SALINITY INDUCED PHYSIOLOGICAL RESPONSES IN JUVENILE DUSKY KOB, *ARGYROSOMUS JAPONICUS* (SCIAENIDAE)

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ABSTRACT

Fisheries management regulations for dusky kob *Argyrosomus japonicus*, an important commercial and recreational fisheries species, have failed and the stock is considered collapsed. It is important to take an ecosystems approach to management which includes understanding the effect of environmental factors on recruitment, abundance and distribution. The distribution of early juveniles (20-150 mm TL) in the wild appears to be restricted to the upper reaches of estuaries at salinities below 5 psu. Food availability could not explain the distribution of early juveniles. The aim of this study was to investigate the role of salinity on the distribution of early juvenile dusky kob (<150 mm TL) by examining physiological responses of juveniles exposed to a range of salinities under laboratory conditions. The hypothesis was that the physiological functioning of early juveniles would be optimised at the reduced salinities which they naturally occur at. The objectives of this study were to investigate the effect of salinity on: i) plasma osmolality; ii) growth, food conversion ratio and condition factor; and iii) gill histology with emphasis on chloride cell size and number.

A preliminary study was undertaken to determine whether the use of 2-phenoxyethanol had an effect on plasma osmolality. Juveniles pithed prior to blood sampling were used as the control. Plasma osmolality was not affected by exposure or duration of exposure (2, 4, 6, 8, 10 min) to 2-phenoxyethanol.

The ability of teleosts to regulate plasma osmolality over a wide range of salinities indicates their degree of 'physiological euryhalinity'. Plasma osmolality of juveniles exposed to 5, 12 and 35 psu was measured every two weeks over a total of six weeks. Although juveniles were able to regulate plasma osmolality over the duration of the experiment, plasma osmolality at 5 and 12 psu was significantly lower than in fish maintained at 35 psu.

Growth is used as an indicator of the relative energy used for osmoregulation at different salinities, as the energy used for osmoregulation becomes unavailable for growth. A nine-week growth experiment was conducted on juveniles exposed to 5, 12 and 35 psu. Juveniles grew and survived at all three salinities. However, growth of juveniles at 5 psu was significantly lower than at 12 and 35 psu. Other than a significantly greater weight gain at 35 psu relative to 12 psu, there was no significant difference in specific growth and length gain between juveniles at 12 and 35 psu. Food conversion ratio and condition factor at 12 and 35 psu were not significantly different, but food conversion ratio and condition factor at 5 psu was significantly greater and lower than at 35 psu respectively.

In fish, gills are considered the major organ involved in osmoregulation. Within the gills, chloride cells are the predominant site of ion exchange which is driven by the Na^+, K^+-ATPase enzyme. Gill samples of juveniles exposed to 5, 12 and 35 psu for six weeks were examined histologically using light microscopy. Chloride cells of juveniles maintained at 5 psu were significantly more abundant than in juveniles at 12 and 35 psu. Chloride cells of juveniles at 5 psu were significantly larger than in juveniles kept at 12 psu, but not significantly different to those of juveniles kept at 35 psu.

The ability of the juvenile fish to regulate plasma osmolality indicates that they are 'physiologically euryhaline', but the reduced growth and proliferation of chloride cells at 5 psu suggests that energy expenditure for osmoregulation is increased at hypoosmotic salinities. Salinity induced physiological responses could therefore not explain the natural distribution of early juvenile dusky kob and it is proposed that other environmental factors (e.g. temperature) are also important. It is also hypothesised that the high conductivity of an estuary in South Africa, to which our understanding is limited, may negate the effect of reduced salinity. Although freshwater input into estuaries is an important factor, further investigations to explain the distribution and abundance of early juveniles is required to make management recommendations. Dusky kob is also becoming an increasingly popular aquaculture species in South Africa. In this regard, early juvenile dusky kob can be grown at salinities as low as 12 psu without negatively affecting growth and production.

TABLE OF CONTENTS

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CHAPTER 1

GENERAL INTRODUCTION

Dusky kob *Argyrosomus japonicus*, a large sciaenid species (maximum size 1.8 m, 75 kg), occurs in both the northern and southern hemispheres. In southern Africa it is found along the east coast, from Cape Point to Mozambique (Griffiths and Heemstra 1995). It is an important estuarine, coastal and offshore fisheries species that is targeted by subsistence, recreational and commercial fishery sectors throughout its distribution range (Griffiths 1996). In South Africa the stock of this highly sought after species is overexploited and the spawner biomass per recruit ratio is estimated to be between 1% and 4.5% of the pristine level (Griffiths 1997b). Fisheries regulations have recently been amended to reduce the fishing pressure on the species. The bag limit was reduced from five to one fish per person per day and the minimum size limit was increased from 40 to 60 cm TL (Griffiths and Fennessy 2000). However, it is generally recognised that conventional fisheries management has failed to prevent or mitigate overexploitation due to ineffective governance, poorly communicated or inadequate science and the failure to account for multispecies effects and climate variability (Pauly *et al*. 2002, Garcia and de Leiva Moreno 2003). Hence the need to adopt an ecosystem approach to fisheries management, which includes understanding the effect of environmental factors on abundance, recruitment and distribution (Beck *et al.* 2001, Alber 2002, Peterson *et al.* 2004).

Prior to 1995, *A. japonicus* was mistakenly classified as *A. hololepidotus* and the taxonomic confusion within the genus in southern Africa was rectified by Griffiths and Heemstra (1995). Aspects of the biology and ecology of the species, as well as the culture technology for aquaculture, have subsequently been researched. Adults spawn in the near-shore marine environment (beyond the surf to depths of approximately 100 m) and early juveniles (> 20 mm TL) recruit into estuaries and migrate to the upper reaches where salinity ranges between

0 to 5 psu. Early juveniles (< 150 mm TL) appear to be restricted to the upper reaches. Larger juveniles (> 150 mm TL) migrate into the middle and lower reach of estuaries, into the surf zone and eventually out to sea (Griffiths 1996, Ter Morshuizen *et al.* 1996).

The confinement of early juveniles (20 – 150 mm TL) to the upper reaches of estuaries has been ascribed to food availability and/or predator avoidance (Griffiths 1996). However, Griffiths (1997a) showed that the most important prey item of early juvenile dusky kob (<100 mm TL) in the Great Fish River Estuary (South Africa, Figure 1.1) was the mysid *Mesopodopsis slabberi* which was most abundant in the middle and lower reaches of the estuary. It has also been suggested that the distribution of early juveniles in the upper reaches may be indirectly influenced by the high freshwater inputs during spring/summer (Griffiths 1997a). High freshwater inputs are known to increase the abundance of primary prey organisms such as calanoid copepods, mysids, insects, *Gilchristella aestuaria*, and other early juvenile teleosts (Whitfield 1994, Whitfield *et al.* 1994). The hypothesis of predator avoidance playing an effective role in the distribution of early juvenile dusky kob is based on theoretical studies on cost-benefit analyses and the evidence to support it is sparse. For other juvenile sciaenids (Louisiana coast), red drum *Sciaenops ocellatus* and spotted seatrout *Cynoscion nebulosus*, the value of estuarine nursery areas, in terms of growth, was more related to variation in physio-chemical variables (temperature, salinity and dissolved oxygen) than diet or extrinsic factors (Baltz 1998).

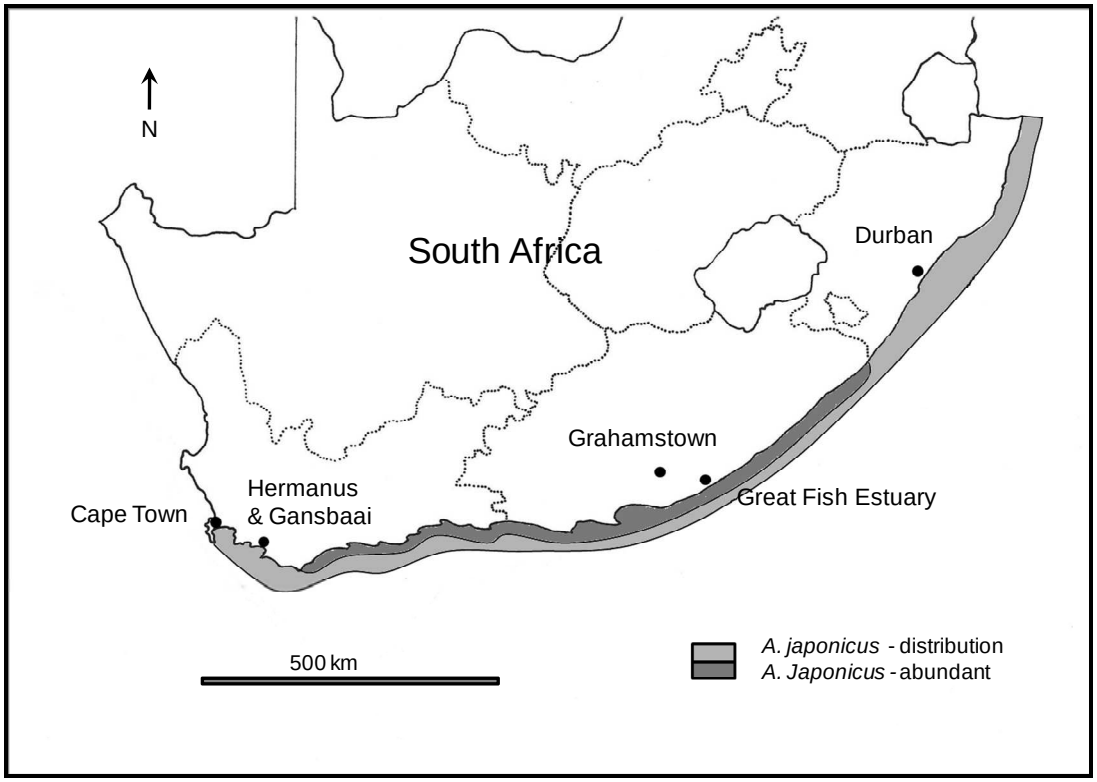

Figure 1.1: Map of South Africa showing the natural distribution of dusky kob, where experimental fish were sourced from, experiments were conducted and other localities referred to in the thesis.

Ecophysiology, a concept introduced by F. E. J. Fry in the 1940s, describes the connection between the ecology and physiology of organisms which are both influenced and regulated by environmental conditions. Ecophysiology improves our understanding of the niche requirements of fish, such as the range of environmental factors that fish can tolerate and populations that can be sustained within (Rankin and Jensen 1993). Fry (1971) classified environmental factors as controlling, limiting, masking, directive and lethal effects on metabolism and indirectly growth, where i) controlling factors (such as temperature and body size) govern metabolic rates, ii) limiting factors (such as dissolved oxygen and food) constrain or feed metabolic rates, iii) masking factors (e.g. salinity and pollutants) increase or load metabolic rate, iv) directive factors (e.g. photoperiod, light intensity and dissolved oxygen) elicit behavioural responses which direct organisms to more favourable conditions and hence reduce load on standard metabolic rate and v) lethal factors are extreme

environments that cause a breakdown in metabolic functioning and ultimately death (Yamashita 2001).

Habitat selection in estuarine associated fish is seldom influenced by a single environmental variable (Gauch 1991). The distribution and abundance of a species is usually affected by a complex relationship between salinity, temperature, turbidity, dissolved oxygen, habitat and food availability (Marshall and Elliot 1998). However, salinity and temperature have been determined as key factors influencing the distributions of fish in estuaries (Whitfield 1994, Thiel *et al.* 1995, Marshall and Elliott 1998, Beck *et al.* 2001, Childs *et al.* 2008). Salinity and temperature were the primary abiotic factors affecting the structure of fish assemblages in the Humber Estuary (England) (Marshall and Elliott 1998). Similarly, fish assemblages in the Elbe Estuary (Germany) were shaped primarily by salinity and temperature, although time of day and tidal cycle were also important (Thiel *et al.* 1995). Whitfield (1994) determined that axial salinity gradients in Eastern Cape estuaries (South Africa) was the single most important factor associated with abundance of larval and juvenile marine fish. On the other hand, it has also been shown that salinity does not play an important or any role in influencing the distribution of fish in estuaries. Although salinity contributed in part to the structural variations of fish distributions between 10 Eastern Cape estuaries, the structural variations were primarily ascribed to the mouth status (open or closed) and the size of estuaries (Vorwerk *et al*. 2003). Salinity could not explain the distinct fish communities associated with the mouth, lower and middle reaches of the Kariega Estuary (South Africa) as there was an absence in a longitudinal salinity gradient (Whitfield and Paterson 2003).

Salinity induced physiological responses under laboratory conditions can generally be explained by the ecology or life history of a species. Cardona (2000) determined that salinity was a key factor in understanding the distribution of juvenile flathead mullet *Mugil cephalus* in estuaries on the Island of Minorca. Juveniles of this species had the fastest growth and lowest metabolic rate in fresh and oligohaline waters, which explained their estuarine distribution (Cardona 2000). In a later study Cardona (2006) also showed that the distribution and growth of other mullet species were similarly affected by salinity. *Lisa ramado* also occurred and grew best at salinities below 15 psu. *L. aurata* and *L. saliens* concentrated in polyhaline and euhaline sites where growth was improved. *Chelon labrosus* was the exception as growth was not affected by salinity although it occurred primarily at salinities

below 15 psu (Cardona 2006). Southern flounder *Paralichthhys lethostigma* predominantly occurs in the low salinity conditions of the upper reaches of estuaries, whereas summer flounder *P. dentatus* occurs more frequently in the high salinity estuarine mouth region (Burke *et al.* 1991). Juvenile southern flounder also grow best at low salinities (Peters and Kjelson 1975) and summer flounder at high salinities (Peters and Angelovic 1973). In some cases, the distribution of fish cannot be explained by physiological responses. For example, although salinity fluctuations were proposed as an important determinant in the distribution of juvenile sciaenids, spot *Leiostomus xanthurus* and croaker *Micropogonias undulates*, Moser and Gerry (1989) established that the juveniles were able to withstand extreme changes in salinity.

Physiological responses to salinity vary between species but are also dependent on life history stages or size and age (Madon 2002, Moser and Gerry 1989). Large juvenile California halibut *Paralichthys californicus* (237-310 mm TL) are less tolerant of variations in temperature and salinity than smaller juveniles (118-172 mm TL). The differences in energetic and water balance responses of the two size groups corresponded with their respective habitat preferences. Small juvenile halibut experience wide fluctuations in temperature and salinity in the estuaries they occur in, whereas larger juveniles migrate into open-coast environments where variations in temperature and salinity are reduced (Madon 2002). Similarly, growth of anadromous juvenile green sturgeon *Acipenser medirostris* at different salinities reflected their life history strategy (Allen and Cech 2007). At a certain age/size they migrate from freshwater environments to brackish and marine water environments (Nakamoto *et al.* 1995) and growth in seawater improves with age/size (Allen and Cech 2007).

Early juvenile dusky kob (mulloway) in Australia showed improved growth at low salinities (Fielder and Bardsley 1999) and in South Africa their distribution appears to be restricted to low salinity (0-5 psu) areas (Griffiths 1996) and to estuaries with high freshwater inputs (Whitfield and Paterson 2003). This suggests that salinity may be the driving force in the distribution of early juveniles. Whitfield and Paterson (2003) found that juvenile dusky kob were scarce in the freshwater deprived Kariega Estuary in contrast with the nearby freshwater enhanced Great Fish Estuary. They postulated that if freshwater was abstracted from a system and the river-estuary interface (salinity < 10 psu) was reduced it would diminish suitable

nursery area for dusky kob. Telemetry studies on juvenile dusky kob (250-400 mm TL) in the Great Fish Estuary indicated that they made frequent longitudinal excursions up or down the estuary and some of the movements corresponded with the tidal cycles (Cowley *et al.* in press, Næsje *et al.* in prep.). Preferred habitat, home ranges and activity patterns of Australian sub-adult mulloway (330 – 730 mm TL) in the Georges River were also investigated using telemetry. Key habitats of mulloway were identified as discrete holes or basins up to 20 m deep. The characteristic salinity of each hole was hypothesised to provide a signature to facilitate homing (Taylor *et al.* 2006). Fielder and Bardsley (1999) established that the juvenile mulloway had the highest growth and the lowest food conversion ratio at 5 psu (Fielder and Bardsley 1999). The results, however, were not significant and this was attributed to the low statistical power of the experiment. Hence it appears that the availability of low salinity areas in estuaries along the east coast of southern Africa may govern the recruitment success of dusky kob and viability of the local dusky kob stock.

