Metabolic basis of growth variation in juvenile sole (*Solea senegalensis*, Kaup 1858)

Mestrado em Aquacultura e Pescas

Especialização em Aquacultura

Maria Filipa Bento de Oliveira Falcão Castanheira

Faro, 2009

Universidade do Algarve
Faculdade de Ciências e Tecnologia

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Dissertação orientada por:
Doutor Luís Conceição, CCMAR
Doutora Sofia Engrola, CCMAR

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ACKNOWLEDGEMENTS

It is a pleasure to thank the many people who made this thesis possible.

I would like to express sincere gratitude to my supervisors Doctor Luís Conceição and Doctor Sofia Engrola who accepted me in the Aquaculture Research Group.

A special thank to Doctor Sofia Engrola, which has been abundantly helpful and has assisted me in numerous ways. Thank you for the unreserved support, infinity patience, for revising, and for your optimism.

Thanks for being a great person as a human being, Doctor Luís Conceição you are the perfect example of a scientist. I am very glad for the chance provided by you for me to grow as a student in this unique research environment. The discussions I had with you were valuable. I specially thank for your warm support, for revision the manuscript and infinite patience.

Thus, many, many thanks to you both. I am not sure that you realize just how important you have been to this phase of my life.

This work was financed by Project ECOAQUA, Programme POCTEP.

A special thanks and appreciation to João Reis, for your precious help concerning everything that you had to do in the rearing systems and all the technical solutions that you found to solve the problems. Many thanks for the conception of Oxilogger program.

I am very grateful to all my colleagues at the Ramalhete. The atmosphere was always been a perfect source of motivation. In particular very special thanks to Lena for constant help with hard work and also for the friendship. I also thanks to Miguel, his background work guaranteed that the experimental facilities were functioning perfectly. Thanks Rita for the help during the initial phase of the experiment. I also thank to all others who were always willing to help, discuss some topic, or just go out for a funny time.

Special thanks also to the Aquagroup Research Group, working with you all was an immense pleasure for me.

I am grateful to João Sendão for the office furniture. ☺

I must also acknowledge to Benjamím Costas for the cortisol analysis.

I would like to give thanks to my friends for making my spare time enjoyable and helping me when it was needed. Special tanks my friends the funny lunch time in the past months.

My deepest gratitude goes to my family. Thank you for all your strength, love and for always supporting me in following my dreams!

Finally, for Rui Alexandre. For everything! Your support, optimism, patience, understanding and endless love. Thank you SO MUCH.

À minha irmã,

Isabel

RESUMO

O objectivo geral deste estudo é compreender os factores intrínsecos responsáveis pela variação individual no crescimento de juvenis de linguado senegalês (*Solea senegalensis*). O Linguado senegalês é uma espécie de elevado valor comercial e com elevado interesse em Aquacultura na Europa, por isso seria importante perceber essa variação individual. Compreender as causas para a variação individual de crescimento e relacionar essas causas com o consumo de alimento, comportamento alimentar e resposta ao stress, contribui para maximizar a eficiência produtiva, reduzir os desperdícios alimentares e melhorar a qualidade da água. Isto poderá resultar no desenvolvimento de novas práticas de gestão e melhoramento produtivo, contribuindo para o desenvolvimento do sector da Aquacultura. De modo a identificar os possíveis factores responsáveis pela variação individual no crescimento do linguado, foi determinado o crescimento e a sua relação com o metabolismo, resposta ao stress e consumo de alimento. Doze peixes com peso inicial de 18.49 ± 2.94g foram mantidos em condições standard e alimentados uma vez por dia, durante um período de seis meses. As medições individuais do consumo de oxigénio em juvenis de linguado foram realizadas através de respirometria em condições standard, após o peixe ser alimentado e após stress por emersão. Foram também retiradas amostras de sangue para cortisol de todos os peixes no início da experiência (controle, indicativo das condições basais) e após exposição ao stress. A quantificação do consumo alimentar individual foi realizada por incorporação de ballotinis na ração que foi administrada aos peixes. Após a alimentação com ração contendo ballotinis foi feita uma radiografia para quantificar o número de grânulos ingeridos por cada peixe. Os linguados apresentaram individualmente uma variação acentuada do crescimento (peso final entre 30.45 a 67.53g), do consumo de oxigénio em jejum (entre 108.0 a 447.4 μmol / g / h), do consumo de oxigénio após alimentação (entre 174.7 a 813.5 μmol / g / h), do consumo de oxigénio após stress por emersão (186.8 a 376,7 μmol / g /h), e do cortisol plasmático após o stress por emersão (4.81 e 46.45 ng/ml). Neste estudo, as diferenças individuais na taxa metabólica não contribuiram para explicar as diferenças individuais no crescimento. No entanto, diferenças individuais na resposta após stress por emersão parecem desempenhar um papel relevante no esclarecimento das diferenças individuais de crescimento, talvez resultante dos peixes apresentarem diferentes estilos comportamentais. A medição do consumo de alimento pelo método de raio-X não parece ser um bom indicador da taxa de crescimento. O consumo de oxigénio em peixes alimentados e sob um stress por emersão aumenta, o que se deve a custos aditivos de crescimento e de stress, em relação ao custo de manutenção. Em conclusão, este estudo sugere que a variação individual de crescimento do Linguado senegalês é essencialmente devido a variação genética, e como tal programas genéticos devem ser adoptadas para diminuir a variação individual no crescimento. Um estudo com um maior número de peixes e durante um maior período de tempo seria importante para se verificar algumas das tendências encontradas nesta tese entre o potencial de crescimento, taxa metabólica e consumo de alimento no linguado.

Palavras-chave: *Solea senegalensis*, metabolismo, consumo de alimento, bioenergética, variação de crescimento.

ABSTRACT

The general aim of this study was to understand the underlying factors responsible for the individual variation in growth of juvenile Senegalese sole (*Solea senegalensis*). Senegalese sole is a species of high commercial value and high interest for aquaculture in Europe, therefore it would be important to understand these individual variations. Understanding the causes for individual variation in growth in relation with the feed intake, metabolic rate and response to stress contributes to maximize the production efficiency by reducing food waste and improving water quality. In order to identify the possible factors responsible for the individual variation in sole growth, its relationship with metabolic rate, stress response and feed intake was determined. Twelve fish with 18.49 ± 2.94g of initial weight were kept in standard conditions and feed once a day, during a period of six months. Individual oxygen consumption measurements in Sole juveniles were determined by flow-through respirometry at standard conditions, after the fish were fed a single meal and after a stressful condition. Blood samples for plasma cortisol, were taken from all fish at the start of the experiment (control, indicative of basal levels) and after stress exposure. Quantification of individual feed intake was performed by incorporation with radio-opaque ballotinis glass beads in a dry feed, followed by radiography to quantify the amount of ingested pellets. Individual fish exhibited pronounced variation in growth (final weight ranging from 30.45 to 67.53g), oxygen consumption of fasted fish (108.0 to 447.4 μmol/g/h), oxygen consumption of fed fish (174.7 μmol/g/h to 813.5 μmol/g/h), oxygen consumption after emersion stress (186.8 μmol/g/h to 376.7 μmol/g/h), and plasma cortisol after emersion stress (4.81 ng/ml to 46.45 ng/ml). Individual differences in metabolic rate do not contribute to explain individual differences in growth. However, after emersion stress individual differences in stress response seem to play a role in explaining individual differences in growth, what may result from different copying styles. Sole feed intake as measured by X-ray does not seem to be a good indicator of growth rate. The oxygen consumption increases both when fish are fed and after an emersion stress, as result of additive costs of growth and stress to the cost of maintenance. All together, this study suggests that individual variation of growth in *Solea senegalensis* is essentially due to genetic variation, and its reduction may accomplished when genetic selection programs are adopted. Furthermore, a study with a larger number of fish and over a larger time period would be important to check some of the trends found in this thesis between growth potential, metabolic rate and coping styles of Senegalese sole.

Key words: *Solea senegalensis*, metabolic rate, feed intake, bioenergetics, growth variation.

CONTENTS

1. INTRODUCTION

1.1. Production in aquaculture

Aquaculture has seen a worldwide expansion over the past 20 years and it seems that growth is set to continue (Ashley, 2007). World total demand for fish and fishery products is projected to expand by almost 50 million tonnes to 183 million tonnes by 2015, and it is expected that out of this increase, 73% will come from aquaculture, accounting for 39% of global fish production (FAO, 2008).

In Europe, the aquaculture industry has expanded extensively in the last few decades, scientific and technical interests have focused on high value native species whose biological cycle can be reproduced using currently available breeding techniques (Imsland *et al.*, 2003). The rapid increase in the production volume of some species, for example the gilthead seabream (*Sparus aurata*) and the European seabass (*Dicentrarchus labrax*), especially from the late 1980s, caused some market saturation and a lowering of the prices, which provided an incentive for diversification into new marine fish species (Shields, 2001).

Senegalese sole (*Solea senegalensis*) has been considered a promising candidate for marine aquaculture in Europe since the nineties (Dinis *et al.*, 1999). The current market price in the EU is around 14,5 €/ kg (FAO, 2008). The high interest in this species has led to major achievements in larval nutrition and rearing techniques, despite weaning success still being highly variable (Conceição *et al.*, 2007, Engrola *et al.*, 2007).

In sole culture, there are still obstacles in the development of feeding and ongrowing systems, which are mainly due to the peculiar feeding behaviour of this species (Engrola *et al.*, 2001). Nonetheless, weaned sole presents high growth during ongrowing in earthponds. Therefore, ongrowing in outdoor ponds in policulture with gilthead seabream appears promising (Dinis *et al.*, 1999).

Nevertheless, growth and survival from juvenile to market-size fish in intensive farming systems is often problematic, due to lack of knowledge with regard to rearing technology and husbandry conditions, feeding behaviour and nutritional requirements (Rema *et al.*, 2007). Among the different factors that may induce high mortality during the juvenile stage, stress might be one of the key issues (Costas *et al.*, 2008).

Furthermore, growth dispersion is also a problem (Imsland *et al.*, 2003) as in many other species. Therefore, information of growth variation and its relation with the feed intake, feeding behaviour and response to stress needs to be clarified.

1.2. Senegalese sole (Solea senegalensis)

The Senegalese sole, *Solea senegalensis*, is a common fish in the Western Mediterranean and in the Southeast coast of the Atlantic. This species is a high value flatfish in the Southern Europe, commonly reared in extensive aquaculture production in Portugal and Spain (Dinis *et al.*, 1999).

The Senegalese sole is a teleost fish and classified as:

Class: Actinopterygii
Order: Pleuronectiformes
Family: Soleidae
Genus: Solea
Species: *Solea senegalensis* (Kaup,1858)

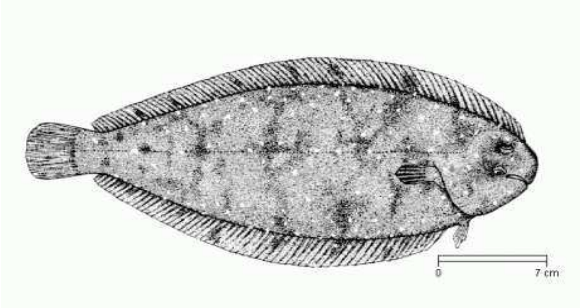

Fig.1 *Solea senegalensis*

Senegalese sole is a flatfish with oval and asymmetric body, eyes on the right side (dorsal side), dorsal fins from head to caudal fin and pectoral fin on the eye side. Its natural habitat is the sandy or muddy bottoms, off coastal areas up to 100m depth. The maximum length of this species is 60cm on Atlantic waters, however in Mediterranean waters is less than 35cm (Whitehead *et al.*, 1986). Sole is a gonochoric species, and females normally mature at age 3 + with a total length around 32cm (Dinis, 1986, Ramos and Roures, 1983). In nature, Senegalese sole feeds mainly on benthonic invertebrate living in the sediment, such as polychaet larvae, bivalve molluscs, smalls fish and crustaceans (Vinagre and Cabral, 2008, Whitehead *et al.*, 1986).

1.3. Individual variation in the growth of fish

Growth is a complex process and is defined as an increase in size over time. Growth can differ between different species, strains or populations within the same species and between individuals within the same population (Irwin *et al.*, 1999b). Most of the fish species reared in aquaculture exhibit considerable growth (weight) variation within a given batch (Martins *et al.*, 2005). Many research into the causes of such intraspecific variation in growth have been undertaken, particularly in commercially important species in aquaculture, where uniform sized fish groups are desirable (Irwin *et al.*, 1999b). As earlier suggest by Butts and Litvak (2007) in Winter flounder (*Pseudopleuronectes americanus*), the parental effects on survival and growth in different populations may allow for selection of the most suitable broodstock for aquaculture production. Intraspecific variability in the size of fish is associated with spawning time with a seasonal decline in egg size having been described in marine fish (Bagenal, 2006).

Knowledge about the maternal age has recently been considered a factor of variability in growth. The older females of black rockfish (*Sebastes melanops*) had growth rates more than three times faster compared with fish from the younger females. The apparent underlying mechanism is a greater provisioning of larvae with energy-rich triacylglycerol (TAG) lipids as female age increases. The volume of the yolk lipid (composed primarily of TAG) present in larvae at spawning increases with maternal age and is correlated with subsequent growth (Berkeley *et al.*, 2004).

Under aquaculture conditions, individual variation in growth can result from external factors (eg, time and frequency of feeding and social interactions) and internal factors (eg metabolic rate, digestibility, energy expended in swimming) (Martins *et al.*, 2006a). For example in African catfish (*Clarias gariepinus)* the individual growth rate was significantly affected by feeding time and frequency. Day time feeding with three equal size meals a day gave the lowest growth and highest food wastage. The growth rates of fish fed continuously or during night time following their feed demand were significantly higher with lowest food conversion ratios and food wastage (Mostafa *et al.*, 2002).

The pronounced inter-individual variations in food consumption, growth, and growth efficiency observed in juvenile hybrid sunfish have been associated with the establishment of social hierarchies (Wang *et al.*, 1998). Dominant fish can monopolize a disproportionate share of available resources, resulting in faster growth of these fish compared to subordinates (Jobling and Baardvik, 1994).

However others factors are responsible for variation in growth. Information of dietary protein requirements for maximum growth in *Solea senegalensis* (Rema *et al.*, 2007) is important in this respect. Response to stressors involves a series of biochemical and physiological responses, which may affect growth variation (Costas *et al.*, 2008). Some variation in growth has also been related to stocking density (Ambrosio et al., 2008).

Studies evaluating growth potential have usually focused on feeding of the tank and the inherent changes in biomass, and do not take individual variation into account (Jobling and Baardvik, 1994). The scarceness of data on individual growth performance is sturdily related to difficulty in measuring it, usually a mean value is used to demonstrate the rank of the inter-individual variation in growth (Martins *et al.*, 2005).

However, understanding the individual variation in feed consumption and growth contributes to maximize the production efficiency by reducing food waste and improving water quality (Millot *et al.*, 2008).

1.4. Causes of Individual variation in growth

Variation in growth of individuals is a common feature of fish populations, and such variation is a drawback in commercial fish culture (Barki *et al.*, 2004). In order to reduce this variation it is essential to have an understanding of the extent of the phenomenon and then to investigate how growth can be promoted and individual variation reduced.

1.4.1. Feed intake

Individual variation in growth may result from individual differences in feed intake. It is thought that a elevated feeding frequency maximizes growth and reduces

growth dispersion in fish juveniles and larvae (Haylor, 1993). In many species, differential growth arises when food is a limiting factor and there is monopolization of food resources by a small number of dominant individuals (Irwin *et al.*, 2002). Competition resulting in an inadequate food supply could persist in older fish even when food supply is adequate (Jobling and Baardvik, 1994). If feed supply is unlimited and competition reduced, increasing feeding frequency may increase growth and reduce dispersion by improving food intake of more individuals in a tank. Furthermore, (Dwyer *et al.*, 2002) observed that when rainbow trout is feed at low frequency fish tend to eat more per meal and may develop a larger gut capacity over time. In addition, pellet size and the feed delivery rate within a meal may also promote a more efficient digestion (Andrew *et al.*, 2004b). However, if feed is limited and competition for feed increases, fish consume as much feed as possible in a short period of time and conserve energy during feeding (Andrew *et al.*, 2004a).

In short, optimising growth rates and feed efficiency, and reducing growth variation, in fish depends upon the way in which food is made available, the feeding method, feeding frequency, the duration of each feeding period, the amount of food delivered and the characteristics of the diet (Ambrosio *et al.*, 2008).

1.4.2. Feeding behaviour

Pronounced variation in feeding behaviour within a population has been described for many fish species (Martins *et al.*, 2006b, Qian *et al.*, 2002, Wang *et al.*, 1998). Some variations in growth can be attributed to genetic differences between individuals but social interactions, specifically competition for food are believed to be a important contributor to differential growth (Irwin *et al.*, 2002). Moreover studies for several fish species showed differential growth arises where feed is restraining and there is monopolization of food resources by dominant individuals (Carter *et al.*, 1992). Dominant individuals present differences in the ability to obtain the meal faster by eating faster and being at the most favourable areas of the tank. When there is more competition for food African catfish exhibited the highest swimming activities and waiting-in-feeding-area what may induce differences in individual growth (Martins *et*

al., 2005). Low and large inter-individual variability in feed intake seem related to feeding motivation. Differences in feeding behaviour during first feeding could explain differences in growth between fast and slow growing strains (Sundström *et al.*, 2003). Different fish species have distinct feeding behaviour associated with their life history and habits therefore, the importance of feeding behavior in growth variation varies from species to species (Wedemeyer, 2001).

