UNIVERSIDADE DO ALGARVE

FACULDADE DE CIÊNCIAS E TECNOLOGIA

Effect of type of food and culture density on the rearing of *Seriola dumerili*

TIAGO ESTEVES JORGE VERÍSSIMO

Dissertation to obtain the degree in

Masters in Aquaculture and Fisheries

Specialty in Aquaculture

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Thesis coordinated by:

António de Vilhena Andrade Ferreira Sykes, PhD

Virginia Martín, PhD

Salvador Jerez, PhD

Covadonga Rodríguez González, Senior Lecturer

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EFFECT OF TYPE OF FOOD AND CULTURE DENSITY ON THE REARING OF *SERIOLA DUMERILI*

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"Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referência incluída"

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(Tiago Esteves Jorge Veríssimo)

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Nenhum Homem é uma Ilha"

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"Bora equipa"

“Viver é fácil, o difícil é saber viver”

Aos meus pais, irmão e cão

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Maria Luísa Jorge

Vasco Veríssimo

Baite

Resumo

Esta tese teve o intuito de estudar o efeito de três tipos de alimento diferentes em duas densidades diferentes para seleccionar um regime de alimentação ideal para o crescimento, a sobrevivência, os parâmetros plasmáticos, e para a composição lipídica do músculo e fígado dos juvenis de *Seriola dumerili* (Risso 1810) de maneira a cobrir todas as necessidades nutricionais e optimizar o custo-produção. A esse respeito, um total de 145 juvenis de *Seriola dumerili* com um peso médio de 117.54 ± 54.98 g, foram distribuídos aleatoriamente em três grupos de 15 peixes / tanque (densidade baixa, LD) e 3 grupos de 25 peixes / tanque (alta densidade, HD) e alimentados com três diferentes tipos de alimentos: ração comercial para Sparidae, Alfa Biomar dieta (SP), ração comercial para *S. dumerili* (Skretting) (SE) e cavala congelada (MA). O estudo foi conduzido por 86 dias. As variáveis estudadas foram o tipo de regime alimentar e a densidade de produção.

A sobrevivência foi distinta e maior nos grupos alimentados com SE, enquanto que a Taxa de Crescimento Específico (SGR) não mostrou estatisticamente nenhuma diferença (P> 0,05) entre os grupos. Os grupos alimentados com a dieta MA tiveram os maiores valores de Ingestão Diária de Alimento (DFI), durante o estudo (9,59 ± 3,56 g / peixe em HD e 8,46 ± 5,00 g / peixe em LD), que embora com uma maior Taxa de Conversão de Alimento (FCR) (9,59% em LD e 8,46% em HD), aparentemente não conseguiram transformar eficientemente a ingestão em biomassa, demonstrado pelo respectivos valores de B% de 0,18% em LD e -0,35% em HD, sendo menor do que os grupos alimentados com SE a LD (0,41%).

No presente estudo, a composição bioquímica do *S.dumerili* foi semelhante à relatada em trabalhos anteriores. Quanto maior o teor de humidade, menor o teor de lípidos. Apesar de a dieta à base de MA ter um maior conteúdo de gordura em bruto (38,11%) que todas as outras dietas, os grupos alimentados com dieta MA, em ambas as densidades, apresentaram um menor valor total de lípido.

O Total de Lípidos Polares (TPL) e o Total de Lípidos Neutros (TNL) não mostraram quaisquer diferenças significativas (P <0.05) entre dietas em ambos os tecidos (P>0.05). Entre as classes de TNL, apesar de DAG, CHO e FFA terem sido significativamente diferentes (P <0.05), TAG foi a classe de lípido mais abundante, e onde nenhuma diferença significativa (P>0.05) entre os diferentes grupos foi encontrada.

Relativamente à composição de ácidos gordos, nomeadamente ao 18:1n-9, os grupos alimentados com MA foram os únicos significativamente diferentes (P<0.05), e obtiveram os

mais baixos valores (P<0.05), mesmo tendo em consideração que na natureza a dieta do *S. dumerili* é composta principalmente por vários peixes ósseos ricos em 18:1n-9. Pode-se deduzir que, ou houve uma degradação da qualidade do MA, enquanto armazenado para a respectiva alimentação dos grupos, que afectou directamente a qualidade e quantidade de 18:1n-9 acumulado nos seus tecidos.

O facto de os valores mais baixos de 18:2n-6 terem sido detectados nos grupos alimentados com MA, enquanto os valores mais elevados foram detectados nos grupos alimentados com SE (P<0.05), e tendo em conta o facto de o 18:2 n-6 ser o representativo mais elevado de n-6 nos ácidos gordos da dieta, que é o ácido gordo responsável pelas modificações mais prejudiciais para a composição de ácidos gordos em peixes de cultura, sugerem que este ácido gordo está em excesso na composição da dieta SE.