Freshwater flow into estuaries is seriously impacted by the increased demand of water for agricultural use through the construction of large dams and numerous small agricultural dams, vast interbasin transfer and irrigation schemes. These anthropogenic influences can result in a loss (impoundment) or increase (inter-basin transfer) in water flow and alter the pattern of supply in terms of seasonality, quantity and quality (Whitfield 1998, Alber 2002). The Great Brak Estuary on the south coast of South Africa was used as a case study to determine the effect of reduced freshwater input on fish communities. A recruitment index, the indicator, was based on the preferred recruitment of 27 fish species and the degree of dependence of these species on estuaries. The recruitment index also integrated the status of the estuary mouth (open or closed) and the longitudinal salinity differences to provide an indication of recruitment potential. It was found that a reduction in freshwater input resulted in a distinct decrease in recruitment index (Quinn *et al.* 1999). Similarly, the diversion of the Colorado River flow from the Gulf of California had a dramatic effect on the life history of the sciaenid, *Totoaba macdonaldi* (Rowell *et al.* 2008). Growth in this species subsequent to the river diversion was significantly reduced, which delayed maturation and consequently impacted abundance (Rowell *et al.* 2008). By contrast, Strydom *et al.* (2002) proposed that larval and juvenile abundance of *G. aestuaria* was negatively affected by high river flow in the Great Fish River Estuary. It is therefore imperative for the management of the species to understand the role of anthropogenic influences on the conservation of estuarine nursery

areas. Examining the physiological responses of species to salinity is a move towards improving our understanding of the consequences of changes in freshwater flow and pattern into estuaries on the ichthyofauna.

Juvenile dusky kob are dependent on estuaries and hence are highly vulnerable to angling pressure, which results in a high mortality (Griffiths 1997b, Whitfield 1998). This was shown by Cowley *et al.* (2003) who found that 93% of undersized dusky kob that are caught in the Great Fish Estuary were kept by anglers. Due to the suppressed state of the dusky kob stock, stock enhancement using hatchery reared juveniles provides a means to contribute towards rebuilding the stock. However, optimal release locations need to be identified in order to restock natural nursery areas (Taylor *et al.* 2006, Bell *et al.* 2008). Taylor *et al.* (2006) suggested that hatchery reared sub adult mulloway (300-500 mm TL) should be stocked directly into deep holes to minimise movement and migration. The reason for the occurrence of juvenile dusky kob in low salinity environments in the upper reaches of estuaries is not yet fully understood. By developing a better understanding of the degree of euryhalinity and the osmoregulatory capacity of juvenile dusky kob this study will contribute towards the development of stock enhancement protocols for this species.

Dusky kob has been identified as a suitable species for aquaculture due to its fast growth rate, high flesh quality and good market value (Gray and McDonall 1993, Griffiths 1996). The culture of dusky kob in South Africa is in pilot scale production and the rearing technology is being optimised (Hecht 2000, Schoonbee and Bok 2006). Optimising the environmental conditions for growth, feed conversion and survival of a culture species is imperative as it directly affects production (Quéméner *et al.* 2002). Collett (2008) determined the optimum temperature (Collett *et al.* 2008), light intensity (Collett *et al.* in press), feeding frequency, and stocking density of juvenile dusky kob. Fry (1971) classified salinity as a masking factor, in other words the amount of energy used for osmoregulation becomes unavailable for growth. Although the proportion of total metabolic energy used for osmoregulation is controversial, the cost of osmoregulation can increase or decrease with changing salinity (Brett 1979). Fielder and Bardsley (1999) reported that juvenile mulloway grew best at 5 psu, the salinity at which they occur naturally (Griffiths 1996). However, Doroudi *et al.* (2006) suggested that the optimum salinity range for juvenile mulloway was between 15 to 33 psu. Survival rates of juveniles were negatively affected below 15 psu and salinity did not have a

significant effect on growth between 15 and 35 psu. The effect of salinity on growth of southern African juvenile dusky kob has not been determined to date and literature on the effect of salinity on the juvenile mulloway is contradictory.

The aim of this study was to investigate whether salinity influenced the distribution of early juvenile dusky kob (< 150 mm TL) by examining various physiological responses of juveniles to a range of salinities under laboratory conditions. It was hypothesised that the physiological functioning of juvenile dusky kob would be optimised at salinities at which they occur naturally (i.e. around 5 psu). The objectives were to investigate the effect of salinity on: i) plasma osmolality; ii) growth, food conversion ratio and condition factor; and iii) gill morphology (histology). The findings of this research will contribute towards the ecological understanding of the estuarine habitat use of early juvenile dusky kob and ultimately assist with the management of this important fishery species. Furthermore, information on the physiological responses to salinity will assist the development of aquaculture and stock enhancement protocols for this species.

In order to address the objectives of the study, the thesis unfolds by broadly discussing the general methods and experimental systems used in all the experiments (Chapter 2), although specific methods and experimental designs are described in each chapter. Before addressing the primary objectives, it was established whether the sampling protocol would mask the effect of salinity on plasma osmolality in Chapter 3. The ability of fish to regulate plasma osmolality over a wide range of salinities provides an indication of their 'physiological euryhalinity' and this was tested on early juvenile dusky kob in Chapter 4. As growth provides an indication of energy expenditure at different salinities, the growth of early juveniles at 5, 12 and 35 psu is examined in Chapter 5. The sixth chapter deals the morphological adaptation of early juvenile dusky kob, by examining the gill morphology, specifically the size and abundance of chloride cells at different salinities. The last chapter (Chapter 7) links all the results from the previous chapters and discusses the overall implications.

CHAPTER 2

GENERAL METHODS

This study made use of first generation hatchery reared juvenile dusky kob that were obtained from either the Espadon Marine Hatchery in Hermanus or the Irvin and Johnson Hatchery in Gansbaai (at an age of approximately three months), depending on availability (Figure 1.1). The fish were purged prior to transport to minimise ammonia production. Fish were transported to the Marine Hatchery of the Department of Ichthyology and Fisheries Science at Rhodes University (Grahamstown, Figure 1.1) by road in 200-L blue plastic drums at a stocking density not exceeding 20 g L^{-1} (< 1000 fish per drum). Temperature was monitored continuously and kept below 20ºC by adding sealed ice bags to the water. Oxygen saturation was kept above 150% by the addition of medical grade oxygen through diffusers in each drum. Upon arrival, fish were first acclimatised to the water of the holding tanks by the slow addition of water.

Fish were acclimatised and grown to a size at which blood could be drawn in a 'holding' system at the Marine Hatchery of the Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown. The 'holding' system consisted of six 290-L tanks. Outflow from the tanks flowed into a 1000-L sump by gravity. Water was pumped from the sump through a sand filter and then partially through the 11-L protein skimmer, the 282-L biological trickle filter and into the tanks. Fish were maintained at a salinity of 35 psu, a temperature of 21 ± 1 °C, a stocking density ≤ 3 kg m^{-3} and a fixed photoperiod of 12L/12D. Initially fish were fed on trout crumble pre-starter pelleted feed (50% Crude protein, min 14% fat, max 4% fibre, max 10% ash and max 10% moisture, by Indian Ocean Aquafeeds Ltd, Johannesburg, South Africa) to apparent satiation five times a day. When fish had reached a size of approximately 6 g the feed was changed to a 2.2-mm pelleted feed (45% crude protein, 12.5% fat, max 4% fibre, max 10% ash and max 10% moisture, by Indian Ocean Aquafeeds Ltd, Johannesburg, South Africa) and fed three times a day to apparent satiation. Juvenile dusky kob exhibit high rates of cannibalism and hence fish were manually size graded every two weeks.

Experiments were undertaken in 12 independent re-circulating tanks (Figure 2.1a and 2.3) in a temperature-controlled room with a fixed photoperiod of 12L/12D. Light was supplied by six 1.5 m Biolux® fluorescent tubes situated along the centre of the room (Figure 2.2). Each re-circulating tank consisted of a 265-L tank, a Resun SP-6600 submersible pump, a 30-L

trickle filter and a 1.4-L counter current protein skimmer. Approximately 60% of the flow (260 L hour^{-1}) was pumped through the biological trickle filter, and 40% through the protein skimmer (Figure 2.2b) attached to the side of the trickle filter. Water was pumped into the trickle filter through a Gardenia sprinkler to increase surface area of spray. The trickle filter was filled with fibre wool (to remove solids), shredded plastic and abalone shells (for biological filtration). Each tank had removable covers, made of 10 mm conduit pipe and 80% percent shade cloth, to reduce light (163-204 lux) and prevent fish from jumping out. Air was supplied by a SE 22 Elektror airsystems gmbh blower and added through an airstone in the tank and the protein skimmer of each tank. Each tank had two 300 watt heaters, suspended in the water with a square piece of Styrofoam. Room temperature was controlled by an air-conditioning system. Salinity treatments were randomly assigned to twelve tanks (Figure 2.2).

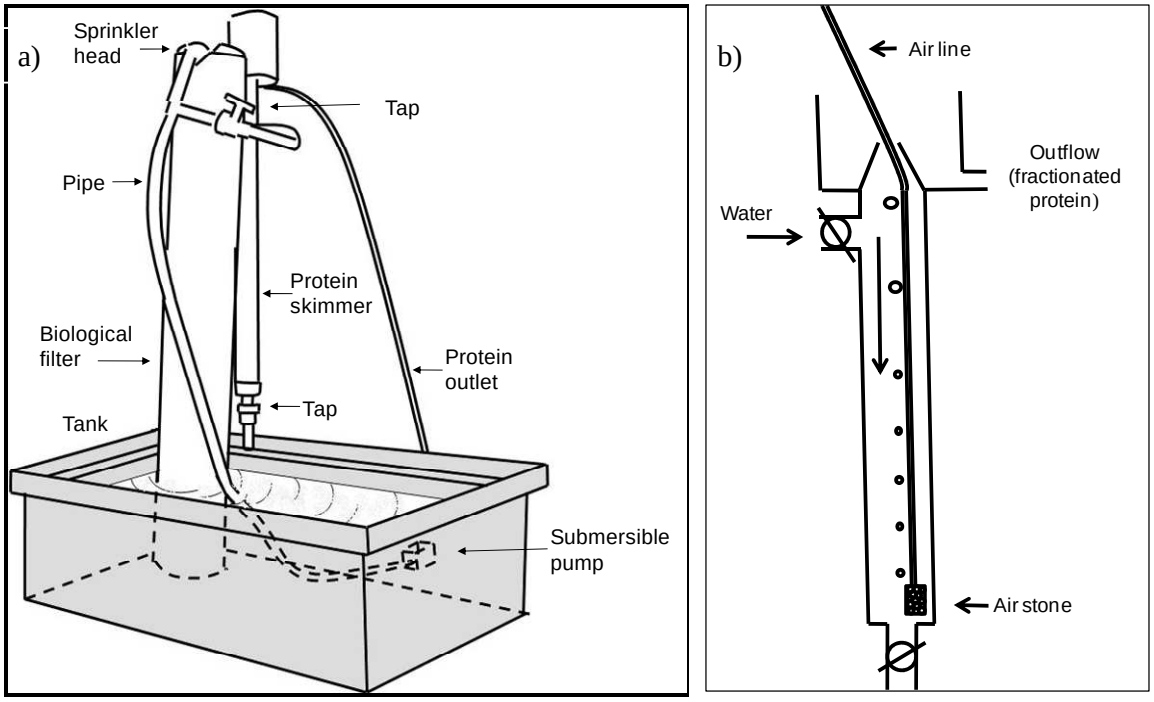

Figure 2.1: a) Schematic of the independent re-circulating system and b) of the protein skimmer that was used during experiments

During experiments temperature, salinity, oxygen and conductivity were recorded on alternate days. Temperature and conductivity was measured with a multi-probe (water

checker U-10, Horiba), oxygen with a portable dissolved oxygen meter (HI-9145, Hanna Instruments) and salinity with a refractometer. Tanks were topped up with rain water to compensate for evaporation. Prior to adding rainwater, it was filtered through a 5µ mesh bag. Total ammonia, nitrite and pH were measured twice a week and maintained by adding fresh water at the respective salinity as and when required. The seawater was diluted with rainwater to the respective salinity in 100-L plastic containers prior to adding it to the tanks. Uneaten food and faeces were siphoned off the bottom of the tanks once a day. Temperature was maintained between 22-23ºC, which falls in the lower range of optimum temperature for growth and upper range for optimum food conversion ratio for juvenile dusky kob (Bernatzeder and Britz 2007, Collett *et al.* 2008). Salinity was changed by reducing the volume of the tank and adding pre-calculated volumes of rain water to decrease salinity by the desired amount.

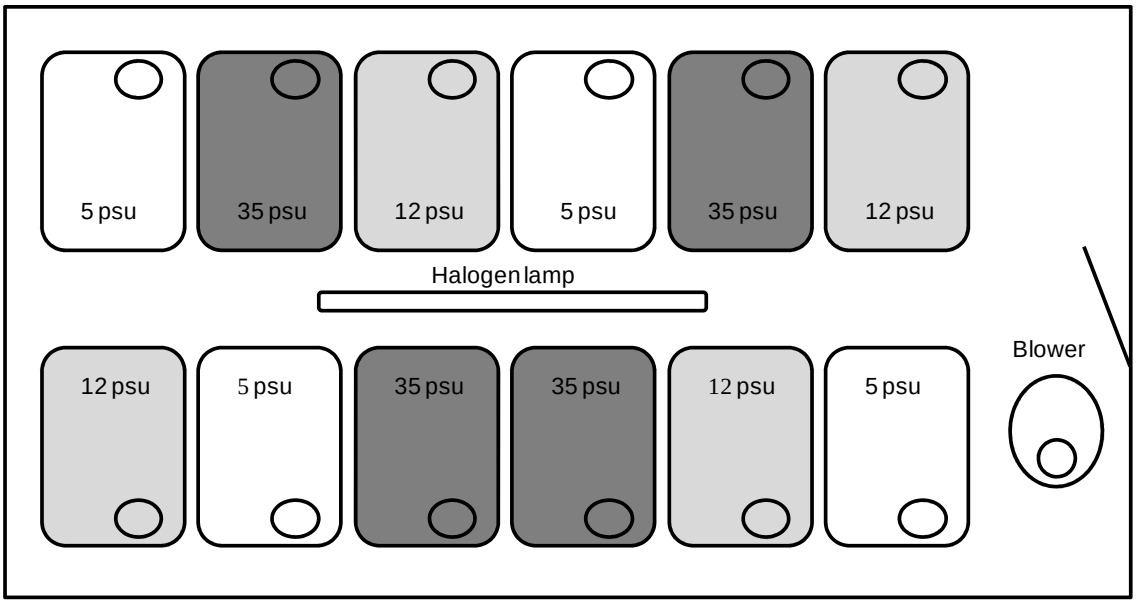

Figure 2.2: Schematic overview of temperature controlled room and the placement of the independent re-circulating tanks with the randomly assigned salinities

Details of the specific methods and treatments used for each experiment are given in the respective chapters below. The methods used for measuring plasma osmolality described in Chapter 3 also apply to Chapter 4. The experimental design described in Chapter 4 also applies to Chapter 6.

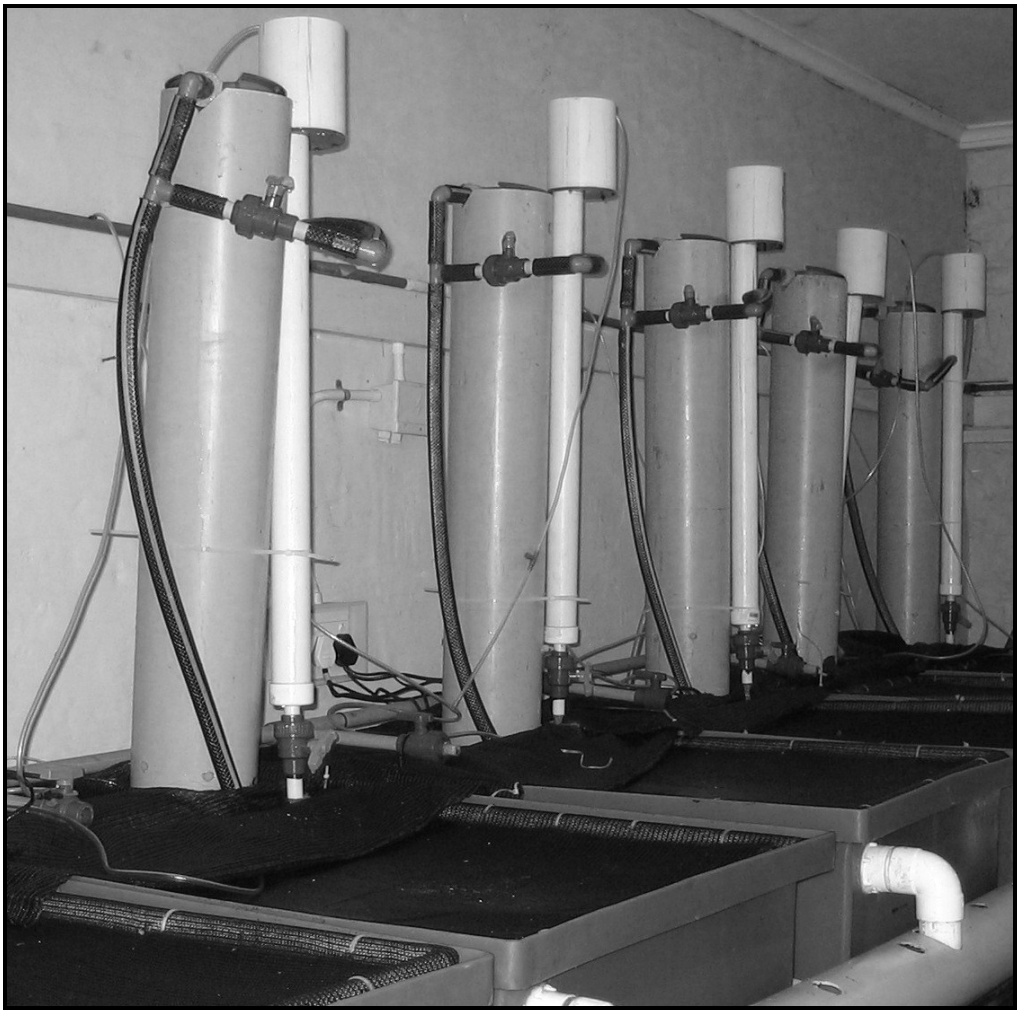

Figure 2.2: Photograph of the independent re-circulating tanks used in experiments.

