fed the imported feed increased under high density conditions (i.e. density 50 kg m^{-3}) from 2.3 ± 0.1 to 2.9 ± 0.2 RBC x 10^{12} L^{-1} and 3.2 ± 0.2 to 5.1 ± 0.4 mmol L^{-1}, respectively (Student's *t*-test: p=0.04 and p=0.00, respectively). The haematological characteristics did not differ for the fish fed the prototype diet low density and high density conditions.

Table 4: Mean (± standard error) of blood variables for dusky kob kept at a high density (i.e. 50 kg m^{-3}). Means with different superscripts in the same line are significantly different and means with no superscripts in the same line are not significantly different (Student's *t*-test: p<0.05).

	Prototype Diet	Commercial Feed
Haematocrit %	46 ± 2.80	44 ± 1.90
Haemoglobin g 100 mL^{-1}	7.78 ± 0.54	7.98 ± 0.21
RBC $x10^{12}$ L^{-1}	2.23 ± 0.20	2.32 ± 0.09
Glucose mmol L^{-1}	9.50 ± 0.45[a]	3.21 ± 0.20[b]

Water Chemistry over Eight Hour Post Feeding Trial and BOD

Ammonia concentration (Student's *t*-test: t=0.17, p=0.60), pH (Student's *t*-test: t=0.63, p=0.97), TDS (Student's *t*-test: t=-0.97, p=0.67) and dissolved oxygen (Student's *t*-test: t=0.36, p=0.97) did not differ between treatments over the eight hour period postprandial (Table 5). Biological oxygen demand (BOD) did not differ between treatments, with a combined mean of 1.5 ± 0.2 mg L^{-1} (Student's *t*-test: t=-1.66, n=6, p=0.13; Table 5).

Table 5: Mean (± standard error) pH, total ammonia nitrogen (TAN), total dissolved solids (TDS) and dissolved oxygen (DO) for individual treatment tanks over an eight hour period postprandial of dusky kob fed either an experimental prototype diet formulated for dusky kob or a commercial feed. There were no significant differences between treatments (Student's *t*-test: p>0.05).

	Prototype Diet				Commercial Feed			
Time (h)	**pH**	**TAN (mg L^{-1})**	**TDS (g L^{-1})**	**DO (mg L^{-1})**	**pH**	**TAN (mg L^{-1})**	**TDS (g L^{-1})**	**DO (mg L^{-1})**
0	7.51 ± 0.03	0.10 ± 0.02	1.47 ± 0.14	5.52 ± 0.12	7.46 ± 0.05	0.13 ± 0.04	1.33 ± 0.03	5.41 ± 0.07
2	7.32 ± 0.05	0.18 ± 0.01	0.73 ± 0.03	5.93 ± 0.34	7.29 ± 0.07	0.17 ± 0.01	0.87 ± 0.24	5.95 ± 0.45
4	7.24 ± 0.07	0.20 ± 0.02	0.63 ± 0.09	5.72 ± 0.15	7.24 ± 0.02	0.17 ± 0.01	0.47 ± 0.22	5.77 ± 0.30
6	7.19 ± 0.07	0.14 ± 0.02	0.37 ± 0.12	5.65 ± 0.08	7.19 ± 0.04	0.16 ± 0.02	0.53 ± 0.18	5.59 ± 0.15
8	7.13 ± 0.04	0.16 ± 0.02	0.33 ± 0.09	5.71 ± 0.13	7.12 ± 0.04	0.19 ± 0.02	0.30 ± 0.06	5.71 ± 0.10

DISCUSSION

The survival rate of the experimental fish was affected by a parasitic infection and there was a high mortality rate within the first two weeks of the trial. The parasite was identified as the monogenean, *Diplectanum oliveri* and was treated with the tremicide praziquantel and the mortality rate was zero post treatment. The initial mortality was however similar between treatments, the fish appeared to recover quickly, and there was no evidence the experimental aims were compromised in any way.

The growth rate and feed conversion ratio (FCR) of the dusky kob fed the prototype diet was similar to the fish fed the commercial feed.

There were no significant differences in visceral and liver weights between the fish fed the two treatments. The hepatosomatic index (HSI) values (1.6 – 2.5 %) were similar to levels reported for other Sciaenid species, visibly the brown meagre, *Sciaena umbra*, (1.8 %) (Chatzifotis *et al*. 2006) and red drum, *Sciaenops ocellatus,* (1.75 – 2.28 %) (McGoogan and Gatlin 1999).