Os grupos alimentados com MA apresentaram as maiores quantidades de ARA (P<0.05) e os valores mais baixos de EPA (P<0.05), sendo que os grupos alimentados com SE apresentaram as menores quantidades de ARA e DHA (P<0.05). A menor quantidade de EPA e o menor ratio EPA/ARA (P<0.05) foram encontrados em grupos alimentados com MA. Devido à competição pelas mesmas enzimas para esterificar ácidos gordos em estruturas fosfolipídicas, entre EPA e DHA, a acumulação excessiva de EPA pode ser prejudicial, e terá provavelmente afectado a produção de DHA nos grupos alimentados com SE, que teve as mais baixas quantidades de ARA e DHA, o que deverá ter provocado uma maior ratio de EPA/ARA (P<0.05), sugerindo que o ratio entre eles está desequilibrado e existe demasiada acumulação de EPA, afectando a produção de ARA.

No presente estudo, apesar de o grupo alimentado com a MA a HD ter obtido a maior quantidade de DHA (P<0.05) no tecido do fígado, a relação DHA/ARA mesmo assim não apresentou diferenças significativas (P>0.05), indicando um equilíbrio estável entre as quantidades disponíveis de DHA e ARA nas dietas.

Este estudo sugere que o perfil de ácidos gordos dos diferentes grupos refletiu a composição de ácidos gordos das dietas, ou seja, as quantidades de 18:1n-9, 18:2-6 e 20:5n-3, 20:4n-6 e 22:6n-3 que têm efeitos nos ratios entre DHA, EPA e ARA, afectando o equilíbrio entre eles, o que afectou directamente o crescimento

No que diz respeito à análise de plasma sanguíneo, a proteína total foi o único parâmetro onde foram detectadas diferenças estatisticamente significativas (P<0.05) entre as dietas e densidades. O facto de os grupos alimentados com dieta SE e MA, mostrarem maior teor de proteína, pode estar relacionado com o facto de estas duas dietas terem níveis mais elevados de proteína (48 g / l e 51,25 g / l, respectivamente).

A partir dos resultados do presente estudo, podemos deduzir que a composição de ácidos gordos das rações afectaram o perfil lipídico do *S.dumerili*, assim como o desenvolvimento do mesmo, o que pede um melhor equilíbrio entre a ácidos gordos essenciais na composição da dieta, para garantir melhor eficiência energética e promover o crescimento.

Considerando os resultados obtidos relativamente aos grupos alimentados com SE, tudo indica que esta dieta parece ter sido a mais adequado para *S.dumerili* no presente estudo.

Palavras-chave: Crescimento, ácidos gordos essenciais, *Seriola dumerili;* densidade, DHA, ARA, EPA

Abstract

This thesis intended to study the effect of three different types of food and two different densities, on growth, survival, plasmatic parameters and lipid composition of muscle and liver of *Seriola dumerili* (Risso 1810) juveniles, in order to select an optimal feeding regime for growth, covering the nutritional requirements, and optimizing the commercial cost-effectiveness of the production. Regarding this, a total of 145 juvenile of *Seriola dumerili* with and average weight of 117.54 ± 54.98 g, were randomly distributed in 3 groups of 15 fish / tank (low stocking density LD) and 3 groups of 25 fish / tank (high stocking density HD) and fed with three different types of food: commercial pellets for Sparidae, the Alpha Biomar diet (SP), commercial pellets for *S. dumerili* (Skretting) (SE) and frozen Mackerel (MA). The study was conducted for 86 days. The study variables were the feeding regime and stocking density.

The survival rates were higher in the groups fed with SE, while the Specific Growth Rate (SGR) showed no statistical differences ($P>0.05$) between groups. The groups fed with MA diet had the higher amounts of Daily Food Ingested (DFI), (9.59±3.56 g/fish at HD and 8.46±5.00 g/fish at LD) during the study, although with a higher Food Conversion Rate (FCR), (9.59 % at LD and 8.46 % at HD), not efficiently turn that ingestion in biomass, as for example the value of B% at groups fed with MA at LD (0.18 %) and at HD (-0.35%) show, being lower than the groups fed with SE at LD (0.41%).

The biochemical composition of the fish in the present study was similar to the one reported in previous works, explaining that the more moisture content in the tissue, the less lipid it has in it.

