
Toward the development of a rearing protocol for juvenile dusky
kob, *Argyrosomus japonicus* (Pisces: Sciaenidae)

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ABSTRACT

The South African mariculture industry is developing the rearing technology of indigenous fish species. Dusky kob (*Argyrosomus japonicus*) has high-quality flesh and a good market demand. Research is needed to determine the environmental requirements under which growth of dusky kob is optimised. This study assessed the effect of temperature, light intensity, feeding frequency and stocking density, respectively, on growth, feed conversion ratio (FCR) and survival of juvenile dusky kob within a weight range of 10-60 g fish⁻¹) in a series of four growth trials.

The effect of temperature on growth and FCR was assessed at 14 temperatures from 17 to 28°C. The temperature for best growth was estimated to be 25.3 °C, while 21.4 °C was the temperature at which the best FCR was achieved. A growth trial testing the effect of light intensity on growth showed that light intensity did not affect growth in the range of 23 – 315 lx. Fish fed a restricted ration of 3.6% body weight per day (chapter 2) or 3.41 % body weight per day (chapter 4) had a better FCR than fish fed to satiation. A trial to assess the effect of feeding frequency on growth and FCR showed that fish fed both two or three times daily grew better than those fed once daily. FCR was best in fish that were fed once or twice daily. Preliminary analysis of the results from a stocking density trial showed that stocking density in the range of 10 – 50 kg m⁻³ did not affect growth of juvenile dusky kob.

It is recommended to culture dusky kob at a temperature of 25.3 - 21.4 °C at stocking densities up to 50 kg m⁻³ to maximise growth. However, a study is needed to determine the environmental conditions needed to maximise profit under commercial conditions. The results allow the South African industry the opportunity to assess the culture potential of this species. In addition, the results will help develop protocols that can be used in other South African candidate aquaculture species such as silver kob, yellowtail, white-stumpnose and sole.

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PREFACE

This thesis is submitted as a collection of four papers (each as a separate chapter), preceded by a General Introduction and followed by a General Discussion. A degree of repetition in the introductions, methods and discussions for each of the papers was unavoidable. In the case of dual authorship, the senior author was responsible for all data collection, data analysis, hypotheses and conclusions.

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CHAPTER 1

GENERAL INTRODUCTION

In order to evaluate the potential of a candidate mariculture species, it is necessary to determine the economic viability of culturing the species (Deacon 1996). Growth, feed conversion ratio (FCR) and survival are fundamental biological factors that affect the production of a species and hence the economics of its culture. Weight and length gain of a species are governed by a complex series of interactions between the species and its environment. In order to assess the growth potential of a species, any growth-reducing effects of the culture environment must be minimised so that growth is maximised (Deacon 1997). Therefore, any evaluation of the culture potential of a candidate mariculture species should begin with the determination of the best possible environmental conditions for growth (Fry 1971, Deacon 1997).

One way of determining the environmental requirements of a species is by observation of the conditions in their natural environment (Deacon 1996, Stickney & Liu 1999, Laing 2000). It is assumed that fish will be found in their zone of environmental preference (Person-Le Ruyet 1990). However, this method of determination is not suitable for fish associated with estuaries. Estuaries are naturally fluctuating systems, varying in, for example, temperature, salinity, turbidity and dissolved oxygen (Whitfield 1998). This dynamic environment provides little clue as to the best possible environmental conditions for growth of fish under culture conditions. The only insight that can be gained is an estimation of the limits of environmental tolerances.

Environmental variables affect the growth of fish by influencing both the metabolism and the level of activity of fish (Jobling 1981). The metabolism of fish is the sum-total of all biochemical reactions. The activities of fish are the processes by which energy is used (Fry 1971). Environmental factors influence both metabolic and catabolic processes. It is hypothesised that best environmental conditions should lead to efficiency of use of nutrients and metabolism leading to fast growth. The effect of the environment is divided into five functional categories (Fry 1971) according to their respective influence of fish metabolism. These have been defined by Fry (1971) and Deacon (1997). Controlling factors govern the rate of metabolic processes.

Temperature is an example of a controlling factor as it influences the rate of metabolism and hence controls growth. Limiting factors restrict the supply or removal of metabolites. Feeding, dissolved oxygen concentration, water quality and crowding density are examples of limiting factors of growth. Masking factors interact with other variables, hence masking their effect and potentially acting as confounding factors. Many factors can act as masking factors under different circumstances. For example, salinity can act as a masking factor when interacting with temperature. Directive factors cue the animal to a certain physiological response due to an environmental stimulus. Light intensity and photoperiod are examples of directive factors as illustrated by their effect on fish behaviour and reproductive physiology. Lethal factors lead to breakdown of metabolic functioning and subsequent death. Examples are high temperatures or salinities outside of the range the species tolerates.

Determination of all the environmental requirements of a species simultaneously would be theoretically convenient, but it is impractical because of the multifactorial nature of such an experiment. Simultaneous testing of all potentially important environmental variables would require extensive experimental facilities to facilitate a very large number of tanks. Based on the model by Fry (1971), a protocol for individually determining the best possible environmental conditions for the culture of juvenile marine species can be suggested. Since dusky kob (*Argyrosomus japonicus*) has been successfully spawned and reared in captivity, the best environmental conditions for growth should be determined. A protocol has been suggested by Deacon (1997) to evaluate the mariculture potential of spotted grunter (*Pomadasys commersonnii*) which, like *A. japonicus*, is an estuarine species occurring along the east coast of South Africa. This study aims to make a contribution to optimising the environmental conditions for growth of juvenile dusky kob by first testing the effect on growth, feed conversion rate and survival of temperature, followed by the testing of light intensity, feeding methods and population density, the latter being studied under farming conditions. The dusky kob, *A. japonicus* will be the candidate species as it is being farmed and marketed.

The South African mariculture industry has grown over the last decade although production is limited to a few species, in particular the abalone, *Haliotis midae* (Britz 2005). Recent cage-culture of Atlantic salmon, *Salmo salar*, off Danger Point in the Western Cape is an example of commercial finfish mariculture. Some indigenous fish species, including *A. japonicus*, have been

identified as candidate mariculture species (Hecht 2000). The potential for mariculture of this species has only been subjected to preliminary examination as part of a nutritional study (Daniel 2004) and further investigation will benefit commercialisation in South Africa.

A. japonicus is a member of the family Sciaenidae, one of the largest perciform families, consisting of 70 genera and 270 extant species (Nelson 1994). Sciaenid species are becoming increasingly important to aquaculture worldwide (Fitzgibbon *et al.* 2007), and are the dominant marine fish family cultured in China, the world's leader in aquaculture products (Hong & Zhang 2003). They are found in the northern hemisphere along the coast of China, South Korea and Japan (Griffiths 1997a), and in the southern hemisphere along the eastern seaboard of Australia (Starling 1993), South Africa and Mozambique (Griffiths & Heemstra 1995). Throughout their distribution, they are an important food species and a key commercial and recreational linefish species in South Africa and Australia (Griffiths 1997a).

On the east coast of South Africa, adult *A. japonicus* are most abundant in the nearshore marine environment, but frequently visit estuaries and surf zones (Griffiths & Hecht 1995). A large proportion of the adult population are believed to migrate up the east coast to the subtropical waters of KwaZulu Natal to spawn and then to return to the Cape region post-spawning (Griffiths & Hecht 1995). Early juveniles of about 30 mm total length move into estuaries where they remain until they are about 150 mm total length (Griffiths 1996) at which stage they are found both at the mouth of estuaries and in the surrounding surf zones (Griffiths 1996). Wild *A. japonicus* are a relatively fast growing during their juvenile phase and they reach sexual maturity at a length of almost 100 cm (Griffiths 1996).

Due to their value as a commercial and recreational fishing species, *A. japonicus* has been severely overfished. In 1997, the spawner biomass per recruit was believed to be as low as 2.3% of pristine levels (Griffiths 1997c). The poor state of wild of *A. japonicus* stocks has caused an interest in the aquaculture potential of this species (Lee & Ostrowski 2001). *A. japonicus* has attributes that make it a suitable aquaculture candidate, including their high market value, fast initial growth rate (Griffiths 1996), salinity tolerance (Whitfield 1998), and tolerance of poor water quality and low oxygen levels (Fitzgibbon *et al.* 2007). As a result, this species is under

evaluation to determine their potential for finfish mariculture in Australia (Battaglione *et al.* 1994; O'Sullivan & Ryan 2001; Pirozzi *et al.* 2008) and South Africa (Schoonbee & Bok 2006).

The aim of the current study was to investigate the effect of selected culture variables on growth of juvenile *A. japonicus*, through a series of four growth trials (Chapters 2 – 5). Salinity was kept constant at 35 g L⁻¹ for all growth trials as this was determined by Bernatzeder *et al.* 2008 to allow maximum growth. Dissolved oxygen concentration, flow rate and nitrogenous waste levels were monitored in all trials.

The first experiment (Chapter 2) reports on the effect of temperature on growth in the absence of limiting factors such as food or dissolved oxygen. Food was provided in excess of the metabolic requirements and stocking density was kept low to ensure that it did not have a limiting effect on growth. The second experiment (Chapter 3) investigates the effect of light intensity on growth and was conducted within the temperature range found for best growth in the first experiment (Chapter 2). Light intensity was chosen as this species exhibits aggressive behaviour that can potentially be controlled by low light intensity. In addition, two feeding methods were used in this study to determine whether behavioural changes as a result of light intensity affected feeding intensity. The third experiment (Chapter 4) incorporated the results of the first two experiments (Chapter 2 and 3) by testing the effect of feeding regimen on growth under the best temperature and light intensity conditions for growth. The fourth experiment (Chapter 5) investigates the effect of stocking density on growth and was conducted at the best temperature, light intensity and feeding regimen for growth as estimated under laboratory conditions. In chapter 6, the results of all experiments will be combined to summarise the effects of the environmental variables tested in this study on growth, survival, feed conversion ratio and feeding intensity.

CHAPTER 2

DETERMINATION OF THE OPTIMAL WATER TEMPERATURE FOR THE CULTURE OF JUVENILE DUSKY KOB *ARGYROSOMUS JAPONICUS*

Introduction

Worldwide the growth of the aquaculture industry depends on the development and testing of protocols for the breeding and rearing of new aquaculture species. The dusky kob *A. japonicus*, family Sciaenidae, is a fast-growing marine finfish species that has a high consumer demand (Griffiths 1997; Fielder, Bardsley & Allan 1999; O'Sullivan & Ryan 2001). As this is a relatively new candidate species, its environmental requirements under mariculture conditions, in particular the temperature for best growth, need to be determined to optimise growth and food conversion ratio (FCR).

Fish growth increases with water temperature up to a species-specific maximum after which it rapidly decreases (Jobling 1996). Studies on the marine teleosts *Pollachius pollachius* L. 1758 (Person-Le Ruyet *et al.* 2006), *Pagrus auratus* Forster 1801 (Fielder 2005), *Sciaenops ocellatus* L. 1766 (Douillet & Pickering 1999), and *P. commersonnii* Lacepède 1801 (Deacon 1997) showed that growth is maximised at an optimum temperature within a species-specific thermal tolerance range. Australian-farmed *A. japonicus* grew at approximately 1 mm d⁻¹ at 15 – 30 °C for adults and 18 – 25 °C for juvenile fish (O'Sullivan & Ryan 2001) which was considered to be a wide temperature range (O'Sullivan & Savage 2004).

The temperature range in which a species naturally occurs is thought to represent the zone of thermal tolerance (Katersky & Carter 2005). *A. japonicus* on the South African east coast occur within a temperature range of 12 - 28 °C depending on locality and season (Whitfield 1998). It is hypothesised that this range represents the zone of thermal tolerance of South African dusky kob. The optimal temperature for best growth and lowest FCR still needs to be established.

One method to estimate the optimal temperature for a species is through temperature preference studies (Jobling 1981). The preferred temperature range of juvenile dusky kob (20 ± 3.45 g) kept

in a temperature gradient tank was 25 to 26.4 °C (Bernatzeder & Britz 2007). However, preference studies are based on fish behaviour and the preferred temperature under these experimental conditions may not correspond to the best temperature for growth (Kellogg & Gift 1983).

The objective of this study was to quantify growth, FCR and feeding intensity of juvenile dusky kob reared at temperatures ranging from 17.5 - 28.5 °C.

Materials and methods

Fourteen days before the start of the experiment juvenile dusky kob from a single spawning of broodstock kept at Espadon Marine Hatchery in Hermanus (Western Cape, South Africa) were transported to the departmental marine hatchery and acclimated to the experimental closed recirculating system. The experimental system consisted of fourteen 100-L (0.44 x 0.50 x 0.45 m) green plastic tanks. Water flow rate was set to 100 L h⁻¹ tank⁻¹. Water leaving the tanks passed through a 100-L sand filter, a 200-L biological trickle filter and a counter-current foam-fractionator. Two 100-L tanks were connected to the system filled with oyster shell to buffer water pH.

Environmental factors within the system were controlled to ensure that temperature was the only variable to account for differences between treatments. Total ammonia and nitrite were determined photometrically twice weekly using a Hach spectrophotometer (method analogous to EPA 354.1, US Standard Methods 4500-N02- B, and EN 26777). These levels were kept low to minimise differences in water quality between treatments. Un-ionised ammonia did not exceed 0.01 mg L⁻¹ and nitrite did not exceed 0.7 mg L⁻¹ in the highest temperature treatment during the study.

Water was chilled by a 550-W Aquamedic Titan-2000 cooler and an evaporative cooler (wet wall). Water was heated to the experimental temperature of each tank using thermostatically controlled 300-W aquarium heaters in each tank. Room temperature was maintained at 20 °C with two wall-mounted air conditioners.