The blood chemistry (i.e. glucose) differed between the fish fed the prototype diet and those fed the commercial feed. The blood glucose levels, 2.7 -10.3 mmol L^{-1}, were in the normal range compared to Atlantic salmon, *Salmo salar*, fed an artificial diet, with glucose levels between 4.0 – 8.0 mmol L^{-1} (Skjervold *et al*. 1999). The glucose levels in the fish fed the prototype diet (mean 9.5 mmol L^{-1}) were significantly higher than the fish fed the commercial feed (mean 3.2 mmol L^{-1}). The prototype diet had a high percentage of starch which is a ready source of glucose and energy to fish (Thodesen and Storebakken 1998). The results of the glucose test suggest that the prototype diet contained more readily digestible carbohydrate than the commercial feed. In contrast, the liver glycogen levels were higher in the fish fed the commercial feed compared to the fish fed the prototype diet. Glycogen is the stored form of carbohydrate within animal cells, and it is broken down into glucose, through glycogenolysis, when blood glucose levels are

low (Bowman *et al*. 1968). This suggests that the fish fed the commercial feed store more glycogen whereas the fish fed the prototype diet have no need store glycogen as there is more readily available glucose source in the diet. The haematological values for dusky kob reared under low density conditions, 25 kg m^{-3}, fed the two treatments were comparable to the values reported for Atlantic salmon, *S. salar* (Skjervold *et al*. 1999). The haemoglobin values (6.2 – 9.0 g 100 mL^{-1}) were similar to levels recorded for salmon, 8.9 – 10.4 g 100 mL^{-1}, and the haematocrit values (38.6 – 51.2 %) are comparable to levels for salmon, 44 – 49 % (Sandes *et al*. 1988). The haematological values here suggest there is no abnormal affect by the nutrition on the blood chemistry of dusky kob fed the prototype diet.

The blood chemistry (i.e. glucose and red blood cell count, RBC) differed between the fish fed the prototype diet and those fed the commercial feed when the stocking density was increased to 50 kg m^{-3}. In commercial farm production, high stocking densities of fish is needed for recirculating systems to be cost effective but increasing stocking densities may be stressful to the fish (Masser et al. 1999, Erikson *et al*. 1997). Glucose levels in the blood have in the past been used as an indicator of stress: Atlantic salmon under high stocking density had higher levels of blood glucose, i.e. levels above 10 mmol L^{-1} (Skjervold *et al*. 1999). The fish fed the commercial feed had significantly higher glucose levels (mean 5.0 mmol L^{-1}) when kept at an increased density of 50 kg m^{-3} compared to 25 kg m^{-3}, but levels were still within levels recorded for other marine finfish. The RBC also increased at the higher stocking density for the fish fed the commercial feed, but values remained within reported values for Atlantic salmon. The haematocrit and haemaglobin values, for fish fed both treatments, were not affected by the increase in stocking density. The growth, survival and FCR of dusky kob was not affected when kept at a density of 50 $kg.m^{-3}$ (Collett 2007) and the haematological results suggest that there is no abnormal affect on the fish health when kept under high stocking density conditions and fed the prototype diet.

In recirculating systems good water quality must be maintained for maximum fish growth (Davis 1990). Ammonia, at high concentrations of above 1.0 mg TAN L^{-1}, is lethal to fish (Rodrigues *et al*. 2007). The recorded total ammonia nitrogen levels were low (< 0.20 mg TAN L^{-1}) for systems fed both treatments over the eight hour period. The water quality in the isolated systems was good, the results of the biological oxygen demand (BOD) test was less than 2.0 mg L^{-1}, hence there was little organic matter present in the water and few bacteria present to decompose it. Thus, the dissolved oxygen levels were high (Delzer and McKenzie 2003). The dissolved oxygen ranged between 5.3 and 6.8 mg L^{-1} throughout eight hour post feeding trial. The most crucial component of water quality is dissolved oxygen. Dissolved oxygen should be maintained above 5.0 mg L^{-1} for optimum fish growth in warm water systems (Masser *et al*. 1999). Growth of red drum, *S. ocellatus*, was reduced when oxygen levels dropped below 4.0 mg L^{-1} (Davis 1990). Thus, the feeding of both the prototype diet and commercial feed had no negative effects on the dissolved oxygen levels within the recirculating systems. The fish were stocked at a density of 25 kg m^{-3}, which is commonly practiced in the commercial farming of dusky kob (Landman 2006, Collett 2007), with no negative effects on the water quality parameters, thus the prototype diet would be suitable for use in recirculating systems.