The Total Polar Lipids (TPL) and Total Neutral Lipids (TNL) did not show any significant differences ($P<0.05$) between different diets in both tissues ($p>0.05$). Among the total neutral lipids (TNL), despite DAG, CHO and FFA were significantly different ($P<0.05$), TAG was the most abundant lipid class, which is generally the major lipid class in the diet of marine fish, and there was no significant differences ($P<0.05$) between the different groups.

Regarding fatty acid composition, namely 18:1n-9, the groups fed with MA were the only significantly different ($P<0.05$), and the lowest amounts in this study ($P<0.05$), even taking in consideration that, in the wild, the *S. dumerili* diet is composed mainly by several finfish rich in 18:1n-9. We can deduce that or there was a degradation of the quality of the

MA while stored to be fed to the groups, which affected directly the quality and quantity of the 18:1n-9.

The fact that 18:2n-6 had its lower amounts in groups fed with MA, and the higher amounts in groups fed with SE ($P<0.05$), and taking in account that 18:2n-6 is the higher representative of n-6 fatty acid in diets, that is the fatty acid responsible for the most detrimental modifications to the fatty acid composition of cultured fish, suggest that this fatty acid is in excess in SE diet composition.

The groups fed with MA presented the highest amounts of ARA ($P<0.05$) and the lower amounts of EPA ($P<0.05$), and the groups fed with SE presented the lower amounts of ARA and DHA ($P<0.05$). The lower amount of EPA and lower EPA/ARA ratio ($P<0.05$) found in groups fed with MA, could positively affect certain physiological functions. Due to the EPA and DHA competition for the same enzymes to esterify fatty acids into phospholipid structures, excessive accumulation of EPA could be prejudicial. In the groups fed with SE, the production of DHA was probably affected by it. The fact they had the lower amounts of ARA and DHA, inducing to a higher EPA/ARA ratio ($P<0.05$), suggests that the ratio is unbalanced and there is too much EPA accumulation, to insure ARA production

Even though the group fed with MA at HD had the higher amount of DHA in this study ($P<0.05$), still the ratio DHA/ARA had no significant differences ($P<0.05$), indicating a stable balance between DHA and ARA amounts.

This study suggests that the fatty acid profile of the different groups reflected the fatty acid composition of the diets, namely regarding 18:1n-9, 18:2n-6 and 20:5n-3, 20:4n-6 and 22:6n-3. that had effects in the ratios of DHA, EPA and ARA, affecting the balance between them, which had directly affected the growth.

Regarding the plasma analysis, total protein was the only displaying statistical differences between diets and densities, and the fact that the groups fed with SE and MA diet, showed higher protein content, could be related to the fact those diets have higher protein levels (48g/l and 51.25 g/l respectively).

From the present results it seems that the fatty acid composition of the pellets and frozen atlantic mackerel, affected the fatty lipid profile of *S. dumerili*, and so it development, so better balance between EFA diet composition while probably insure better energy efficiency and promoting growth.

Considering the results obtained with SE, it seems to be the most appropriated for *S. dumerili* in the present study.

Keywords: Growth, essential fatty lipids, *Seriola dumerili;* stocking density, DHA, ARA, EPA

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1. Introduction

Figure 1 –*S. dumerili* adult in a culture tank, Spanish Institute of Oceanography

1.1 Current State of World Fisheries and Aquaculture

The global population is increasing and, in order to maintain at least the current level of per-capita consumption of aquatic foods, the world will require an additional 23 million tons by 2020 (FAO, 2012). According to FAO (2012), the fisheries and aquaculture sector supplied 148 million tons of fish in 2010, of which about 128 million tons was used as food for human consumption, and preliminary data for 2011 indicate an increased in production to 154 million tons, being China the main leading country owning to the substantial increase in its fish production, particularly from aquaculture, reach 31.9 kg per capita in 2009, due to a growing domestic income and an increase in the diversity of fish available. (FAO, 2012). World fish food supply increased from an average 9.9 kg per capita (live weight equivalent) in the 1960s to 18.4 kg in 2009.

Aquaculture production is vulnerable to adverse impacts of disease and environmental conditions. Disease outbreaks in recent years have affected farmed Atlantic salmon in Chile, oysters in Europe, resulting in partial or sometimes total loss of production. Most of the cases, as a consequence, production has contracted or stagnated in Japan, the United States of America, Spain, France, the United Kingdom of Great Britain and Northern Ireland, Canada and Italy. An exception is Norway, where, thanks to the farming of Atlantic salmon in marine cages, aquaculture production grew from 151 000 tonnes in 1990 to more than one million tonnes in 2010, at an average growth rate of 12.6 percent in the 1990s and 7.5 percent in the 2000s.