1.4.3. Stress response

In intensive fish culture, environmental and physical stressors associated with stocking densities of fish can affect many physiological functions thereby affecting fish growth. In response to a stressor, a fish will undergo a series of biochemical and physiological changes in an attempt to compensate for the challenge imposed upon it, and so cope with the stress (Costas *et al.*, 2008, Arjona *et al.*, 2009). One of the most accepted primary indicators of stress is the increase in plasma cortisol levels (Van Weerd and Komen, 1998). Fish respond to a stress by several alterations (endocrine and physiological), which may influence the hability of fish to survive, increase the incidence of diseases, or limit growth (Pickering, 1998).

Stress in teleost fishes is manifested as primary, secondary and tertiary responses. The primary stress response of fish involves the realease of catecholamines into the circulatory system from chromaffin cells. This stress response also stimulates the hypothalamic-pituitary-interrenal (HPI) axis to release corticosteroids (e.g.,cortisol) from the interregnal tissue (Barton, 2002). In turn, these primary effects cause a number of physiological changes known as “secondary effects”. The secondary response as changes in range of biochemical, haematological and immunological factors (e.g., hight plasma glucose, low muscle protein and low potassium) (Frisch and Anderson, 2005)

The secondary responses may eventually advance to tertiary stress responses, which include reduced growth rate, reduced metabolic range for activity, decreased disease resistance, decreased reproductive capacity, changed behaviour and survivability (Barton, 2002, Barton and Iwama, 1991, Mommsen, 2001). The extend of tertiary responses may be directly related to the severity and duration of the stressor

(Portz *et al.*, 2006). Chronic stress, even at low levels may impair performance by diverting energy resources that might otherwise be used for routine activities, growth, immune function, and/or reproduction (Barton, 2002, Barton and Iwama, 1991). Environmental stress is often long-lasting (chronic) and therefore, non- adaptive. It is assumed that this must, in the long run, influence growth (Van Weerd and Komen, 1998). In addition, stress leads to an increase in metabolic requirement for maintenance leading to a reduction of the growth potential (Barton and Iwama, 1991). This results from increasing demands for defence and repair processes (e.g., heat-stock proteins, increased protein turnover) which require energy results in increased maintenance costs. Individual differences in the mobilization and allocation of energy under stress response are related to individual differences in growth (Maltby, 1999).

Under stress conditions cortisol may also influence amino acid metabolism, as suggested by the decrease in free amino acid concentrations in Senegalese sole under repeated handling stress (Aragao *et al.*, 2008).

1.4.4. Social hierarchy

Social rank has great consequences for individual growth variation. Individual differences in competitive ability, aggressiveness and/or size can lead to the establishment of dominance hierarchies within groups of fish (Metcalfe *et al.*, 1989). The variation in growth rates in groups of fish are related to dominance hierarchies and preferential access to food resources by dominant individuals (Carter *et al.*, 1992). Aggressive behaviour as well as recordings of inter-individual variation in growth rate and food intake, suggest that in many species the social hierarchies become less distinct with increasing stocking densities (Cubitt *et al.*, 2008).

Knowledge of social hierarchies under high stocking densities is scarce. However, such information would be relevant to understand species biology, to optimize production, and improve animal welfare (Martins *et al.*, 2006a).

Furthermore, the traditional theory that individuals grow faster because they are dominant has been questioned (Wang *et al.*, 1998). These authors suggest that genetic factors may be more important in explaining differences in individual growth

rate. In fact, individual differences in feed intake and feed efficiency were found in fish housed individually, thus in the absence of social interaction (Qian *et al.*, 2002).

1.5. Metabolism and bioenergetics in fish

Growth is an energy demanding process and it accounts for a large portion of the energy expenditure (Jobling *et al.*, 1989). The distinction between energetic costs of growth and maintenance is problematic, because costs of maintenance also include costs of synthesis of macromolecules to balance breakdown of body constituents, including turnover of proteins (Jørgensen, 1988). According to Conceição (1997) the cost of maintenance may be approximated by the oxygen consumption of a fasted fish with routine activity, and is mainly due protein turnover and cell volume regulation. In general, the cost of protein turnover accounts for around 40% of the cost of maintenance (Houlihan *et al.*, 1995).

Growth rates per unit of body mass decrease as animals increase in size. In addition, increasing body mass gives rise to a diminishing mass-specific oxygen consumption (Conceição *et al.*, 1998). The cost of growth, defined as the energy expenditure above the maintenance metabolism, was estimated to be 66 mmol ATP g^{-1} dry weight (DW) deposited for animals growing faster than 16.8% body dry weight (BDW) day^{-1} (Wieser, 1994), and generally increasing as growth rate diminishes. However, there is no evidence so far that the cost of growth changes in such a pattern for individuals within a given species growing at different rates.

Growth of an organism is based on the utilization of available energy to increase body tissue. Energy gained from food consumption may also be used for processes other than growth. Studies in bioenergetics at the individual level deal with the partitioning of ingested food energy based on a balanced energy budget:

$$C=G + R + E + F$$

where C =consumption of food energy, G =growth, R =respiration, E =excretion (nitrogenous wastes) and F =faecal output (Shearer *et al.*, 1997).

There are three general metabolic states for which oxygen consumption has been defined: standard, routine, and active metabolism. The standard rate is when

there is no spontaneous fish activity, the routine rate is when spontaneous activity occurs, and the active rate is induced by forcing the fish to swim (Merino *et al.*, 2009).

The cost of growth is defined by the energy expenditure above the maintenance metabolism. This cost includes the deposition of proteins and lipids, the protein turnover on the maintenance level and the cost of food search (capture and assimilation) that leads to an increase in oxygen consumption comparable to the increased in prostprandial metabolism. A significant part of cost of growth may be due to energy spent in control processes involved in the regulation of metabolism in general and of protein synthesis in particular (Pedersen, 1997).

A higher cost of growth is associated with reduced growth rates, and reflected in lower gross food conversion efficiencies. Decreasing growth rates are a result of reduction in relative rates of feed-intake and are accompanied by a reduction in oxygen consumption (Conceição *et al.*, 1998).

1.6. Objectives of the study

The general aim of this study was to understand the underlying factors responsible for the individual variation in growth of juvenile Senegalese sole (*Solea senegalensis*).

Most of the species reared in aquaculture exhibit considerable growth variation. Understanding the causes for individual variation in growth in relation with feed intake, feeding behaviour and response to stress contributes to maximize the production efficiency by reducing food waste and improving water quality. That may result in the development of new management practices and improved production, contributing to the development of the aquaculture sector.

In order to identify the possible factors responsible for the individual variation in sole growth, growth and it relationship with metabolic rate, stress response and feed intake was studied in a group of twelve animals.

2. MATERIAL AND METHODS

2.1. Experimental procedures

2.1.1. Fish stocks and rearing conditions

Twelve Senegalese sole juveniles (*Solea senegalensis*), with 18.49 ± 2.94 g of initial weight were kept at the Ramalhete facility at the CCMAR/University of Algarve (Faro, Portugal). Fish were obtained from natural spawning of wild broodstock and reared according to standard larval and juvenile rearing protocols (Dinis *et al.*, 1999). Fish were maintained in a 21L flat-bottomed fibreglass tank (70cm length x 30cm width x 6cm depths) in a semi-closed recirculation system equipped with a biofilter and UV filter, at a constant flow rate in the tanks of 109 L/h, nearly five renovations per hour (Fig. 2). The water renewal in the recirculation system was 19.5% per day. The initial stock was divided in two tanks during the experiment, in order to avoid further disturbance of sole once most measurements were done in six fish per day. The tanks were covered with a net, and placed behind a black curtain, in order to avoid stress due to human presence. The sole were reared at 19.9 ± 1.3 ° C water temperature; dissolved oxygen was above 90% of saturation, and salinity around 36‰. The water parameters were monitored daily. The quality of water was determined by weekly measurements of nitrite and ammonia.

The photoperiod was maintained 14 h dark and 10 h light with a light intensity of 12 lux. During a period of six months, the fish were fed once a day a commercial diet (Aquagold 2mm, Sorgal SA, Ovar, Portugal; proximate feed analyses was 44% crude protein, 14% crude fat, 8% ash, 2.5% crude fibres, 1.0% phosphorus; starting at 9:30. The feed excess (feed not eaten) was removed from the tanks after three hours, around 12:30.

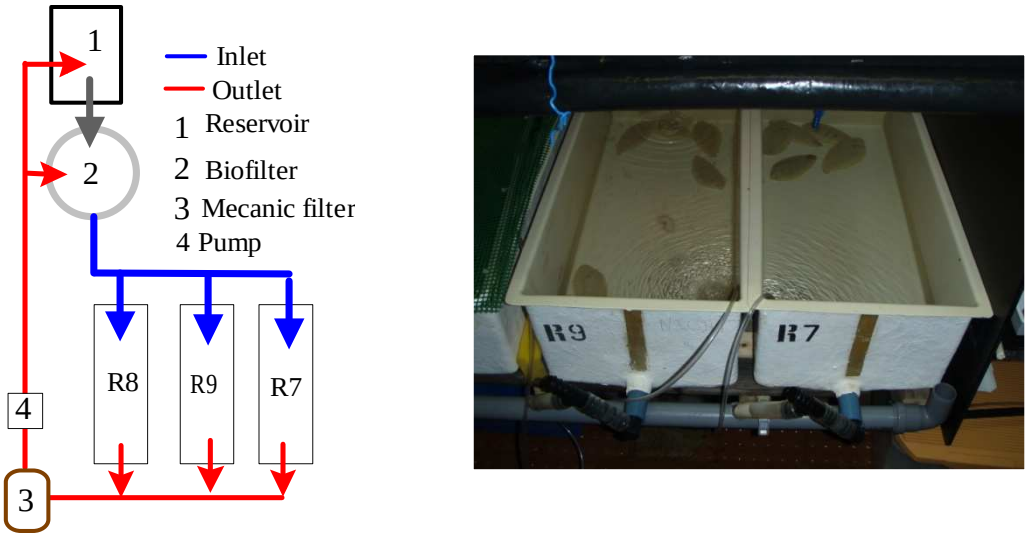

Fig. 2- Recirculation system and tanks used throughout the experimental period.

Fish were tagged with colour codes in order to be able to identify each fish individually throughout the experimental period. Before tagging, fish were randomly transferred to a 10L bucket filled with 5L of sea water and anaesthetic, 2-phenoxyethanol (Sigma-Aldrich, Germany; 1:150 v/v). Anaesthetized fish were tagged individually with colour marks in abdomen, weighted and distributed over two 21L flat-bottomed fibreglass tanks. Three different colours (blue, green and pink) of water-based paint (Acualux TITAN "al agua" colores satinados, Industrias TITAN, S.A., Spain) were used for colour marks. The colours were injected in order to form three lines placed on the blind side between skin and muscle and parallel to muscle direction. The paint was injected using sterile syringes (ONCE 1ml) and non-pyrogenic needles (Terumo Neolus 25G x 5/8''; 0.5 x 16mm, Terumo Europe N.V., Belgium). This procedure was repeated four times during the experiment, as marks tended to become faint with time.

2.1.2. Experimental design

Individual fish growth was recorded during the whole experimental period. In addition, the experiment was divided into two distinct parts. A first part where fish were grown undisturbed in order to analyse the growth potential of individual fish,

and a second part where repeated handling of fish occurred, in order to perform measurements of oxygen consumption, feed intake and/or plasma cortisol levels under different conditions: fasting fish, fed fish and fish subjected to a 3 min emersion stress.

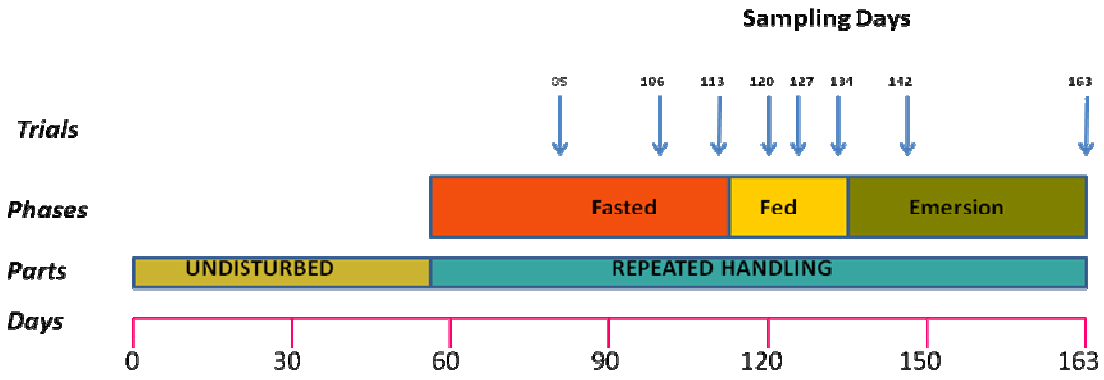

Fig. 3- Chronogram of the experiment. The phases refer to treatment during the trial (sampling) days. Sampling days are marked with blue arrows. The remainder of the (non-sampling) days fish were let undisturbed and fed normally.

2.1.2.1. Individual measurements at fasting conditions

This phase aimed to quantify the individual differences in Senegalese sole juveniles, in terms of cortisol levels and routine metabolism. It was also intended to establish eventual relationships between those variables and growth. Three trials performed in different days, with a minimum of one week interval, were performed for every fish. In each trial, oxygen consumption in individual Sole juveniles was determined by flow-through respirometry. Subsequently, blood samples from all fish were taken to determine the routine levels of cortisol. This phase lasted from day 59 to day 113 of the experiment and measurements were performed at days 85, 106 and 113 (Fig. 3). In the remaining days fish were let undisturbed and fed normally.

2.1.2.2. Individual measurements in fish fed a single meal

The goal of this phase was to quantify if feed consumption and metabolism of Senegalese sole juveniles were affected after a single meal. It was intended to establish the possible relationships between these variables and growth. Three trials performed in different days, with a minimum of one week interval, were performed for every fish. Quantification of individual consumption of food, was performed by offering feed incorporated with (radio-opaque) dense ballotini glass beads (rate of incorporation: 1%) to fish during a period of 30 minutes. After this period fish were removed from the tank, anaesthetized and exposed to X-rays in order to quantify the amount of ingested food. Subsequently the oxygen consumption in individual Senegalese sole juveniles was determined by flow-through respirometry. This phase lasted from day 114 to day 134 of the experiment and measurements were performed in days 120, 127 and 134 (Fig. 3). In the remaining days fish were let undisturbed and fed normally.

2.1.2.3. Individual measurements after stressful condition

This phase was designed to assess if an acute stressful condition could influence the Senegalese sole individual response in relation to oxygen consumption and cortisol levels. Two trials performed in different days, with twenty one days interval, were performed for every fish. The stress test consisted of holding each fish individually in a net outside the water for three minutes. One hour after the stress test, and before starting the oxygen consumption measurements, blood was collected from each fish for cortisol analysis. Subsequently, the oxygen consumption in individual Sole juveniles was determined by flow-through respirometry. This phase lasted from day 135 to day 163 of the experiment and measurements were performed in days 142 and 163 (Fig.3). In the remaining days fish were let undisturbed and fed normally.

2.1.3. Sampling methods

2.1.3.1. Growth

Growth was determined by weighting (g) all fish at the beginning of the experiment and at the end of each oxygen consumption measurement. Fish were individually weighed in a scale (KERN- EW 600-2M, 0.01 g).

2.1.3.2. Feed intake

Feed intake was quantified by fish exposure to X-rays after a single meal, in order to determine the amount of ingested feed.

Fish were fed in excess during 30 minutes before the radiography determination. The feed was previously incorporated with known quantities of X-ray dense ballotini glass beads at 1% incorporation in feed dry matter. After the meal, the fish were taken out from each tank, three at a time and anaesthetized with 2-phenoxyethanol (Sigma-Aldrich, Germany; 1:300 v/v). The X-ray was taken with a Multimage X-ray (Microbloc 20, Italy) with a focus of 1.8mm; the time of exposure to X-ray was 2´´ (Fig. 4). Green film (Kodak, France) was used, and developed with (Kodak, France) developer.

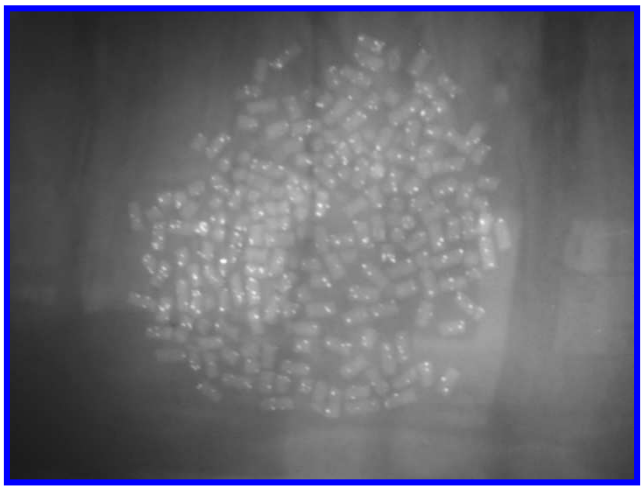

Fig. 4- X-ray of feed with incorporated ballotinis.