Light was provided by ten 1.5-m Biolux® fluorescent tubes situated 1.3 m above the fish tanks. Photoperiod was maintained at a 12L:12D cycle with a timer-switch. Water was vigorously aerated using airstones connected to an oil-free rotary blower. Dissolved oxygen was recorded daily using an Oxyguard Gamma portable oxygen meter and kept above 5 mg L⁻¹ in all tanks. Salinity was maintained at 31 - 35 g L⁻¹ through the addition of freshwater when necessary.

Experimental design

The effect of temperature on growth, FCR and feeding intensity was assessed within the range of 17.5 to 28.5 °C in a 42-day growth trial. Each tank was stocked with 23 fish (average weight: 23.7 ± 2.6 g fish⁻¹). Temperature was recorded daily in each tank with a mercury thermometer to an accuracy of 0.1 °C. The experiment was designed to test seven temperatures at two replicates per treatment. All tanks were randomly assigned to the experimental system.

Every 14 days all fish in each tank were anaesthetised using 0.2 mL L⁻¹ 2-phenoxyethanol after they had not been fed for 24 hours. The standard length of each fish was measured using a measuring board with an accuracy of 1 mm, and fish were weighed individually (to 0.1 g) by placing them in a shallow dish containing seawater on a digital balance.

Fish were fed to apparent satiation twice daily at 08:00 and 17:00 with a 1.8-mm pelleted feed (450 g kg⁻¹ crude protein, 140g kg⁻¹ crude lipids, 40g kg⁻¹ crude fibre, up to 100 g kg⁻¹ ash and 100 g kg⁻¹ moisture, manufactured by Indian Ocean Aquafeeds, Johannesburg, South Africa). The diet and feeding frequency were in accordance with recommendations by Daniel (2004) and current industry practice for *A. japonicus* of this size range. Satiation was assumed when pellets reached the bottom of the tank before being eaten, thus reducing food wastage. Any food wasted would have been standard across all treatments. Food containers were assigned to each tank and weighed weekly to determine the amount of food fed. Tanks were cleaned twice daily by siphoning faeces from the tank bottom and scrubbing the tank walls.

Data analysis

Weight gain was expressed as percentage daily weight gain for each of three 14-day growth periods using the following formula: $G = [(\log(W_e) - \log(W_0)) / t] \times 100$; where G = percentage

daily weight gain, W_0 and W_e = average weight of all fish in a tank at the beginning and end of a growth period, respectively, and t = time in days of the growth period. Length gain was given as percentage length gain over each 14-day period. Food conversion was obtained by dividing the average amount of dry food fed per fish by the average wet weight gain of the fish in each tank during the respective growth period. Feeding intensity was calculated in % body weight d^{-1} by multiplying the FCR by the daily percentage growth rate (G).

Although the experimental design included two replicates for each of the seven experimental temperatures, average temperature per growth period differed between replicates and was thus considered a continuous variable that was taken as the independent variable in the regression model. The dependent variables were daily percentage weight and length gain, FCR, and feeding intensity. For each two-week growth period these dependent variables were related to the average temperature of that period using different regression analysis techniques. For this, a comparison of statistics, derived from both linear regression and a second-order polynomial regression model, was used to decide which model provided the best fit for the data. The decision for the choice of the model was based on a comparison of the p-values to test the significance of the regression coefficients, the F-statistics of the model, and the distribution of residuals. The type I – error used to reject the alternative hypothesis was set to 5% ($P \leq 0.05$). The first derivative of the polynomial model was used to estimate the point of inflection which gave the temperature that produced the highest growth rate and the lowest FCR, respectively. Finally, average weight and average length of fish per tank were plotted against time.

Results

The temperature that resulted in the maximum daily percent growth rate was estimated as 23.9, 25.6, and 26.3 °C for the three growth periods, respectively (Figure 1.1). In the first growth period percent length gain (%) increased linearly with temperature within the range of 17.9 - 28.4 °C. For growth periods 2 and 3 the optimal temperature for length gain ranged between 25.5 °C and 24.9 °C (Figure 1.1).

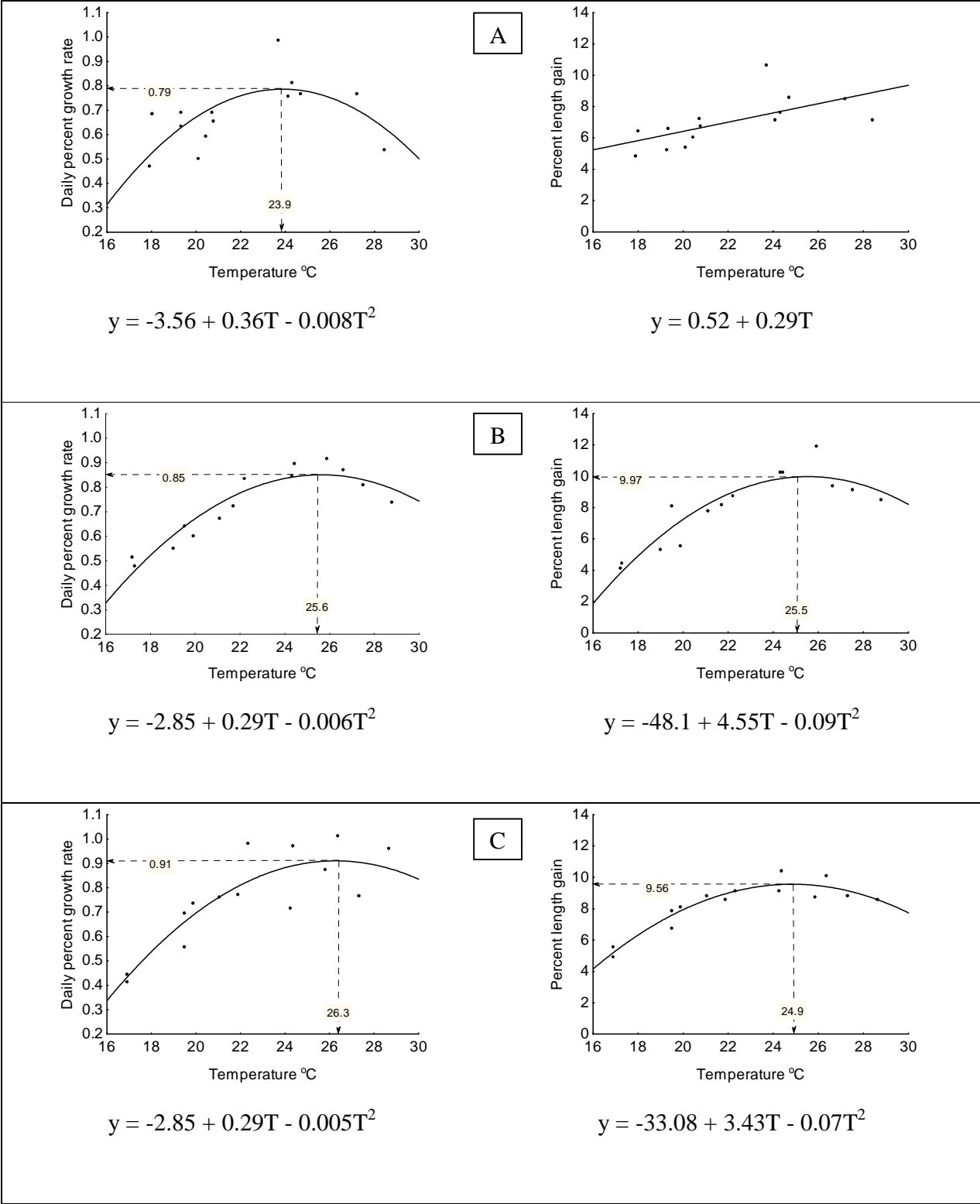


Figure 1.1 (page 9): Daily growth rate in percent of body weight per day (left column) and percentage length gain (right column) of juvenile dusky kob in relation to average water temperature for three 14-day growth periods (A=first, B=second, and C=third row of graphs, respectively.) Regression models for daily percentage growth rate and percentage length gain (y) and temperature (T) are given for each growth period.

FCR was best at 21.4 °C in period 2 and 21.9 °C in period 3. In the first growth period no significant relationship could be established between temperature and FCR ($F_{1, 12} = 0.24$; $p = 0.63$; $r^2 = 0.02$) (Figure 1.2). For all three growth periods feeding intensity was linearly related to temperature within the temperature range of 17.9 – 28.5 °C (Figure 1.2).

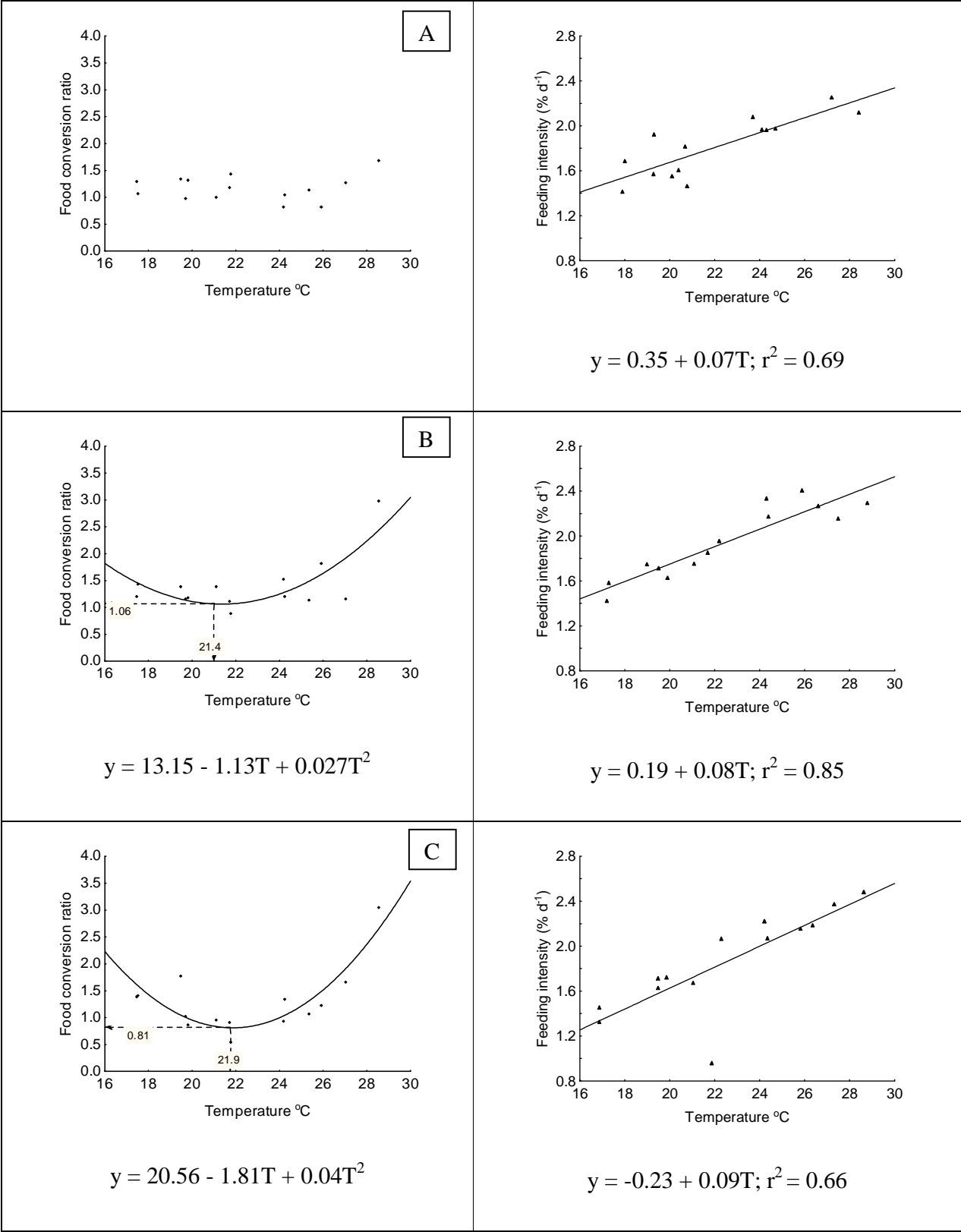


Figure 1.2 (page 11): FCR and feeding intensity in percent of body weight per day of juvenile dusky kob as a function of temperature for three 14-day growth periods (A=first, B=second, and C=third row of graphs, respectively.) Regression models for the relationship between the variables are given for each growth period. No regression model with significant coefficients could be given for the relationship between FCR and temperature for the first growth period.

Percentage daily weight gain (y) for the entire 42-day growth period was related to temperature (x) as follows: $y = -3.17 + 0.319x - 0.0063x^2$. Thus, the optimum temperature for growth was 25.3 °C. The average percentage daily weight gain for the treatment with the lowest temperature (17.5 °C) was 1.2% while the two tanks of the treatment closest to the optimum temperature (25.4 - 25.9 °C) grew at 2.05%, a 71% increase over the tank with the lowest temperature (Table 1.1, Figure 1.3).

FCR (y) for the entire 42-day growth period was related to temperature (x) as follows: $y = 13.86 - 1.18x + 0.027x^2$. Thus, the temperature that achieved the best FCR was 21.7 °C. The average FCR of the two tanks kept at 21.7 and 21.8 °C, respectively, was 0.72 kg feed per kg gain, a 94% improvement over the treatment kept at 17.5 °C with an average FCR of 1.40 kg · kg gain⁻¹ (Table 1.1).

There was no effect of temperature on mortality and average survival over all tanks was 95.6%.