Aquaculture is evolving regarding to technological innovation and adaptation, in order to face the new world challenges. According to Cairo (2004) the introduction of a new species, must fulfill some criteria such like the high market price, high nutritional value and growth rate, tolerance to rearing densities and knowledge of their biological needs, in order to improve the production. The culture of new species could be one of the keys to the future development of this sector.

Still according to FAO (2012), in 2010, global production of farmed food fish was 59.9 million tons, up by 7.5% from 55.7 million tons in 2009 (excluding aquatic plants and non-food products). One-third of the global production was achieved without the use of feed, mainly through the production of bivalves and filter-feeding carps.

In the last three decades (1980-2010), world food fish production of aquaculture has expanded by almost 12 times, at an average annual rate of 8.8%, and its contribution to world total fish production raise from 20.9% in 1995 to 40.3% 2010. This means a contribution to world food fish production for human consumption of 47% in 2010 compared with only 9% in 1980, which is an amazing achievement. On the top of the 10th biggest producers in the world, China produced 36 million tons per year, representing 61.35% of total production. In Europe, Norway is leading the table with an annual production around 1 million tons, representing 39,95% of the total production, followed by Spain and France, being the 7th biggest producer in the World (FAO, 2012).

According to Portuguese National Statistical Institute fisheries report (2011), in Portugal, on the year of 2012 the production was 8013 tons, which means an increase of 0.2% since 2009, where sea bream, turbot and clam represent, the most produced species. With Norway leading the table, in 2 th place is Spain with a production of 253784 tons that is almost 32 fold the Portuguese production, (Eurostat, 2011) showing the huge gap between both countries, even tought Spain has more territory to production.

1.2 *Seriola dumerili*

a. Biology, Ecology and Distribution of species

The *Seriola* gender is an highly active carnivore pelagic marine fish in Canary Islands in Spain, but also distributed worldwide in temperate and tropical water (Rodriguez-Barreto et al 2012). *Seriola dumerili* (Risso, 1810) is a teleostei perciforme fish, from the Carangidae family. The species is known to be an epibenthic and pelagic fish, surrounding reefs or offshore shallows, generally between 18 and 72 meters depth, but can occur at greater depths (Smith-Vaniz, 1986).

b. Growth

According to Kozul et al (2001), wild individuals with one year old and having 32-45 cm in total length, triple its size at 4 years (93-106 cm) in nature. In culture conditions, a size of 35-45 cm at 1 year old and 85-95 cm at 4 years were reported by Garcia and Diaz, (1995). The pre-fattening and fattening of the juveniles in captivity, using commercial pellets for turbot, have shown some good results, such as an average weight of 453.5 g at 8 months of production, and average weights of 2.5 and 8.5 kg with 1, 2 and 3 years respectively. (Jerez et

al 2003). Overall, *S. dumerili* has shown a good adaptation to captivity, as well as high growth and survival rates fed on low value-fish or dry pellets (Jerez *et al*., 2003, 2006).

Stocking density (D) is a key factor in determining the productivity and profitability of commercial fish farms. It's also important to recognize that the welfare of farmed fish can represent the physical and health state of an animal in relation to its environment. Fish must be stocked at appropriated densities to their size, water temperature and flow, available oxygen, production cycle stage and fish holding unit type to minimize the risk of poor water quality, physical damage, stress and disease. Considering the previous, commercial farmers are using a combination of intuition and experience to decide which D (kg m^{-3}) is more appropriate, when it comes to finding a balance between welfare and productivity (Ellis et al, 2002). The season is crucial on the amount of D. For example, during winter months, when the water temperature is lower, there will be higher dissolved oxygen. The correct D, will also depend on the correct water exchange rate and/or additional aeration (Buss et al, 1970).

c. Reproduction

S. dumerili is a dioecious species; both sexes are separated, with a sex ratio of 1:1 (Lazzari and Barbera, 1989). The individuals can be sexual differentiated at 4 to 5 months old (Marino et al, 1995a), and sexually mature males and females are detected at the minimum standard length of 61 cm (24 months) and 80 cm (36 months), respectively. The spawning season takes place with the water temperature rise in spring, although its duration depends on the geographical area (Lazzari and Barbera, 1989). In captivity conditions, 4 to 5-year-old fish (80 cm length) display the start of vitellogenesis in December and the deposition of vitellogenin in May. Eggs are pelagic, have 1mm in diameter, and are fertilized by males in the water, known as broadcast spawners, being ovapartity reproducers. At 22ºC and after 48 hours, eggs originates larvae with 3mm in length. However, reproduction and spawning in captive conditions have been achieved in a spontaneous way (Jerez 2006). Due to the difficulties in reproduction of *S. dumerili* in captivity, in general commercial culture have been based to date, mainly, on the capture of fingerlings from the wild to be fattened until reaching marketable size. Successful maturation and spawning has been achieved more recently with the use of gonadotropin-releasing hormone agonist (GnRHa) loaded into IGled-release devices (Mylonas et al. 2004).

d. Food and Nutrition

Due to its carnivorous nature, the first juvenile captured wild stocks were fed on raw frozen fish, such as *Sardinella aurita*. However, this type of feeding is not environmentally sustainable, much less in a more intensive industrial regime (Garcia, A. et al. 1995).

