Determining production characteristics of dusky kob, *Argyrosomus japonicus*, grown in sea cages under commercial conditions in Richards Bay, South Africa

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Abstract

With aquaculture in South Africa being in its infancy there are many questions regarding the production characteristics of potential candidate aquaculture species, one such species being the dusky kob (*Argyrosomus japonicus*). Production results were obtained from the DST SU KZN Aquaculture Development Project that was implemented and managed by Stellenbosch University from 2015 to 2017 in Richards Bay, KwaZulu-Natal. The project assessed the technical, environmental and financial feasibility of farming dusky kob in sea cages. In August 2015, 25000 dusky kob fingerlings of 9 grams each were stocked into the cages. Two size classes emerged in the growth trial; the fish reached average weights of 1580 grams and 1082 grams respectively. Total FCR calculated over the course of the production period (23 months) was 2.25 at an average water temperature of 21°C.

Fish size had no effect on fillet yield (%), but had a significant effect on fillet proximate composition. As fish size increased, lipid content increased and moisture content decreased. Initially, protein content increases as fish grew in size, although the rate of increase also declined.

Using a copper alloy net cage to farm marine fish in an attempt to reduce or eliminate biofouling caused no significant difference in metal concentrations in meat samples derived from fish grown in traditional polyester versus copper alloy net cages. The maximum concentrations measured for the various metals in meat samples were below the upper limits as set by South African, EU and USA regulations for safe human consumption.

A summary of harvesting methods used to harvest dusky kob from sea cages and the subsequent logistical cold chain, as well as general observations and recommendations regarding the production of dusky kob is provided.

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Opsomming

Akwakultuur in Suid Afrika is ‘n jong en ontwikkelende industrie met verskeie potensiële kandidaat spesies wat ondersoek word om produksie verwante vrae te beantwoord; een sodanige spesie is die kabeljou (*Argyrosomus japonicus*). Produksie resultate is verkry vanaf die DST SU KZN Aquaculture Development Project wat deur Stellenbosch Universiteit geïmplementeer en bestuur was gedurende 2015 tot 2017 in Richardsbaai, KwaZulu-Natal. Die projek het die tegniese, omgewings en finansiële uitvoerbaarheid van kabeljou produksie in hokke geassesseer. Gedurende Augustus 2015 is 25000 kabeljou vingerlinge van gemiddeld 9 gram elk in ‘n hok geplaas. Tydens produksie (23 maande) het twee grootte klasse ontstaan wat onderskeidelik ‘n gemiddelde massa van 1580 gram en 1082 gram bereik het. Die voeromset bereken oor die totale produksie tydperk was 2.25 by ‘n gemiddelde water temperatuur van 21°C.

Vis grootte het geen effek gehad op die filet uitslag-persentasie nie, alhoewel, dit het ‘n noemenswaardige effek gehad op die filet vleis-samestelling. Soos vis grootte toegeneem het, het lipied inhoud toegeneem en vog inhoud afgeneem. Aanvanklik neem proteien inhoud toe met ‘n toename in vis grootte, maar die tempo van toename verminder.

Die gebruik van ‘n koper allooi hok vir die uitgroei van die vis as ‘n poging om die ontwikkeling van bio-organismes “biofouling” te verminder of verhoed het geen noemenswaardige verskil veroorsaak in die metaal konsentrasies in vleis monsters afkomstig van vis in ‘n koper allooi hok teenoor vis in ‘n tradisionele polyester hok nie. Die maksimum konsentrasies wat gemeet is, was laer as die voorgeskrewe maksimum limiete vir veilige menslike gebruik soos gespesifiseer deur die Suid Afrikaanse, EU en USA regulasies.

Die oes metodes wat gevolg was om die kabeljou te oes met die daaropvolgende verkoeling en vervoer is opgesom, asook algemene aanmerkings en voorstelle rakende kabeljou produksie.

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Chapter 1: General Introduction

1.1. Overview

The South African aquaculture industry is still in its infancy, although significant development has been made in certain sectors of the industry. According to the Food and Agriculture Organization of the United Nations (FAO) the total aquaculture production in South Africa during 2015 and 2016 was 7430 tons and 7994 tons respectively. The total value of aquaculture production during these years were USD 52 million for 2015 and USD 46 million for 2016.

During 2016 freshwater aquaculture accounted for 1834 tons (23 % of total production) and a value of USD 4.6 million with production being dominated by rainbow trout (*Oncorhynchus mykiss*, 1500 tons), followed by Mozambique tilapia (*Oreochromis mossambicus*, 330 tons) and marron crayfish (*Cherax tenuimanus*, 4 tonnes).

On the marine side production is dominated in volume by aquatic plants and in value by abalone. During 2016 total marine aquaculture production in South Africa amounted to 6160 tons with a value of USD 42 million. Of this aquatic plants contributed 2500 tons valued at USD 459 000. Abalone production during 2016 amounted to 1500 tons with a value of USD 37 million. Abalone production only contributed 24 % in quantity but 89 % in value to the total aquaculture production in South Africa. Other contributors to the total marine aquaculture production during 2016 were mussels (*Mytilus galloprovincialis*, 1800 tons), oysters (*Crassostrea gigas*, 280 tons) and finfish (various species, 80 tons).

This study reports on the results obtained during the DST SU KZN Aquaculture Development Project that was implemented and run by Stellenbosch University during 2015 and 2017 (Chapter 3). Emphasis was placed on the infrastructure used, fish husbandry procedures throughout the production period, fish performance, data collection and environmental parameters monitored.

The effect that fish size might have on the proximate composition of fish flesh and fillet yield (%) was examined in Chapter 4.

As part of the DST SU KZN Aquaculture Development Project, a copper alloy net cage was tested to determine its effectiveness in reducing or eliminating the growth of biofouling organisms (Chapter 5). Biofouling has a profound effect on aquaculture operations, as it is costly to remove and affects the operation in a negative way.

The harvest procedure and cold chain followed during the project is described in Chapter 6.

Chapter 7 contains general observations and recommendations regarding the farming of dusky kob (*Argyrosomus japonicus*) in particular, with special reference to their feeding behavior.

1.2. References

FAO. 2018. (Food and Agriculture Organization of the United Nations). Fisheries and Aquaculture Department, Global Aquaculture Production Statistics (online query). http://www.fao.org/fishery/statistics/global-aquaculture-production/en

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Chapter 2: Literature Review

2.1. Commercial culture of dusky kob (*Argyrosomus japonicus*)

Research into developing techniques for the farming of dusky kob in South Africa was initiated in 2000. Three private companies have participated in the development of kob aquaculture. During 2014 approximately 160 tons of kob was produced in South Africa (DAFF, 2016). Generally, the commercial hatchery production of fingerlings appears to be well established in South Africa; the grow-out technologies are, however, at the research and development and pilot commercial level.

Dusky kob and other *Argyrosomus* species are also produced on large scale in other parts of the world, especially in Egypt and Europe. In 2010, Egypt produced 12 246 tons of *Argyrosomus regius*, however this figure declined to 5 884 in 2014. Turkey was the second largest producer of *A. regius* with a production of 3 281 tons in 2014. In Mozambique a total of 150 tons of *A. japonicus* was produced during 2014 (FAO, 2017).

Generally, *Argyrosomus* species appear to be well suited to marine aquaculture as it matures at a late age (Griffiths, 1996; Heemstra and Heemstra, 2004), and are tolerant to various conditions of temperature and salinity (PIRSA, 2001; Collet, 2007). Kob species appears to have typically robust larvae. Biologically kob are suited for aquaculture, with relatively fast growth rates and acceptable food conversion ratios in experimental systems. Processed kob produce a good fillet size with a high yield and the flesh is of excellent quality (Guy and Nottingham, 2014).

The use of sea cages appears to be the preferred method for the grow-out of kob in both Australia and Europe. As the species can withstand reasonably low salinities, they also appear to be suitable candidates for inland saltwater aquaculture using ponds and tanks.

2.2. Classification of dusky kob

Dusky kob, *Argyrosomus japonicus*, has the following classification:

Phylum:	Chordata
Subphylum:	Vertebrata
Class:	Actinopterygii
Order:	Perciformes
Family:	Sciaenidae
Genus:	*Argyrosomus*
Subgenus:	*japonicus*

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2.3. External anatomy of dusky kob

A. japonicus closely resemble *A. inodorus* (silver kob) which makes it difficult to distinguish between the two species on external features alone. Dusky kob reach a larger size and has a deeper and shorter tail-fin peduncle (Branch *et al.* 2007). The overall body colour is silvery with a pearly-pink sheen on the head, flanks and dorsal area (Figure 2.1). The double dorsal fin has 10 spines followed by one spine and 26 – 29 rays. The anal fin has 2 spines and 7 rays. The body is covered with thick scales along the lateral line (Van der Elst, 2012). The axillary flap on the pectoral fin of dusky kob has a brown colour and lack scales (Heemstra and Heemstra, 2004; Van der Elst, 2012).

On the first gill arch there are 12 – 17 well developed gill rakers. Each jaw is set with strong canine teeth. The inside of the mouth is white in juveniles and more yellow in larger adults. Underwater the fish normally show a series of silver spots along the lateral line.

Figure 2.1: A dusky kob (*Argyrosomus japonicus*) specimen farmed at the DST SU KZN Aquaculture Development Project in Richards Bay.

2.4. Biology and distribution of dusky kob

In South Africa, *A. japonicus* occurs along the coast from Cape Town to northern KwaZulu-Natal. Globally kob occur in Australia, India, Pakistan, China, Korea and Japan (Figure 2.2) (Griffiths and Heemstra, 1995). They can be found in coastal environments, including the lower reaches of rivers, in estuaries, on rocky reefs, around ocean beaches, inside bays and on the continental shelf to a depth of 100 m (Griffiths, 1996). Adults are normally found in near-shore environments around shallow coastal reefs, rocky shores and inside estuaries. Juvenile kob are primarily found in estuaries and in the surf zone (Griffiths, 1996).

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Figure 2.2: Map displaying the distribution of *Argyrosomus japonicus* (Silberschneider and Gray, 2008).

Kob can grow to a large size and live to a relatively old age. The largest confirmed size is 181 cm with a weight of 75 kg (Griffiths and Heemstra, 1995). Griffith and Hecht (1995) reported a maximum age of 42 years based on an otolith study. Male dusky kob reach sexual maturity when 5 years old with a median total length of 92 cm. Females reach sexual maturity at 6 years of age and a median length of 107 cm (Griffiths, 1996). From August to November, kob spawn along the KwaZulu Natal coastline predominantly in near-shore environments. Spawning also takes place in the Southern and Eastern Cape areas with the highest activity from October to January (Griffiths, 1996). Kob are predatory and feed all through the water column consuming a variety of fish and squid. Juvenile kob also consume crustaceans (Silberschneider and Gray, 2008).

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2.5. Recommended environmental parameters for the production of dusky kob

Table 2.1 indicates recommended values of the important water quality parameters for the production of dusky kob as specified in the referenced literature.

Table 2.1: The recommended environmental parameters for the production of dusky kob

Parameter	Tolerance	Optimal range	References
Water temperature	15 – 30°C	24 – 26°C	PIRSA, 2001; Collet, 2007
Dissolved oxygen	> 6 mg/L	> 6 mg/L	Fielder and Heasman, 2011
Salinity	5 – 35 ppt	15 – 35 ppt	Doroudi *et al*., 2006; Fielder and Heasman, 2011
pH	7.6 – 8.2	7.6 – 8.2	Fielder and Heasman, 2011

2.6. Production systems used to culture dusky kob

2.6.1. Cages

In South Africa the use of cages to produce fish is a relatively new concept and the aquaculture industry itself is still in its infancy. Only surface gravity type cages are used in the South African aquaculture industry, both in the fresh water and marine sectors. To date, commercial offshore cage systems from Fusion Marine Limited (Fusion Marine Limited, Scotland, UK), AKVA Group (Bryne, Norway) and a few different designs originating from China have been predominantly used in SA.

The South African coastline is very dynamic with strong current and wave action, although there are some quieter bays as well. However, the entire coastline can become very rough with large swells and strong currents during stormy conditions. It is therefore imperative that the cage system used will be strong enough to withstand these conditions to provide a safe haven for the cultured fish and a stable, safe working platform for personnel.

The technology and systems to make cage aquaculture work in more challenging environments exists, although it comes at a high cost. Compared to land based aquaculture using recirculating technology, cage aquaculture also has a high capital outlay but the running costs of such an operation is far less per production output.

Cage culture operations are exposed to the elements and offer little protection to the cultured fish against parasites and diseases that could be carried by wild fish in the vicinity. Good husbandry practices and biosecurity measures are therefore necessary to ensure the health of the cultured fish and subsequently the money invested in them.

Only two operations in South Africa have cultured dusky kob using offshore cages. Both operations were research pilot projects implemented and run by Stellenbosch University. Fusion Marine Limited cages and Jeyco mooring systems were used in both operations. One operation was located 2 km offshore in Algoa Bay, Port Elizabeth. The other operation was based in Richards Bay and the cages

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located within the Richards Bay harbour. During both operations, the cage and mooring systems performed flawlessly. The system in Algoa Bay was also exposed to a one in fifty-year storm and suffered no significant damage proving the strength and resilience of these systems.

2.6.2. Recirculating aquaculture systems

Recirculating aquaculture systems (RAS systems) are used worldwide for the commercial production of aquatic products. It is used in the production of tropical and marine fish for the hobby industry as well as in large grow-out and hatchery systems for the production of food fish.

RAS systems can be divided into categories based on their complexity and water use strategies. Systems can vary from flow-through, to partial flow-through, to a complete water recirculation system. RAS systems are used for the production of both freshwater and saltwater species. The systems typically incorporate a water treatment plant that recirculates and cleans the water mechanically, biologically and sometimes chemically to maintain a high level of water quality. The high cost of operation is the biggest challenge with using a RAS system. To maximise profitability mostly high value species are farmed using these systems.

In South Africa only two operations, Oceanwise (Pty) Ltd (later called OceanChoice (Pty) Ltd) and Pure Ocean Aquaculture (Pty) Ltd, have produced dusky kob commercially using RAS systems. Both these operations were located in East London within the East London Industrial Development Zone. Unfortunately, both these operations have since closed down.

2.6.3. Ponds

Earthen ponds are used to produce fish and other aquatic organisms and plants in various parts of the world, especially in third world countries. It is considered to be more of an extensive production method and is often used for polyculture, thus farming more than one species in the same pond. Often the ponds are fertilized to increase the natural productivity of the water. The increased nutrient load supports the increased growth of algae. Fish species such as tilapia can feed and survive on the algae. Fertilization is predominantly achieved by the administering of animal manure into the water. Some ponds might even have pigsties or chicken coops suspended above the water.

In South Africa one company, Zini Fish Farms (Pty) Ltd (previously Mtunzini Aquaculture) located in Mtunzini KwaZulu-Natal, is producing dusky kob using earthen ponds. The ponds were initially constructed and used for the production of prawns since the 1980’s. For the production of kob, the ponds were dug deeper and aerator paddlewheels were added to the ponds to increase the dissolved oxygen concentration. Water to the ponds is supplied from a beach-well pump located on the Mtunzini beach just above the wave zone, as well as a pump located in the estuary nearby. Due to the relatively shallow depth, the ponds are prone to water temperature and dissolved oxygen fluctuations, especially during the warmer summer months. The company has recently upgraded their facilities to incorporate a marine finfish hatchery. They have a total of 54 dusky kob broodstock fish in their holding tanks and have started producing their own fingerlings. They have also started to produce spotted grunter

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(*Pommadasys commersonni*) as an additional species. At the time of writing, the farm was up for sale and their future existence is uncertain (personal communication, Neil Stallard from Zini Fish Farms).

2.7. Fish size and its influence on chemical composition

2.7.1. Introduction

The nutritional content of fish is generally referred to as the fish composition. It is also termed as a sign of general health, corpulence, or gonad development of an individual or group of individuals (Brown and Murphy, 1991). Proximate body composition can be defined as the analysis of water, protein, fat and ash (mineral) contents of fish (Ali *et al.*, 2005). Body composition is influenced by both internal and external factors. Internal factors refer to the biological properties of the fish, i.e. sex, life cycle, size, and state of maturity (Shearer, 1994). Environmental conditions and variations are generally regarded as external factors influencing the body composition of fish. Examples of these external factors are season and seasonal change, temperature, water quality and the availability of feed. These internal- and external factors will influence the biological processes in the fish body that determines the body composition.

For the majority of fish, the live weight proportions are roughly 70 - 80 % water, 20 - 30 % protein, and 2 - 12 % lipid (Love 1980, Weatherley and Gill 1987) although these constituent values might be well beyond these ranges in extremities (Weatherley and Gill 1987). These values can nonetheless vary between species, fish size, sexual condition, feedings, season, and activity to name but a few intrinsic and extrinsic factors (Weatherley and Gill 1987; Ali et al., 2005). Differences in the distribution of these constituents among the different tissues and organs have been noted (Weatherley and Gill 1987). Due to these differences in distribution, the proportion of visceral content, muscles and bones will also differ. These different proportions define the physiological state of an individual fish and determine its ability to successfully compete in the aquatic environment, be it through optimal foraging, reproduction, growth, tissue repair, or coping with environmentally induced stresses (Brown and Murphy, 1991).