In conclusion, the prototype diet performed on a par (i.e. fish growth and FCR) with the commercial salmon diet. The haematological values of the fish fed the prototype diet were comparable to those of other cultured marine finfish. There was no indirect evidence, i.e. evidence based on haematology, blood glucose and liver glycogen values that the fish were under any physiological stress when the stocking density was increased. Nonetheless, blood glucose and liver glycogen of dusky kob appear to be diet dependent, varying significantly with dietary carbohydrate level. The prototype diet did not affect water quality in the recirculating systems. The prototype diet, formulated using ingredients that are readily available and manufactured locally, offers a practical option to farmers

using closed recirculating systems in the growing mariculture industry in South Africa.

CHAPTER 6

CONCLUDING DISCUSSION

In South Africa there is a lack of a locally produced diet for the growing marine finfish farming industry. A practical diet must be acceptable to dusky kob and support rapid growth, and must also have limited wastes. Thus, the aim of this study was to develop a practical diet based on previous research into the nutritional requirements of dusky kob and similar cultured species as well as using the existing abalone aquaculture feed technology already well established in South Africa. This work resulted in a practical marine finfish diet that utilizes local ingredients and technologies that performed equally well as international finfish feeds.

In commercial farming the stability of the pellet within the water is crucial to deliver important dietary components to the fish and limit wastes from uneaten food (Halver and Hardy 2002). Chapter 2 investigated the effect of an additional binder and moisture content on the growth of dusky kob as well as the stability of the pellet. Binders are essential in pellet formation and reduce waste in both dry and moist feeds (Storebakken 1985). The experimental feeds tested here, utilising starch-binding technologies developed for the abalone aquaculture industry, resulted in a pellet with water stability equivalent to that of the commercial reference diets. All treatments had a comparative loss of less than 1 % of initial weight after 15 minutes submersed in water. Abalone weaning diets containing 20 % gelatin had very good water stability over six hours (Knauer *et al*. 1993). The inclusion of the gelatin at a rate of 3.0 % did not significantly improve pellet water stability of either the hard or the soft diet treatments. It was therefore concluded the starch binding was adequate, and that the addition of the gelatin binding agent was not required.

The high moisture diets produced similar growth rates as the dry diets, thus dusky kob fed diets containing 10 and 40 % moisture had no significant difference in growth rates. However, the high moisture diet realized a significantly poorer FCR.

The high moisture diets required refrigeration and frequent preparation which is not as convenient for commercial operations as a low moisture diet, which is far more practical to manufacture and store. The presence of the binder did not improve pellet water stability in either the high moisture or dry diets and it was concluded that the starch binding was adequate to manufacture a practical pelleted feed for dusky kob.

Chapter 3 investigated various dietary protein to energy ratios. It tested the effect that two dietary protein diets (42 and 46 %), each with increasing dietary lipid (i.e. 6, 12 and 18 %) had on the amount of nitrogenous waste produced by the fish, as lowering ammonia excretion is critical in recirculating mariculture systems. The inclusion of a high protein level (i.e. 46 %) in this study as well as studies conducted by Daniel (2004) show that a 46 % protein level is close to the optimal level for the commercial culture of dusky kob. This is similar to the optimal protein levels required for other carnivorous, culture species such as red drum, *Sciaenops ocellatus*, which requires protein levels between 40 – 45 % (Daniels and Robinson 1986, Serrano *et al*., 1992). There is scope for further research in this area, particularly into the substitution of fishmeal with plant based proteins to test the cost benefits and varying protein source ratios which yield the best possible economic growth. However, these results suggest that formulations using less fishmeal may not be profitable as fishmeal yields high performance (i.e. growth and FCR) and it promotes low waste. Growth performance and FCR was higher for dusky kob fed diets with higher dietary protein (46 % protein) levels compared to those fed diets with lower dietary protein (42 % protein) levels. The protein efficiency ratio (PER) increased in fish fed the high protein (46 % protein), high energy diets (18 % lipid) compared to the low protein (42 % protein), high energy diet. Thus, an increase in dietary energy by lipid allows for improved protein utilization at a higher dietary protein level.

