EFFECT OF SALINITY ON OXYGEN CONSUMPTION AND GROWTH OF JUVENILE WHITE STEENBRAS, *Litohognathus lithognathus*

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by

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……dedicated to my ailing father and in loving memory of my late brother, Robert ‘Uaraa’ Kandjou……

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ABSTRACT

A stress-induced increase in metabolic rate of fish consumes energy within the metabolic scope of a fish that could otherwise be used for such functions as growth and reproduction. By estimating the degree of the metabolic response under given salinity levels and sudden changes thereof, it could be tested whether growth under given culture conditions could be predicted. Using intermittent respirometers, this study investigated the metabolic response of juvenile *Lithognathus lithognathus* following gradual acclimation to 5, 25 and 35‰ and, as a result of abrupt change from 35‰ to 5‰ or from 35‰ to 25‰ at 20˚C. . The main aim of the study was to establish whether the magnitude of such responses could be used to predict growth of juvenile *L. lithognathus* under culture conditions. Hence, in addition to the respirometry study, two growth studies were conducted at 5, 10, 25 and 35‰ salinities. The baseline metabolic rates of juvenile *L. lithognathus* were also determined.

Oxygen consumption measurements over 24-hours showed that most fish exhibited a diurnal peak in metabolic rates. The standard and active metabolic rates calculated from juvenile *L. lithognathus* with a diurnal peak in oxygen consumption were $0.06\pm0.001 mgO_2g^{-1}h^{-1}$ (mean±SEM, n = 5), and $0.11\pm0.01 mg\ O_2g^{-1}h^{-1}$, respectively. The standard and active metabolic rates of juvenile *L lithognathus* showing a nocturnal peak in metabolic activities were $0.04\pm0.001 mgO_2g^{-1}h^{-1}$ (n = 1), and 0.12 ± 0.003 mg O_2 g^{-1} h^{-1}, respectively. Routine metabolic rate of these fish calculated over a 3-h measurement period was $0.09\pm0.005 mgO_2g^{-1}h^{-1}$ (n = 6). Juvenile *L. lithognathus* showed a relationship between metabolic rate (mo_2) and body weight (W) following the equation: $mo_2 = 0.62\ W^{-0.53}$.

The effect of salinity changes on oxygen consumption rates of juvenile *L. lithognathus* revealed that abrupt change in salinity from 35‰ to 5‰ was the only treatment in which oxygen consumption was affected by time of measurement, and this response to a change in salinity contributed to the significant interaction between time and treatment (two-way within-subjects ANOVA, $F_{13,18} = 2.60$; $p = 0.003$). The lack of significant difference in oxygen consumption over time with and without salinity acclimation in any of the other treatments may suggest that oxygen consumption of juvenile *L. lithognathus* may have been influenced by other untested physiological processes in addition to energy required for osmoregulation.

In this study, no significant difference in weight gain and specific growth rate was observed between three salinity treatments throughout the 56-day rearing period of experiment 1 or during the 28-day rearing period in experiment 2. Despite these findings, some mortalities were recorded in both growth experiments at 5‰ salinity. Future research should be undertaken to investigate the interdependence of oxygen consumption, behavioural processes and physiological events in juvenile *L. lithognathus* under various salinities.

CHAPTER 1

General Introduction

The term bioenergetics refers to the ways in which animals make use of the energy they require (Ney, 1993). Bioenergetics models are essentially mass-balance equations in which energy in consumed food is partitioned for various purposes, i.e. growth, metabolism, and excretion of waste products (Winberg, 1956). Studies of bioenergetics have focused on those teleosts that are major sport fishes, have significant commercial harvests, and have potential as aquaculture species. While emphasis has been given to evaluating nutrition and efficiency of food conversion under various dietary regimes and activity patterns, influences of environmental factors on metabolism have been studied in an ecologically diverse group of fishes (Nordlie *et al*., 1991).

Bioenergetics are broadly employed to the management of living marine resources, environmental protection and aquaculture (MacIsaac *et al.*, 1997; Lemos *et al.*, 2001). Energy budget equations can be used to evaluate energy required for growth of fish either under experimental or under aquaculture conditions (Zhou *et al*., 2005). Thus, bioenergetic studies have become a part of aquaculture research and development. Quantifying the excretion of nitrogen compounds, which is an important component of a bioenergetic budget, is also suitable for evaluating the contribution of fish to recycling nutrients in the aquatic environment (Cockcroft and Du Preez, 1989). Quantifying the excretion of nitrogen compounds is also important in aquaculture context to systems loads, especially in recirculating

systems where biofiltration is required to handle ammonia conversion to nitrite and nitrate, and in open systems where nutrients loading may impact the environment.

In commercial aquaculture knowledge and an understanding of the effects of the environmental factors on the physiology of a candidate aquaculture species are prerequisites for successful aquaculture (Deacon, 1997; Bacela, 2002). Environmental salinity is one of the most important factors in fish physiology (Morgan and Iwama, 1991; Boeuf and Payan, 2001) particularly in estuarine-dependent and marine aquaculture candidates. Physiological rates can be measured across a salinity gradient and converted to energetic equivalents to determine the effect of salinity on certain components of the energy budget of fish (Bayne, 1975). For example, energy expenditure of fish can be estimated as a function of salinity by measuring metabolic rate and the release of nitrogenous compounds (Nordlie *et al*., 1991; Woo and Kelly, 1995; Frick and Wright, 2002). An increase in energy expenditure on excretion could influence energy balance and therefore growth efficiency (Altinok and Grizzle, 2004). Metabolism, which includes all processes where transfer of energy is involved, can be quantified on the basis of the energy expenditure, the energetic cost of life (Hogendoorn *et al.*, 1981). The scope for growth, or the energy available for growth and reproduction, can be estimated from these data, allowing for predictions of growth rates of fish (Guerin and Stickle, 1997).

Despite many reports about the influence of salinity on aspects of growth performance of fish (Woo and Wu, 1982; Gaumet *et al*., 1995; Claireaux and Lagardere, 1999; Resley *et al*., 2006) accounts of the effects of salinity on oxygen consumption and growth of estuarine-dependent and marine teleosts in southern Africa are lacking. Some information on the ecology, osmoregulation and reproductive biology of the euryhaline marine white steenbras, *Lithognathus lithognathus*, is available, especially

concerning juveniles in estuaries. *Lithognathus lithognathus* is endemic to southern Africa, occurring between the mouth of the Orange River and Natal (Smith and Smith, 1986). Before 1983 they were also an important component of commercial beach-seine catches (Penny, 1991). Both recreational and commercial catches have, however, declined markedly (Bennett, 1991; Penny, 1991). The species is reported as a good osmoregulator in its natural environment, for its presence has been recorded from predominantly freshwater lakes which had become cut off from their estuarine water source (Mehl, 1973).

A comprehensive study on the occurrence, spawning and growth rate of *L. lithognathus* was conducted by Bennett (1993). *Lithognathus lithognathus* has a restricted spawning period which appears to peak in August. Spawning takes place along the Eastern Cape and Transkei coasts of Southern Africa. After spawning, the fish return southwards and westwards and, by late summer, a large proportion of the mature population congregate off the South Western Cape. *Lithognathus lithognathus* exhibits characteristics of a typical estuarine fish spending its first years maturing in the estuary and migrating seaward to spawn (Wallace *et al*., 1984). Juvenile recruitment to estuaries shows a temporal trend, commencing in the Eastern Cape in August/September (Beckley, 1984), in the Southern Cape in September/October (Whitfield and Kok, 1992) and in the Western Cape in October/November (Bennett, 1989a). Modal progressions in monthly samples of juvenile *L. lithognathus* from the Kleinemond and Palmiet estuaries (Bennett, 1989a) suggested growth rates of approximately 13 mm $month^{-1}$ with total lengths of 160 and 230 mm being attained after 12 and 18 months, respectively (Bennett, 1993).

Mehl (1973) undertook an extensive micro- and macroscopic study of the reproductive biology of *L. lithognathus*. Unfortunately, the collection he used consisted almost entirely of small, immature specimens (less than approximately 35 cm in length), obtained from the Heuningnes Estuary. He was only able to examine 68 marine individuals from False Bay, the majority of which were less than 65 cm in length. Mehl (1973) established that *L. lithognathus* is rudimentary hermaphrodite with genetically determined sexes, and, although some mature fish have approximately equal ovarian and testicular development in the gonads, individuals function as either males or females.

The diet of *L. lithognathus* in various estuaries in the Southern and South-Western Cape has been described by Mehl (1973), Whitfield (1985) and Bennett (1989b). Small juveniles (< 30 mm total length (TL)) consume mainly copepods, ostracods and amphipods. Individuals between 30 to 100 mm TL had more diverse diets, adding polychaetes, insect larvae and decapods. The largest size class (> 100 mm TL) examined in estuaries consumed a wide variety of invertebrates and an appreciable quantity of seaweed (Bennett, 1989b). Bennett (1989b) therefore suggested that the species is omnivorous in estuaries, possibly because of the short supply of larger invertebrates in that environment. In the marine environment, the predominant food items were the macruran *Macropetasma africanus*, the sand dwelling bivalves *Donax spp.* and some polychaete species (Lasiak, 1984a).

A study to establish the relationship between temperature, fish size and ration size and growth of *L. lithognathus* under aquaculture conditions was conducted by Harris *et al*. (1991). *Lithognathus lithognathus* grew well under culture conditions with efficient utilization of a formulated diet. For small *L. lithognathus* (26.5 to 29.6 g) the food conversion efficiencies were good at a relatively high

ration size at temperatures of 16 and 20 °C. For the same ration size, the efficiency was lower at the higher temperature. Absorption efficiencies for larger *L. lithognathus* (234.8 to 280.1 g) were lower at the higher ration size and for the same ration size the food conversion efficiency was also lower at the higher temperature. Nevertheless, all recorded food conversion efficiencies, particularly the nitrogen assimilation, were high ranging from 87-97 %.

The effects of photoperiod, fish body size, temperature, feeding and starvation on the oxygen consumption of *L. lithognathus* were studied by Du Preez *et al.* (1986a). *Lithognathus lithognathus* showed a diurnal peak in oxygen consumption rate. The mean value of *b* in the equation $R = aM^b$ that describes the relationship between routine oxygen consumption (R) and body size of *L. lithognathus* (M) was 0.31, and this value was temperature-independent. The maximum rate of oxygen consumption of *L. lithognathus* induced by feeding was 1.40-2.97 times the maintenance metabolism for fish that had been starved for 40 and 162 h, respectively. Starving the fish resulted in a maximum decrease in active, routine and standard metabolism of 51, 47 and 56 %, respectively.

Work on nitrogen and energy loss via non-faecal and faecal excretion in *L. lithognathus* has been reported by Cockcroft and Du Preez (1989). Non-faecal-nitrogen excreted by starved and fed *L. lithognathus* consisted mainly of ammonia with urea and amino acids as secondary excretory products. Ammonia excretion was temperature-dependent with the excretion rate of starved fish being significantly lower than those of fed fish, at all three experimental temperatures of 15, 20 and 25 °C. The mass component *b* of the mass/ammonia excretion equation was temperature-independent and ranged from 0.651 to 0.700 $mg.NH^{4+}h^{-1}$ and 0.589 to 0.635 $mg.NH^{4+}h^{-1}$ for starved and fed fish, respectively. The average percentage of food energy lost via dissolved non-faecal excretory products

(exogenous plus endogenous) was 6.11±6.07 %. Assimilation efficiency (%) calculated using the following two methods: 1) 1 – [Mass of egested material / Mass of ingested material (g)] × 100, and [Energy intake – (Energy in faeces/Energy intake)] × 100, ranged from 70.8 to 99.3 % for dry matter and from 95.7 to 99.6 % for energy. The combined non-faecal and faecal energy was calculated at 11.9 % of the ingested energy.

Salinity that differs from the internal osmotic concentration of the fish must impose energetic regulatory costs for ion transport (Laiz-Carrion *et al*., 2005) and such energy expenditure can be estimated from the metabolic rate (Morgan and Iwama, 1991). Because salinity is an easily controlled variable and ion transport is an energy demanding process, fish growth may be maximized by selecting salinities which would decrease energy expended to maintain homeostasis (Sampaio and Bianchini, 2002). Along the east coast of southern Africa, the relationship between salinity, growth, food conversion and protein efficiency has been studied in juvenile spotted grunter, *Pomadasys commersonnii* (Deacon and Hecht, 1999). However, there has not been any report on simultaneous measurements of growth and energy metabolism of estuarine-dependent and marine teleosts in relation to salinity. It is this gap in our knowledge of the effects of salinity and salinity changes on metabolism, growth and survival under culture conditions that this research aims to address.

It is generally considered desirable to have rapid growth in fish reared for stock enhancement or commercial aquaculture (Shearer *et al*., 2006). The energetics of fish under various environmental conditions must, therefore, be evaluated and optimal conditions that promote the general health and growth of a candidate species must be determined and tested under aquaculture conditions. Reducing the energetic cost of maintenance of fish is relevant to any fish farming operation and fundamental to

this is knowledge of specific environmental requirements needed to obtain the best survival and good growth.

Respirometers of different designs have been used to measure the respiration rate, or oxygen uptake rate, i.e. the rate at which biomass takes up dissolved oxygen (DO) from water (Steffensen, 1989, Tsoris *et al.*, 2002). Traditional designs of respirometers demand a considerable period of measuring time in order to give an indication of the respiratory activity of the animal species under investigation. Respirometers are tailored to suit not only specific research objectives, but also different sizes and species of fish, taking into consideration their behaviour and general biology. Thus, most investigators construct their own respirometers depending on the study hypothesis, the accuracy desired and the construction cost (Radull, 2002). Three methods have been used to measure respiratory gas exchange. In closed respirometers the change in gas content of the water over time is measured in a closed chamber containing the experimental animal (Steffensen, 1989). In open respirometers the difference in gas content between the inlet and outlet and the flow rate of water through the chamber is measured (Ege and Krogh, 1914). An intermittent flow respirometer works like a closed system respirometer with repeated measures over time (Radull, 2002).

The disadvantages of a closed system include the decline in oxygen concentration, the accumulation of metabolites and the formation of an oxygen gradient as a result of water stagnation (Kamler, 1969). Many types of flow-through respiration systems have been used in oxygen consumption studies on various aquatic animals (Marais, 1978; Hart, 1980; Emmerson and Strydom, 1984; Wrona and Davies, 1984). Problems encountered include complexity of construction and operation (Klekowski and Kamler, 1968), difficulty in calibration of the methods and instruments (Berg and Jonasson, 1965) and

operations, which limit experimental duration (Caulton, 1978; Hart, 1980). The intermittent-flow system retains some of the simplicity and low cost of closed system respirometry while it is still possible to manipulate the duration of a measurement period (Quetin, 1983) by replacing water when oxygen levels drop too low. By regularly flushing the chamber the concentration of dissolved oxygen is maintained at levels suitable for the species tested (Kaufmann *et al.*, 1989) and repeated measurements can be conducted on the same specimen.

In an experiment lasting hours or days, bacterial oxygen consumption may cause a significant error in the determination of oxygen consumption (Steffenson, 1989). To correct for this bacterial metabolism, blanks should be run and subtracted from the calculated metabolic rates of the fish (Claireaux and Lagardere, 1999; Chipps *et al*., 2000).

Stratification of gas content in the water, the accumulation of carbon dioxide and other metabolites as well as nitrogenous excretory products may influence the oxygen consumption of the test animal (Keys, 1930). The use of a pump for recirculating and mixing the water will help solve the problem of stratification (Steffenson, 1989). By flushing the chamber at defined intervals as is done in intermittent flow systems, the accumulation of waste products can be reduced (Radull, 2002).

One of the conditions of measuring oxygen consumption of the experimental fish is that the fish should be able to swim freely in the chamber without any disturbance (Radull, 2002). There is also a need to minimize the water volume in respirometry studies when measuring small fish or those having low metabolic rates (Hove *et al.*, 2000). Hence, it is important to work with optimum ratios of fish size to respirometer volume, in order to make measurements of oxygen consumption over short time

intervals (Steffensen, 1989). The flow rate of water through the respirometer should be maintained as low as possible in order to achieve continuous replacement of the water without disturbing the fish (Sloman *et al.*, 2000).

There is a need to study tolerance to salinity change based on short- and long-term adaptive capability of juvenile *L. lithognathus*. Since salinity tolerance of fish is often positively correlated to growth capacity (Lemarie *et al*., 2004), and metabolism is a major factor influencing the proportion of energy intake allocated to growth (Sun *et al*., 2006), understanding salinity tolerance based on measurement of metabolic rate and growth of juvenile *L. lithognathus* can help evaluate the effects of salinity fluctuations and conditions on metabolism and growth.

The first objective of this study was, therefore, to quantify the metabolic response of juvenile *L. lithognathu*s to salinity changes by measuring oxygen consumption (Chapter 4). The study was designed to first evaluate the effects of day/night cycles and body size on oxygen consumption, thus assessing their effects on the metabolic rate of the species (Chapter 3). The second objective was to evaluate the effect of salinity, using 5, 10, 25 and 35 ‰ on growth performance of juvenile *L. lithognathus* (Chapter 5). Establishing the effect of salinity conditions on growth of *L. lithognathus* will provide important information for the development of future aquaculture efforts in coastal and estuarine regions, including inland areas and those adjacent to low salinity bays along the coast of southern Africa. It will also contribute to a database on the effect of salinity on metabolism and growth in estuarine-dependent aquaculture candidate species. Different methods used in measuring metabolic rate of fish and some sources of errors and possible solutions were reviewed in this chapter. In Chapter 2 the experimental system and the general method used for measuring metabolic rate will be

described. Specific methods used to measure metabolic rate at different salinity treatments will be outlined in the relevant chapters.

CHAPTER 2

Experimental systems and general methods

Recirculating system

All experiments were conducted in a temperature-controlled laboratory at Rhodes University, Grahamstown. A 2360-L seawater recirculating system used in this study comprised of six 300-L holding tanks, two 15-L glass tanks, one 200-L settlement tank, two 110-L biological filter boxes filled with finely shredded plastic strips, a 110-L sump and a pump to circulate the water (Fig 2.1). The glass tanks were filled with water in which a respirometer chamber was kept. The holding tanks were used to acclimate the fish to a selected salinity prior to testing their oxygen consumption at that salinity in the respirometer chambers. The respirometer chambers were linked to the seawater recirculating system via 5-mm plastic tubes. The waterflow entering the respirometer chambers and holding tanks was controlled by clamps attached to the tubes and PVC pipes. The water exiting the respirometer chambers drained into the 15-L glass tanks in which the respirometers were kept. Overflow from the water baths and holding tanks was gravity-fed into settlement tanks before reaching the biological filters. Outflowing water from the biological filters was returned to the respirometer chamber, glass tanks and the holding tanks by means of a pump connected to the sump. A 90 kg oxygen cylinder connected to the recirculating system by means of plastic tubes was used to aerate the water in the sump prior to entering the chambers. Excess delivery from the pump was directed back into the pump chamber. The water temperature of the system was maintained at $\pm 2^{\circ}C$ using a thermostatically controlled heating element located in one of the biological filters. Water quality

variables such as ammonia, nitrite and pH were monitored and recorded every third day, and temperature and salinity were monitored every day. Airstones were used to aerate the water in the holding tanks. Photoperiod was controlled to achieve a 12L:12D cycle using a programmed timer with 06:00 h being the phase of transition from scoto- to photophase.