The greater amberjack fingerlings don't have the ability to shift from live prey, with high moisture content, to commercial dry diets (pellets) so easily, resulting in low growth rates during weaning (Masumoto 2002). Some studies, as Mazzola (2000), have shown that the greater amberjack is able to accept pellets, but when making a direct comparison of moist against extruded pellets, fish fed on dry pellets were found to display inferior growth results.

According to Garcia and Diaz (1995), the nutritional demand in terms of proteins, lipids, carbohydrates, vitamins, minerals and energy content of *S. dumerili* is currently unknown. Research is needed to determine a suitable and healthy type of food.

e. Plasma biochemical parameters in culture fish

At the 70's, a metabolic profile was created to better understand the nutritional aspects and digestion of fish, making possible to see nutritional changes that were not possible to identify in any other way (Cappa, 1988), that made possible to avoid health problems and enhance a balance feeding scheme (Pavlidis et al., 1997), allowing a better production.

One of the the primary stress response in fish involves the release of catecholamines and activation of the hypothamalic-pituitary-interenal (HPI) axis (Ashley et al., 2007). HPI activation results in energy source mobilization, depletion of glycogen stores, and an increase in plasma levels of glucose, along with high muscle activity, anaerobic glycosis and an increase in plasma lactate. Therefore, the levels of both glucose and lactate in the plasma are often used alongside cortisol to assess stress levels. (Acerete, L. et al 2004). This is due to an increase of glucose concentration in the liver where the glycogen is quickly converted to glucose, increasing the glucose concentration, which is a result of energy demand that occurs under stress conditions (Barton and Iwama, 1991). These stress conditions are normally due to confinement conditions, namely density (Melotti et al., 2004), that could also promote a decline in plasma protein levels on teleosts (Jeney et al, 1997). On the other hand, the variation of plasma triglycerides and cholesterol concentration are more related to adequate food and changes of energy metabolism (Pfeffer, 1995).

Plasma analysis can also bring a lot of knowledge when it comes to nutrition. An incrising of plasma glucose concentration and low levels of triglycerides and cholesterol can

be related to an high concentration of carbohydrates in the diet (Montero et al., 1999), which is probably due to an unbalanced diet or of low quality food (Mazeaud et al. 1997). Diets with high concentration levels of lipids and carbohydrates promote fish growth, but also increase cholesterol concentration in plasma, that can influence the physiological status and immune system of fish (Torstensen et al. 2000).

f. Nutritional requeriments for *S. dumerili*

Even tough successful maturation and spawning has been achieved more recently with the use of gonadotropin-releasing hormone agonist (GnRHa) loaded into IGled-release devices (Mylonas et al. 2004), the research group (IEO: Research Group of Aquaculture - Canary Islands Oceanographic Centre – Spanish Institute of Oceanography), has also obtained the natural spawning of this species without hormonal induction at its facilities (Jerez et al., 2006). This research at IEO began in 1996, by capture of wild juveniles that were kept in captivity, and after 6 years, the stock of broodstock spawned naturally in the spring-summer of 2002. The annually spawns obtained since then, have allowed the development of several studies concerning reproduction, larval culture and weaning, and growth performance of this species, as well as the formation of an important stock of potential captive bred broodstock.

The studies concerning nutritional requirements for *S. dumerili* are scarce, but there are several studies on the effect of different dietary formulations for other *Seriola* species, including *Seriola quinquerradiata, Seriola lalandi* and *S. dumerili* (Rodríguez-Barreto et al., 2012) and most of them related to the optimization of dietary protein inclusion rates, or substitution of fishmeal with other animal or plant protein sources.

According to Sargent (2002), highly unsaturated fatty acids (HUFA) revealed influence on growth, reproduction and egg quality, and among the components of a diet, lipids have an essential role on the fish's nutrition. They are important to provide energy through oxidation of fatty acids, as on the synthesis of structural lipids, such as cholesterol and phospholipids, main components of the cell membranes, which regulate their fluidity and functions. (Sargent et al 2002).