The ammonia excretion by the fish fed the experimental diets was not significantly reduced by varying the dietary protein to lipid levels. Ammonia production was only slightly reduced in diets with lower protein levels and increasing lipid levels.

The protein efficiency ratio (PER) increased in the fish fed the high protein diets. There was greater protein retention, lowering the amount of deaminated amino acids and reducing the elevation of ammonia production. Webb and Gatlin (2003) found increased protein retention reduced ammonia excretion by red drum, *S. ocellatus*.

Nutritionally, fish meal is the most important ingredient as it is the major source of dietary protein (Cho and Kaushik 1990). The main costs associated with intensive aquaculture are largely dietary so any attempts to lower the amount of protein, in essence the amount of fish meal, would be profitable (Gatlin 2002). In this study, the inclusion of higher levels of energy, as lipid, with high dietary protein level improved fish growth without negatively affecting pollution by ammonia excretion. However, increasing dietary energy level in the diets with lower dietary protein lowered the growth performance of the fish. Thus, any saving made through lower protein levels in the diet would be negated by the additional costs associated with extra feeding and longer growth periods of the fish.

In South Africa, there is increasing interest in open sea- cage culture as a method for the grow out of dusky kob where ongoing preliminary studies are investigating the effectiveness of pilot, small-scale commercial cages used as grow out facilities (Schoonbee and Bok 2006). A disadvantage of cage farming is the possible increased amount of dietary wastes from uneaten feed as pellets are able to sink through the cage mesh where they are no longer available to the fish (Thorpe *et al*. 1990). Chapter 4 investigated the effect of pellet shape on settling velocity and the feeding response of dusky kob fed two different shape pellets in cages, to determine if a pellet shape with a slower sinking rate would produce better FCR. The settling velocities between the two experimental pellets shapes, a round, cylindrical pellet and the flat square pellet, were significantly different. The square pellet spent significantly more time sinking within the water column.

This had an effect on the feeding period by the fish in the cages, the fish fed the cylindrical pellet had a shorter feeding period and the fish fed the square pellet spent a longer period of time feeding within the water column of the cage. The fish

fed the square pellet realized better growth compared to the fish fed the cylindrical pellets. The improved growth could be due to the longer feeding period; the square pellet sank at a slower rate and hence was more accessible to the fish in the cages. The fish fed the slower sinking pellet also realized a better FCR compared to the fish fed the pellet with a faster sinking rate. It is recommended the cage mariculture farmers make use of an artificial pellet that has the slowest possible settling velocity as these results suggest pellets with slower settling velocities produce better growth and FCR performance.

Chapter 5 investigated the overall growth of fish stocked at commercial density, overall fish health and the effect on water quality in recirculating systems of a prototype diet, formulated from the results of the previous studies, which was directly compared to the overall performance of fish fed the commercial imported feed. The fish fed the prototype diet (46 % protein; 18 % lipid) produced growth rates and FCR equal to those realized by the fish fed the commercial feed.

Heamatology is commonly used to evaluate overall health of fish, as heamatological characteristics are sensitive to changes in fish health (Blaxhall and Daisley 1973). The glucose levels were higher in the fish fed the prototype diet then the fish fed the commercial feed. The increase glucose levels may be due to the high levels of starch in the diet, as starch is used for binding and as the carbohydrate portion of the diet.

The stocking density of the fish was increased after the feeding trial to determine if crowding stress affected haematological characteristics of the fish fed the prototype and commercial diets. The stocking density was increased from 25 kg m^{-3} to 50 kg m^{-3} for 12 days after which blood samples were drawn and analysed. The haematology of the fish fed the prototype was not affected by an increase in stocking density. The fish fed the commercial diet had elevated levels of glucose and an increase in RBC, although values were still within ranges reported for Atlantic salmon, *Salmo salar* (Skjervold *et a*l. 2001).

In recirculating systems good water quality must be maintained for maximum fish growth (Davis 1990). Thus, the fish fed the two treatments were kept in isolated recirculating systems to determine the effect on water quality parameters. Water quality was not negatively affected by fish fed either of the two treatments. Water quality was good for all systems and the 5-day biological oxygen demand (BOD_5) test values were low and dissolved oxygen values were high (Delzer and McKenzie 2003). The total ammonia nitrogen levels were low, and well within acceptable limits for the survival and growth of culture marine finfish (Dosdat et al. 1996). Water quality is an essential component for both recirculating systems and open sea cages, where wastage negatively affects both forms of aquaculture production (Masser et al. 1999). The prototype diet is practical to use within recirculating systems as biological bacteria loads and nitrogen wastes were low and high levels of dissolved oxygen were maintained.