2.7.2. Defining the nutritional content of fish

The nutritional content or composition of fish are similar to those of other animals and consists of water, proteins, fats, carbohydrates and minerals (generally referred to as ash) (Weatherley and Gill 1987). Carbohydrates and minerals normally occur in small amounts (Weatherley and Gill 1987). As mentioned earlier the general composition of fish consists of 70 - 80 % water, 20 - 30 % protein, and 2 - 12 % lipid (Love, 1980; Weatherley and Gill, 1987). The water content of a fish is a good indicator of the relative content of energy, proteins and lipids, the lower the percentage of water, the greater the protein and lipid contents and higher the energy density of the fish (Salam and Davies, 1994). Thus by measuring the relative amount of water in the fish, one can obtain relatively good estimates of the energy, fat and lipid contents (Salam *et al*., 2001).

Generally, fish store or reserve surplus energy in the form of visceral fat (Love, 1970). A change in the proximate composition generally reflects the storage or depletion of these fat reserves (Brown and

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Murphy, 1991). Each fish species differs in the way that they store lipids for energy and can be categorized accordingly. Lean fish primarily store lipids in the liver, and the fatty species store lipids in fat cells throughout the body (FAO, 1995). According to George and Bhopal (1995), white fleshed marine fish (cod, coley, plaice and haddock) have a very low fat content compared to oily fish species (trout, salmon, mackerel, herring, pilchard and sardine) which can have a fat content up to 25 percent. George and Bhopal (1995) further state that the fat content depends on whether the fish is reared through aquaculture or wild. Farm raised fish is fed a specific feed formulation to promote optimal growth and a reduced feed conversion ratio (George and Bhopal, 1995). The different feed formulations will affect the body composition properties. The nutritional value of the fat is determined by the amount of fat and by its fatty acid composition. Both of these parameters are influenced by the diet of the fish. Typically, fish lipids contain long chain fatty acids of which the polyunsaturated omega-3 fatty acids are of special interest to consumers (Mustafa *et al.*, 2006; Tang *et al.*, 2008). The polyunsaturated fatty acids in fish (especially marine fish) have important health benefits for humans and can prevent the onset of various diseases if they form part of the human diet (FAO, 1995).

The vitamin and mineral composition of fish varies greatly between species and can also differ during the different seasons. Most fish are generally a good source of the B vitamins. Fatty fish species contain more of the fat soluble A and D vitamins in their flesh than lean fish species. Also, vitamins A and D are found in relatively high concentrations in the livers of species such as cod and halibut. Fish are also regarded as a good source of phosphorus, calcium, iron, copper and selenium. Marine fish species generally have a higher content of iodine. Fish contain relatively low concentrations of sodium and are therefore suitable for people who need to follow a low sodium diet. (FAO, 1995)

2.7.3. Fish size as influencing factor

Fish go through several life stages when they grow from egg to mature fish. Salmon species generally have more growth phases than most fish. When energy intake is limited through the diet, juvenile fish tend to use most of the energy for growing and very little for energy storage. This is evident when comparing wild and farm raised juvenile fish of equal weight, where the composition of the wild fish consists of a lower percentage of lipids and a higher percentage of moisture (Shearer, 1994). In juvenile steelhead trout (*Salmo gairdneri,* later changed to *Oncorhynchus mykiss*) it was observed by Sheridan *et al.* (1985) that the lipid level declined before the smoltification stage started. It was thought that the decline in lipid level was due to energy use during migration and the mobilization of energy reserves for osmoregulation as the decline occurs prior to saltwater entry. In contrast, Plotnikoff *et al*. (1984) found no association between body lipid content and seawater tolerance in chinook salmon. Research by Fujioka *et al*. (1991) on different salmon species concluded that the change in lipid level preceding the smoltification phase was due to energy use during migration and due to the anticipation and pre-adaptation for seawater entry.

The smolts show an increase in total body protein after the smoltification phase and sea water entry. The protein content continues to rise as the fish grows and new muscle cells are formed. Existing muscle cells increase in diameter as the fish grows thereby contributing to the continual increase of

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total body protein (Shearer, 1994). As the fish gets larger, the rate of the increase in percentage protein in relation to the increase in weight slows down up to a point that the percentage protein becomes relatively constant (Shearer, 1994).

Compared to the increase in protein content, the increase in percentage lipid in relation to the decrease in percentage water can be much greater. During this stage of fish growth, the fish grows faster in weight than in length (Shearer, 1994), consequently muscle mass increase at a faster rate than bone mass. The result is a decline in phosphorus and calcium concentrations although the total actual mineral content is still increasing (Shearer, 1994). The findings by Salam and Davies (1994) correspond with these as they also concluded that as the fat and protein percentages increase, the water percentage decrease while the ash percentage remains constant. Their results below demonstrate the relationship between wet body weight and the different constituents for the northern pike, *Esox Lucius* (Figure 2.3).

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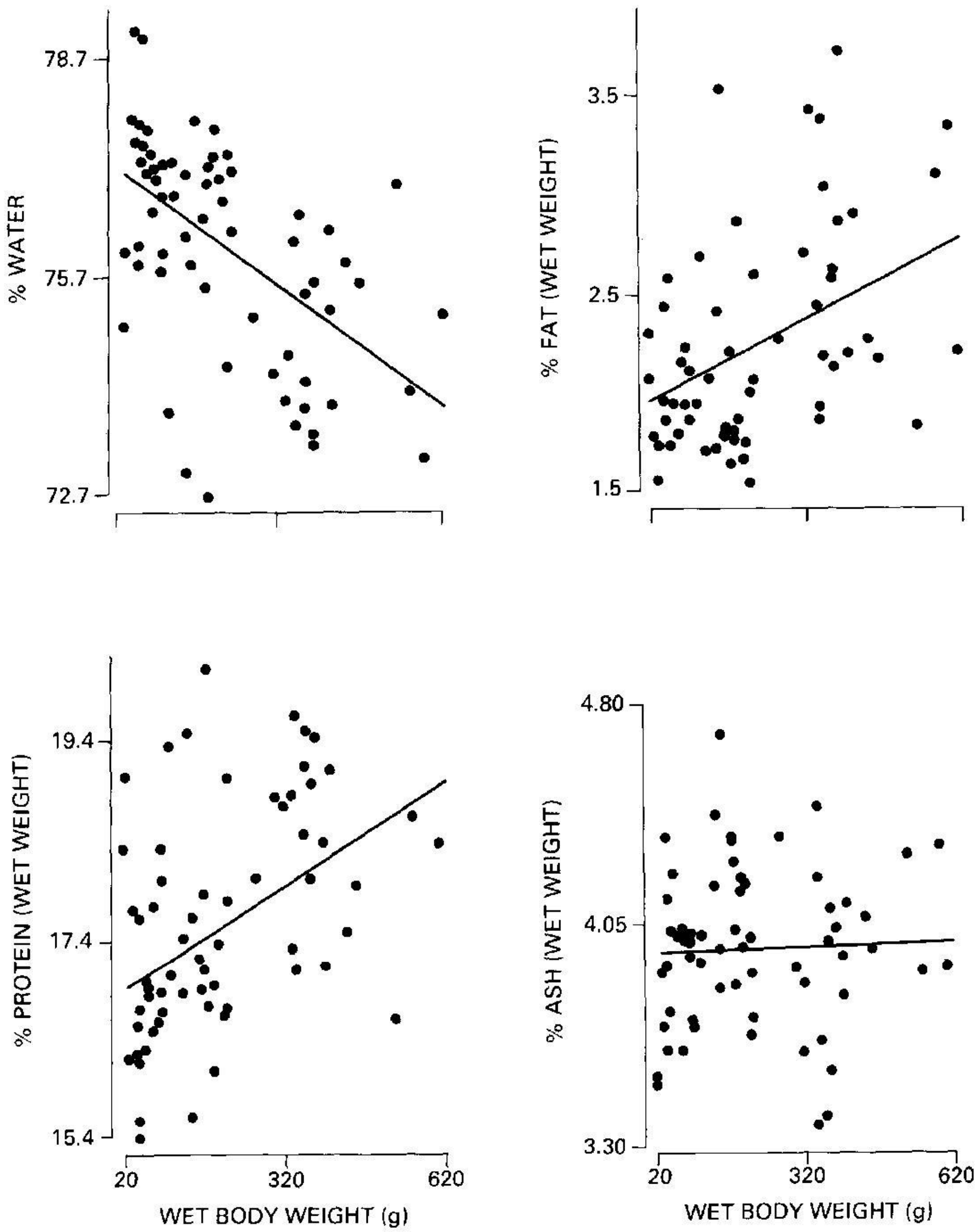

Figure 2.3: The relationship between wet body weight and the different constituents, water, fat, protein and ash (Salam and Davies, 1994).

Fish size also has an indirect effect on the nutritional content of fish. As fish reach a certain size and become sexually mature they start to use some of the energy and protein to produce eggs and milt. Saliu *et al*. (2007) found that larger fish species (with a greater mean standard weight and length) had higher percentage values of both protein and fat compared to smaller fish species (with a smaller mean standard weight and length). This difference due to specific species size was also noted in *O. mossambicus* by Salam *et al*. (2001) and they concluded that body weight and length significantly affect the respective water, fat, protein and ash constituents.

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2.7.4. Other factors influencing body composition of fish

All fish species experience seasonal changes in certain bodily characteristics. Fish may appear thinner, floppier and less lively than normal, especially after spawning (Pedrosa-Menabrito and Regenstein, 1990). The utilization of tissue reserves during sexual maturation has been documented in several fish species in the natural environment (Nassour and Léger, 1989). This causes the flesh to be more watery, less firm and generally have a lower protein and/or fat content (Pedrosa-Menabrito and Regenstein, 1990). Tissue reserves are transferred to the gonads to produce eggs and milt in the different sexes respectively. According to Love (1970), protein and fat accumulate in the growing oocytes during ovarian growth. The protein and fat comes from the fish's diet and are also mobilized from the carcass, liver and gut; but the specific origin for mobilization differs from species to species (Love, 1970; Nassour and Léger, 1989; Shearer, 1994).

Different diets as well as the composition of the diets can have a significant effect on both fresh water and salt water fish species (Tang *et al*., 2008). This is especially noticeable when comparing wild and cultured fish. The difference in body composition can therefore be ascribed to the differences in diet types. Cultured fish are fed formulated pellet feeds whereas wild fish's diet consist of algae, insect larvae, crustaceans, invertebrates, fish, etc. (Tang *et al*., 2008). According to Shearer (1994), it appears to be the energy intake and not the dietary lipid level that influence the whole body lipid stores. As mentioned earlier, ash levels do not vary greatly and is present in relatively small amounts. If ash levels appear to be lower than normal it could be due to a mineral deficiency in the diet (Shearer, 1994).

Starvation can lead to significant changes in body composition due to tissue reserves being used. Glycogen stores in the liver initially increases and then become depleted very rapidly. Muscle protein increases for a short while as liver proteins are moved to the muscles to be stored. The lipids contained in the muscles of fatty fish is replaced by water and used for energy. Proteins are catabolized and utilized as energy once the lipid stores have declined (Love, 1970; Shearer, 1994).

2.8. Aim of the study

This study will firstly report on the production performance of dusky kob grown under commercial cage culture conditions in South Africa (Chapter 3). The production results were obtained from The DST SU KZN Aquaculture Development Project that was implemented and managed by Stellenbosch University and was based in Richards Bay, KwaZulu-Natal. The pilot project specifically investigated growth rates; food conversion ratios; survival rates; the technical, financial and environmental feasibility of cage farming in Richards Bay; training opportunities and local aquaculture industry development. The current study reports on some of these aspects.

In addition, the study aimed to investigate the effect of dusky kob fish size on fillet yield (%) and proximate composition, as both attributes have an effect on product quality (Chapter 4).

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The study also evaluated the possible metal bioaccumulation effect of keeping fish in an aquaculture cage net made from a copper alloy versus an industry standard polyester net. The copper alloy net was tested as part of The DST SU KZN Aquaculture Development Project to evaluate its effectiveness in reducing the growth of biofouling organisms and thus reducing the operational costs associated with the cleaning of nets (Chapter 5).

Implementing and adhering to an effective cold chain ensures product quality is maintained from the production site to the processing facility. Chapter 6 examined the methods and procedures followed during the harvesting of dusky kob and the subsequent cold chain thereafter at the DST SU KZN Aquaculture Development Project in Richards Bay.

Chapter 7 summarized the general observations and recommendations relating to the production of dusky kob in sea cages, with specific reference to the DST SU KZN Aquaculture Development Project.

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Chapter 3: Dusky kob production – Performance under cage culture conditions

3.1. Introduction

This chapter reports on the results of the DST SU KZN Aquaculture Development Project that was run by Stellenbosch University and based in Richards Bay, KwaZulu-Natal (KZN), South Africa. The project was a collaborative undertaking between the Department of Science and Technology (DST), the Department of Agriculture, Forestry and Fisheries (DAFF) and Stellenbosch University (SU) to determine the technical, environmental and financial feasibility of farming dusky kob, *Argyrosomus japonicus*, in sea cages in Richards Bay in KwaZulu-Natal. This pilot project was funded by DST and production commenced in August 2015 with a stocking of 25000 dusky kob fingerlings. The project was accepted as an Operation Phakisa project in 2014.

The project involved the grow-out of a single batch of fish to a targeted weight of 2.0 kg. The estimated production target during the pilot project was 50 tons (over a 19-month period).

The specific objectives of the pilot project were:

- To evaluate dusky kob growth rates, food conversion ratio (FCR) and survival under commercial sea cage culture conditions
- To demonstrate the suitability of high density polyethylene (HDPE) sea cages, mooring technology and husbandry procedures for application in Richards Bay in KZN
- To demonstrate the environmental sustainability of sea cage aquaculture in Richards Bay
- To provide a platform for the training of personnel in all fish and cage husbandry methods
- To catalyze the development of commercial marine finfish sea cage aquaculture in KZN

The project was successfully completed and has since been decommissioned; however, there is a possibility of a follow-up project and potential commercial development by an established aquaculture industry company in partnership with a local investor group.

This chapter will report on some of the production results acquired at the pilot project, with special reference to: dusky kob growth, FCR, survival and husbandry. In addition, this chapter will also report on the water quality parameters measured at the cages during the production trial.

3.2. Material and Methods

3.2.1. Cage equipment and project location

Four Fusion Marine Aquaflex surface gravity type cages (Fusion Marine Limited, Scotland, UK) were installed on a nearshore site leased by SU from Transnet National Ports Authority in the Port of Richards Bay (Figure 3.1). The cages had a circumference of 50 m, the nets were 5 m deep and had a volume of 1000 m^3. One three-ring cage and three two-ring cages were deployed, with cages utilising 250 mm diameter HDPE flotation pipes (Petzetakis Africa, South Africa). Cages were moored using an orthogonal paired, drag embedment mooring system (Jeyco, Australia) arranged in a 4 x 1 grid. Anchor chains and mooring lines were supported by separate cushion buoys. Flipper Delta anchors (Anchor Industries, South Africa) were used to anchor the mooring system. The anchor rope lengths were altered to facilitate installation between a sand bar and the shipping channel (Figure 3.2). The cage site had an average water depth of 18 m and a maximum tidal fluctuation of 2.47 m.

Figure 3.1: The four Fusion Marine Aquaflex cages installed on the project site.

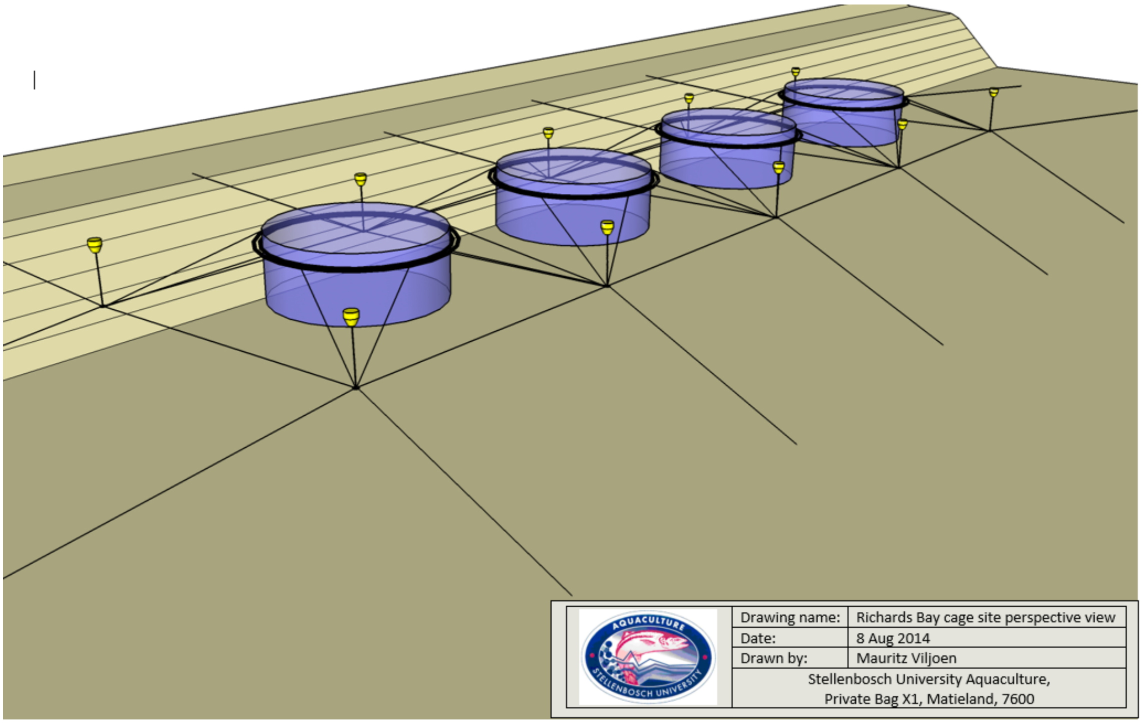

Figure 3.2: A perspective view of the cages and mooring system as moored next to a sand bar.