The essential fatty acid (EFA) requirements of marine fish species have been extensively studied over the past 20 years and are known to vary both qualitatively and quantitatively, having a crucial role on the reproduction process, embryo ontogeny, vitellum composition and early stages of larval development (Sargent et al., 1989, 2002). The dietary essentiality of the n-3 highly unsaturated fatty acids (HUFA), docosahexaenoic acid (22:6n-3),

(DHA), and eicosapentaenoic acid (20:5n-3), (EPA), is of major importance for successful production of marine fish. Considering the higher biological value for DHA than EPA during first feeding of marine species such as red seabream and turbot (Watanabe et al., 1989) it is suggested that the n-3 HUFA requirements might not only be a function of the total amount of these fatty acids in the diet, but also of the relative proportions of DHA and EPA.

In marine fish, EPA and, especially, DHA are regarded as EFA due to their requirement for good growth and the inability of all marine fish studied to date being barely able to convert 18:3n-3 to EPA and DHA if at all (Sargent et al., 1989, 2002). EPA and DHA are the major HUFA in cell membranes, involved in maintaining their structure and function, although EPA is selectively catabolized with respect to DHA to provide energy during ovary maturation prior spawning (Tocher, 2003).

Also considered as an EFA, ARA has been largely neglected, and is in part due to the assumption that the ARA requirement would be very low and would be met by the small, but significant amount of ARA found in marine fish meals and oils. It is known that eicosanoids derived from ARA are physiologically active in fish, as series-2 prostaglandins derived from ARA have long been used to induce spawning in fish (Sargent et al., 1989, 1995), and have critical role in many areas of cellular signal transductions (Sargent et al., 1989).

These three EFA with their eicosanoids derivatives, have a wide range of functions in fish reproduction, i.e. EPA along with ARA, act as precursors in the formation of eicosanoids that act as autocrine hormones, regulating many physiological processes, such as osmoregulation, and immune and inflammatory response (Tocher, 2003); and DHA that specifically plays an important role in neural tissue membranes (brain and retina) in which reaches high concentrations.

Many of the difficulties in understanding essential fatty acid biochemistry and optimising the essential fatty acid content of the diet stems from competitive interactions between a different series of fatty acids, such as competitions between n-6 and n-3 polyunsaturated fatty acids (PUFA) and also monosaturated fatty acids; and between fatty acids of different chain lengths and degrees of unsaturation within a given series, such as competitions between EPA and DHA (Sargent et al., 1999). The DHA and EPA competition results from both molecules using the same enzyme to esterify fatty acids into phospholipid structures (Mourente et al., 1991; Sargent et al., 1999).

ARA and EPA compete for the same enzymatic complex to generate different series of prostanoids with different biological activities. These two EFA, ARA and EPA, compete for the cyclo-oxygenases and lipoxygenases that produce, respectively, 2-series prostanoids and

4-series leukotrienes from ARA, and 3-series prostanoids and 5-series leukotrienes from EPA (Sargent et al., 1999). The difference is that eicosanoids produced from ARA are generally more biologically active than those produced from EPA and both compete for the same cell membrane receptors. Considering this, it is essential for the a good development of the fish, that a adequate EPA/ARA ratio exists.

As mentioned before, the requirements of fish for their growth vary qualitatively and quantitatively. Considering that, in this study to better understand the *Seriola dumerili* needs, different diets were characterized to study the performance of those, in the growth and quality of the fish, in different densities.

2. Objectives

The aim of this study was to evaluate the effect of three different types of food **(**commercial pellets for Sparidae, SP, commercial pellets for *S. dumerili*, SE, and frozen Mackerel, MA) and two stocking densities on:

a) Growth and survival;

b) Lipid composition of muscle and liver focusing on the lipid classes and fatty acid composition

c) Plasma biochemical parameters

3. Methods

3.1. Experimental Conditions

Figure 2 –*S. dumerili* adult in a culture tank, Spanish Institute of Oceanography

A total of 145 juveniles of *S. dumerili* bred and kept in captivity in the experimental facilities of the Spanish Institute of Oceanography (Tenerife, Canary Islands, Spain), with an average weight of 117.54 ± 54.98 g were used. Individuals were randomly distributed in 3 groups of 15 fish / tank (low stocking density LD) with 1900±74 g, and 3 groups of 25 fish / tank (high stocking density HD) with 2800±66 g. All fish were anaesthetized with clorobutanol (200 mg/l) prior to tagging with a Passive Integrated Transponder, commonly known as PIT tag . Fish were anaesthetized and individually tagged with PIT tags of 13,3 x 2,1 mm in size and 0,1 g in weight. PIT tagging was carried out with tags intramuscularly introduced using a sterile syringe. PIT codes were detected by an ISO MAX V portable reader (Datamars S.A., Lugano, Switzerland).