The experimental diets were all compared to an imported salmon feed which is currently used by the local fish farmers. The experimental diets performed on a par with the commercial diet. Both treatments produced similar growth and FCR results in all the growth trials. The water stability of the pellets and the effect on water quality were similar between treatments. The imported feed is very costly to import and the every changing currency exchange rates make it an uneconomical option for long term use. The estimated cost of the experimental diet, excluding manufacture and marketing costs, would be US $ 890 per ton, based on the cost of the raw ingredients: maize US $ 180 per ton, fishmeal US $ 1 800 per ton and fish oil US $ 1 200 per ton (FAO Globefish 2008). At the time of purchase, the commercial diet cost approximately US $ 1 800 per ton.

The prototype diet, formulated with 46 % dietary protein and 18 % dietary lipid levels, was manufactured using ingredients that are sourced locally and using technology that is well established in the local aquaculture industry. The prototype diet yielded high growth rates and good FCR results when used within recirculating systems. The diet was also successfully used in a cage culture environment and by producing a different shape of pellet the growth and FCR was

improved. Thus, the prototype diet offers a practical solution for the growing mariculture industry in South Africa.

Further research into nutritional requirements and feeding studies is recommended to determine improved diets for dusky kob. While the results of these diets are promising, research into least cost formulations will be imperative to sustain the economic viability of culturing this and other similar finfish species. Further studies into heat extruded pellets are also recommended for cage culture where floating pellets may be advantageous. In addition, investigations into the dietary requirements of different size classes of juvenile dusky kob will be useful in optimising diets at the various stages of grow out.

REFERENCES

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

Andrew J.E., Holm J. and Huntingford F.A. (2004) The effect of pellet texture on the feeding behaviour of gilthead sea bream (*Sparus aurata* L.) *Aquaculture* 232: 471 – 479.

Aquaculture SA (2001) *Mulloway aquaculture in South Australia*. Primary Industries and Resources South Australia. 6 pp.

Ballestrazzi R., Lanan D., D'Agaro E. 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.

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

Benjamin S., Britz P.J. and Jones C.L.W. (2007) The effect of pellet size on growth and feeding behaviour of juvenile dusky kob (*Argyrosomus japonicus*). *Proceedings of the 8th conference of the Aquaculture Association of Southern Africa.* p 27.

Bernatzeder A. and Britz P.J. (2007) Temperature preference of juvenile dusky kob *Argyrosomus japonicus* (Pisces: Sciaenidae). *African Journal of Marine Science* 29: 539 – 543.

Blaxhall P.C. and Daisley K.W. (1973) Routine haematological methods for use with fish blood. *Journal of Fish Biology* 5: 771 – 781.

Booth M.A., Allan G.J. and Anderson A.J. (2005) Investigation of the nutritional requirements of Australian snapper, *Pagrus auratus* (Bloch and Schneider 1801): Apparent digestibility of protein energy sources. *Aquaculture Research* 36: 378 – 390.

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.

Bowman W.C. and Rand M.J. (1968) *Textbook of Pharmacology*. Blackwell Scientific Publications, Oxford and Edinburgh. p. 46 and 386 – 387.

Britz P.J., Hecht T., Knauer J. and Dixon M.G. (1994) The development of an artificial feed for abalone farming. *Suid-Afrikaanse Tydskrif vir Wetenskap* 90: 7 – 8.

Britz P.J. (1995) The *nutritional requirements of Haliotis midae and development of a practical diet for abalone aquaculture.* PhD Dissertation, Rhodes University, Grahamstown, South Africa, 150 pp.

Cai Y., Wermerskirchen J. and Adelman I.R. (1996) Ammonia excretion indicates protein adequacy for fish. *The Progressive Fish-Culturist* 58: 124 – 127.

Chaiyapechara S., Casten M.T., Hardy R.W. and Dong F.M. (2003) Fish performance, fillet characteristics, and health assessment index of rainbow trout (*Oncorhynchus mykiss*) fed diets containing adequate and high concentrations of lipid and vitamin E*. Aquaculture* 219: 715 – 738.