Respirometer design

Two intermittent-flow respirometer chambers were used to measure oxygen consumption of the experimental fish. The chambers were operated in an open (flow-through) mode during the overnight acclimation of the fish and when the chambers were flushed and in a closed mode when oxygen consumption was recorded. Each chamber was a 2-L plastic container placed on top of a magnetic stirrer plate (Fig. 2.2 and 2.3). A magnetic bead generated a current within each chamber to ensure homogeneous distribution of oxygen. The speed of the current was controlled by the stirrer and it was kept the same for all trials. Clamps fitted to the inflow and outflow tubes of the respirometers facilitated flow rate adjustment through the chambers. Flow rate of water out of the two chambers was determined by measuring the volume of water captured in a graduated cylinder at the outflow of each chamber for 60 seconds. The average flow rate of water to the two chambers was ±0.25 L min^{-1}. To minimize visual disturbance, the glass tanks containing the respirometers were covered with black plastic throughout the acclimation period and during the recording phase but the transparent top of the tanks could allow the experimental fish to experience changes in photoperiod. The chambers were submerged into the glass tanks with only the lid (size of the lid: height = 20 mm, diameter = 100 mm) exposed (Fig. 2.2). Dissolved oxygen levels in each chamber were monitored using an OxyGuard (Handy Gamma) probe inserted into the lids through a rubber stopper. To ensure an airtight fitting, a rubber stopper was glued into the lid using Q-Bond super glue. Prior to measuring oxygen the probe

was calibrated in the air at sea level to 102% saturation according to the manufacturer's instruction. The fish were introduced through the lid of the respirometer. During the closed mode of the system, the chambers were sealed with an insulating tape located around the walls of the lids.

Validation of the method and determination of the accuracy of measurement

Bacterial oxygen consumption may cause an error in the estimation of the oxygen consumption of the fish (Steffensen, 1989, O'Connor *et al.*, 2000). Hence, after constructing the respirometer chambers, a trial was conducted using chambers without fish to quantify the amount of oxygen that could be lost from the system due to bacterial metabolism (Radull, 2002). These measuring chambers were connected to the recirculating system in the evening before the measurements were conducted. The recirculating system was left in flow-mode throughout the night. At the beginning of the experimental trial, water flow through the chamber was turned off, and oxygen concentration was recorded every five minutes for a period of 3 h. Drift was calculated from the following equation as described by Radull (2002):

Drift = $\delta Cwo_2 / Cwo_2 * 100$, where:

Drift is the amount of oxygen (% saturation) lost from the system due to systematic (using Polarographic Oxygen Sensors) and non-systematic (biological) reasons.

Cwo_2 is the dissolved oxygen concentration (mg L^{-1}) in the respirometer chamber at the beginning of the measurement. δCwo_2 is the difference between the initial dissolved oxygen concentration and the concentration ($mg.L^{-1}$) at the end of the measurement period. The drift is expressed as a percent of the mean of the two chambers.

The average dissolved oxygen concentration of the water in the two respirometer chambers was 6.61 $mg.L^{-1}$. Drift accounted for 0.09 $mg.L^{-1}$ over three hours. Using the above formula, the 'oxygen loss'

as a result of the effects of drift, bacterial activity, or system artefacts was less than 1.4%. This 'loss' was assumed to be negligible. It was thus considered an estimate of random measuring error. Thus, any measured changes in the oxygen concentration of the chambers were attributed to the consumption of oxygen by the fish. In addition, since measurement of oxygen levels lasted only 20 minutes before the water in the chamber was flushed, the drift values obtained were assumed to be the maximum error.

Collection and acclimation procedures of the experimental fish

Juvenile *L. lithognathus* (>10 cm TL) were obtained by beach seine netting from the Great Fish River estuary (33° 30'S, 27°07'E) between May and November 2004. The fish were transported in 110-L plastic containers filled with aerated estuarine water to Grahamstown and kept in the temperature controlled laboratory of the Department of Ichthyology and Fisheries Science (DIFS) where they were acclimated to captive conditions for six weeks. Juvenile *L. lithognathus* were generally caught in brackish estuarine water and salinity levels ranged between 3-17‰. In the laboratory, the fish were acclimated to a higher salinity (35‰). Salinity acclimation was achieved by gradually mixing seawater from the holding tanks with estuarine water for 3 h until the desired salinity was reached. The fish were size-graded to avoid dominance hierarchies and then placed into six 300-L holding tanks at approximately 15 fish per tank. Two days after arrival juvenile *L. lithognathus* were fed freshly chopped burrowing bivalve, *Donax spp*., with commercial 2-mm trout pellets at each meal. Juvenile *L. lithognathus* were fed twice per day between 08:00 h and 16:00 h, and ration size was kept to 2% of their total body mass throughout the acclimation and experimental period. Within 14 days the experimental fish readily accepted trout food pellets. The pellets consisted of protein (480 $g.kg^{-1}$) moisture (120 $g.kg^{-1}$), lipid (120 $g.kg^{-1}$), fibre (40 $g.kg^{-1}$), phosphorus (7 $g.kg^{-1}$) and calcium (30 $g.kg^{-1}$).

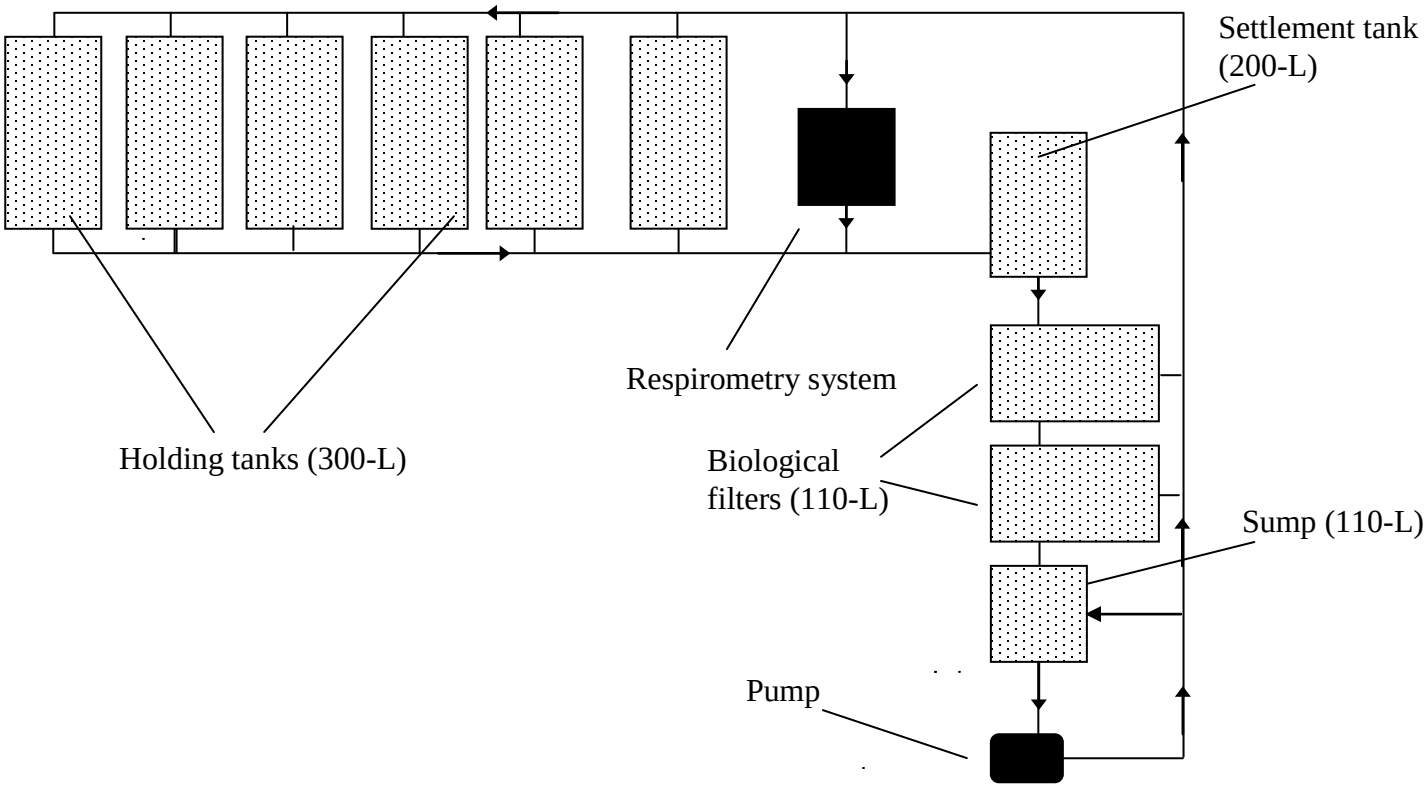

Figure 2.1: The 2360-L recirculating system used to acclimate and measure oxygen consumption of juvenile *L. lithognathus*. The arrows indicate direction of water flow.

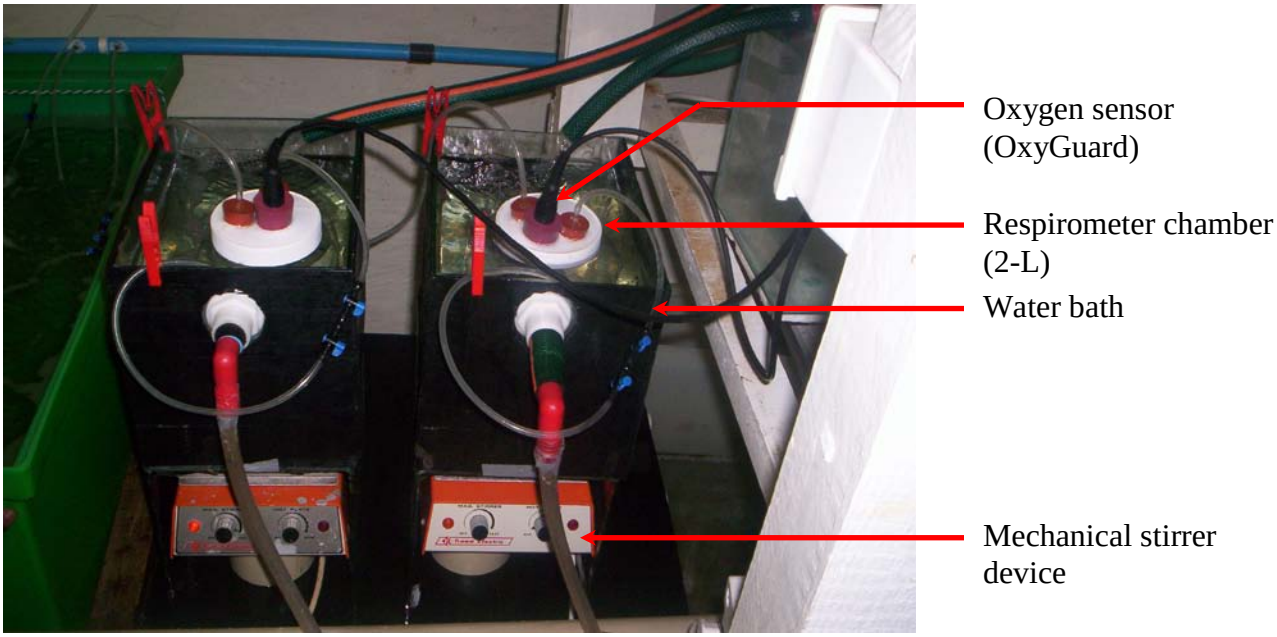

Figure 2.2: The respirometry system used to measure oxygen consumption of juvenile *L. lithognathus*.

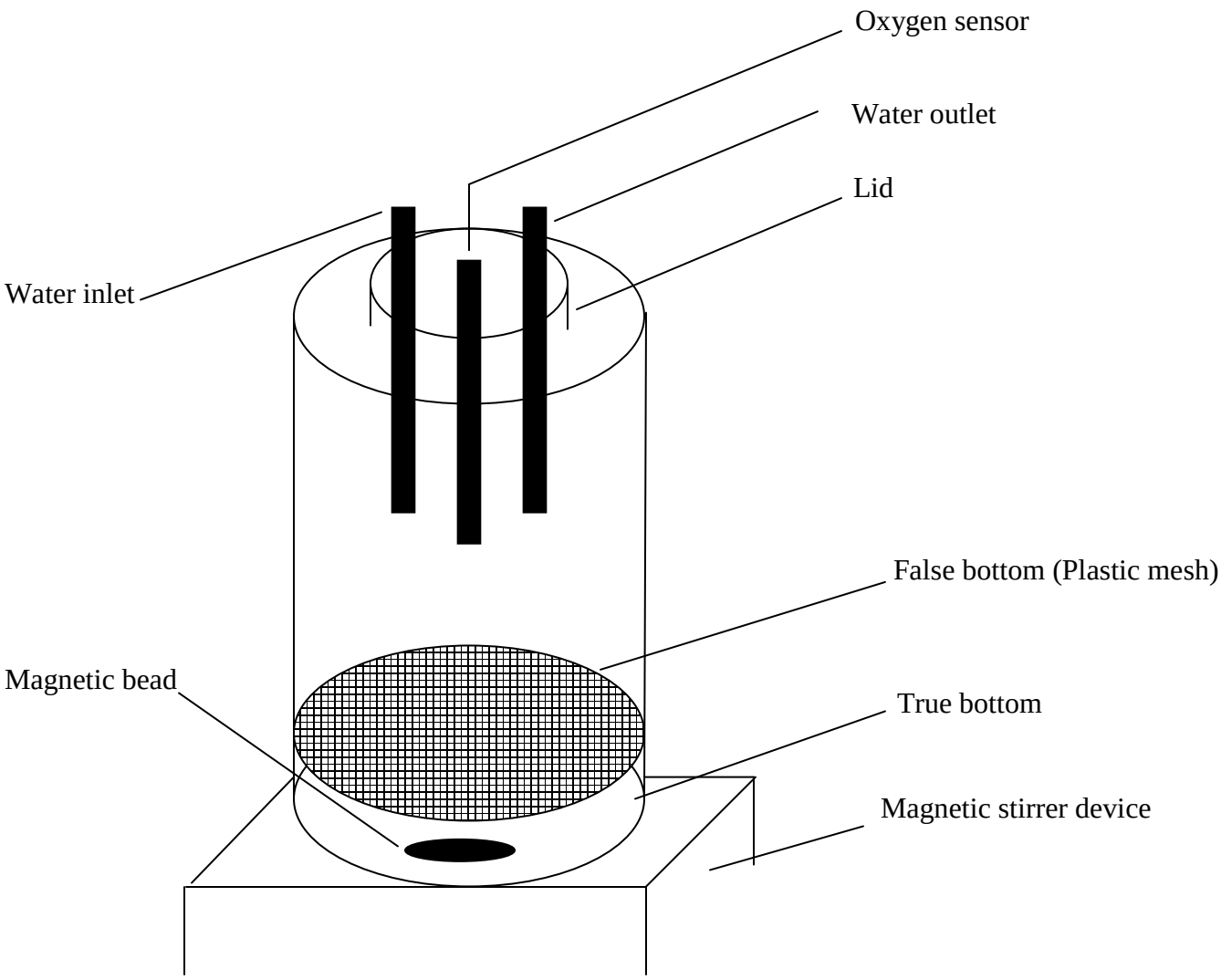

Figure 2.3: A schematic diagram of the 2-L respirometer chamber used to measure oxygen consumption of juvenile *L. lithognathus*.

Measurement of oxygen consumption

General procedures

To eliminate the possibility of diurnal fluctuation in oxygen consumption (Sparks *et al.*, 2003; Wuenschel *et al*., 2004), measurements were always taken in the morning at 09:00 h as outlined in Chapter 3. A single fish was randomly selected from the holding tanks and introduced for overnight acclimation into the respirometer chamber at least 12 h before measuring its oxygen consumption, and the chamber was immediately connected to the recirculating system. The fish were not fed at least 12 h before introduction into the chamber to ensure that all the experimental fish were in an equally post-absorptive nutritional state as suggested by Sparks *et al.* (2003). Since oxygen consumption rates cannot be maintained independent of environmental oxygen-levels indefinitely (Pichavant *et al.*, 2000), the oxygen concentration in the chamber was not allowed to fall below 4 $mg.L^{-1}$. During the recording of oxygen concentration the respirometer chamber was flushed with oxygen-saturated new water for 10 minutes between the measurement intervals to prevent any build-up of metabolites (Steffensen, 1989) and to oxygenate the system. At the end of each trial, the fish were anaesthetized using 2-phenoxyethanol (0.2 $ml.L^{-1}$ of seawater), blot-dried on paper tissues, weighed (to the nearest 0.01g) and measured (total length to the nearest mm). All fish recovered upon placement into aerated water in their tanks and no mortality was recorded following any of these experiments.

Measuring oxygen consumption

Before measuring oxygen consumption of fish, water flow through the chambers was stopped, and oxygen concentration of the water was measured over time using the OxyGuard probe. A regression analysis was conducted using oxygen concentration ($mg\ L^{-1}$) as dependent variable and time as

independent variable. Using the least-squares model starting and ending levels of the measurement period of 20 minutes were estimated. The estimated values were subtracted from each other, divided by the number of minutes that had passed between the two estimates, and multiplied by 60. The resulting value was dissolved oxygen consumption per hour (DOC h^{-1}). This value was then divided by the mass of the fish (g) to arrive at a mass-corrected value for oxygen consumption. The values for DOC/g biomass/h were correlated with the biomass of the fish in order to arrive at a model that predicts DOC as a function of biomass.

Data analysis

Data were presented as means ± standard error of the means as calculated within each treatment group. Least-square regression analysis relating oxygen consumption to fish weight was calculated using the equation $MO_2 = aW^b$, where MO_2 is oxygen consumption in $mgO_2g^{-1}h^{-1}$, W is the mass of the fish in g and *a* and *b* are constants determined from the least-square regression model. Comparisons of the treatment values obtained were described in the respective chapters.

CHAPTER 3

Standard, active and routine metabolism, mass-specific oxygen consumption and daily variations in oxygen consumption rate of juvenile *L. lithognathus*

Introduction

Many methods have been used to study fish metabolism, each offering advantages and disadvantages depending on the species, life stage, and the type or rate of metabolism studied (Wuenschel *et al.*, 2005). Three levels of metabolism have been investigated: (1) standard metabolic rate (SMR), defined as respiration of resting, unfed fish; (2) routine metabolic rate (RMR), defined as respiration of unfed fish showing spontaneous swimming; and (3) active metabolic rate (AMR), defined as the maximum sustainable aerobic rate (Winberg, 1956). Standard metabolic rate is the minimum metabolic rate required to sustain life (Cech, 1990) and therefore gives an indication of the overall physiological condition of the animal. Although SMR is difficult to measure, determination of the oxygen consumption of a resting fish is thought to provide a close approximation (Sloman *et al.*, 2000). Standard metabolic rate represents the energy demand of basic metabolic processes but not those associated with swimming, digestion, and catabolism (Bennetti *et al.*, 1995).

Standard metabolic rate can be determined indirectly by extrapolating oxygen consumption to a state of zero activity (Beamish and Mookherjii, 1964) or by recording metabolic rate over a long period and assuming the minimum to be SMR (Du Preez *et al.*, 1986b; Bennetti *et al*., 1995). Fish observed for

several days under conditions of low stress can display both very low and high activity, SMR and AMR, respectively (O'Connor *et al.*, 2000). The AMR can be measured in fish swimming at maximum sustainable speed (Radull, 2002). The elevated oxygen consumption that coincides with sustained activity of fish can be taken as AMR (Schuurmann and Steffensson, 1997). Routine metabolic rate measurements include some level of activity and the activity level of fish should be accounted for or controlled (Fry, 1971), because individual differences in spontaneous activity may contribute to the variation in oxygen consumption (Zimmermann and Kunzmann, 2001).

Metabolic rate changes with body size and this depends on surface area to volume ratio of the animals (Brett and Groves, 1979). Spark *et al*. (2003) observed negative linear correlations between RMR and body mass in tilapia, *Oreochromis mossambicus*, indicating that RMR decreased as the fish grew. SMR of a juvenile dogfish, *Scyliorhinus canicula*, of 5 g was 82.4 $mgO_2kg^{-1}h^{-1}$ while that of a 500 g adult was 42.2 $mgO_2kg^{-1}h^{-1}$ (Sims, 1996). Therefore, fish mass must be taken into account when comparing metabolic rate within and between species. Dominance hierarchies, spontaneous activity and interaction between individuals could also influence oxygen consumption of fish when measurements are conducted on a group of fish (Sloman *et al*., 2000). Wirtz and Davenport (1976) demonstrate that oxygen consumption rates increased in the blenny, *Blenny pholis*, in response to a conspecific, while measurements of growth led Abbot and Dill (1989) to suggest that there was a metabolic disadvantage, associated with subordinance in juvenile steelhead trout, *Oncorhynchus mykiss*. Consequently, in this study, oxygen consumption rate of juvenile *L. lithognathus* was measured using a single fish in an intermittent flow respirometer.