EFFECT OF SHORT TERM EXPOSURE TO THE ANAESTHETIC 2-PHENOXYETHANOL ON PLASMA OSMOLALITY OF JUVENILE DUSKY KOB*

*****Bernatzeder AK, Cowley PD, Hecht T (2008) Effect of short term exposure to the anaesthetic 2-phenoxyethanol on plasma osmolality of juvenile dusky kob, *Argyrosomus japonicus* (Sciaenidae). *Journal of Applied Ichthyology* **24**: 303-305

3. 1. INTRODUCTION

The general protocol of measuring plasma osmolality, used as an indicator of the physiological responses of fish to salinity, includes anaesthesia of fish prior to blood extraction (Imsland *et al.* 2003, Magill and Sayer 2004, Sardella *et al.* 2004). Plasma osmolality can be affected by stressors (Korcock *et al.* 1988, Hunn and Greer 1991) through changes in blood cortisol and adrenaline levels (Tort *et al.* 2001). Cortisol may affect osmolality by increasing membrane permeability to ions (Wedemeyer 1969, Ross and Ross 1984), and increased adrenalin levels in the blood can influence blood Na^+ and Cl^- concentrations (McDonald and Milligan 1997). As a secondary stress response, fish that are acutely or chronically stressed may undergo hemodilution in fresh water and hemoconcentraton in seawater (Wedemeyer *et al.* 1990). This concept is supported by the studies of Robertson *et al.* (1988), Marino *et al.* (2001) and Cataldi *et al.* (2005).

Anaesthetics are used to reduce stress; however, certain anaesthetics used can influence the magnitude of the stress response in fish (Hunn and Greer 1991, King *et al.* 2005). 2-phenoxyethanol (2-PE) is an inexpensive and commonly used anaesthetic. 2-PE was found to be one of four anaesthetics, out of 16 chemicals tested by Gilderhus and Marking (1987) on rainbow trout *Oncorhynchus mykiss*, to meet all the criteria for efficacy. It was found to anaesthetise rainbow trout (73g) to a handleable state within 3 min at a concentration of 0.2ml L^{-1}. However, little is known about the anaesthetic mechanism of 2-PE on fish (Deacon *et al.* 1997). It has been shown that 2-PE can result in an increase in cortisol in gilthead sea bream *Sparus aurata* L. (Molinero and Gonzalez 1995, Tort *et al.* 2002), rainbow trout (Tort *et al.* 2002) and black sea bass *Centropristis striata* (King *et al.* 2005). Thus, if 2-PE has an effect on plasma cortisol, it is hypothesised that it may affect plasma osmolality. Hseu *et al.* (2005) found that 2-PE did not reduce stress response in black porgy *Acanthopagrus schlegel*

when exposing them to air for 3 min. Osmolarity of the control fish, where blood was extracted directly, was significantly lower than those exposed to air with or without prior anaesthesia. The osmolarity of fish anaesthetised prior to air exposure was marginally higher than those exposed to air for 3 min without prior anaesthesia. Consequently, responses to environmental stimuli could be masked by the stress response to the sampling procedure (Di Marco *et al.* 1999). Thus, the possible influence of the anaesthetic and the duration of exposure to the anaesthetic on plasma osmolality should be known.

The mariculture potential of dusky kob, *Argyrosomus japonicus*, has been realised in Australia and is currently in pilot-scale production in South Africa (Hecht 2000, O'Sullivan and Savage 2004). It is therefore vital to understand stress responses of dusky kob to general handling and anaesthetics. In addition, dusky kob can tolerate a wide range of salinities (Whitfield *et al.* 1981, Fielder and Bardsley 1999); consequently, the physiological responses to salinity are of interest to aquaculturalists. There is also a paucity of information on the influence of blood extraction methods and the use of anaesthetics on blood plasma osmolality. The aim of this study was to investigate i) the effect of 2-PE on plasma osmolality and ii) the effect of duration of exposure to 2-PE on plasma osmolality.

3.2. METHODS

Two-month-old hatchery-reared juvenile dusky kob were acquired from Espadon Marine Hatchery in Hermanus (South Africa) in April 2006. Fish were held in 290-L rectangular tanks in a recirculating system, at a stocking density of 2.8 kg/m^3. They were fed Indian Ocean Aquafeed trout pellets (50% protein, 14% fat, 4% crude fibre). The light cycle was kept at 12h dark: 12h light. Water temperature was maintained at approximately 23.2°C (+/- 0.8°C, n=14) and salinity was kept at 35 psu.

Prior to the experimental trials, the weight (g), standard length (SL) and plasma osmolality of the juvenile dusky kob (average size of 56.9g and 150.8 mm S L (n = 60)) was measured. The first trial involved measuring the plasma osmolality of 15 fish that had been exposed to 2-PE for 2, 4, 6, 8 and 10 min. This was done by placing five fish in a bucket with 10-L of seawater and a 0.2 ml L^{-1} concentration of 2-PE. Blood was drawn from these fish in succession, with two-minute intervals between each fish sampled. This was repeated three

times each with different fish. In the control treatment blood was drawn from fish that had been pithed directly after they were netted and blood was drawn immediately. Fish were pithed by the insertion of a rigid metal rod (diameter 2 mm) through their nostrils into their brain.

The method of blood sampling was the same for all trials. 150 µl of blood was drawn from the caudal vein using 0.5-ml heparinised syringes. The blood samples were then transferred into 1.5-ml transparent Eppendorf tubes and centrifuged at 3000 rpm for 5 min. The plasma layer was transferred into new Eppendorf tubes and covered with a thin layer of chemically pure liquid paraffin to prevent evaporation of the samples. Plasma osmolality was measured by a freeze-depression osmometer (Advanced Instruments, Model 3320) from 20 µl of the plasma samples.

Weight (g) and length (mm standard length) of the initial 60 fish sampled were plotted against plasma osmolality. The Friedman test was used to test the hypothesis that the duration of exposure to 2-PE affected plasma osmolality. Plasma osmolality of anaesthetised fish (n = 15) was compared to the pithed fish (n = 15) using a t-test for independent samples on square-root-transformed data, after testing for homogeneity of variance and normality of residuals.

3.3. RESULTS

There was no significant relationship between plasma osmolality and fish weight ($r^2 = 0.065$ and $p < 0.05$), and length ($r^2 = 0.088$ and $p < 0.05$) of the sampled fish. Duration of exposure to 2-PE (0.2 ml L^{-1}) over the five time intervals (2, 4, 6, 8 and 10 min) was not significantly different, however, the highest variability was found after 2 min of exposure (Figure 3.1: $p = 0.976$). There was no significant difference between plasma osmolality of anaesthetised fish and those sampled after pithing (Figure 3.2: $p = 0.99$).

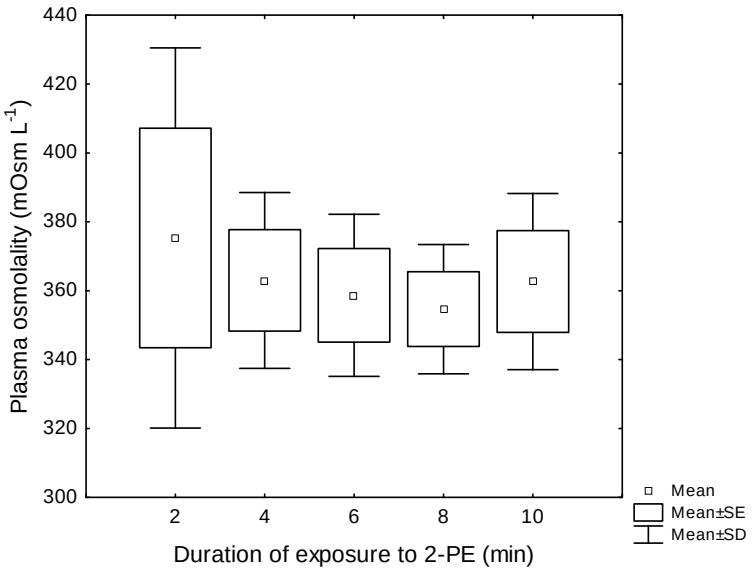

Figure 3.1: Effect of 2-PE on plasma osmolality (mOsm L^{-1}) of dusky kob after 2, 4, 6, 8 and 10 min exposures (n = 15)

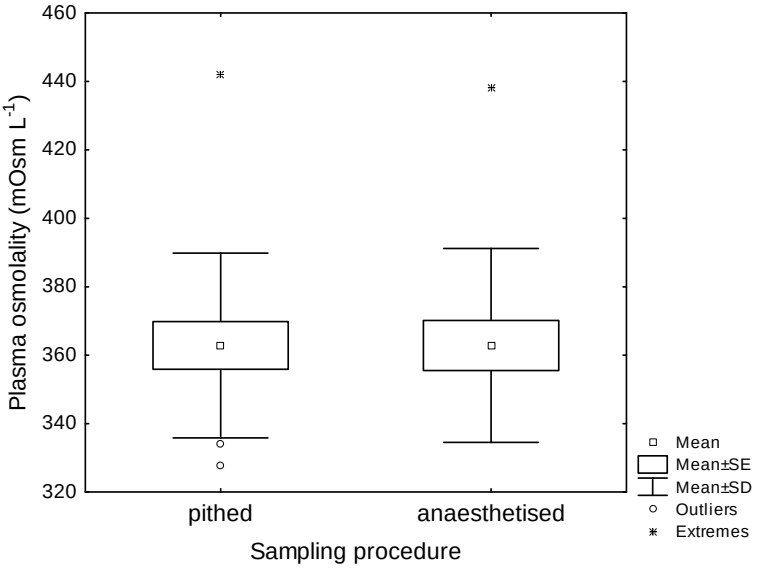

Figure 3.2: Comparison of plasma osmolality (mOsm L^{-1}) between fish pithed (control, n = 15) prior to drawing the blood and those anaesthetised with 2-PE (n = 15)

3.4. DISCUSSION

Determining the haematological parameters of a completely unstressed fish is difficult as sampling procedures are potentially stressful to fish (Kreiberg 2000). It is, however, possible to eliminate the stress of the anaesthetic and drawing blood by euthanising the fish directly after netting and before sampling. The results of this study revealed no difference in plasma osmolality between pithed (control) and anaesthetised fish. Following this, the exposure to 2-PE did not affect plasma osmolality of juvenile dusky kob. Similarly, Bystriansky *et al.* (2006) established that 2-PE did not compromise the osmoregulatory ability of artic charr *Salvelinus alpinus*.

The duration of exposure to certain anaesthetics can itself induce a stress response. Molinero and Gonzalez (1995) showed that both the dose and exposure to 2-PE evoked a cortisol response in gilthead sea bream. Thomas and Robertson (1991) found that both cortisol and glucose increased up to a point, the longer red drum *Sciaenops ocellatus* were exposed to MS-222 (80 mg L^{-1}) and quinaldin sulphate (20 mg L^{-1}). In contrast, King *et al.* (2005) found an initial cortisol response by juvenile and adult black sea bass due to handling of the fish but no significant difference in cortisol levels over the 10 – 30-min exposure to 2-PE following this. The results of this study showed that exposure to 2-PE over 2, 4, 6, 8 and 10 min exposure intervals did not elicit a significant change in plasma osmolality. This indicates that one is able to anaesthetise dusky kob in 'batches' as the duration of exposure over short time intervals does not affect osmolality. However, the high variance after 2 min exposure to 2-PE, which may have been significant with a larger sample size, could be explained by the fact that it takes approximately 3 min to anaesthetise fish with 2-PE (Gilderhus and Marking 1987, Bystriansky *et al.* 2006) hence it is recommended that blood is only extracted after 4 min exposure to 2-PE.

Hunn and Greer (1991) found that plasma osmolality in small Atlantic salmon *Salmon salar* was higher than in larger salmon. However, this was not the case in this experiment. The results may suggest species specific differences or because Hunn and Greer (opt. cit.) analysed a larger size range of fish (70 - 290 mm).

In conclusion, exposure and duration of exposure to 2-PE did not affect the plasma osmolality of dusky kob. Therefore 2-PE is a suitable anaesthetic for investigating osmoregulatory responses in this species.

EFFECT OF SALINITY ON PLASMA OSMOLALITY OF JUVENILE DUSKY KOB

4.1. INTRODUCTION

Euryhaline teleosts are known to regulate their internal body fluid to salinities between 10-15 psu (Holmes and Donaldson 1969, Brett 1979). In order for fish to maintain the small range in extracellular osmolality they need to hyper-osmoregulate at salinities levels below isosmotic (e.g. freshwater) and hypo-osmoregulate at salinities levels above isosmotic (e.g. seawater) (Marshall and Grosell 2006). The less the extracellular osmotic concentration of fish is affected by exposure to a wide range of salinities, the more euryhaline the species is considered (Jobling 1995). For example, Allen and Cech (2007) found that the effect of salinity on the osmolality of juvenile green sturgeon *Acipenser medirostris* decreased as size/age increased. Concurrently, growth and survival of juvenile green sturgeon at high salinities improved with age/size.

Fish exposed to a change in salinity generally undergo an adaptive followed by a regulatory physiological response. During the adaptive phase, the osmolality of fish changes and then gradually levels off close to the original values (Jobling 1995). Blood plasma osmolality of euryhaline fish appears to adapt to abrupt changes in salinity within 48 hours. Juvenile red drum *Sciaenops ocellatus* were found to adapt their blood serum osmolality to a steady state after abrupt changes in salinity within 24 hours (Wakeman and Wohlschlag 1983). In the regulatory phase, on the other hand, fish finely regulate plasma osmolality and attain ionic homeostasis (Jobling 1995). Previous studies have shown that changes in plasma osmolality over time in fish exposed to different salinities, is manifested in decreased growth, mortality and uncommon morphological changes. Cataldi *et al.* (1995), for example, found that plasma osmolality of juvenile Italian sturgeon *Acipenser naccarii* increased with increased length of exposure to seawater which resulted in kidney degeneration. Similarly, Rodriguez *et al.* (2002) found plasma osmolality of juvenile Siberian sturgeon *Acipenser baerii* was significantly higher than freshwater when exposed to above 9 psu. Plasma osmolality also increased over time at 9 psu and above, which led to negative affects on growth and gill morphology.

Early juvenile dusky kob (30-150 mm TL) occur in the upper reaches of estuaries where the salinity is generally between 0-5 psu (Griffiths 1996). It is natural for juvenile dusky kob in

estuaries to be subjected to short term fluctuations in salinity due to tidal effects and rainfall. Anthropogenic impacts such as river impoundment and inter-basin transfer schemes, can lead to long term or permanent alterations in the salinity regime of an estuary and ultimately influence the abundance and life history strategy of estuarine dependent species (Whitfield 1998). This has been reported for another Sciaenid, *Totoaba macdonaldi*, where dramatic changes in abundance, growth and reproduction were attributed to the diversion of the Colorado River (Rowell *et al*. 2008)

The status of the dusky kob stock along the South African east coast is considered as "collapsed" and this has led to the implementation of stringent output controls such as a bag limit of one fish per angler per day and a minimum size limit of 60 cm TL (Griffiths and Fennessy 2000). Hence it is important for management to understand the role that man-made perturbations in the catchment have on the conservation of the habitat of estuarine dependent species, the impact of short and long term changes in salinity on the physiology of fishes and the concomitant effects on recruitment into the fishery. Given the collapsed status of the stock along the coast the possibility of re-seeding is now seriously being considered. An understanding of the degree of euryhalinity and the osmoregulatory capacity of juvenile kob is therefore imperative. The aim of this study was to determine if, and to what degree, early juvenile dusky kob are able to osmoregulate efficiently during long term exposure to a wide range of salinities. The objective was to test the effect of a wide range of salinities on plasma osmolality over time. The trial was run over a six-week period using hatchery reared juvenile dusky kob. The salinities tested were 5 psu (the salinity at which juveniles normally occur in the wild), 12 psu (assumed isosmotic level) and 35 psu (reference salinity at which they were spawned and reared).