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The project utilized 5 m deep cage nets (Alnet (Pty) Ltd, South Africa; Universal Nets (Pty) Ltd, Australia) manufactured from knotless polyester netting fitted with a lead line. Predator and bird nets (Alnet (Pty) Ltd, South Africa) manufactured from knotted polyethylene material were also used. The bird nets were supported over the cages by floating HDPE bird net support structures. Additionally, a single copper panel net was custom built (Advance Africa, South Africa) and deployed from the three ring cage for an evaluation of its potential to reduce bio-fouling and thereby reducing the requirement for net changes or net cleaning.

3.2.2. Fingerlings and Stocking

Fingerlings for the project were spawned from wild broodstock held in a recirculation aquaculture system operated by Pure Ocean Aquaculture (Pty) Ltd in East London. Metamorphosed juveniles of 0.3 g were transported by road in 1500 L tanks to the Mtunzini Aquaculture (Pty) Ltd (now called Zini Fish Farms (Pty) Ltd) hatchery in Mtunzini for on-growing in an indoor green-water tank system to 9 g. From here they were transported by road and boat to the project site for stocking.

The 25000 fingerlings of 9 g (Figure 3.3) were initially stocked into a 5 m diameter inflatable cage (manufactured by ARK Inflatables, South Africa) positioned within one of the larger cages over a 20-day period that commenced on the 14th of August 2015 (Figure 3.4). The small fish were then transferred into the bigger cage on the 10th of September 2015. All the fish remained in this cage until grading started on the 6th of October 2016 (14 months later) where the fish were divided between the four cages according to size. The grading (Figure 3.5) resulted in cage 1 and 2 being stocked with smaller fish with an average weight of approximately 570 g and cage 3 and 4 being stocked with bigger fish with an average weight of approximately 850 g. Due to the dusky kob's high susceptibility to handling stress, grading was limited to only one event, i.e. each fish was only graded once during the production period.

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Figure 3.3: The stocked fingerlings at an average size of 9 grams.

Figure 3.4: One of the project workers, Mr Tsepo Vilakazi, feeding the fingerlings in the ARK inflatable cage positioned within one of the bigger cages.

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Figure 3.5: Dusky kob being graded in small and large size classes.

3.2.3. Feed and Feeding

In this study only feed manufactured locally in South Africa were used. A kob feed supplied by Avi-Products (Pty) Ltd (Pietermaritzburg) were used for the first 2 months since the stocking of the fingerlings. During the hatchery phase the fingerlings were weaned onto the Avi feed and were therefore accustomed to this feed when released into the cages. For the rest of the production cycle the fish were fed feed manufactured by Montego Pet Nutrition (Pty) Ltd in Graaff-Reinet. The compositions of both feeds as supplied by the manufacturer are displayed in Table 3.1. The feed size was increased periodically from an initial crumble to a 9 mm pellet. The fish were fed by hand, twice a day, at first and last light (Figure 3.6). Feed use was recorded on a daily basis with the feed being weighed on either a platform scale (AFS-150, UWE Scales, South Africa) or hanging scale (OCS-500, UWE Scales, South Africa).

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Figure 3.6: One of the project workers, Mr Tsepo Vilakazi, feeding the fish at first light.

Table 3.1: The feed composition (%) of both feeds used during the DST SU KZN Aquaculture Development Project as provided by suppliers.

Diet	Kob pellet by Avi-Products (Pty) Ltd	Kob Grower pellet by Montego Pet Nutrition (Pty) Ltd
Crude protein	40.0	44.0
Crude fat	10.0	11.0
Ash	6.8	7.5
Fibre	2.8	2.3

3.2.4. Growth Sampling, Cage Maintenance and Harvesting

Growth samples (on average 68 fish) were taken approximately every 4 to 6 weeks depending on weather and sea conditions. The fish were collected from the cages using a cast net (Figure 3.7), placed in holding tanks supplied with oxygen and freshly pumped sea water and subsequently weighed individually on an electronic scale (Ohause Valour 2000 XW 6 kg, Weighcomm Consultancy (Pty) Ltd, South Africa). After weighing the fish were returned to the cages. During the latter part of the trial, growth samples were taken less frequently due to time constraints and unfavorable weather conditions.

Maintenance activities on the mooring system and the cleaning of nets (Figure 3.8) were done periodically as required. The initial cleaning of the first net was accomplished by doing a net change which unfortunately induced high levels of stress in the fish. A high number of mortalities were experienced in the days following the event. Since then nets were cleaned *in-situ* using scuba diving equipment (Figure 3.9) and a high pressure washer (B16-250, Kränzle South Africa (Pty) Ltd, South Africa).

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Figure 3.7: The project manager and author, Mr Mauritz Viljoen, collecting dusky kob for growth sampling using a cast net.

Figure 3.8: A predator net being cleaned by the project staff next to an empty cage using a high pressure washer.

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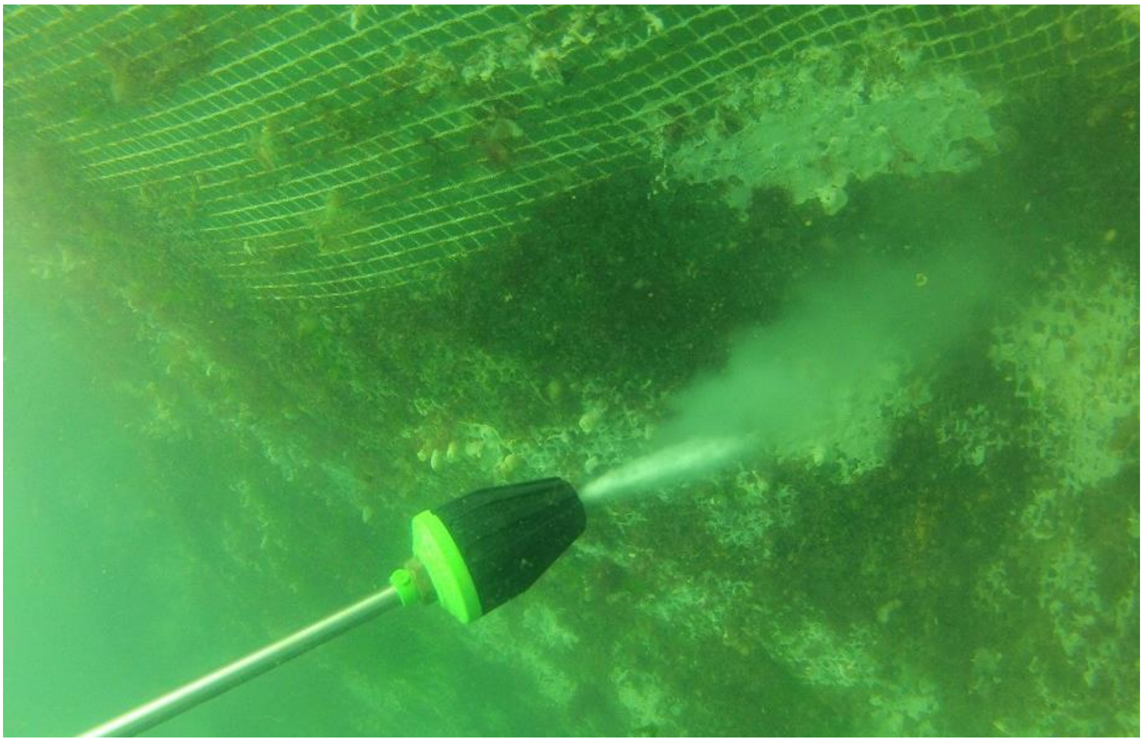

Figure 3.9: A cage net being cleaned *in-situ* using scuba diving equipment and a high pressure washer.

All the fish were harvested between June 2017 and July 2017 (24 months old) which amounted to a total of 21.8 tons. A sweep net (Alnet (Pty) Ltd, South Africa) was used to crowd the fish (Figure 3.10) during grading and harvesting. When crowding the fish, oxygen was added to the water by means of an air-ring placed inside the net. Fish were harvested out of the sweep net using a braile net (Alnet (Pty) Ltd, South Africa and Fusion Marine Limited, Scotland, UK) attached to a crane mounted on the work boat (Figure 3.11). From here the fish were placed into a harvesting bin containing AQUI-S anesthetic (Aqui-S New Zealand Ltd, Lower Hutt, New Zealand) at a concentration of 15 mg/L. The fish were then rinsed in clean seawater and transferred to an ice-slurry (Figure 3.12).

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Figure 3.10: A sweep net used to crowd the dusky kob during grading and harvesting.

Figure 3.11: Dusky kob being harvested out of the sweep net using a braile net attached to a crane on the work boat.

Figure 3.12: The project staff with some of the dusky kob harvested.

3.2.5. Data Collection and Analysis

Feed conversion ratio (FCR) was calculated using the following formula:

$$Feed\ conversion\ ratio = \frac{Feed\ consumed\ (kg)}{Weight\ gained\ (kg)}$$

Weight gained was calculated using the following formula:

$$Weight\ gained\ (kg) = Harvest\ weight\ (kg) - Stocking\ weight\ (kg)$$

Stocking density was calculated using the following formula:

$$Stocking\ density\ (kg/m^3) = \frac{Total\ weight\ of\ fish\ in\ cage\ (kg)}{Total\ cage\ volume\ (m^3)}$$

Water temperature was measured using a Hoboware automatic water temperature data logger (Onset, Bourne, Massachusetts, USA) installed at 6 m below the water surface. The logger was programmed to record water temperature at 30 minute intervals.

Dissolved oxygen was measured on a daily basis inside each of the cages using an Oxyguard Polaris hand held oxygen meter at a depth of approximately 2.5 m below the water surface.

Water turbidity was measured on a daily basis using a secchi disc attached to a line marked with depth measurements. The secchi disc was made from acrylic plastic, cut into a circular shape with a diameter of 250 mm. The alternating quadrants of the circle were black and white. A weight was attached to the middle of the circular shape to ensure that it will remain horizontal when lowered into the water and facilitate a clear observation of the disc and the alternating colours as it is lowered (Figure 3.13). The disc was lowered into the water until the quadrants were only just visible. The depth at this point was recorded.

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Salinity was measured using a hand held Hanna Salintest HI98203 salinity meter (Hanna Instruments (Pty) Ltd, Bedfordview, Johannesburg, South Africa) with measurements taken every 2 weeks. pH was measured using an Eutech Instruments pH Tester 30 (Thermo Scientific, Eutech Instruments Pte Ltd, Singapore) with measurements also taken every 2 weeks. Both salinity and pH was measured on the water surface.

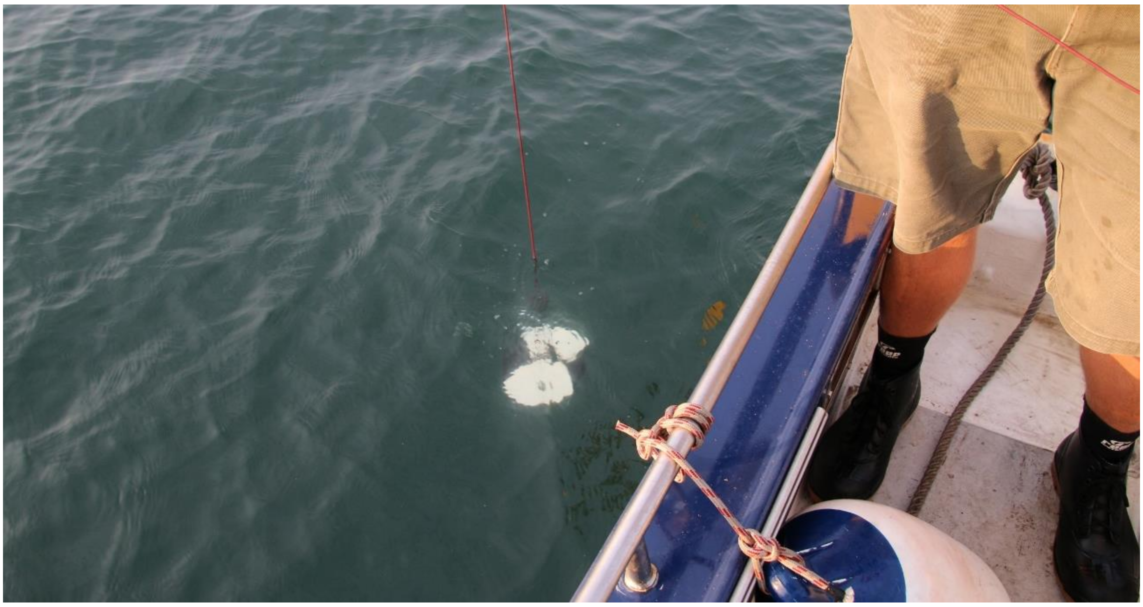

Figure 3.13: Measuring water turbidity using a secchi disc. (Photo credit: Gert le Roux)

3.3. Results

In this study the stocked fingerlings grew from an initial weight of 9 g to a maximum weight of 1580 g in 23 months. All the fish were initially stocked into cage 2, this specific growth period is displayed on both the growth graphs for the specific cages as the period before grading took place (up until 25 August 2016). The graded smaller fish in cages 1 and 2 attained an average weight of 1082 g in 23 months (Figure 3.14). The larger fish were stocked into cages 3 and 4 after grading and reached a maximum average weight of 1580 g after 23 months (Figure 3.15). Figure 3.2 displays the growth of cages 3 and 4 compared to a predictive growth model at a constant water temperature of 22°C developed by Pirozzi *et al*. (2010a).

A total feed conversion ratio (FCR) of 2.25 was calculated over the entire course of the growth period (mortalities taken into account). This value was obtained using the total weight of fish harvested and total weight of feed used, also taking into account the weight of mortalities. A maximum stocking density of 13.72 kg/m^3 was obtained in cage 2 during August 2016 prior to the commencement of grading. After grading, stocking densities varied between 2.50 kg/m^3 and 6.48 kg/m^3 in the respective cages.

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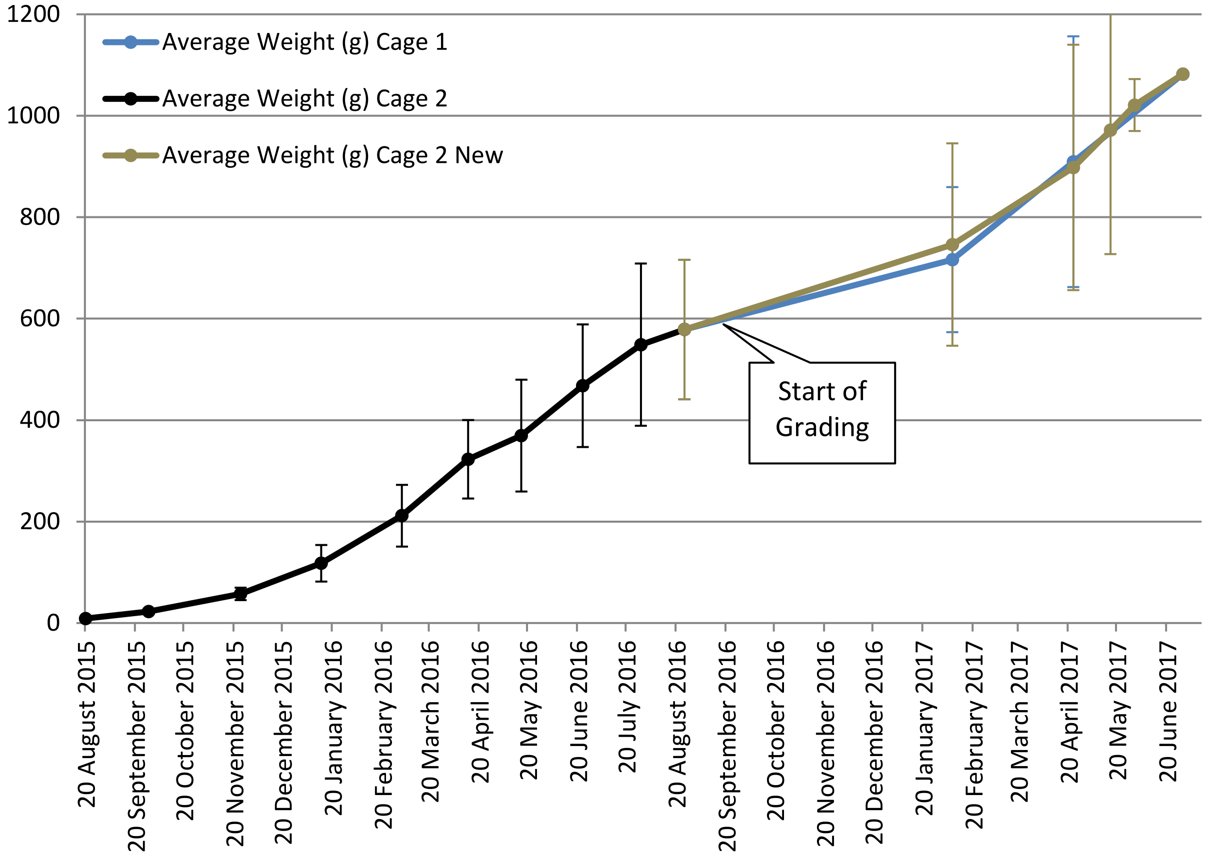

Figure 3.14: Fish growth displayed as average fish weight (g) of the smaller fish from cage 1 and 2 during the DST SU KZN Aquaculture Development Project.