Periodic regular changes in the environment, such as circadian, diurnal, tidal and lunar cycles can have an effect on the rate of physiological processes in organisms (Shekk, 1986). Circadian rhythms have been reported in many fish species and in mammals (Sedikki *et al*., 1996; Chipps *et. al*., 2000; Pavlov and Kasumyan, 2000; Bolliet *et al*., 2001; Imre and Boisclair, 2004). Some authors have observed diurnal variation in oxygen consumption for several fish species (Smart, 1981; Guinea and Fernandez, 1991; De la Gandara *et al*., 2002). Metabolic rate of Nile tilapia, *Oreochromis niloticus* L. varied over 24-h periods with peaks during daylight hours and low metabolic activity at night (Ross and Kinney, 1988). The diurnal variation in the respiratory activity of Nile tilapia varied approximately 20% around the mean value, implying a diel variation in metabolic rate in this species. Marias (1978) who recorded higher oxygen consumption in mullet, *Mugil cephalus, Liza dumerili* and *Liza richardsonii*, during the first two hours after sunrise and after sunset, also reported on the existence of circadian rhythms.

Peak oxygen consumption rates in white sturgeon, *Acipenser transmontanus*, were measured to be as much as twice the mean daily values and occurred in response to feeding in fish fed a ration of less than 2.6% of body mass per day (Thomas and Piedrahita, 1997). The diurnal variation in oxygen consumption, particularly under fasting conditions in which the effect of feeding activity was absent, suggested a nocturnal activity pattern in Atlantic halibut, *Hippoglossus hippoglossus* (Jonassen *et al*., 2000).

The study of circadian rhythms is therefore of practical value, as data on energy metabolism over a long period (24 h) can lead to erroneous estimates if diel variation in oxygen consumption of the experimental fish is not accounted for. Establishing a suitable time for recording that takes into

account the circadian rhythm in oxygen consumption is, therefore, essential in order to obtain representative measurements of the oxygen consumption rate of a species (Radull, 2002). The above-mentioned findings show that estimates of metabolic rate in fishes may be dependent upon the fish species, size and condition as well as time of measurement. Thus, the metabolic rate of aquaculture candidate species should be known in order to determine optimum water-flow, stocking density, oxygenation requirements and feeding times and frequencies that could maximise production and reduce feed waste.

In the present study it was tested whether the metabolic activity of juvenile *L. lithognathus* varies over time and how it is correlated to fish mass. This study was designed to: (1) establish standard, active and routine metabolic rate of this species which were needed as reference values to assess the stress response to changes in salinity, (2) quantify mass-specific oxygen consumption of juvenile *L. lithognathus* with the aim of presenting a species-specific metabolic rate estimation and (3) determine the 24-h variation in oxygen consumption rate of juvenile *L. lithognathus* in order to establish the time of lowest metabolic activity for taking oxygen consumption measurements as a response to salinity changes.

Materials and methods

Daily pattern of oxygen consumption

The experimental fish were obtained from the Great Fish River estuary, transported to Grahamstown, and acclimated to captive conditions at least six weeks before experimentation (Chapter 2). During the acclimation, juvenile *L. lithognathus* were fed to apparent satiation twice daily, between 08:00 h and

10:00 h and between 14:00 h and 16:00 h. All experiments were conducted in a temperature-controlled room and six fish were used for this study. The average weight and total length (±SEM) of the fish were 41.8±2.28 g and 121.8±1.96 mm, respectively. The average water temperature for this study was 20.1±0.1 °C. Water samples were collected from the outflow of each respirometer chamber prior to commencement of the experiment for water quality analysis. Unionised ammonia and nitrite levels remained below 0.1 $mg.L^{-1}$. Salinity was kept constant at 35‰ through the addition of new water to correct for evaporation. Salinity was measured using the *BS* Eclipse refractometer. Photoperiod was set to a 12L:12D cycle with 06:00 h being the phase of transition from scoto- to photophase.

Two intermittent-flow respirometers were used concurrently to measure oxygen consumption of individual juvenile *L. lithognathus* (see Chapter 2). Fish were randomly selected from the holding tanks, introduced into the respirometers where they were acclimated to experimental conditions for at least 12 h, while the respirometers were left in a flow-through mode overnight. Fish had not been fed for 24 h prior to the introduction into the chamber. After the 12-h overnight acclimation period, water flow through the respirometers was stopped, and dissolved oxygen concentration in the chamber was recorded at half-hourly intervals for 24 hours. The measurements of oxygen consumption for each fish were conducted once. At each measurement interval, oxygen concentration was recorded every minute for 20 minutes, resulting in 20 measurements per half-hourly interval, and the remaining 10 minutes were used to flush the respirometer. The hourly oxygen consumption of a fish was determined from the mean of the measurements obtained per hour (20 measurements X 2 intervals = 40 measurements per hour). Oxygen consumption of a single fish was determined following the protocol outlined in Chapter 2. The mean 24-h measurements of all fish were pooled together, resulting in 24 data points, and these values were used to study diel variation in oxygen consumption of juvenile *L. lithognathus*.

The 24-h variations in oxygen consumption obtained from these measurements were used to determine the time of lowest metabolic activity that could be used in subsequent trials to assess metabolic stress response of the fish at different salinity levels. To test the difference in oxygen consumption between scoto- and photo phase, within-subject analysis of variance was used with photophase being the independent variable and oxygen consumption as the dependent variable. Analysis was conducted on average values of oxygen consumption per fish over time to avoid the bias of pseudoreplication. The p-value at which the null hypothesis was rejected was set at 0.05.

SMR, AMR and RMR

Oxygen consumption measurements of juvenile *L. lithognathus* obtained during the 24-h period were divided into active and standard metabolic rate. Since the lower level of metabolism of fish can be referred to as SMR, and the higher level as AMR (Von Herbing and White, 2002), SMR was taken as the average of the lowest 5 recorded values, and AMR was taken as the average of the highest 5 recorded values.

Routine metabolic rate can be calculated as the mean metabolic rate obtained from the 24-h measurements of oxygen consumption (Du Preez *et al.*, 1986a). For this study, RMR was calculated from 3-h measurements of oxygen consumption using a group of six fish by following the method by Radull (2002). The average weight and total length (±SEM) of the fish for this experiment were 40.4±2.69 g $fish^{-1}$ and 145.0±3.42 mm, respectively. By measuring oxygen concentration continuously for 3 h between 09:00 h and 12:00 h, six data points were obtained for each fish. Routine metabolic rate was determined from the mean of the half-hourly data points of the six fish. Water temperature in

the respirometers was 20.1±0.1 °C, unionised ammonia and nitrite remained below 0.1 $mg.L^{-1}$, and a 12L:12D photoperiod cycle was used.

Mass-specific oxygen consumption

The oxygen consumption of 24 fish ranging in mass from 19.8 to 76.8 g $fish^{-1}$ was measured to study the effect of fish size on oxygen consumption. The experimental fish were acclimated to captive conditions for at least six weeks before experimenting. Individual fish selected from the holding tanks were introduced into the respirometer for at least 12 h for overnight acclimation when the chamber was left in a flow-through mode. Prior to measurement of dissolved oxygen concentration, water-flow through the respirometer was turned off and dissolved oxygen concentration was recorded following the protocol explained in Chapter 2. Dissolved oxygen concentration was recorded every minute for 20 minutes for 3 h, resulting in six data points per fish. The effect of body size on oxygen consumption was calculated using the formula: $mo_2 = aW^b$, where *a* and *b* are constants determined from least square regression analysis, W is the mass of fish in g, and mo_2 is metabolic rate in $mgO_2g^{-1}h^{-1}$.

Results

Daily pattern of oxygen consumption

Time of day had a significant effect on oxygen consumption of juvenile *L. lithognathus*. During the photophase, mean oxygen consumption of five fish was significantly higher ($F_{1,36} = 12.83$; $p = 0.0001$) than during the scotophase (Fig. 3.1). The mean hourly oxygen consumption was 0.09±0.003 mgO_2g^{-1} $^{-1}$ during the photophase and 0.07±0.002 $mgO_2g^{-1}h^{-1}$ during the scotophase. Oxygen consumption of one fish was characterized by a nocturnal peak over the 24-h measurement (Fig. 3.2). During the

photophase the mean hourly oxygen consumption of this specimen was 0.05±0.002 $mgO_2g^{-1}h^{-1}$ and during scotophase it was 0.08±0.006 $mgO_2g^{-1}h^{-1}$.

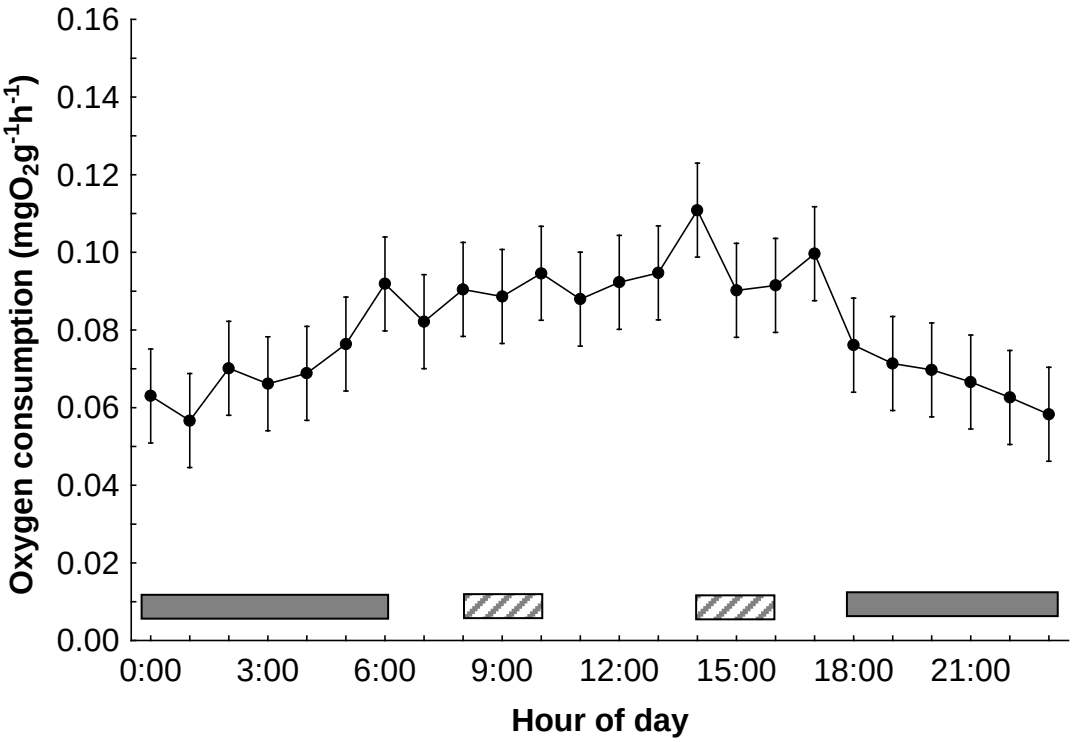

Figure: 3.1. The diel pattern of oxygen consumption of juvenile *L. lithognathus* with means and standard deviation (*n* = 5) of the hourly oxygen consumption rates measured over 24 h. Diagonal bars indicate 'subjective' meal times and the solid bars indicate scotophase.

Standard metabolic rate, active metabolic rate and routine metabolic rate

The SMR and AMR calculated from five fish exhibiting a diurnal peak in oxygen consumption were 0.06±0.001 $mgO_2g^{-1}h^{-1}$ and 0.11±0.01 $mgO_2g^{-1}h^{-1}$, respectively (Fig. 3.3). The SMR and AMR of the fish showing a nocturnal peak in metabolic activities were 0.04±0.001 $mgO_2g^{-1}h^{-1}$ and 0.12±0.003 $mgO_2g^{-1}h^{-1}$, respectively (Fig 3.4). When RMR was estimated for a 3-h-period during the day, time had no significant effect ($F_{5, 30}$ = 1.22, p = 0.32) on RMR of juvenile *L. lithognathus* (Fig. 3.5), and

analysis of variance showed no significant difference ($F_{5,30} = 0.70$, $p = 0.63$) in RMR between the six fish. RMR of these fish was 0.09 ± 0.005 $mgO_2g^{-1}h^{-1}$ during the 3-h measurement period.

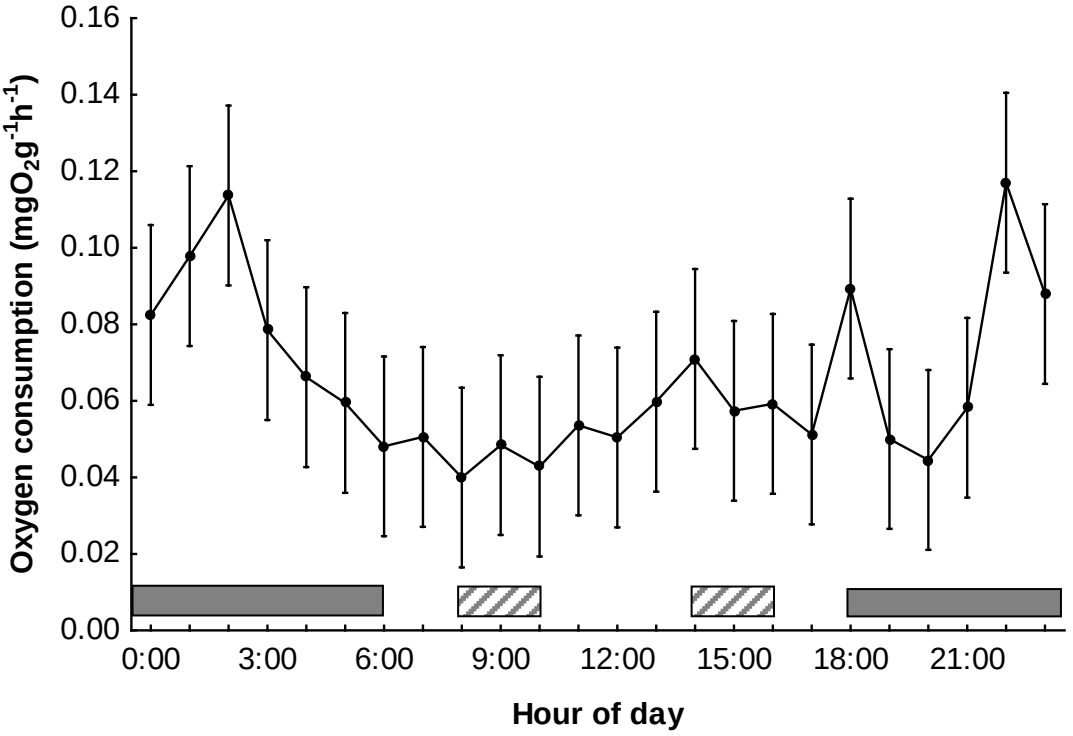

Figure: 3.2. The diel pattern of oxygen consumption of one juvenile *L. lithognathus* with means and standard deviation of the hourly oxygen consumption rates measure over 24 h. Diagonal bars indicate 'subjective' meal times and the solid bars indicate scotophase.

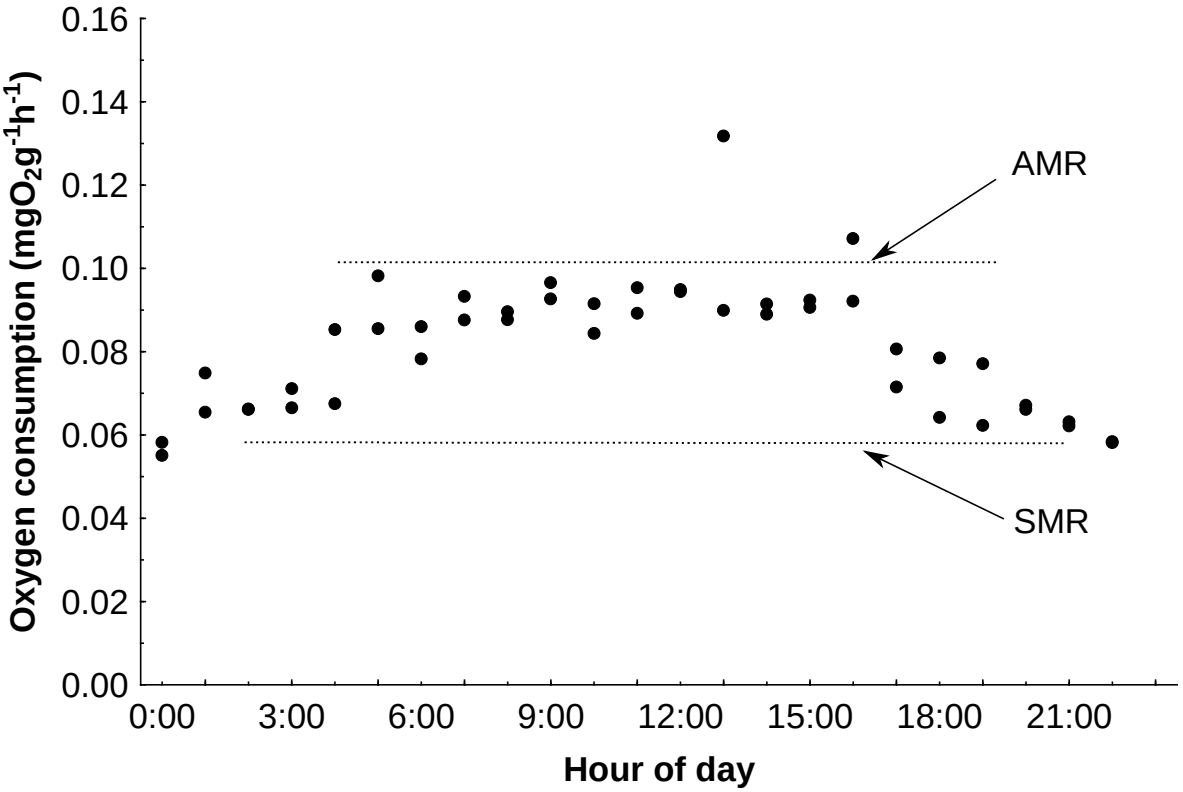

Figure: 3.3. The standard metabolic rate (SMR) and active metabolic rate (AMR) of juvenile *L. lithognathus* exhibiting a diurnal pattern in oxygen consumption. Data points are mean oxygen consumption rates of five fish measured during a 24-h period.

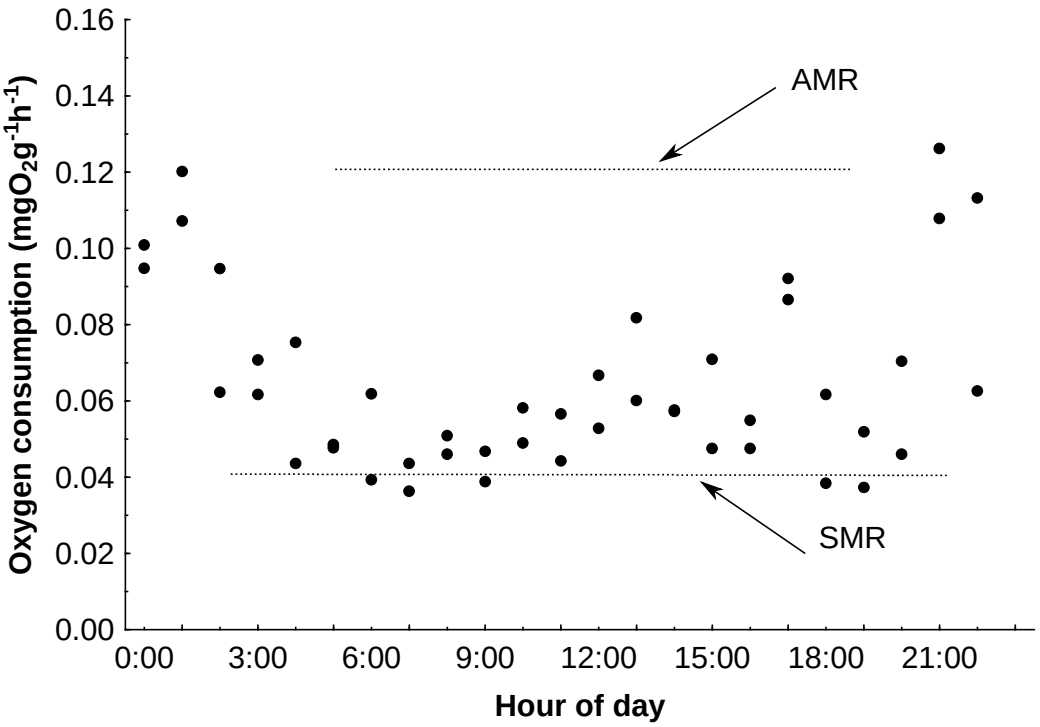

Figure: 3.4. The standard metabolic rate (SMR) and active metabolic rate (AMR) of one fish exhibiting a nocturnal pattern in oxygen consumption.