Chatzifotis S., Villamor Martin-Prat A. Limberis N., Papandroulakis N. and Divanach P. (2006) First data on growth of cultured brown meagre *Sciaena umbra* using diets with different protein and fat contents. *Fisheries Science* 72: 83 – 88.

Chen Y.S., Beveridge M.C.M. and Telfer T.C. (1999) Physical characteristics of commercial pelleted Atlantic salmon feeds and consideration of implications for modeling of waste dispersion through sedimentation. *Aquaculture International* 7: 89-100.

Cho Y.C. and Bureau D.P. (1997) Reduction of waste output from salmonid aquaculture through feeds and feeding. *The Progressive Fish-Culturist* 59: 155 – 160.

Cho Y.C. and Kaushik S.J. (1990) Nutritional energetic in fish: protein and energy utilization in rainbow trout. *World Review of Animal Nutrition* 61: 132 – 172.

Collett P.D. (2007) *Toward the development of a rearing protocol for juvenile dusky kob Argyrosomus japonicus (Pisces: Sciaenidae)*. MSc Thesis. Rhodes University, Grahamstown. 60 pp.

Collett P.D. Vine N.G. and Kaiser H. (2008) Determination of the optimal water temperature for the culture of juvenile dusky kob *Argyrosomus japonicus* Temminck and Schlegel 1843. *Aquaculture Research* 39: 979 – 985.

Daniel S.J. (2004) *Investigation into the nutritional requirements of juvenile dusky kob Argyrosomus japonicus (Pisces: Sciaenidae), under ambient culture conditions.* MSc Thesis, Rhodes University, Grahamstown. 117 pp.

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

Davis J.T. (1990) Red drum: Production of food fish. *Southern Regional Aquaculture Centre, United States department of Agriculture. June 1990: Publication No. 322.* 4 pp.

Delzer G.C. and McKenzie S.W. (2003) *Five-day biological oxygen demand: U.S. Geological survey techniques of water-resources investigations Book 9, chapter A7 (3rd Edition)*, section 7.0, accessed 21/08/2008, from http://pubs.water.usgs.gov/twri9A/.

De Silva S.S., Rasanthi M. and Shim G.K.F. (1991) Interactions of varying dietary protein and lipid levels in young red tilapia: evidence of protein sparing. *Aquaculture* 95: 305 – 318.

Dosdat A., Metailler S.R., Huelvan C. and Desbryeres E. (1996) Comparison of nitrogenous losses in five teleost fish species. *Aquaculture* 141: 107 – 127.

Elberizon I.R. and Kelly L.A. (1998) Empirical measurements of parameters critical to modeling benthic impacts of freshwater salmonid cage aquaculture. *Aquaculture Research* 29: 669-677.

El-Shebly A.A., El-Kady M.A.H., Hussin A.B. and Hossain Y. (2007) Preliminary observations on the pond culture of meagre, *Argyrosomus regius* (Asso, 1801) (Sciaenidae) in Egypt. *Journal of Fisheries and Aquatic Science* 2: 345 – 352.

Erikson U., Sigholt T. and Seland A. (1997) Handling stress and water quality during live transportation and slaughter of Atlantic salmon (*Salmo salar*). *Aquaculture* 149: 243 – 252.

FAO (2004) *Cultured Aquatic Species Information Programme: Argyrosomus regius (Asso, 1801)* 17 pp.

FAO (2008) *FAO Fisheries Department, Fishery Information, Data and Statistics Unit. Fishstat Plus: Universal software for fishery statistical time series. Aquaculture production: quantities 1950-2006, Aquaculture production: values 1984-2006; Vers. 2.30* p. 42.

FAO Globefish (2008) *FAO Globefish Commodity Report June 2008: Small Pelagics.* Rome, Italy. p. 67.

Farmanfarmaian A., Lauterio T. and Ibe M. (1982) Improvement of the stability of commercial feed pellets for the giant shrimp (*Macrobrachium rosenbergii*) *Aquaculture* 27: 29 – 41.

Fielder D.S., Bardsley W. and Allan G. (1999) Enhancement of mulloway (*Argyrosomus japonicus*) in intermittently opening lagoons. *NSW Fisheries report Series No. 14.* 27 pp.

Fielder D., and Bardsley W. (1999) A preliminary study of the effects of salinity on growth and survival of Mulloway *Argyrosomus japonicus* larvae and juveniles. *Journal of the World Aquaculture Society* 30: 380 – 387.