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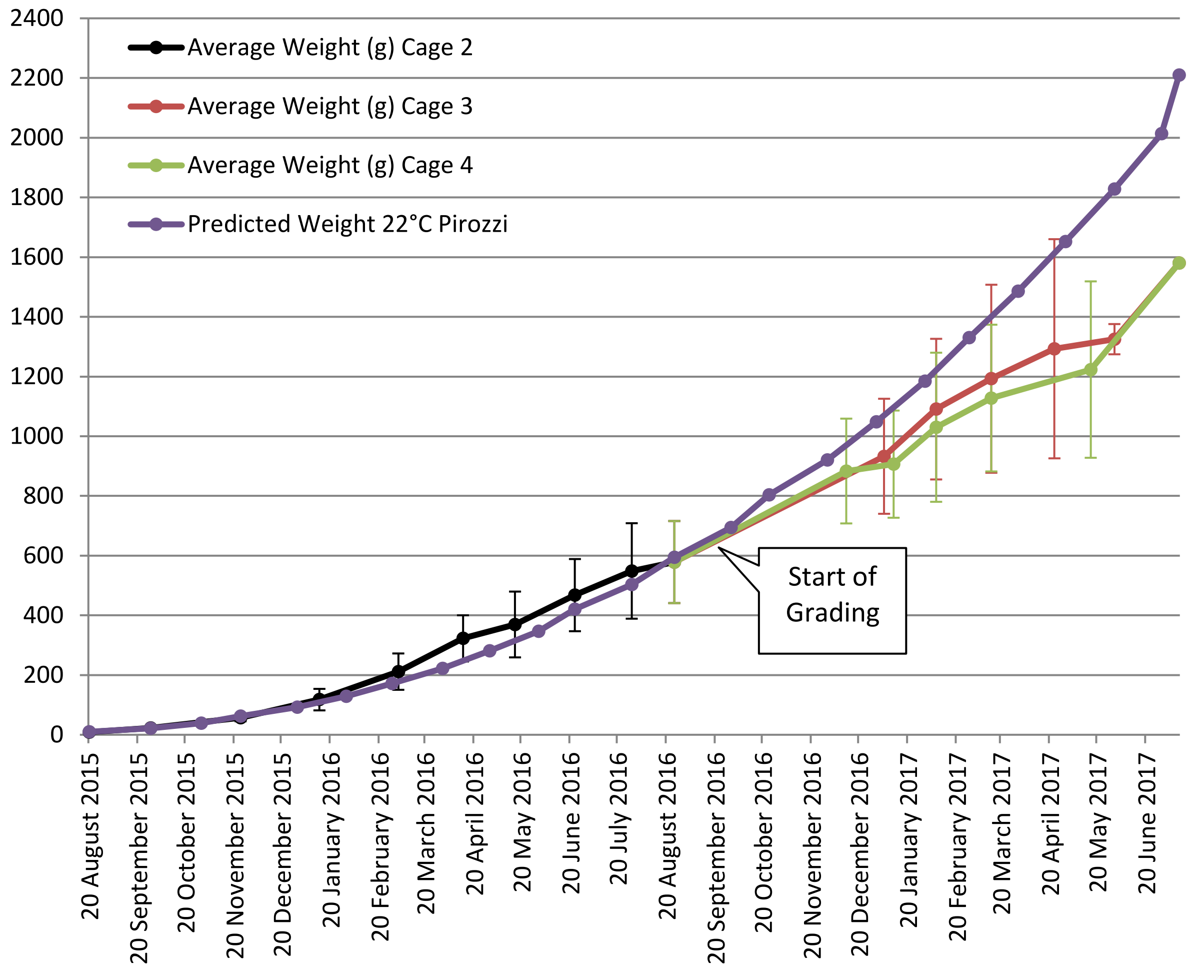

Figure 3.15: Fish growth displayed as average fish weight (g) of the larger fish from cage 3 and 4 during the DST SU KZN Aquaculture Development Project compared to a predictive growth model calculated at a constant water temperature of 22°C (Pirozzi *et al.*, 2010).

During the course of the growth trial, the number of mortalities (2.00 ± 3.59) encountered on an average day was accepted as being normal (Figure 3.16). However, a substantial number of mortalities was experienced on days following grading, net changing, harvesting and sometimes net cleaning activities (displayed as spikes in Figure 3.16).

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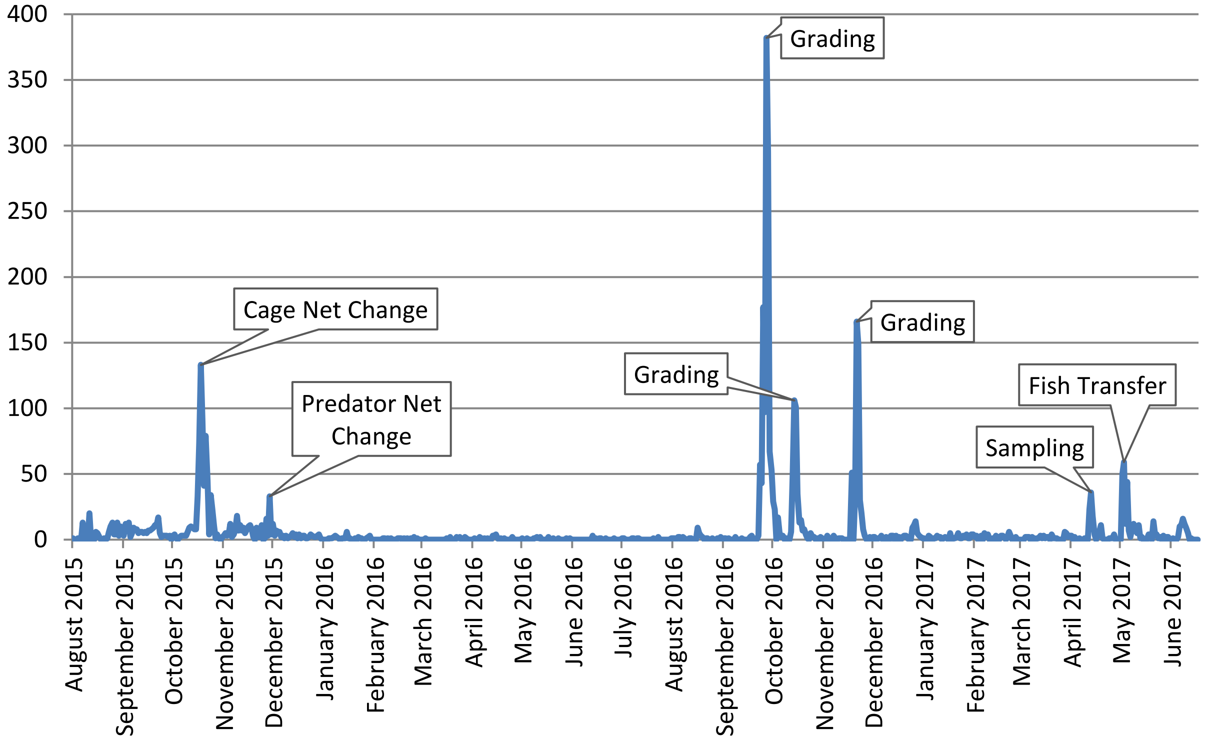

Figure 3.16: Mortalities experienced during the course of the DST SU KZN Aquaculture Development Project.

The average water temperature, at a depth of 6 m, during the course of the production cycle was 22.6°C (Figure 3.17). The minimum and maximum water temperatures were 18.9°C and 27.8°C respectively.

The dissolved oxygen level in the water was measured on a daily basis using an Oxyguard Polaris oxygen meter at a depth of approximately 2.5 m below the water surface and is displayed in Figure 3.18. From the 10th of October 2015 measurements were taken inside the cages. Prior to this the measurements were taken outside of and next to the cages. For this reason, there is a sharp decline in the dissolved oxygen level displayed on the graph. The cage net was also fouled with marine biofouling at the time explaining the lower oxygen levels inside the cage. The average dissolved oxygen level during the course of the production cycle was 6.3 mg/L. The minimum and maximum dissolved oxygen levels were 3.0 mg/L and 9.9 mg/L respectively.

In Figure 3.19 the daily secchi disc readings are displayed in meters as a measure of the water turbidity. The average secchi disc reading was 3.2 m, with the minimum and maximum being 1.5 m and 7.5 m respectively.

Salinity and pH was measured approximately every 2 weeks using hand held field equipment. During the latter part of the production cycle measurements were taken only a few times resulting in the specific graph curves as displayed in the salinity (Figure 3.20) and pH (Figure 3.21) figures. The average salinity

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during the course of the production cycle was 32.16 g/L, with the minimum and maximum being 18.0 g/L and 39.0 g/L respectively. The respective corresponding pH values were 8.14, 7.24 and 8.93.

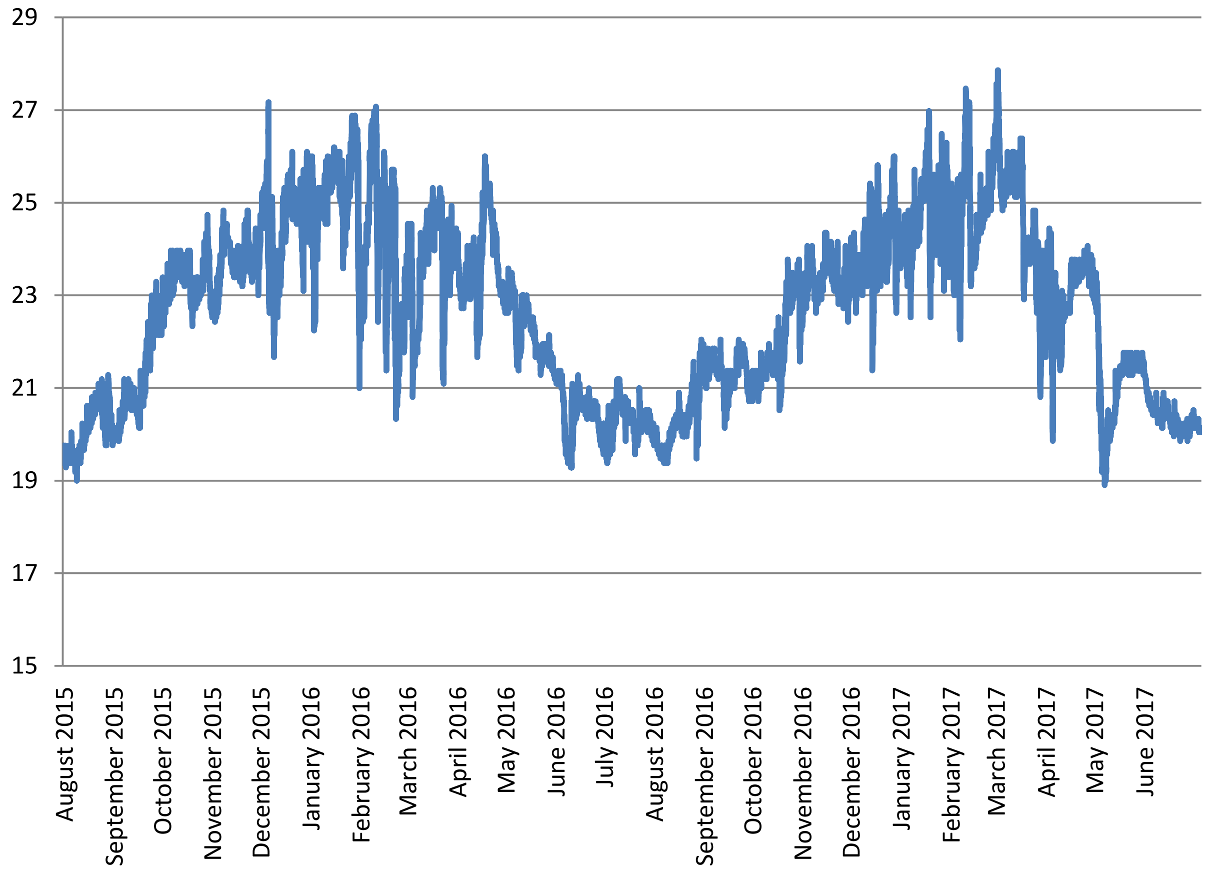

Figure 3.17: Water temperature (°C) taken at 6 m deep next to cage 2 using an underwater HoboWare data logger during the course of the production cycle.

Table 3.2 displays the production data collected and calculated at sampling events during the course of the project. The fish were fed twice a day, at first and last light, to satiation. No feed response was observed when feeding was done at mid-day. Following this feeding regime FCR's between 0.96 and 1.53 was achieved during the first 15 months. During this time stocking density increased from 0.57 kg/m^3 to 13.72 kg/m^3 before grading and the subsequent splitting of fish commenced in month 15. After grading and until harvesting the stocking densities varied between 2.50 kg/m^3 and 6.48 kg/m^3 in the respective cages. In month 22 the fish from cage 3 and 4 were combined which increased the stocking density to 12.80 kg/m^3 in cage 3. From month 16 to harvest the FCR's varied between 1.30 and 8.48 in the respective cages.

Some cages were difficult to sample, especially using a cast net, which resulted in seemingly skewed data for slightly smaller fish. Sample size was increased in an effort to acquire more accurate data. Whenever sample data seemed inaccurate, the sampling was repeated. At the time of harvesting the smaller fish in cages 1 and 2 attained an average weight of 1082 g, while the larger fish from cages 3 and 4 reached an average weight of 1580 g.

Table 3.2: Production data collected during sampling events throughout the course of the production trial.

Date	Average Weight (g) Cage 1	Nr of Fish	Feed Consumed (kg)	FCR	Biomass (kg)	Stocking Density (kg/m^3)
Cage 1						
24 October 2016	578.53	5226	-	-	3023.39	3.05
16 February 2017	716.38	4488	871.4	1.34	3215.11	3.24
23 April 2017	909.45	4463	1935.1	2.25	4058.86	4.09
Cage 2						
Date	**Average Weight (g) Cage 2**	**Nr of Fish**	**Feed Consumed (kg)**	**FCR**	**Biomass (kg)**	**Stocking Density (kg/m^3)**
20 August 2015	8.93	25007	-	-	223.31	2.84
28 September 2015	22.93	24767	331.25	0,96	567.91	0.57
24 November 2015	57.45	23934	1068.85	1.29	1375.01	1.39
13 January 2016	117.77	23698	2184.1	1.53	2790.91	2.81
03 March 2016	211.53	23627	2986.65	1.35	4997.82	5.04
13 April 2016	322,82	23600	3233.56	1.23	7618.55	7.68
16 May 2016	369.46	23578	2971.6	2.70	8711.10	8.78
23 June 2016	467.82	23564	2975.21	1.28	11023.71	11.11
29 July 2016	548.81	23550	2695.55	1.41	12924.45	13.03
25 August 2016	578.53	23525	1895.65	2.71	13609.90	13.72
Grading						
03 December 2016	578.53	4294	-	-	2484.21	2.50
16 February 2017	746.14	4264	932.25	1.37	3181.54	3.21
23 April 2017	898.15	4251	1432.65	2.22	3818.04	3.85
16 May 2017	971.83	4194	533,8	1.73	4075.86	4.11
30 May 2017	1021.12	3905	249.55	1.30	3987.49	4.02

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Cage 3

Date	Average Weight (g) Cage 3	Nr of Fish	Feed Consumed (kg)	FCR	Biomass (kg)	Stocking Density (kg/m^3)
24 October 2016	578.53	5166	-	-	2988.68	3.01
05 January 2017	932.96	5000	2360.50	1,33	4664.80	4.70
07 February 2017	1090,86	4993	1260.30	1.60	5446.68	5.49
14 March 2017	1192.65	4977	1466.50	2.89	5935.79	5.98
23 April 2017	1293.07	4970	1308.55	2.62	6426.57	6.48
Fish from cage 4 moved into cage 3						
30 May 2017	1325.18	9583	1648.65	5.36	12699.22	12.80

Cage 4

Date	Average Weight (g) Cage 4	Nr of Fish	Feed Consumed (kg)	FCR	Biomass (kg)	Stocking Density (kg/m^3)
03 December 2016	578.53	5056	-	-	2925.04	2.95
12 December 2016	883.47	5032	-	-	4445.60	4.48
11 January 2017	906.56	5004	980.2	8.48	4536.43	4.57
07 February 2017	1030.11	4994	1026.8	1.66	5144.36	5.18
14 March 2017	1127.90	4980	1546.54	3.18	5616.95	5.66
11 May 2017	1223.37	4945	1925.3	4.08	6049.54	6.10