4.2. METHODS

Thirty juvenile fish (17.5 ± 1 g, 100 ± 1.1 mm SL) were placed into each of the twelve independent 265-L tanks in the temperature controlled lab (Chapter 2). Fish were acclimatised for seven days at 35 psu. The twelve tanks were randomly assigned to the three salinities (5, 12 and 35 psu) and the four replicates of each. Fish were then progressively acclimated to the relevant salinities during daylight hours, over two days, at a rate of 2 psu

every two hours until the relevant salinities (5 and 12 psu) were reached. Taylor *et al*. (2005) found that the Australian juvenile *A. japonicus* were able to survive a change in salinity from 35 to 5 psu in 1.5 hours. The first blood samples were taken the day after the target salinities had been reached and then at two-week intervals thereafter. Fish were exposed to the three salinities for a total of six weeks. During the course of the experiment fish were fed on pellets (45% crude protein, 12.5% fat, max 4% fibre, max 10% ash and max 10% moisture) to apparent satiation three times a day. Temperature, oxygen, salinity and conductivity were measured every second day. Temperature (22-23ºC) was kept within the range of optimum temperature for growth (25.3ºC) and FCR (21.4ºC) of juvenile dusky kob (Collett *et al.* 2008) and photoperiod was fixed at 12L/12D. Total ammonia, pH, and nitrite were measured twice a week and water was replaced accordingly (Un-ionised ammonia 0.007 ± 0.01 mg L^{-1}, Nitrite 0.06 ± 0.06 mg L^{-1}).

During each sampling period, blood samples were taken from five fish per salinity and per replica. Fish were anaesthetised prior to the blood being extracted. This was achieved by transferring the fish into a 10-L bucket containing a 0.2 mL L^{-1} of 2-phenoxyethanol solution of water at the equivalent salinity. The juveniles were exposed to the anaesthetic for a minimum of 3 minutes and a maximum of 10 minutes. In Chapter 3 (Bernatzeder *et al.* 2008) it was shown that anaesthetising the fish with 2-phenoxyethanol had no effect on blood osmolality. Blood was extracted and the plasma osmolality measured as described in Chapter 3. To establish osmolality of the treatment salinities, nine salinity concentrations between 0 – 35 psu (Table 4.1) was made up with rain water and sea water and osmolality was measured with the same Freeze depression osmometer as the blood samples.

Data Analysis

Average plasma osmolalities after two weeks exposure, were subjected to regression analysis against the osmolality of the treatment salinities. The intersection of the regression line with the isoionic line was used to estimate the salinity isosmotic to the osmolality of juvenile dusky kob. One Way Analysis of Variance (ANOVA) was used to test whether there was a significant difference between environmental variables, such as oxygen and temperature, between individual systems and treatments. If the data did not meet the assumptions of normality or homogeneity of variance then the non-parametric Kruskal-Wallis test was used instead. Mean temperature over the duration of each two-week sampling interval was plotted

against time, on the same graph as total osmolality over time. Difference in osmolality over time and between salinities was tested with a Repeated Measures ANOVA. Assumptions of normality of residuals and sphericity were tested and where sphericity was not met (epsilon < 0.7) the Greenhouse-Geisser correction was used. The Tukey HSD test was used to determine where the significant differences lay. All the analyses were done with STATISTICA 7^{TM}.

4.3. RESULTS

Isosmotic

There was a significant linear relationship between the treatment water osmolality and plasma osmolality of the fish at the different salinities (r^2 = 0.848, p < 0.00). The regression relationship

$$P_{osmo} = 320.14 + 0.0309 \times W_{osmo}$$

was slightly positive with a slope of 0.0309 and intersected with the isoionic line at 330.35 mOsm L^{-1} (Figure 4.1). P_{osmo} = osmolality of plasma and W_{osmo} = osmolality of treatment water.

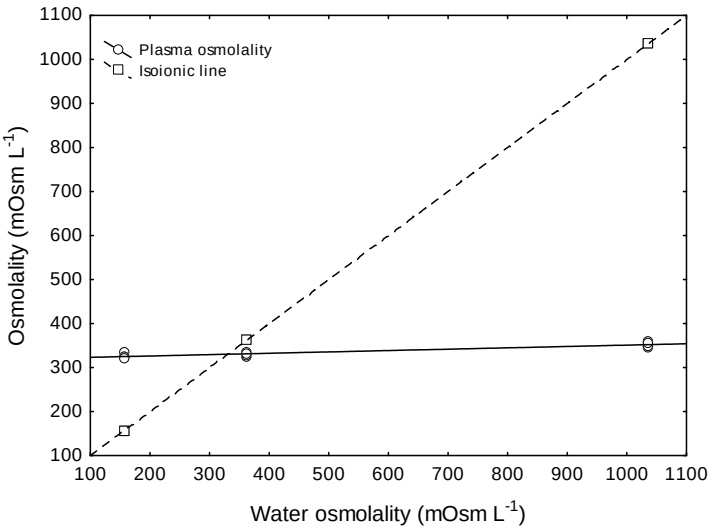

Figure 4.1: Regression of plasma osmolality of juvenile dusky kob at 5, 12 and 35 psu after 14 days of exposure (solid line) and intersect with isoionic line (dotted)

The isosmotic level of 330.35 mOsm L^{-1} corresponded to a salinity of 11 psu which was calculated with the following equation derived from osmolality of different concentrations of seawater (Table 4.1).

$$W_{osmo} = 29.327 \times Salinity + 10.151$$

Table 4.1: Osmolality of different concentrations of seawater

Salinity (psu)	0	5	10	12	15	20	25	30	35
Osmolality (mOsm L^{-1})	15	152	303	357	443	616	740	887	1036

Plasma osmolality

Osmolality changed significantly over time ($F_{3,27} = 3$, $p < 0.05$). However, epsilon was < 0.7, and the Greenhouse-Geisser correction suggested that osmolality did not change over time ($F_{1.9, 17.4} = 2.99$, $p = 0.07751$). Salinity had a significant effect on osmolality ($F_{2,9} = 44.68$, $p < 0.00$) (Figure 4.2). The Tukey HSD test revealed that osmolality was significantly different between salinities 5 and 35 psu ($p < 0.00$), 12 and 35 psu ($p < 0.00$) but not between 5 and 12 psu ($p = 0.124$). The same test showed that the only significant change in osmolality over time was between the beginning and the fourth week of the trial ($p < 0.05$).

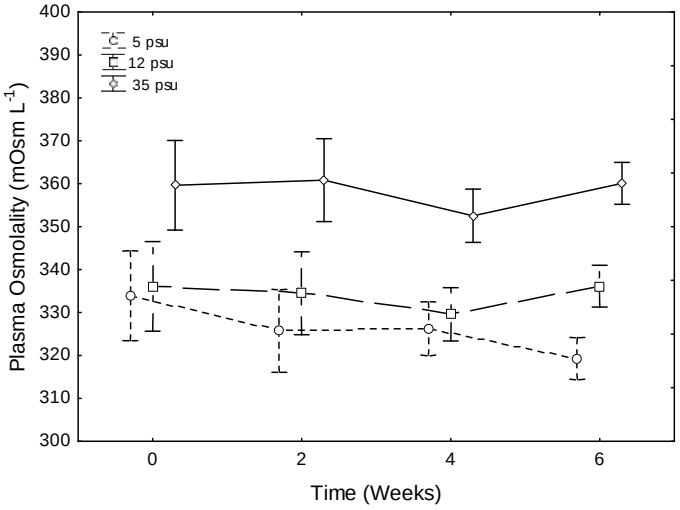

Figure 4.2: Plasma osmolality of juvenile dusky kob exposed to 5, 12 and 35 psu over the six week trial duration. (Bars denote 95% confidence interval)

Table 4.2: Means (± Stdev) of abiotic variables in the all treatments

Treatment (psu)	Salinity (psu)	Oxygen saturation (%)	Temperature (ºC)	Conductivity (mS)	Oxygen (mg L^{-1})
5	5.09 ± 0.06	88.82 ± 1.3	21.96 ± 0.08	9.12 ± 0.048	6.99 ± 0.1
12	12.14 ± 0.03	88.47 ± 1.2	22.08 ± 0.21	17.60 ± 0.064	6.67 ± 0.2
35	35.04 ± 0.15	86.69 ± 0.6	22.03 ± 0.20	44.13 ± 0.116	5.73 ± 0.05

Abiotic conditions

Temperature was not significantly different between all twelve replicas ($F_{11,228}$ = 0.5, p = 0.882) or between treatments ($H_{11,240}$ = 6.197, p = 0.452). There was no significant difference in oxygen concentration between replicates at 5 psu ($F_{3,60}$ = 0.9, p = 0.44), 12 psu ($F_{3,60}$ = 1.01, p = 0.39) and 35 psu ($F_{3,60}$ = 0.24, p = 0.865) (Table 4.2). However, there was a significant difference in oxygen concentration between treatments ($F_{2,189}$ = 162.54, p < 0.00) but this was primarily due to the different oxygen saturation levels at different salinities. There was also a significant difference in percent oxygen saturation between salinities ($H_{2,12}$

= 7.73, $p < 0.05$), but mean percent oxygen saturation of all treatments exceeded 85% (Table 4.2).

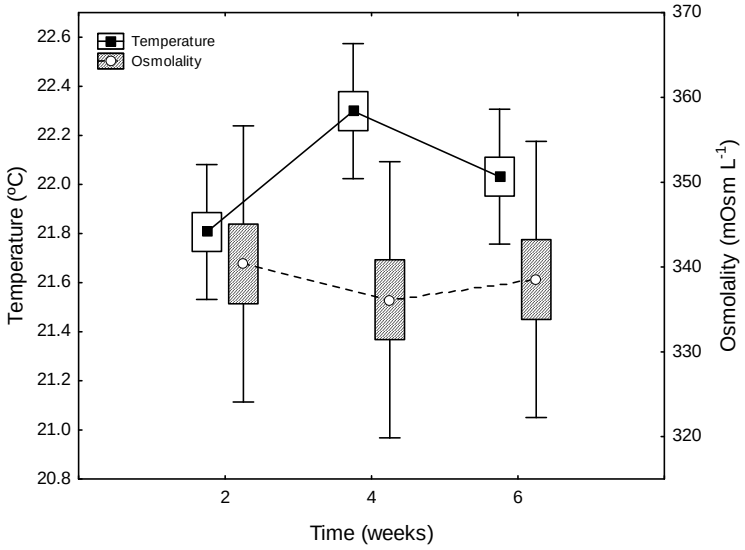

Figure 4.3: Mean osmolality and mean temperature (of two week intervals between sampling) against after 2, 4 and 6 weeks. (Bars denote 95% confidence intervals)

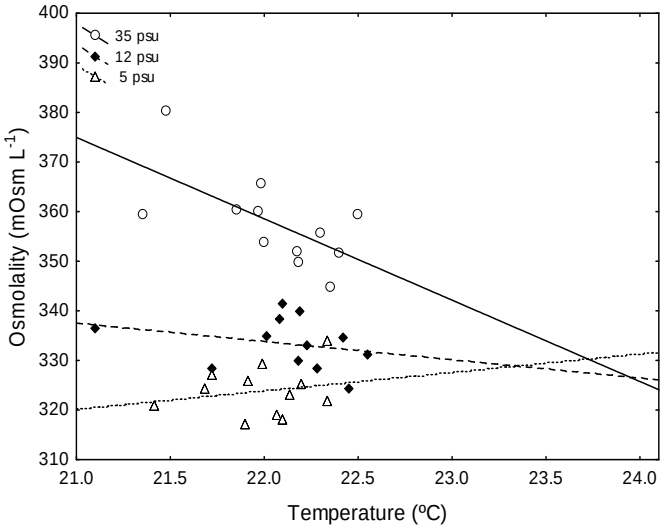

Figure 4.4: Average osmolality of juvenile dusky kob per tank exposed to 5, 12 and 35 psu against respective mean temperature (two week interval between sampling) per tank.

To investigate the significant difference in osmolality at week four, mean temperatures of the time intervals between blood extractions was plotted on the same axes as mean osmolality at each time interval (Figure 4.3). Interestingly at week four, the temperature over all the treatments peaked. When mean temperatures, per tank over the two weeks prior to blood extraction, was graphed against plasma osmolality there appeared to be an interaction effect between temperature and salinity (Figure 4.4). At 35 psu (strongly hyperosmotic) there was a strong negative relationship. However, at 5 psu (hypoosmotic) there was a slight positive relationship and at 12 psu (marginally higher than the isosmotic level of 11 psu) there was a weak negative relationship. Due to the repeated measures design of the experiment, the values were not independent of one another and consequently the results could not be analysed statistically as it would have led to pseudoreplication (Hurlbert 1984).

4. 4. DISCUSSION

The isosmotic point of juvenile dusky kob was calculated to be 11 psu, which is close to the 12 psu treatment that was chosen to represent the isosmotic level. The calculated isosmotic level is similar to isosmotic values for many other euryhaline species (Sampaio and Bianchini 2002). The regression analysis showed that plasma osmolality was influenced by salinity. There was a significant positive relationship between plasma osmolality and water osmolality. The slope of the regression was very low (b=0.0308) and according to Franklin and Forster (1992), who concluded that osmotic imbalance indicated ‘non-adaption’ to salinities tested, may indicate that juvenile dusky kob are adapted to ambient salinities between 5 and 35 psu. Sampaio and Bianchini (2002) found a similar low slope (b=0.041) for the euryhaline flounder *Paralichthys orbignyanus.* In contrast, the slope of the regression between salinity (2-14 psu) and osmolality of cobia *Rachycentron canadum*, a marine teleost, was markedly higher (1.9048) and there were high mortalities of cobia at 1 psu (Burkey and Young 2007). Cobia were also found to grow significantly slower at 5 psu compared to 15 and 30 psu (Denson *et al.* 2003). This supports the hypothesis that the closer the slope is to zero the more ‘physiologically euryhaline’ a species is.

The low slope of plasma osmolality against salinity and the ability of juvenile dusky kob to maintain their internal plasma osmolality over the six weeks (except for a significant

difference at week four) suggests the species is 'physiologically euryhaline' (Jobling 1995). The plasma osmolality at 5 psu (319-334 mOsm L^{-1}) was equivalent to the upper range of osmolality for stenohaline freshwater fish (260 – 330 mOsm L^{-1}) and at 35 psu (352-361 mOsm L^{-1}) the osmolality fell just below the range of stenohaline marine species (370 – 480 mOsm L^{-1}) (Jobling 1995, Sampaio and Bianchini 2002). This supports the findings that juvenile dusky kob are strongly euryhaline. However, there was a significant difference in plasma osmolality between 5 psu and 35 psu, and 12 psu and 35 psu. Rodriguez *et al.* (2002) found that although there were only minor significant differences in plasma osmolality for juvenile Siberian sturgeon *Acipenser baerii* exposed to 0, 9 and 14 psu, significant differences in growth were observed. It is unknown whether the metabolic cost of osmoregulation in juvenile dusky kob differs at the different salinities. Therefore, an independent growth experiment was conducted (See Chapter 5).

The plasma osmolality of juvenile dusky kob remained unchanged over time, except for the significant difference at week four. This variation could have been influenced by environmental variables such as temperature. Although it was not possible to statistically analyse the relationship between temperature and osmolality, there appeared to be an interaction between temperature and salinity on plasma osmolality. The trend suggests that active enzyme driven ion transport exceeded passive fluxes with an increase in temperature (Reynolds and Casterlin 1980). In other words, at 35 psu fish actively excrete more ions than the passive osmotic gain of ions or loss of water in order for extracellular osmolality to decrease. Conversely, at 5 psu fish actively took up more ions than the passive osmotic loss of ions or gain of water for extracellular osmolality to increase. Interestingly, plasma osmolality of euryhaline pupfish *Cyprinodon salinus* (Stuenkel and Hillyard 1981) was similarly affected by an interaction between temperature and salinity. It was found that plasma osmolality of pupfish decreased at 35 psu and increased in 5 psu when the fish were exposed to higher temperatures. Lower temperatures can inhibit active transport mechanisms of osmoregulation and higher temperatures generally increase passive fluxes such as ion loss and water gain (Crawshaw 1979). Furthermore, temperature increases activate enzyme-driven ion transport, which is theoretically greatest at the optimum temperature for enzyme activity (Reynolds and Casterlin 1980). Passive and active mechanisms are not equally sensitive to temperature and one may outweigh the other and hence osmoregulation may be affected by temperature (Crawshaw 1979, Reynolds and Casterlin 1980, Metz *et al.* 2003). This is

supported by Kidder *et al.* (2006), who examined water flux of the euryhaline killifish *Fundulus heteroclitus* at different salinities and temperatures. They found that low temperatures (4ºC) inhibited active osmoregulation transport mechanisms, leading to a net passive water gain, which was reversed in warmer water. Similarly, the acclimation of antartic fish, *Trematomus bernacchii* and *T. newnesis*, to warmer temperature (4ºC) led to a decrease in plasma (serum) osmolality, which increased the seawater-to-extracellular fluid (EFC) osmotic gradient. This coincided with an increase in the active ion excretion enzyme (Na^+/K^+ ATPase) in the osmoregulation organs, the gills and the kidney (Gonzalez-Cabrera *et al.* 1995).