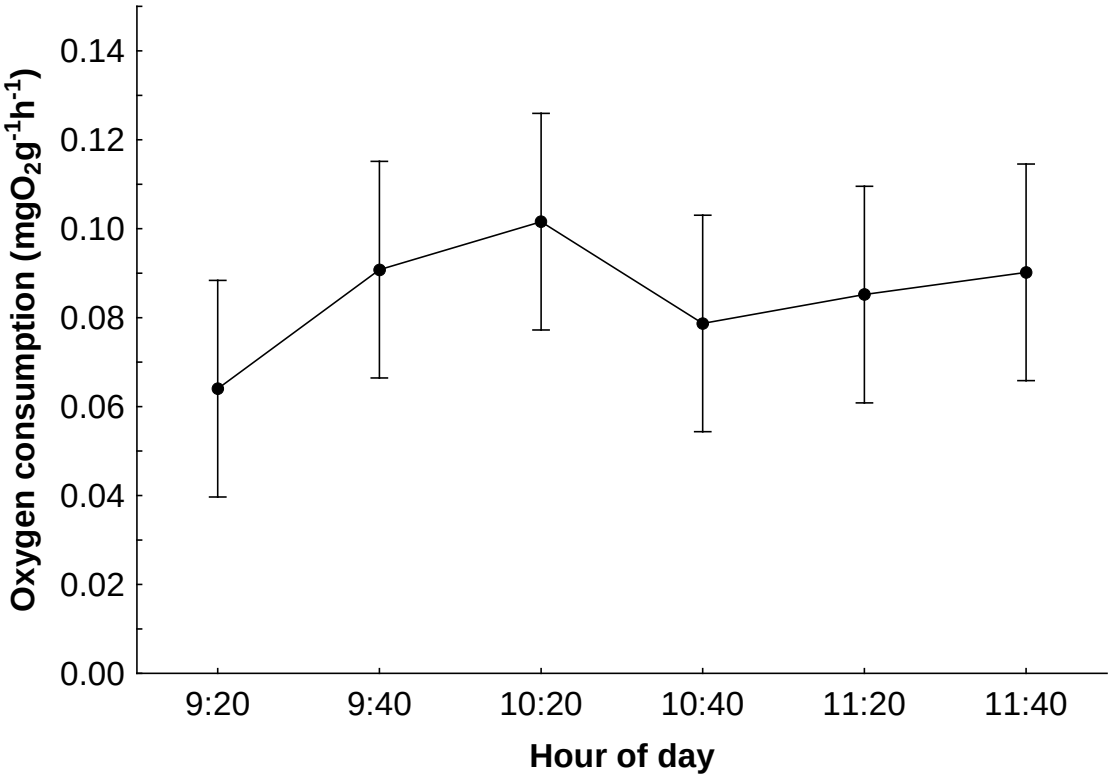

Figure: 3.5. The routine metabolic rate (RMR) of juvenile *L. lithognathus*. Data points are the mean of the half-hourly oxygen consumption of six fish.

Mass-specific oxygen consumption

Least-square regression analysis suggested that oxygen consumption decreased with an increase in body mass of fish and was best described by the following equation:

$mo_2 = 0.62\ W^{-0.53}$ ($r^2 = 0.42$)

The oxygen consumption of the smallest fish was about 44.7% higher than that of the largest fish.

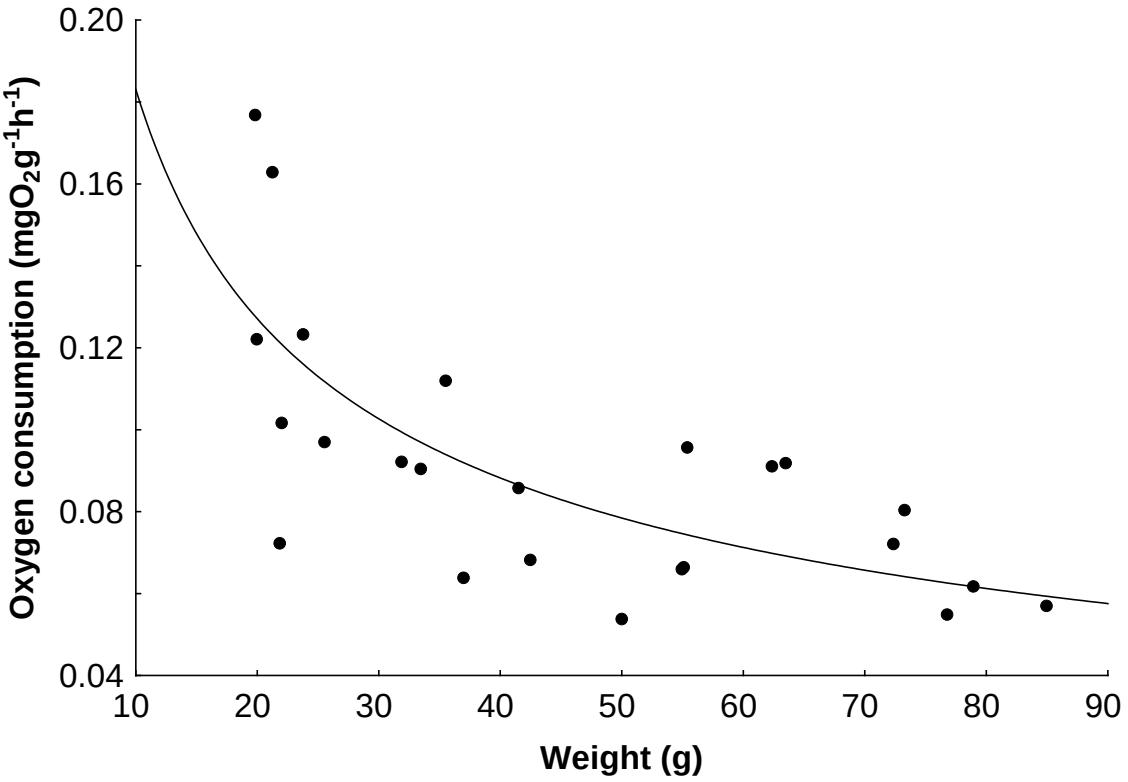

Figure: 3.6. Relationship between fish mass and oxygen consumption of juvenile *L. lithognathus*. Each data point represents one individual. The least-square regression model, $mo_2 = 0.62\ W^{-0.53}$ was fitted through the data points.

Discussion

Mass specific metabolism

Body mass (W) of juvenile *L. lithognathus* was negatively correlated with oxygen consumption (mo_2) when expressed per unit mass, and was best described by the equation: $mo_2 = 0.62W^{-0.53}$. The relationship between metabolism and body size changes with body mass, concomitant with developmental changes (Post and Lee, 1996). Schuurmann and Steffensen (1997) compared the metabolic rate of juvenile and adult Atlantic cod, *Gadus morhua*, and used a coefficient of 0.82 in the

exponent of the equation. Results of this study suggest a lower value of 0.53 for juvenile *L. lithognathus*. These coefficients in the mass/metabolism equation for the routine oxygen consumption of the two species *Lithognathus mormyrus* and *Lithognathus lithognathus* at 20 ºC were 0.34 and 0.31, respectively (Du Preez *et al*., 1986a). However, these fish ranked in size between 14.5 g and 201 g, and 50.5 g and 2338 g for *L. mormyrus* and *L. lithognathus*, respectively.

Values for *b* in the equation $mo_2 = aW^b$ may decrease from ~1.0 in fish larvae to values close to 0.75 in juveniles and adults (Peck *et al*., 2003). The *b*-value obtained for juvenile *L. lithognathus* falls within the range (0.4-1.0) reported for many studies (Schmidt-Nielsen, 1984). The ¾ power scaling law (*b* = 0.75) is associated with a variety of biological phenomena (West *et al*., 1997). Three hypotheses have been put forward to explain the b-value of the relationship between metabolic rate and body mass: 1) changes in the relative size of respiratory surfaces, 2) changes in swimming efficiency and 3) changes in the relative proportion of different organs and tissues which vary in metabolic activity (Post and Lee, 1996).

Metabolic scope

In the present study the ratio of active to standard metabolic rate as an index of routine metabolic scope in fish showing a diurnal peak in oxygen consumption was 1.8 and in fish showing a nocturnal peak in metabolic activities it increased to 3. In Atlantic salmon, *Salmo salar*, the ratio was about 1.5 for both par and smolts during the experimental period, except for smolts in freshwater after peak smolting when it decreased to 1.2 (Maxime *et al*., 1989). Calculations of this ratio from literature data was 1.33 at 20 °C for *L. lithognathus* (Du Preez *et al*., 1986a), 1.35 at 15 °C for *Lichia amia* (Du Preez, 1987), and 1.76 at 25 °C for *Oreochromis niloticus* (Ross and McKinney, 1988). The SMR

value of 0.05 $mgO_2g^{-1}h^{-1}$ measured in juvenile *L. lithognathus* (size range: 26.54 to 29.55 g) at 16 °C by Harris *et al*. (1991) falls within the SMR range of 0.04±0.001 to 0.06±0.002 $mgO_2g^{-1}h^{-1}$ determined from the fish in the present study. However, the AMR value estimated for *L. lithognathus* in this study is only 50% of approximately 0.2 $mgO_2g^{-1}h^{-1}$ value reported by Harris *et al.* (1991) in juvenile *L. lithognathus*.

Juvenile *L. lithognathus* had a diel pattern in oxygen consumption. The mean daily oxygen consumption rate was 1.4 times higher than the mean oxygen consumption during the scotophase. For one fish the mean oxygen consumption during the scotophase was 1.6 times higher than during the photophase. Du Preez *et al*. (1986a) reported a diurnal peak in oxygen consumption of *L. lithognathus* with some fish showing nocturnal peaks. Comparison of the frequency of occurrence of *L. lithognathus* in the surf zone of a sandy beach with catch statistics over the diel cycle revealed that this species was present irrespective of day/night cycle although the number of fish caught peaked just after twilight (Lasiak, 1984b) when some teleosts move closer inshore to feed (Du Preez *et al*., 1986a). Oxygen consumption of juvenile spotted grunter, *Pomadasys commersonnii*, was significantly higher at night than during the day (Radull *et al*., 2002). In juvenile Atlantic turbot, *Scophthalmus maximus*, oxygen consumption of the 24L:0D treatment during the night-time period was higher than the 12L:12L and 16L:8D treatments (Imsland *et al*., 1995). The findings of this study together with those of Du Preez *et al*. (1986a) suggest that future studies are needed to determine other factors that may influence oxygen consumption patterns in this and potentially other estuarine species.

The activity of fish is reflected in their oxygen consumption and energy demand (Dalla Via *et al.*, 1999), and continuous exposure of fish to light may affect oxygen consumption by altering the level of

activity (Imsland *et al*., 1995). Like higher vertebrates (Johnson, 1992), fish can display free-running rhythms of feeding activity (Boujard *et al*., 2000). Some cyprinids such as goldfish, *Carassius auratus* have been reported to concentrate their activity around feeding time (Sanchez-Vazquez *et al.*, 1997) and juvenile cyprinids, *Rutilus rutilus*, deprived of food for about 24 h, showed higher locomotor activity and rates of oxygen consumption in a respirometer than well-fed fish (Wiese *et al*., 1988). Increased locomotor activity prior to the critical time limit of food absence raises the chance of finding a new food patch (Mendez and Wieser, 1993). The feeding activity of tilapia hybrids (*Oreochromis niloticus* X *O. mossambicus*) reared in ponds was continuous during daytime, with a single feeding peak at around afternoon-dusk (Yousuf Haroon *et al*., 1998). Moreover, an increased oxygen consumption rate of fish after feeding has been reported for various fish species (Jobling, 1981). For example, the daily metabolic rate of the mullet, *Mugil saliens*, was closely linked to feeding regime, with maximum rates developing after feeding, independent of feeding time and light-dark cycle (Guinea and Fernandez, 1991). In the present study, fish were not fed 24 h before experimenting, therefore, it is unlikely that the metabolism was affected by digestion processes. However, five fish exhibited a diurnal pattern in oxygen consumption comprised of elevated levels in the hours before 'subjective' feeding time. Patterns in oxygen consumption rate such as in the present study were reported for mullet by Marais (1978) and Shekk (1986) and juvenile flounder by Liu *et al.* (1997).

Diurnal pattern

Although larvae of some fish species have a non-visual sense for feeding (Batty and Hoyt, 1995) most fish larvae are visual predators (Porter and Theilacker, 1999) and environmental conditions represent a key factor in feeding success (Sabates, *et al.*, 2003). For example, the incidence of feeding in myctophid larvae, *Benthosema glaciale* increased at sunrise to reach a maximum at midday and then

decreased before sunset. Photoperiod is considered the most important synchroniser of biological rhythms, but periodic food access is also known to synchronise behaviour (Boulos and Teman, 1980; Mistlberger, 1994). Sanchez-Vazquez *et al*. (1995) reported that periodic food access in sea bass could synchronise the demand-feeding rhythm. These authors also suggested that food availability might be one of the factors inducing a shift in phasing and that, as reported in mammals, two different oscillators, one synchronised by photophase and the other by food, might entrain the rhythmic pattern of feeding activity in this species. In the present study while the highest peaks in oxygen consumption of juvenile *L. lithognathus* occurred a few hours before subjective feeding time (between 08:00 h and 10:00 h, and between 14:00 h and16:00 h)., a switch from scoto- to photophase and vice versa also affected oxygen consumption of juvenile *L. lithognathus*. Juvenile *L. lithognathus* started increasing and decreasing their oxygen consumption rates a few hours before photophase and scotophase period, respectively. Thus, in this study both the physiological needs (e.g. feeding/starving) of juvenile *L. lithognathus* and environmental conditions encountered (e.g. scoto- and photophase) may have provided the experimental fish with a potential means for tuning their metabolic rate.

Despite the variability at individual level in oxygen consumption pattern observed in juvenile *L. lithognathus* in the present study and that found by Du Preez *et al*. (1986a) for the same species, the results of this study point to the existence of oxygen consumption pattern in juvenile *L. lithognathus*. It was not tested whether the pattern was regulated by photoperiod, feeding anticipatory effects or an endogeneous system. A circadian rhythm in oxygen consumption reported in unfed young Mediterranean yellowtail, *Seriola dumerili*, was attributed to an endogeneous origin (De la Gandara *et al.* 2002), as previously reported in the river puffer fish, *Takifugu obscurus*, (Kim *et al.*, 1997). Future studies should investigate the regulation by photoperiod combined with restricted feeding cycles of the

rhythmic pattern in oxygen consumption of juvenile *L. lithognathus*. Trials of longer duration can be conducted in replicates to investigate whether juvenile *L. lithognathus* will keep showing the same pattern in oxygen consumption during the day when not fed, and during 24D:0L and 0D:24L cycles.

This thesis was designed to test to what extent the magnitude and rate of change of the environmental variable salinity dictates the metabolic cost of acclimation of fish to salinity changes. As a prerequisite to allow the evaluation of the metabolic costs of variable salinity conditions, reference data regarding the metabolic rate of unstressed juvenile *L. lithognathus* were needed. There was also a need to investigate diel activity pattern as they should be taken into account when studying fish metabolism, or handling and feeding of fish in aquaculture facilities. A pattern in oxygen consumption of juvenile *L. lithognathus* was observed with fluctuations depending on scoto- and photophase. These fluctuations in oxygen consumption of juvenile *L. lithognathus* were used to determine the different levels of metabolism. Some fish undergo metabolic compensation as an anticipatory response to variable environmental conditions (Chipps *et al*., 2000). Thus, the different rates of metabolism as observed in this study form the basis for determining the amount of energy needed by juvenile *L. lithognathus* for maintenance and growth. The RMR measurements conducted on fish between 09:00 h and 12:00 h for 3 h indicated that RMR did not differ significantly over time. Consequently, all oxygen consumption measurements for the remaining experiments in which salinity changes were tested were conducted during the photophase over a period of 3 h, between 09:h00 h and 12:00 h to minimize the effects of the observed 24-h variation in oxygen consumption of juvenile *L. lithognathus*.

CHAPTER 4

Salinity tolerance and metabolic response of juvenile *L. lithognathus*

Introduction

Salinity is one of the main environmental factors exerting a selective pressure on aquatic organisms. Some species spend their whole life cycle in a habitat where salinity is stable while others experience variable conditions. Both estuarine and marine species may accomplish ontogenetic migrations submitting the successive stages of their life cycle to different salinity regimes. The ability of each developmental stage to cope with salinity changes depends on the ability to osmoregulate, resulting in salinity tolerance that enables the organism to occupy various habitats (Varsamos *et al*., 2005). To describe the complexity of environmental effects on fish, Fry (1971) proposed a conceptual model, which has recently been implemented and supplemented (Neill *et al*., 1994). This model categorises environmental factors according to their physiological effects on fish, e.g., lethal, controlling, limiting, masking and directive. Salinity was classified in Fry's model as a masking factor. According to the definition, a masking factor may channel a portion of the available metabolic energy toward organismic regulations, which can be of mechanical, physiological or behavioural nature (Claireaux and Lagardere, 1999).

Salinity changes present a challenge to be overcome by both metabolic control and behavioural responses (Abud, 1992). The mechanisms of osmoregulation in fishes are well understood (Evans, 1984, 1993). Fishes incur energetic costs for active ion transport at salinities that differ from that of

their internal osmotic conditions (Wuenschel *et al.*, 2004). Osmoregulation, therefore, results in the maintenance of almost constant or slightly variable blood osmolality depending on the species osmolality ranges from 280 to 360 mOsM kg^{-1} (Varsamos *et al*., 2005). Marine fish species regulate their blood plasma ions so that the osmotic pressure of body fluids is between 10-15‰ salinity but energy must be spent to meet the cost of osmo-ionic regulation (Brett, 1979; Jobling, 1994). Below this salinity, especially in fresh water, a fish hyper-osmoregulates thereby experiencing passive influx of water and diffusive loss of ions, mainly Na^+ and Cl^-. Above the isosmotic salinity of approximately 10-12‰ the animal hypo-osmoregulates and is exposed to ion invasion and dehydration (Varsamos *et al*., 2005). Marine teleosts must continuously drink water to compensate for water loss across the gills. Intestinal Na^+/K^+-*AT*Pase pumps move ions from the ingested water into the blood, allowing for the water to follow osmotically. The excess ions are pumped out of the plasma at the gills by branchial Na^+/K^+-*AT*Pase (Webb *et al.*, 2001).

Estimates of the magnitude of the costs of ion transport in fish vary (Febry and Lutz, 1987; Morgan and Iwama, 1991; Nordlie *et al*., 1991; Swanson, 1996), and little information is available on the related energetic and physiological consequences of life in changing or different salinities (Eddy, 1982; Swanson, 1998; Boeuf and Payan, 2001). An estimate of the energetic cost of osmoregulation in salmonids suggests that it amounts to up to 1% of resting metabolism (Eddy, 1982). Changes in metabolic rate of such low magnitude are difficult to measure accurately (Morgan and Iwama, 1991). Bullivan (1961) reported no significant difference in metabolic rate of yearling Chinook salmon, *Onchorhynchus tshawytscha*, reared in fresh water, half strength seawater, and full strength seawater. Standard metabolic rate measured in seawater Atlantic cod, *Gadus morhua*, at 14‰ and 28‰ did not decrease or increase with salinity changes (Dutil *et al*., 1997). Similar results were obtained for this

species with and without salinity acclimation. In contrast, oxygen consumption in rainbow trout, *Onchorhynchus mykiss* showed a reduction in metabolic rate (20-28%) under conditions of isosmotic salinity relative to both fresh water and seawater (Rao, 1968). Bushnell and Brill (1992) estimated that osmoregulation might consume as much as 54-68% of the non-swimming metabolic demand in two species of tuna. Oxygen consumption of the silver sea bream, *Sparus sarba*, was the lowest at isosmotic salinity (15‰) relative to both low salinity water (7‰) and seawater (35‰) (Woo and Kelly, 1995). Such studies support the hypothesis that the energetic cost of ion regulation is lower in an isosmotic environment, where the ionic gradients between blood and water are minimal (Morgan and Iwama, 1991).