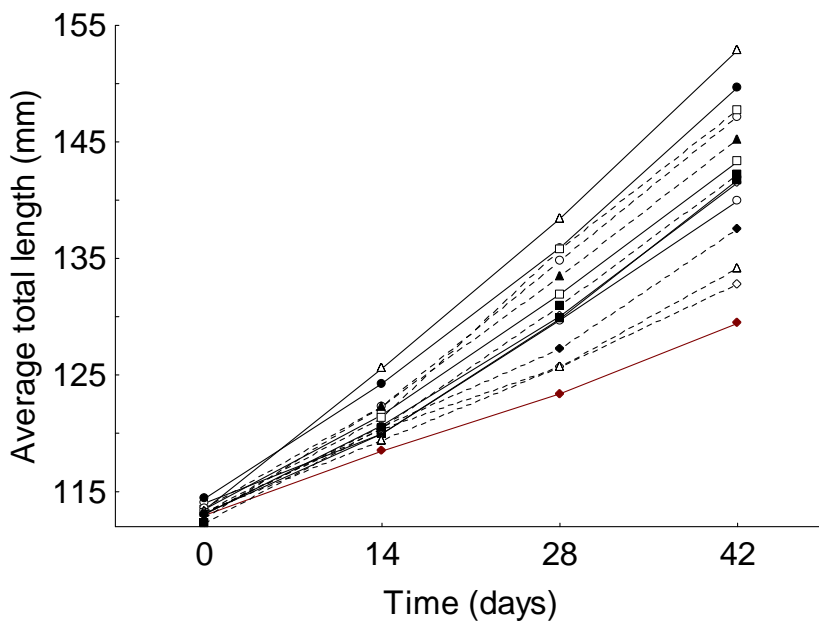
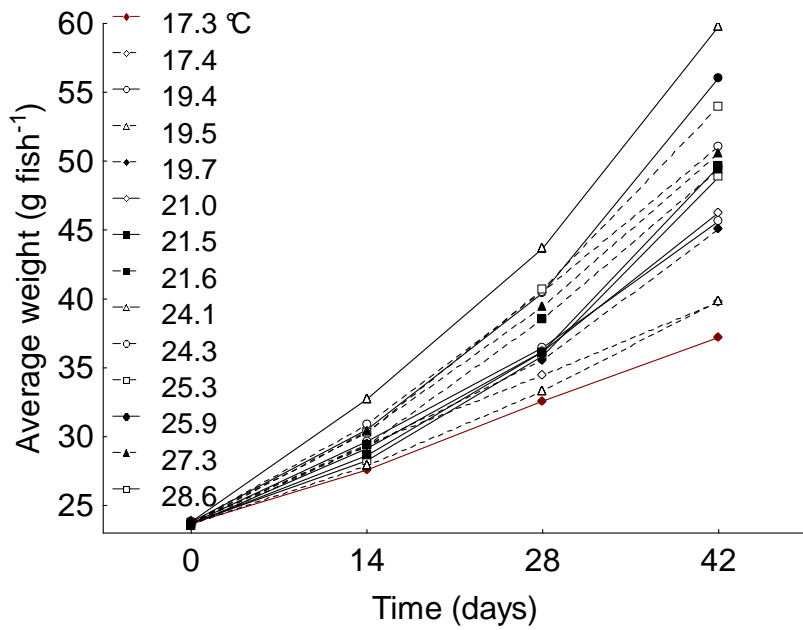


Figure 1.3: Average weight (g fish⁻¹) and average total length (mm) of dusky kob kept at different water temperatures during a 42-day experiment. Average temperatures for the growth period and markers in the legend refer to both graphs.

Table 1.1: Growth, FCR and feeding intensity of juvenile dusky kob during a 42-day growth experiment. T = average water temperature (°C); W₀, W_e and L₀, L_e = average weight (g fish⁻¹), length (mm) at the beginning and end of the experiment; G = percentage daily growth rate (% body weight d⁻¹); FCR = food conversion ratio; dL = daily length increase (mm d⁻¹); FI = feeding intensity (% body weight d⁻¹); S = survival (%). Errors are standard deviation of the mean.

T	W ₀	W _e	L ₀	L _e	G	FCR	dL	FI	S
17.5	23.7±2.9	37.2±7.1	112±4.6	129±7.8	1.1	1.39	0.4	1.5	100
17.5	23.6±2.4	39.7±6.7	113±4.4	132±7.5	1.3	1.41	0.5	1.8	100
19.5	23.7±2.5	39.8±6.3	113±4.2	134±5.8	1.3	1.78	0.5	2.3	95.6
19.7	23.7±2.4	45.6±7.1	114±4.9	140±7.0	1.6	1.02	0.6	1.6	100
19.8	23.8±2.6	45.0±7.3	113±4.6	137±7.9	1.6	0.86	0.6	1.3	100
21.1	23.6±3.0	46.3±8.3	113±4.2	141±8.3	1.7	0.96	0.7	1.6	95.6
21.7	23.7±2.9	49.6±7.4	113±5.1	141±7.7	1.8	0.91	0.7	1.7	100
21.8	23.5±2.9	49.4±7.8	112±4.8	142±7.6	1.8	0.53	0.7	1.0	100
24.2	23.8±1.9	59.8±6.6	113±3.6	152±6.1	2.3	0.92	0.9	2.1	95.6
24.3	23.7±2.7	51.0±14.4	114±6.5	147±10.7	1.9	1.34	0.8	2.5	100
25.4	23.7±2.5	53.9±9.4	113±4.7	147±9.5	2.0	1.08	0.8	2.2	100
25.9	23.8±2.7	56.0±9.0	114±5.8	149±9.2	2.1	1.23	0.8	2.6	91.3
27.1	23.7±2.8	50.5±8.01	112±5.0	145±8.6	1.9	1.65	0.8	3.0	91.3
28.5	23.7±2.2	48.8±13.6	113±4.4	143±14.2	1.8	3.03	0.7	5.4	69

Discussion

This study identified the optimal temperature for growth and best FCR of juvenile dusky kob reared under conditions when food supply was not restricted.

Juvenile dusky kob grew fastest between 24 and 26 °C. Bernatzeder (2005) suggested that the thermal preference of South African hatchery-reared juvenile dusky kob was within the temperature range of 25 - 26.4 °C. The similarity in results illustrates that behavioural thermoregulation observed in a thermal gradient could be used to estimate the optimum temperature for growth of dusky kob, but that temperature preference was higher than the temperature required for best FCR.

The optimal temperature for growth of dusky kob found in this study falls within the temperature range of their natural distribution. Juveniles of the size used in this study are predominantly found in the upper reaches of estuaries in the warm-temperate and subtropical region of the east coast of South Africa (Griffiths 1996). Temperatures in these estuaries range from 12 - 28 °C depending on the season (Whitfield 1998).

The specific growth rate of 2.05% per day achieved by juvenile *A. japonicus* (initial weight 23.7 g fish⁻¹) in this study compares to that of another sciaenid species, *Pseudosciaena crocea* Richardson 1846 (initial weight 17.82 g fish⁻¹), that grew at a rate of up to 1.94% per day (Qinghui *et al.* 2006). This growth rate is however, lower than that found for juvenile *Lates calcarifer* (initial weight 40 g fish⁻¹) which had a specific growth rate of up to 4.1% per day (Katersky & Carter 2005).

The high survival of 95.6% is similar to the 96% found in *L. calcarifer* (Katersky & Carter 2005) and the 85 - 98% in *P. crocea* (Qinghui *et al.* 2006) of similar size. These similarities to well-established aquaculture species suggest that *A. japonicus* is a promising aquaculture candidate.

The temperature at which food conversion efficiency was maximised, i.e., at the lowest FCR, was lower than the optimal temperature for growth. Similarly, a study on European sea bass *Dicentrarchus labrax* L. 1758 showed that the optimal temperature for growth (26 °C), was higher than the temperature for best FCR (23.9 °C) (Person-Le Ruyet *et al.* 2004). Growth in

fishes is generally maximised at a higher temperature than food conversion efficiency (Jobling 1996, Deacon 1997, Van Ham *et al.* 2003) due to two factors. First, as water temperature increases, oxygen solubility in the water decreases. This decrease in oxygen availability results in an increased energy requirement for ventilation and hence an increased total maintenance energy requirement (Jobling 1996). The increased maintenance energy expenditure reduces the proportion of energy available for growth, thus reducing the food conversion efficiency (Larsson & Berglund 2005). Second, as temperature increases, so does ingestion rate due to a higher metabolic requirement (Jobling 1996). The increased feed uptake can correspond to a decrease in the efficiency of feed absorption of up to 15% (Elliott 1982, Jobling 1994). This was also seen in the present study in which there was a positive linear relationship between temperature and feeding intensity.

The South African mariculture industry may use the results from this study as a guideline for dusky kob farming operations. By keeping the temperature between the optimal temperature for growth (25.3 °C) and the optimal temperature for feeding efficiency (21.7 °C), farmers are likely to minimize the cost of feeding with little negative effect on growth.

Determination of the temperature range that does not limit growth of dusky kob is a prerequisite for the assessment of the relationship between growth and other environmental variables such as light intensity, feeding regime and stocking density. Consequently, future experiments to determine the levels of those variables that maximize growth of dusky kob should be conducted at 24 - 26 °C.

CHAPTER 3

THE EFFECT OF LIGHT INTENSITY AND FEEDING METHOD ON GROWTH OF JUVENILE DUSKY KOB *ARGYROSOMUS JAPONICUS*

Introduction

The South African mariculture industry is investigating the farming potential of dusky kob *A. japonicus*. This requires research to establish rearing protocols aimed at maximising fish growth and profit. In this context, the effect of light intensity on growth of juvenile dusky kob under culture conditions has not been investigated.

Juvenile dusky kob of about 30 mm total length move into estuaries where they remain until they have grown to a length of about 150 mm (Griffiths 1996). In South Africa, juvenile dusky kob appear to be more abundant in turbid estuaries with freshwater influx than in clear, marine-dominated estuaries (Ter Morshuizen *et al.* 1996). For example, juvenile *A. japonicus* were not recorded in the non-turbid South African Swartvlei and Knysna estuaries even though they fall within their natural distribution (Whitfield & Kok 1992). Abundance of dusky kob may be influenced by temperature, salinity, food supply, and turbidity with the latter influencing light intensity. Many fish species need a minimum light intensity to develop and grow. Below this level, feeding and spontaneous swimming do not occur (Blaxter 1968). The effect of light intensity on growth in fish needs to be studied (Oppedal *et al.* 1997, Boeuf & Le Bail 1999), in particular its importance for the culture of juvenile dusky kob as they are abundant in turbid estuaries (Ter Morshuizen *et al.* 1996). Turbidity can reduce light penetration into water, thus creating an environment different from that experienced by the fish under culture conditions in mechanically filtered clear water.

Visually oriented species may defend a territory more actively under high light intensity than under dark conditions and may become less aggressive as light intensity decreases (Valdimarsson & Metcalfe 2001). Depending on species and rearing conditions, light intensity may moderate both the stress response of cultured fish (Boeuf & Le Bail 1999) and their activity level (Reynolds & Casterlin 1976). An example is the level of swimming activity, territoriality

and cannibalism in *Clarias gariepinus* (Burchell 1822) (Almazán-Rueda *et al.* 2004). Since energy requirement in fish is directly related to activity, the effect of light intensity on growth and FCR (FCR) of aquaculture candidate species should be tested under culture conditions (Deacon 1997).

Light is a directive factor (Fry 1971) as it can stimulate behavioural responses such as feeding activity. Light intensity influenced feeding behaviour of larval marine fish (Blaxter 1986, Batty 1987, Puvanendran & Brown 1998, Downing & Litvak 1999), but more studies are needed to understand the importance of light intensity for the feeding behaviour of juvenile marine fish species that spend parts of their life in estuaries. Such a study should include testing the effect of light intensity at two feeding methods in order to establish whether light intensity may have an effect on feeding behaviour, fish metabolism or both.

The aim of this study was to test the effect of a) three light intensity levels, and b) the combined effect of two light intensities at two feeding methods on growth, condition factor, FCR and survival of juvenile dusky kob in a 56-day experiment.

Materials and methods

Captive-bred juvenile *A. japonicus* were transported from Espadon Marine Hatchery in Hermanus (Western Cape, South Africa) to the departmental hatchery and acclimated to a closed recirculation system for 14 days. This system consisted of fifteen 100-L (0.44 x 0.50 x 0.45 m) green plastic tanks with a water flow rate of 150 L h⁻¹ tank⁻¹. The tank effluent passed through a 100-L sand filter, a 200-L biological trickle filter and a counter-current foam-fractionator. A 100-L tank filled with crushed oyster shell was added in-situ to buffer water pH. Each fish tank was stocked with 11 fish (average weight 7.2 ± 1.6 g fish⁻¹). Tanks were cleaned by scrubbing algae off the tank walls and siphoning debris twice daily.

Twice weekly total ammonia-nitrogen and nitrite concentrations were determined photometrically using a spectrophotometer (Hach Ltd.) according to methods analogous to EPA

354.1, US Standard Methods 4500-N02- B, and EN 26777, respectively. Un-ionised ammonia did not exceed 0.01 mg L^{-1} and nitrite did not exceed 0.09 mg L^{-1} .

Water temperature was maintained at $25.8 \pm 0.3^\circ\text{C}$ by a 1.2 kW thermostatically controlled heater. This temperature was selected as it falls within the range reported for the optimal temperature for growth (Collett *et al.* 2008a) and the thermal preference (Bernatzeder & Britz 2007) of juvenile dusky kob. Room temperature was maintained at 20°C with two wall-mounted room air conditioners.

Light was provided by ten 1.5-m Biolux® fluorescent tubes situated 1.3 m above the tanks and the light intensity in each tank was controlled using shade net covers. Light intensity was measured at the water surface with a LX 101 digital light meter. Light-dark cycle was maintained at a 12L:12D program by a timer-switch. Water was aerated with airstones connected to an oil-free rotary blower to maintain at least 5 mg L^{-1} dissolved oxygen. Salinity was kept at 35 g L^{-1} through the addition of freshwater.