Not all studies on temperature and salinity show similar interaction effects. Woo (1990) found that the osmolality of red sea bream *Chrysophrys major* increased with increasing temperature. Davis and Parker (1990) found no significant difference in osmolality of striped bass *Morone saxatilis* over a wide range of temperatures. Osmolality of Mozambique tilapia *Oreochromis mossambicus* in seawater and double-strength seawater increased with increasing temperature and hence the passive gain of ions (or loss of water) outweighed active osmoregulatory mechanisms as temperature increased (Fiess *et al.* 2007). There were, however, large intervals between the temperatures tested (20, 28 and 35ºC) and the optimum metabolic temperature may have been ommitted. In addition, Sardella *et al.* (2004) found that low temperatures (15ºC), not tested by Fiess *et al.* (2007), distinctly reduced the salinity tolerance of Mozambique tilapia. They also found that plasma osmolality of tilapia at 35 psu was highest at 15ºC, lowest at 25ºC and increased again at 35ºC. Hence it is possible that the optimum tempertaure for active enzymes in ion transport for the species lies between 20-28ºC, which was not tested by Fiess *et al*. (2007). The interaction effect of temperature and salinity appears to be species specific and may vary with the stenohaline vs. euryhaline adaptation of different species, in addition to the optimum temperatures.

In conclusion, it is clear that juvenile dusky kob are able to osmoregulate in salinities between 5 and 35 psu and maintain their extracellular osmolality over long periods, which indicates that they are ‘physiologically euryhaline’. However, there was a significant difference in plasma osmolality at the different salinities. Whether this difference manifests in different growth rates (an indication of metabolic energy expenditure) at the different salinities or metamorphological changes is explored in later chapters.

SALINITY EFFECTS ON GROWTH, FOOD CONVERSION RATIO, FEED INTAKE AND CONDITION FACTOR OF JUVENILE DUSKY KOB

5.1. INTRODUCTION

Fish require energy to osmoregulate in either hypo- or hyperosmotic environments (Brett 1979, Jobling 1994, Boeuf and Payan 2001). There is considerable debate about the percentage of metabolic energy used for osmoregulation, and estimates range from as low as 1-2% (Jobling 1994), while other studies have shown that osmoregulation can account for between 10-50% of total energy expenditure (Boeuf and Payan 2001). Irrespective of the percentage of energy used, that used for osmoregulation becomes unavailable for growth. Consequently, fish theoretically grow best at a salinity at which energy required for osmoregulation is reduced (Brett 1979).

It is hypothesised that juvenile fish, in particular, grow best at isosmotic salinities where the osmotic gradient between the internal osmolality and the environment is reduced and hence the energy expenditure for osmoregulation is minimised (Jobling 1994). Juveniles of numerous species have been shown to grow best at isosmotic salinities (Boeuf and Payan 2001). Juvenile turbot *Scophthalmus maximus* grew best at 15 psu (Imsland *et al.* 2001), a salinity at which Na^+, K^+- ATPase activity was minimal (Imsland *et al.* 2003). Similarly, juvenile gilthead seabream *Sparus aurata* grew best at 12 psu in comparison to 6 psu and 38 psu (Laiz-Carrión *et al.* 2005), and this corresponded to reduced gill Na^+-K^+-ATPase activity. Results from previous research investigating the "isosmotic hypothesis" are somewhat ambivalent. Partridge and Jenkins (2002) found that there was no significant difference in the growth of juvenile black bream *Acanthopagrus butcheri* between 0 – 60 psu and that they grew best at 24 psu, which is markedly higher than isosmotic salinities. Salinity had a significant effect on the growth of juvenile cobia *Rachycentron canadum* and they grew best at 30 psu, which is also markedly higher than isosmotic salinities (Denson *et al.* 2003).

It has been proposed that salinity tolerance is a function of adaptation to the natural environment (Morgan and Iwama 1991, Deacon and Hecht 1999, Allen and Cech 2007) and this may explain the above discrepancies within the "isosmotic hypothesis". Freshwater and eurohaline (anadromous) species tend to grow best at salinities below isosmotic levels. The optimum salinity for growth of common carp *Cyprinus carpio*, steelhead trout *Oncorhynchus mykiss*, chinook salmon *Oncorhynchus tshawytscha*, striped bass *Morone saxatilis* and

sharptooth catfish *Clarias gariepinus* was below isosmotic (Britz and Hecht 1989, Morgan and Iwama 1991, Brown *et al.* 1992, Wang *et al.* 1997). Optimum salinity for growth of true marine and euryhaline, such as spotted grunter *Pomadasys commersonnii,* black bream, cobia, brown spotted grouper *Epinephelus tauvina* and European sea bass *Dicentrarchus labrax,* is predominantly above the isosmotic level (Akatsu *et al.* 1983, Dendrinos and Thorpe 1985, Deacon and Hecht 1999, Partridge and Jenkins 2002, Denson *et al.* 2003, Conides and Glamuzina 2006). Salinity responses are also dependent on the size/age of fish and life history stages. Green sturgeon *Acipenser medirostris* are a semi-anadromous species and at a certain age/size juveniles migrate from freshwater to brackish- and even seawater (Allen and Cech 2007). Similarly, the survival and growth of juvenile green sturgeon in seawater increases with size/age (Allen and Cech 2007). In the wild, juveniles of the Mediterranean flathead mullet *Mugil cephalus* are highly dependent on areas of low salinity. Cardona (2000) found that these juveniles had the lowest metabolic rate and highest growth at the same low salinities.

Juvenile dusky kob occur mainly in low salinities environments (0-5 psu) (Griffiths 1996) and, in a preliminary study, Fielder and Bardsley (1999) showed that the optimum salinity for juvenile dusky kob was 5 psu. Juveniles had the highest growth and lowest food conversion ratio (FCR) at 5 psu. Salinities between 5 and 35 psu, however, did not have a significant effect on growth or FCR, which may have been the result of a low statistical power (Fielder and Bardsley 1999). Recently, Doroudi *et al.* (2006) suggested that optimal salinities for maximum growth and survival of juvenile *A. japonicus* were between 15 and 33 psu. Although they tested survival of juveniles between salinities 5 and 35 psu, they did not test growth below 15 psu. Additionally, they kept temperatures (20 ± 1ºC) below the optimum temperature for growth of juvenile dusky kob (Bernatzeder and Britz 2007, Collett *et al.* 2008) which may have influenced the results. The hypothesis of this study was that juvenile dusky kob would grow best at around 5 psu, the salinity at which they occur in the wild. The aim of this experiment was to determine the effect of three salinities (5, 12 and 35 psu) on growth, food conversion ratio, condition factor and survival of juvenile dusky kob.

5.2. METHODS

Juvenile dusky kob (7.2 ± 0.4 g) were moved from the marine hatchery to a temperature controlled experimental laboratory two weeks prior to the start of the experiment. Twenty-two juveniles (stocking density = 0.88 g L^{-1}) were randomly placed into each of the twelve independent 265-L tanks. Juveniles were acclimatised to the tanks, at 35 psu, for one week. Tanks were randomly assigned to three different salinities with four replicas of each salinity treatment. The chosen salinities were 5, 12 and 35 psu, where 5 psu represented the salinity at which juveniles normally occur in the wild (Griffiths 1996), 12 psu represented the isosmotic level and 35 psu the salinity at which the fish are spawned and reared. Fish were progressively acclimatised to the respective salinities, by reducing salinity at a rate of 5 psu a day. The trial began once reference salinities were obtained and ran for a total of nine weeks. Wet weight and standard length of all the juveniles was recorded at the start of the trial and at three-week intervals thereafter. The initial length and weight of the juveniles were 81 ± 1.5 mm SL and 9.6 ± 0.5 g. Standard length of each fish, to the nearest mm, was measured on a measuring board as sibling aggression sometimes compromised total length. The wet weight of each fish, to the nearest 0.1 g, was measured by placing fish in a shallow dish, with water at the relevant salinity, on a digital scale. Prior to handling, fish were anaesthetised for a minimum of 4 minutes with a 0.2 mL L^{-1} concentration of 2-phenoxyethanol in an aerated 20-L bucket. Anaesthesia with 2-phenoxyethanol, within the range of concentration 0.2-0.5 mL L^{-1}, does not affect growth (Deacon *et al.* 1997).

Fish were fed pelleted feed (45% crude protein, 12.5% fat, max 4% fibre, max 10% ash and max 10% moisture) to apparent satiation three times a day. Apparent satiation was defined as feeding the fish until the first pellets sank to the bottom of a tank without being eaten. Food containers were assigned to each tank and the total food consumed per tank was weighed (to 0.1 g) at the end of each three-week growth interval. Tanks were siphoned clean half an hour after the morning and late afternoon feeds.

Temperature, oxygen, salinity and conductivity were measured every second day. Temperature (22.5 ± 0.5ºC) was kept within the range of optimum temperature for growth and FCR of juvenile dusky kob (Collett *et al.* 2008) and photoperiod was fixed at 12L/12D. Total ammonia, nitrate and pH were measured twice a week and kept within acceptable levels (Nitrite 0.13 ± 0.08 mg L^{-1}, unionised ammonia 0.0018 ± 0.001 mg L^{-1}).

Data Analysis

Length gain, weight gain, specific growth rate, food conversion ratio, feed intake (% body weight per day), mortality and condition factor were analysed with a Repeated Measures ANOVA (Analysis of Variance). The assumptions of normality and sphericity of the Repeated Measures ANOVA were tested. If the assumption of normality (Kolmogorov-Smirnov test) was not met, the data were transformed accordingly and the Greenhouse-Geiser correction was applied if epsilon was less than 0.7. The Tukey HSD test was used to determine where the significant differences lay. The One Way ANOVA and the Repeated Measures ANOVA was used to test for significant differences in oxygen and temperature between individual systems and treatments. If the data or the residuals of the data did not meet the assumptions for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test) a non-parametric Kruskal-Wallis test was used.

Specific growth rate (SPG) was calculated using the equation,

$$G = 100 \times \left(\left(\frac{W_f}{W_i} \right)^{\left(\frac{1}{d-1} \right)} - 1 \right)$$

where G = specific growth rate, W_f = final weight, W_i = initial weight and d = the number of days in the growth period (Focht 1984). Food conversion ratio (FCR) was derived from the total amount of dry food fed per tank divided by the total wet weight gain per tank for each growth period (3 weeks). Feed intake (Fi) was calculated as % BW d^{-1} by multiplying FCR by SPG. Condition factor (Cf) was calculated using a modification of Fulton's condition factor equation:

$$Cf = 10^5 \times \left(\frac{W}{L^{-3}} \right)$$

where W = wet weight (g) and L = standard length.

5.3. RESULTS

Abiotic conditions

There was no significant difference in oxygen saturation ($F_{11,24}$ = 1.52, p = 0.189) or temperature ($F_{11,24}$ = 0.1, p = 0.999) between all twelve replicates. Similarly, there was no significant difference in oxygen saturation ($F_{2,18}$ = 2.57, p = 0.131) or temperature ($F_{2,18}$ = 3, p = 0.077) between salinity treatments. Oxygen saturation remained above 75% in all treatments over the entire duration of the trial (Table 5.1). There was a significant change in mean temperature ($F_{2,18}$ = 425, p < 0.00) and mean oxygen saturation ($F_{2,18}$ = 14.03, p < 0.00) between growth periods. Mean temperature in the second growth period (week 3-9) was higher than in the third growth period (week 6-9), which in turn was higher than the mean temperature over the first growth period (week 0-3). As percent oxygen saturation is temperature dependent, the change in mean percent oxygen saturation paralleled the change in temperature (Table 5.1).

Table 5.1: Mean (± SD) values for abiotic variables (temperature, oxygen saturation, conductivity and salinity)

Treatment (psu)	Time (weeks)	Temperature (ºC)	Oxygen saturation (%)	Conductivity (mS)	Salinity (psu)
5	3	22.28 ± 0.03	80.44 ± 2.54	9.89 ± 0.46	5.75 ± 0.42
	6	23.02 ± 0.07	84.53 ± 2.61	9.60 ± 0.55	6.05 ± 0.71
	9	22.84 ± 0.05	82.11 ± 2.75	9.69 ± 0.40	5.42 ± 0.23
12	3	22.50 ± 0.09	79.50 ± 2.14	16.73 ± 0.38	11.97 ± 0.25
	6	23.11 ± 0.07	83.58 ± 3.14	16.79 ± 0.37	12.18 ± 0.54
	9	22.79 ± 0.06	80.83 ± 1.20	17.13 ± 0.20	12.08 ± 0.06
35	3	22.44 ± 0.10	77.72 ± 1.35	42.64 ± 0.17	34.81 ± 0.17
	6	23.07 ± 0.06	81.35 ± 2.01	43.12 ± 0.40	35.25 ± 0.49
	9	22.78 ± 0.04	80.38 ± 0.30	43.72 ± 0.18	35.31 ± 0.14

High mortalities (60.87%) occurred in one of the 5 psu replicates. The cause was unknown and the data from this replicate were not considered in the analysis. Overall, however, there were no significant differences in mortality between salinities ($H_{2,12} = 4.197$, $p = 0.122$).

Growth

Salinity had a significant effect on specific growth rate ($F_{2,9} = 42.91$, $p < 0.00$). SPG rate at 5 psu was significantly lower than in the 12 ($p < 0.00$) and the 35 psu treatments (Figure 5.1a: $p < 0.00$), but there was no significant difference in SPG between the 12 and 35 psu treatments ($p = 0.435$). There was no significant difference in SPG among all treatments in the third growth period (week 6–9) (Figure 5.2). SPG decreased significantly over the duration of the trial (Figure 5.2b: $F_{1.27,11.43} = 16.15$, $p < 0.00$).

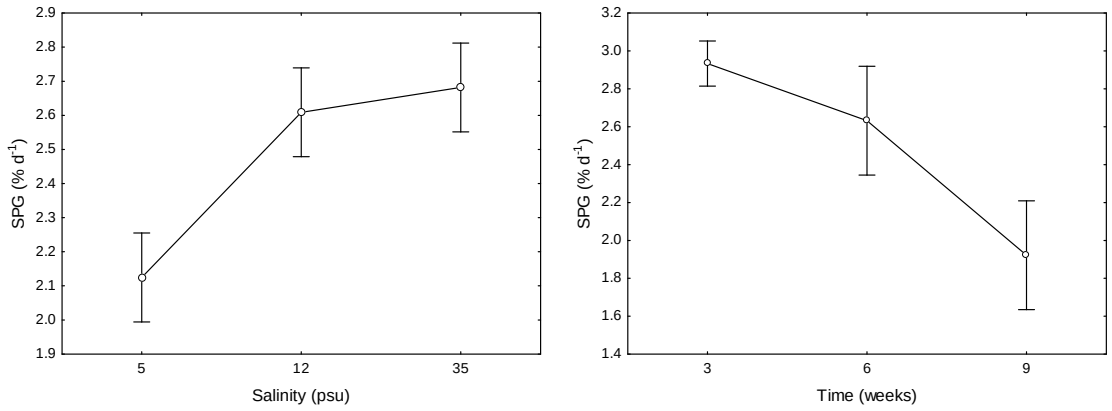

Figure 5.1: (a) Effect of salinity on mean SPG of juvenile dusky kob for the entire trial, and (b) effect of time on mean SPG for juvenile dusky kob in all salinity treatments (vertical bars denote 95% confidence interval)

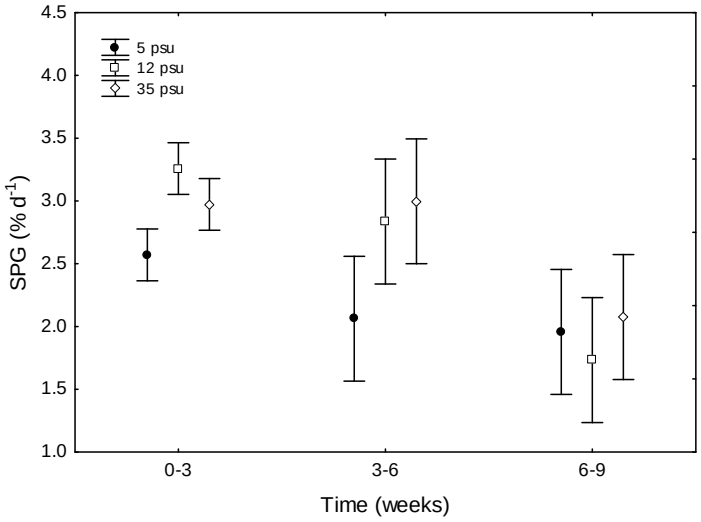

Figure 5.2: Effect of salinity on specific growth rate (SPG) of juvenile dusky kob over time (vertical bars denote 95% confidence intervals)

Table 5.2: Mean (± SD) of specific growth rate (SPG), length gain (Lg), weight gain (Wg), food conversion ratio (FCR), feed intake (Fi), condition factor (Cf) and mortality. Different superscripts denote significant differences within time intervals between treatments