Analyses of metabolic rate in swimming fish revealed that the cost of osmoregulation was higher in freshwater than in seawater in the red hybrid tilapia (*Oreochromis mossambicus* X *Oreochromis hornorum*) (Febry and Lutz, 1987), whereas it was more expensive in seawater (30‰) than in freshwater in the Nile tilapia, *Oreochromis niloticus* (Farmer and Beamish, 1969). In the Mozambique tilapia, *Oreochromis mossambicus*, routine metabolic rate in fish acclimated to seawater was lower than in fish in freshwater or hypersaline seawater (Iwama *et al.* 1997). However, when Mozambique tilapia were transferred from freshwater to 75% seawater, oxygen consumption of the swimming fish was significantly elevated four days after transfer compared with those in freshwater or at iso-osmotic salinity (Morgan *et al*., 1997). Thus, the effect of environmental salinity on oxygen consumption may vary, depending on the methods of determination, stage of acclimation to the environment, species, age and body mass of the fish (Sparks *et al*., 2003). Tolerance to reduced salinities in marine teleosts varies within a single family or even genus (Wu and Woo, 1983). For example, the black sea bream, *Mylio macrocephalus*, appeared to be the most euryhaline and the rabbitfish, *Siganus oramin*, was

found to be the most susceptible to dilution of the ambient medium (Wu and Woo, 1983). More data are, therefore, needed on metabolic changes following salinity adaptation in teleosts in order to better understand osmoregulation in teleosts, in particular estuarine species with life cycles that expose fish to different salinities at different ages or developmental stages.

Since salinity can potentially act as a stressor under both natural and aquaculture conditions (Varsamos *et al*., 2004), modelling the metabolic response to changes in salinity may provide a bioenergetic basis to evaluate performance of this species under culture conditions. This is also relevant for the culture of salinity-tolerant aquaculture candidates such as *L. lithognatus* which could be reared at different salinities. The objectives of the present study were, therefore to (1) evaluate the influences of a wide range of ambient salinities occurring in its natural habitat on the metabolic response of juvenile *L. lithognathus* by using oxygen consumption as an indicator, and to (2) evaluate the short-term and long-term responses of juvenile *L. lithognathus* to an abrupt and gradual salinity decrease, respectively.

Materials and methods

1. Experimental system, general maintenance of the fish and acclimation procedures to different salinities

The metabolic response to salinity was studied in juvenile *L. lithognathus* that were subjected to either a gradual or abrupt salinity decrease. Fish were collected from the Great Fish River estuary at the end of November 2004. The experimental fish were acclimated to the laboratory conditions at the Department of Ichthyology and Fisheries Science in Grahamstown for at least eight weeks. Fish were

kept in four 300-L plastic tanks and acclimated to seawater (35‰) of a recirculating system in the laboratory. Two 300-L plastic tanks served as experimental tanks to further acclimate the fish to the selected salinity before measuring their oxygen consumption. A 300-L glass tank with a submersible pump (Resun, SP-6600) was used to effect salinity changes. During measurement of oxygen consumption at a selected salinity, the glass tank was connected to the respirometry system via 10-mm PVC pipes with valves. When in flow mode, the outflowing water from the glass tank was pumped into the respirometer chambers and water leaving the chambers drained into the glass tank via water baths. Average ammonia (NH_3) and nitrite (NO_2^-) levels of the experimental tanks and glass tank were kept at or below 0.04±0.02 mg L^{-1} and 0.03±0.01 mg L^{-1}, respectively, by daily replacing 50% of the water with new water of the same salinity as that of the tanks. All tanks were aerated using airstones and the average water temperature of the two experimental tanks and the glass tank was maintained at 20.1±0.1˚C using a 150-W aquarium heater in each tank.

Fish were fed to satiation twice daily. Uneaten feed was siphoned from the tanks 30 min after each meal and faecal material was removed from each tank before feeding. Satiation was defined as the stage when the fish no longer accepted single pellets dropped into the tanks. Fish ranged in body mass from 51.1 g to 97.6 g with no significant difference (one-way ANOVA, $F_{4, 54} = 2.40$; $p = 0.062$) between treatments.

Two intermittent-flow respirometer chambers with one fish per chamber were used concurrently to measure oxygen consumption of juvenile *L. lithognathus*. Fish were not fed for at least 24 h prior to measurements of oxygen consumption and each fish was introduced into a chamber at least 12 h before the start of measurements. This was done to acclimate the fish to the experimental conditions.

Withholding feeding for 24 h ensured that all fish were in a similar post-absorptive nutritional state. After measurement of oxygen consumption, the fish were weighed following the protocol outlined in Chapter 2 and returned to the holding tanks at a salinity of 35‰.

2. Salinity change

A series of experiments was designed to test the effect of three levels of salinity: 5‰, 25‰ and 35‰ on metabolic rate of juvenile *L. lithognathus*. A measurement period during which oxygen levels were measured in 20-minute intervals lasted 3 h. Using this approach the metabolic rate of the fish was studied by measuring oxygen consumption following either a gradual or an abrupt change of salinity. Juvenile *L. lithognathus* were kept at 35‰ to acclimate them to captive conditions for approximately eight weeks, and oxygen consumption of 12 fish was recorded at this level of salinity. Two groups of 12 fish were tested to study their oxygen consumption following gradual and abrupt change from 35‰ to 5‰ and from 35‰ to 25‰. Oxygen consumption of the fish was first measured as a result of gradual decrease to 5‰ or 25‰, and the fish were thereafter returned to the 300-L plastic tanks and kept at 35‰, fed to satiation and allowed to acclimate for 14 days. After this, oxygen consumption of juvenile *L. lithognathus* was determined as a result of abrupt change from 35‰ to 5‰ or from 35‰ to 25‰. The salinity change was effected after 10 minutes.

Gradual decrease in salinity

Two fish were randomly selected from four 300-L plastic tanks and transferred to an isolated experimental tank at 35‰. In this tank, salinity was lowered to 5 or 25‰ by replacing approximately 10% of seawater in the tank with fresh water over 3 or 9 h, respectively, until the desired salinity was reached. Fish were allowed to acclimate to this salinity for 2 days before being introduced into the of

the respirometer chamber at the same temperature and salinity as that of the holding tank. The respirometer was fed by continuously flowing water during overnight acclimation. After the overnight acclimation on day 3, oxygen consumption was measured at the selected salinity. Before measurement of oxygen consumption, water flow through the chamber was stopped and oxygen consumption was recorded every 20 minutes over a period of 3 h following the protocol outlined in Chapter 2.

Abrupt decrease in salinity

Metabolic rate of juvenile *L. lithognatus* was studied using fish acclimated for 14 days in 300-L plastic tanks of the recirculating system at 35‰. To study metabolic rate as a result of abrupt decrease, individual fish were introduced into the respirometer chamber with flowing water from the recirculating system at 35‰ for at least 12 h overnight. To measure oxygen consumption after change to 5 or 25‰, the chamber was flushed with water of the selected salinity for 10 min until the test salinity was reached. During the salinity reduction, the water was monitored continuously at the outflow of each chamber and as soon as the desired level was measured, recording of oxygen consumption commenced. At a flow rate of 0.25 $L.min^{-1}$, the water in the chambers was exchanged approximately 2.5 times in 10 min. Water flow was stopped intermittently, and oxygen consumption was every 20 minutes over a period of 3 h.

3. Statistical analysis

Since oxygen consumption of each fish was repeatedly measured over time there were twelve replicates for each of the five treatments with repeated measures. A treatment was defined as the way salinity changed, i.e., either slow or fast to either 25 or 5 ‰, with the fish kept at 35‰ throughout the study being the control treatment. Within-subject analysis of variance was used for both the change in

oxygen consumption over time within one treatment, and by using two independent variables, i.e., time and treatment. Thus, it was possible for check for interactions between time and treatment as well as to describe the changes in oxygen consumption over time within an experiment. After testing for sphericity, the Greenhouse-Geisser adjustment for degrees of freedom and F-statistic was performed, and values reported are adjusted F- and p-values.

Results

No mortality was recorded during the 3-day acclimation period and/or during the 3-h exposure of juvenile *L. lithognathus* to salinity tests. There was a significant time-treatment interaction (two-way within-subjects ANOVA, $F_{13,18} = 2.60$; $p = 0.003$). This suggests that oxygen consumption over time changed depending on the treatment. At 35‰ oxygen consumption of fish ranged from 0.06±0.01 to 0.08±0.01 $mgO_2g^{-1}h^{-1}$ with no change in oxygen consumption over time (within-subjects analysis of variance, $F_{3,28} = 2.63$; $p = 0.07$). However, in fish exposed abruptly to a change in salinity to 5‰, mean oxygen consumption decreased significantly from a high level of 0.05±0.02 $mgO_2g^{-1}h^{-1}$ in the first measurement interval to 0.03±0.004 $mgO_2g^{-1}h^{-1}$ in the second measurement interval ($F_{3.28} = 12.04$; $p = 0.0001$, Tukey's multiple range test $p = 0.0001$) and it remained at the lower level until the end of the measurement period. Since this was the only treatment in which oxygen consumption was affected by time of measurement, this response to a change in salinity contributed to the significant interaction between time and treatment. A gradual decrease in salinity from 35‰ to 25‰ did not effect a significant change ($F_{2,22} = 1.07$; $p = 0.36$) in oxygen consumption over time and mean oxygen consumption was 0.06±0.003 $mgO_2g^{-1}h^{-1}$ (Fig. 4.2). Similarly, following an abrupt change in salinity from 35‰ to 25‰ mean oxygen consumption of juvenile *L. lithognathus* did not change significantly

over time ($F_{3,28}$ = 1.21; p = 0.32) and remained at 0.06±0.002 $mgO_2g^{-1}h^{-1}$ (Fig. 4.3). A gradual decrease in salinity from 35 to 5‰ resulted in a mean oxygen consumption of 0.03±0.001 $mgO_2g^{-1}h^{-1}$ with no significant difference in oxygen consumption ($F_{3,28}$ = 0.71; p = 0.53) over the 3-h period (Fig. 4.4).

Juvenile *L. lithognathus* showed the highest coefficient of variations (CV) in oxygen consumption between and within measurement periods in the 25‰ treatments following the 72-h acclimation and in the 5‰ treatments following abrupt decrease from 35‰ (CV: following the 72-h acclimation at 25‰ = 42.94%; following abrupt decrease to 5‰ = 38.56%) (Fig. 4.2 and Fig. 4.5). The lowest coefficient of variations in oxygen consumption of the fish between and within measurement periods were obtained in the 35‰ treatments following the 72-h acclimation and in the 25‰ treatments following abrupt decrease from 35‰ (CV: following the 72-h acclimation at 35‰ = 32.62%; at 25‰ following abrupt decrease from 35‰ = 34.20%) (Fig. 4.1 and Fig. 4.3). A comparison of treatment means showed that the average oxygen consumption of fish kept at 5‰ independent of the speed at which salinity changed was significantly lower than in those fish kept at 25 ‰ and 35 ‰ (Two-way within-subject analysis of variance followed by Tukey's post-hoc multiple range test, $p<0.05$).

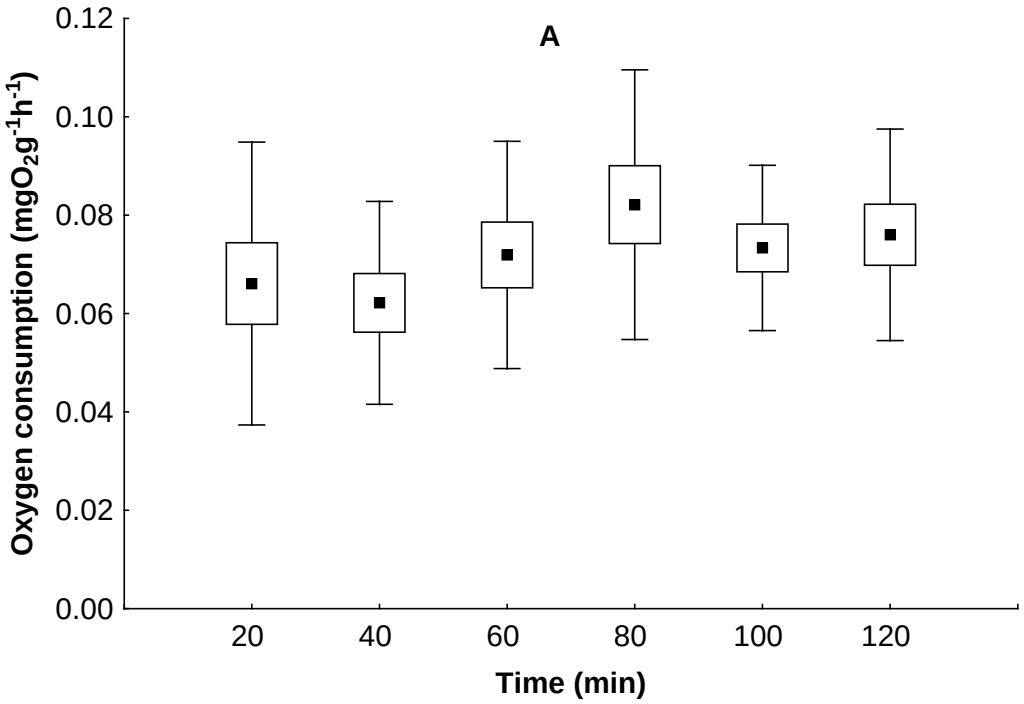

Figure 4.1: Oxygen consumption ($mgO_2g^{-1}h^{-1}$) of juvenile *L. lithognathus* at 35‰ following a 72-h acclimation. Each datum is the mean of 12 fish and the vertical bars are standard errors.

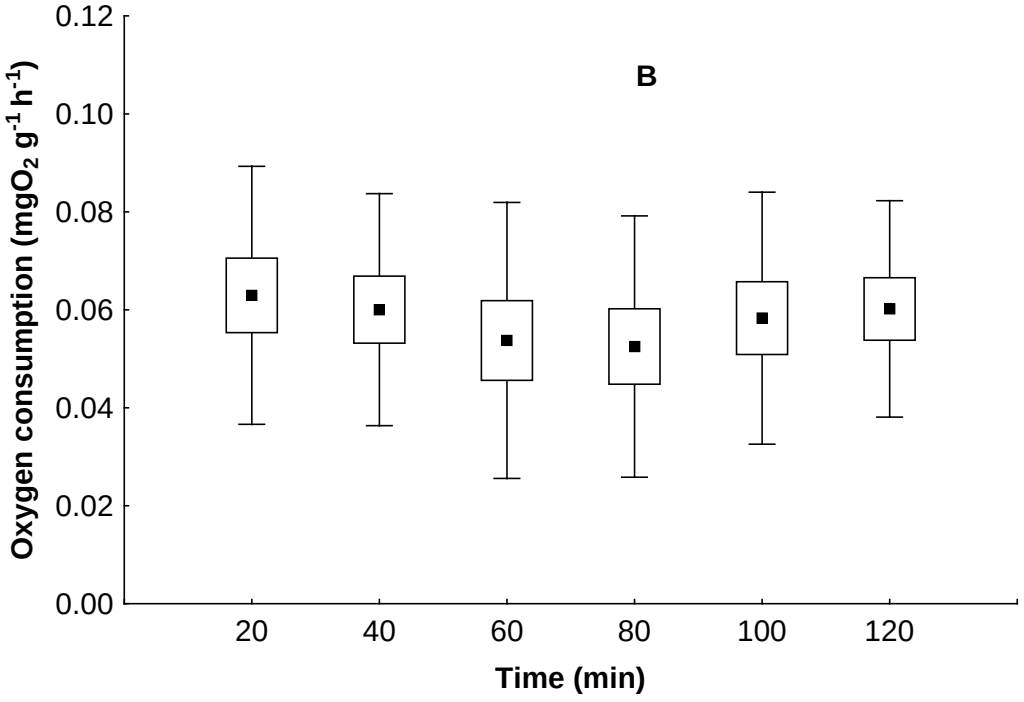

Figure 4.2: Oxygen consumption ($mgO_2g^{-1}h^{-1}$) of juvenile *L. lithognathus* at 25‰ following a 72-h acclimation. Each datum is the mean of 12 fish and the vertical bars are standard errors.

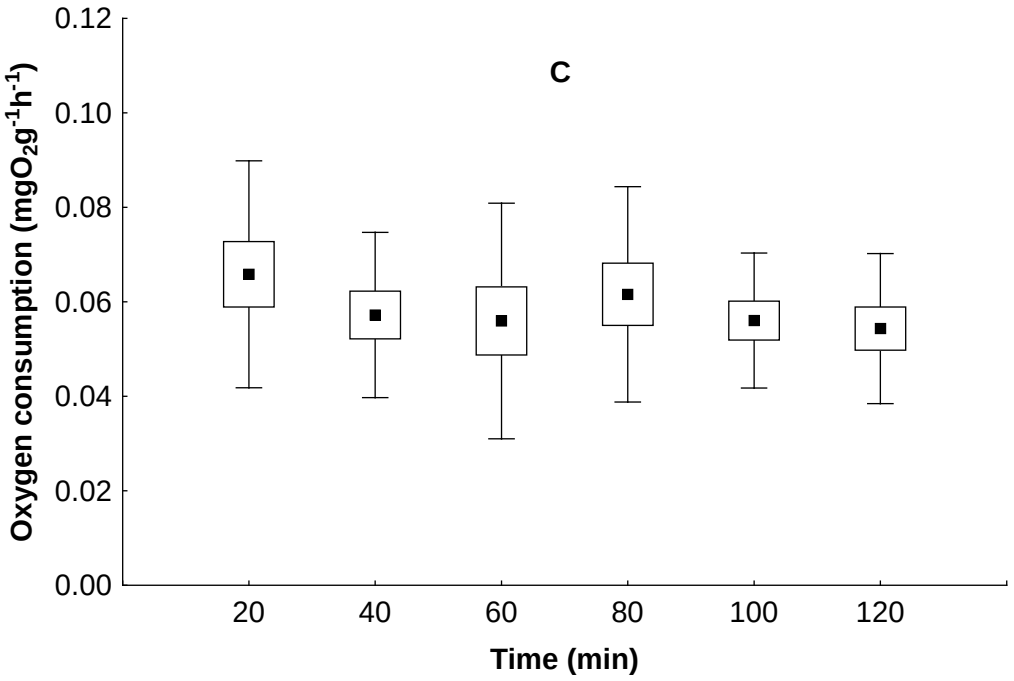

Figure 4.3: Oxygen consumption ($mgO_2g^{-1}h^{-1}$) of juvenile *L. lithognathus* at 25‰ following abrupt decrease from 35‰. Each datum is the mean of 12 fish and the vertical bars are standard errors.