The feed intake measurements were calculated using a calibration curve with ballotini-incorporated feed (Fig. 5). The different amounts of feed (0.25g; 0.5g; 1.0g; 1.5g; 2.0g; 3.0g and 4.0g), were weighed in triplicate samples. Radiography was done to the feed in order to quantify the number of ballotinis per gram of feed. The equation obtained was: y=92.36x-12.62; R^2=0.98.

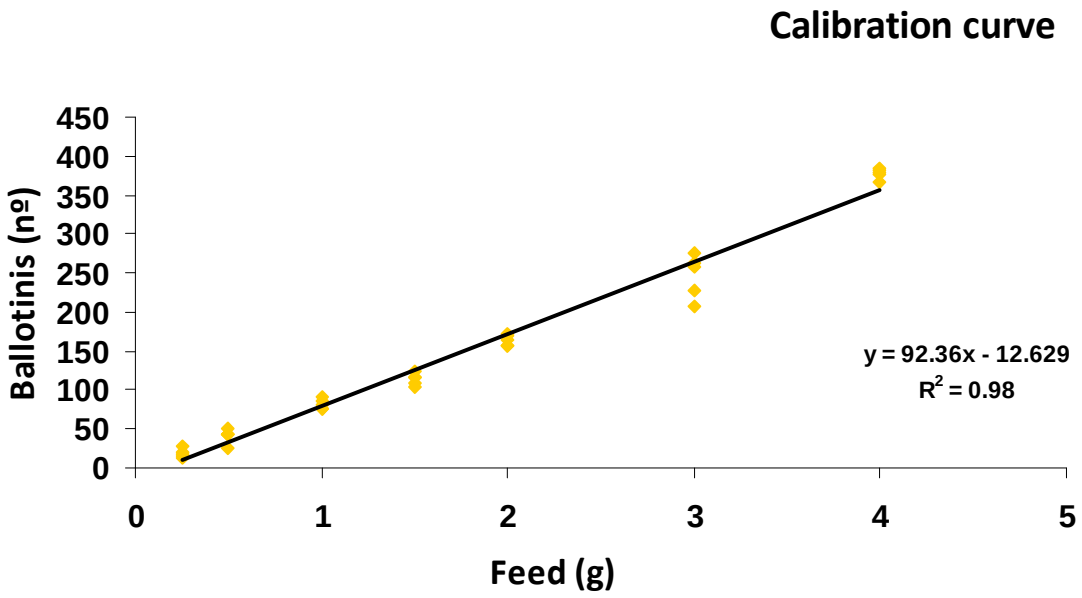

Fig. 5- Calibration curve of feed with incorporated ballotinis.

2.1.3.3. Plasma cortisol levels

Plasma cortisol levels were measured in fasted fish and after stressful condition (emersion for three minutes).

Blood sampling was performed in anaesthetized fish (2-phenoxyethanol, Sigma-Aldrich, Germany; 1:500 v/v). Blood was withdrawn from caudal vein (Fig. 6) using heparinised syringes (ONCE 1ml) and non-pyrogenic needles (Terumo Neolus 25G x 5/8″; 0.5 x 16mm, Terumo Europe N.V., Belgium).

Fig. 6- Withdrawing blood

(Benjamím Costas courtesy)

A heparin solution (1000U/mL; grade I-A: from porcine intestinal mucosa; Sigma- Aldrich, Germany) was prepared in NaCl 0.9% and used as anticoagulant in the syringes and needles. The blood collection lasted less than 3 minutes to avoid a cortisol increase due to manipulation during sampling.

After sampling, blood was centrifuged in a HERMLE centrifuge (Z 233 M-2) with HERMLE rotor (220.87 VO 5/6) at 1500G during 8 minutes at room temperature. Plasma was removed with micropipettes and stored in micro-tubes. Samples were stored at -80ºC until further analysis.

2.1.3.3.1 Cortisol analysis

Plasma cortisol levels were measured with a commercially available competitive binding Coat-A-Count®cortisol kit (SIEMENS Medical Solutions Diagnostics, Los Angeles, CA, USA) as described by (Irwin *et al.*, 1999a). Briefly, 50 µl of each sample to be assayed was pipetted into an Ab-Coated tube and 1 ml of 125I Cortisol added. The tubes were then incubated for 45 min at 37 ºC in a water bath. The contents of all tubes were decanted, and allowed to drain for 5 min before being read on a gamma counter (2470 WIZARD/2 _TM , PerkinElmer, Turku, Finland) for 1 min. A calibration curve was constructed on logit-log graph paper and used to convert results from percent binding cortisol to concentration (ng/ml).

2.1.3.4. Respirometry

Oxygen consumption of individual Sole juveniles was determined by flow-through respirometry. The saturation level of oxygen was maintained in the water reservoir and a peristaltic pump (ISMATEC, model ISM920A, Switzerland) (Fig. 7), controlled the water flow of each chamber. The flow-through respirometry system consisted in 6 individual metabolic chambers (2.3L). Each fish was placed individually in a chamber (Fig. 8).

Fig. 7- Peristaltic pump

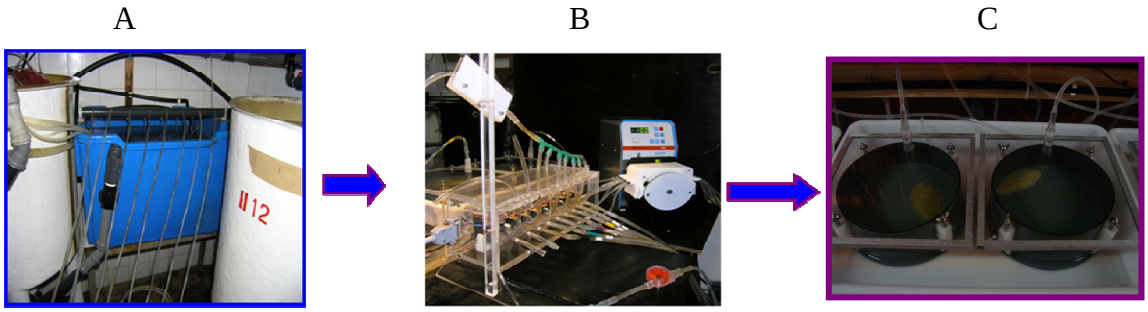

Fig. 8- Flow-through respirometry system. A – Water reservoir; B – Peristaltic pump and magnetic valve system; C – Respirometry chambers

Each chamber had a water inlet and water outlet. Water inlet was always at oxygen saturation level and oxygen concentration at outlet was measured by a Poligraphic microelectrode model 8-730 (Microelectrodes Inc., USA). Each respirometry trial consisted in measurements from all the chambers. The magnetic valves determined in which chamber the oxygen consumption was being measured (Fig. 9). This was controlled by a PC using the Oxilogger 2009 software (João Reis, CCMAR, Faro, Portugal) which also registered the microelectrode measurements.

At the beginning of each cycle the oxygen dissolved in water was measured during a 30 seconds period in order to calibrate the software. This calibration was followed by a 120 seconds washing step of seawater from the next chamber before the start of the measurement period (30 sec). This step was always done before and after the measurement of each chamber. The data was collected through a dynamic mean of 6 measurements (5 sec each) during a total period of 30 sec. A complete cycle of measurements was done during an 18 minutes period, and comprised the calibration of the oxygen probe, a washing step, measurement of dissolved oxygen in the water chamber, washing step, and so forth for a total of six chambers. The water temperature was always measured in the outlet water of each chamber by a temperature probe.

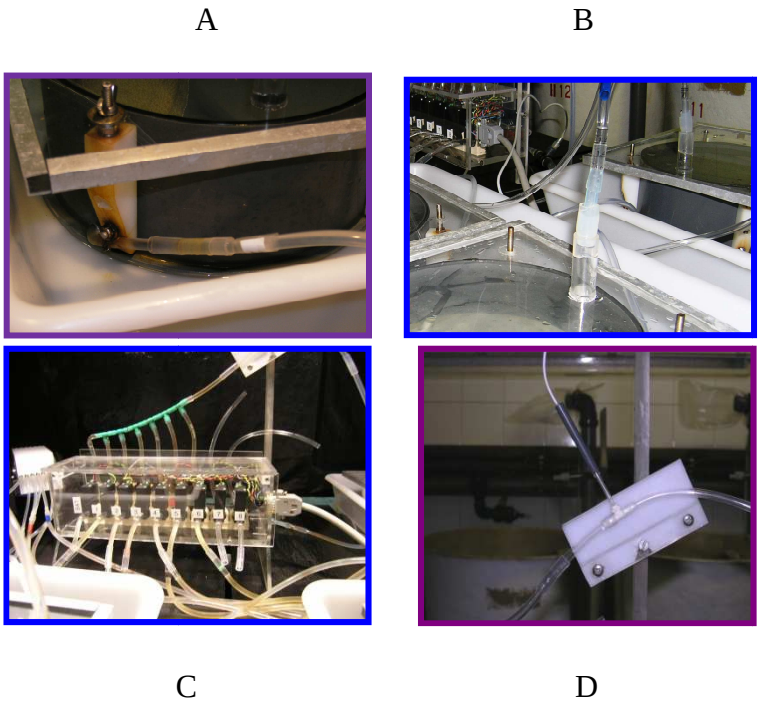

Fig. 9- Flow-through respirometry system. Water inlet (A) and outlet (B), magnetic valves (C) and oxygen microelectrode (D)

The individual measurements of oxygen consumption in Senegalese sole juveniles were monitored in 6 chambers containing one fish each. Measurements were taken in all the three distinct phases (Fasted, Fed and Emersion). Oxygen consumption was measured before the meal except in the Fed group. The Fed group was allowed to

feed for 30 minutes, and after transferred to the metabolic chambers. The oxygen calibration was performed each time a measurement cycle started.

2.2. Data analysis

Nearly three hours of measurements (11 cycles of 30 seconds of measurements per fish) were performed in each trial for quantification of the oxygen consumption in individual Senegalese sole juveniles. All measurements were done in the light phase of the photoperiod. The experimental units used were the mean values for each of the 11 cycles; however the values 15% or more above the mean were excluded from analyses (considered as outliers).

Growth, expressed as relative growth rate (RGR, %/day), was calculated using the formula: $(e^{g}-1) \times 100$, with g = [(ln final weight – ln initial weight)/time] (Ricker, 1958), where, W_1 and W_2 were the initial and final wet weight, respectively.

Feed intake (% body weight) was analysed by X-ray and expressed by the number of ballotinis per gram of feed.

The results were expressed as mean ± standard deviation (S.D.). Coefficient of variation was calculated as follows: CV = standard deviation / mean x 100.

Data was analyzed by t-test for dependent samples, two-way analysis of variance (ANOVA) and regression using the computer packages STATISTICA.
The level of significance used was $P<0.05$ for all statistical tests. For regression analysis, P values ranging from 0.05 to 0.15 were considered as showing a trend.

3. RESULTS

3.1. Individual variation in growth performance

At the beginning of the experiment, the Senegalese sole (*Solea senegalensis*) juveniles had 18.49 ± 2.94g of wet weight, the mean weight was significantly superior (t- test, P < 0.001) by about two-fold to final body weight

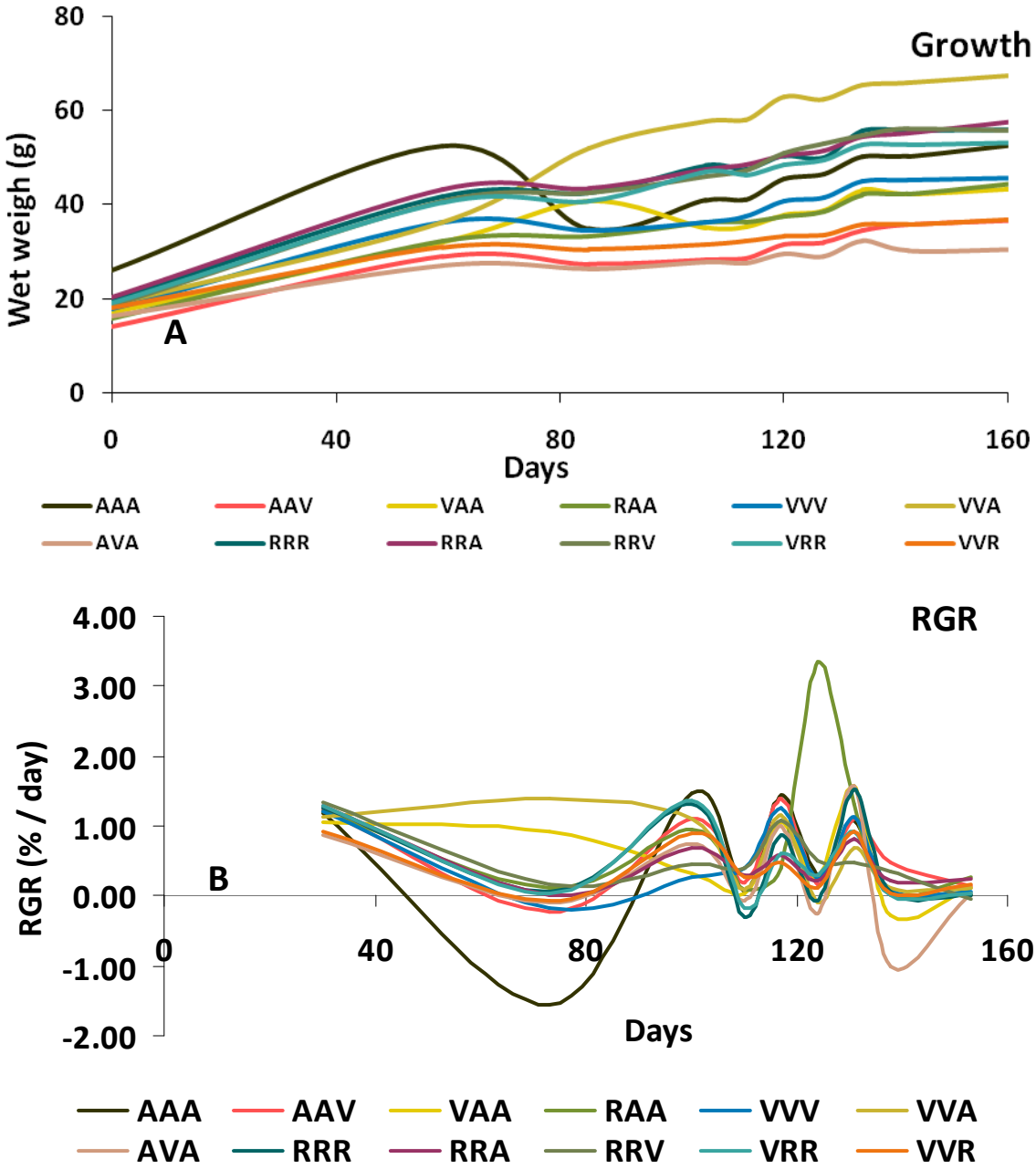

Fig. 10- Individual wet weigh (WW) (A) and relative growth rate (RGR) (B) of Senegalese sole juveniles during the 163 days of the experiment (n = 12).

48.45 ± 10.64g. The comparison of performance data, growth and relative growth rate (RGR), during the 163 days of the experiment is present in Fig.10.

The mean weight of Sole juveniles were between 27.68 ± 10.82g, at the end of the Undisturbed part of the experiment at day 59; 39.37 ± 8.53g at the end of the "Fasted" phase at day 113; 44.52 ± 10.04g at the end of the "Fed" phase at day 134 and 47.87 ± 10.34g at the end of the "Emersion Stress" phase at day 163 of the experiment. The comparison between the different experimental periods (Undisturbed, Fasted, Fed and Emersion Stress) revealed an increase in mean sole weight during the experiment, despite some variations (Fig.10).

Over the experimental period, fish showed a high variation in relative growth rate (RGR). The mean RGR (%/day) values ranged from 1.167 ± 0.149% during the Undisturbed part of the experiment (days 0-59), 0.144 ± 0.426% during the Fasted phase (days 59-113), 0.745 ± 0.954 during the Fed phase (days 113-134), and 0.055 ± 0.215 during the Emersion Stress phase (days 134-163). The RGR were considerably elevated in the undisturbed phase compared with the other phases. In the Emersion Stress phase the RGR showed a low mean.