To address objective b) fish were fed twice daily according to two feeding methods, i.e., apparent satiation and at a restricted ration of 4% of body weight d^{-1} at a low and a high light intensity. Satiation was assumed when pellets that reached the bottom of the tank were not immediately eaten. Tank covers were removed during feeding. Food containers were assigned to each tank and weighed weekly to determine the amount of food fed. The restricted ration was determined according to the growth of the fish estimated from the previous growth period. As growth projection was an estimate, the feeding intensity as percentage body weight was calculated at the end of the growth period. On average, fish realised a feeding intensity of 3.6% body weight d^{-1} .

The diet was a 1.8-mm pelleted feed (450 g kg^{-1} crude protein, 140 g kg^{-1} crude lipids, 40 g kg^{-1} crude fibre, less than 100 g kg^{-1} ash and less than 100 g kg^{-1} moisture, by Indian Ocean Aquafeeds Ltd., Johannesburg, South Africa).

Every 14 days all fish were purged for 24 hours and anaesthetised with 0.2 mL L^{-1} 2-phenoxyethanol. The standard length of each fish was determined using a measuring board with

an accuracy of 1 mm. All fish were weighed individually at a precision of 0.1 g by placing them in a shallow dish containing seawater on a digital balance.

Experimental design

The effect of light intensity on growth, FCR and survival was assessed at three light intensity levels. The ranges were 23-34 (low), 163-204 (medium) and 270-315 (high) lux, respectively, and fish were fed to apparent satiation twice daily.

The combined effect of light intensity and feeding method was tested using a factorial study testing the two light intensity ranges of 23-34 lx and 270-315 lx, each at two feeding methods, i.e., a daily ration of 3.6% of body weight fed in two equal portions, and feeding fish to apparent satiation twice daily, respectively.

Data analysis

Percentage daily weight gain was calculated using the formula: $G = ((W_0/W_e)^{(1/t-1)} - 1) \cdot 100$; where G = percentage daily weight gain, W_0 and W_e = average weight of fish (g fish^{-1}) in a tank at the beginning and end of a growth period, respectively, and t = time in days of the growth period. Length gain was expressed as the average increase per tank during a growth period. FCR was calculated by dividing the mass of dry food fed per fish, by the wet weight gain per fish in each tank for that feeding period. The condition factor (CF) of each fish was calculated as $CF = (W L^{-3}) \cdot 106$; where W is the weight (g fish^{-1}) and L the length (mm fish^{-1}).

Within-subjects analysis of variance was used to compare the average values of the dependent variables over time. These variables were daily percentage growth rate, length gain and FCR for each 14-day growth period. There were three replicates of the medium light intensity treatment and six replicates of the high and low light intensity treatments, respectively.

To test the effect of light intensity, low, medium and high light intensity treatments were compared using one-way analysis of variance with repeated measures. A 2x2 factorial within-subjects analysis of variance was used to test for the main effects 'light intensity' (low and high) and 'feeding method' (satiation and restricted) and their interaction. In this study, treatments

were done in triplicate. For all tests, an error level of 5% ($p < 0.05$) was selected to detect significant differences between treatments.

Results

All fish survived the experiment.

Light intensity had no significant effect on growth rate ($F_{8, 48} = 1.36$, $p = 0.29$); length gain ($F_{8, 48} = 1.49$, $p = 0.26$); FCR ($F_{6, 36} = 0.07$, $p = 0.93$) and condition factor ($F_{8, 48} = 1.50$, $p = 0.26$) (Table 2.1). During the 56-day study, average fish weight increased from 7.2 ± 1.6 to 41.9 ± 10.2 g fish⁻¹ at a growth rate (G) of 3.25% of body mass per day (Figure 2.1). Total fish length increased at a rate of 1.05 mm day⁻¹. FCR values averaged 1.39 ± 0.47 over the 56-day study period.

Table 2.1: The effect of three light intensity ranges on fish growth over a 56-day growth trial: Δ Length and Δ Weight = average length gain (mm fish⁻¹) and weight gain (g fish⁻¹ over the 56-day period; CF = condition factor, G = growth rate (% body weight d⁻¹); FCR = feed conversion ratio; FI = feeding intensity (% body weight d⁻¹). Errors are standard deviation of the mean. Superscript letters indicate significant differences between treatment means

Light intensity	Δ Length	Δ Weight	CF	G	FCR	FI
Low (23–34 lx)	58.9 \pm 3.7a	34.4 \pm 3.2b	1.65 \pm 0.1c	3.31d	1.40 \pm 0.50e	4.52f
Medium (160–204 lx)	58.6 \pm 2.4a	35.5 \pm 1.3b	1.62 \pm 0.1c	3.24d	1.43 \pm 0.39e	4.54f
High (270–315 lx)	59.2 \pm 3.8a	34.6 \pm 2.8b	1.65 \pm 0.1c	3.20d	1.36 \pm 0.49e	4.43f

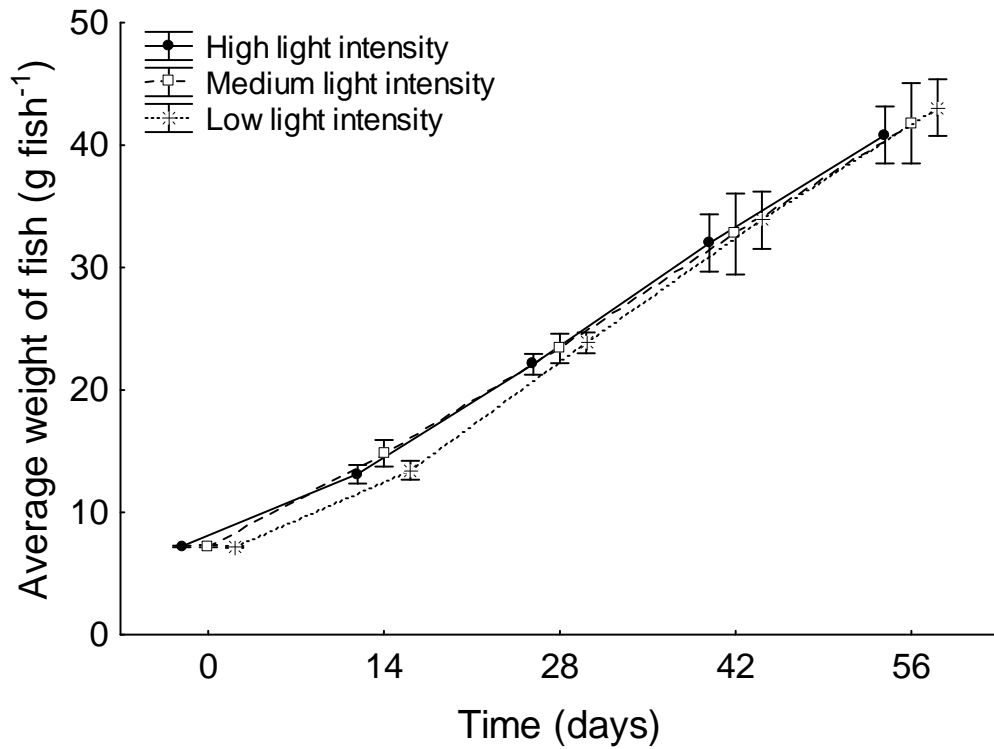


Figure 2.1: Average weight (g fish^{-1}) of juvenile *A. japonicus* kept at three light intensities during a 56-day experiment. Vertical bars are weighted standard deviations of the mean.

Factorial analysis of variance showed that neither light intensity ($F_{1, 8} = 0.006$, $p = 0.94$) nor feeding method ($F_{1, 8} = 2.3$, $p = 0.16$) affected growth rate significantly and there was no significant interaction between these two main effects ($F_{1, 8} = 0.03$, $p = 0.86$) (Table 2.2).

Table 2.2: The effect of two feeding methods at high and low light intensities on growth over a 56-day growth trial: Satiation ration = apparent satiation twice daily, restricted ration = 4% of body weight d^{-1} divided into two portions, dL and dW = average length (mm fish $^{-1}$) and weight increase (g fish $^{-1}$), over the 56-day period; C = condition factor, G = percentage daily growth rate (% body weight d^{-1}); FCR = food conversion ratio; FI = feeding intensity (% body weight d^{-1}). Errors are standard deviation of the mean. Superscript letters indicate significant differences.

Ration	dL	dW	C	G	FCR	FI
Satiation	61.1±3.5a	35.8±3.3b	1.63±0.1c	3.29d	1.62±0.46e	5.09g
Restricted	57.0±2.5a	33.2±1.7b	1.66±0.1c	3.19d	1.14±0.46f	3.61h

Average FCR-values were significantly better in fish fed a ration than in those fed to satiation ($F_{1,8} = 70.1$, $p = 0.003$) (Figure 2.2). These fish had a 42% better FCR than those fed to satiation. There was no significant interaction between the two main effects feeding method and light intensity ($F_{1,8} = 0.083$, $p = 0.78$).

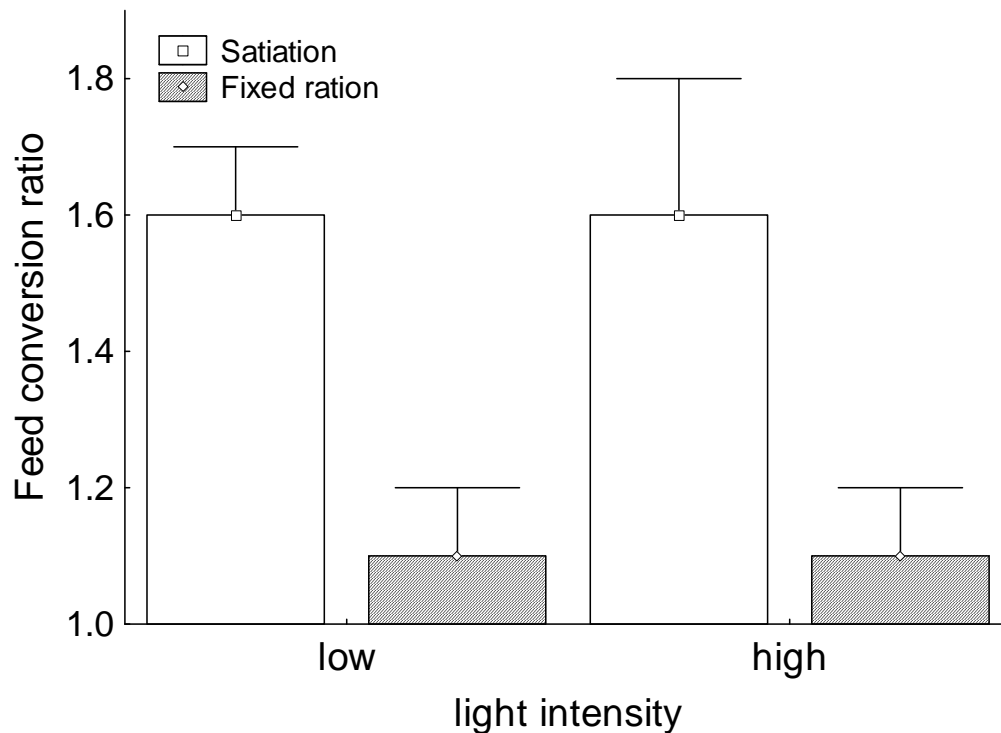


Figure 2.2: Average FCR (kg feed kg weight gain⁻¹) of juvenile *A. japonicus* kept at low (23 – 34 lx) and high (270 – 315 lx) light intensities and two feeding methods during a 56-day growth experiment. Satiation = fish fed to apparent satiation twice daily, restricted ration = 4% body weight d⁻¹ divided into two portions. Vertical bars indicate range of data set.

Discussion

This is the first study to test a) the effect of light intensity and b) the combined effect of light intensity and feeding method on the growth of South African juvenile dusky kob. Light intensity ranging from 23 to 321 lx and feeding method did not influence growth. However, FCR was significantly better in fish fed a restricted daily ration than in those fed to satiation.

Few studies have determined the effect of light intensity on growth of juvenile marine or estuarine fish species and the results of these studies suggest that the effects vary according to species. Light intensity had no effect on growth of juvenile salmon *S. salar* (L. 1758) within the

range of 27–715 lx (Stefansson *et al.* 1993), but Wallace *et al.* (1988) observed better growth in juveniles (0.7 g fish^{-1}) of this species at 700 lx than at 200, 50 and 10 lx. Light intensity affected growth and survival of juvenile spotted grunter *P. commersonnii* (Lacepède 1801) (Deacon 1997), a South African estuarine-dependent species (Whitfield 1998). Mortality was higher and growth rate was lower at the lowest light intensity ($2.32 \times 10^{-4} \mu\text{E}\cdot\text{sec}^{-1}\cdot\text{cm}^2$) than at both the intermediate ($1.95 \times 10^{-3} \mu\text{E}\cdot\text{sec}^{-1}\cdot\text{cm}^2$) and high ($6.31 \times 10^{-3} \mu\text{E}\cdot\text{sec}^{-1}\cdot\text{cm}^2$) level. Condition factor was best at the intermediate light intensity, and FCR-values did not differ between treatments (Deacon 1997). The units of light intensity used by Deacon (1997) cannot be converted to lux but were similar to those tested in this study.