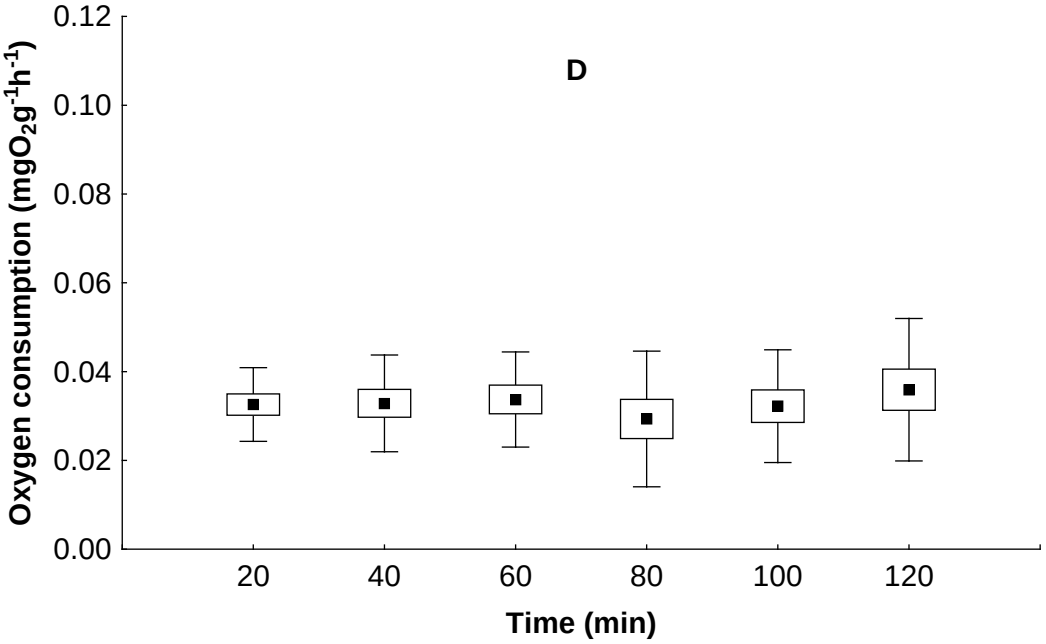

Figure 4.4: Oxygen consumption ($mgO_2g^{-1}h^{-1}$) of juvenile *L. lithognathus* at 5‰ following a 72-h acclimation. Each datum is the mean of 12 fish and the vertical bars are standard errors.

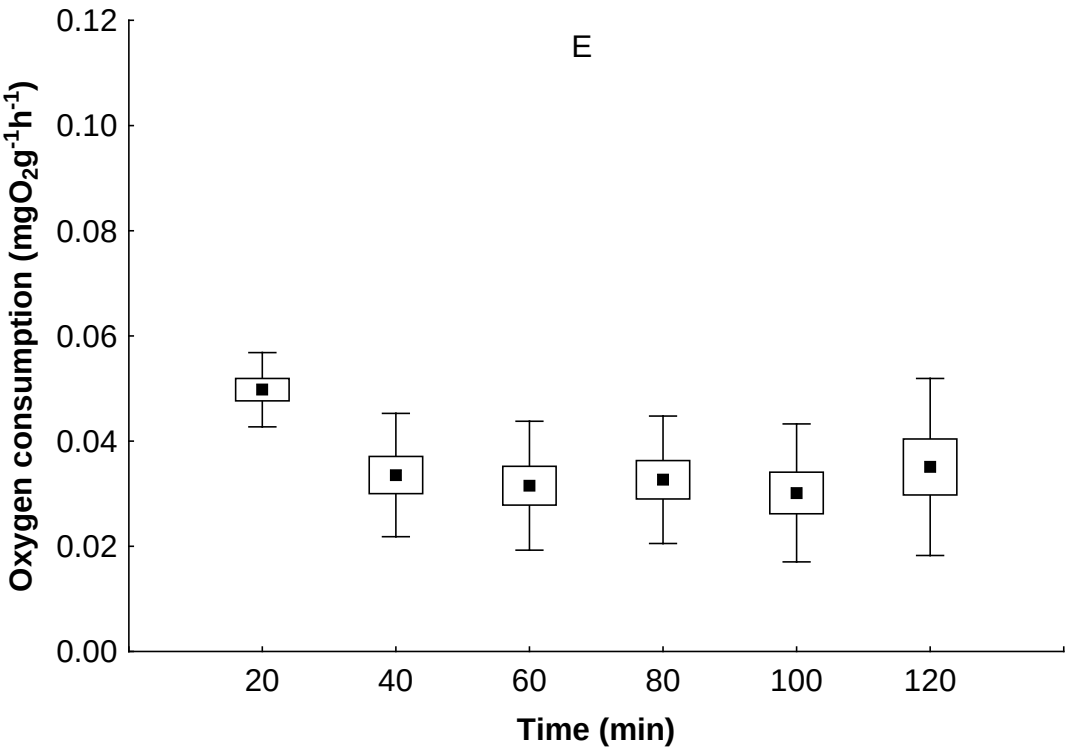

Figure 4.5: Oxygen consumption ($mgO_2g^{-1}h^{-1}$) of juvenile *L. lithognathus* at 5‰ following abrupt decrease from 35‰. Each datum is the mean of 12 fish and the vertical bars are standard errors.

Discussion

There were no mortalities in juvenile *L. lithognathus* following abrupt or gradual exposure to the selected salinities. The juvenile phase of the life cycle of *L. lithognathus* is considered to be entirely dependent on estuarine habitats for its first year of life (Wallace *et al*., 1984). The occurrence of this species has also been recorded from predominantly freshwater lakes which had become cut off from their estuarine water sources for considerable time (Mehl, 1973) and has been reported to occur at salinities of 0.001‰ (Whitfield *et al.*, 1981; Marais, 1982), while there was mortality when salinities remained below 0.003‰ for prolonged periods (Bennett, 1985). The ability to maintain the experimental fish over a wide salinity range (5-35‰) in the present study without mortality supports

findings about their salinity tolerance. The salinity tolerance of juvenile *L. lithognathus* in the present study is comparable to data obtained by Kelly and Woo (1999) who reported no mortality of silver sea bream, *Sparus sarba,* following direct exposure to a salinity of 6‰. This is also consistent with the observations on osmoregulatory capacity of other species of sea bream (Woo and Wu, 1982; Mancera *et al*., 1993) and some species of marine fish (Dutil *et al*., 1992; Munro *et al*., 1994). Direct exposure of Atlantic cod, *Gadus morhua* to salinities below 7‰ resulted in increased mortality (Provencher *et al.,* 1993) but gradual decreases in salinity over several months caused mortality only at salinities below 3‰ (Odense *et al*., 1966).

Osmoregulation in fish has been estimated to consume between 25 and 50% of total metabolic energy (Febry and Lutz, 1987; Morgan and Iwama, 1991). This seems to be what has occurred to juvenile *L. lithognathus,* as the speed of salinity change may have affected energy utilization. Based on the routine metabolic rate, defined as oxygen consumed by a fish whose activity is spontaneous (Spark *et al*., 2003), oxygen consumption of juvenile *L. lithognathus* at 5‰ following abrupt salinity decrease from 35‰ was influenced by the combined effect of time passed since salinity change and salinity. In this study fish generally displayed a significantly reduced rate in oxygen consumption at the lower salinity of 5‰. Once juvenile *L. lithognathus* at 35‰ had been exposed to the salinity of 5‰ for longer than 20 minutes their metabolic rate was significantly lower than at any of the other two salinities tested.

The lowest metabolic rate in response to salinity is associated with the environments in which the species are most commonly found, and presumably most physiologically adapted to for a particular life stage (Morgan and Iwama, 1991). Thus, the differences in oxygen consumption between a salinity

of 5‰ and the other tested salinities may be explained by the fact that juvenile *L. lithognathus* are estuarine fish (Whitfield *et al.*, 1981; Whitfield and Kok, 1992), and the experimental fish used for the present study were sampled from an estuarine environment in the salinity range of 4-17‰ (see Chapter 2). In future studies it could be tested if in juvenile *L. lithognathus*, the metabolic response to salinity is dependent on its developmental state, and if the lowest metabolic rate will be found in that environment that is natural for this particular species and its life-history stage. This physiological response has also been reported for juvenile rainbow and steelhead trout, *Onchorhynchus mykiss* and fall Chinook salmon, *Onchorhynchus tshawytscha* (Morgan and Iwama, 1991, 1998).

The amount of energy devoted to osmoregulation should be lowest at isosmotic salinities, where the osmoregulatory gradient is negligible; in other salinities energy demand for osmoregulation may be proportional to the ionic gradient and ionic permeability (Febry and Lutz, 1987). On the assumption that energy demands in juvenile *L. lithognathus* were related solely to ion and osmotic regulation, the metabolic rate data in the present study suggest that the energetic cost of ion-osmotic regulation in juvenile *L. lithognathus* was the lowest at a salinity of 5‰ relative to 25 and 35‰ following a 72-h acclimation. However, the rate and magnitude of salinity decrease did not have a significant influence on energy demands for osmoregulation of juvenile *L. lithognathus* when salinity dropped to 25‰.

The mechanisms of ion-osmotic regulation are similar among fish species (Evans, 1984), and the metabolic responses to salinities depending on species, habitats, and life stages suggest that these responses are influenced by physiological processes in addition to the energy required for ion-osmotic regulation (Febry and Lutz, 1987). McCormic *et al.* (1989a), for example, reported that changes in the metabolic capacity of isolated gill and kidney tissues of Atlantic salmon in different salinities did not

account for the magnitude of changes seen in whole-body metabolism. During acclimation to different salinities, hormonal changes occur in fish which, in addition to their role in ionic regulation, may also have effects on metabolism not directly related to ion exchange processes (Forskett *et al*., 1983; McCormic *et al.* (1989b).

The higher level in metabolic rate at 35‰ following the 72-h acclimation and the decrease at 5‰ following an abrupt salinity decrease from 35‰ in this study may have been related to an increase in metabolism of juvenile *L. lithognathus* related to increasing salinity rather than the energetic cost of ion-osmotic regulation as reported by Morgan and Iwama (1991). An established method in the salmon farming industry for testing osmoregulatory capacity of salmon smolts, is the standardized seawater challenge test (Blackburn and Clarke, 1987) in which blood plasma chloride or sodium levels after 24 h in seawater are used to assess seawater tolerance. Further studies should, therefore, be designed to investigate to what extent the responses to salinity changes expressed by the oxygen consumption may have been accompanied by physiological responses other than those needed for osmoregulation. Short and long-term changes in plasma growth hormone levels, kidney and gill Na^+/K^+-ATPase activity, plasma chloride levels and muscle water content can be measured in juveniles exposed to different salinities following the salinity change protocol outlined in this study. This approach can help evaluate possible relationships between the various responses to better understand the costs of osmoregulation in this and in other species. Moreover, it should be evaluated if juvenile *L. lithognathus* will have a lower rate of oxygen consumption in an iso-osmotic environment, a condition reported to have the lowest metabolic demand (Febry and Lutz, 1987, Jobling, 1994), than in lower and higher salinities.

The results demonstrate that juvenile *L. lithognathus* had a high osmoregulatory capacity. This is in agreement with Bennett (1989a), who reported that the species is found in estuaries at reduced salinities. Fishes use flexible strategies for the allocation of energy for maintenance, activity and growth in response to changes in both environmental conditions and physiological status (Wieser and Medgyesy, 1991). The cost of osmoregulation could increase as the activity of fish increases and its effects would be different in different salinities (Perez-Pinzon and Lutz, 1991). Swanson (1998) observed a reduction in oxygen consumption at 55‰ salinity relative to 35‰ in milkfish, *Chanos chanos*, and attributed it to a reduction in activity level, allowing for greater use of metabolic energy for osmoregulation. When oxygen consumption of sheepshead minnow, *Cyprinodon variegatus*, decreased nearly 33% over a range of salinities from 40 to 100‰, Haney and Nordlie (1997) suggested that the drop was related to a change in the permeability of the branchial epithelium, as described by Kultz and Onken (1993). Such a reduction in branchial permeability could have resulted in less oxygen diffusion into the animal. These hypotheses are not mutually exclusive, and it should be tested if both physiological events play a role in the change of oxygen consumption in juvenile *L. lithognathus* under conditions of variable salinity.

Successful movement of fish between a hypo- and hyperosmotic environment requires an ability to tolerate and/or correct disturbances in multiple physiological and metabolic functions (Madsen *et al*., 1996). Transient elevation in plasma cortisol after abrupt transfer is a phenomenon shown to occur in fish species regardless of transfer direction (Ishioka, 1980; Madsen *et al*., 1994). Cortisol, a principal corticosteoid in teleost fishes is associated with the mobilization of energy reserves for increased metabolic requirements during environmental stresses (Wendelaar Bonga, 1997). Elevated cortisol levels have been linked to higher rates of oxygen consumption in fresh water fish (Barton and Schreck,

1987). Some of the increases in oxygen consumption rate in juvenile salmonid parr in seawater were associated with the glucocorticoid effects of cortisol, in addition to energy required to maintain ionic and osmotic homeostasis (Morgan and Iwama, 1991, 1996). Given this relationship between cortisol and oxygen consumption in fish, it should be tested to what degree cortisol affected the different patterns in oxygen consumption of juvenile *L. lithognathus* observed under various salinities in this study. Therefore, both metabolic rate and cortisol measurements could be used in future experiments to complement each other as indicators of the costs of osmoregulation in juvenile *L. lithognathus*.

This study contributes to the understanding of the salinity tolerance of juvenile *L. lithognathus* following exposure to salinity levels from 5-35‰ and two rates of change. The rate of the reduction in ambient salinity may affect the cost of osmoregulation in this species over time. In an aquaculture context, this observation may be transformed into practical application as juvenile *L. lithognathus* could be selected for farming in estuaries and lagoons, regions in which salinity is usually lower than in the open sea (Saillant *et al*., 2003) and are subjected to greater variation in salinity (Wallace *et al*., 1984). Moreover, short-term freshwater exposure benefits sea lice-infected fish (Wagner *et al.*, 2004) but it is not clear how stressful this may be to marine fish. Studying the metabolic response of juvenile *L. lithognathus* to reduced salinities may, therefore, reveal if this method can be used to treat fish diseases in this species. In conclusion, findings of the present study provide a good starting point to add other studies of behaviour, physiology and biochemistry of osmoregulation in juvenile *L. lithognathus* using growth studies at different salinity levels.

CHAPTER 5

The effect of salinity on growth of juvenile *L. lithognathus*

Introduction

Salinity and growth

Growth of fish depends on consumption of feed and its assimilation and conversion into body tissues (Brett and Groves, 1979; Burel *et al*., 1996). However, growth and food intake are controlled by internal factors involving the central nervous system, endocrine system, and neuroendocrine system, and also by environmental factors such as, for example, temperature, photoperiod, salinity, oxygen level, ammonia and pH. Salinity, like other environmental factors specific to aquatic habitats, has prompted many studies on its influence on fish growth (Rubia *et al*., 2005). Most studies showed that water salinity affects the growth of both marine (Tandler *et al*., 1995; Peterson *et al*., 1999; Imsland *et al*., 2001) and freshwater species (Arunachalam and Reddy, 1979; Lilongwe *et al*., 1996); and there appear to be few species whose growth is not affected by salinity (Saunders and Henderson, 1970; Buckel and Steinberg *et al*., 1995). Salinity can modify a number of aspects related to growth including metabolic rate (Nordlie *et al*., 1991; Woo and Kelly, 1995), (b) total food intake (Dendrinos, and Thorpe, 1985; Imsland *et al*., 2001), feed conversion efficiency, (Alava, 1998; Imsland *et al*., 2001), and the balance of hormones involved in metabolism (Handeland *et al*., 2000, Boeuf and Payan, 2001).

It has been proposed that the energetic cost for homeostasis (i.e. ionic and osmotic regulation) is reduced in iso-osmotic environments and that saved energy translates into growth (Gaumet *et al*.,

1995; Woo and Kelly; 1995, Dutil *et al.*, 1997; Boeuf and Payan, 2001, Imsland, 2001). A review by Boeuf and Payan (2001) attributed the growth enhancement at iso-osmotic salinities in stenohaline and euryhaline fish species to several causes, i.e., reduction of energetic costs for ionic and osmotic regulation, increased food intake, food conversion and stimulation of hormones supporting growth. Foss *et al*. (2001) working on spotted wolffish, *Anarhichas minor*, did not report any effect of salinity on feeding rate, food conversion or protein efficiency ratio, although indications of enhanced growth at iso-osmotic salinity were discussed. Reports of a slower intestinal transit of nutrients at the intermediate salinities in the Atlantic wolffish, *Anarhichas lupus*, (Francois *et al*., 2004) could suggest, as supported by Ferraris *et al*. (1986), a prolonged digestion time for nutrient absorption that could translate into better digestibility and increased energy available for somatic growth. Marine species such as Atlantic cod, *Gadus morhua*, and turbot, *Scophthalmus maximus*, have also been shown to exhibit increased growth rates (cod), food conversion ratios (cod), and food intake (turbot) when cultured at intermediate salinities of 12-19‰ (Lambert *et al*., 1994; Gaumet *et al*., 1995; Imsland *et al.*, 2001). However, cobia, *Rachycentron canadum*, reared at a salinity of 5‰ grew as well or better than conspecifics reared at 15 and 30‰ (Resley *et al*., 2006). Likewise, a range of salinity. i.e., 0.1, 10 and 20‰ did not influence growth of Atlantic salmon, *Salmo salar* (Shaw *et al*., 1975).

Oxygen consumption, salinity and growth

Oxygen consumption is often taken as a measure of metabolic expenditure during osmoregulation (Farmer, 1969), and can be determined as a function of salinity (Nordlie, 1978; Claireaux and Lagardere, 1999; Wuenschel, 2004). It has been hypothesised that if the fish's environment ensures a reduction of metabolic costs of ion-osmoregulation growth and food utilisation of fish would be improved (Jobling, 1994). Despite better growth and food conversion at intermediate salinity in

juvenile cod, *G. morhua*, Dutil *et al.* (1997) reported similar standard metabolic rates and protein digestibilities for a range of experimental salinities. However, the highest growth rate, associated with high food intake in juvenile fat-snook, *Centropomus parallelus*, acclimated at the salinity of 20‰ for a 15-day period coincided with the lowest metabolic energy demand (Da Silva Rocha *et al*., 2005). Cardona (2000) showed a better growth performance of juvenile flathead grey mullet *Mugil cephalus* associated with a low metabolic rate.

The best salinity for rearing juvenile *L. lithognathus* has not been defined. In this study it was hypothesised that energy saved at a favourable salinity during osmoregulatory processes may be available to increase somatic growth. Thus, in the present study it was assumed that growth performance of juvenile *L. lithognathus* could be enhanced in salinities exhibiting the lowest level of metabolic expenditure. The objective of the study was, therefore, to evaluate the effect of water salinity on growth of juvenile *L. lithognathus* during two 56-day growth trials. The first trial tested growth of juvenile *L. lithognathus* at salinities of 5, 25 and 35‰. In the second test salinities of 5, 10 (approximate iso-osmotic salinity) and 35‰ were investigated.

Materials and Methods

Acclimation of fish, system maintenance and experimental procedures

Two 56-day growth studies were carried out to evaluate the effects of salinity on growth of juvenile *L. lithognathus*. Fish were obtained by beach seine netting from The Great Fish River estuary between July 2004 and November 2005. The fish were either acclimated to captive conditions for at least 60 days at the field station of the Department of Ichthyology and Fisheries Science (DIFS) in Port Alfred,

or transported to the laboratory in Grahamstown where acclimation to captive conditions commenced. At the field station, juvenile *L. lithognathus* were kept under a natural light and temperature regimen in two 300-L rectangular tanks supplied with seawater at a salinity of 35‰. Fish were initially fed on finely chopped *Sardinops* spp. before being moved to the Grahamstown laboratory. At the Grahamstown laboratory, juvenile *L. lithognathus* were kept in four 300-L holding tanks within a 2200-L seawater (35‰) recirculating system and acclimated to the laboratory conditions for at least forty-five days. They were weaned onto semi-dry commercial trout pellets, and for the duration of the acclimation period, fish were fed to satiation twice per day. The nutritional composition of the feed pellets is described in Chapter 2. Prior to the weighing and stocking of the tanks, the experimental fish were not fed for 24 h to ensure evacuation of the gut. Individual juvenile *L. lithognathus* were anaesthetised in a 0.02% solution of 2-phenoxyethanol in a 10-L glass tank filled with 3-L of water from the recirculating system. Each fish was blot-dried, weighed (to the nearest 0.01g) and total length was measured to the nearest mm. The fish were size-selected for each growth tank to assure similar mean weights in all tanks. Salinity was gradually lowered from 35‰ to 5, 10 and 25‰ by replacing ~ 10% of seawater in the growth tanks with fresh water per hour until the desired salinity was reached. All growth estimates were based on the biomass in each tank estimated from the average fish weight. This was done to avoid the bias caused by pseudoreplication, i.e., using the data from fish belonging to the same tank. Freshwater was added to the tanks when required to adjust for evaporation, to maintain treatment salinities and to keep unionized ammonia (NH_3) concentrations at or below 0.1 $mg.L^{-1}$. Water temperature in each tank was controlled using an automatic aquarium heater (*Resun*, 150W), and aeration was supplied to each tank through airstones. A 12L:12D photoperiod was maintained throughout the acclimation period and during the experiments.