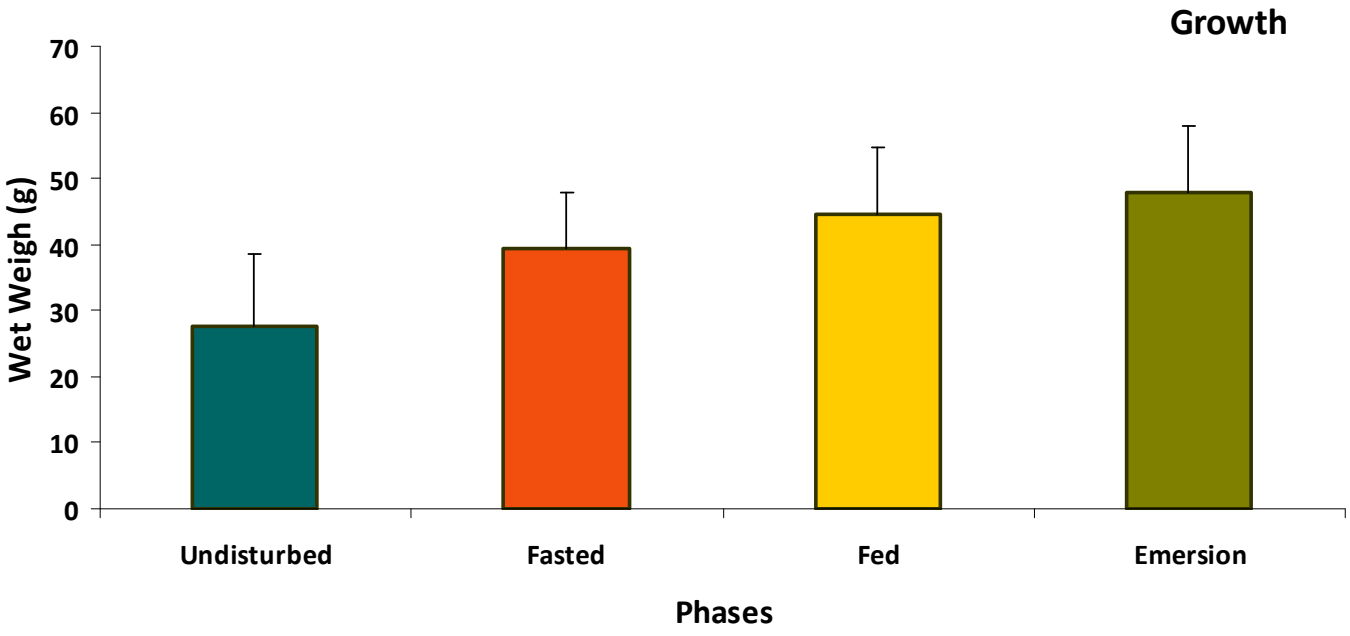

Fig. 11- Mean (±SD) wet weight (WW) of Senegalese sole juveniles at the end of the different phases of the experiment (n = 12).

The individual variation in weight during the repeated handling period lead to CVs of 21.66%; 22.56% and 21.56%, in Fasted, Fed and Emersion Stress phases, respectively. During the undisturbed part of the experiment the CV was 30.08%.

The RGR of individual fish during the experiment was analyzed in two distinct parts. The first undisturbed part of the experiment before de handling at day 59 indicates the growth potential without chronic stress. The second part of the experiment, with repeated handling until day 163, represents growth with possible chronic stress conditions (Table 1).

Table 1 – Senegalese sole juveniles Relative growth rate (RGR, %/day) during the Undisturbed and Repeated handling parts of the experiment.

Fish ID	Undisturbed RGR (%/day)	Repeated handling RGR (%/day)
AAA	1.194	0.012
AAV	1.230	0.225
VAA	1.063	0.288
RAA	1.217	0.317
VVV	1.204	0.223
VVA	1.121	0.601
AVA	0.859	0.111
RRR	1.268	0.281
RRA	1.286	0.281
RRV	1.342	0.299
VRR	1.289	0.264
VVR	0.925	0.174
Mean	1.167	0.256
SD	0.149	0.140
CV	12.779	54.666

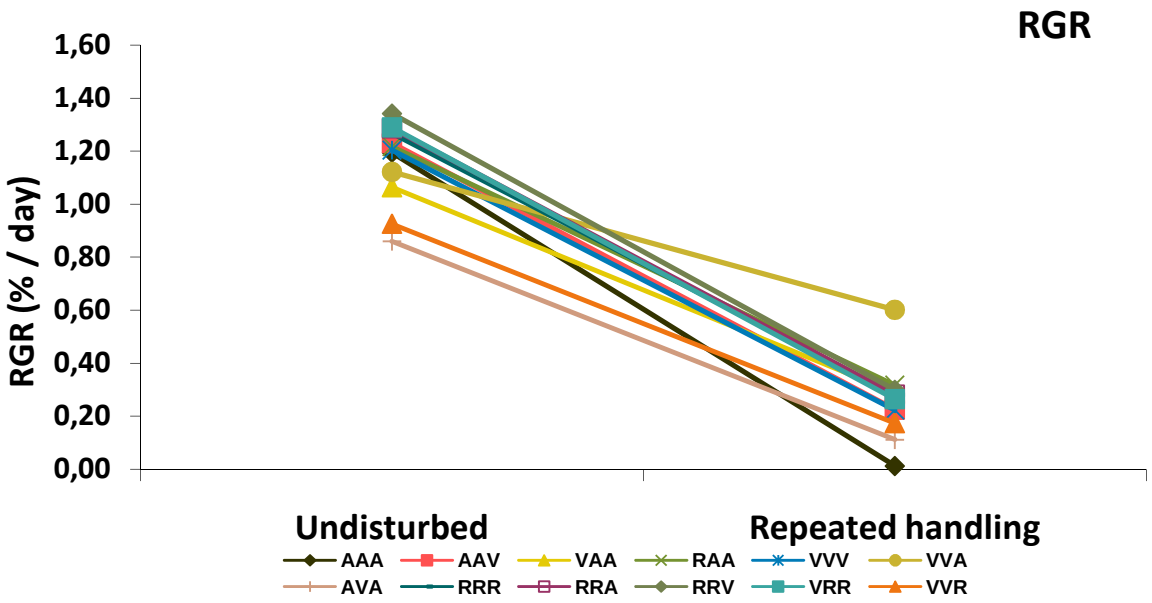

Fig.12- Relative growth rate (RGR, %/day) of individual Senegalese sole juveniles during the Undisturbed part of the (until day 59) and during the Repeated handling part (days 59 to 163) (n=12)

In the beginning of the experiment (Undisturbed phase) the fish had a mean RGR of 1.167 ± 0.149 %/day, however during the Repeated handling phase the mean RGR was significantly reduced (t- test, $P < 0.001$) by about five-fold to 0.256 ± 0.140 %/day (Table. 1).

3.2. Individual variation in oxygen consumption

3.2.1. Oxygen consumption in fasted fish

Mean oxygen consumption on fasted fish in the Trial 1, Trial 2 and Trial 3 was respectively around 186.9 ± 14.3, 188.4 ± 17.9, 241.3 ± 15.4 µmol O_2/g/h. In every trial the mean oxygen consumption in fasted fish was significantly different (ANOVA two-way, $P < 0.001$). The CV was 8.018% Trial 1, 9.311% Trial 2 and 6.849% Trial 3. Fish VVA had the maximum mean oxygen consumption (327.7µmolO_2/g/h) Trial 1, whereas fish AAV had the minimum mean oxygen

consumption (121.8µmolO_2/g/h). In Trial 2 fish RRR had the maximum mean oxygen consumption (266.8µmolO_2/g/h), and fish VVR the minimum mean oxygen consumption (108.0µmolO_2/g/h), while in Trial 3 fish VRR had the maximum mean oxygen consumption (447.4µmolO_2/g/h), and fish VVV the minimum mean oxygen consumption (146.2µmolO_2/g/h).

A significant effect on oxygen consumption (ANOVA two-way, $P < 0.001$) was observed between individuals and also for the interaction individuals x trials (Fig. 13).

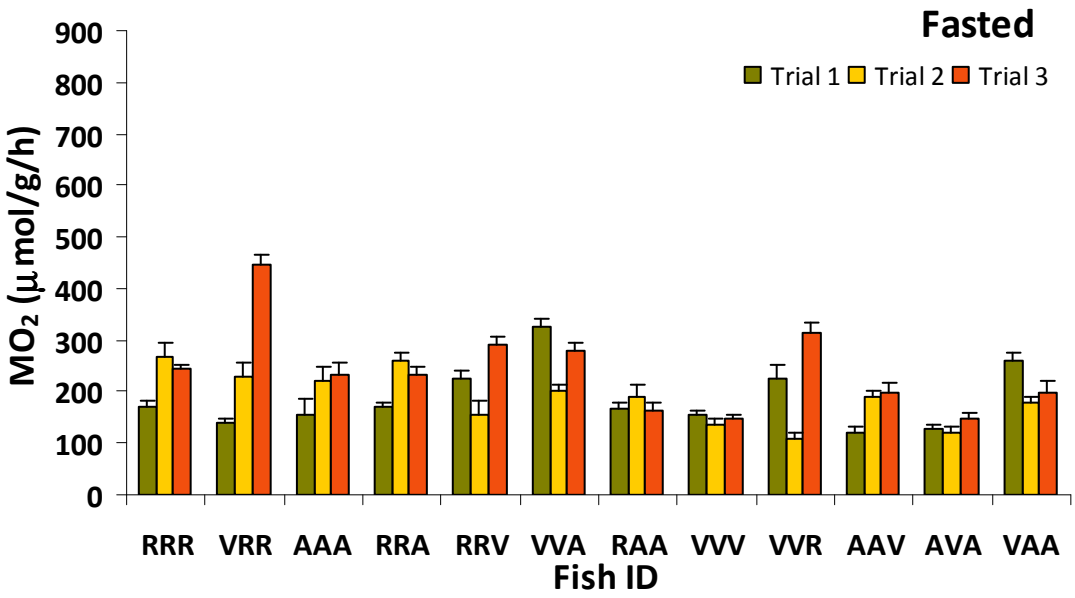

Fig. 13- Individual measurements of oxygen consumption (µmol O_2/g/h) in Senegalese sole juveniles during the Fasted phase at days 85, 106 and 113 (n =11).

3.2.2. Oxygen consumption in fed fish

Mean oxygen consumption on fed fish in the Trial 1, Trial 2 and Trial 3 was respectively 279.6 ± 20.7, 284.5 ± 23.1, 341.0 ± 34.6 µmol O_2/g/h (Fig. 14). In every trial the mean oxygen consumption in fed fish was significantly different (ANOVA two-way, $P < 0.001$). The CV was 7.499% Trial 1, 8.050% Trial 2 and 10.42% Trial 3. Fish VVA had the maximum mean oxygen consumption

(359.7µmolO_2/g/h) Trial 1, whereas fish AAV had the minimum mean oxygen consumption (221.7µmolO_2/g/h). In Trial 2 fish RRV had the maximum mean oxygen consumption (285.2µmolO_2/g/h), and fish AVA the minimum mean oxygen consumption (174.7µmolO_2/g/h), while in Trial 3 fish VRR had the maximum mean oxygen consumption (813.5µmolO_2/g/h), and fish AVA the minimum mean oxygen consumption (224.8µmolO_2/g/h).

A significant effect on oxygen consumption (ANOVA two-way, $P < 0.001$) was observed between individuals and also for the interaction individuals x trials (Fig. 14).

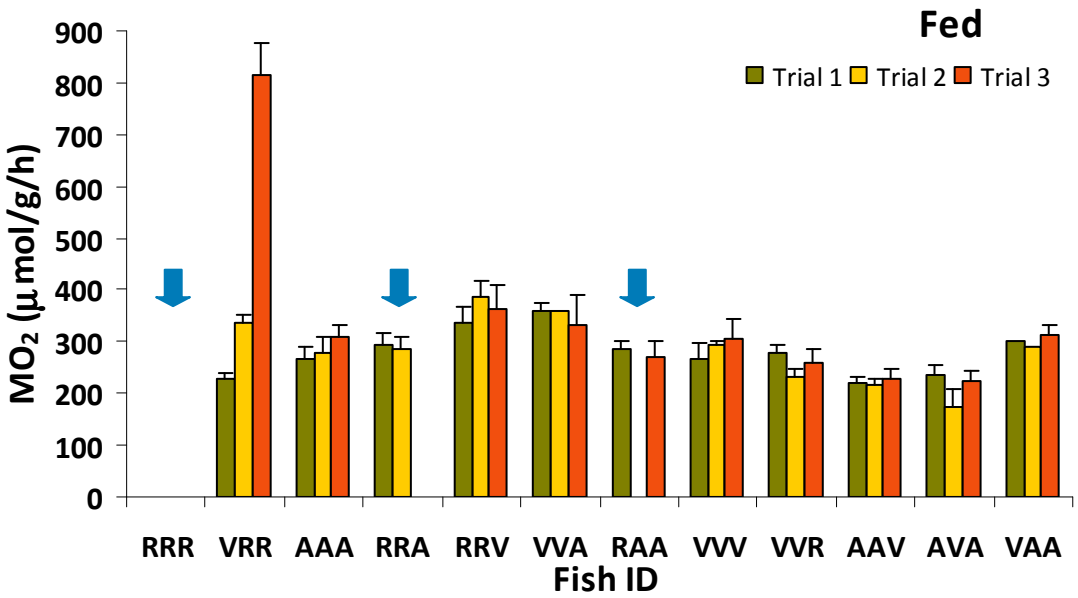

Fig. 14 -Individual measurements of oxygen consumption (µmol O_2/g/h) in Senegalese sole juveniles during the Fed phase at days 120, 127 and 134 (n =11). Fish that did not consume feed, during all trials (RRR) or in one trial (RRA and RAA) were removed from the remaining data analysis and are indicated with the blue arrows.

3.2.3. Oxygen consumption in fish after Emersion stress

The mean results obtained for oxygen consumption in the two trials were 280.6 ± 28.2 µmol O_2/g/h in Trial 1 and 261.2 ± 25.2 µmol O_2/g/h in Trial 2 (Fig. 15). The CV in the Trials 1 and 2 was 10.18% and 9.79%, respectively. Fish VVA

had the maximum mean oxygen consumption (376.7µmolO_2/g/h) Trial 1, whereas fish AVA had the minimum mean oxygen consumption (186.8µmolO_2/g/h). In Trial 2 fish RRV had the maximum mean oxygen consumption (347.3µmolO_2/g/h), and fish VVR the minimum mean oxygen consumption (193.2µmolO_2/g/h).

A significant effect on oxygen consumption (ANOVA two-way, $P < 0.001$) was observed between individuals and also for the interaction individuals x trials (Fig. 15).

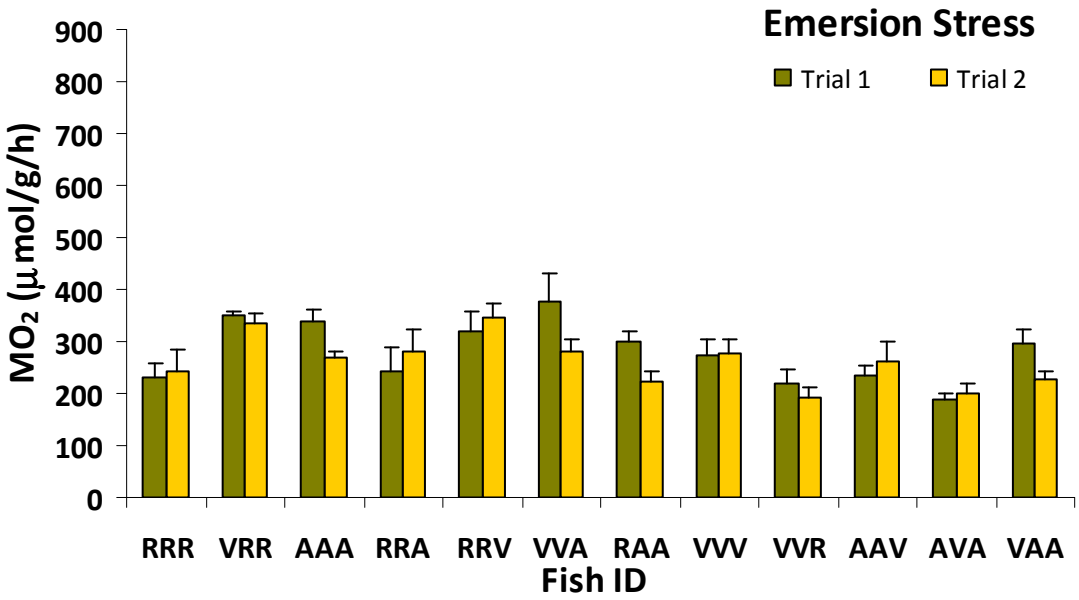

Fig. 15-Individual measurements of oxygen consumption (µmol O_2/g/h) in Senegalese sole juveniles during the Emersion stress phase at days 142 and 163 (n =11).

3.2.4. Comparison of oxygen consumption in different conditions

During the Fasted conditions the mean result obtained for oxygen consumption was 205.5 ± 15.9 µmol O_2/g/h and CV was 8.059%. During the Fed conditions the mean result obtained for oxygen consumption increased to

299.9± 25.9 µmol O_2/g/h, while CV was 8.637 %. The mean result obtained for oxygen consumption per fish in the Emersion stress phase was 270.9 ± 26.7µmol O_2/g/h and CV was 19.98%.