Garb H.R. and Gab-Alla A.F.A. (2007) Comparison of biochemical composition and organoleptic properties between wild and cultured finfish. *Journal of Fisheries and Aquatic Sciences* 2: 77 – 81.

Gatlin III D.M. (2002) Red drum, *Sciaenops ocellatus*. In: Webster C.D. and Lim C.E., *Nutrient requirements and feeding of finfish for aquaculture*. CABI Publishing, Wallingford, UK. pp. 147 – 158.

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

Griffiths M.H. (1997) Feeding behaviour 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. and Hecht T. (1995) Age and growth of dusky kob *Argyrosomus japonicus* (Sciaenidae) based on otoliths. *South African Journal of Marine Science* 16: 119 – 128.

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

Halver J.E., and Hardy R.W. (2002) *Fish Nutrition*, 3rd Ed. Academic Press. California, USA. p. 15 and 110.

Hargis P.S. and Van Elswyk M.E. (1993) Manipulating the fatty acid composition of poultry meat and eggs for the health conscious consumer. *World's Poultry Science Journal* 49: 251 – 264.

Hecht T. (2000) Considerations on African aquaculture. *World Aquaculture* 31: 12 – 15.

Hilton J.W., Cho C.Y. and Slinger S.J. (1981) Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption, and the physiological response of rainbow trout (*Salmo gairdneri* R.) *Aquaculture* 25: 185 – 194.

Horn M. H., Maihiot K.F., Fris M.B. and McClanahan L.L. (1995) Growth, consumption, assimilation and excretion in the marine herbivorous fish *Cebidichthys violaceus* (Girard) fed natural and high protein diets. *Journal of Experimental Marine Biology and Ecology* 190: 97 – 108.

Hunter B.J. and Roberts D.C. (2000) Potential impact of the fat composition of farmed fish on human health. *Nutrition Research* 20: 1047-1058.

Jobling M. and Miglavs L. (1993) The size of lipid depots – a factor contributing to the control of food intake in Arctic charr, *Salvelinus alpinus*? *Journal of Fish Biology* 43: 487 – 489.

Juell J.E. (1991) Hydroacoustic detection of food waste – a method to estimate maximum food intake of fish populations in sea cages. *Aquaculture Engineering* 10: 207 – 217.

Knauer J., Britz P.J. and Hecht T. (1993) The effect of seven binding agents on 24-hout water stability of an artificial weaning diet for the South African abalone, *Haliotis midae* (Haliotidae, Gastropoda). *Aquaculture* 115: 327 – 334.

Landman R. (2006) *Development plan*. Irvin & Johnson Holding Company (Pty) Ltd. Cape Town, South Africa. 28 pp.

Lazo J.P., Davis 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., 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 rockfish (*Sebastes schlegeli*). *Aquaculture* 211: 227 – 239.

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

Masser M.P., Rakocky J. and Losordo T.M. (1999) Recirculating aquaculture tank production systems: management of recirculating systems. *Southern Regional Aquaculture Centre, United States department of Agriculture. June 1999: Publication No. 452.* 11 pp.

McGoogan B. and Gatlin D. (1999) Dietary manipulations affecting growth and nitrogenous waste production of red drum, *Sciaenops ocellatus* I. Effects of dietary protein and energy levels. *Aquaculture* 178: 333 – 348.

McGoogan B. and Gatlin D. (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.

Mortensen A., Toften H. and Aas K. (2007) Cage culture of spotted wolffish (*Anarhichas minor* Olafsen). *Aquaculture* 264: 475 – 478.

Naylor R.L., Goldberg R.J., Primavers J.H., Kautsky N., Beveridge M.C.M., Clay J., Folkes C., Lubchenco J., Mooney H. and Troell M. (2000) Effect of aquaculture on worldwide fish supplies. *Nature* 405: 1017-1024.

National Research Council (NRC) (1993) *Nutrient requirements of fish*. National Academy Press, Washington, DC. 114 pp.

Nelson J.S. (1994) *Fishes of the world. 3rd Edition.* John Wiley and Sons, New York. 416 pp.

Official Methods of Analysis of AOAC International (2003) 17th Edition, Revision 2. AOAC International, Gaithersburg, USA. Official methods 920.39, 934.01, 942.05 and 990.03.

O'Sullivan D. and Ryan M. (2001) Mulloway trials suggest opportunities for brackish water ponds. *Austasia Aquaculture* 15: 22 – 24.