Boeuf & Le Bail (1999) suggested that a high light intensity may be stressful or lethal to some fish species in captivity due to its effect on behaviour. African catfish *C. gariepinus*, for example, spent more time swimming at high light intensity (150 lx) than at a low light level of 15 lx (Almazán-Rueda *et al.* 2004). Observations made in this study suggested that dusky kob exhibit cover-seeking behaviour at a high light intensity, but behavioural differences between treatments were not quantified. However, as there was no difference in growth, any changes in behaviour may not affect the aquaculture potential of juvenile dusky kob within the light intensity ranges tested here. There was also no difference in growth of dusky kob between the two feeding methods. This suggests that, unlike in studies on larval fishes (Blaxter 1986, Batty 1987, Puvanendran & Brown 1998, Downing & Litvak 1999) light intensity did not influence feeding behaviour and growth of juvenile dusky kob.

Fish that were fed to satiation consumed more food than those receiving a ration of 3.6% body mass d^{-1} . It is thus hypothesised that under conditions where food supply is not limited, fish will consume more food than they require while using the diet less efficiently (Jobling 1994). An increase in feed uptake may reduce FCR for two reasons. First, an increased rate of ingestion reduces gut evacuation time, thereby reducing absorption of nutrients and increasing nutrient loss in faeces (Jobling 1994). Second, feeding results in a post-prandial increase in metabolic rate of two to three times the pre-prandial level. Large rations extend the time that metabolic rate remains elevated above the pre-prandial level (Jobling 1994). This leads to a high energy requirement for metabolism, reducing FCR. European sea bass *D. labrax* (L. 1758) fed at a ration of 4.5% body mass d^{-1} showed poorer FCR than those fed at 3.1% body mass d^{-1} (Paspatis

et al. 1999). The better FCR in fish fed a restricted ration in the current study suggests that a ration of 3.6 % of body mass d⁻¹ allowed good growth and FCR of juvenile dusky kob.

In conclusion, light intensity did not affect growth rate, condition factor or FCR of juvenile *A. japonicus*. Thus, although juvenile wild dusky kob appeared to prefer turbid estuaries (Ter Morshuizen *et al.* 1996) a light intensity range of 23 to 315 lx may not have a negative effect on performance of kob under culture conditions.

CHAPTER 4

THE EFFECT OF FEEDING REGIMEN ON GROWTH AND FEED CONVERSION RATIO OF JUVENILE DUSKY KOB *ARGYROSOMUS JAPONICUS*

Introduction

Dusky kob *A. japonicus* (family: Sciaenidae) is a South African mariculture species. Collett *et al.* (2008a) determined the best temperature range for growth and feed conversion ratio, but the effect of feeding rate and feeding frequency on growth of kob under aquaculture conditions requires further research. Inefficient feeding reduces profit in intensive aquaculture (Goddard 1996), and food intake is an important variable controlling fish growth and metabolism (Fry 1971, Brett 1979).

Fish require a species and size-specific daily ration for maximum growth (Chua & Teng 1982). If the daily ration cannot be provided in one feeding, there is a need to determine the minimum species-specific feeding frequency required to achieve the daily ration and hence best growth (Deacon 1997). However, increasing the feeding frequency above the level of the required daily food intake may not improve growth and may reduce feed conversion ratio (FCR) (Brett & Groves 1979, Jobling 1994). The best feeding regimen is the lowest feeding frequency at which the daily ration can be provided without reducing growth or FCR (Chua & Teng 1982).

Daniel (2004) investigated the effect of feeding frequency and feeding intensity on growth of wild-caught juvenile *A. japonicus* (34.6 ± 17.3 g fish⁻¹) kept at 18.9 ± 3.9 °C. Fish fed once daily grew slower than those fed more often and it was concluded that juvenile *A. japonicus* should be fed twice daily (09:00 and 18:00) at 4% body weight per day. However, since the temperature for best growth of juvenile *A. japonicus* has been suggested to be between 24 and 26 °C (Collett *et al.* 2008a), growth and metabolism of dusky kob may have been reduced due to the low temperature used by Daniel (2004). In this study, juvenile *A. japonicus* that had been spawned in a hatchery were grown within the temperature range known to maximise growth.

The feeding regimen required for maximum growth has been determined in related fish species. Red drum *S. ocellatus*, also a sciaenid species, is cultured on the United States east coast (Lee & Ostrowski 2001) and in China (Hong & Zhang 2003). Juvenile *S. ocellatus* (7.7 g fish⁻¹) should be fed 5 to 6% body weight per day in two meals (morning and evening) (McGoogan & Gatlin 1999). Juvenile spotted grunter *P. commersonii* occur in South African estuaries (Whitfield 1998). Deacon (1997) recommended feeding juvenile spotted grunter (4.2 g fish⁻¹) at 3.6% body weight per day over two feedings per day (morning and evening) in order to optimise FCR, growth and condition factor.

The aim of this study was to test the effect of feeding frequency and feeding intensity on growth, FCR, condition factor and survival of hatchery-reared juvenile *A. japonicus* cultured within their recommended temperature range.

Methods and materials

Juvenile *A. japonicus* were obtained from Espadon Marine Pty. Ltd. in Hermanus (Western Cape, South Africa). Fish were acclimated to the experimental facility for 14 days prior to the experiment. This system consisted of sixteen 100-L (0.44 x 0.50 x 0.45 m) green plastic tanks connected through one filtration system as described in Collett *et al.* 2008a. Each tank was stocked with 20 fish (average weight \pm SD: 7.5 \pm 1.0 g fish⁻¹).

Twice weekly total ammonia-nitrogen and nitrite concentrations were determined using a spectrophotometer (Hach Ltd.) according to methods analogous to EPA 354.1, US Standard Methods 4500-NO₂- B, and EN 26777. Un-ionised ammonia did not exceed 0.03 mg L⁻¹ N and nitrite did not exceed 0.5 mg L⁻¹ N. Once a week 50% of the system water was exchanged with seawater to control the nitrate level.

Water temperature was maintained at 24.8 \pm 0.6 °C by a 1.2-kW thermostatically controlled heater. This temperature falls within the range reported for the best temperature for growth (Collett *et al.* 2008a) and the thermal preference (Bernatzeder & Britz 2007) of juvenile *A.*

japonicus. Room temperature was maintained at 20 °C with two wall-mounted room air conditioners.

Light was provided by ten 1.5-m Biolux® fluorescent tubes placed 1.3 m above the tanks. Light-dark sequence was maintained at a 12L:12D cycle by a timer-switch. Water was aerated with airstones connected to an oil-free rotary blower to maintain at least 5 mg L⁻¹ dissolved oxygen. Salinity was kept at 35 g L⁻¹ through the addition of freshwater.

Depending on the treatment, fish were fed either a restricted ration three times daily or to apparent satiation once, twice or three times daily. Satiation was assumed when pellets that reached the bottom of the tank were not immediately eaten. The amount of food fed per tank was recorded weekly. When feeding the restricted ration, the feeding intensity of 4% of body weight per day was calculated according to the growth of the fish estimated from the previous growth period. As growth projection was an estimate, feeding intensity was determined at the end of the growth period based on the growth rate of the fish and the feed conversion ratio for each tank. Feeding intensity for the restricted ration treatment averaged 3.4% body weight per day.

The diet was a 1.8-mm pelleted feed manufactured by Indian Ocean Aquafeeds Ltd., Johannesburg, South Africa. It contained 450 g kg⁻¹ crude protein, 140 g kg⁻¹ crude lipids, 40 g kg⁻¹ crude fibre, less than 100 g kg⁻¹ ash and less than 100 g kg⁻¹ moisture.

Every 14 days all fish were purged for 24 hours and anaesthetised with 0.2 mL L⁻¹ 2-phenoxyethanol. The standard length of each fish was determined using a measuring board with an accuracy of 1 mm. All fish were weighed individually at a precision of 0.1 g by placing them in a shallow dish containing seawater on a digital balance.

Experimental design and data analysis

The effect of feeding frequency and feeding intensity on growth, FCR, condition factor and survival was tested using four feeding regimens. These were a restricted ration fed three times daily and apparent satiation at three feeding frequencies. There were four replicates per treatment. Fish fed once daily were fed at 08:00; fish fed twice daily were fed at 08:00 and 16:00 and fish fed three times daily were fed at 08:00, 12:00 and 16:00.

Percentage daily weight gain was calculated using the formula: $G = ((W_e/W_0)^{(1/(t-1))}-1) \cdot 100$; where G = percentage daily weight gain, W_0 and W_e = average weight of fish (g fish^{-1}) in a tank at the beginning and end of a growth period, respectively, and t = time of the growth period (days). FCR was calculated by dividing the mass of dry food fed per fish by the wet weight gain per fish in each tank for that feeding period. Feeding intensity (FI) was calculated as $FI = FCR \cdot G$. Condition factor (C) of each fish was determined from its weight (W) (g fish^{-1}) and length (L) (mm) using the formula $C = (W L^{-3}) \cdot 105$.

Within-subjects ANOVA (Ott 1988) was used to compare the average values for the length and weight gain per tank over time. Percentage daily growth rate, condition factor, FCR and feeding intensity over the 42-day period were compared using a one-way ANOVA and differences among the means were tested using Tukey's Multiple Range Test. For all tests, an error level of 5% ($P < 0.05$) was selected to test for significant differences between treatments.

Piecewise linear regression was used to model the relationship between FCR and feeding intensity by using average values for the experimental period for each of the 16 tanks. Residuals were normally distributed and the coefficient of correlation was higher than that obtained from a linear least-squares regression model, a polynomial model, or an exponential function. The regression analysis estimated a breakpoint feeding intensity and provided two models. At feeding intensities below the breakpoint ($FI < 3.85$) there was no relationship between FCR and feeding intensity, while there was a significant slope of a least-square linear model using the values above the breakpoint ($FI > 3.85$).

Results

Fish fed once daily had the lowest weight gain (Figure 3.1) and length gain over time ($F_{9, 36} = 19.83$, $P < 0.01$, and $F_{9, 36} = 16.18$, $P < 0.001$, respectively; within-subjects ANOVA). Percentage daily growth rate of fish fed once daily (Table 3.1) was lower than in each of the treatments in which fish were fed more than once daily ($F_{3, 12} = 18.24$, $P < 0.001$, ANOVA) with no differences between the other three treatments. FCR was significantly better in the treatment fed the restricted ration ($F_{3, 12} = 19.39$, $P < 0.001$, ANOVA) than in any of the treatments fed to

apparent satiation. However, average FCR did not differ between treatments fed to apparent satiation once or twice daily, and it was worst in fish fed to apparent satiation three times daily (Table 3.1). Feeding intensity did not differ between the treatment fed the restricted ration three times daily and the treatment fed to apparent satiation once daily. Treatments fed to apparent satiation twice or three times daily differed from each other with the latter having the highest feeding intensity. These two treatments had a higher feeding intensity ($F_{3, 12} = 68.41$, $P < 0.001$, ANOVA) than those fed to apparent satiation once daily or a restricted ration three times daily (Table 3.1). There was no difference in condition factor between any of the treatments ($F_{3, 12} = 0.37$, $P = 0.774$, ANOVA).

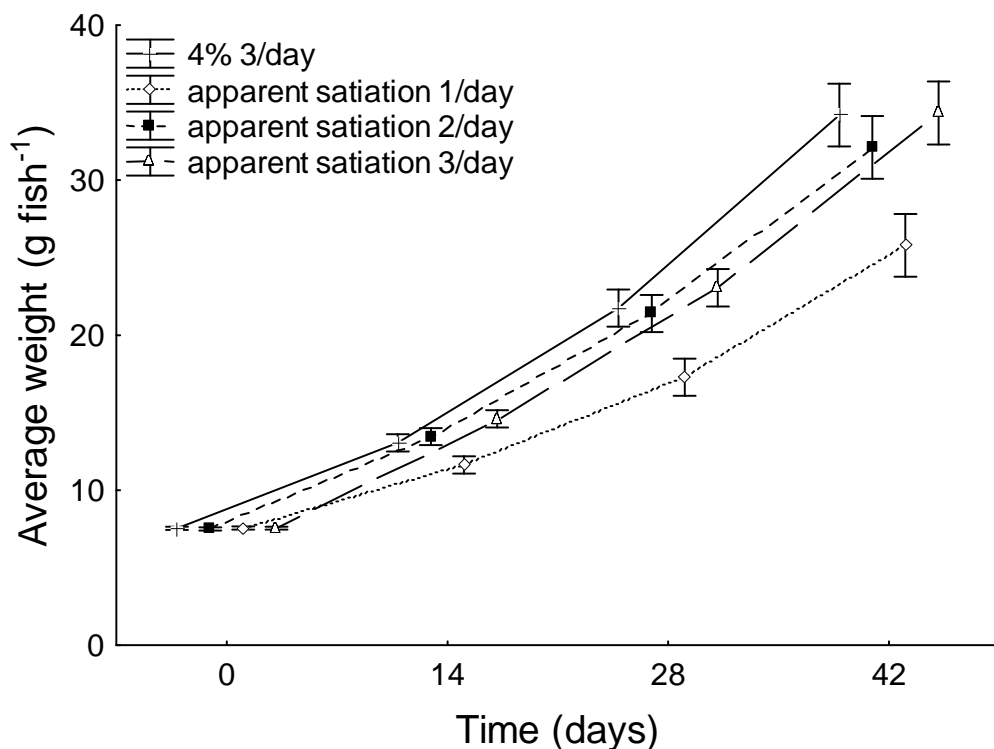


Figure 3.1. Average weight (g fish^{-1}) of juvenile *A. japonicus* fed at four feeding regimens during a 42-day experiment. Vertical bars are weighted standard deviations of the mean.

Table 3.1. The effect of four feeding methods on fish growth during a 42-day growth trial: dL and dW = average length gain (mm fish⁻¹) and weight gain (g fish⁻¹), respectively, over the experimental period; C = condition factor, G = growth rate (% body weight per day); FCR = feed conversion ratio; FI = feeding intensity (% body weight per day). There was no fish mortality in this study. Errors are standard deviation of the mean. Different superscript letters within a column indicate significant differences between treatment means.