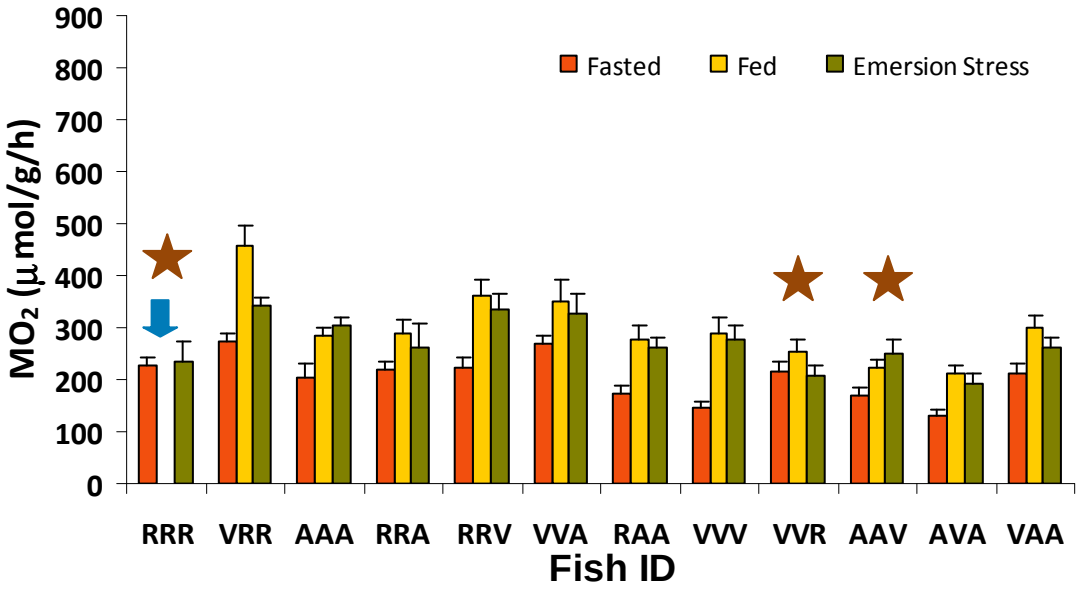

Fig. 16- Individual measurements of Senegalese sole juveniles (n=11) oxygen consumption (µmol O_2/g/h) during the three distinct phases of the experiment (Fasted, Fed and Emersion Stress) 163 days. Fish that did not have food intake, during all trials (RRR) in the phase Fed were removed from the data analysis and are indicated with the blue arrow. The fish were no significant differences were detected are indicated with the brown star.

A significantly difference in oxygen consumption (two-way ANOVA, $P < 0.001$) between the Fasted and Fed phase was observed (Fig. 16). However, no differences were observed in two fish (Newman-Keuls test) in fish AAV, P= 0.109 and in the fish VVR, P = 0.167 (appendix). Comparing the Fasted and Emersion stress phases there was also a significant difference (two-way ANOVA, $P < 0.001$). However, no difference was observed (Newman-Keuls test) in fish RRR, P= 0.300. It be emphasised that values observed for oxygen consumption in the Fasted phase were always higher for the Emersion stress phase with the exception of one fish (VVR).

3.2. Feed intake

During this experiment no variation was observed for feed-intake at days 120, 127 and 134 (one-way ANOVA, P = 0.322). The feed-intake in the Trial 1, Trial 2 and Trial 3 was respectively 1.195 ± 0.390, 1.078 ± 0.404, 0.712 ± 0.396 % of fish body weight. The CV was 32.58% Trial 1, 37.51% Trial 2 and 55.26% Trial 3 (appendix). Individual variation in feed-intake ranged between 0.392% and 1.892% of fish body weight. Fish (RRR) was removed from data analysis because did not consume any feed.

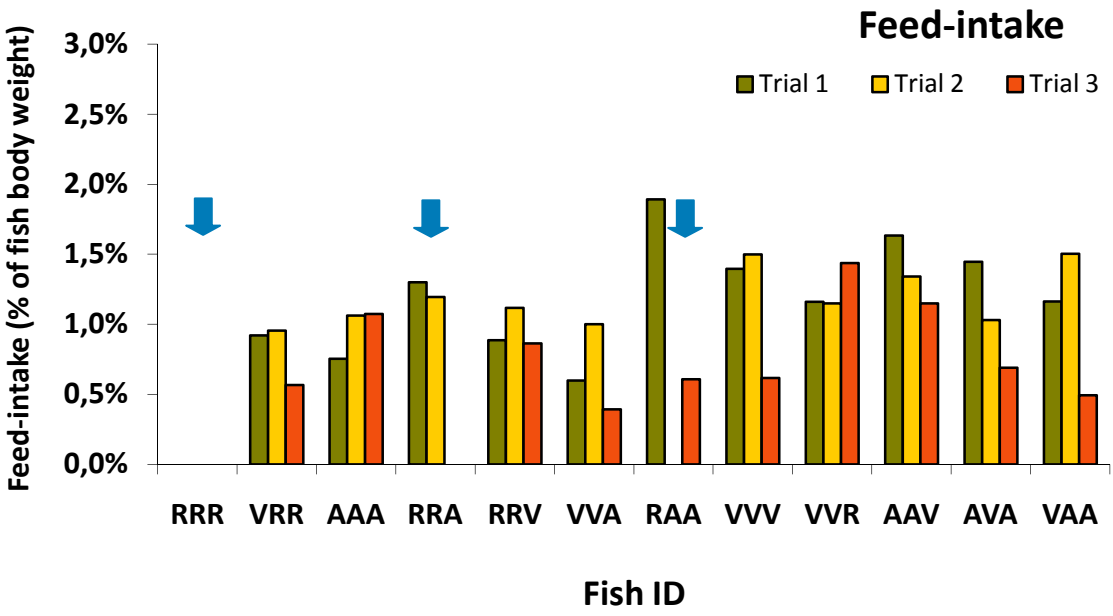

Fig. 17- Individual measurements of feed-intake (% of fish body weight) in Senegalese sole juveniles during the Fed phase at days 120, 127 and 134. Fish that did not had food intake, during all trials (RRR) or in one trial (RRA and RAA) were removed from the data analysis and are indicated with the blue arrows.

3.3. Stress indicators (Cortisol levels during emersion)

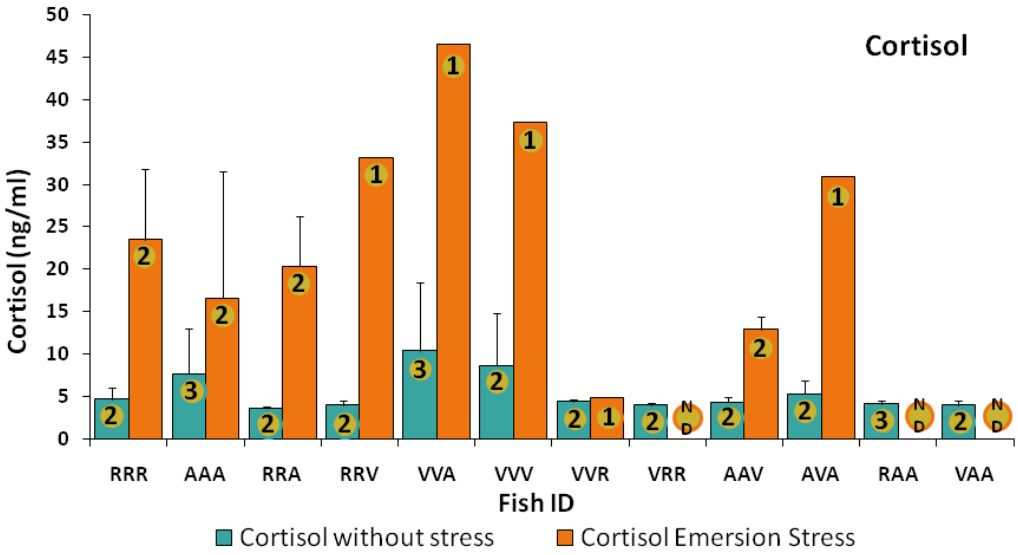

Fig. 18- Individual measurements of Cortisol (ng/ml) in Senegalese sole juveniles with (n=9) and without (n=12) Emersion stress. ND- No Determinations available due to plasma thickning. N values range from 0 to 3 (n values given inside bars) due to plasma thickning.

During the experiment Senegalese sole juveniles exhibited a wide variation in cortisol levels, in fasted fish (days 106, 113 and 127), but mostly in fish after emersion stress (days 142 and 163). There was also a significant difference (t- test, $P < 0.001$) between these two conditions (Fig. 18). It should be noted that the cortisol levels of fasted fish were low and similar in all fish, except in fish AAA, VVA and VVV. VVA showed the maximum mean cortisol levels without stress, 10.39 ± 8.05 ng/ml. The minimum mean value was in RRA, 3.556± 0.195 ng/ml. The levels of cortisol with stress were considerably higher, with a maximum level of 46.45 ng/ml in fish VVA.

3.4. Correlations between variables

Table 2 – Summary of correlation and regression analysis between O_2 consumption ($\mu molO_2/g/h$), cortisol levels (ng/ml), growth (%/day) and feed-intake (%) in individual Senegalese sole juveniles.

Independent variable	Dependent variable	Regression	P-value	R^2	Equation	Figure number
Oxygen consumption Fed	RGR Undisturbed phase (%/day)	**(+)**	0.084	0.294		**19**
Oxygen consumption Fed	RGR Handling phase (%/day)	**NS**	0.200	0.175		**20**
Oxygen consumption Fasted	Oxygen consumption Fed	**+**	0.003	0.632	y=1.2204x + 51.39	**21**
Oxygen consumption Fasted	Oxygen consumption Emersion Stress	**+**	0.002	0.390	y=0.6845x + 130.19	**23**
Oxygen consumption Fasted	Oxygen consumption Δ Fed	**NS**	0.495	0.053		**22**
Oxygen consumption Fasted	Oxygen consumption Δ Emersion Stress	**NS**	0.270	0.120		**24**
Feed-intake	Oxygen consumption	**-**	0.017	0.482	y=-385.39x + 17.409	**25**
Feed-intake	Oxygen consumption Fasted	**NS**	0.270	0.436		**25**
Cortisol without stress	Cortisol with emersion stress	**(-)**	0.070	0.393		**26**
Feed-intake	Cortisol with emersion stress	**(-)**	0.108	0.372		**27**
Feed-intake	Δ Cortisol	**(-)**	0.117	0.358		**28**
Cortisol with emersion stress	RGR Undisturbed phase	**NS**	0.733	0.017		**29**
Cortisol with emersion stress	RGR Handling phase	**(+)**	0.077	0.379		**29**
Cortisol with emersion stress	Oxygen consumption Emersion Stress	**(+)**	0.144	0.278		**30**
Feed-intake	RGR Undisturbed phase	**NS**	0.635	0.040		**31**

"Regression" column gives the result as: **NS** not significant; **+** positive correlation ($P<0.05$); **-** negative correlation; (**+**) tendency for increase ($P<0.15$); (**-**) tendency for decrease; O_2Fed = O_2 consumption fed fish - O_2 consumption fasted fish; ΔO_2stress = O_2 consumption stressed fish - O_2 consumption fasted fish; Δcortisol= cortisol emersion stressed fish - cortisol fasted fish.

3.4.1. *Correlation between oxygen consumption and growth*

There were no significant relationships between relative growth rate (RGR) of individual sole during the Undisturbed part (P=0.084; R^2=0.294) or the Repeated Handling part (P=0.200; R^2=0.175) and results obtained for oxygen consumption on the same fish when Fed (Table 2, Fig. 19 and 20).

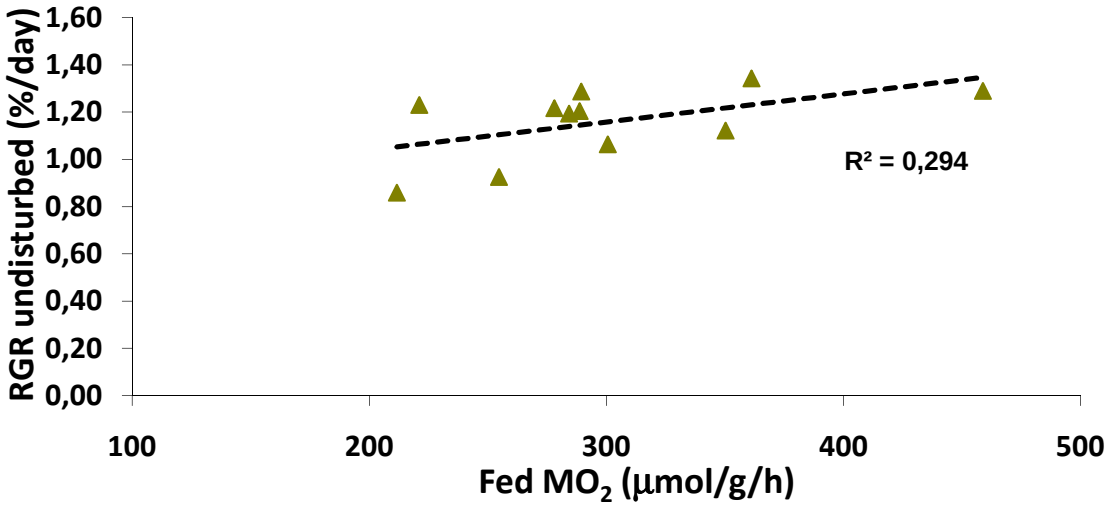

Fig. 19- Relation between RGR during the Undisturbed phase (%/day) and oxygen consumption (µmol O_2/g/h) in fed Senegalese sole juveniles (n = 11).

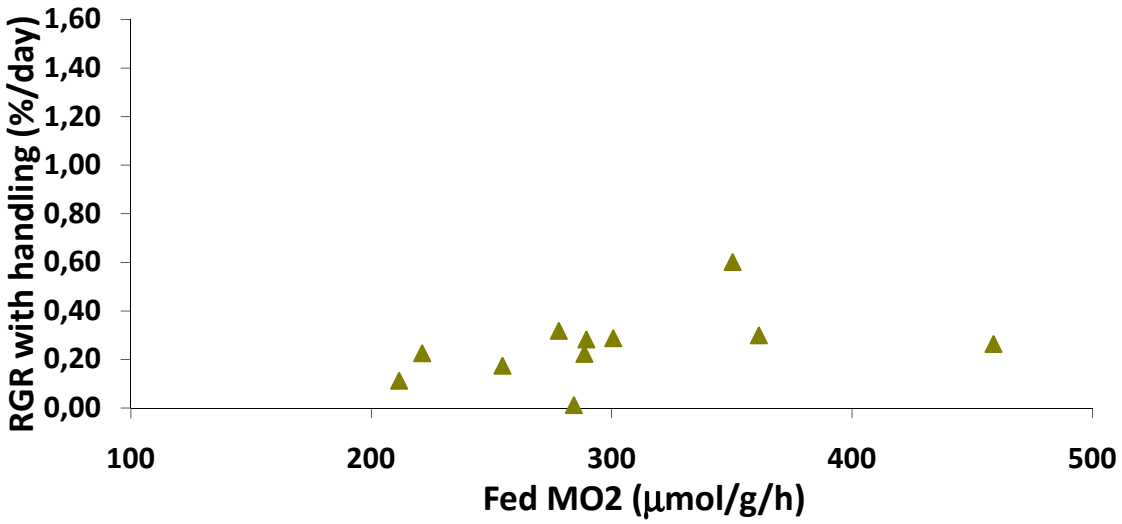

Fig. 20- Relation between RGR with Repeated handling phase (%/day) and oxygen consumption (µmol O_2/g/h) in fed Senegalese sole juveniles (n = 11).

There were no significant relationships between relative growth rate (RGR) of individual sole during the Undisturbed part (P=0.072; R^2=0.287) or the Repeated Handling part (P=0.250; R^2=0.129) and results obtained for oxygen consumption on the same fish when Fasted.

3.4.2. Correlation between oxygen consumption in Fasted and Fed fish

The oxygen consumption when individual sole were Fasted was positively correlated (P=0.003) with oxygen consumption on the same fish when Fed. This relation is described by the regression equation $y=1.2204x + 51.39$, R^2=0.632 (Fig. 21). Furthermore, statistical differences were found in the phases mentioned above (two-way ANOVA, P<0.001). However, no differences were observed in two fish as reported earlier. Those fish (AAV and VVR) were represented in brown colour in Fig. 21.

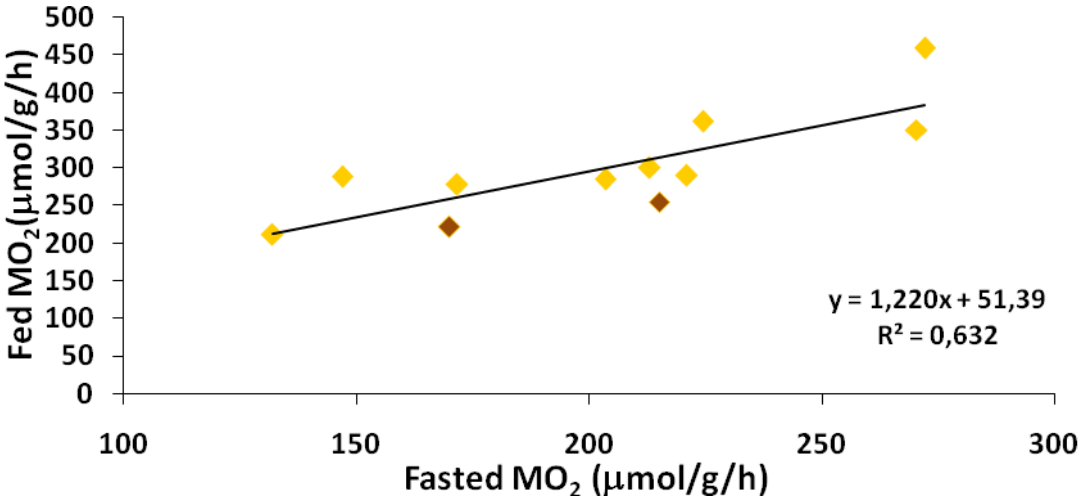

Fig. 21- Relationship between measurements oxygen consumption (µmol O_2/g/h) in Fed and Fasted Senegalese sole juveniles (n = 11; P=0.003).