Each group of fish was stocked in a 1 m^3 circle and balck indoor tank, in a closed system, with a continuos seawater input flow of 15 L/min to ensure an oxygen level close to saturation. Fish were reared under a natural photoperiod, water temperature of 21.70±0.86ºC and salinity of 37.5‰. As shown by Figure 3, three different types of food fish groups were established, itwo different stocking densities (High Density and Low Density): commercial pellets for Sparidae (Alpha Biomar - SP), commercial pellets for *S. dumerili* (Skretting - SE)

and frozen Atlantic mackerel *Scomber sconbrus* (MA). Fish were fed on a daily basis, twice a day, and at a 3% tank biomass. The Initial group (IG), the control diet for this study, was being fed by a pellet for sparidae, which is the same type of SP.

Table 1 and 2 show the proximate composition and fatty lipid profile of diets, respectively.

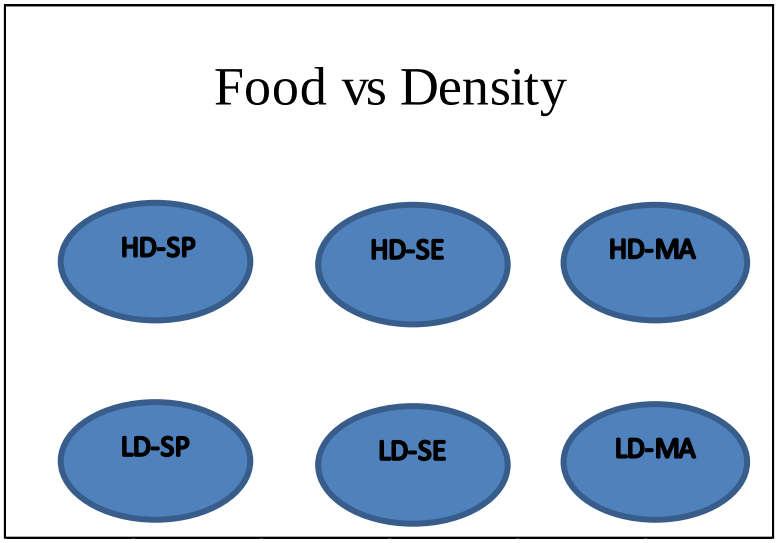

Figure 3 –*S. dumerili* adult in a culture tank, Spanish Institute of Oceanography

Table 1 – Proximate composition and raw materials of SP, SE and MA

	SP	SE	MA
Proximal Composition			
Crude protein (%)	44	48	18.6
Crude fat (%)	12	16	13.89
Carbohydrates (%)	28.2	26.5	65.25
Crude cellulose (%)	3.4	2.8	5.42
Ashes (%)	6.4	9.5	1.35
Total Fósforo (%)	0.9	1.3	0.2
Crude Phosphorus (MJ/kg)	20.6	-	0.63
Additives			
Vitamin A (I.U/kg)	5250	5000	0.33
Vitamin D3 (I.U/kg)	1050	750	0.16
Vitamin E (mg/kg)	175	150	0.002
Anti-oxidant	Propyl gallate	BHT	-
Copper (copper sulphate) (ppm)	8	-	-
Size of pellet (mm)	4.5	4	-
Raw Material			
Fishmeal (%)	-	28	-
Extracted toasted soy flour (%)	-	17	-
Corn gluten (%)	-	11	-
Lima beans (%)	*	9	-
Fish oil (%)	-	9	-
Wheat gluten (%)	*	8	-
Soy protein concentrated (%)	-	6	-
Pea protein (%)	*	6	-
Wheat (%)	-	4	-
Rapessed meal	-	-	-
Hemoglobin	-	-	-
Soy oil (%)	-	2	-

(-) Not present; (*) The values were not available.Information supplied by manufacter