Piccolo G., Bovera F., De Riu N., Marono S., Salati F., Cappuccinelli R., and Moniello G. (2008) Effect of two different protein / fat ratios of the diet on meagre (*Argyrosomus regius*) traits. *Italian Journal of Animal Science* 7: 363 – 371.

Pirozzi I., Booth M.A., and Pankhurst P.M. (2008) Defining the dietary protein and energy needs of mulloway: Investigations using factorial bioenergetic approaches to nutrient requirements. *Proceedings of the 13th International Symposium on Feed Nutrition and Feeding.*

Poli B.M., Parisi G., Mecatti M., Lupi P., Iurazan F., Zampacavallo G. and Gimozzi M. (2001) The meagre (*Argyrosomus regius*), a new species for Mediterranean aquaculture. 1. Morphological, merchantable and nutritional traits in a commercial wide size-range. *European Aquaculture Society Special Publication* 29: 209-210.

Poston H.A. (1974) Effect of feeding brown trout (*Salmo trutta*) a diet pelleted in dry and moist forms. *Journal of Fish Research Board Canada* 31: 1824 – 1826.

Rodrigues R., Schwarz M.H., Delbos B.C. and Sampaio L.A. (2007) Acute toxicity and sublethal effects of ammonia and nitrite for juvenile cobia *Rachycentron canadum*. *Aquaculture* 271: 553 – 557.

Rowland S.J., Mifsud C., Nixon M. and Boyd P. (2006) Effects of stocking density on the performance of Australian freshwater silver perch (*Bidyanus bidyanus*) in cages. *Aquaculture* 253: 301 – 308.

Sandnes K., Lie Ø. and Waagbrø R. (1988) Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon, *Salmo salar*. *Journal of Fish Biology* 32: 129 – 136.

Schoonbee W. and Bok A. (2006) *Experimental Sea-cage operation plan*. Irvin & Johnson Holding company (Pty) Ltd. Cape Town, South Africa. 29 pp.

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

Shyong W.J., Haung C.H. and Chen H. (1998) Effects of dietary protein concentration on growth and muscle composition of juvenile *Zacco barbata*. *Aquaculture* 167:35 – 42.

Skjervold P.O., Fjæra S.O. and Østby P.B. (1999) Rigor in Atlantic salmon as affected by crowding stress prior to chilling before slaughter. *Aquaculture* 175: 93 – 101.

Statsoft (2004) Statistical software package, STATISTICA™ 7.0.

Storebakken T. (1985) Binders in fish feeds. I. Effect of alginate and guar gum on growth, digestibility, feed intake and passage through the gastrointestinal tract of rainbow trout. *Aquaculture* 47: 11 – 26.

Sureshkumar S. and Madhusoodana Kurup B. (1999) variations in hepatosomatic index and biochemical profiles among the male morphotypes of *Macrobrachium rosenbergii*. *Aquaculture* 176: 285 – 293.

Thodesen J. and Storebakken T. (1998) Digestibility of diets with precooked rye or wheat by Atlantic salmon, *Salmo salar*, L. *Aquaculture Nutrition* 4: 123 – 126.

Thorpe J.E., Talbot C., Miles M.S., Rawlings C. and Keay D.S. (1990) Food consumption in 24 hours by Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture* 90: 41 – 47.

Tuan L.A. and Williams K.C. (2007) Optimum dietary protein and lipid specifications for juvenile Malabar grouper (*Epinephelus malabaricus*). *Aquaculture* 267: 129 – 138.

Tyrer L. (2006) *Mariculture walking fine line between providing protein and threatening biological diversity*. *Engineering News*. 3 November 2006.

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

Wedemeyer G.A. (2000) *Physiology of fish in intensive culture systems*. Chapman and Hall. 135 pp.

Whitfield A.K. (1998) Biology and ecology of fishes in South African estuaries. *Ichthyological Monographs of the J.L.B. Smith Institute of Ichthyology*. 223 pp.

Woodcock S.H. and Bekendorff K. (2008) The impact of diet on the growth and proximate composition of juvenile whelks, *Dicathais orbita* (Gastropoda: Mollusca). *Aquaculture* 276: 162 – 170.

Yang S.D., Liou C. and Liu F. (2002) Effects of dietary protein level growth performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*). *Aquaculture* 213: 363 – 372.