Time (weeks)	Treatment (psu)	SPG	Lg (mm)	Wg (g)	FCR	Fi (% BW d-1)	Cf	Mortality (%)
0	5	-	-	-	-	-	1.80 ± 0.02	-
	12	-	-	-	-	-	1.79 ± 0.02	-
	35	-	-	-	-	-	1.79 ± 0.04	-
3	5	2.57 ± 0.14^{a}	16.76 ± 0.75^{a}	6.39 ± 0.30^{a}	1.40 ± 0.17^{a}	3.60 ± 0.53^{a}	1.69 ± 0.07^{a}	10.87 ± 10.35^{a}
	12	3.26 ± 0.25^{b}	20.47 ± 1.37^{a}	8.13 ± 0.80^{a}	1.18 ± 0.17^{a}	3.82 ± 0.25^{a}	1.71 ± 0.03^{a}	0 ± 0^{a}
	35	2.97 ± 0.14^{c}	18.88 ± 1.56^{a}	7.96 ± 0.43^{a}	1.17 ± 0.12^{a}	3.49 ± 0.33^{a}	1.73 ± 0.01^{a}	0 ± 0^{a}
6	5	2.06 ± 0.73^{e}	111.31 ± 4.90^{e}	24.40 ± 3.52^{e}	1.52 ± 0.76^{e}	2.72 ± 0.29^{e}	1.88 ± 0.10^{e}	5.56 ± 11.11^{e}
	12	2.84 ± 0.12^{f}	118.80 ± 2.04^{f}	29.99 ± 1.48^{e,f}	0.96 ± 0.04^{f}	2.73 ± 0.16^{e}	1.73 ± 0.02^{e}	1.09 ± 2.17^{e}
	35	2.99 ± 0.19^{f}	122.06 ± 1.85^{g}	32.40 ± 1.14^{f}	0.94 ± 0.06^{f}	2.81 ± 0.17^{e}	1.78 ± 0.06^{e}	4.35 ± 3.55^{e}
	5	1.96 ± 0.58^{h}	127.64 ± 1.50^{h}	35.57 ± 1.50^{h}	0.99 ± 0.21^{h}	1.86 ± 0.33^{h}	1.71 ± 0.04^{h}	12.42 ± 15.94^{h}
9	12	1.73 ± 0.40^{h}	135.17 ± 2.70^{i}	42.31 ± 2.70^{i}	0.88 ± 0.23^{h}	1.46 ± 0.13^{h}	1.71 ± 0.04^{h}	2.17 ± 4.35^{h}
	35	2.08 ± 0.28^{h}	141.15 ± 1.30^{j}	48.86 ± 1.30^{j}	0.70 ± 0.05^{h}	1.44 ± 0.11^{h}	1.74 ± 0.07^{h}	0 ± 0^{h}

There was a significant effect of salinity on weight gain ($F_{2,9}$ = 40.270, p < 0.00). Weight gain in all three treatments was significantly different (Figure 5.3, p < 0.05) and increased with increasing salinity. Unlike specific growth rate, weight gain at 5 psu in the third growth period (week 6-9) was significantly lower than in the 12 (p < 0.05) and 35 psu treatments (p < 0.00). Salinity similarly affected length gain ($F_{2,9}$=31.14, p < 0.00). However, in contrast to weight gain, there was no significant difference in length gain between the 12 psu and 35 psu treatments (p = 0.12). Length gain in the 5 psu treatment was significantly lower than in both the 12 (p < 0.00) and the 35 psu treatments (p < 0.00) (Table 5.2).

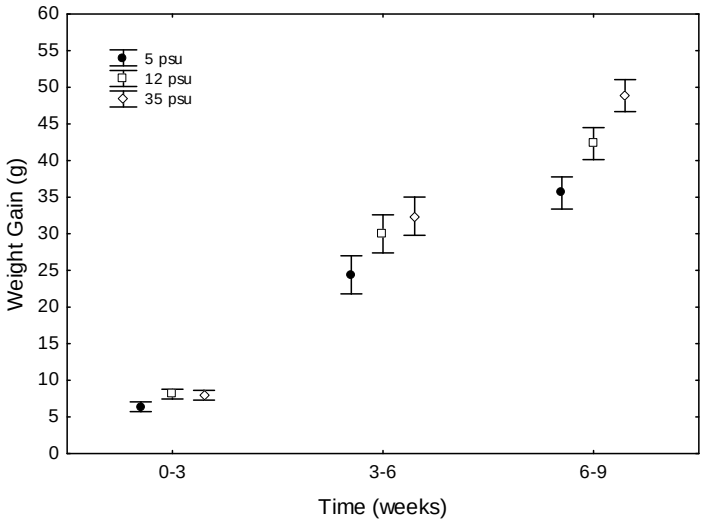

Figure 5.3: Effect of salinity on weight gain of juvenile dusky kob over time (Bars denote 95% confidence intervals)

Food conversion ratio and condition factor

Salinity also had a significant effect on food conversion ratio (FCR) (Figure 5.4: $F_{2,9}$ = 5.263, p < 0.05). FCR at 5 psu was significantly higher than at 35 psu (p < 0.05) but not significantly higher than at 12 psu (p = 0.083). FCR at 12 psu was not significantly different than at 35 psu (p = 0.823). There was a significant decrease in FCR over time ($F_{1.3,11.7}$ = 6.094, p < 0.05). The FCR during the first growth period was significantly higher during the third growth period (p < 0.05), while the FCR of the second growth period was not

significantly different to either the first (p = 0.611) or the third growth period (p = 0.64). Salinity had no significant effect on feed intake (% BW d^{-1}) (Table 5.2: $F_{2,9}$ = 0.403, p = 0.679). Condition factor was not significantly affected by salinity (Table 5.2: $F_{2,9}$ = 1.46, p = 0.2816).

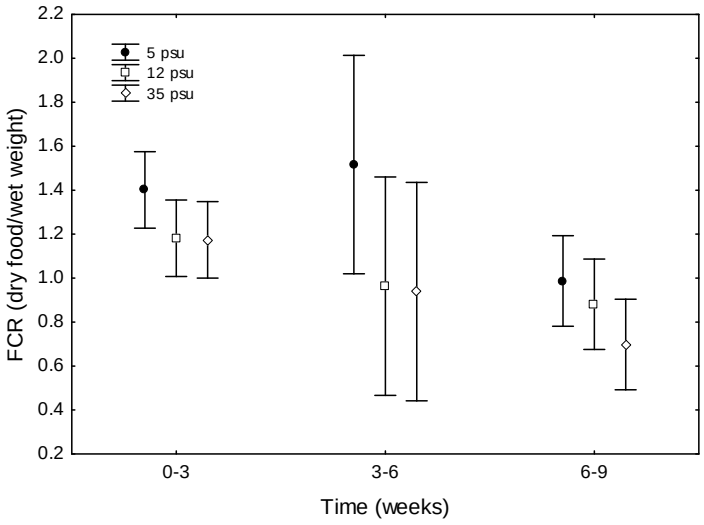

Figure 5.4: Effect of salinity on FCR of juvenile dusky kob over time (Bars denote 95 % confidence intervals)

5.4. DISCUSSION

Contrary to what was hypothesised, juvenile dusky kob responded poorly to 5 psu in all aspects. The juveniles had a lower growth rate and a higher food conversion ratio (FCR) at 5 psu than at the higher salinities. Although juvenile dusky kob gained significantly more weight at 35 than at 12 psu, there was no significant difference in specific growth rate, length gain and FCR. Doroudi *et al.* (2006) similarly indicated that the optimal salinity range for survival and growth of juvenile *A. japonicus* was between 15 and 35 psu. However, they did not test growth below 15 psu and the conclusion was primarily based on survival. Fielder and Bardsley (1999) concluded that optimal salinity of juvenile *A. japonicus* was 5 psu but found salinity (5-35 psu) did not have a significant effect on growth or FCR. They also tested a smaller size range of juveniles (W_i 6 g - W_f 11 g) than was tested in this study (W_i 10 g – W_f

42 g) and the trial ran for a shorter period (only 28 days). According to Boeuf and Payan (2001) anomalies related to duration of experiment and/or the developmental stage of an animal can be expected.

While the percent of total metabolic energy used for osmoregulation is debatable, numerous studies have shown that optimum salinities for growth correspond to reduced metabolic rate or gill Na^+, K^+-ATPase activity (NKA). Juvenile turbot had reduced gill NKA and higher growth rates at intermediate salinities (Imsland *et al.* 2001, Imsland *et al.* 2003). The growth and metabolic rate of juvenile flathead mullet were both negatively affected by high salinity levels (Cardona 2000). In rabbitfish *Siganidae rivulatus* an increase in gill NKA corresponded to a slight decrease in growth, at lower salinities (Saoud *et al.* 2007). The higher growth of gilthead seabream at 12 psu was attributed to lower metabolic rates at that salinity (Laiz-Carrión *et al.* 2005). Consequently, the reduced growth of juvenile dusky kob at 5 psu could, in part, be due to a higher energy expenditure associated with a higher gill NKA activity at this salinity.

Differences in growth as a response to salinity are not necessarily a reflection of changes in metabolic costs of osmoregulation. It has been suggested that salinity may affect feed intake, digestion and absorption processes which would lead to net affects on growth (Jobling 1994). In this study there was no significant difference in feed intake between salinities. Similarly, no difference in feed intake was found for Atlantic cod *Gadus morhua* exposed to different salinities, although growth was significantly affected by salinity (Lambert *et al*. 1994). Changes in feed intake, as a response to different salinity, have been reported for other species. Feed intake and growth of eurohaline species, such as European bass *Dicentrarchus labrax* and flathead mullet *Mugil cephalus*, was found to increase with an increase in salinity (De Silva and Perera 1976, Dendrinos and Thorpe 1985). In freshwater species, such as carp *Cyprinus carpio* and rainbow trout *Oncorhynchus mykiss*, feed intake and growth increased with decreasing salinity (McKay and Gjerde 1985, Wang *et al.* 1997). Although not properly understood, it is possible that changes in the digestibility of feed with salinity are a consequence of an interaction between nutrient and salt uptake in the gut (Usher *et al*. 1990). It was not within the scope of this study to look at digestibility or absorption of food at different salinities. The lower growth and higher FRC of juvenile dusky kob at 5 psu, however, cannot be attributed to differences in feed intake between the salinities.

Table 3: Summary of key results and finding of the current study and previous studies on *A. japonicus*

Study	Present study			Collett *et al.* 2008		Fielder and Bardsley 1999			Doroudi *et al.* 2006	Partridge *et al.* 2006	Flowers and Hutchinson 2004	
Initial weight (g)	9.6 ± 0.5			7.2 ± 1.6	23 ± 2.7	6.1 ± 0.1			7.2 ± 1.1	116	32.5	32.3
Temperature (°C)	22.3-23.1			25.8 ± 0.3	24.3	17.5-22.6			20 ± 1	21	22-24	
Salinity (psu)	5	12	35	35	35	5	10	35	15, 25 and 35	14	16	17
Conductivity (mS)	9.7	17	43	-	-	-	-	-	-	-	26.9	28.6
SPG (% BW d^{-1})	2.2 ± 0.5	2.6 ± 0.7	2.7 ± 0.5	3.2-3.3	2.1	2.4*	2.2*	1.6*	0.8-1.5	0.68	0.99 ± 0.1	1.34 ± 0.1
FCR	1.3 ± 0.5	1 ± 0.2	0.9 ± 0.2	1.36-1.4	0.9	1.1 ± 0.1	1.6 ± 0.4	1.9 ± 0.3	2.1	1.39	1.1-1.8	1.2-1.6
Water source	Seawater (Port Alfred), rainwater			Seawater (Port Alfred)		Seawater and groundwater (0.6 psu, 66 mg L^{-1} hardness)			Artificial saltwater and potassium	Saline bore water (supplemented with K)	Saline ground-water (K deficient)	Seawater and tap water
Conclusion	Growth, FCR, etc negatively affected at 5 psu			Optimum temp for growth = 25.3, for FCR = 21.4		Optimum growth at low salinities			Optimum salinities 15-33 psu, K conc. > 40 %	Growth improved at temp > 20ºC	Growth increased in saline groundwater with K supplementation	

*Calculated from mean initial and final weight in Fielder and Bardsley (1999).

The decrease in feed intake with an increase in fish size has also been reported for European bass *Dicentrarchus labrax* (Dendrinos and Thorpe 1985), Dab *Limanda limanda* (Pandian 1970), European plaice *Pleuronectes platessa* (Birkett 1972), common sole *Solea solea* (Bromley 1974), Tarpon *Megalops cyprinoides* and murrel *Ophiocephalus striatus* (Pandian 1967). Similarly, specific growth rate (SPG) is known to decrease with fish size (Jobling 1994). This explains the decrease in specific growth over the duration of the trial and the lack of a significant difference in SPG in the third growth period of the trial. At the end of the second growth period (week 3-6), the juveniles at 5 psu (23.3 ± 2.1 g) were significantly smaller than at the higher salinities (31.2 ± 1.8 g). Thus, although weight gain for juveniles in 5 psu was significantly lower in the third growth period, SPG was not.

FCR of juvenile dusky kob improved at salinities at which growth was optimum, indicating that a higher percentage of available energy of the feed was used towards somatic growth as opposed to osmoregulation. This in turn reinstates that early juveniles used less energy for osmoregulation at 12 and 35 psu. There was however, no significant difference in condition factor. Salinity similarly had a significant effect on growth but not on condition factor of gilthead sea bream (Laiz-Carrión *et al.* 2005). Condition factor values for this study are comparable with those recorded by Collett *et al.* (2008). Overall, the higher FCR and reduced condition factor at 5 psu, reinforced that juvenile dusky kob did not respond well to 5 psu.

In aquaculture, high SPG and low FCR are favourable as it directly and indirectly maximises production and reduces cost (Quéméner *et al.* 2002). The findings of this study indicate that juveniles can be grown out at salinities as low as 12 psu, without negative effects on growth and FCR. The mean SPG and FCR values recorded at 12 and 35 psu, were notably better than previous studies on juvenile *A. japonicus* and comparable to values reported by of Collett *et al.* (2008) (Table 5.3). In the case of Doroudi *et al.* (2006) and Partridge *et al.* (2006), the high FRC and reduced SPG is most likely due to the suboptimal temperature that juveniles were grown at (Collett *et al.* 2008). The discrepancies with other studies are probably due to differences in initial fish size, food and water source. With regard to water source, potassium deficiency in saline groundwater has been found to negatively affect growth (Fielder *et al.* 2001, Flowers and Hutchinson 2004, Doroudi *et al.* 2006).

In conclusion, although juvenile dusky kob can survive and grow at 5 psu, growth and FCR was negatively affected at this low salinity. This suggests that the metabolic cost of

osmoregulation is greater below isosmotic levels. These results are in contrast to our understanding of the life history of juvenile dusky kob. The low salinity at which juveniles occur in estuaries (Griffiths 1996) may be mitigated by higher conductivity levels estuaries (see final discussion). Further studies are needed to test changes in salinity adaptation with size/age of dusky kob and to determine a more specific optimum salinity for growth.

CHAPTER 6

EFFECT OF SALINITY ON GILL MORPHOLOGY, SPECIFICALLY CHLORIDE CELLS, OF JUVENILE DUSKY KOB

6.1. INTRODUCTION

In regulating extracellular osmolality over a wide range of salinities, euryhaline teleosts are known to undergo behavioural and physiological adaptations (Jobling 1995). In freshwater, teleosts have to combat a diffusive ion loss and osmotic water gain to maintain extracellular osmolality greater than the environment. This osmotic gradient is sustained by the production of a large volume of dilute urine by the kidney and urinary bladder and the active uptake of ions. Ion loss is compensated for by the active uptake of ions across the gills, the absorption of salts across the intestine and ion uptake from ingested food (Jobling 1995, Marshall and Grosell 2006). In seawater, teleosts have to hypo-osmoregulate to compensate for the osmotic loss of water across the gills. Extracellular osmolality is regulated below environmental concentration by high drinking rates that facilitate the absorption of water across the gastrointestinal tract. The excess ion load is secreted actively across the gills and the skin epithelial. In addition, the kidney and the urinary bladder produce minimal volumes of isotonic urine (Jobling 1995, Marshall and Grosell 2006).

In teleosts, gills are considered the major organ involved in osmoregulation (Evans *et al*. 2005). Chloride cells or “mitochondria rich” cells in the gills are the primary site for the active discharge of excess ions in hyperosmotic environments and uptake of ions in hypo-osmotic environments (Maetz 1971, Perry 1997, Uchida *et al.* 2000, Wilson and Laurent 2002). Chloride cells are characterised by having numerous mitochondria and an extensive tubular system (Sakamoto *et al.* 2001, Wilson and Laurent 2002). The regulation of chloride cells is critical in the adaptation of eurohaline teleosts to hyper and hypo-osmotic environments (Evans *et al.* 1999, Sakamoto *et al.* 2001). Changes in environmental salinity are known to alter the number, size and location of chloride cells (Kelly and Woo 1999, Caberoy and Quinitio 2000). The extent and pattern of change in chloride cells depends on the degree of salinity change, the species and age of fish (Kelly and Woo 1999, Caberoy and Quinitio 2000). Of the teleosts examined to date, the majority showed an increase in chloride cell number and/or size with an increase in salinity (Sakamoto *et al.* 2001).