Correlation between oxygen consumption Fed phase (ΔO2Fed) and Fasted phase

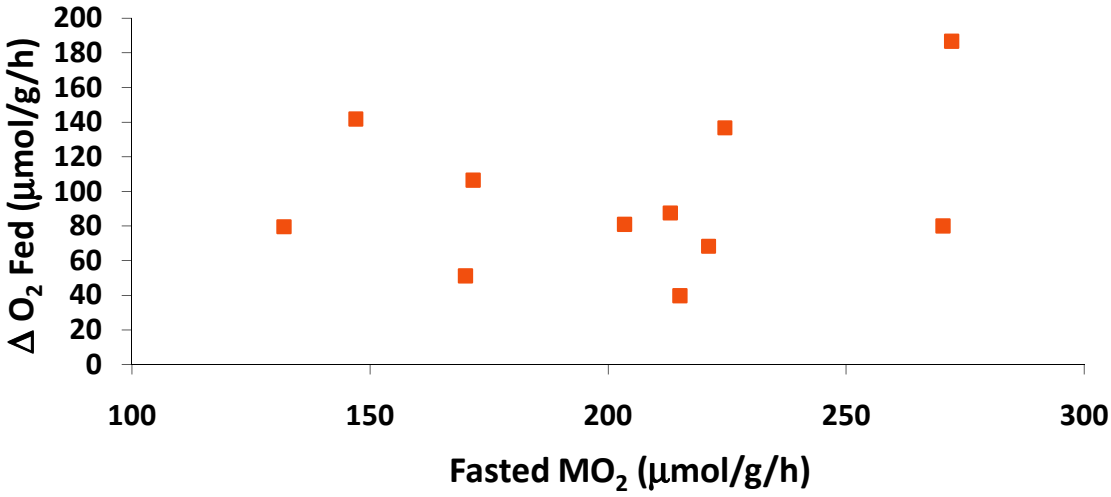

Fig. 22- Correlation between measurements oxygen consumption (µmol O_2/g/h) in Fed (ΔO_2Fed) and Fasted Senegalese sole juveniles (n=11).

There was no significant relationship between oxygen consumption increase due to feeding (ΔO_2Fed = O_2 consumption fed fish - O_2 consumption fasted fish) of individual sole and results obtained for oxygen consumption on the same fish when Fasted; P=0.495 and R^2=0.053 (Table 2, Fig. 22).

3.4.3. Correlation between oxygen consumption in Fasted and Stressed fish

The oxygen consumption when individual sole were Fasted was positively correlated (P=0.002) with oxygen consumption on the same fish when Emersion stress. This relation in described by the regression equation y=0.6845x + 130.19, R^2=0.390 (Fig. 23). Furthermore, statistical differences were found in the phases mentioned above (two-way ANOVA, P<0.001). However, no differences were observed in (RRR) fish as reported earlier. This fish was represented in brown colour in Fig. 23.

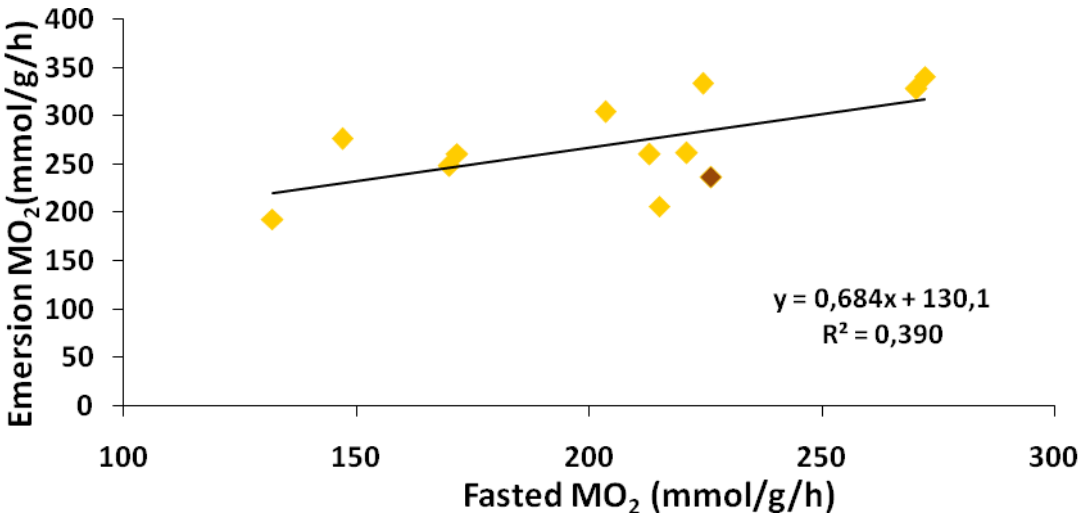

Fig. 23- Relationship between measurements oxygen consumption (µmol O_2/g/h) in Emersion stress and Fasted Senegalese sole juveniles (n = 12; P=0.002).

Correlation between oxygen consumption Emersion Stress phase (ΔO2Fed) and Fasted phase

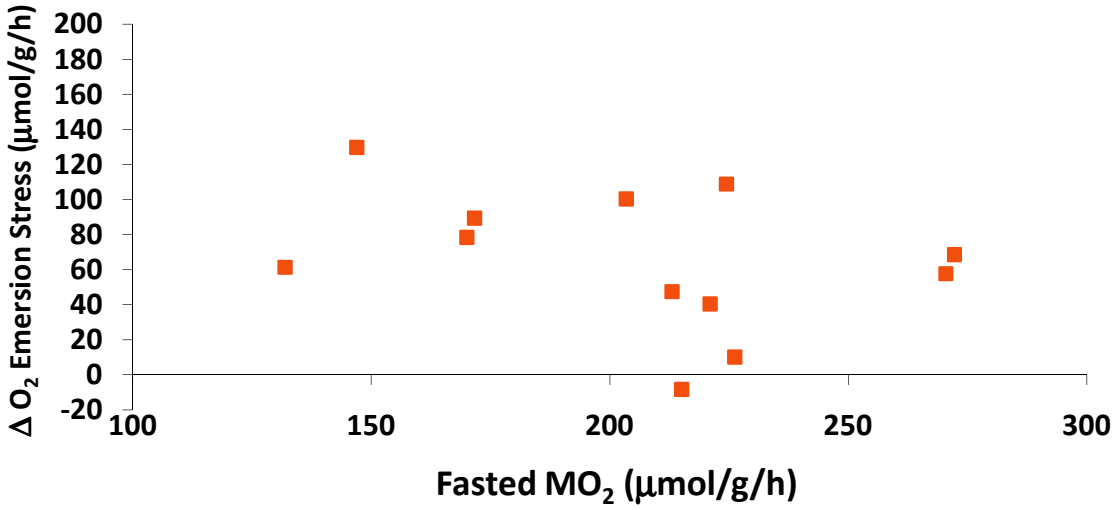

Fig. 24- Correlation between the measurements of oxygen consumption (µmol O_2/g/h) in Emersion Stress (ΔO_2Emersion stress) and Fasted Senegalese sole juveniles (n=12).

There was no significant relationship between oxygen consumption increase due to Emersion stress (ΔO_2stress = O_2 consumption stressed fish - O_2 consumption fasted fish) of individual sole and results obtained for oxygen consumption on the same fish when Fasted, P=0.270 and R^2=0.120 (Table 2, Fig.24).

3.4.4. Correlation between oxygen consumption and feed intake

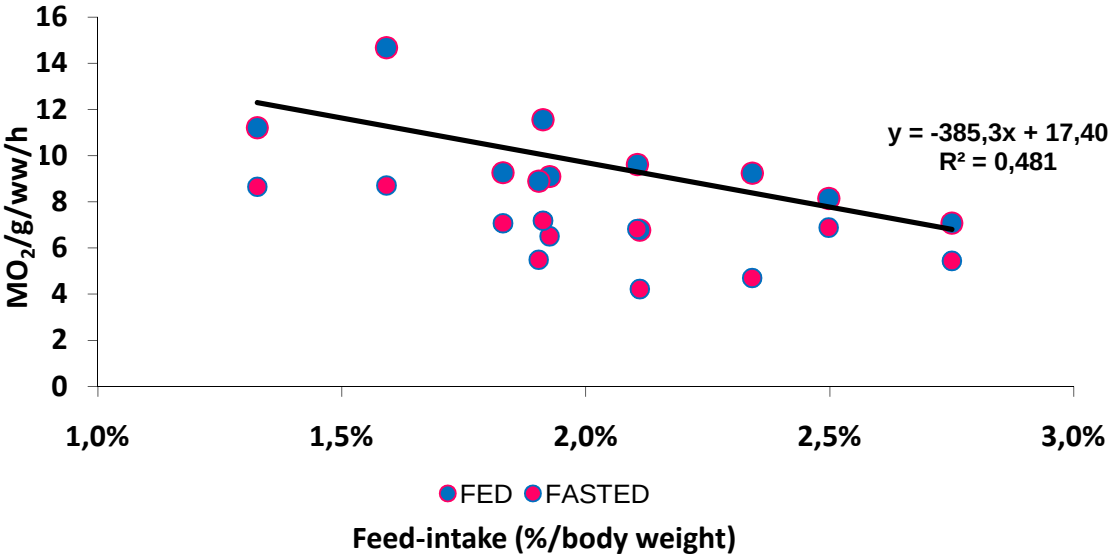

Fig. 25- Correlation between the measurements of oxygen consumption (g/WW/h) in Fasted (n=12) and Fed (n=11) Senegalese sole juveniles and their feed-intake (% fish body weight).

The feed-intake when individual Senegalese sole were Fed was negatively correlated (P=0.017) with oxygen consumption on the same fish when Fed, this relation being described through the regression equation y=-385.39x + 17.409, R^2=0.482 (Table 2, Fig. 25).

There was no significant relationship between the feed intake when individual Senegalese sole were Fasted with oxygen consumption on the same fish when Fasted, P=0.027, R^2=0.436 (Table 2, Fig. 25).

3.4.5. Correlations between cortisol levels and other variables

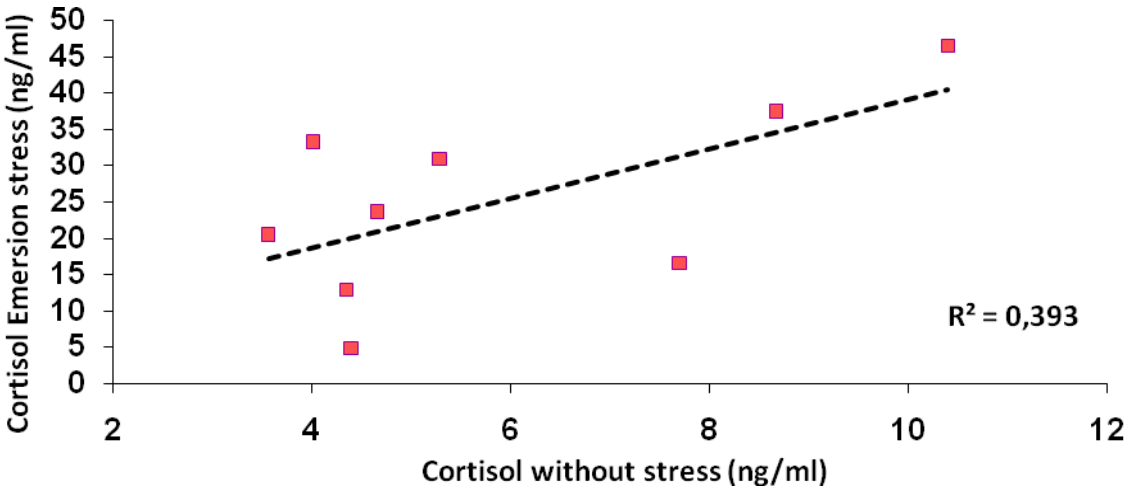

Fig. 26- Relation between Cortisol (ng/ml) with and without Emersion stress in Senegalese sole juveniles (n=9).

The cortisol when individual Senegalese sole where undisturbed showed a positive tendency (P=0.070) for a positive correlation with levels of cortisol on the same fish when under emersion stress (R^2=0.393, Fig. 26).

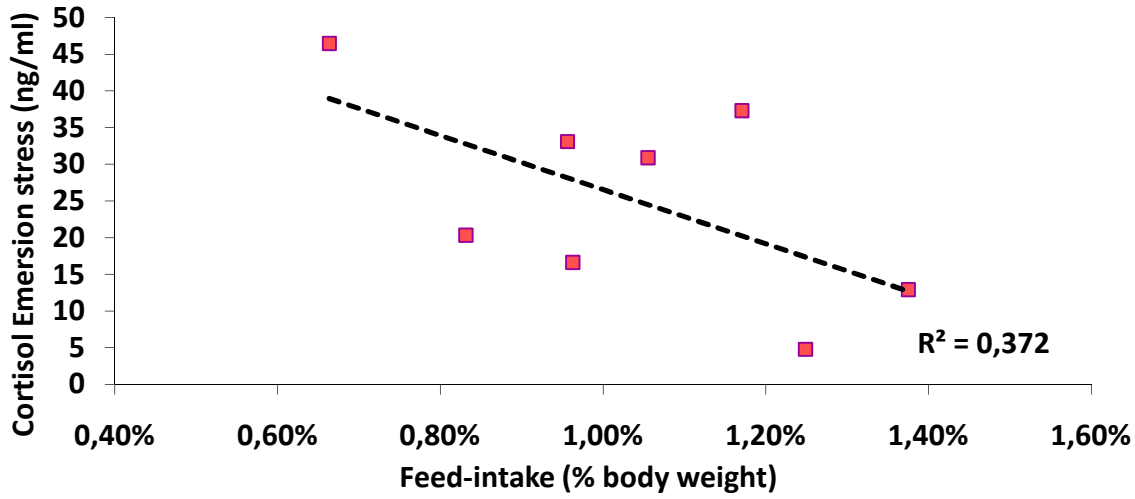

Fig. 27- Correlation between the measurements of Feed/intake (% body weight) in Fed Senegalese sole juveniles (n=8) and cortisol after emersion stress (ng/ml).

The Feed-intake in individual Senegalese sole showed a negative tendency (P=0.108) for correlation with cortisol levels on the same fish emersion stress (R^2=0.372, Fig. 27).

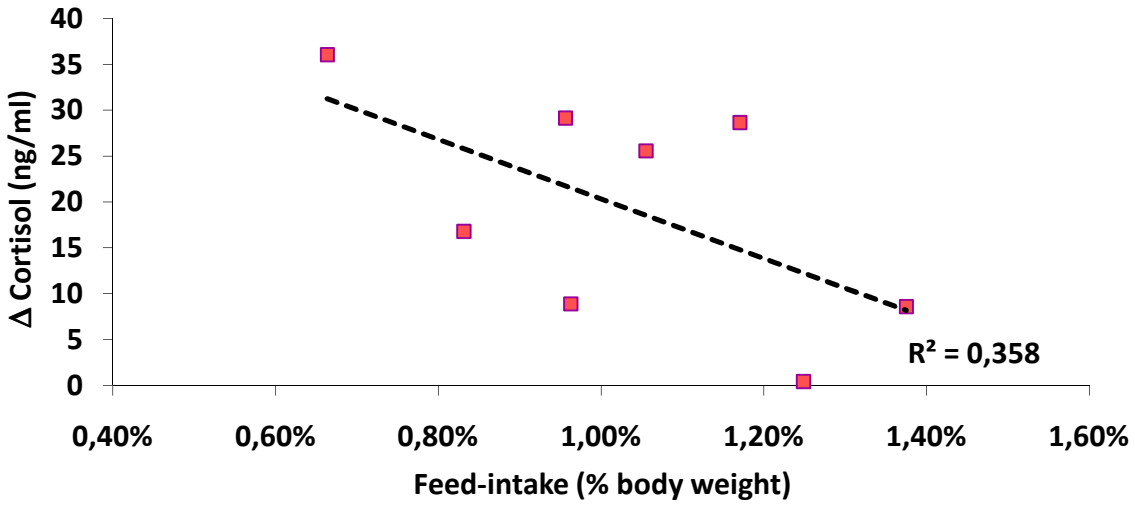

Fig. 28- Correlation between the measurements of Feed/intake (%) in Fed Senegalese sole juveniles (n=8) and Δcortisol (ng/ml). Δcortisol = Δcortisol with emersion stress - Δcortisol without emersion stress.

The Feed-intake in individual Senegalese sole showed a negative tendency (P=0.117) for correlation with Δ levels of cortisol on the same fish, (R^2=0.358, Fig. 28).