Growth experiment 1

Three salinity treatments, 5, 25‰ and 35‰, with four replicates each were allocated randomly to twelve 90-L glass tanks to study growth of juvenile *L. lithognathus*. Due to efforts to arrive at the same biomass per tank and the number of fish available, each tank was stocked with eight to nine fish from the holding tanks. Each tank was a closed re-circulating system operated with an airlift-driven biological filter (Fig. 5.1). Biological filters were filled with a combination of shredded plastic, granular activated charcoal and oyster shells. The tanks were partially covered with black plastic sheets to minimize disturbance of the experimental fish. Fish were fed to satiation twice per day on commercial pellets (pellet size = 2 mm) supplied by AQUANUTRO, Cape Town. The pellets consisted of protein (480 g..kg^{-1}), moisture (120 $g.kg^{-1}$), lipid (120 $g.kg^{-1}$), fibre (40 $g.kg^{-1}$), phosphorous (7 $g.kg^{-1}$) and calcium (30 $g.kg^{-1}$). Satiation was defined as the stage when the fish no longer accepted single pellets dropped into the tanks. Uneaten pellets from each tank were collected and counted within 30 min after feeding to determine the amount of feed consumed. To determine the weight of uneaten pellets, the average weight of dry pellets had been determined from 100 pellets to the nearest milligram. Daily food consumption (in g) by fish in each tank was recorded as the difference in weight of a feed container before and after feeding, minus the estimated weight of uneaten pellets. Average water temperatures for treatment salinities 5, 25 and 35**‰** were 19.8±0.05, 20.2±0.07 and 20.0±0.08 ºC, respectively, while unionized ammonia for the same treatments were 0.1±0.02, 0.1±0.04 and 0.1±0.01 $mg.L^{-1}$, respectively.

Growth experiment 2

This experiment was conducted using three salinity treatments, i.e., 5, 10 and 35‰. Nine 300-L opaque plastic tanks were used for this study. Three replicate recirculating tanks per treatment, each

comprising a trickle-filter with shredded plastic material and oyster shells were used (Fig. 5.2). Water flow through the recirculating system was maintained by a submersible pump (SP-2500, 18W). Each tank was stocked with eight to nine fish. Initial attempts into feeding the experimental fish indicated that the fish were not feeding readily on the feed pellets of the size used in experiment 1 because uneaten pellets accumulated at the bottom of the tanks within 30 minutes after feeding. Subsequently, juvenile *L. lithognathus* were fed a finer commercial trout pellet (pellet size = 1 mm). Due to the small size of the pellets in this experiment, counts of the number of uneaten pellets after feeding was not considered accurate and, based on the results from experiment 1, fish were fed at feeding intensity of 3% body mass daily throughout the experiment. The ration size for each tank was increased every two weeks based on the average weight of the fish at the end of a growth period. By day 42 there were mortalities across the tanks of the 5‰ treatment, and this treatment was discontinued. The fish in the 10 and 35‰ salinity treatments were reared for the entire 56-day period. Average water temperatures for treatment salinities 5, 10 and 35‰ were 19.5±0.03, 19.2±0.02 and 19.9±0.03 ºC, respectively, and unionized ammonia for the same treatments were 0.1±0.04, 0.04±0.05 and 0.1±0.01 $mg.L^{-1}$, respectively.

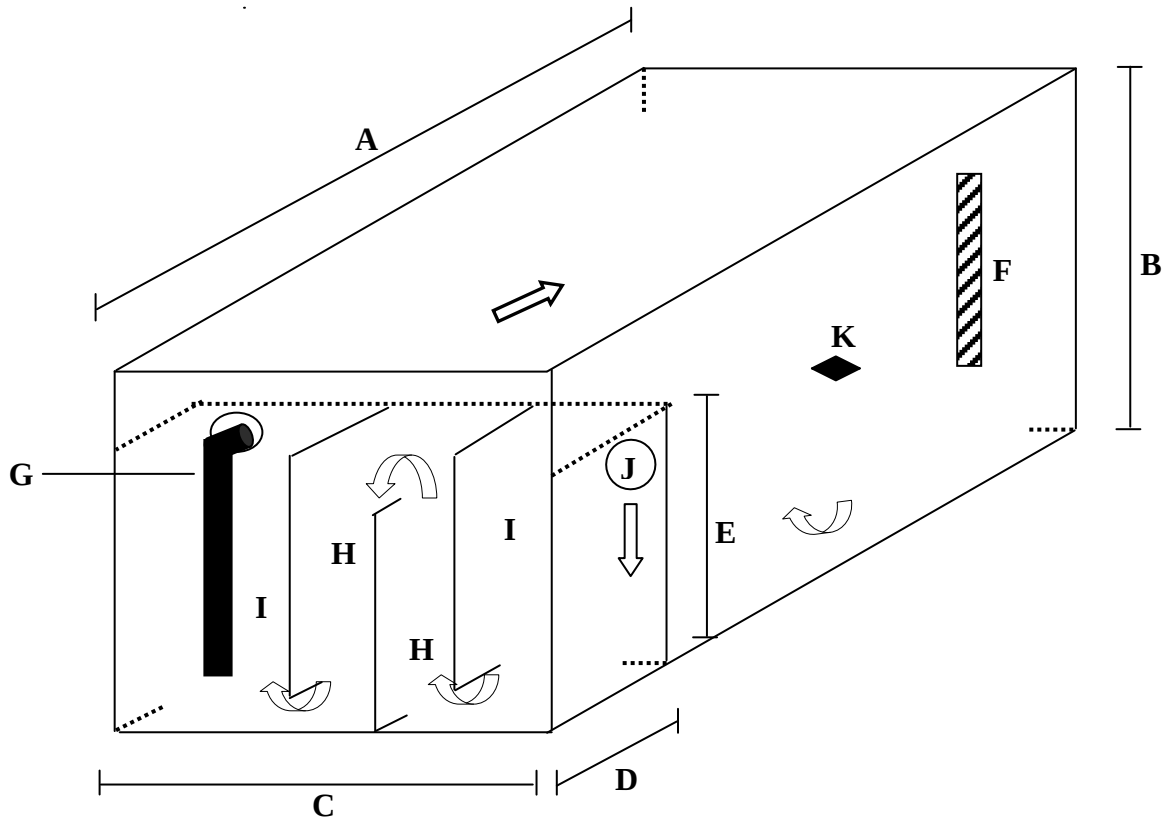

Figure 5.1: The biological filter partitioning in a fish tank used for the rearing of juvenile *L. lithognathus* (Growth experiment 1). Arrows show direction of water flow.

A: Length of fish tank (900 mm), B: Height of fish tank (320 mm), C: Width of biological filter and fish tank (313 mm), D: Length of biological filter (200 mm), E: Height of biological filter (280 mm), F: Submersible heater (*Resun*, 150W), G: Air-lift driven outflow from biological filter, H: Biological filter partitions (filled with oyster shells), I: Biological filter partitions (filled with shredded plastic materials), J: Gravity-fed inflow into biological filters, K: Airstone

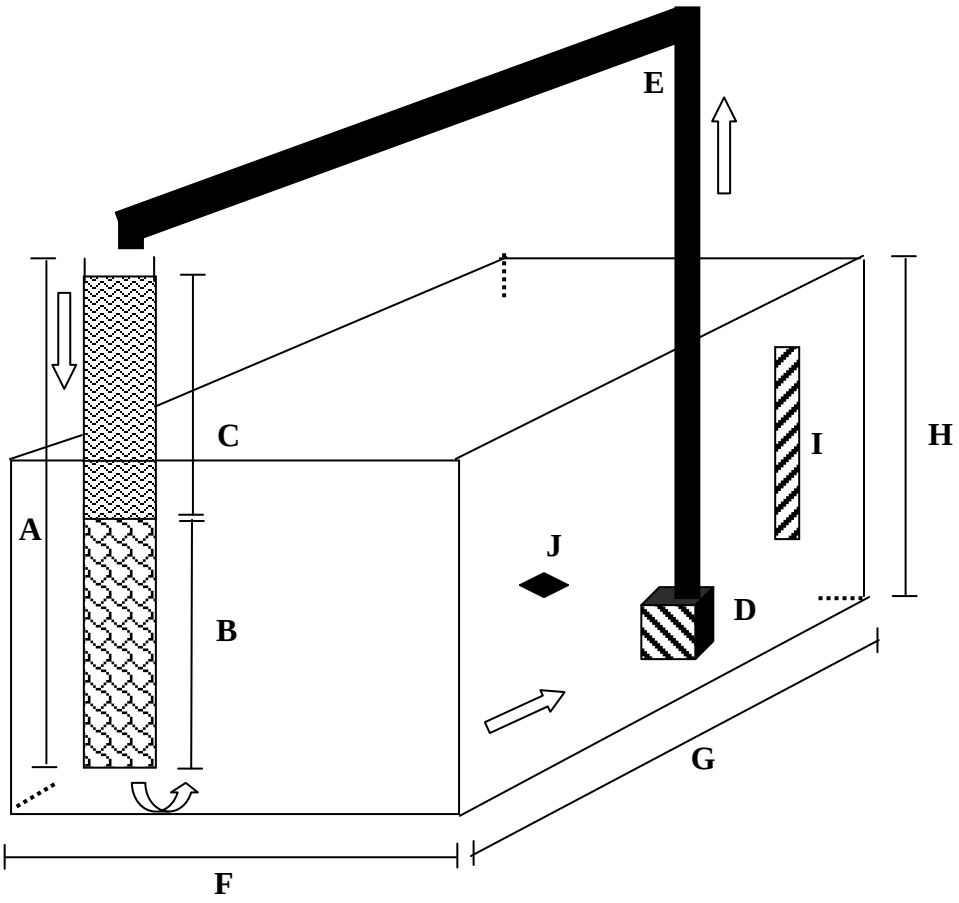

Figure 5.2: Fish tank with trickle filters used for the rearing of juvenile *L. lithognathus* (Growth experiment 2). Arrows show direction of water flow.

A: Height of trickle filter (1200 mm), B: Height of oyster shells in trickle filter (600 mm), C. Height of shredded plastics in trickle filter (600 mm), D: Pump (SP-2500, 18W), E: Pipe carrying water to trickle filter, F: Width of fish tank (530 mm), G: Length of fish tank (910 mm), H: Height of fish tank (640 mm), I: Submersible heater (*Resun*, 150W), J: Airstone, Diameter of trickle filter, 140 mm.

Data analysis and statistics

Specific growth rate (SGR) of the fish was calculated as follows: $SGR = ((W_e/W_o))^{1/d-1}-1]\times100$, where SGR is the percentage daily growth rate, W_e is final average weight (g), W_o is average weight (g) of the fish at the beginning of a growth period and d is the number of days between two weighing events. Feed conversion ratio (FCR) was calculated as the relationship between wet weight gain of the fish in a tank and the mass of pellets consumed in that tank (Da Silva Rocha, *et al*., 2005). Feeding intensity (FI) in percentage of body weight per day was determined by multiplying the feed conversion ratio with the percentage daily growth rate (SGR). Weight and length values of fish in a tank were averaged before being used in the analysis. One-way analysis of variance (one-way ANOVA) was used to detect the differences in the mean values of weight gain, SGR, FCR and FI between and within treatments at a Type 1 error level of $p \leq 0.05$.

Results

Experiment 1

Mean initial weight of juvenile *L. lithognathus* did not differ significantly ($F_{2, 9} = 2.81$; $p = 0.11$) between treatments (Fig. 5.3 and Table 5.1). Analysis of variance (ANOVA) showed no significant differences in the final body weight and total length of the fish between replicates within each salinity treatment at the end of the 56-day rearing period. The data did not show a significant difference in the mean weight and total length between salinity treatments at the end of the 56-day rearing period (weight: $F_{2, 8} = 2.24$; $p = 0.17$, total length: $F_{2, 8} = 2.78$; $p = 0.12$) (Table 5.1 and Fig. 5.3). Survival was 100% in all treatments for the first 42 days of the rearing period. At the end of the growth study, survival was 100% in the 25‰ and 35‰ treatments while all fish died in one of the four replicates of

the 5‰ treatment. The remaining fish readily consumed the feed from the start to the end of the experiment. Average feeding intensity ranged from 2.22±0.06% at 5‰ to 2.12±0.08% of wet body weight per day at 35‰ with no significant difference ($F_{2, 9}$ = 0.45; p = 0.65) between the treatments (Table 5.2). Percentage weight gain of fish did not differ significantly between the salinity treatments ($F_{2, 8}$ = 0.90; p = 0.44). Average feed conversion ratio ranged from 2.22±0.057 in fish reared at 5‰ to 2.12±0.084 in fish at 35‰ but these values did not differ significantly between the different treatments ($F_{2, 9}$ = 1.21; p = 0.34) (Table 5.2).

Table 5.1: Initial and final body weight (g), final total length (mm) and weight gain (%) of juvenile *L. lithognathus* reared at three salinities for 56 days. Data are means±standard errors of the mean.

Salinity (‰)	Mean initial weight (g)	Mean final weight (g)	Final total length (cm)	Weight gain (%)
5	11.59±0.69	22.73±1.40	11.88±0.22	98.13±8.83
25	11.93±0.65	24.54±1.47	12.25±0.21	106±10.97
35	10.99±0.63	22.10±1.44	11.76±0.23	101.54±6.38

Table 5.2: Means ± standard errors of the mean of feeding intensity, food conversion ratio (FCR) and specific growth rate (SGR) of juvenile *L. lithognathus* reared at three salinities for 56 days.

Salinity (‰)	Feeding intensity (% body weight/day)	Food conversion ratio	Specific growth rate (%/day)
5	2.22±0.057	1.88±0.15	1.20±0.074
25	2.15±0.091	1.64±0.08	1.32±0.096
35	2.12±0.084	1.67±0.11	1.28±0.057

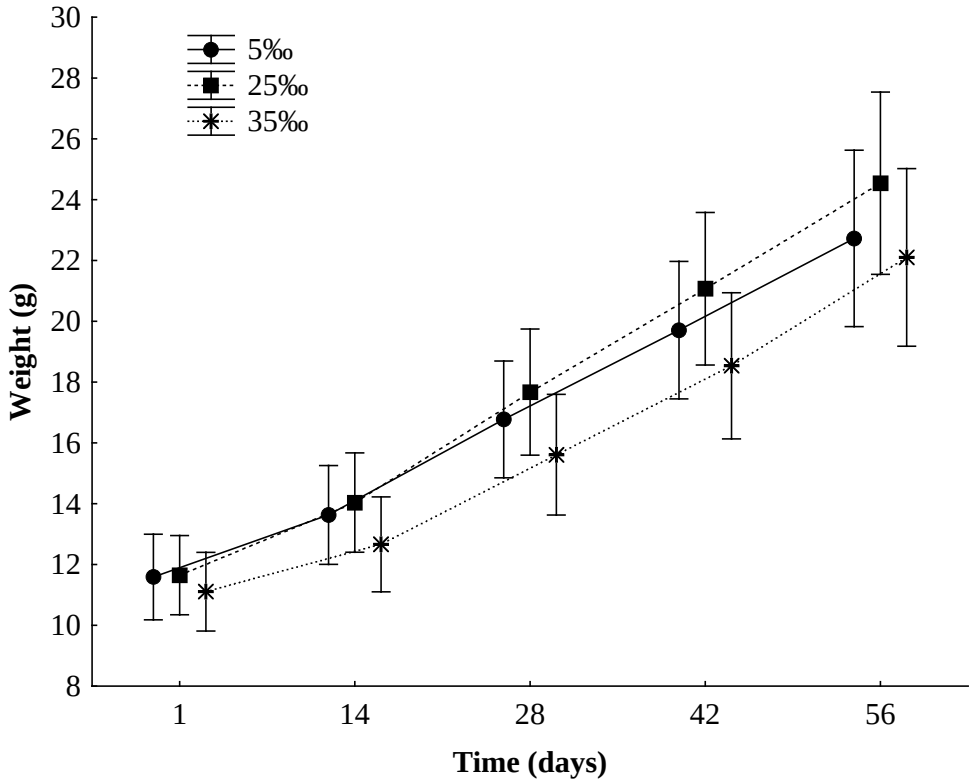

Figure 5.3: Means plot of change in average wet weight (g) of juvenile *L. lithognathus* reared in different salinities over 56 days. The vertical bars represent the 95% confidence intervals.

Experiment 2

Mean initial weight of fish did not differ significantly between the three salinity treatments ($F_{2, 6}$ = 1.43; p = 0.31) (Fig. 5.4 and Table 5.3). Average values for all replicates of all treatments were used for the analysis. Following the 28-day rearing period, no significant difference ($F_{2, 6}$ = 0.18; p = 0.84) in the mean weight was observed between salinity treatments (Table 5.3). Survival was 100% in all treatments during the first 28 days of rearing. Between the fifth and sixth week, eight fish died in the three 5‰ replicates, accounting for approximately 30% of the experimental fish in that treatment, while survival in the 10‰ and 35‰ treatments remained at 100% until the end of the 56-day rearing period. The rearing of fish at 5‰ was, therefore, discontinued. Data from the 28-day rearing period did not show a significant difference in weight gain ($F_{2, 6}$ = 0.12; p = 0.89) of juvenile *L. lithognathus* between the three treatments (Table 5.3 and Fig. 5.2). During that period specific growth rate of juvenile *L. lithognathus* ranged from 0.90±0.077 for fish in 10‰ to 0.82±0.054 for fish in 35‰ with no significant difference between the three treatments ($F_{2, 6}$ = 0.13 p = 0.88) (Table 5.4). There was also no significant effect of salinity on food conversion ratio of juvenile *L. lithognathus* over 28 days ($F_{2, 6}$ = 1.11; p = 0.39). At the end of the 56-day rearing period, fish in the 10‰ and 35‰ treatments did not show any significant difference in percentage weight gain ($F_{1, 4}$ = 0.10; p = 0.77), specific growth rate ($F_{1, 4}$ = 0.72; p = 0.80) and food conversion ratio ($F_{1, 4}$ = 0.0043; p = 0.95) (Table 5.4 and Fig. 5.4).

Table 5.3: Initial and final body weight (g), final total length (mm) and weight gain (%) of juvenile *L. lithognathus* reared under three salinities for 28 days. Data are means±standard errors of the mean.

Salinity (‰)	Mean initial weight (g/fish)	Mean final weight (g)	Final total length (cm)	Weight gain (%)
5	3.63±0.42	4.68±0.52	7.03±0.24	25.38±4.10
10	3.70±0.41	4.60±0.50	7.12±0.23	27.57±4.10
35	3.77±0.43	4.64±0.52	7.06±0.24	24.85±4.10

Table 5.4: Means±standard errors of the mean of food conversion ratio (FCR) and specific growth rate (SGR) of juvenile *L. lithognathus* reared at three salinities for 28 and 56 days, respectively.