Na^+, K^+-ATPase (NKA) is the key enzyme involved in ion transport of chloride cells. The extensive tubular system of chloride cells is the site of expression for the transport enzyme NKA (Perry 1997, Sakamoto *et al.* 2001, Wilson and Laurent 2002, Nebel *et al.* 2005). The proliferation of chloride cell size and number generally accompanies an increased expression and activity of NKA (Dang *et al.* 2000, Sakamoto *et al.* 2001). For example, an increase in both chloride cells and NKA activity was observed during seawater adaptation in tilapia *Oreochromis mossambicus* (Uchida *et al.* 2000), grouper *Epinephelus coioides* (Caberoy and Quinito 2000) and brown trout *Salmo trutta* (Seidelin and Madsen 2000). This was similarly observed in European sea bass *Dicentrarchus labrax* during freshwater acclimation (Nebel *et al.* 2005) and in killifish *Fundulus heterclitus* exposed to calcium deficient water (Katoh and Kaneko 2002). Jensen *et al.* (1998) found that NKA activity increased with chloride cell proliferation during the acclimation of European sea bass to both freshwater and concentrated seawater (50 psu). NKA activity and abundance has been used as an indicator of the osmoregulatory ability of teleosts where minimum NKA activity corresponds to optimum salinity (Imsland *et al.* 2003). The aim of this experiment was to further investigate the osmoregulatory ability of juvenile dusky kob by examining the effect of salinity on the gill morphology of early juveniles. It was hypothesised that chloride cell number or size would decrease at reduced salinities.

6.2. METHODS

Juvenile dusky kob (127 ± 4.4 mm SL, 33.4 ± 3.5 g) were sampled for histological analysis after six weeks of exposure to 5, 12 and 35 psu. Fish were sampled upon the completion of the plasma osmolality experiment (for experimental protocol see Chapter 4). One fish per tank was anaesthetised with 0.2ml/l of 2-phenoxyethanol for a minimum of 10 min and subsequently killed with a single sharp incision through the vertebral column behind the head. The gills were dissected out and immediately fixed in bouin's solution for 24 hours. Spear and Ferguson (1988) established that bouin's fixative, rather than 10% formalin, minimised epithelial capillary separation and epithelial hypertrophy artefacts. Post fixing, tissue samples were transferred through serial dilutions of ethanol (25%, 50% and 70%). The 70% ethanol was replaced after 24 hours to remove any excess bouin's solution.

Recent immunochemical staining techniques have been successfully used in staining chloride cells for identification and analysis. Due to practical and cost limitations this staining method was not used in this study. The effectiveness of the standard haematoxylin and eosin stain in identifying chloride cells was confirmed by preliminarily staining the gills of two fish exposed to 5 psu and two fish exposed to 35 psu. To standardise the processing of tissue samples for histology, samples were sent to the National Health Laboratory Services (NHLS) in Port Elizabeth. At NHLS tissue samples were dehydrated in increasing concentrations of ethanol from 70% to absolute and subsequently transferred through serial solutions of xylene to remove the ethanol. Tissue samples were embedded in wax, sectioned at 3 µm thick sections and transferred onto glass slides.

The slides were transferred through serial solutions of xylene to remove the wax and rehydrated in serial ethanol grades from absolute to 70%. Slides were stained with haematoxylin and rinsed first with distilled water and then 70% ethanol before being counter stained with eosin. Stained slides were rinsed again in distilled water, dehydrated in serial ethanol grades from 70% to absolute and transferred through a series of xylene solutions. Finally the cover slip was mounted with DPX.

Tissue samples were analysed using a Nikon (Eclipse E400) light microscope and pictures were taken with a digital camera (Canon, PowerShot S80). The images were analysed with a computer image analysing programme (Digimizer 3.4.1.0). Chloride cells were identified as large eosinophilic granular cells with large rounded nuclei (Carmona *et al.* 2004). The area of thirty chloride cells per fish was measured with the image analysing tool to determine average chloride cell size per fish. Chloride cell numbers were counted along five randomly photographed filaments (500µm) per fish and averaged. General observations of the structure of the gill filaments and lamella were also noted. The size and number of chloride cells were compared using a one-way ANOVA (Analysis of Variance) after the assumptions of homogeneity of variance and normality of data were met.

6.3. RESULTS

Chloride cells

Salinity had a significant effect on the size ($F_{2,9}$ = 26.8, p < 0.00) of chloride cells (Figure 6.1a). The size of chloride cells of fish maintained at 5 psu was significantly greater (p < 0.00) than those of fish at 12 and 35 psu. There was no significant difference in chloride cell size of fish at 12 and 35 psu (p = 0.95). Salinity also had a significant effect on the number of chloride cells (Figure 6.1b: $F_{2,9}$ = 5.16, p < 0.05). There was a significantly higher number of chloride cells in fish at 5 psu than at 12 psu (p < 0.05). There was no significantly difference in number of chloride cells between fish at 5 psu and 35 psu (p = 0.09) and fish at 12 psu and 35 psu (p = 0.79).

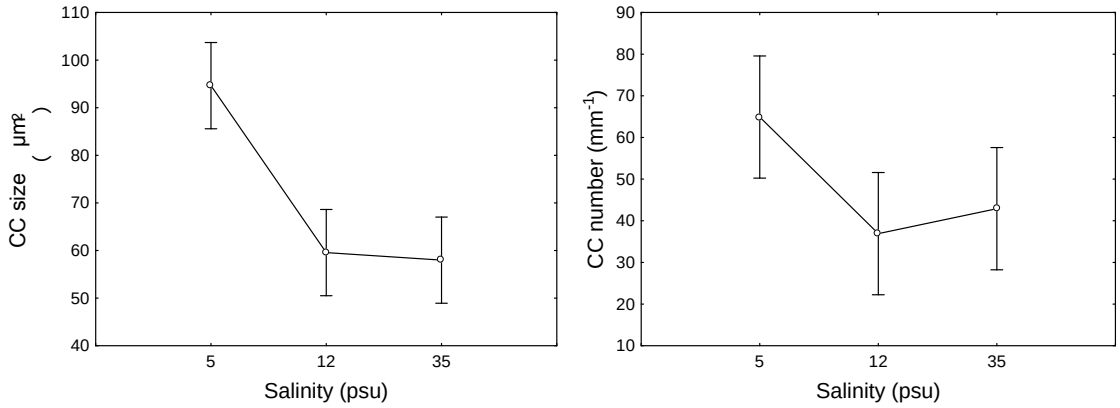

Figure 6.1: Effect of salinity on a) chloride cell (CC) size and b) chloride cell number along filaments (vertical bars denote 95% confidence interval)

Histological observations

It was observed that the gills of fish maintained at 5 psu had a high degree of proliferation of both chloride and epithelial cells (Figure 6.2a). Despite some proliferation in the gills of fish at 12 and 35 psu it was not extensive and localised to short sections of the filaments (Figure 6.2b). Chloride cells occurred on the filament and lamellar epithelia of the gills of fish acclimatised to 5 psu, whereas in fish maintained at 12 and 35 psu they were found exclusively on the filaments, in the filament interlamellar spaces (spaces between the bases of the lamellae) (Figure 6.3).

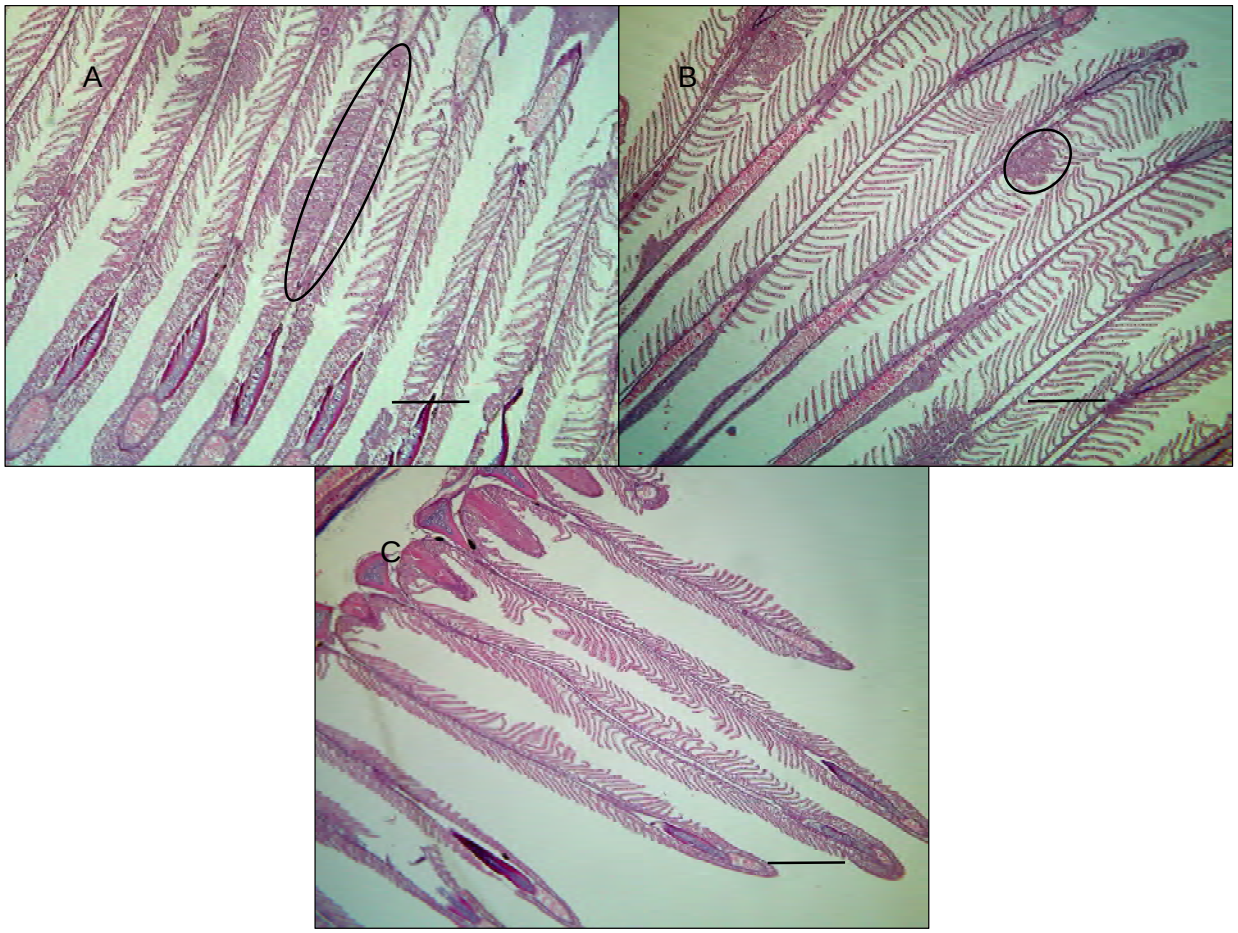

Figure 6.2: Light micrographs of gills of juvenile dusky kob maintained at 5 psu (A), 12 psu (B) and 35 psu (C). Circles indicate highlight examples of cell proliferation. Haematoxylin and eosin stained. Scale bar = 200 µm.

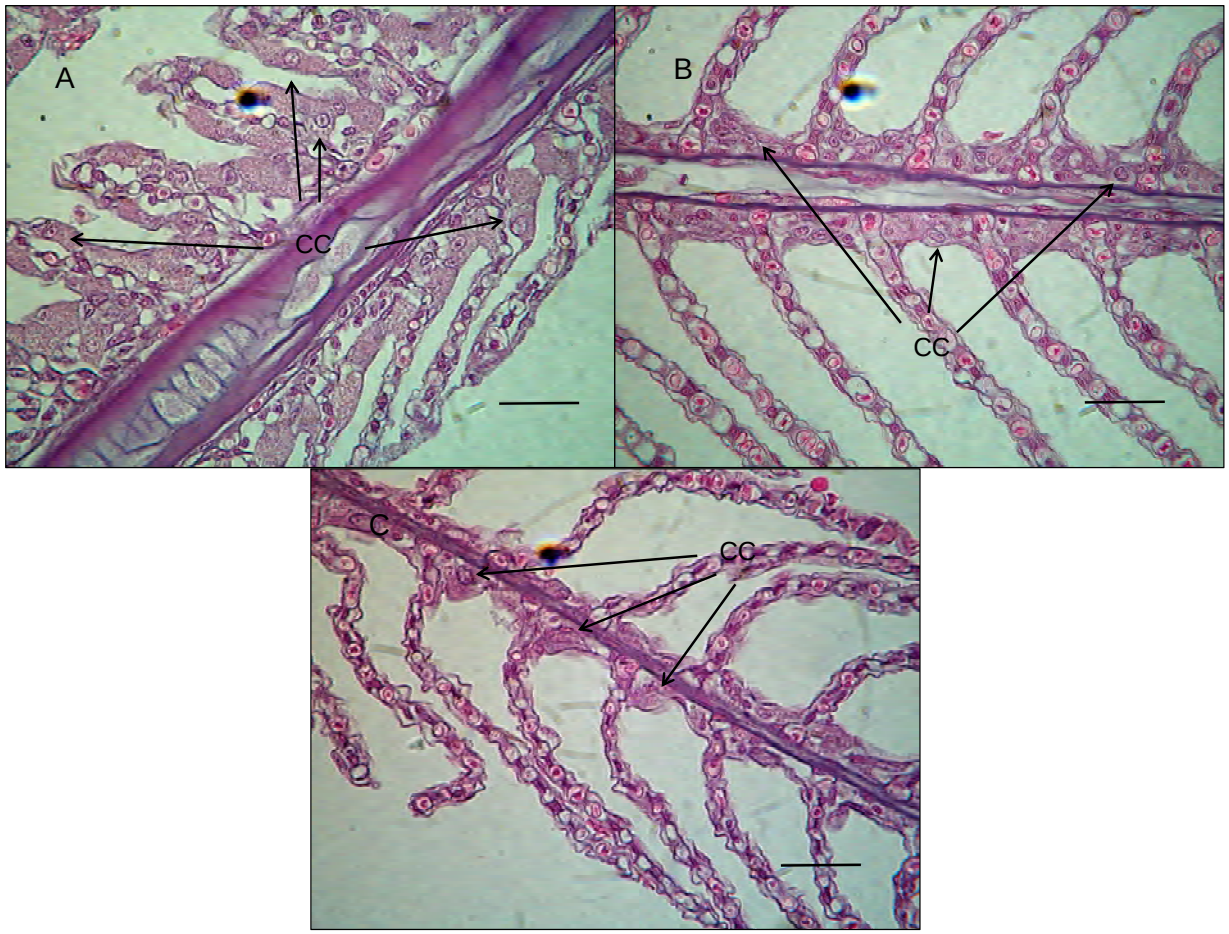

Figure 6.3: Light micrographs of gills of juvenile dusky kob maintained at 5 psu (A), 12 psu (B) and 35 psu (C). Examples of chloride cells (CC) are indicated with arrows. Haematoxylin and eosin stained. Scale bar = 20 µm.

6.4. DISCUSSION

The acclimation of teleosts to different salinities can elicit a significant change in the size and number of chloride cells (McCormick 1995, Hirai *et al.* 1999, Sakamoto *et al.* 2001). Juvenile dusky kob exposed to 5 psu had significantly more branchial chloride cells than juveniles exposed to 12 psu and those kept at 35 psu. The size of chloride cells of fish kept at 5 psu was also significantly larger than those acclimated to 12 psu. Chloride cells are associated with the Na^+, K^+-ATPase (NKA) enzyme and an increase in chloride cell size or number is generally associated with an increase in NKA activity (Sakamoto *et al.* 2001). This suggests that juvenile dusky kob experience higher levels of NKA activity at 5 psu or hypo-osmotic salinities than at isosmotic and hyperosmotic salinities.

In support of this study, there are several examples where chloride cell number and size in teleosts increased with adaptation to reduced salinities or hypo-osmotic environments. The adaptation of killifish *Fundulus heterclitus* to freshwater caused an increase in chloride cell size (Marshall *et al.* 1997, Katoh and Kaneko 2003). Similarly, both the number and fractional area chloride cells in silver sea bream *Sparus sarba* increased after a transfer from 33 to 6 psu (Kelly and Woo 1999). Exposure of European sea bass *Dicentrarchus labrax* to freshwater also produced a significant increase in the number of branchial chloride cells (Giari *et al.* 2006). However, in contrast, most teleosts examined to date underwent an increase in size and/or number of chloride cells at increased salinities and not at reduced salinities (Sakamoto *et al.* 2001). Compared to individuals maintained in freshwater, seawater adapted Adriatic sturgeon *Acipenser naccarii* showed a marked increase in the number and size of chloride cells (Carmona *et al.* 2004). The size of chloride cells in the gills of tilapia *Oreochromis mossambicus* were twice as large in seawater and four times larger in concentrated seawater (180% seawater) than in freshwater (Uchida *et al.* 2000). The transfer of juvenile Australian snapper *Pagrus auratus* from 30 psu to 45 psu also caused an increase in filament chloride cell size, whereas the size of filament and lamellar chloride cells decreased after transfer to reduced salinity (15 psu) (Fielder *et al.* 2007). Chloride cells in seawater and brackish water adapted juvenile Gulf of Mexico sturgeon *Acipenser oxyrinches de sotoi* were more numerous and larger in size than freshwater adapted juveniles (Altinok *et al.* 1998). It has been hypothesised that certain teleosts maintain a higher gill permeability and ion flux in freshwater and hence require higher levels of NKA and consequently more

numerous and larger chloride cells (McCormick 1995). Varsamos *et al.* (2002) found that the number of chloride cells of sea bass kept at 35 psu increased after gradual acclimation to both freshwater and double strength seawater (70 psu) which suggests that changes in chloride cell abundance may be related to salinity extremes.