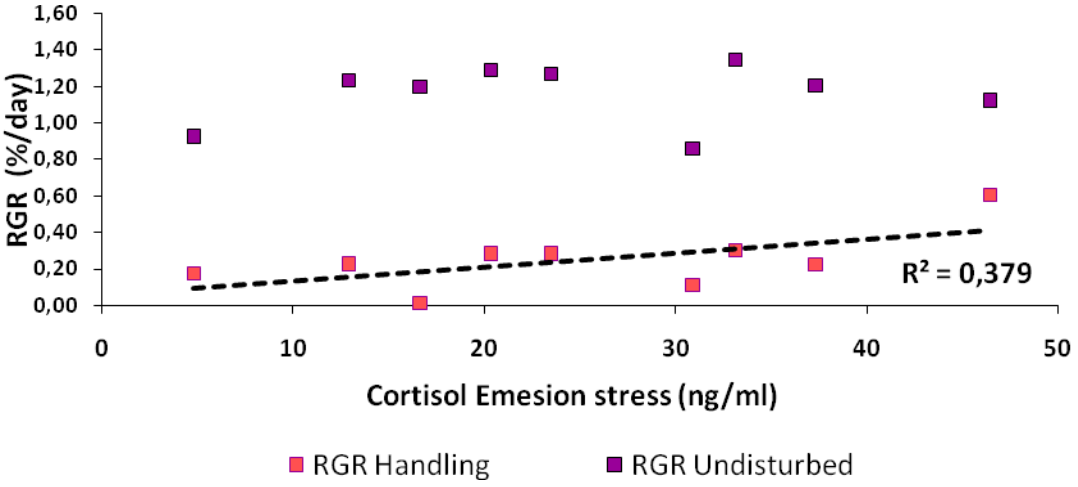

Fig. 29- Correlation between the measurements of cortisol with emersion stress (ng/ml) in Senegalese sole juveniles and RGR (%/day) Undisturbed or after repeated Handling (n=9).

There was no significant relationship between cortisol levels after emersion stress and RGR Undisturbed of individual Senegalese sole (Fig. 29, P=0.733; R^2=0.0176). However, cortisol levels after emersion stress showed a positive tendency (P=0.077; R^2=0.379) for correlation with RGR after repeated Handling (Table 2, Fig. 29).

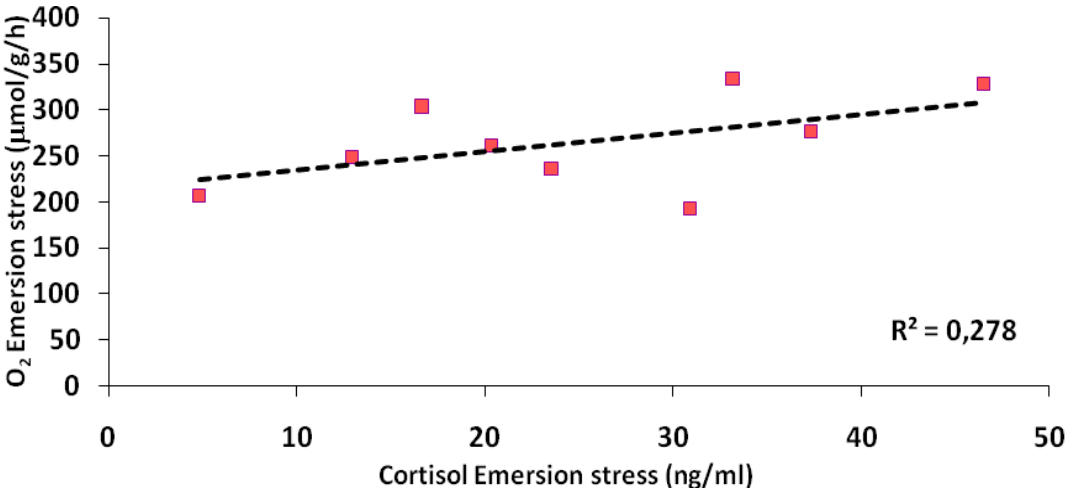

Fig. 30- Correlation between the measurements of cortisol with emersion stress (ng/ml) in Senegalese sole juveniles and measurements oxygen consumption (µmol O_2/g/h) in Emersion stress (n=9).

In this study there was no significant relationship between cortisol levels after emersion stress and oxygen consumption on the Emersion stress of individual Senegalese sole. However, cortisol levels after emersion stress showed a positive tendency (P=0.144; R^2=0.278) for correlation with measurements of oxygen consumption (Table 2, Fig. 30).

3.4.6. Correlations between feed intake and growth

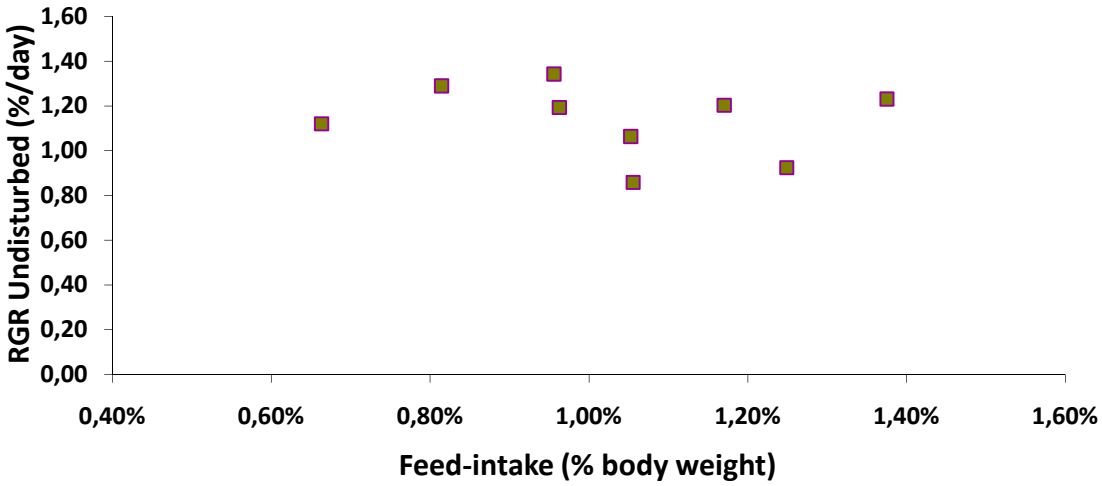

Fig. 31- Correlation between the measurements of Feed-intake (% Body weight) in Fed Senegalese sole juveniles (n=8) and RGR Undisturbed (%/day).

There were no significant relationships between feed intake of individual sole (P=0.635; R^2=0.040) and results obtained for RGR on the same fish when Undisturbed (Table 2, Fig. 31).

4. DISCUSSION

4.1. How variable are individual growth, feed intake and metabolic rate of sole juveniles?

In the present study fish exhibited a pronounced variation in performance. From 18.49 ± 2.94g of wet weight at the beginning of the experiment, the Senegalese sole juveniles increased body weight by about two-fold 48.45 ± 10.63g. In the Undisturbed part of the experiment the fish had a mean RGR of 1.167 ± 0.149 %/day, while during the Repeated handling part the mean RGR was significantly reduced by about five-fold to 0.256 ± 0.140 %/day (Fig.10 A and B).

The difference in growth performance may be explained by several factors, like social hierarchy, feeding behaviour, stress and genetics (Irwin *et al.*, 1999b, Jobling and Baardvik, 1994). In *Tilapia zilii* the hierarchy effect contributes to higher individual growth dispersion. The dominant fish is able to efficiently defend the food supply, resulting in an higher growth of the dominant relative to the subordinates (Koebele, 1985). However, the present study showed that fish with higher initial weight was not necessarily the same fish with higher weight in the end of the experiment. This is possible to verify in the fish AAA and VVA for example. This result suggests that probably social hierarchy is not playing a major role in explaining weight variation in Sole.

There is an evidence of a genetic basis for inter-individual variation in food consumption and growth of hybrid sunfish, housed individually suggesting that these differences have a genetic basis (Wang *et al.*, 1998). This study suggest that the individual variation in growth is not dependent of social hierarchy but may be instead genetical, in line with results obtained by Martins (2005) in African catfish. On the other hand Irwin *et al.*, (2002) attributed some variations in growth of Turbot to genetic differences. However, social interactions are believed to be a major contributor to differential growth.

Several studies have shown that stressful conditions may result in reduced growth (Van Weerd and Komen, 1998). Under intensive fish culture stressful conditions (30kg/m^3) brook trout (*Salvelinus fontinalis*) showed a decrease in final body weight (Vijayan *et al.*, 1990). In the present work marked differences in individual

RGR between the undisturbed and handling phase were observed. Probably this is related to a chronic stress situation induced by repeated handling.

However, physiological variables, also influence growth of individual fish and may also explain some of the variation in growth not accounted for by differing rates of consumption. Rates of protein synthesis are correlated with growth rate and account for a large proportion of total energy expenditure in fish (Houlihan, 1991). In grass carp and rainbow trout individual differences in protein turnover contribute to the individual variation in growth (Carter *et al.*, 1992).

The observed differences in growth rate could be explained by differences in feed intake, as suggested earlier for African catfish (Martins *et al.*, 2006b). In the present study, fish exhibited an average feed intake in Trial 1, Trial 2 and Trial 3 around 1.195 ± 0.390, 1.078 ± 0.404, 0.712 ± 0.396% body weight. This is still lower than reported feeding levels of 1.39% for Senegalese sole on similar conditions (Coutteau *et al.*, 2001). The CV for feed intake was 32.58% in Trial 1, 37.51% in Trial 2 and 55.26% in Trial 3. A high coefficient of variation in fish may arise from aggressive behavior and/or reduced availability of food to less competitive animals (Jobling and Wandsvik, 1983). Despite the absence of apparent aggressive behavior in our study, the high coefficients of variation suggest that a feeding hierarchy may have existed. Experiments with turbot indicate that larger juveniles cause stress to smaller fish, preventing them from obtaining a normal feed intake (Carter *et al.*, 1996).

The growth rate of fish is strongly influenced by metabolic rate (Jobling *et al.*, 1989). Metabolic rates can be quantified as rates of oxygen uptake. Metabolic rate is highly variable since it is influenced by environmental factors, body size and composition, nutritional status and activity (Chappel *et al.*, 1999). Some of the above factors mainly individual body composition and nutritional status were not controlled in the present study, and may influence the individual metabolic rates. In Chinese sturgeon metabolic rate was a determinant factor for differences in individual growth rate and had a genetic influence (Qian *et al.*, 2002). In this study Senegalese sole showed a high variability in oxygen consumption of fasted fish, suggesting a possible relation with genetic basis.

In addition, the individual variation in oxygen consumption showed higher oxygen consumption on fed fish. This suggests that oxygen consumption may increase

as a result of the digestive process and/or postprandial metabolism. In rainbow trout and African catfish the cost of maintenance reduced with increasing feeding level to accommodate the cost of growth, what shows that the costs of maintenance and growth may not always be additive (Conceição *et al.*, 1998). Therefore, it cannot be excluded that also in Senegalese sole the cost of maintenance is variable depending on feeding level.

4.2. Does growth rate correlate with feed intake?

In relation to feed intake, the development of a noninvasive X-radiographic technique for measuring food consumption by fish has greatly facilitated the investigation of meal sharing among individual fish within groups (Rombough, 1994). Several studies have shown that most of variation in growth of fish is due the variation in feed intake (McCarthy *et al.*, 1992). Radiography (X-ray) has been used to measure individual consumption rates for groups of fish and has demonstrated considerable inter-individual variation in food intake (Carter *et al.*, 1992). For juvenile turbot using data from individual fish a strong positive correlation in consumption and growth has been shown (Jobling *et al.*, 1989, Thodesen *et al.*, 2001). According to Irwin *et al.*, (2002), in grass carp the higher feed intake was associated to higher growth, with variations up to 14%. In Chinese sturgeon the individual differences in feed intake were positively correlated to individual differences in growth, suggesting that those individuals that consumed the highest quantities of feed were also those that had the highest rates of weight gain (Carter *et al.*, 1992).

However, no significant correlation between feed intake and RGR was seen in the present study. The fish with high feed intake were not necessarily the fish with higher growth. This suggest that feed intake is not a good indicator of the growth rate and others factors such as digestibility, metabolic rate or other genetic factors may play an important role in determining growth rate. Alternatively, the measurement of feed intake by the method of X-ray may not be a good indicator of feed intake for an extended period.

4.3. Does growth rate correlate with fish metabolic rate?

Understanding how metabolic rate affect the individual growth is crucial to maximize production in aquaculture. It is difficult to define a basal oxygen consumption rate, as the experimental conditions imposed on the fish during the oxygen consumption measurements processes may significantly affect fish metabolic rate. Therefore, it is necessary to specify clearly the environmental and experimental conditions in which oxygen consumption measurements are done (Qian *et al.*, 2002).

Metabolic rate has been found to correlate with fish growth (Mitz and Newman, 1989). Estimation of the costs of growth relies on the assumptions that the cost of growth and maintenance are additive, i.e. the costs of maintenance will not change with different sizes and/or feeding levels, and the cost of growth is constant. Above the cost of growth, further increases in growth rate were not matched by an increase in the rate of respiration (Cutts *et al.*, 1998). No correlation was found between oxygen consumption in Fasted or Fed fish and RGR (Undisturbed or with Handling) and RGR in the present study. This suggests that the relative growth rate is not directly influenced by metabolic rate. This also suggests that a difference in sole growth potential is not due to differences in energy costs of maintenance, or to the efficiency of utilization of food. Instead, fish that grow more are probably those who have a higher consumption of food and / or increased digestive efficiency. In fact, an increase in metabolic rate does not necessarily compete with growth, as both processes are complementary and indissociable (Wootton, 1998).

4.4. Does stress affect growth and feed intake of sole juveniles?

An intriguing question is to understand how stress affects the individual growth rate and feed intake of juveniles of Senegalese sole. Chronic stress normally leads to an increase in plasma cortisol (Cunha *et al.*, 2007). Plasma cortisol level is a common stress indicator of stressful conditions in fish because an increase in plasma cortisol promotes various secondary physiological responses (Barton, 2002). This response may possibly decrease growth rate and feed intake (Barton, 2002, Costas *et al.*, 2008). In

the current experiment, it seems clear that the fish after emersion were stressed, based on the results of plasma cortisol, despite the variability in plasma cortisol values responses among individual Senegalese sole. The present results revealed no significant correlation between plasma cortisol values and RGR of undisturbed fish. However, the results from this study revealed a positive tendency between plasma cortisol values after emersion stress and RGR under repeated handling. These findings suggest that the stress susceptibility seems independent of the individual growth. During the undisturbed phase the fish with high cortisol values were not necessarily the fish with lower growth. Nevertheless in fish after emersion stress the slight tendency observed suggests a possible relation with growth under chronic stress. However, further studies should be done with a higher number of fish to confirm (or not) this tendency.

The physiological response of a fish to chronic stress results in different responses in feed intake in different species. A 91% decrease in feed intake was found in brook trout (*Salvelinus fontinalis*) exposed to a low environmental pH during 60 days (Arjona *et al.*, 2009). But in brown trout (*Salmo trutta*) that had been subjected to handling stress during 28 days, only a suppression of feeding for the first three days was observed (Mackett *et al.*, 1992). The present study suggests a negative tendency between feed intake and plasma cortisol levels in individual Senegalese sole juveniles. This may indicate that feed intake is a reasonable predictor of susceptibility to stress in Senegalese sole juveniles, but this should be confirmed in future studies.

4.5. Does stress affect metabolic rate of sole juveniles?

This study presented a significantly different metabolic rate in fasted and Senegalese sole juveniles after emersion stress. Under stressful conditions the fish needs energy substrates in tissues such as brain, gills and muscles, in order to cope with the increased energy demands (Barton *et al.*, 1987). Moreover, the results from the present study revealed a positive tendency for correlation between plasma cortisol

values after emersion stress and measurements of metabolism after emersion stress. This tendency is in line with the generally accepted idea that metabolic rate increases under stressful conditions, but further studies should be done with a higher number of fish.

4.6. How does individual variation relate with stress response?

In this study, individual variation in stress response was distinct and may relate with different coping styles. Coping styles seem to have a genetic base and indicate individual difference within a population; these differences represent adaptative individual differences in resource use and response to a stressful condition (Seppãnen *et al.*, 2008). Various authors use the term shyness and boldness to indicate individual differences in personality traits in fish (Koolhaas *et al.*, 1999). The present results suggest an individual difference in stress response as fish with high cortisol levels has a tendency to have higher growth and present a lower metabolic rate. Probably this is an intrinsic characteristic of the fish and possibly it is related to personal traits. Bold individual may present a lower stress response than shy conspecifics and this may be reflected in growth and metabolic rate. However, this hypothesis needs further investigation in sole. It would be interesting to see to what extent coping styles impact on resistance of pathogens and/or situations of additional acute stress.

5. CONCLUSIONS

It is hypothesized that the pronounced individual growth of Senegalese sole (*Solea Senegalensis*) is probably the result of interaction between genetic and environmental factors.

Senegalese sole feed intake (as measure by radiography) does not seem to be a good indicator of growth rate. In fact, the measurement of feed intake by the method of X-ray may not be a good indicator of feed intake for an extended period.

Oxygen consumption of individual fish increases both when sole are fed or subjected to emersion stress, showing that at least part of the maintenance costs are additive to the cost of growth and the cost of stress.

Individual differences in metabolic rate do not contribute to explain individual differences in growth.

Individual differences in stress response seem to play a role in explaining individual differences in growth. In this study, the most efficient individual were a high growth and low stress responders. It could well be that more efficient individuals have an active coping style and less efficient animals a passive coping style.

Altogether, this study suggests that individual variation of growth in *Solea senegalensis* is essentially due to genetic variation, and its reduction may accomplished when genetic selection programs are adopted. Furthermore, a study with a larger number of fish and over a larger time period would be important to check some of the trends found in this thesis between growth potential, metabolic rate and coping styles of Senegalese sole.