Salinity (‰)	Rearing period (days)	Food conversion ratio	Specific growth rate (%/day)
5	28	3.35±2.20	0.83±0.19
10	28	2.81±0.26	0.90±0.077
35	28	3.27±0.27	0.82±0.054
10	56	2.40±0.28	1.00±0.095
35	56	2.38±0.067	0.98±0.031

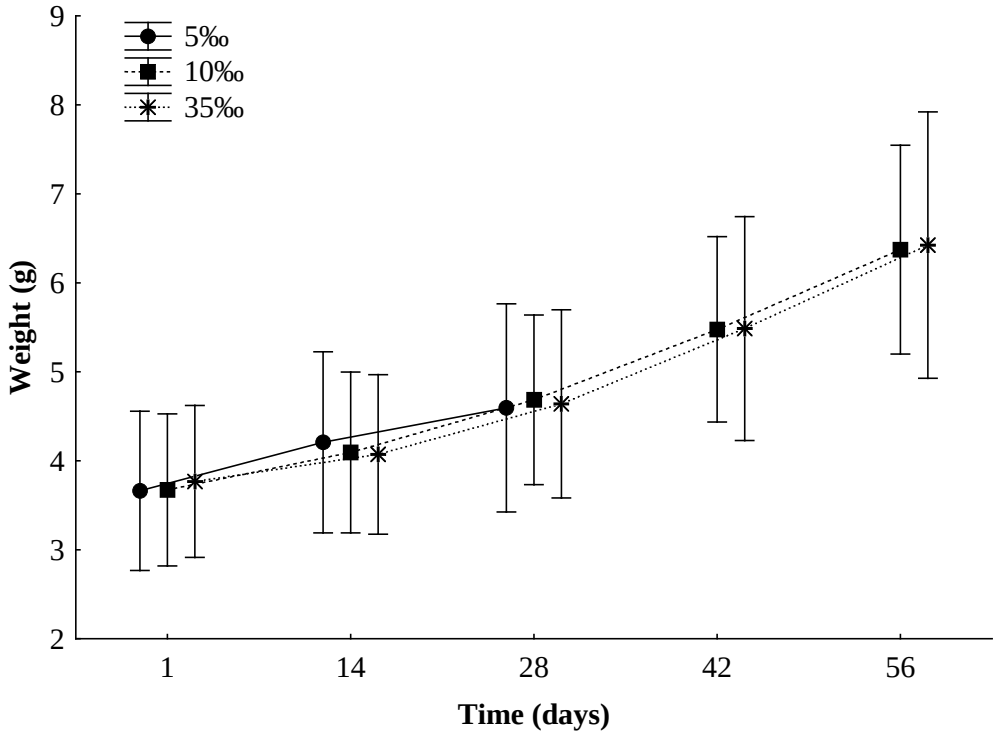

Figure 5.4: Means plot of change in average wet weight (g) of juvenile *L. lithognathus* reared at different salinities for 56 days. The 5‰ values are excluded from day 28 onwards as a result of mortality. The vertical bars represent the 95% confidence intervals.

Discussion

Salinity tolerance and growth

In this study isosmotic salinity (10‰ ± 2) was selected because several studies (Canagaratnam, 1959, Otto, 1971, De Silva and Perera, 1976, Gaumet *et al*., 1995, Dutil *et al*., 1997, Boeuf and Payan, 2001, Laiz-Carrion, 2005) reported growth enhancements at isosmotic salinity in stenohaline and euryhaline fish species. Disturbance of plasma osmolality suggests disruption of the Na^- and K^+ homeostatic balance which precedes metabolic disfunction and ultimately results in mortality (Woo and Fung, 1981). Juvenile *L. lithognathus* tolerated a wide salinity range at 20C. Survival was high in both

experiments with the exception of the fish in the 5‰ treatment in experiment 2 following 28 days into the study. Despite mortalities in both experiments at 5‰, no significant difference in weight gain and specific growth rate was observed between the salinity treatments throughout the 56-day rearing period of experiment 1 or during the 28-day rearing period in experiment 2. One possible explanation for these mortalities in the 5‰ treatments, although not examined in the present study, could be that juvenile *L. lithognathus* are more susceptible to disease or increased virulence of parasites at a lower salinity. Gilthead seabream, *Sparus auratus*, cultured at a salinity of 6‰ had a significantly reduced immune responses such as peroxidase content and complement activity compared to fish reared at higher salinities (Cuesta *et al*., 2005). Resley *et al*. (2006) reported that juvenile cobia, *Rachycentron canadum*, reared at salinities of 5 or 15‰ could grow equally well as fish reared at 30‰ despite the incidence of higher mortalities at 5‰. The authors identified a coccidian parasite infection and noted a lack of mucus on the skin in fish reared at 5‰ relative to fish reared at 15 and 35‰. This may be caused by a lowered resistance to opportunistic pathogens. However, Deacon and Hecht (1999) reported significantly lower survival and growth rate of juvenile spotted grunter, *Pomadasys commersonnii*, a member of the marine sparids utilising estuarine environments during the juvenile stage of their life history, at 5‰ relative to 12, 25 and 35‰. It was assumed that these effects were not caused by physiological dysfunction, i.e. disruption of the homeostatic balance. The authors suggested other causes but did not measure or quantify them.

Many juvenile fish species choose intermediary water salinities of estuaries and coastal lagoons where they find advantageous conditions for their growth (Da Silva Rocha *et al*., 2005). Although it has been hypothesised that maximum growth should occur in an iso-osmotic environment of 10±2‰ due to a low osmoregulatory energy demand (Brett, 1979), interspecific differences in salinity optima have

been documented. The Arctic cisco, *Coregonus autumnalis*, showed no significant difference in growth between 6 and 30‰ (Fechhelm *et al*., 1993). The euryhaline cichlid, *Oreochromis mossambicus*, and many of its hybrids are either not affected by salinity (Jauncey, 1982) or exhibit better growth above the isosmotic level (Canagaratnam, 1959). Two common coastal fishes found throughout the gulf of Mexico and along the Atlantic coast of the south-eastern U.S.A., the code goby, *Gobiosoma robustum*, and clown goby, *Microgobius gulosus*, exhibited better growth at a salinity of 5‰ than at 35‰ salinity (Schofield, 2004). Results of this study demonstrate that juvenile *L. lithognathus* tolerate a wide range of salinities and can grow well in the 5-35‰ salinity range when maintained at 20°C, while best survival can be expected above 5‰. Many studies reported by Morgan and Iwama (1991) failed to show that maximum growth rates were obtained in an isosmotic environment. In this study growth at 10‰ was not better than that at 5‰ or 25 and 35‰. In true marine fish, i.e. species that spawn at sea, growth generally increases above isosmotic salinity to a maximum at a salinity of 35‰ (De Silva and Perera, 1976; Akatsu *et al*., 1983; Dendrinos and Thorpe, 1985).

Salinity can influence both food consumption and food conversion ratio (FCR), however, results differed between species (Kilambi, 1980; Conides, 1997; Partridge and Jenkins, 2002). Growth of black bream, *Acanthopagrus butcheri*, was greatest at 24‰ due to a high food intake and the most efficient FCR in this salinity as compared to 12, 36 and 48‰ (Partridge and Jenkins, 2002). Klaoudatos and Conides (1996) obtained a similar result with gilthead sea bream, *Sparus auratus*, with the highest food intake and best food conversion efficiency resulting in optimum growth at 28‰. In contrast, optimal growth in Atlantic cod, *Gadus morhua*, at 14‰ was due only to improved food conversion efficiency, and not due to differences in food intake (Lambert *et al*., 1994). FCR of

juvenile flounder, *Platichthys flesus* L., (Gutt, 1985) and turbot, *Scophthalmus maximus* (Imsland *et al*., 2001) reached a peak at intermediate salinities (14-28‰). In the present study, FCR was not affected by tested salinities. Appetite depression at suboptimal salinities has been observed for many species including mullet, *Mugil cephalus*, (De Silva and Perera, 1976), red drum, *Sciaenops ocellata*, (Crocker *et al*., 1981) and European sea bass, *Dicentrarchus labrax*, (Dendrinos and Thorpe, 1985). However, in this study food intake expressed as daily feeding intensity, was not affected by salinity as juvenile *L. lithognathus* consumed similar ration sizes when fed to satiation.

Behavioural and physiological adjustment

The relationship between growth and salinity is complex, as it is species-specific, and may differ between populations or life-history stages (Morgan and Iwama, 1991; Secor *et al*., 2000). For, example, Nordlie and Haney (1998) reviewed results from studies on twelve euryhaline fish species that utilise habitats from fresh water to marine waters and found that different species with similar habitat utilisation showed different combinations of physiological responses to ranges of ambient salinity. Additionally, in a review of the osmotic regulatory ability of euryhaline fishes, Nordlie (1985) concluded that the affinity of a species for fresh water, brackish and marine habitats is not directly related to their ability to maintain their plasma osmotic concentration. Acclimation to changes in environmental salinity involves several hormonal and osmoregulatory adjustments in order to re-establish ionic homeostasis (Sparks *et al*., 2003). Salinity may influence physiological mechanisms involved in growth such as the secretion of growth hormone or prolactin (Saillant *et al*., 2003). In the present study the role of these hormones could not be established. However, the following hypothesis may be offered to explain why salinity did not influence growth of juvenile *L. lithognathus*. While activity of fish may have affected osmoregulatory costs, salinity may also have affected activity in

ways that compensate for elevated maintenance costs and/or control or minimise activity-related costs of osmoregulation. This has been reported in milkfish, *Chanos chanos*, by Swanson (1998). The development of telemetric techniques has allowed activity of fish to be studied *in situ* (Lucas *et al*., 1993). One of the best indicators of activity levels are integrated electromyograms (EMG*i*'s) from axial musculature (Kaseloo *et al*., 1992). EMG*i* activity is proportional to locomotory activity and can also be correlated to oxygen consumption using respirometry (Cooke *et al*., 2000). Future studies could, therefore, be conducted to examine the difference in locomotory muscle activity in juvenile *L. lithognathus* under variable salinities using axial muscle electromyogram signals. Juvenile *L. lithognathus* could be implanted with activity (electromyogram; EMG*i*) transmitters and randomly assigned to different salinities and the relationship between the EMG*i* activity, oxygen consumption and growth in juvenile *L. lithognathus* could be evaluated in order to better understand the physiological state, activity, the associated energy metabolism and their potential impacts on growth in juvenile *L. lithognathus* under variable salinities. Video recording and infra-red sensors could also be used in the process to measure locomotor activity of experiment fish under salinities to be tested.

Other physiological processes unrelated or only indirectly related to the energetics of osmoregulation such as tissue permeability to water and ions (Rankin and Bolis, 1984), gill ventilation, perfusion, functional surface area and permeability (Jones and Randall, 1978) play an important role in salinity adaptation. Osmo-ionic adjustments of juvenile *L. lithognathus* could be evaluated after transfer of fish to different salinity treatments by measuring the physiological variables such as plasma osmolality, sodium (Na^+), chloride (Cl^-), potassium (K^+) and water content of skeletal muscle at regular intervals following the methods described by Dutil *et al.* (1992) and Audet *et al.* (1993). In addition to a transfer experiment, a growth study of the same duration and under environmental conditions similar to that of

the transfer experiment could be conducted concurrently to establish the relationship between growth and physiological response of juvenile *L. lithognathus* under different salinities.

In defining the growth response of juvenile *L. lithognathus* to different salinities, future work should, therefore, attempt to evaluate the interdependence of osmoregulation costs, behavioural compensation and physiological constraints. Salinity adaptation by euryhaline fishes involves the coordination of all these aspects and investigation of any one aspect of salinity response should take the other components into account. In mariculture context, salinity tolerance with regard to growth of juvenile *L. lithognathus* indicates adaptation to unstable estuarine environmental conditions during the juvenile phase and suggests that the rearing of juvenile *L. lithognathus* may be extended to estuarine coastal environments with potential positive effects on productivity.

CHAPTER 6

General discussion and conclusions

Estuaries and coastal areas are often suitable for fish farming because many areas are enclosed and sheltered. However, in the tropics and sub-tropics seasonal variation in water salinity may be large (Wu and Woo, 1983). The rearing of fish in these systems can expose fish to changes of salinity, thus, studies about the metabolic rates and growth of fish under conditions of fluctuating salinity levels are needed to predict their culture potential. Juvenile *Lithognathus lithognathus* seem to be able to tolerate a wide range of salinity (Whitfield *et al*., 1981). However, energetic costs associated with occupying different salinities require further study in this species. The aim of this study was to determine if the metabolic response expressed through oxygen consumption rate of juvenile *L. lithognathus* under variable salinity conditions could be used to predict growth and survival of this species under aquaculture conditions.

To address this aim, the objective of the first experiment was to evaluate diurnal oxygen consumption patterns of juvenile *L. lithognathus*. It was hypothesised that differences in oxygen consumption between photo- and scotophase could point to a change from resting metabolism to active metabolism. The different rates of metabolism of fish represent an animal's aerobic rate of converting food energy into energy needed for body maintenance and other activities (Brick *et al*., 2002). Thus, oxygen consumption rate may be viewed as a reference that can be used to measure the bioenergetic effects of salinity changes. Diurnal patterns in the rate of metabolism may also be used to determine when during the period of reduced activities oxygen consumption measurements should be taken. This was

followed by a study to quantify the mass-specific oxygen consumption rate of juvenile *L. lithognathus*. The metabolic rate of fish ($mgO_2g^{-1}h^{-1}$) declines with an increase in body mass both within and among species (Schmidt-Nielsen, 1983). However, the relationship between metabolic rate and body mass is species-specific and, as this aspect has not been investigated for this species, this experiment aimed at modelling metabolic rate in *L. lithognathus* as a function of body mass. Using the metabolic weight as a reference point, the aim of the third experiment was to quantify the metabolic costs of acclimation to various salinities in juvenile *L. lithognathus* following different rates and magnitudes of salinity changes as well as salinity levels. Growth rate is expected to decrease with increasing size and mass of fishes, but there is poor understanding of how energy is allocated to growth and metabolism of fish (Von Herbing and White, 2002). Two growth experiments were conducted to determine growth, feed conversion and feed uptake of juvenile *L. lithognathus* at salinities ranging from 5‰ to 35‰.

Circadian rhythm in oxygen consumption rates

Time of day significantly influenced the metabolic rate of juvenile *L. lithognathus* (Chapter 3). The peak in oxygen consumption of juvenile *L. lithognathus* occurred during the photophase in five fish and during scotophase in one fish. Considering that the experimental fish in the present study were exposed to similar conditions during the acclimation period and during the experiment, the individual variations in the peak of oxygen consumption between the fish in this study could not be explained by feeding entrainment or photoperiod as noted by Du Preez *et al.* (1986a) in their study of *L. mormyrus* and *L. lithognathus*, and by Imsland *et al*. (1995) for juvenile turbot, *Scophthalmus maximus*. Thus, the variability in the circadian rhythm of oxygen consumption could not be explained using available data. It should be tested whether oxygen consumption patterns in juvenile *L. lithognathus* vary within the species as a result of a genetic basis. However, such studies show the need to establish species-specific

methods for respirometry and account for possible individual variations of oxygen consumption. Bang *et al.* (2004) suggested that a large difference in metabolic rate between zebrafish embryos, *Danio rerio*, reflected genetic differences in their metabolic capacity. In support of this, a two-fold difference in oxygen consumption rate during early development between two different genotypes of the killifish *Fundus heteroclitus* (L.) has been found by Paynter *et al.* (1991).

This study showed that by measuring oxygen consumption of fish continuously it was possible to assign the oxygen consumption rate of juvenile *L. lithognathus* into the following levels of metabolism: standard metabolic rate (SMR), routine metabolic rate (RMR) and active metabolic rate (AMR). Attempts to investigate oxygen consumption of fish under variable environmental conditions should account for these variables and differences between them. Results suggested that the remaining trials under variable salinities should be conducted between 09:00 h and 12:00 h in order to minimise any confounding effect of a diurnal rhythm on the interpretation of the results.

Mass-specific oxygen consumption rates

Modelling the effect of body mass on oxygen consumption of juvenile *L. lithognathus* predicted that oxygen consumption decreased with an increase in body mass and was best described by the following equation: $mo_2 = 0.62W^{-0.53}$ ($r^2 = 0.42$), where mo_2 was oxygen consumption in $mgO_2g^{-1}h^{-1}$, W was the weight of the fish in g, and 0.62 and - 0.53 were coefficients from the least-square regression model. The results suggested that there was a need to take the relationship between size of fish and oxygen consumption in consideration under aquaculture conditions. This model provided a method of predicting oxygen consumption rate in this species in order to provide juvenile *L. lithognathus* of different sizes with the best culture conditions, i.e., oxygenation, flow rate, loading density or

crowding density. Thus, the model makes a contribution to both an understanding of the physiology of this species and aquaculture.

Salinity changes and oxygen consumption rates

Acclimation state of juvenile *L. lithognathus* to 25-‰ salinity did not affect their metabolic response. Abrupt salinity change from 35 ‰ to 5 ‰ induced a rapid response in fish expressed by a reduction in their oxygen consumption, and a 72-h acclimation to 35‰ elicited an increase in oxygen consumption of juvenile *L. lithognathus* (Chapter 4). Oxygen consumption of juvenile *L. lithognathus*, therefore, suggested an elevation in the metabolic costs of ion regulation at 35‰ and a temporary decrease of these costs at 5‰. Osmoregulatory energy demands should be the lowest in the isosmotic environment of 10±2‰ (Brett, 1979). In the 5‰ salinity treatment of the present study during an abrupt decrease from 35‰ the costs of ion transport should continued to increase as the difference between internal concentration and ambient concentration increases (Nordlie *et al*., 1991). Findings of the present study did not support the hypothesis by Brett (1979) and Nordlie *et al*. (1991) and could suggest that such costs of ion transport were decreasing at 5‰. Decreased activity has been reported for some species at salinities near the upper end of their tolerance range ((Swanson (1998) using *Chanos chanos* (Forsskal) > 35‰, and Plaut (2000) using *Aphanius dispar* (Ruppell) > 70‰). Information on the activity level of fish species at salinities near the lower end of their salinity tolerance range has been obtained in fish in their early stage of development. Holliday (1965) noted that activity levels of fish embryos and larvae were reduced at low salinities. Due to the experimental design of the present study, it was difficult to accurately monitor activity levels of juvenile *L. lithognathus* under various salinities over long durations. Thus, other studies could address whether any acclimation of juvenile *L.*

lithognathus to lower salinities could be accompanied by a reduction in activity levels to compensate for the metabolic costs of osmoregulation.

Salinity and growth

Salinity did not affect fish growth although mortality was high at 5‰ (Chapter 5). While oxygen consumption demonstrated a reduction and an elevation in metabolic energy demand at 5‰ and 35‰, respectively, there did not seem to be a relationship between metabolic rate at different salinities and growth rate of juvenile *L. lithognathus* at these salinities. Juvenile *L. lithognathus* reduced or increased their metabolic rate significantly under variable salinities (i.e. 5‰ and 35‰, respectively), but this would not explain the lack of differences in growth between the tested salinities of this study. Contrary to the theory by Woo and Kelly (1995) that there is a reduction of osmoregulatory energy demand at an isosmotic salinity and the metabolic energy saved can be channelled to benefit other functions, growth of juvenile *L. lithognathus* in 10‰ salinity was not significantly better than at 5‰, 25‰ and 35‰. The findings of this study were similar to work by Morgan and Iwama (1991) who did not find metabolic or growth advantages of os-osmotic salinity to juvenile rainbow and steelhead trout, *Onchorhynchus mykiss* and fall Chinook salmon, *Onchorhynchus tshawytscha*.

Recommendations for research and conclusions

Since this study did not account for the activity level of fish under the tested salinities, future work should attempt to investigate the behavioural component of juvenile *L. lithognathus* under different rates and magnitudes of salinity changes when studying the metabolic activity in this species. It should also be assessed what percentage of oxygen consumption can be attributed to active ion transport process in the gills. In addition, the contributions to whole-animal oxygen consumption rates of

changes in the metabolic activities of tissues such as the gastrointestinal tract and kidneys in response to acclimation to different water salinities needs further investigation.

Although oxygen consumption data suggested that the energetic cost of ion-osmotic regulation expressed as oxygen consumption was not similar under different salinity changes, attempts to quantify this cost with regard to growth could have been affected by other metabolic processes related to changes in salinity. The results suggested that juvenile *L. lithognathus* can tolerate a salinity decrease of between 35‰ and 5‰, but from an aquaculture point of view, the 35‰ to 10‰ range of salinity may probably be the most ideal for the rearing of juvenile *L. lithognathus* because long-term exposure to 5‰ salinity was followed by mortalities. Yet, it should be possible to expose fish to fast changes in salinity during the treatment of fish diseases, i.e., removal of parasites. In addition, the findings provided estimates of metabolic rates of *L. lithognathus* that could be used to estimate oxygen requirements under aquaculture conditions.

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