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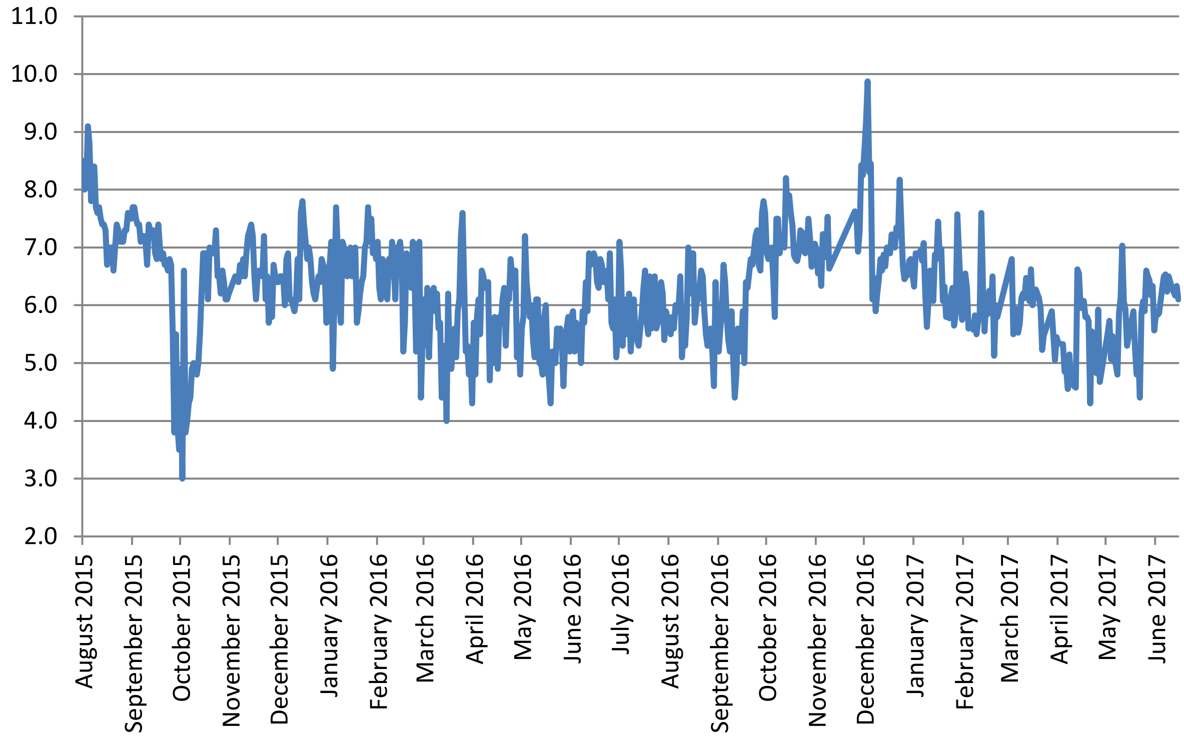

Figure 3.18: The average dissolved oxygen level (mg/L) measured on a daily basis during the production cycle.

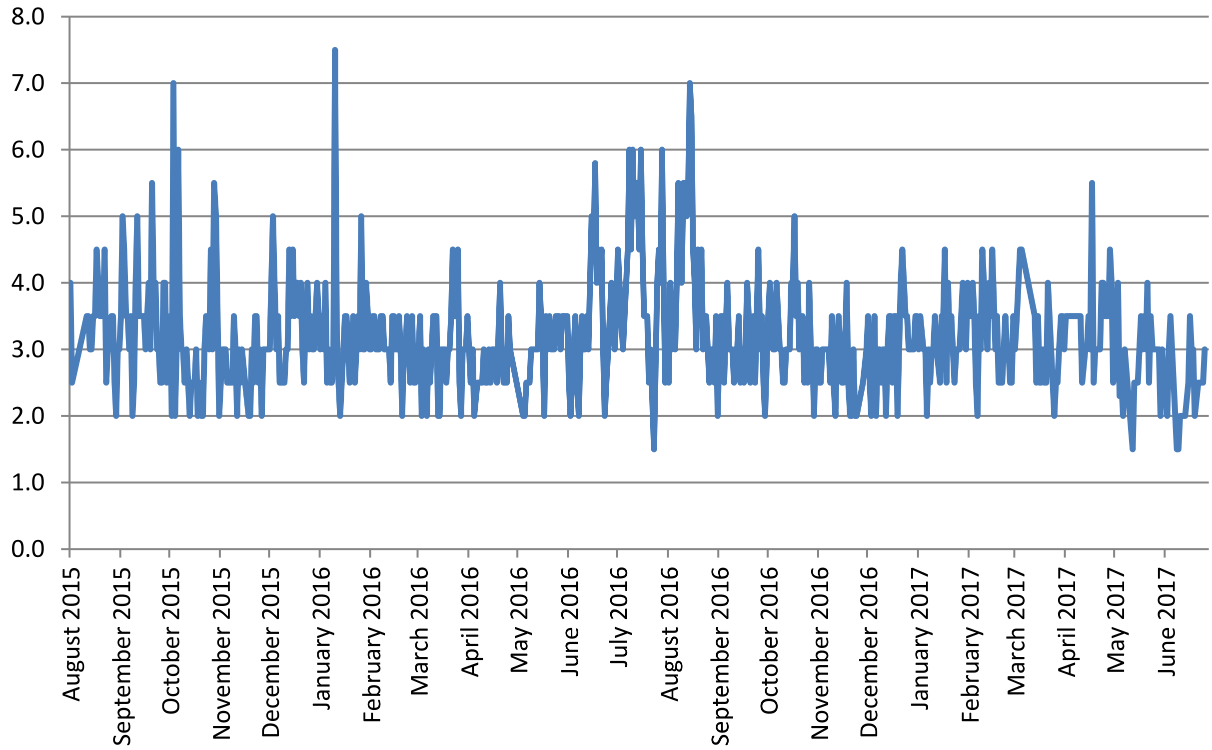

Figure 3.19: The secchi disc reading (m) measured on a daily basis during the course of the production cycle as a measure of water turbidity.

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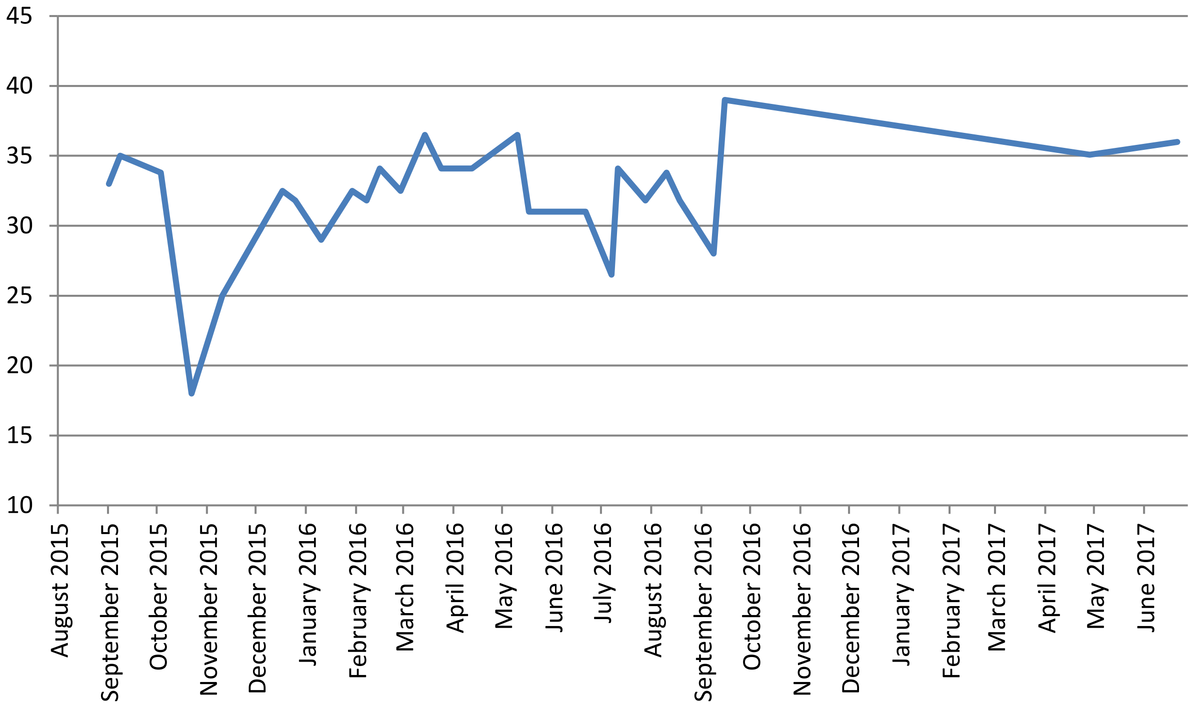

Figure 3.20: Salinity (g/L) of the sea water as measured next to the cages.

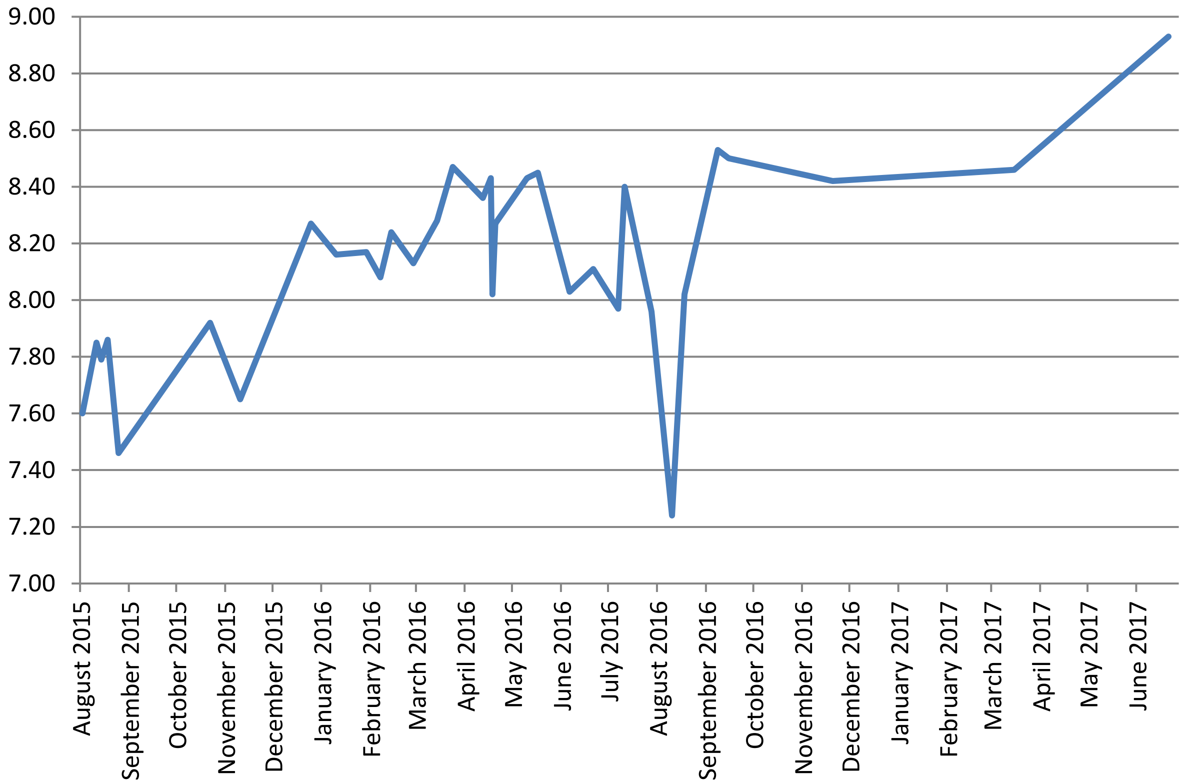

Figure 3.21: pH as measured at the water surface every 2 weeks during the course of the production cycle.

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3.4. Discussion

The DST SU KZN Aquaculture Development Project achieved its primary objectives as set out at the beginning of the project. The project proved the suitability of the HDPE sea cages and the associated mooring technology as installed in Richards Bay. During the course of the project all project personnel were trained on fish husbandry methods pertaining specifically to the dusky kob species and the use of sea cages.

The dusky kob seemed to adapt well to the commercial sea cage culture conditions, although optimal feeding posed a challenge. Adopting a feeding regime of feeding twice a day, at first and last light, to satiation resulted in acceptable FCR's. Attempts were made to administer a third feeding during mid-day but the fish would not respond. Personal communication with Neil Stallard from Zini Fish Farms confirmed the fish's behavior as they also adopt a similar feeding regime on their farm. During the latter part of production there was a big variation in FCR. It was difficult to determine exactly why the FCR range was so big as FCR is influenced by many factors.

Collet (2007) tested the effect of feeding regime on the growth and FCR of dusky kob. The experiment was conducted at a constant temperature of 24.8°C and showed that the highest growth rate and best FCR was achieved by administering 3 daily feedings. However, there was no significant difference reported in growth between the three daily feedings and two daily feedings administering the same amount of total feed fed. Collett (2007) recommended that a restricted ration is fed at least twice daily according to the size-specific daily requirements of the fish in order to achieve maximum growth and the best FCR. Similarly, Daniel (2004) found both feed intake and growth increased from one feeding a day to two feedings a day. However, further increases in feeding frequency from two to four feedings a day did not result in better growth or improved feed efficiency. He concluded that the optimum feeding frequency seems to be twice a day.

From stocking, the fingerlings grew very well for the first 12 months (Figures 3.1 and 3.2). At this time there was a big variation in the size of the sampled fish and it became evident that the fish needed to be graded. After grading the stocking densities in each of the cages were relatively low and a reduction in both the feed response and growth was observed. Collet (2007) also tested the effect of stocking densities on the growth of juvenile dusky kob. The study found fish of approximately 37 g could be stocked at 10 – 50 kg/m^3 without any significant negative effects on their growth. Booth *et al*. (2010b) reported that stocking densities up to 22 kg/m^3 is possible with juvenile mulloway although it might lead to higher FCR's. They recommend using densities of 10 – 13.5 kg/m^3. Importantly they also showed there is a reduction in growth and an increase in FCR if a stocking density below 5.9 kg/m^3 is adopted. This lower stocking density threshold value is also confirmed by Pirozzi *et al*. (2010b). In the current study, the effect of this lower stocking density on the growth and FCR of the fish became evident after grading. Figure 3.2 displays the growth of the larger fish in cages 3 and 4 compared to a predictive growth model developed by Pirozzi *et al.* (2010). The model assumes a constant water temperature of 22°C. The average water temperature during the course of the project was 22.6°C. Compared to the growth model it is clear that the fish grew well for the first 12 months. After grading it is evident that

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there was a reduction in growth. This was probably due to the combination of stress after the grading event and the effect of the subsequent low stocking density in each cage.

The oxygen concentration, pH, and salinity levels were all within the acceptable range for dusky kob during the production period. The water temperature was within and periodically slightly lower than the optimal temperature range for dusky kob during the production period.

3.5. Conclusion

Overall the fish performed well, taking into account that the fingerlings were produced from undomesticated wild broodstock where no prior genetic selection has been made to increase the performance of the fish. Overall the fish had acceptable growth and FCR. During the latter part of production there was a big variation in FCR. It was difficult to determine exactly why the FCR range was so big as FCR is influenced by many factors. The fish adapted well to aquaculture conditions with an acceptable survival rate, however there were isolated events of significant mortalities due to stress. The project proved that dusky kob can be successfully grown in sea cages in Richards Bay and demonstrated the technical feasibility of the farming equipment and cages. The water quality in the Richards Bay harbour was also suitable for the production of dusky kob. The data obtained from this project is invaluable to the local aquaculture industry, especially as it is still in its infancy. The results of this project will hopefully stimulate commercial interest on this topic and subsequent commercial development.

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Chapter 4: Effect of dusky kob (*Argyrosomus japonicus*) size on fillet yield (%) and proximate composition

4.1. Introduction

This chapter examines the effect of fish size on both fillet yield (as a % of body weight) and proximate composition in cultured dusky kob (*Argyrosomus japonicus*), both attributes have an effect on product quality.

The nutritional content or composition of fish is affected by numerous factors. These factors can be of endogenous or exogenous origin. Fish size is one of the endogenous factors together with sex, life cycle stage and genetics (Shearer, 1994). These factors all influence the composition of the fish flesh. This leads to changes in the physiological state of an individual fish and ultimately determines its ability to survive and successfully compete in the aquatic environment. From a market point of view, the nutritional content contributes to the product quality, which in turn, directly affects market demand and price (profit). For fish farmers it is important to know at what size product quality is at its highest.

Fillet yield is an important marketing measure in fish production and is influenced by several factors. Apart from fish size, other factors include age, sex, body shape, head size and the weight of the viscera, skin and fins (Souza, 2015). The efficiency of the fillet machine or person cutting the fillets also influences the fillet yield. When fish are processed and marketed in fillet form, the fillet is obviously the only part of the fish that returns an income (profit) to the fish farmer. Fish with a higher fillet yield (%) thus have a higher potential of maximizing income. If fish size influences fillet yield (%), the potential profits earned from farming fish will also be influenced.

Fish size also affect other production parameters such as feed conversion ratio (FCR) and specific growth rate (SGR). As fish size increase, FCR increases and SGR decreases thereby influencing the profit. Both FCR and SGR also increase with an increase in water temperature (Pirozzi *et al*., 2010a) in relation to the optimal range for a particular species.