Feeding method	dL	dW	C	G	FCR	FI
3 x daily, 4% BW	48.8±1.2 ^a	26.6±1.3 ^c	1.74±0.05 ^e	3.75±0.10 ^f	0.91±0.04 ^h	3.41±0.06 ^k
1 x daily, satiation	37.9±6.2 ^b	18.2±3.2 ^d	1.73±0.05 ^e	3.02±0.30 ^g	1.09±0.10 ⁱ	3.28±0.14 ^k
2 x daily, satiation	47.4±1.0 ^a	24.6±0.9 ^c	1.71±0.02 ^e	3.61±0.09 ^f	1.10±0.07 ⁱ	3.96±0.17 ^l
3 x daily, satiation	49.5±0.6 ^a	26.8±0.6 ^c	1.74±0.05 ^e	3.76±0.04 ^f	1.31±0.08 ^j	4.93±0.28 ^m

Below a feeding intensity of 3.85% body weight per day there was no significant relationship between FI and FCR ($P = 0.32$, $r^2 = 0.16$, least square regression). At feeding intensities between 3.85 and 5.15% of body weight per day, FCR was related to feeding intensity (FI), i.e.: $FCR = 0.18 + 0.23 FI$ ($P = 0.01$, $r^2 = 0.96$, least-square linear regression) (Figure 3.2).

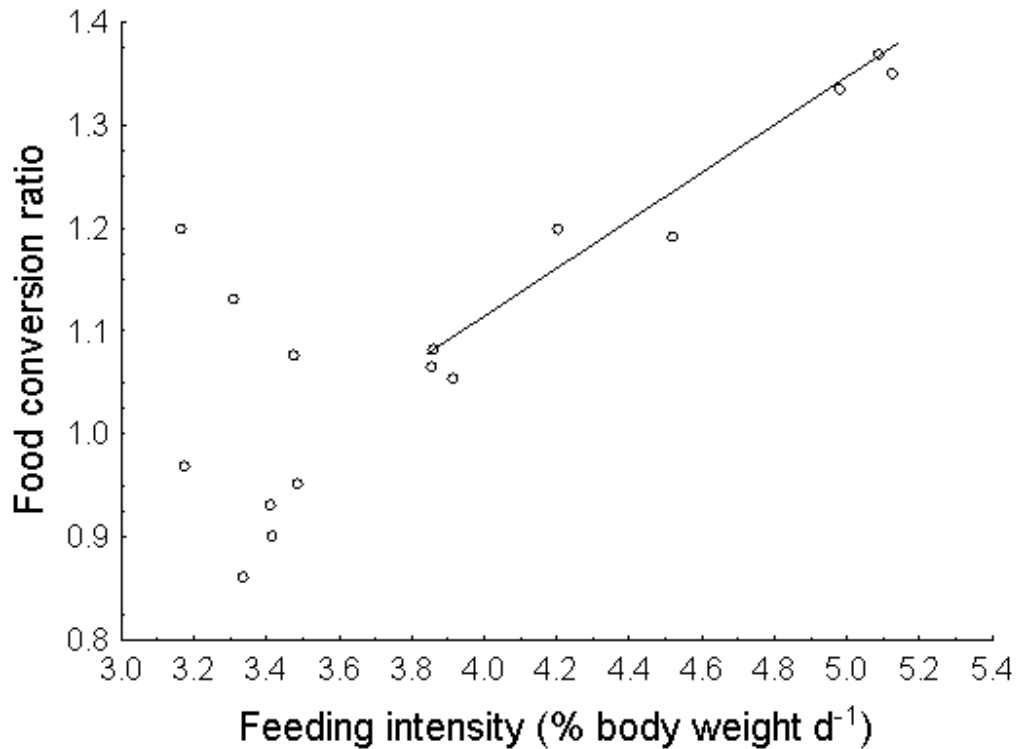


Figure 3.2. The effect of feeding intensity (FI), in % body weight per day, on food conversion ratio (FCR). The regression line at FI-values above 3.85 indicates a significant relationship between the two variables ($FCR = 0.18 + 0.23 FI$, $r^2 = 0.96$). At feeding intensity levels below 3.85% there was no significant relationship between FCR and feeding intensity ($p = 0.32$, $r^2 = 0.16$).

Discussion

Feeding frequency had a significant effect on growth rate, FCR and feeding intensity of juvenile *A. japonicus*. Fish fed once per day grew slower than those fed more than once per day. There was no significant difference in growth between treatments in which fish were fed more than once per day, but the fish fed a restricted ration had a lower feeding intensity and a better FCR than those fed to apparent satiation more than once per day. Thus, fish fed a restricted ration

consumed less food in percentage body weight per day than those fed to apparent satiation, but did not grow slower.

Of the treatments in which fish were fed to apparent satiation, those fed twice or three times daily consumed more food than those fed once daily. Individual meal size must, however have been disproportionately smaller in fish fed more frequently because the feeding intensity of fish fed twice and three times daily was only 20% and 50% greater, respectively, than in fish fed once daily. Frequent feeding can reduce gut evacuation time, thus decreasing the efficiency of feed absorption (Elliott 1982, Liu & Liao 1999) by up to 15% (Jobling 1994). Therefore, it is recommended to determine the lowest feeding frequency that achieves a feeding intensity sufficiently high to produce good growth without leading to a lowering of the FCR. In this study, fish fed the restricted ration three times daily grew at the same rate as treatments fed to apparent satiation twice daily. However, the former treatment had a 16% lower feeding intensity and a 20% better FCR than the latter.

As feeding intensity increased above 3.85% body weight per day, food conversion efficiency decreased linearly. Thus, to achieve good food conversion, the feeding intensity for juvenile *A. japonicus* within a size range of 7.5 – 35.0 g fish⁻¹ should not be higher than 3.85% body weight per day. Similarly Collett *et al.* 2008b showed that good growth and FCR were achieved in juvenile *A. japonicus* under culture conditions comparable to this study at a restricted ration of 3.6% body weight per day.

Limiting the fish to one feeding per day resulted in a 24% reduction in growth rate and a 20% increase in FCR relative to feeding the restricted ration three times daily. Therefore, in this species, the effect of feeding frequency on growth was not only a function of food intake, as suggested by Chua & Teng (1982), but of both the effect of food deprivation time and the quantity of food that can be efficiently metabolised by the fish from a single feeding. The low growth rate of fish fed once daily suggests that a food deprivation time of 24 hours reduces growth despite a daily food intake similar to that of the restricted ration treatment. The similarity in growth rate of fish fed twice or three times daily suggests that a short-term food deprivation of less than eight daylight hours does not reduce growth of fish fed to apparent satiation. Therefore

juvenile *A. japonicus* within the size range tested here should be fed more than once daily at a minimum feeding intensity of 3.4% body weight per day.

The suggestions from the current study are similar to those made by Daniel (2004) who tested growth at a lower temperature but concluded that juvenile dusky kob should be fed twice daily at an interval of nine hours to 4% body weight per day. However, growth rate and FCR in the current study were 2.5 and 2 fold better than those reported by Daniel (2004). The daily feed requirement of juvenile *A. japonicus* was lower than in juvenile *S. ocellatus* (7.7 g fish⁻¹) fed at 5 to 6% body weight per day, (McGoogan & Gatlin 1999) and similar to that required by juvenile *P. commersonnii* (4.2 g fish⁻¹) of 3.6% body weight per day (Deacon 1997).

The improved FCR in the treatment fed a restricted ration relative to fish fed to apparent satiation has applications for industry. Feeding according to demand or allowing unrestricted access to food may reduce feed conversion efficiency and hence increase cost of production. In addition, the determination of a feeding method that allows fast growth of juvenile *A. japonicus* is important for the design of experiments on this species as the effect on growth of environmental variables such as population density should be tested at rearing conditions allowing maximum growth.

CHAPTER 5

THE EFFECT OF CROWDING DENSITY ON GROWTH AND FOOD CONVERSION RATIO OF JUVENILE DUSKY KOB *ARGYROSOMUS JAPONICUS*

Introduction

Fish stocking density influences survival, growth, aggressive and feeding behaviour, and health of farmed fish (Rowland *et al.* 2006), but stocking density requirements vary depending on the fish species, fish size and culture method (Beveridge 2002). Thus, the effect of stocking density on growth, survival and feed conversion ratio needs to be determined for each species to enable efficient management and maximise production and profit.

The term stocking density has been used in connection with both loading density and crowding density. Loading density has been expressed in fish biomass per unit of water flow rate. It takes into account the oxygen consumption of the fish species and its tolerance to metabolic waste such as ammonia and carbon dioxide. Crowding density has been defined as fish biomass per unit water volume (Wedemeyer 2000). Of these two factors loading density is considered the more important limiting factor for fish production due to metabolic requirements, in particular the need for a constant availability of dissolved oxygen (Wedemeyer 2000). However, depending on the species, intraspecific aggression or territorial behaviour may limit growth and feed conversion efficiency independent of loading density. For example, a greater frequency of 'predator-prey' interactions as a result of high crowding densities resulted in an increase in cannibalistic behaviour in *Oreochromis niloticus* (Fessehaye *et al.* 2005), and the development of a social hierarchy which reduced the feeding rate and growth of low-ranking fish in *Salmo salar* (Fenderson & Carpenter 1971). Competitive behaviour may reduce feeding rate of subordinate fish and may lead to a metabolic cost that reduces growth (Brett 1979). The effect of crowding density on growth and survival of dusky kob, *A. japonicus*, under South African production conditions in closed intensive recirculating systems has not been studied, but it has been suggested that crowding density influenced cannibalistic behaviour in this species (O'Sullivan & Ryan 2001).

In Australia, *A. japonicus* is farmed in intensive systems with densities of up to 25 kg m⁻³ (O'Sullivan & Dobson 2000, O'Sullivan & Savage 2004), in extensive culture in saline ponds (O'Sullivan & Ryan 2001), and as part of a lagoon stock enhancement program (Fielder & Bardsley 1999). *A. japonicus* reared in Australia showed no difference in growth rate at crowding densities between 8 and 16 kg m⁻³, provided good water quality was maintained, while growth was significantly lower at 4 kg m⁻³ than at the higher densities (Pirozzi *et al.* 2008).

South African kob farmers employ intensively managed land-based facilities, and there are plans to test sea-cage culture methods (Schoonbee & Bok 2006). Data from studies testing crowding density as a growth-influencing factor are needed for business planning. In addition, while many studies have tested this and other variables in laboratory trials on aquaculture candidate species, there is a need to establish the importance of crowding density on fish production under commercial conditions (Stickney & Liu 1999, Lee & Ostrowski 2001) and hence produce results applicable to local farming ventures.

This study investigated the effect of crowding density on growth, FCR and survival of juvenile *A. japonicus* reared in a closed recirculating system at 10, 30, and 50 kg m⁻³.

Methods and materials

Juvenile *A. japonicus* were obtained from Irvin & Johnson (Pty) Ltd. in Gansbaai (Western Cape, South Africa). They were size-graded according to a protocol used by industry. Fish were acclimated to the experimental tanks for 30 days prior to the experiment. The system consisted of twelve 600-L circular green plastic tanks (radius = 0.6 m, height = 0.53 m) with a central drain. Each tank was stocked with the required number of fish of a starting weight of 35.4 ± 1.7 g fish⁻¹ to obtain crowding densities of 10, 30 and 50 kg m⁻³. The flow rate in each tank was set according to the mass of fish in each tank so as to maintain a loading density of 33 kg fish · m⁻³ water flow · h⁻¹. To maintain a constant population density in each tank, the water level was adjusted weekly by increasing the height of the central drain pipe according to the fish biomass in each tank. Effluent water from all tanks passed through a 3000-L swirl separator, 100-L sand filter, a 5000-L semi-submerged trickle filter and a 1000-L counter-current foam fractionator.

The system operated on a partial recirculation scheme and the total volume of the system was replaced with seawater (35 g L^{-1}) every 30 hours for the duration of the study.

Twice weekly total ammonia-nitrogen and nitrite concentrations were determined by the phenolhypochlorite method of Solorzano (1969) using a spectrophotometer (Biochrom Novaspec II; Cambridge). Absorbance values were converted to total ammonia-nitrogen concentrations using a linear regression model derived from calibration with known concentrations of stock solutions of ammonium chloride ($n = 30$ concentrations, $r^2 = 0.95$). Using water pH, water temperature and salinity the concentration of $\text{NH}_3\text{-N}$ was calculated according to Bower and Bidwell (1978). Nitrite concentrations were determined according to methods analogous to EPA 354.1, US Standard Methods 4500- $\text{NO}_2\text{-B}$, and EN 26777. $\text{NH}_3\text{-N}$ values did not exceed 0.035 mg L^{-1} and nitrite values did not exceed $0.2 \text{ mg L}^{-1} \text{ NO}_2\text{-N}$.

Water temperature was maintained at $24.5 \pm 0.6^\circ\text{C}$ by three thermostatically controlled 3-kW immersion heaters (Metalquip: Hot Rod) placed in the swirl separator. This temperature falls within the range reported for the best temperature for growth (Collett *et al.* 2008) and the thermal preference (Bernatzeder & Britz 2007) of juvenile *A. japonicus*. The system was housed in a greenhouse building with asbestos roofing alternating with transparent roofing sheets to allow natural light penetration. Room temperature fluctuation at night was reduced by twelve 500-W wall-mounted panel-heaters. Oxygen was supplied through a diffuser into each tank fed from an oxygen cylinder. Oxygen flow rate was adjusted for each tank in an attempt to keep dissolved oxygen concentration equal in all tanks.