6. REFERENCES

Ambrosio, P.P., Costa, C., Sánchez, P. & Flos, R. (2008) Stocking density and its influence on shape of Senegalese sole adults. *Aquaculture International,* **16,** 333-343.

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

Andrew, J.E., Holm, J., Kadri, S. & Huntingford, F.A. (2004b) The effect of competition on the feeding efficiency and feed handling behaviour in gilthead sea bream (Sparus aurata L.) held in tanks. *Aquaculture,* **232,** 317-331.

Aragao, C., Corte-Real, J., Costas, B., Dinis, M.T. & Conceicao, L.E.C. (2008) Stress response and changes in amino acid requirements in Senegalese sole (Solea senegalensis Kaup 1858). *Aquaculture Research,* **34,** 143-148.

Arjona, F.J., Vargas-Chacoff, L., Ruiz-Jarabo, I., Gonçalves, O., Páscoa, I., Martín del Río, M.P. & Mancera, J.M. (2009) Tertiary stress responses in Senegalese sole (Solea senegalensis Kaup, 1858) to osmotic challenge: Implications for osmoregulation, energy metabolism and growth. *Aquaculture,* **287,** 419-426.

Ashley, P.J. (2007) Fish welfare: Current issues in aquaculture. *Applied Animal Behaviour Science,* **104,** 199-235.

Bagenal, T.B. (2006) The interrelation of the size of fish eggs, the date of spawning and the production cycle. *Journal of Fish Biology,* **3** 207 - 219.

Barki, A., Harpaz, S., Hulata, G. & Karplus, I. (2004) Effects of larger fish and size grading on growth and size variation in fingerling silver perch *Aquaculture International,* **8,** 391-401.

Barton, B.A. (2002) Stress in Fish: A Diversity of Responses with Particular Reference to Changes in Circulating Corticosteroids In *Integrative and Comparative Biology* Vol. 42, 517-255

Barton, B.A. & Iwama, G.K. (1991) Physiological changes in fish from stress in aquaculture with emphasis on the reponse and effects of corticosteroids *Annual Rev Fish Diseases,* Vol. 1, 3-26.

Barton, B.A., Schreck, C.B. & Barton, L.D. (1987) Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. *Diseases of Aquatic Organisms* **2,** 173–185.

Berkeley, S.A., Chapman, C. & Sogard, S.M. (2004) Maternal Age as a determinant of larval growth and survival in marine fish, *Sebastes melanops Ecology,* **85,** 1258-1264.

Butts, I.A.E. & Litvak, M.K. (2007) Parental and stock effects on larval growth and survival to metamorphosis in winter flounder (Pseudopleuronectes americanus). *Aquaculture,* **269,** 339-348.

Carter, C.G., Houlihan, D.F., McCarthy, I.D. & Brafield, A.E. (1992) Variation in the food intake of grass carp, Ctenopharyngodon idella (Val.), fed singly or in groups. *Aquatic Living Resources* **5,** 225-228.

Carter, C.G., Purser, G.J., Houlihan, D.F. & Thomas, P. (1996) The effect of decreased ration on feeding hierarchies in groups of greenback flounder (*Rhombosolea tapirina*: Teleostei). *Journal Marine Biology,* **76**, 505-516.

Chappel, M.A., Bech, C. & & Buttermer, W.A. (1999) The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *Experimental Biology,* **202,** 2269-2279

Conceição, L.E.C. (1997) Growth in early life stages of fishes: an explanatory model In *Fish Culture and Fisheries,*Wageningen Agricultural University, The Netherlands, 209pp.

Conceição, L.E.C., Dersjant-Li, Y. & Verreth, J.A.J. (1998) Cost of growth in larval and juvenile African catfish (Clarias gariepinus) in relation to growth rate, food intake and oxygen consumption. *Aquaculture,* **161,** 95-106.

Conceição, L.E.C., Ribeiro, L., Engrola, S., Aragão, C., Morais, S., Lacuisse, M., Soares, F. & Dinis, M.T. (2007) Nutritional physiology during development of Senegalese sole (Solea senegalensis). *Aquaculture,* **268,** 64-81.

Costas, B., Aragão, C., Mancera, J.M., Dinis, M.T. & Conceição, L.E.C. (2008) High stocking density induces crowding stress and affects amino acid metabolism in Senegalese sole Solea senegalensis (Kaup 1858) juveniles. *Aquaculture Research,* **39,** 1-9.

Coutteau, P., Robles, R. & Spruyt, W. (2001) Ongrowing feed for Senegalese sole. *INVE - Aquaculture division - Farm nutrition.*

Cubitt, K.F., Winberg, S., Huntingford, F.A., Kadri, S., Crampton, V.O. & Øverli, Ø. (2008) Social hierarchies, growth and brain serotonin metabolism in Atlantic salmon (Salmo salar) kept under commercial rearing conditions. *Physiology & Behavior,* **94,** 529-535.

Cunha, I., Conceição, L.E.C. & Planas, M. (2007) Energy allocation and metabolic scope in early turbot, Scophthalmus maximus, larvae. *Marine Biology,* **151,** 1397–1405.

Cutts, C.J., Metcalfe, N.B. & Taylor, A.C. (1998) Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard metabolic rate. *Journal Fish Biology,* **52,** 1026–1037.

Dinis, M.T. (1986) Quatre Soleidae de l'estuaire du Tage. Reproduction et croissance. Essai d'elevage de Solea senegalensis Kaup 1858. Université de Bretagne Occidentale, France, 348 pp.

Dinis, M.T., Ribeiro, L., Soares, F. & Sarasquete, C. (1999) A review on the cultivation potential of Solea senegalensis in Spain and in Portugal. *Aquaculture,* **176,** 27-38.

Dwyer, K.S., Brown, J.A., Parrish, C. & Lall, S.P. (2002) Feeding frequency affects food consumption, feeding pattern and growth of juvenile yellowtail flounder (Limanda ferruginea). *Aquaculture,* **213,** 279-292.

Engrola, S., Cação, P., Conceição, L.E.C. & Dinis, M.T. (2001) Ongrowing of sole (Solea senegalensis) juveniles at diferent densities In *Book of Abstracts of the World Aquaculture 2001 WAS Conference* (2001, B.o.A.o.t.W.A. ed. Book of Abstracts of the World Aquaculture 2001, Lake Buena Vista, Florida, USA.

Engrola, S., Conceicao, L.E.C., Dias, L., Pereira, R., Ribeiro, L. & Dinis, M.T. (2007) Improving weaning strategies for Senegalese sole: effects of body weight and digestive capacity. *Aquaculture Research,* **38,** 696-707.

FAO (2008) *The State of world fisheries and aquaculture*.

Frisch, A. & Anderson, T. (2005) Physiological stress responses of two species of coral trout (Plectropomus leopardus and Plectropomus maculatus). *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology,* **140,** 317-327.

Haylor, G.S. (1993) Controlled hatchery production of Clarias gariepinus (Burchell 1822): an estimate of maximum daily feed intake of C. gariepinus larvae. *Aquaculture and Fisheries Management,* **24,** 473-482.

Houlihan, D.F., Carter, C.G. & McCarthy, I.D. (1995) Protein Turnover in Animals In *Nitrogen metabolism and Excretion* (Wright, P.J.W.a. ed.). CRC Press, Boca Raton, 32pp.

Imsland, A.K., Foss, A., Conceição, L.E.C., Dinis, M.T., Delbare, D., Schram, E., Kamstra, A., Rema, P. & White, P. (2003) A review of the culture potential of Solea solea and S. senegalensis. *Reviews in Fish Biology and Fisheries,* **13,** 379-407.

Irwin, S., Kenny, A.P., O'Halloran, J., Fitz, G. & Duggan, P.F. (1999a) Adaptation and validation of a radioimmunoassay kit for mesuarings plasma cortisol in turbot *Compendio Biochemestry Phisiology,* **C124,** 27-31.

Irwin, S., O'Halloran, J. & FitzGerald, R.D. (1999b) Stocking density, growth and growth variation in juvenile turbot, Scophthalmus maximus (Rafinesque). *Aquaculture,* **178,** 77-88.

Irwin, S., O'Halloran, J. & FitzGerald, R.D. (2002) The relationship between individual consumption and growth in juvenile turbot, Scophthalmus maximus. *Aquaculture,* **204,** 65-74.

Jobling, M. & Baardvik, B.M. (1994) The influence of environmental manipulations on inter- and intra-individual variation in food acquisition and growth performance of Artic charr, Salvelinus alpinus. *Journal of Fish Biology,* **44,** 1069-1087.

Jobling, M., Baardvik, B.M. & Jørgensen, E.H. (1989) Investigation of food-growth relationships of Arctic charr, Salvelinus alpinus L., using radiography. *Aquaculture,* **81,** 367-372.

Jobling, M. & Wandsvik, A. (1983) Effect of social interactions on growth rates and conversion efficiency of Artic charr, Salvelinus alpinus L. *Journal of Fish Biology,* **22,** 577-584.

Jørgensen, C.B. (1988) Metabolic costs of growth and maintenance in the toad, *Bufo bufo*. *Journal Experimental Biology* **138,** 319-331.

Koebele, B.P. (1985) Growth and the size hierarchy effect: an experimental assessment of three proposed mechanisms; activity differences, disproportional food acquisition, physiological stress. *Environmental Biology of Fishes,* **12,** 181-188.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W. & Blokhuis, H.J. (1999) Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews,* **23,** 925-935.

Mackett, D.B., Tam, W.H. & Fryer, J.N. (1992) Histological changes in insulin-immunoreactive pancreatic b-cells, and suppression of insulin secretion and somatotrope activity in brook trout (*Salvelinus fontinalis*) maintained on reduced food intake or exposed to acidic environment. *Fish Physiology and Biochemistry* **10,** 229–243.

Maltby, L. (1999) Studying stress: the importance of organism-level responses *Ecological Applications,* **9,** 431-440

Martins, C.I.M. (2005) Individual variation in growth of African catfish Clarias gariepinus: a search for explanatory factors In *Institute of Animal Sciences*. Wageningen University, The Netherlands, 166 pp.

Martins, C.I.M., Aanyu, M., Schrama, J.W. & Verreth, J.A.J. (2005) Size distribution in African catfish (Clarias gariepinus) affects feeding behaviour but not growth. *Aquaculture,* **250,** 300-307.

Martins, C.I.M., Schrama, J.W. & Verreth, J.A.J. (2006a) The effect of group composition on the welfare of African catfish (Clarias gariepinus). *Applied Animal Behaviour Science,* **97,** 323-334.

Martins, C.I.M., Trenovski, M., Schrama, J.W. & Verreth , J.A.J. (2006b) Comparison of feed intake behaviour and stress response in isolated and non-isolated African catfish. *Journal of Fish Biology,* **69,** 629-636.

McCarthy, I.D., Carter, C.G. & Houlihan, D.F. (1992) The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal Fish Biology,* **41,** 257–263.

Merino, G.E., Piedrahita, R.H. & Conklin, D.E. (2009) Routine oxygen consumption rates of california halibut (Paralichthys californicus) juveniles under farm-like conditions. *Aquacultural Engineering,* **In Press, Corrected Proof**.

Metcalfe, N.B., Huntingford, F.A., Graham, W.D. & Thorpe, J.E. (1989) Early social status and the development of live-history strategies in Atlantic salmon *Proceedings of the Royal Society* **B 236,** 7-19.

Millot, S., Bégout, M.L., Person-Le Ruyet, J., Breuil, G., Di-Poï, C., Fievet, J., Pineau, P., Roué, M. & Sévère, A. (2008) Feed demand behavior in sea bass juveniles: Effects on individual specific growth rate variation and health (inter-individual and inter-group variation). *Aquaculture,* **274,** 87-95.

Mitz, S.V. & Newman, M.C. (1989) Allometric relationship between oxygen consumption and body weight of mosquitofish, *Gambusia affinis Environmental Biology of Fishes,* **24** 267-273.

Mommsen, T.P. (2001) Paradigms of growth in fish. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology,* **129,** 207-219.

Mostafa, A.R.H., Graham, S.H. & Malcolm, C.M.B. (2002) Effect of feeding time and frequency on the growth and feed utilization of African catfish *Clarias gariepinus* (Burchell 1822) fingerlings. *Aquaculture Research* **32,** 999-1004.

Pedersen, B.H. (1997) The cost of growth in young fish larvae, a review of new hypotheses. *Aquaculture,* **155,** 259-269.

Pickering, A.D. (1998) Stress response of farmed fish. In: Black K, Pickering A (eds) Biology of farmed fish. *SheffieldAcademic Press,* Sheffield, 222-382.

Portz, D.E., Woodley, C.M. & Chech, J.J.J. (2006) Stress-associated impacts of short-term holding on fishes. *Journal Fish Biology,* **16,** 125-170.

Qian, X., Cui, Y., Xie, S., Lei, W., Zhu, X., Xiong, B. & Yang, Y. (2002) Individual variations in growth, food intake and activity in juvenile Chinese sturgeon Acipenser sinensis Gray. *Journal of Applied Ichthyology,* **18,** 695-698.

Ramos, J. & Roures, S. (1983) Estudio del estado larvario y postlarvario del lenguado, Solea solea (Linneo, 1758) (Pisces, Soleidae). *Inf Técn Inst Inv Pesq,* **110,** 3-14.

Rema, P., Conceição, L.E.C., Evers, F., Castro-Cunha, M., Dinis, M.T. & Dias, J. (2007) Optimal dietary protein levels in juvenile Senegalese sole (Solea senegalensis). *Aquaculture Nutrition,* **13,** 1-7.

Rombough, P.J. (1994) Energy partitioning during fish development: additive or compensatory allocation of energy to support growth? *Functional Ecology,* **8,** 178–186.

Seppãnen, E., Piironen, J. & Huuskomen, H. (2008) Standard metabolic rate, growth rate and smolting of the juveniles in three Atlantic salmon stocks. *Boreal Environment Research,* **14,** 369-381.

Shearer, K.D., Silverstein, J.T. & Dickhoff, W.W. (1997) Control of growth and adiposity of juvenile chinook salmon (Oncorhynchus tshawytscha). *Aquaculture*, **157,** 311-323.

Shields, R.J. (2001) Larviculture of marine finfish in Europe. *Aquaculture,* **200,** 55-88.

Sundström, F., Devlin, R.H., Johnsson, J.I. & Biagi, C.A. (2003) Vertical Position Reflects Increased Feeding Motivation in Growth Hormone Transgenic Coho Salmon (*Oncorhynchus kisutch*) *Ethology,* **109** 701-712.

Thodesen, J., Gjerde, B., Grisdale-Helland, B. & Storebakken, T. (2001) Genetic variation in feed intake, growth and feed utilization in Atlantic salmon (Salmo salar). *Aquaculture,* **194,** 273-281.

Van Weerd, J.H. & Komen, J. (1998) The effects of chronic stress on growth in fish: a critical appraisal. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology,* **120,** 107-112.

Vijayan, M.M., Ballantyne, J.S. & Leatherland, J.F. (1990) High stocking density alters the energy metabolism of brook charr, *Salvelinus fontinalis*. *Aquaculture*, **88,** 371-381.

Vinagre, C. & Cabral, H.N. (2008) Prey consumption by the juvenile soles, Solea solea and Solea senegalensis, in the Tagus estuary, Portugal. *Estuarine, Coastal and Shelf Science,* **78,** 45-50.

Wang, N., Hayward, R.S. & Noltie, D.B. (1998) Variation in food consumption, growth, and growth efficiency among juvenile hybrid sunfish held individually. *Aquaculture,* **167,** 43-52.

Wedemeyer, G. (2001) Fish Hatchery Management (Edition, n. ed.), pp. 720-733. American Fisheries Society, Washington.

Whitehead, P.J.P., Bauchot, M.L., Hureau, J.C., Nielsen, J. & Tortonese, E. (1986) Fisches of the North-eastern Atlantic and the Mediterrnean, Vol. 3, pp. 1445-1473. Unesco, Paris.

Wieser, W. (1994) Cost of growth in cells and organisms: general rules and comparative aspects, *Biological Reviews,* **68,** 1–33.

Wootton, R.J. (1998) Ecology of teleost fishes (edition, S. ed.), Kluwer Academic Publishers, Netherlands, 72-76 pp.

7. APPENDIX

Senegalese sole juveniles food intake during the experiment (n = 12).

Fish ID	Trial 1	Trial 2	Trial 3			
	Food Intake (%)	Food Intake (%)	Food Intake (%)	Average	SD	CV
RRR	0.000	0.000	0.000	0.000	0.000	0.000
VRR	0.921	0.955	0.567	0.814	0.215	26.384
AAA	0.754	1.062	1.073	0.963	0.181	18.783
RRA	1.300	1.194	0.000	0.831	0.722	86.840
RRV	0.886	1.118	0.864	0.956	0.141	14.731
VVA	0.597	1.001	0.392	0.663	0.310	46.765
RAA	1.892	0.000	0.608	0.833	0.966	115.923
VVV	1.395	1.500	0.617	1.170	0.482	41.216
VVR	1.159	1.149	1.438	1.249	0.164	13.134
AAV	1.634	1.342	1.149	1.375	0.244	17.762
AVA	1.447	1.029	0.689	1.055	0.380	35.977
VAA	1.163	1.503	0.492	1.053	0.514	48.858

Newman-Keuls test: Fasted vs Fed

Newman-Keuls test: Fasted vs Emersion Stress