4.2. Material and Methods

The fish used in this study were obtained from the DST SU KZN Aquaculture Development Project in Richards Bay. The fish were spawned from wild broodstock at the Pure Ocean (Pty) Ltd hatchery that was located in East London. The fish larvae were transported by road to the Zini Fish Farms (Pty) Ltd hatchery located in Mtunzini where they were grown to fingerlings.

When the fingerlings reached an average weight of 9 grams they were transported by road to Richards Bay to be stocked into the fish cages. The stocking took place on 15 August 2015 and the fish remained in the cages until July 2017 when all the fish were harvested. During this production period all the fish received the same type and size feed and was fed at first and last light. Dusky kob feeds manufactured by Avi-Products (Pty) Ltd (Pietermaritzburg, South Africa) and Montego Pet Nutrition (Pty) Ltd (Graaff-

Reinet, South Africa) were used during the study. In Chapter 3 the production aspects of this study were reported, including the nutritional composition of the feeds used.

During harvesting the fish were crowded using a sweep net (Alnet (Pty) Ltd, Cape Town) and removed from the cage using a braile net (Fusion Marine, Scotland) attached to a crane mounted on the work boat. The fish were transferred from the cage to a harvesting bin containing an AQUI-S anesthetic (Aqui-S New Zealand Ltd, Lower Hutt, New Zealand) at a concentration of 15 mg/L. Once the fish showed no sign of movement, they were transferred into an ice slurry.

For this study fish were sourced from different size classes. Fish were randomly selected from the ice slurry, weighed, measured (total length) and allocated to a specific sized group. The three size classes used were 800 g – <1000 g, 1000 g – <1200 g and 1200 g – <1400 g. For ease of reference the size classes will be referred to as small (800 g - <1000 g), medium (1000 g - <1200 g) and large (1200 g - <1400 g). Twelve (n=12) fish were selected from each size group. The fish were subsequently frozen whole at -18°C.

For preparation of the fish samples the fish were defrosted overnight at a temperature of 5°C. The fish were weighed individually to determine total fish weight (g). Filleting was done following the procedure explained in US EPA (2000). Both the fillets were removed (pectoral fins and belly-flap were excluded) with skin-on and weighed individually (Fig. 4.1a). The skins were then removed and the fillets weighed individually again (Fig. 4.1b). The liver was also removed and weighed.

For each fish sample, two strips of meat were cut from one of the fillets as indicated in Figure 4.1c,d to be homogenized in a food processor, vacuum packed (Fig 4.1e,f) and re-frozen at -20°C for further analysis.

a) 

b) 

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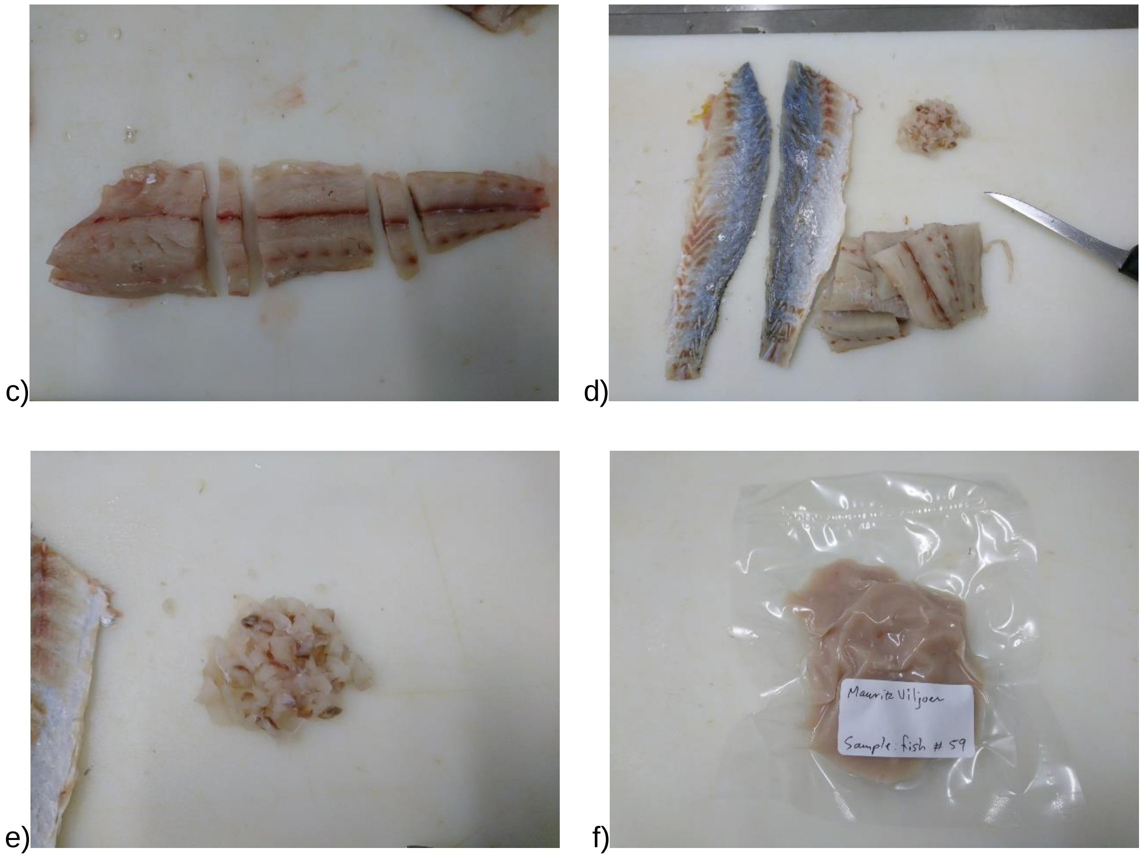

Figure 4.1: The filleting and processing procedure that was followed to prepare a homogenized sample. The two smaller strips in c) were homogenized.

Determination of moisture content

The moisture content of the fish samples was determined according to the AOAC official method 934.01 (AOAC International, 2016). Porcelain crucibles were cleaned and subsequently dried for a minimum period of two hours at 100°C. The crucibles were then placed into desiccators to allow them to cool down for a period of 30 minutes. Each individual crucible was then weighed and the mass recorded. A 2.5 g sub-sample was taken from the homogenized fish fillet samples and placed into the crucibles. The crucibles (with the fish samples) were then placed into an oven at a temperature of 100°C for a period of 24 hours. After 24 hours the crucibles were removed from the oven and placed into a desiccator and allowed to cool down for a period of 30 minutes. The crucibles (containing the moisture free sample) were then weighed again and the weights recorded.

The percentage moisture in each individual sample was calculated as follows:

$$\%\,Moisture = \frac{(A+B)-C}{B} \times \frac{100}{1}$$

Where

A = the weight of the empty, clean crucible

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B = the weight of the sample

C = the weight of the crucible and dry sample

Determination of ash content

The ash content of the fish samples was determined according to the AOAC official method 942.05 (AOAC International, 2016). The oven dried samples acquired after determining the moisture content (still in the original crucibles) were placed in an incinerator oven for a period of six hours, at a temperature of 500°C. After six hours the oven was turned off and the samples left in the oven for a further two hours. The crucibles were then carefully removed and placed inside a desiccator to cool down for a period of 30 minutes. The crucibles, containing the ash, were then weighed and the weights recorded.

The percentage ash in each individual sample was calculated as follows:

$$\% \, Ash = \frac{D - A}{B} \times \frac{100}{1}$$

Where

A = the weight of the empty, clean crucible

B = the weight of the sample

D = the weight of the crucible and ash

Determination of fat content

The fat content of the fish samples was determined according to the method described by Lee *et al*. (1996). Glass fat-beakers were cleaned and subsequently dried overnight in an oven at a temperature of 100°C. The fat-beakers were then removed from the oven and placed in a desiccator and allowed to cool down for a period of 30 minutes. The fat-beakers were then individually weighed and the weights recorded. Sub samples of 5 g were taken from the homogenized fillet meat samples and placed in the respective fat-beakers (800 ml) after which 50 ml of a chloroform/methanol (1:2, v/v) solution was added to the meat sample and mixed with a Bamix® stick blender (Bamix of Switzerland) for 1 minute. The mixed solution was then filtered through a Whatman no.1 filter paper into a separation funnel. The used filter paper was dried and the defatted muscle sample used for protein analysis.

To each separation funnel, 20 ml of 0.5% (w/v) NaCl solution was added after which each separation funnel was shaken four times to mix the solution. The separation funnels were then left to stand for a period of 30 minutes to allow the separation to occur, which was clearly visible at the end. The bottom layer of the separated solution was then tapped off into a 100 ml Erlenmeyer flask. Using a pipette, 5 ml of this liquid was extracted and placed into the pre-weighed fat-beakers and subsequently placed on a sand plate (setting 4) for a period of approximately 45 minutes. The fat-beakers were then carefully removed (ensure no sand residue was present on the fat beakers) from the sand plate and placed inside a desiccator and allowed to cool down for a period of 30 minutes. The fat-beakers were then individually weighed and the weights recorded.

The percentage total fat in each individual sample was calculated as follows:

$$\% \, Fat = \frac{(fat\_beaker + fat) - (fat\_beaker)}{sample \; mass} \times \frac{(chloroform \; volume)}{5} \times \frac{100}{1}$$

Determination of protein content

The protein content of the fish samples was determined following the Dumas combustion method 992.15 (AOAC, 2016). The defatted and dried fish samples were ground in a mortar and pestle to a fine powder. A sub-sample of 0.150 g was taken and inserted into a foil wrap designed for the Leco protein analyser (LECO FP-528) to be analysed. Results of the analysis were expressed as % Nitrogen (N). To calculate the protein concentration in the respective samples, the nitrogen content was multiplied by the prescribed factor of 6.25. With each batch of samples an EDTA calibration sample (LECO Corporation, 3000 Lake View Avenue, St. Joseph, MI 49085-2396, USA, Part number 502-092) was also analysed to ensure accuracy and recovery rate.

Data analysis

All statistical analyses were conducted using SigmaPlot Version 10.0 (2006) (SyStat Software, San Jose, California, USA) with significance set at $\alpha = 0.05$. Means for fillet yield (% of body weight) and proximate composition were compared between size classes using T-tests.

4.3. Results and Discussion

As expected, the weight of the fillets increased with an increase in fish size (Table 4.1). However, there was no significant difference ($P > 0.05$) in fillet yield (%) as a result of differing fish size. The fillet yields obtained were similar to other studies of the same or similar species (Monford, 2010; Shearer, 2001; Guy and Nottingham, 2014; Martelli *et al.*, 2013). Crouse *et al.* (2018) studied the growth of six different strains of rainbow trout (*Oncorhynchus mykiss*) and concluded that fillet yield (%) increased as the fish grew in size. This is also confirmed by Shearer (2001) and Schmitz (1999). Martelli *et al.* (2013) found as body weight of meagre (*A. regius*) increased, fillet weight (g) increased proportionally.

Luzanna *et al.* (2002) conducted a study on sunshine bass (*Morone chrysops x Morone saxatilis*) comparing the fillet yield (%) of small, medium and large fish. Their results showed the fillet yield in medium sized fish were significantly higher than that of small and large fish, however the fillet yields from small versus large fish were not significantly different. The larger fish in their study did have a higher percentage of visceral fat following the same dietary intake, which may have accounted for the decrease in fillet yield (%).

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Fogaca (2011) compared the fillet yield of pirarucu (*Arapaima gigas*) in three different size classes and found no significant difference in yield. Similarly, Santos *et al*. (2000) found no significant difference in fillet yield between different size classes of triara (*Hoplias malabaricus*).

Poli *et al*. (2003) reported that head percentage (as a percentage of total body weight) in meagre increased significantly ($P < 0.01$) with an increase in fish size. This is a limiting market trait of meagre (Piccolo *et al.*, 2008; Monfort, 2010) and could possibly be the case for *A. japonicus* as well. The fish used in this trial could also be susceptible to this as they were offspring from wild broodstock with no prior selection for growth or body conformity.

Saillant *et al*. (2009) examined the genetic variation in carcass quality traits in cultured sea bass (*Dicentrarchus labrax*) and concluded that head percentage (as a percentage of total body weight) was strongly negatively correlated to body weight. They further state that genetic selection for increased body weight would lead to a decrease in the relative size of fish heads and an increase in viscera and fillet yields (%).

Haffray *et al*. (2012) predicted genetic selection for body weight (or fillet yield %) in rainbow trout (*Oncorhynchus mykiss*) will decrease the relative head development by 25 – 30 % in approximately 10 generations, however, their conclusion was mathematically determined and they recommended further investigation.

In South Africa, the legal minimum size (measured as total length) for wild dusky kob caught by recreational anglers from the shore West of Cape Agulhas is 500 mm and East of Cape Agulhas is 600 mm. Dusky kob caught by boat at sea East of Cape Agulhas up to the Umtamvuna River has a minimum legal size of 500 mm, while dusky kob caught by boat at sea in the province of KwaZulu-Natal has a minimum legal size of 400 mm. Average total lengths for the small, medium and large fish in this study are indicated in Table 4.1. Wild dusky kob of the same average length would have weighed slightly less at 785 g, 1000 g, and 1210 g respectively (Griffiths, 1996; ORI) compared to the fish obtained in this study.

Table 4.1: Average dusky kob (*Argyrosomus japonicus*) weight (g) and fillet yield (%) of the fish in the different size classes.

Fish size class	**Small** **800 g – <1000 g**	**Medium** **1000 g – <1200 g**	**Large** **1200 g – <1400 g**
Average fish weight (g)	$870.83^{a} \pm 52.89$	$1131^{b} \pm 36.12$	$1274.33^{c} \pm 71.06$
Average total length (mm)	$424.42^{a} \pm 11.31$	$459.83^{b} \pm 12.43$	$489.00^{c} \pm 17.10$
Average fillet yield_skin on (%)	$43.17^{a} \pm 2.22$	$42.47^{a} \pm 1.84$	$43.21^{a} \pm 2.37$
Average fillet yield_skin off (%)	$35.80^{a} \pm 1.96$	$35.10^{a} \pm 1.79$	$35.85^{a} \pm 2.01$

Values are means ± standard deviation (n = 12).

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[a-c] Means with different superscripts within rows differs significantly at $P \leq 0.05$.

Fish size also affect other production parameters such as feed conversion ratio (FCR) and specific growth rate (SGR). FCR increase with an increase in fish size, whereas SGR decrease. However, both FCR and SGR increase with an increase in water temperature, in relation to the optimal range for a particular species. Figure 4.2 displays the FCR of mulloway (*Argyrosomus japonicus*) as a function of body weight at three water temperature ranges (Pirozzi *et al*., 2010). These water temperatures are similar to the minimum, maximum and average water temperatures encountered during this study. Figure 4.3 display the SGR of mulloway (*Argyrosomus japonicus*) with increasing fish size at different water temperatures, 19°C , 22°C and 28°C respectively (Pirozzi *et al*., 2010).

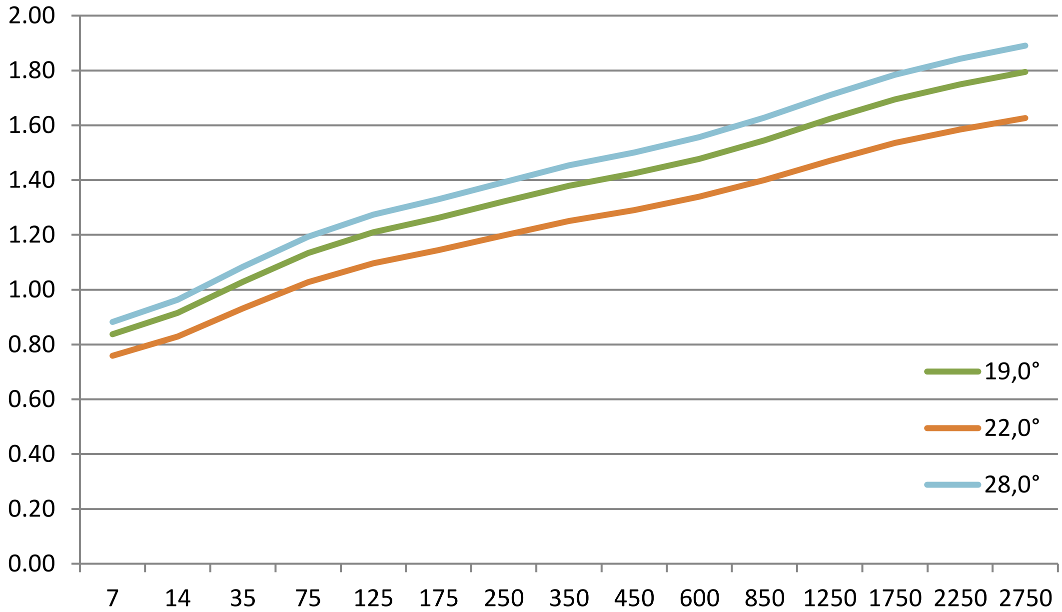

Figure 4.2: The feed conversion ratio (FCR) of mulloway (*Argyrosomus japonicus*) displayed as a function of body weight at different water temperatures (adapted from Pirozzi *et al*., 2010).