Fish were fed a daily ration of 4% body mass divided into three portions as suggested by Collett *et al.* (2008c). The ration was calculated according to the growth of the fish estimated from the previous growth period. As growth projection was an estimate, feeding intensity was determined at the end of the growth period based on the growth rate of the fish and the feed conversion rate for each tank. Feeding intensity for the 40-day study averaged $3.1\% \text{ body mass day}^{-1}$. The diet was a 3-mm pelleted feed by Indian Ocean Aquafeeds Ltd., Johannesburg, South Africa. It contained 450 g kg^{-1} crude protein, 140 g kg^{-1} crude lipids, 40 g kg^{-1} crude fibre, less than 100 g kg^{-1} crude ash and less than 100 g kg^{-1} moisture.

Every 14 days all fish were purged for 24 hours and 30 randomly selected fish per tank were removed and anaesthetised with 0.1 mL L⁻¹ 2-phenoxyethanol. The standard length of each fish was determined using a measuring board with an accuracy of 1 mm. Each fish was weighed at a precision of 0.1 g by placing them on a digital balance in a dish containing seawater.

Experimental design and data analysis

Percentage daily weight gain was calculated using the formula: $G = ((W_e/W_0)^{(1/(t-1))}-1) \cdot 100$; where G = percentage daily weight gain, W_0 and W_e = average weight of sampled fish (g fish⁻¹) in a tank at the beginning and end of a growth period, respectively, and t = time in days of the growth period. FCR was calculated by dividing the mass of dry food fed per fish by the average wet weight gain per fish in each tank for that feeding period. Feeding intensity (FI) was calculated as $FI = FCR \cdot G$. Condition factor (C) of the sampled fish was determined from their weight (W) (g fish⁻¹) and length (L) (mm) using the formula $C = (W L^{-3}) \cdot 10^5$.

Within-subjects analysis of variance was used based on the average values for the length and weight gain per tank over time (Ott 1988) at four replicates per density treatment. Percentage daily growth rate, condition factor, FCR, feeding intensity, water quality and dissolved oxygen concentration for the 40-day period were compared using a one-way ANOVA and differences between means were analysed using Tukey's Multiple Range Test. Percentage mortality values were compared between treatments using a Kruskal-Wallis ANOVA by tank test. For all tests, equality of variance and normal distribution of residuals were tested and an error level of 5% ($P < 0.05$) was selected to test for significant differences between treatments. Errors are given as standard deviations of the mean.

Results

Crowding density had no significant effect on weight gain ($F_{6, 27} = 1.24$, $p = 0.33$), percentage daily growth rate ($F_{2, 9} = 0.89$, $p = 0.44$), length gain ($F_{2, 9} = 1.36$, $p = 0.30$), FCR ($F_{2, 9} = 1.02$, $p = 0.40$) and condition factor ($F_{2, 9} = 0.74$, $p = 0.51$) (Table 4.1). Average fish weight increased from 35.5 ± 1.2 to 58.8 ± 2.1 g fish⁻¹ (Figure 4.1) at a growth rate of $1.3 \pm 0.2\%$ of body mass d

¹. Average total fish length increased at $0.7 \pm 0.1 \text{ mm d}^{-1}$ (Table 4.1). FCR values averaged 2.4 ± 0.4 over the 40-day growth period. There was no significant difference in feeding intensity ($F_{2, 9} = 2.96, p = 0.10$), percentage mortality ($H_{2, 11} = 3.74, p = 0.15$) (Table 4.1) or free-ammonia nitrogen concentrations ($F_{11, 63} = 1.50, p = 0.15$) between treatments. There was, however, a significant difference in dissolved oxygen concentration ($F_{2, 9} = 44.51, p < 0.001$; 4.1) between all treatments with oxygen levels decreasing with increasing crowding density.

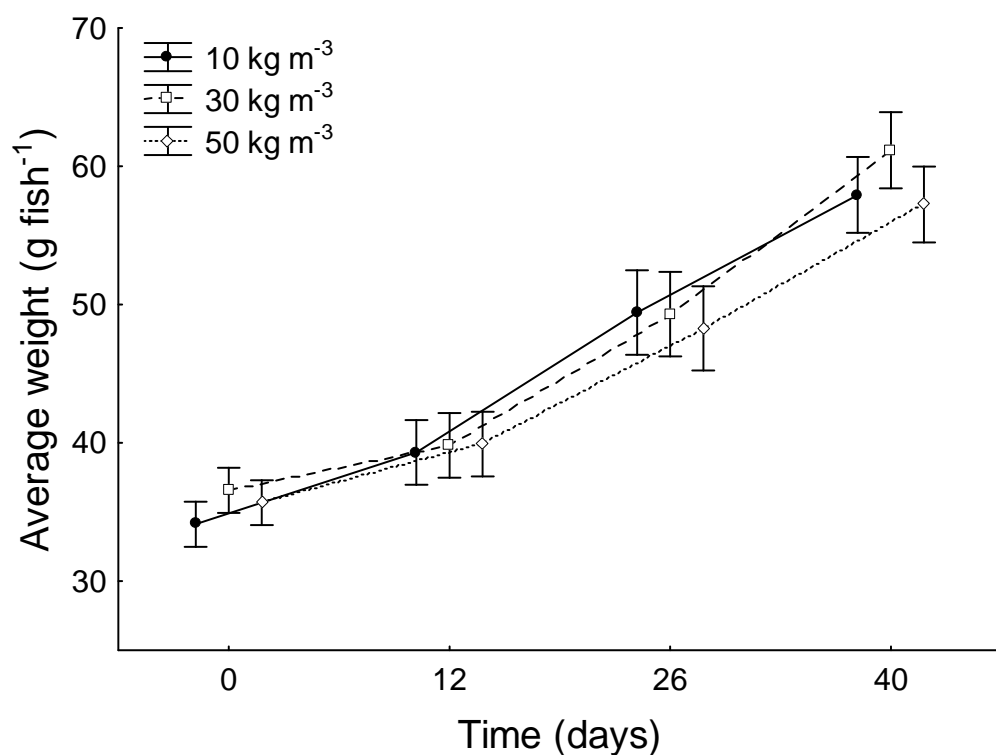


Figure 4.1: Average weight (g fish⁻¹) of juvenile *A. japonicus* kept at three crowding densities during a 40-day experiment. Vertical bars are weighted standard deviations of the mean.

Table 4.1: The effect of three crowding densities on fish growth over a 40-day growth trial: CD = crowding density treatment; dL and dW = average length gain (mm fish⁻¹) and weight gain (g fish⁻¹) over the 40-day period, respectively; CF = condition factor; G = growth rate (% body weight d⁻¹); FCR = feed conversion ratio; FI = feeding intensity (% body weight d⁻¹); Survival = total percentage survival by the end of the study period; O₂ = dissolved oxygen (mg L⁻¹ O₂). Errors are standard deviation of the mean. Superscript letters indicate significant differences between treatment means.

CD	dL	dW	CF	G	FCR	FI	Survival	O ₂
10 kg m ⁻³	26.4±2.1	24.1±1.3	1.6±0.1	1.3±0.1	2.3±0.16	3.1±0.1	99.4±0.6	5.3±0.1 ^a
30 kg m ⁻³	27.5±1.4	24.6±2.9	1.6±0.1	1.3±0.1	2.3±0.30	3.0±0.1	98.5±0.5	4.7±0.2 ^b
50 kg m ⁻³	24.8±3.1	21.6±4.3	1.6±0.1	1.2±0.2	2.6±0.60	3.1±0.1	98.9±0.2	4.4±0.1 ^c

Discussion

As crowding densities within the range of 10, 30 and 50 kg m⁻³ did not lead to differences in growth, FCR or survival of juvenile *A. japonicus*, kob farmers may consider stocking juvenile fish at 50 kg m⁻³ or test higher stocking densities experimentally under on-farm conditions.

The maximum crowding density tested here was higher than that tested previously for *A. japonicus* (Pirozzi *et al.* 2008). Tolerance to high crowding densities may be explained by the fact that *A. japonicus* is a shoaling species (Griffiths 1996) which suggests a preference for high crowding densities (Brett 1979). Pirozzi *et al.* (2008) suggested that stocking densities of 4 kg m⁻³ inhibited growth of *A. japonicus*, while 8 to 16 kg m⁻³ showed similar and faster growth. The similarity in growth rate at densities between 10 and 50 kg m⁻³ and those between the densities tested by Pirozzi *et al.* (2008) suggests that densities above 50 kg m⁻³ may reduce growth in

dusky kob of the size used in this study. The limiting crowding density should be determined in future on-farm experiments under those husbandry conditions considered appropriate for this species based on the findings obtained in chapters 2 to 4.

Results from this trial can provide a basis for studies testing the effect of loading density on dusky kob growth. Compared to the average oxygen level at the inflow to the culture tanks, a loading density of 10 kg m^{-3} resulted in a constant reduction in dissolved oxygen of $2.3 \text{ mg L}^{-1} \text{ O}_2$ while an increase in crowding density from 10 to 30 kg m^{-3} and 30 to 50 kg m^{-3} resulted in additional oxygen depletion of only 0.56 and $0.87 \text{ mg L}^{-1} \text{ O}_2$, respectively, suggesting a non-linear relationship. Dissolved oxygen concentrations across all treatments averaged between 4.4 and 5.3 mg L^{-1} (range: 3.7 to 6.4 mg L^{-1}) depending on crowding density, but as there were no differences between treatments in growth rate or FCR of juvenile kob, it is assumed that oxygen levels were sufficiently high for to allow good growth. Fitzgibbon *et al.* (2007) reported no difference in the standard metabolic rate (SMR) or active metabolic rate (AMR) of *A. japonicus* within this range of dissolved oxygen concentrations; however AMR of juvenile *A. japonicus* was significantly increased above the 75% saturation level. Therefore, *A. japonicus* should be cultured at as close to 100% dissolved oxygen saturation as possible to ensure that metabolic rate and hence growth is not suppressed. *A. japonicus* are reported to tolerate dissolved oxygen concentrations as low as $1.8 \text{ mg L}^{-1} \text{ O}_2$ (Fitzgibbon *et al.* 2007), which is lower than critical levels for *S. ocellatus* of $2.2 \text{ mg L}^{-1} \text{ O}_2$ (Sandifer *et al.* 1993). This study showed that *A. japonicus* was tolerant of dissolved oxygen concentrations as low as $4.4 \text{ mg L}^{-1} \text{ O}_2$ without any negative effects on growth.

The tolerance of juvenile *A. japonicus* to crowding densities of 50 kg m^{-3} has applications for both industry development and research programmes. Determination of the maximum allowable crowding density defines the maximum weight of fish that can be sustained, and sets the limits at which no further production is possible (Brett 1979). This allows the industry to increase production in a limited culture environment and hence use tank space efficiently. The relevance of this is that *A. japonicus* requires large volumes of water to be heated to the temperature for best growth (Collett *et al.* 2008a), hence making efficient use of this water important.

For the development of rearing protocols for mariculture, crowding density should not be limiting when testing the effect on growth of variables such as temperature and feeding regime. Since crowding densities between 10 and 50 kg m⁻³ did not affect growth, researchers can conduct trials within this a range of crowding densities but dissolved oxygen levels need to be controlled. Future studies could be designed to determine the loading density that ensures adequate oxygen supply.

Juvenile kob grew at an average rate of 1.3% of body mass d⁻¹. This is slower than growth rates reported by Collett *et al.* (2008a) of 2.05% of body mass d⁻¹ and Collett *et al.* (2008c) of up to 3.71% of body mass d⁻¹. In addition, the FCR values for the current study of 2.4 were poorer than the value of 0.91 reported under similar environmental conditions (Collett *et al.* 2008c). The feeding intensity and condition factor were, however, very similar to those in the studies mentioned above. A possible explanation for differences between the studies could be the diet. The feed used in the current study was manufactured by the same company and had the same nutritional composition as that used in previous studies, but it was a 3-mm floating pellet instead of a 1.8-mm sinking pellet. Future trials aiming to compare growth rates between studies should use the same diets and pellet sizes in all studies to avoid speculation over the cause of different growth rates.

Aggressive behaviour may be density-dependent in some fish species such as *Salmo salar* (Fenderson & Carpenter 1971) and *Clarias gariepinus* (Kaiser *et al.* 1995) and may be reduced as crowding density increases. Social hierarchies within tanks may influence aggression and suppress normal feeding behaviour (Brett 1979, Fessehaye *et al.* 2006). No behavioural observations were performed but the low mortality in this experiment and the lack of differences in mortality between treatments suggest that any possible density-related change in social hierarchy did not affect cannibalistic behaviour. Furthermore, the similarity in feeding intensity between treatments suggests that feeding behaviour was also not affected by crowding density.

Culture of fish at a high crowding density may increase the risk of mass mortality in the case of system failure. For example, neither the inflow of oxygen-rich water, nor the use of oxygen diffusers in the tanks maintained dissolved oxygen concentrations above 25% saturation at the high crowding densities if used in isolation. The need for good management of water quality, and

particularly dissolved oxygen, may restrict the intensive culture of *A. japonicus* to locations with access to electricity and artificial aeration facilities. Hence the crowding densities suggested here may not be applicable to cage-culture operations and this would need to be tested in future studies. The costs of production and maintenance of the culture environment must be taken into account when developing facilities for culture of dusky kob. If high crowding densities are to be employed in aquaculture, culture technology must be optimised and sufficiently reliable to ensure that farming is economically viable and that fish survive brief system failures.

In conclusion this study showed that crowding densities of up to 50 kg m^{-3} may be employed for the culture of juvenile *A. japonicus* of the size range tested here and that dissolved oxygen concentrations as low as $4.4 \text{ mg L}^{-1} \text{ O}_2$ did not inhibit growth. This tolerance of high crowding densities and relatively low dissolved oxygen concentrations under culture conditions, favours its choice as a candidate aquaculture species.