Exposure to ion deficient freshwater can also trigger a proliferation of chloride cells. The exposure of trout *Oncorhynchus mykiss* to soft water caused an increase in size and number of chloride cells (Perry 1998). Killifish exposed to freshwater with reduced Ca^{2+} concentration had significantly larger chloride cells than those exposed to higher concentrations of Ca^{2+} (Katoh and Kaneko 2002). It is therefore possible that a deficiency in ions other than Na^{+} and Cl^{-1} may have contributed towards the proliferation of chloride cells of juvenile dusky kob maintained at 5 psu.

Long term development and differentiation of branchial chloride cells is a complex process and appears to be regulated predominantly be endocrine factors (McCormick 2001, Sakamoto *et al*. 2001, Sloman *et al*. 2001). Lamellar chloride cells have predominantly been observed in fish acclimatised to freshwater (Uchida *et al.* 1997, Nebel *et al.* 2005, Fielder *et al.* 2007) and it is hypothesised that lamellar chloride cells are primarily involved in hypo-osmoregulation (Sakamoto *et al.* 2001, Nebel *et al.* 2005). Similarly, in this study it was observed that lamellar chloride cells occurred solely in juveniles kept at 5 psu. The lamellae in fish gills are also responsible for respiration, more specifically the exchange of oxygen and carbon dioxide between the blood and external water. Consequently the proliferation of chloride cells on the lamellae may increase the blood-to-water diffusion barrier of respiration, which could negatively affect gas transfer (Perry 1998). Teleosts appear to negate this to some degree with physiological responses such as hyperventilation and increased haemoglobin-oxygen binding affinity, which could, in part, result in increased energy expenditure (Perry 1998).

In conclusion, although juvenile dusky kob were able to survive for six weeks in a wide range of salinities (see Chapter 4), the acclimation of juveniles to 5 psu elicited a significant increase in size and number of branchial chloride cells. This suggests that juvenile dusky kob experience increased NKA activity and hence expend more energy osmoregulating at reduced or hypo-osmotic salinities. The proliferation of both branchial and filament chloride cells may have negatively affected respiration and thus led to compensatory responses, which may

have additionally affected energy expenditure. Future work is required to examine the effect of ion deficiencies at reduced salinities on gill morphology of juvenile dusky kob.

CHAPTER 7

GENERAL DISCUSSION

As for many other fish stocks, conventional fisheries management regulations have failed to protect the stock of the dusky kob from overexploitation. It is now generally recognised that an ecosystem approach is probably an improved approach to the management of fisheries. Amongst others this approach requires an understanding of the effect of environmental factors on recruitment, abundance and distribution of fish species (Peterson *et al.* 2004). From life history studies we understand that adult dusky kob generally occur at sea and spawn in the near-shore environments. Larvae recruit into estuaries and early juveniles (30-150 mm TL) appear to be restricted to the upper reaches of estuaries at salinities below 5 psu, whereas larger juveniles are found throughout the estuary (Griffiths 1996). Food availability could not explain the apparent restriction of early juveniles to the upper reaches at reduced salinities (Griffiths 1997a).

The term ecophysiology describes how the ecology and physiology of a species are influenced and regulated by environmental factors (Fry 1971, Rankin and Jensen 1993). Salinity and temperature have been identified as key environmental factors influencing the distribution of fish in estuaries (Whitfield 1994, Marshall and Elliot 1998). Physiological responses to salinity in laboratory studies have been linked to the environment that species are naturally found in during their various life history stages (Morgan and Iwama 1991, 1998, Deacon and Hecht 1999, Cardona 2000, Allen and Cech 2007).

The isosmotic hypothesis states that juveniles are likely to occur and grow optimally at isosmotic salinities, as the osmotic gradient is reduced and osmoregulation would therefore require less energy (Jobling 1994, Boeuf and Payan 2001). Growth is not always optimised at isosmotic salinities, as the optimum salinity for growth of freshwater and marine species is generally below and above isosmotic levels respectively (Deacon and Hecht 1999). Optimum salinity levels can also change with the life history stage of a species. The aim of this study was to investigate whether salinity influenced the distribution of early juvenile dusky kob (< 150 mm TL) by examining various physiological responses of juveniles to a range of salinities under laboratory conditions. It was hypothesised that the physiological functioning of juvenile dusky kob would be optimised at salinities at which they occur naturally (i.e. around 5 psu). The key findings of the experiment are schematically summarised in Figure 1 and discussed in greater detail below.

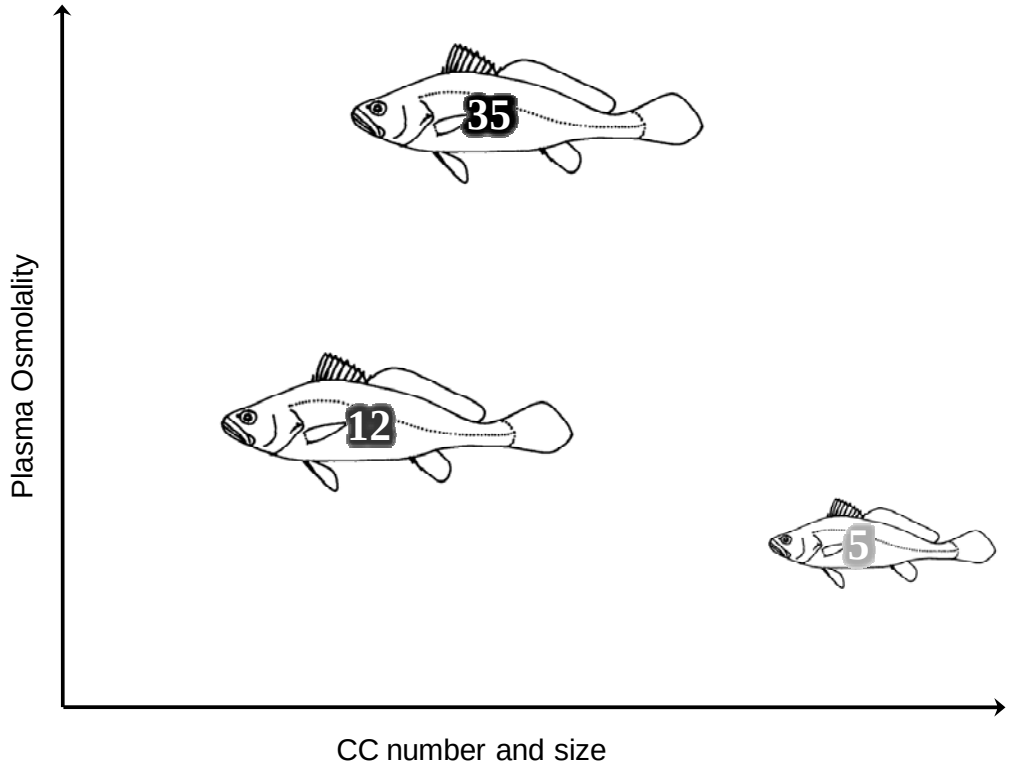

Figure 7.1: Schematic representation of the key findings of this study on juvenile dusky kob exposed to 5, 12 and 35 psu. The relative size and number of chloride cells (CC) are represented on the x-axis, the relative plasma osmolality is represented on the y-axis and growth is represented by the size of fish.

The ability of teleosts to regulate plasma osmolality over a wide range of salinity indicates their degree of 'physiological euryhalinity' (Jobling 1995). Plasma osmolality is measured by anaesthetising fish prior to extracting the blood. The sampling protocol and anaesthetic used can indirectly affect plasma osmolality, by eliciting a stress response. This study found that the exposure and duration of exposure (over 10 min) to 2-phenoxyethanol did not compromise the osmoregulatory ability of juvenile dusky kob (Bernatzeder *et al.* 2008 and Chapter 3). Therefore the sampling protocol and anaesthetic used would not mask the effect of salinity on plasma osmolality of early juvenile dusky kob. There was a significant positive relationship between salinity and plasma osmolality; however, the low slope of the regression line and the ability of juveniles to maintain plasma osmolality over the six-week exposure period suggests that early juvenile dusky kob can be considered 'physiologically euryhaline'

(Chapter 4). However, plasma osmolality of juveniles at 5 psu and 12 psu was significantly lower than in the fish maintained at 35 psu.

Significant differences in plasma osmolality can result in significant differences in growth. (Rodriguez *et al*. 2002, Sampaio and Bianchini 2002). Growth is used as an indicator of the relative energy used for osmoregulation at different salinities, as the energy used for osmoregulation becomes unavailable for growth (Brett 1979). The significant difference in plasma osmolality manifested in significant differences in growth of juveniles at the same salinity levels. Although juvenile dusky kob were able to survive and grow at 5 psu over nine weeks, growth, food conversion ratio and condition factor were negatively affected at this salinity (Chapter 5).

Reduced growth generally corresponds to increased metabolic rate or gill Na^+, K^+-ATPase (NKA) enzyme activity (Cardona 2000, Boeuf and Payan 2001, Imsland *et al.* 2001, 2003, Saoud *et al.* 2007).

In teleosts, the gills are considered the major organ involved in osmoregulation (Evans *et al.* 2005). Within the gills, the chloride cells have been identified as the primary site responsible for ion exchange (Wilson and Laurent 2002) and this is mediated by the NKA enzyme activity. Chloride cells were significantly more abundant and larger in juveniles maintained at 5 psu. An increase in size and/or number of chloride cells is generally associated with an increase in NKA activity (Sakamoto *et al.* 2001). The increase in chloride cells together with the decrease in growth, support the conclusion that early juvenile dusky kob are able to adapt to 5 psu but require increased energy to do so. Despite the apparent restriction of early juvenile dusky kob to the upper reaches of estuaries at salinities below 5 psu, the fish expend more energy osmoregulating at low salinity levels. Consequently the physiological responses of early juvenile dusky kob to salinity cannot explain their distribution patterns within estuaries.

Griffiths (1996) hypothesised that the early juveniles of this species occurred in the upper reaches of the estuary due to food availability and predator avoidance. However, the most important prey item (the mysid *Mesopodopsis slabberi*) of early juveniles was more abundant in the middle reaches (Griffiths 1997a). Griffiths (1997a) also suggested that the juveniles are restricted to the upper reaches of estuaries to avoid predation by larger kob (>400 mm TL)

that dominate the middle and lower reaches of estuaries. However, based on the available information on the feeding ecology of larger kob and other predators in estuaries (Marais 1984, Griffiths 1997a, 1997c), this hypothesis can neither be accepted nor rejected.

This study hypothesised that salinity influenced the estuarine distribution of early juvenile dusky kob. However, temperature is also a key factor influencing the distribution of fish in estuaries (Whitfield 1994, Thiel *et al.* 1995, Marshall and Elliott 1998, Beck *et al.* 2001, Childs *et al.* 2008). Early juvenile dusky kob have a preference for higher temperatures (25-26.4ºC) which corresponds to their optimum temperature for growth (Bernatzeder and Britz 2007, Collett *et al.* 2008). During the summer months, temperatures in the upper reaches of the Great Fish Estuary reach and exceed the preferred and optimum temperatures of juveniles (Childs *et al.* unpublished data). There also appeared to be an interaction between temperature and salinity on the osmoregulatory ability of juvenile dusky kob (Chapter 4). It appeared that osmoregulation improved at temperatures above 23ºC, which is close to their preferred temperature range (Bernatzeder *et al.* 2007), their optimum temperature for growth (Collett *et al*. 2008) and the temperature range that they experience in the upper reaches of estuaries along the east coast of southern Africa (Whitfield 1998).

Early juvenile dusky kob have been recorded in South African estuaries from the Matigulu Estuary in north to the Breede Estuary in the south (Griffiths 1996). However, our understanding of their distribution within estuaries is largely limited to the Great Fish Estuary, which restricts the interpretation of the results of this study. Griffiths' (1996, 1997a) life history study was predominantly based on early juveniles caught in the Great Fish Estuary. However, in the Hawkesbury River (South-eastern Australia), early juvenile dusky kob (mulloway) were most abundant in the middle reaches of the estuary, at salinities around 12 psu (Gray and McDonall 1993). Further investigation is therefore required to establish whether the estuarine distribution of early juvenile dusky kob is limited to the reduced salinity upper reaches. Such investigations can be realised through the advances in technology. Acoustic tags have become increasingly smaller, making it possible to study the distribution and movements of small juvenile fishes. For example, Welch *et al.* (2007) found that survival of juvenile steelhead trout (>12 cm) tagged with relatively small (24 x 8mm) tags was greater than 60%. Additionally, micro-chemical constituents analyses of otoliths,

particularly strontium-calcium ratios, have been used successfully to interpret the distributional history of fish in relation to salinity (Secor and Rooker 2000).

The distribution of early juveniles in the Great Fish Estuary may also be linked to the high conductivity (up to 6 mS cm^{-1}) recorded there, which was negatively correlated to river flow rate (Ter Morshuizen *et al.* 1996, Bate *et al.* 2002). Ter Morshuizen *et al.* (op cit.) found that the abundance of juvenile dusky kob throughout the estuary declined markedly during periods of high river flow (>20 x 10^6 m^3) and increased during periods of low river flow (<10 x 10^6 m^3). The conductivity of a solution of water is highly dependent on the concentration of dissolved salts or ions. Concentration levels of various ions in water can affect osmoregulatory responses and the physiology of fish. The addition of ions, specifically Ca^{+2} and Mg^{+2}, to freshwater or dilute media has been shown to improve survival and growth of the red drum, *Sciaenops ocellatus* (Forsberg and Neill 1997). Similarly, exposure to low calcium concentrations and soft water increased the size and/or abundance of chloride cells in the euryhaline killifish *Fundulus heterclitus* (Katoh and Kaneko 2002) and rainbow trout *Oncorhynchus mykiss* respectively (Perry *et al.* 1996). Deficiencies in potassium also negatively affected growth and survival of juvenile kob (mulloway) (Douroudi *et al.* 2006) and Australian snapper *Pagrus auratus* (Fielder *et al.* 2001). It is therefore hypothesised that the high conductivity in the Great Fish Estuary may mitigate the physiological effects of the reduced salinity on early juvenile dusky kob. This hypothesis needs to be tested by establishing which major ions contribute towards the high conductivity of the Great Fish Estuary and what effect the addition of these ions would have on the osmoregulatory capacity of early juvenile dusky kob.

Research on the recruitment and abundance of estuarine-dependent marine species in South African estuaries has predominantly focused on the effect of reduced river flow as opposed to increased river flow, due to anthropogenic changes (Strydom *et al*. 2002). Although the abundance and distribution of early juvenile dusky kob in estuaries appears to be linked to adequate freshwater inflow (Whitfield and Paterson 2003), this study has shown that they appear to expend more energy osmoregulating at reduced salinities. Further investigation into the distribution of early juveniles is required to make management recommendations but it suggested that although freshwater input is important, high river flow and flooding may negatively affect recruitment and abundance of juveniles.

The potential of dusky kob as an aquaculture species has been recognised in South Africa (Hecht 2000, Schoonbee and Bok 2006) and currently there is an intensive research effort to develop this sector. In aquaculture it is important to determine the environmental requirements of a species to maximise production. Collett *et al.* (2008) recently defined the optimum temperature, light, stocking density and feeding frequency for juvenile dusky kob. However, this is the first study to investigate the effect of salinity on growth of juvenile dusky kob in South Africa. Locally, dusky kob juveniles are currently reared at 35 psu, although the results of this study show that they can be reared equally well at salinities between 12 -35 psu. This corresponds to the optimum salinity range suggested by Douroudi *et al.* (2006) for rearing juvenile dusky kob (mulloway). As a reduction in salinity to 12 psu did not negatively affect growth (specific growth and length gain), food conversion and condition factor, juvenile dusky kob are good candidates for estuarine cage culture. In addition, it would be possible to reduce the artificial salinity of inland recirculating systems that juvenile dusky kob are currently being grown out at (Espadon Marine fish farm, Johannesburg). However, as juveniles gained significantly more weight at 35 psu, there appears to be no need to reduce the salinity they are currently grown out at. The ability of the species to osmoregulate at 5 psu allows for the reduction of salinities to treat certain ectoparasites, should these become problematic under farming conditions.

Due to the practical limitations of catching adequate numbers of early juveniles from the wild, experiments were conducted on hatchery reared juveniles, using wild caught brood stock. All experiments were therefore undertaken on the assumption that there would be no significant variations in the physiological responses between wild and hatchery reared juveniles.

In conclusion, salinity does not appear to play an important role in the distribution of early juvenile dusky kob and it is proposed a suite of both abiotic (e.g. temperature) and biotic factors may instead influence the distribution of early juveniles. The high conductivity in the upper reaches of the Great Fish Estuary may mitigate the effect of low salinity on early juveniles. Further investigation into the distribution of early juveniles in other estuaries along the South African coast and the impact of high conductivity on osmoregulation will assist management efforts in negating the effects of overexploitation by managing the

environmental conditions necessary for the recruitment, growth and survival of early juvenile dusky kob.

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