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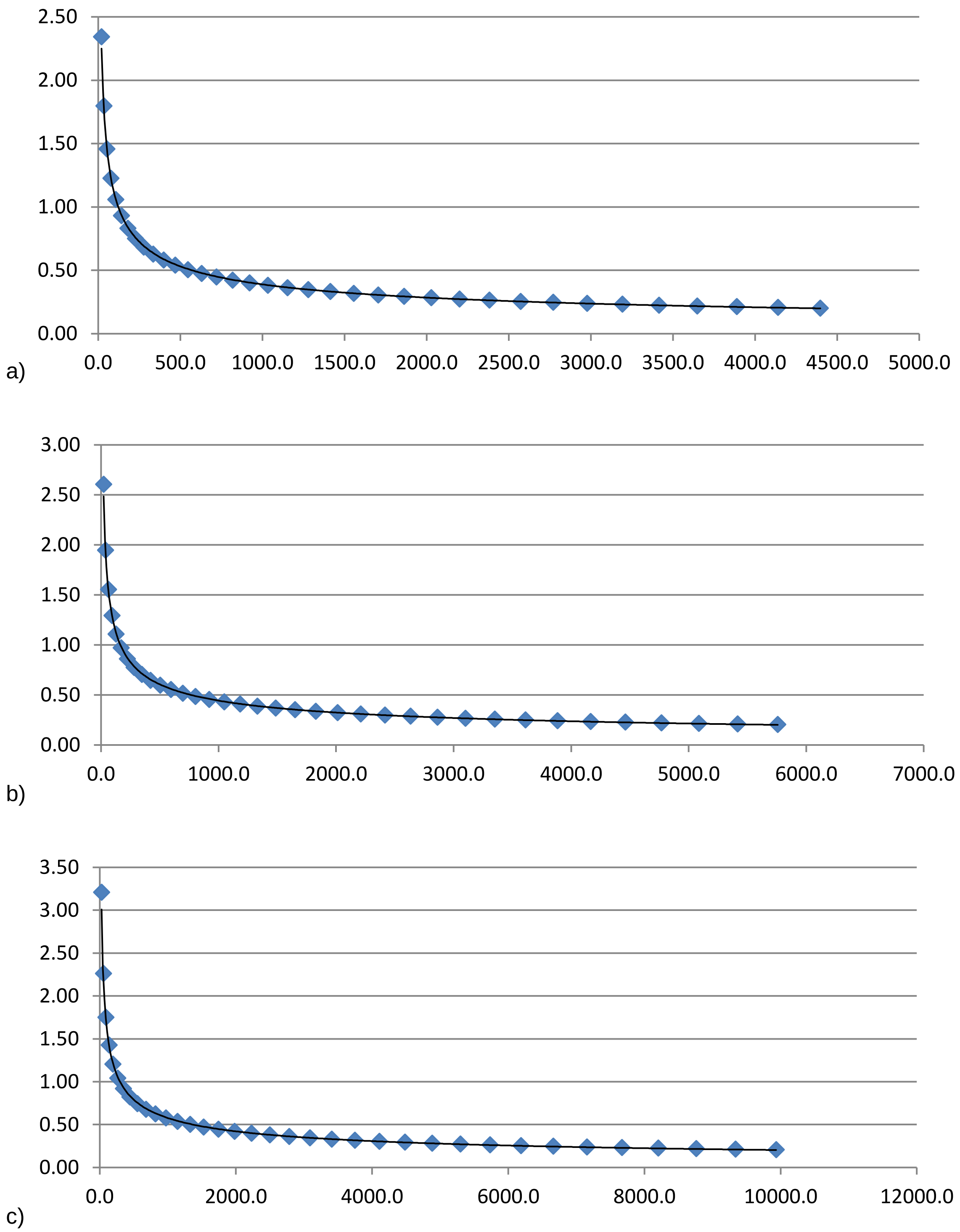

Figure 4.3: The specific growth rate (SGR) of mulloway (*Argyrosomus japonicus*) with increasing body weight at a constant water temperature of 19°C (a), 22°C (b) and 28°C (c) (adapted from Pirozzi *et al.*, 2010).

The comparison of the proximate composition according to the three different size classes is displayed in Table 4.2. Significant differences ($P < 0.05$) were seen in the moisture, protein and fat contents. The moisture content was significantly lower in the large fish compared to both the small and medium fish. The protein content was also significantly lower in the medium fish compared to both the small and large fish, however there was no significant difference between the protein content in the small versus large fish. The fat content was significantly higher in the medium and large fish compared to the small fish. There were no significant differences between the ash contents in the different size classes. Lipid content and fish size had a moderately strong positive correlation (0.65), whereas moisture content had a moderate negative correlation (-0.45) to fish size.

These results correspond with the findings of others (Crouse *et al.*, 2018; Davidson *et al.,* 2014; Shearer, 2001, Martelli *et al.,* 2013) that lipid content increases and moisture content decreases with an increase in fish size. Protein and ash content remains relatively constant with an increase in fish size if sufficient food (nutrition) is available (Shearer, 2001; Crouse *et al.*, 2018).

Evaluating wild versus farmed fish, Sinanoglou *et al*. (2014) found no significant difference in moisture and ash content in meagre (*Argyrosomus regius*), however, farmed meagre had significantly higher lipid content. Similarly, Guy and Nottingham (2014) found farmed mulloway (*Argyrosomus japonicus*) to have significantly higher levels of fat, energy and cholesterol compared to wild mulloway. The wild mulloway did have significantly higher sodium and moisture content. No significant difference was observed in protein, ash, sugar and carbohydrate content. It is still unclear whether the higher lipid content in these studies, and that observed in this study is linked to sufficient quality feed and/or less energy expenditure with fish being in cages. Casual observations of the fish in cages indicated that they were mostly calm, schooling and congregated at the bottom of the cage during daylight hours. During feeding periods activity increased substantially.

Table 4.2: The proximate composition of the three different sized dusky kob (*Argyrosomus japonicus*).

Fish size class	**Small** **800 g – <1000 g**	**Medium** **1000 g – <1200 g**	**Large** **1200 g – <1400 g**
Moisture	$76.88^{a} \pm 0.49$	$77.03^{ac} \pm 0.36$	$76.07^{b} \pm 0.57$
Protein	$22.09^{a} \pm 0.46$	$21.13^{b} \pm 0.52$	$21.98^{ac} \pm 0.86$
Fat	$1.07^{a} \pm 0.24$	$1.82^{bc} \pm 0.27$	$1.86^{c} \pm 0.44$
Ash	$1.28^{a} \pm 0.02$	$1.27^{a} \pm 0.04$	$1.29^{a} \pm 0.07$

Values are means ± standard deviation (n = 12).

$^{a\text{-}c}$ Means with different superscripts within rows differs significantly at $P \leq 0.05$.

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4.4. Conclusion

In this study, no significant effect of dusky kob size was observed on fillet yield (%), although all yields were within range as reported by other studies of the same or similar sized species. This study confirmed that dusky kob size had a significant effect on the fillet's proximate composition. As the fish size increased, the lipid content increased and moisture content decreased. Initially, protein content increases as fish grow in size, although the rate of increase also declines. It would be of interest with this increase in lipid contents in larger fish to quantify what the effect of this would be on consumer taste and acceptance of more fatty fish, although the dusky kob would still be classified as being a lean fish species with a total fillet lipid <2%.

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Chapter 5: Investigating the effect of using a copper alloy mesh cage net for the commercial production of dusky kob (*Argyrosomus japonicus*)

5.1. Introduction

Cage aquaculture in the marine environment is constantly exposed to various factors such as weather and sea conditions, anthropogenic effects, disease from wild fish and attack from predators to name but a few. The effect of biofouling on the cages, cage nets and mooring system is another challenge faced by fish farmers as it is a time-consuming and costly exercise to remove the biofouling. The term biofouling is used collectively to refer to the settlement and growth of spores and propagules of algae and the larvae of invertebrates such as hydroids, ascidians, sponges, bryozoans, barnacles, bivalves and polychaetes (Fitridge *et al*., 2012).

Biofouling affects fish cage culture in various ways. The excessive growth of the biofouling organisms reduces the aperture of the cage net mesh and therefore reduce water exchange. A lower water flow will reduce the flushing effect needed to remove uneaten feed and faeces from the cages, but will also reduce the oxygen concentration inside the cages as less oxygen-rich water flows into the cages. Fish exposed to low oxygen concentrations will continually be under stress and more susceptible to infection by pathogenic microorganisms. The biofouling communities established on the net surfaces increases the risk of the disease to the fish as they act as pathogenic microorganism reservoirs (Fitridge *et al*., 2012). The biofouling organisms also add a substantial amount of weight to the load on aquaculture infrastructure which could lead to structural fatigue and failure (Fernandez-Gonzalez and Sanchez-Jerez, 2017; Fitridge *et al*., 2012).

Fish farmers combat biofouling in several ways. Cage nets get replaced and removed for cleaning off-site or cleaned *in-situ* by divers or by specialized net cleaning equipment. Antifouling coatings can also be applied to nets and mooring ropes to retard and reduce the settlement and growth of biofouling organisms. Antifouling coatings are now predominantly copper based and normally include a range of booster biocides to control copper resistant organisms (Guardiola *et al*., 2012). Alternatively, complete mesh cages can be constructed from copper alloy mesh which are stronger, resistant to biofouling, friendlier to the environment and recyclable. When these copper alloy mesh nets are submerged in the water they form a protective patina layer that inhibits corrosion and resists the attachment of biofouling organisms (Chambers *et al*., 2012).

The aim of this study was to determine if fish farmed in a coper alloy mesh cage have a higher content of bio-accumulated metals compared to fish farmed in a traditional polyester mesh cage net.

5.2. Material and Methods

The dusky kob (*Argyrosomus japonicus*) used in this study was also produced as part of the DST SU KZN Aquaculture Development Project in Richards Bay, implemented and managed by Stellenbosch University (previously discussed in Chapters 3 and 4). All the fish were kept in a single cage for a period of 14 months after which grading commenced and the fish were divided into four cages according to

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average weight. After grading, two of the cages (cage 1 and 2) were stocked with smaller fish with an average weight of approximately 570 g and two cages (cage 3 and 4) were stocked with larger fish with an average weight of approximately 850 g.

Cages 1, 2 and 3 had cage nets installed manufactured from knotless polyester netting (Alnet (Pty) Ltd, South Africa; Universal Nets (Pty) Ltd, Australia) (Figures 5.1 and 5.2) and predator nets manufactured from knotted polyethylene (Alnet (Pty) Ltd, South Africa). None of the polyester or polyethylene nets were treated with antifouling chemicals. Cage 4 was fitted with a custom built copper alloy mesh cage net (Advance Africa, South Africa) (Figures 5.3 and 5.4). No predator nets were installed on cage 4.

Figure 5.1: Close-up view of a typical knotless polyester cage net as used during the trial.

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Figure 5.2: A typical knotless polyester cage net installed on the floating fish cage.

Figure 5.3: A close-up view of the coper alloy mesh used to construct the copper cage net. (Photo credit: Advance Africa)

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Figure 5.4: The completed copper alloy cage net ready for attachment to the floating fish cage. (Photo credit: Advance Africa)

The copper alloy mesh was manufactured by Non Ferrous Metal Works (Durban, South Africa) and the cage net was custom build by a team from Advance Africa Management Services (Johannesburg, South Africa). The copper alloy used is a type of naval brass (CDA486-DZR Brass) and consists of copper (Cu), zinc (Zn), arsenic (As), lead (Pb) and tin (Sn). The addition of tin retards any leaching when submerged in salt water (personal communication, Zane Havemann, Non Ferrous Metal Works).

The fish in both cages received the same size and type of feed throughout the production trial (Kob Grower 48 FF4812 8 mm, Montego Pet Nutrition (Pty) Ltd, Graaff-Reinet, South Africa). The fish were fed by hand, to satiation twice a day, at first and last light.

For this study, the metal composition of the larger fish in cage 3 (traditional knotless polyester cage net) and cage 4 (copper alloy mesh net) were compared to ascertain if fish farmed in copper alloy mesh cages have a higher content of bio-accumulated metals.

Ten (n=10) fish were randomly selected from each cage, weighed, measured (total length mm) and frozen at -18°C. For analysis in the laboratory, the fish were defrosted overnight at 5°C, filleted and the representative samples taken from the fillets, following the exact same procedure as explained in chapter 4. The samples were homogenized in a food processor and re-frozen at -20°C for further analysis. The livers were also removed and weighed before being frozen.

<u>Metal analysis</u>

One meat sample and one liver sample from each fish (n=20), as well as an additional feed sample (n=1) was analysed for metal concentrations of aluminium (Al), manganese (Mn), cobalt (Co), nickel (Ni), molybdenum (Mo), iron (Fe), copper (Cu), chromium (Cr), zinc (Zn), selenium (Se), arsenic (As),

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antimony (Sb), cadmium (Cd), mercury (Hg) and lead (Pb) through inductively coupled plasma mass spectrometry (ICP-MS). To digest the samples, approximately 0.3 g of the homogenised meat samples (n = 20), liver samples (n=20) and single feed sample (n=1) were broken down in 2 ml HCl and 8 ml HNO_3 (Merck Suprapur® acids, Merck, Massachusetts, USA) using a Mars 240/50 microwave digester (CEM Corporation, North Carolina, USA) at 160°C for a period of 20 min. After cooling, the solutions were diluted to 50 ml with deionised water in sample bottles cleaned with 5% HNO_3. The digested samples were then analysed on an Agilent 7700 ICP-MS (Agilent Technologies Inc., California, USA) which was calibrated with NIST-traceable standards (Inorganic Ventures, Virginia, USA).

Data analysis

All statistical analyses were conducted using SigmaPlot Version 10.0 (2006) (SyStat Software, San Jose, California, USA) with significance set at $\alpha = 0.05$. Mean concentrations for each metal were compared between fish from polyester and copper alloy cages using standard T-tests. Tests were done on the fillet meat samples as well as liver samples.

5.3. Results and Discussion

The metals molybdenum (Mo), antimony (Sb) and mercury (Hg) were excluded from the analysis as their concentrations were below the limit of detection for all samples. Due to a technical issue, no analysis was done for tin (Sn). Table 5.1 displays the weight and total length ranges of the fish used in this trial.

Table 5.1: The weight range (g) and the total length range of the dusky kob (*Argyrosomus japonicus*) used in this study.

Cage Net Type	n	Weight Range (g)	Total Length Range (mm)
Copper Alloy Net	10	728 – 1074	411 – 464
Polyester Net	10	744 – 1058	400 – 458

Table 5.2 displays the metal concentrations in meat samples from both the polyester and copper alloy cage nets. No significant difference ($P > 0.05$) was found in the concentration of any of the metals between the two cages. These meat samples represent the edible portion of the fish product; the results are therefore of importance to consumers.

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Table 5.2: The concentration (mg/kg meat) of various metals analysed from meat samples of dusky kob (*Argyrosomus japonicus*) farmed in copper alloy net cages or traditional polyester net cages.

Metal	**Meat Sample Copper Net**	**Meat Sample Polyester Net**	***P* - value**
Al	0.79 ± 0.52	0.65 ± 0.39	0.51
Cr	0.06 ± 0.06	0.04 ± 0.03	0.44
Mn	0.22 ± 0.24	0.13 ± 0.06	0.25
Fe	2.39 ± 0.84	2.15 ± 0.48	0.45
Co	0.02 ± 0.02	0.02 ± 0.02	0.94
Ni	0.07 ± 0.07	0.05 ± 0.02	0.33
Cu	0.39 ± 0.16	0.41 ± 0.19	0.84
Zn	4.56 ± 1.17	3.84 ± 0.29	0.07
As	0.29 ± 0.05	0.31 ± 0.06	0.64
Se	0.15 ± 0.04	0.12 ± 0.05	0.11
Cd	0.00 ± 0.01	0.00 ± 0.00	-
Pb	0.01 ± 0.01	0.01 ± 0.00	0.11

Values are means ± standard deviation (n = 10).

The liver samples from fish in the polyester net cages had significantly ($P < 0.05$) higher concentrations of manganese (Mn) compared to the copper alloy net cages (Table 5.3). On the other hand, the liver samples from fish in the copper alloy cage had significantly ($P < 0.05$) higher concentrations of selenium (Se) compared to the polyester net cages (Table 5.3). Copper, the main ingredient (approximately 60 %) of the copper alloy, was found at a higher concentration in the liver samples from fish in the copper alloy cage compared to the polyester net cage, although not significantly higher ($P > 0.05$). Similarly, Chambers *et al*. (2012) found no significant difference in the copper concentrations in meat, skin and gill samples of Atlantic Cod (*Gadus morhua*) grown in copper versus nylon cage nets. Kalantzi *et al*. (2016) also reported no significantly higher concentrations of copper in gilthead seabream (*Sparus aurata*) as a result of using a copper net cage.

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Table 5.3: The concentration (mg/kg liver) of various metals analysed from liver samples of dusky kob (*Argyrosomus japonicus*) farmed in copper alloy net cages or traditional polyester net cages.