CHAPTER 6

GENERAL DISCUSSION

This study has addressed the effect of temperature, light intensity, feeding regimen and crowding density within the context of evaluating the farming potential of dusky kob, a promising aquaculture candidate species. By summarizing the relevant literature and results from chapter 2 to 5, this discussion will address the relevance of each of these variables for the production of dusky kob for both commercial and research purposes and provide suggestions for their culture in intensive systems.

Temperature

Temperature is an important variable that controls growth of fish both under culture conditions (Fry 1971) and in the wild. Chapter 2 showed that the optimal temperature for growth coincided with the thermal preference of dusky kob (Bernatzeder & Britz 2007) which suggests that the thermal preference may be a good predictor of the temperature at which growth is maximised. The natural distribution range of *A. japonicus* along the east coast of South Africa is within the temperature range of 12 - 28°C (Whitfield 1998). Juvenile *A. japonicus* (< 200 mm) are believed to be most abundant in the upper reaches of estuaries (Griffiths 1996) where temperature frequently ranges from 25 to 28°C (pers. obs.). The best temperature for growth was within this temperature range. Thus, for future tests on estuarine aquaculture candidate species, an understanding of their habitat preference could contribute to the selection of experimental culture conditions.

FCR was best at a lower temperature than that resulting in best growth. Growth in fishes is generally maximised at a higher temperature than that achieving best food conversion efficiency (Jobling 1996; Deacon 1997; Van Ham, *et al.* 2003, Person-Le Ruyet *et al.* 2004). Thus, the testing of both growth and FCR-values under the same conditions can provide an estimate of the best rearing conditions when taking into account economic factors such as time required to grow

fish to market size and production costs. The best temperature for grow-out must be determined based upon an economic trade-off between the increased costs associated with heating water to the required temperature and increased feeding costs as maximum growth at higher temperatures is reached. Keeping fish at 25.3°C, the estimate for best growth, will result in a 11.1% better growth rate and a 18.7% poorer FCR relative to that from fish kept at the temperature at which FCR was best. This would mean that while market size would be reached in shorter time at the temperature for best growth, the increased cost of food may need to be considered. The best temperature should be estimated depending on ambient temperature and food costs.

The results of this experiment may influence both the choice of locality and type of facilities that are developed for *A. japonicus* culture. For research and development, knowledge of the temperature required for best growth of juvenile *A. japonicus* allows experiments to be conducted on other variables using that temperature. In addition, chapter 4 showed that aspects of the feeding regimen, such as feeding frequency and feeding intensity also influenced FCR values and thus need to be taken into account in this and possibly related species when determining the best production strategy.

Light intensity

Following the determination of the effect of temperature (chapter 2) and salinity (Bernatzeder *et al.* 2008) on growth, the effect of light intensity on growth could be determined. Again, the rationale for the choice of this independent variable was influenced by the distribution of this species in South African estuaries. The absence of wild juvenile *A. japonicus* from the non-turbid Swartvlei and Knysna estuaries (Whitfield & Kok 1992) contrasts the relative abundance of this species in the Great Fish estuary in South Africa suggesting a preference for turbid systems as nursery areas (Griffiths 1996). In addition, it was hypothesised that light intensity would affect the growth of juvenile *A. japonicus* due to the difference in light intensity between their highly turbid natural environment and the clear mechanically filtered water characteristic of culture systems. There was no difference in growth or FCR in kob reared at 23 to 315 lux. Juvenile *A. japonicus* may thus be tolerant of both high and low light intensity common to fluctuating estuarine systems. This is supported by records of juvenile *A. japonicus* (synonymous with

Argyrosomus hololepidotus) in the Lake St Lucia, Richards Bay (Wallace & Van der Elst 1975) and Sundays (Beckley 1984) estuaries all of which are low turbidity systems (Griffiths 1996).

However, testing the effect of light intensity on growth in isolation would not allow detection of any possible interaction between this factor and feeding intensity and metabolism. Thus, this study investigated the combined effect of light intensity and feeding method on growth and FCR. It is here hypothesised that light intensity did not have an effect on feeding behaviour or metabolism in such a way that it influenced growth of dusky kob. This suggests that juvenile *A. japonicus* were tolerant of a wide range of light intensities and that the aquaculture environment can be manipulated within this range. The light intensity range included conditions common to indoor temperature-controlled culture facilities, but light intensities outside this range need to be tested in order to avoid errors from extrapolation.

Light intensity did not affect growth or FCR, but although fish fed to apparent satiation grew at the same rate as fish fed a restricted ration, feeding to satiation resulted in a significant reduction in FCR. Thus, as with the effect of temperature on growth (Chapter 2) this experiment provides data for business models related to dusky kob culture. Good growth could be achieved cost-effectively in fish fed a restricted ration. Although, the effect of wasted food on water quality was not tested in this study, it may be suggested that a reduction in feeding rate from 5.1 to 3.6% of body mass d^{-1} for this species could lead to an improvement of water quality resulting in less effort required for the cleaning of tanks because less food is wasted through defecation. This could be tested in future studies on a commercial scale where the results can be quantified from an economic perspective. In addition, this study provided information for the design of the third experiment on the effect of feeding regimen on growth, FCR and survival. Here, both feeding frequency and feeding intensity were tested at the temperature for best growth and at a light intensity within the range used in chapter 3.

Feeding regimen

Identification of the optimal temperature for growth in chapter 2 provided a range within which to study the effect of potentially limiting biotic factors on growth. Of these, the feeding regimen

that allowed best growth and FCR of juvenile *A. japonicus* was tested because results from the previous study suggested that a feeding intensity of 3.41% of body weight d^{-1} had an effect on FCR. Dusky kob were able to consume the required daily ration for good feed conversion in one daily feeding if fed to apparent satiation, but growth was inhibited at this low feeding frequency, probably due to the duration of food deprivation. As a result, it was assumed that growth was limited by feeding frequency rather than by ration size alone, provided that the daily feed requirement was met. A maximum feeding intensity of 3.85% body mass d^{-1} was estimated as the breakpoint above which growth did not increase with increased feeding rate. FCR decreased linearly as feeding intensity increased above the suggested maximum level.

In commercial culture systems fish may have unrestricted access to food or they are fed to apparent satiation. This study suggests that feeding to apparent satiation resulted in reduced feed conversion efficiency. The best growth rate of 3.75% body weight d^{-1} and most efficient FCR of 0.91 of the study were achieved in chapter 4 at the best temperature for growth under a feeding regimen of 3.41% body weight d^{-1} divided into three daily feedings. As there was no significant difference in growth between this treatment and the treatment testing the same ration in two daily feedings, it is recommended based on this experimental design that dusky kob could be fed a restricted ration at least twice daily according to their size-specific daily requirements in order to achieve maximum growth and best FCR. Since the experiment involving a range of crowding densities (chapter 5) showed the potential of low dissolved oxygen levels under culture conditions, it should be tested in on-farm experiments whether feeding kob three times per day can reduce fluctuations in water quality relative to two daily feedings.

Some growth studies may require food supply to be unrestricted to ensure that it does not become a limiting factor. Future studies may therefore use either the size-specific restricted ration recommended in chapter 4, or allow unrestricted access to food to ensure good growth.

Crowding density

Once the temperature and food requirements for maximum growth had been established, the effect of crowding density on growth of juvenile *A. japonicus* was tested using these culture

conditions in a commercial operation. Three stocking densities were tested, ranging from the minimum stocking density recommended for best growth (Pirozzi *et al.* 2008) and that used in chapters 2-4, to the maximum crowding density used by industry to culture juvenile *A. japonicus* within this size range. There were no differences in growth or FCR between stocking densities of 10, 30 and 50 kg m⁻³ but the growth rate in the current study was lower than that found in chapters 2-4. Culture variables were controlled as in the previous experiments with the exception of dissolved oxygen concentration and food pellet size. Dissolved oxygen concentration within the ranges recorded did probably not cause of the differences in growth between this study and those in chapters 2-4 (Fitzgibbon *et al.* 2007). The change in feed type, however, may have caused a relative reduction in growth in this study. Similar observations were made by Bernatzeder (unpublished data) when comparing these two feed types in trials on juvenile *A. japonicus*. Future studies should avoid any changes in culture variables or diet to ensure that conditions are replicated as closely as possible between studies.

Wild populations of *A. japonicus* exhibit shoaling behaviour (Griffiths 1997). In future studies, by observing the behaviour of this species in tanks, it could be tested if shoaling behaviour may be the reason for crowding densities of up to 50 kg m⁻³ allowing good growth under commercial conditions. These findings contribute to culture system design for juvenile *A. japonicus*. In addition, researchers can conduct trials for optimisation of other variables under crowding densities up to 50 kg m⁻³. It is, however, likely that crowding density will limit growth of juvenile *A. japonicus* at densities higher than those tested in this study. This may be as a result of crowding density, or perhaps the combined effect of crowding density and low levels of dissolved oxygen in the water. It is hence important to gain further understanding of the tolerance of dusky kob to hypoxia in order to fully understand the effects of low oxygen levels on metabolism and growth. According to Fitzgibbon *et al.* (2007) maximum growth of *A. japonicus* can only be obtained at dissolved oxygen concentrations close to 100% saturation. Maintaining high dissolved oxygen concentrations in the water was increasingly difficult with increasing crowding density in the current study. Therefore, the practical implications of keeping fish at crowding densities higher than 50 kg m⁻³ should be considered before the effect of these densities on growth can be tested.

Conclusions

The aim of this series of growth trials was to determine culture conditions for juvenile *A. japonicus* and suggest levels of environmental variables to be used for research and development on this species and for its commercial culture. Recommendations can be made both for industry and for research purposes. Juvenile *A. japonicus* of the size range tested grew best at 25.3°C while the temperature for best FCR is 21.7°C. The best feeding regimen for growth was 3.41% of body weight d⁻¹, divided into at least two daily portions. Light intensity and crowding density, within the ranges tested, did not affect growth or FCR. It is, however, recommended that *A. japonicus* be cultured within the ranges tested in these studies until further research is conducted that may determine whether or not these may act as important culture variables under different conditions.

Juvenile *A. japonicus* grew well under aquaculture conditions. They readily accepted an artificial diet, grew at between 1.4 and 3.7% of body weight per day and were tolerant of dissolved oxygen concentrations as low as 4.4 mg l⁻¹ O₂. As an aquaculture candidate, *A. japonicus* compares well to *S. ocellatus*, an established sciaenid cultured on the United States east coast (Lee & Ostrowski 2001) and in China (Hong & Zhang 2003). *S. ocellatus* grew well at between 25 and 28°C (Douillet & Pickering 1999) within a salinity range of 6-28 g L⁻¹ (McGoogan & Gatlin 1999). Optimum feeding regime for this species has been found to be between 5 and 6% body weight per day, divided into two daily portions (McGoogan & Gatlin 1999 and growth rates of 1.8 – 2.2% body weight per day have been reported (Sandifer *et al.* 1993). This study showed that *A. japonicus* has good potential as a candidate for South African mariculture.

Few researchers have had the opportunity to conduct consecutive trials under laboratory and commercial conditions. This study consisted of a series of laboratory-based growth trials which were followed by a commercial-scale study. While differences in dissolved oxygen concentration and diet, i.e., pellet size, made a comparison between the laboratory trials and the commercial trial difficult, some points can be drawn from the results of these trials and the experience gained while conducting them. For commercial culture, securing a sufficiently large number of fish and facilities to perform large-scale trials is expensive and thus the scope of these trials is limited. It is also difficult to work with a large number of replicates due to limitations on farms. Thus, it

will always be challenging to conduct multifactorial studies. These, are, however, important as the results from chapter 3 (light intensity combined with feeding regimen) showed. Thus, as results from large-scale studies may be more applicable to industry than laboratory trials, a combination of laboratory studies and experiments in a commercial setting may be recommended in order to detect possible interactions between independent variables. Another advantage of laboratory studies is the opportunity to reduce costs. For example, the determination of the optimal temperature for growth is important, but culture facilities must be equipped to provide the best conditions by heating large quantities of water. Here, predictions derived from controlled experiments can help reducing the costs for studies on a commercial scale. In addition, it is important to supply sufficient oxygen in the water when farming kob at high stocking densities on a commercial scale. Thus, the results from the commercial-scale study made it possible to quantify this limiting factor, a finding that may have been missed if studies had been done only under controlled laboratory conditions.

Considering that some independent variables interacted with each other as was seen in the experiment using combinations of light intensity and feeding method, future studies should include multifactorial designs where possible. For example, this study did not test a combination of salinity and oxygen levels. Salinity was not tested here because it was considered not to influence growth (Bernatzeder *et al.* 2008), but variations in salinity combined with fluctuations of oxygen levels may influence growth and survival. In addition, examination of the effect of temperature and feeding regimen on growth of different size classes of juvenile *A. japonicus* will be useful in optimising the culture protocol for this species. The combined effect of light intensity and crowding density on feeding behaviour may also be tested to understand if these factors influence feeding behaviour in *A. japonicus* under culture conditions.

In summary, dusky kob had high survival rates under both experimental conditions and on a fish farm. The species tolerates high crowding densities and low oxygen levels, and, depending on the feeding method, juvenile dusky kob can grow at a rate of 3.75% body weight d⁻¹ at an FCR of 0.91.

CHAPTER 7

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APPENDIX A

Papers emanating from this thesis:

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

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Collett, P.D., Vine NG, and H. Kaiser (2008) The effect of crowding density on growth and food conversion ratio of juvenile dusky kob *Argyrosomus japonicus*. ?