Metal	**Liver Sample Copper Net**	**Liver Sample Polyester Net**	***P* - value**
Al	1.12 ± 1.32	1.18 ± 1.15	0.91
Cr	0.11 ± 0.16	0.04 ± 0.03	0.17
Mn	1.20 ± 0.39	2.66 ± 1.43	0.0059
Fe	36.92 ± 9.60	27.18 ± 12.41	0.06
Co	0.17 ± 0.06	0.12 ± 0.04	0.06
Ni	0.10 ± 0.06	0.10 ± 0.02	0.85
Cu	431.94 ± 161.13	316.18 ± 176.92	0.14
Zn	72.53 ± 14.54	65.12 ± 12.01	0.23
As	0.56 ± 0.17	0.48 ± 0.09	0.18
Se	2.59 ± 0.47	1.95 ± 0.56	0.01
Cd	0.09 ± 0.03	0.05 ± 0.03	0.46
Pb	0.01 ± 0.01	0.01 ± 0.01	0.75

Values are means ± standard deviation (n = 10).

The kob grower feed that was fed to the dusky kob during the production trial was analysed and compared to the EU regulations for metal concentrations in animal feeds (Table 5.4). None of the metals exceeded the maximum allowable limits set by the regulations.

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Table 5.4: The concentration (mg/kg) of various metals in the kob grower feed fed to the dusky kob (*Argyrosomus japonicus*) during the trial.

Metal	Feed Sample	EU Regulations[1]
Al	117.01	-
Cr	2.11	-
Mn	72.41	-
Fe	517.3	-
Co	0.94	-
Ni	3.40	-
Cu	15.78	-
Zn	192.13	-
As	1.59	10.00 mg/kg
Se	1.42	-
Cd	0.35	1.00 mg/kg
Pb	0.26	5.00 mg/kg

[1]Commission Regulation 2013

The maximum concentration for each metal measured in the meat samples from either the copper alloy or polyester net cages compared to the upper limits set for safe human consumption are displayed in Table 5.5. None of the measurements exceeded the recommended upper limits. Yigit *et al*. (2018) reported similar results in axillary seabream (*Pagellus carne*) farmed in copper net cages. Their metal analysis from bream inside the copper cages and wild bream that aggregated around the copper net cage, showed metal concentrations below the upper limits set by USA and EU regulations.

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Table 5.5: The maximum concentrations (mg/kg meat) of various metals analysed from meat samples of dusky kob (*Argyrosomus japonicus*) farmed in copper alloy net cages and traditional polyester net cages, compared to upper limits set for safe human consumption.

Metal	Maximum Concentrations mg/kg	RSA Regulations [1]	EU Regulations [2]	USA Regulations [3]
Al	2.20	-	-	-
Cr	0.22	-	-	-
Mn	0.82	-	-	11 mg/day
Fe	3.76	-	-	45 mg/day
Co	0.08	-	-	-
Ni	0.27	-	-	1 mg/day
Cu	0.90	-	-	10 mg/day
Zn	6.75	-	-	40 mg/day
As	0.42	3.00 mg/kg	-	-
Se	0.23	-	-	-
Cd	0.02	1.00 mg/kg	0.05 mg/kg	-
Pb	0.02	0.30 mg/kg	0.30 mg/kg	-

[1] Department of health 2004, 2018

[2] Commission Regulation 2006

[3] Food and Nutrition Board 2001

5.4. Conclusion

This study found no significant difference in metal concentrations of meat samples derived from fish in polyester versus copper alloy net cages. The maximum concentrations measured for the various metals in meat samples were below the upper limits as set by South African, EU and USA regulations for safe human consumption.

Liver samples from fish in the polyester net cage contained significantly higher levels of manganese (Mn), whereas the liver samples from the copper alloy cage contained significantly higher levels of selenium (Se). The copper (Cu) concentration did not differ significantly between any of the samples, however was higher in the liver samples of fish from the copper alloy cage.

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Chapter 6: General description of the harvest and cold chain of dusky kob (*Argyrosomus japonicus)*

6.1. Introduction

Proper harvesting and handling procedures ensure that the quality of fish products are maintained. These procedures strive to maintain food safety and quality characteristics from the time the fish are harvested to when consumed by reducing the spoilage rate as far as possible; preventing contamination with undesirable microorganisms, substances and foreign bodies and avoiding physical damage to the edible parts of the fish (FAO, 1995).

Fish handling procedures such as chilling, washing and gutting have an immediate effect on fish quality that can easily be assessed following a sensory analysis. Shelf-life and food safety of fish products can be severely affected by the presence and growth of unwanted microorganisms, the effect of which will only become apparent later on (FAO, 1995).

This chapter examines the methods and procedures followed during the harvesting of dusky kob (*Argyrosomus japonicus*) and the subsequent cold chain thereafter at the DST SU KZN Aquaculture Development Project in Richards Bay. It is assumed that that these procedures will serve as guidelines for this emerging industry.

6.2. Methods followed at the DST SU KZN Aquaculture Development Project in Richards Bay

Proper planning was necessary to coordinate logistics during the harvest. Ice was not freely available locally in large enough quantities and had to be transported to Richards Bay. A sufficient amount of ice was acquired to ensure rapid cooling; the amount also had to account for the higher water temperature (average of 20.23°C at harvest). Stellenbosch University made arrangements with Komicx Products (Pty) Ltd to supply refrigerated transport and chipped ice to the project site prior to harvesting. The harvested fish were transported using the same trucks. Wild Caught Products (Pty) Ltd was appointed to process and sell the harvested fish.

The Department of Agriculture Forestry and Fisheries (DAFF) required the fish to be independently tested to ensure safety for human consumption prior to harvesting. Fish samples were collected and sent to Mérieux NutriSciences (https://www.merieuxnutrisciences.com/corporate/en/international-network##southafrica) to be tested for the following:

a) Cadmium: Method 1288, Test Code F008890.1
b) Lead: Method 1288, Test Code F023730.1
c) Mercury: Method 1288, Test Code F019640.1
d) Copper: Method 1288, Test Code: F025280.1
e) Inorganic arsenic: Method FDA EAMS ICPMS Canada

f) Dioxin-like and non-dioxin-like polychlorinated biphenyls: Method EPA 1668, Test Code PS0000620

The analysis (Table 6.1) revealed all the substances were present in levels way below the regulatory maximum limits and the fish were classified as being safe for human consumption by DAFF.

Table 6.1: Results of the analysis by Mérieux NutriSciences to test the fish for various parameters as requested by DAFF.

Parameter	**Value**	**Unit**
Cd	None detected	mg/kg
Pb	None detected	mg/kg
Hg	0.0088 ± 0.0038	mg/kg
Cu	0.142 ± 0.44	mg/kg
Inorganic As	< 6.9	ppb (w/w)
Dioxins and dioxin-like PCBs sum (OMS0, PCDD/PCBTEQ)	0.092 ± 0.025	pg/g

Fish were freshened out (no feed) for two days prior to harvest. On the harvest day, the fish were caught and crowded using a sweep net (Alnet (Pty) Ltd, Epping, South Africa). Oxygen was provided by placing one or two oxygen diffusers into the sweep net. A braile net attached to a hydraulic crane fitted on the work boat was used to scoop the fish from the sweep net. The scooping action was done carefully to minimize damage to the fish. The steel frame of the braile net was also covered with sponge to minimize potential fish bruising. Once fish were scooped into the braile net, the fish were lifted out of the water and place into an anaesthetic bath. The fish were anaesthetized in a solution of Aqui-S (Aqui-S New Zealand Ltd, Lower Hutt, New Zealand) at a concentration of 15 mg/L. When no movement was evident anymore, the fish were removed from the anaesthetic, rinsed in clean sea water and placed into an ice slurry. Harvesting bins (Ivy Blue (Pty) Ltd, Cape Town) were filled approximately two thirds with chipped ice and one third with salt water to prepare the ice slurry (Figure 6.1). Filled harvesting bins (approximately 420 kg fish per bin) were covered with lids and transported to shore. The full bins (containing fish and ice slurry) were offloaded on shore and subsequently loaded onto a refrigerated truck. The fish were refrigerated between 0°C and -5°C and transported to Wild Caught Products (Pty) Ltd for processing.

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Figure 6.1: A harvesting bin filled with harvested dusky kob (*Argyrosomus japonicus*) and ice slurry.

Harvesting was done on three occasions (Table 6.2) during June and July 2017. In total 21255 kg were harvested. The fish were gilled and gutted (Figure 6.2) (completely cleaned but with head on) and packed into 10 kg boxes (Figure 6.3) to be sold locally in South Africa. Initially the fish were marketed and sold at R88.00 per kg but the price was later reduced to R60.00 per kg due to strong competition from imported wild caught kob originating from Namibia.

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Figure 6.2: Dusky kob (*Argyrosomus japonicus*) being processed (gilled and gutted with head on) at Wild Caught Products (Pty) Ltd. (Photo credit: Gert le Roux)

Figure 6.3: Processed dusky kob (*Argyrosomus japonicus*) being packed into 10 kg boxes for distribution at Wild Caught Products (Pty) Ltd. (Photo credit: Gert le Roux)

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To test the market acceptability, 200 kg of whole kob were directly supplied to the Beluga Restaurant in Durban. The kob was favourably received by both the chefs and patrons. A small amount of the larger fish was taken to Three Streams Smokehouse (Pty) Ltd for a filleting and portioning trial (Figure 6.4). Their skilled filleters achieved an average fillet yield of 55.28 % and an average portion yield of 49.25 % with a Marel Portioncutter I-cut 10. As a test, some of the portions were vacuum packed to resemble a potential final product (Figure 6.5).

Figure 6.4: Dusky kob (*Argyrosomus japonicus*) portions cut with the Marel Portioncutter I-cut 10 at Three Streams Smokehouse (Pty) Ltd. (Photo credit: Gert le Roux)

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Figure 6.5: Dusky kob (*Argyrosomus japonicus*) portions vacuum packed as a trial to resemble a potential final product at Three Streams Smokehouse (Pty) Ltd. (Photo credit: Gert le Roux)

Table 6.2: Total weights (kg) of the harvested dusky kob (*Argyrosomus japonicus*) and the respective gilled and gutted weights (kg) and yields (%) achieved during the DST SU KZN Aquaculture Development Project in Richards Bay.

Date	Harvest Weight (kg)	Gilled and Gutted Weight (kg)	Gilled and Gutted Yield (%)
20 June 2017	6891	6042	87.68
2 July 2017	8612	7669	89.05
13 July 2017	5752	5036	87.55

6.3. General discussion and Conclusion

During the dusky kob harvest, the fish were challenging to catch and crowd into the sweep net. The fish formed a school and stayed close to the cage net floor and adjoining sides. To ensure all the fish were caught, a diver had to be in the water and drag the sweep net along the bottom of the cage net. Whenever a crevice formed in the sweep net or cage net, some fish would swim into the crevice and

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the school would follow. This behaviour became easier to predict later on but still posed a challenge during the harvest.

The high average water temperature (20-23°C at time of harvest) meant the ice melted fast. A sufficient volume of ice was thus required to ensure the ice slurry temperature was low enough to facilitate the rapid cooling of the fish to bring the core temperature down.

The harvesting methods followed was effective and the subsequent cold chain ensured that a high quality end product was delivered. The product was favourably received by restaurant chefs and patrons. The small filleting and portioning trial revealed dusky kob have a high fillet yield (%) and can be well presented for marketing in portion form if larger fish are used.

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Chapter 7: General observations and recommendations on dusky kob (*Argyrosomus japonicus*) production in sea cages

7.1. Introduction

Marine finfish aquaculture in South Africa is still in its infancy. At present there is only one operating sea cage farm in South Africa, farming rainbow trout in Saldanha Bay on the West Coast. This chapter examines the general observations and recommendations to produce dusky kob (*Argyrosomus japonicus*) in sea cages with specific reference to the DST SU KZN Aquaculture Development Project that was located in Richards Bay, KwaZulu-Natal, South Africa.

7.2. General observations and recommendations

Dusky kob as a species seems to be a good candidate for aquaculture as they are easy to work with, highly fecund, grow relatively fast, is widely accepted by consumers and commands a relatively good market price (Fielder and Heaseman, 2011). They do however require a specific feeding regime and responded best to being fed at first and last light. They do respond well to a floating and sinking feed, however they tend to follow the sinking feed down the water column making observations difficult. A floating feed was preferred during the DST SU KZN Aquaculture Development Project. It was also noted that the fish were stressed during net changing, grading and harvesting events. After these events mortalities would spike for a few days. Feed response would also be less. Higher stocking densities had a positive effect on feed response. The feed response was low in cages with low stocking densities.

Although the fish grew relatively well on the locally produced feeds the quality of the feeds is not on the same standard as imported feeds manufactured by large, long established fish feed companies such as Biomar (http://www.biomar.com) in Denmark or Skretting (https://www.skretting.com) in Norway. It would be of interest to conduct a similar production trial using imported feeds. The imported feeds come at a higher cost, but growth and FCR is generally much better. This increased performance in fish growth and FCR due to imported feeds were also confirmed in the production of rainbow trout in personal communication with Mr Barend Stander (Southern Atlantic Sea Farms (Pty) Ltd) and Mr Paul Lückhoff (Three Streams Aquaculture (Pty) Ltd).

Unfortunately, in South Africa there is a lack of marine finfish hatcheries not only to produce dusky kob, but other marine species (i.e. yellowtail, *Seriola lalandi*) as well. The recent closure of two hatcheries in the Eastern Cape and the hatchery in Mtunzini, KwaZulu-Natal, (Zini Fish Farms) currently being up for sale leaves very few options for potential fish farmers of marine finfish. The lack of hatcheries also means no selective breeding programs have been established to produce fingerlings (and broodstock) of high quality and selected attributes. For the project, all of the fingerlings were acquired from wild broodstock resulting in non-uniform growth as no selection has been done for growth or body conformity.

South Africa has a very dynamic coastline often exposed to strong and stormy sea conditions, leaving very few potential sites suitable for cage culture. As sheltered bays, Saldanha Bay on the West Coast

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and the Richards Bay Harbour in KwaZulu-Natal are good options. Richards Bay proved to be a good site for cage culture and has immense potential for further development. The Harbour is well protected and has a favourable water temperature (18 - 28°C) and depth (max 24 m). The water quality is good and there is well established support infrastructure in terms of vessel repairs/maintenance, equipment supplies, etc. The harbour is also in close proximity (45 km) to Zini Fish Farms (Pty) Ltd in Mtunzini for the possible supply of fingerlings. However, the harbour has a limited potential area available for cage culture and the long-term availability of water rights is uncertain. There are also planned harbour expansions that might influence the water area available. There are some pollution concerns as heavy industry surround the harbour. During the project several attempts were made by local poachers to catch fish from the cages. They also stole rope and netting material. Police presence on the water was not very frequent.

The overall sea cage aquaculture capacity (estimated at approximately 600 tons per annum) of the Richards Bay harbour will have to be divided into smaller water areas in an attempt to stay clear of the shipping lane. The Department of Agriculture Forestry and Fisheries and Transnet National Ports Authority are currently investigating Richards Bay harbour as a potential sea cage aquaculture site and an independent consultant has been appointed to conduct a feasibility study.

7.3. Conclusion

Aquaculture is still a growing industry in South Africa facing many challenges. The lack of marine finfish hatcheries with established long-term breeding programs is a concern for the development of the sector. Dusky kob as a species seems to be a good candidate for aquaculture production in South Africa, especially in the warmer waters of the Richards Bay harbour. The Richards Bay harbour proved to be one of the best sea cage aquaculture sites for finfish production in South Africa, although at a limited capacity estimated at approximately 600 tons per annum.

7.4. References

Fielder, D.S., Heasman, M.P., 2011. Hatchery Manual for the production of Australian Bass, Mulloway and Yellowtail Kingfish. Industry and Investment NSW. ISBN: 978 1 74256 058 8.

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Chapter 8: General Conclusion

The dusky kob (*Argyrosomus japonicus*) produced at the DST SU KZN Aquaculture Development Project in Richards Bay performed relatively well. The growth was less than expected, however the fingerlings originated from wild broodstock with no prior selection for growth. The average water temperature was also slightly less than optimal which also affected the growth rate. After grading, the low stocking densities in the cages had a negative effect on the growth and increased FCR. The feed conversion ratios varied greatly. It is difficult to determine exactly why as FCR is influenced by many factors. The dusky kob responded well to aquaculture conditions and showed an acceptable survival rate, however the fish were susceptible to stress induced events of significant mortality.

Dusky kob fish size had no significant effect on fillet yield (measured as a % of body weight), however it had a significant effect on the fillets proximate composition. As the fish size increased, the lipid content increased and moisture content decreased. Initially, the protein content increased as the fish grew in size, but the rate of increase also declined.

The use of a copper alloy cage net for the production of fish does not increase the risk for the bioaccumulation of metals in the fish meat. The results in Chapter 5 showed all the maximum metal concentrations measured in the fish meat was below the regulatory limits set for safe human consumption by the South African, EU and USA regulations. There is thus no increased risk for consumers eating fish produced in a copper alloy net versus a traditional polyester net.

Chapter 6 and 7 shows that with proper planning, the right equipment and following the correct methods fish can be successfully harvested, chilled and transported for processing. Following an effective cold chain ensures a high quality end product that will be easily marketed. Larger sized fish are also more suited for filleting and portioning. The product was favourably received by restaurant chefs and patrons.

The DST SU KZN Aquaculture Development Project proved the suitability of the Richards Bay harbour for the production of warm water marine finfish. It also proved dusky kob can be successfully farmed in sea cages in South Africa.