Table 2 - Fatty acid composition of SP, SE and MA

	Dry weight Pellet (%)		
	Alpha Biomar	Skretting	Mackerel
C 14: 0	9.07 ± 0.44	6.07 ± 0.13	2.10 ± 0.40
C 16: 0	27.84 ± 1.41	23.10 ± 0.31	27.70 ± 1.50
C 16: 1 n-7	8.46 ± 0.75	6.22 ± 0.19	4.30 ± 1.00
C 16: 1 n-5	1.01 ± 0.36	0.66 ± 0.05	0.00 ± 0.00
C 16: 2 n-4	0.72 ± 0.02	0.58 ± 0.02	0.00 ± 0..00
C 16: 3 n-4	0.70 ± 0.05	0.52 ± 0.02	0.00 ± 0.00
C 16: 4 n-1	0.97 ± 0.09	0.68 ± 0.09	0.00 ± 0.00
C 18: 0	5.47 ± 0.31	5.21 ± 0.06	6.70 ± 0.20
C 18: 1 n-9	13.81 ± 2.45	18.59 ± 0.91	12.20 ± 1.90
C 18: 1 n-7	6.19 ± 1.82	6.64 ± 0.96	4.20 ± 0.60
C 18: 2 n-6	7.46 ± 0.36	13.82 ± 0.51	2.10 ± 0.20
C 18: 3 n-3	0.76 ± 0.06	1.32 ± 0.08	0.68 ± 0.10
C 18: 4 n-3	0.99 ± 0.15	0.52 ± 0.04	0.00 ± 0.00
C 20: 0	0.33 ± 0.00	0.36 ± 0.01	0.00 ± 0.00
C 20: 1 n-9/n-7	0.65 ± 0.47	1.01 ± 0.39	1.80 ± 0.50
C 20: 4 n-6	0.38 ± 0.04	0.32 ± 0.02	2.80 ± 0.30
C 20: 5 n-3	5.03 ± 0.32	4.05 ± 0.24	5.90 ± 0.70
C 22:1 n-11/ n-9	1.48 ± 0.11	2.68 ± 0.05	0.00 ± 0.00
C 22: 5 n-3	0.49 ± 0.01	0.47 ± 0.01	1.00 ± 0.20
C 22: 6 n-3	2.44 ± 0.04	1.79 ± 0.10	25.30 ± 4.50
C 24: 1 n-9	0.36 ± 0.01	0.39 ± 0.01	0.00 ± 0.00
UK.	1.67 ± 0.54	2.47 ± 0.64	0.00 ± 0.00
SFA	44.49 ± 2.17	36.48 ± 0.55	37.20 ± 1.70
MUFA	32.99 ± 1.50	36.65 ± 0.12	63.50 ± 2.10
PUFA	20.86 ± 1.21	24.40 ± 1.31	0.00 ± 0.00
HUFA	8.80 ± 0.42	6.73 ± 0.52	0.00 ± 0.00
n-3	10.40 ± 0.62	8.26 ± 0.62	33.30 ± 4.70
n-6	7.83 ± 0.41	14.13 ± 0.53	5.70 ± 0.30
n-9	16.30 ± 1.14	22.67 ± 0.42	14.00 ± 0.90
n-3/n-6	1.33 ± 0.15	0.58 ± 0.06	5.84 ± 3.11
DHA/EPA	0.49 ± 0.20	0.44 ± 0.10	4.29 ± 2.69
EPA/ARA	13.24 ± 0.20	12.66 ± 0.16	2.11 ± 0.28
DHA/ARA	0.49 ± 0.00	0.44 ± 0.06	4.29 ± 2.97

Results are expressed as means ± SD. N = 2.

One hour after being provided, the non-ingested pellet and MA were periodically took, to determine the real ingestion, taking in consideration that the weight of the feed SE, after their stay in the tank, increased 1.5, while the weight of the feed SP multiplied by 2.5. The growth trial was conducted for 86 days, with individual monthly sampling of Weight and Length parameters. These sampling moments were made at the 1º, 36º , 71º and 86º day of the study. In the last day of experiment (86º day) the remaining fish were sacrified and muscle, liver and blood plasma samples were took from each of them.

Data and sample collection of weight and length, where used for calculate:

- Mean Wet Weight (WW) (g)
- Initial (Bi) and Final (Bf) Biomass (g)
- Mean Biomass Relative Index (B) (%)

$$B\% = 100 \times \frac{Bf - Bi}{Bi \times t}$$

- Specific Growth Rate (%)

$$SGR(\%\, BWday^{-1}) = 100 \times \frac{\ln Final\ Weight\ (g) - \ln Initial\ Weight(g)}{total\ number\ of\ (days)} \times 100$$

- Condition Factor (CF)

$$CF\% = \frac{Weight(g)}{Lenght\ (cm)^3} \times 100$$

- Survival (S %) was determined taking out the dead individuals, daily, and make their identification.
- Daily Food Ingestion (DFI)

W_{TFS} – Weight of total dry feed supplied

$$DFI\ = \frac{W_{TFS}}{(\frac{B_f + B_i}{2}) \times T} \times 100$$

- Food Conversion Rate (%) (FCR)

$$FCR = \frac{W_{TFS}(g)}{Bf(g) - Bi(g)}$$

At the beginning of the experiment, a 7 fish representative samples were sacrificed. After 86 days, all the other live fish were sampled and sacrificed. For blood sampling, the fish was previously lightly anesthetized and blood was collected from the caudal vein with a

heparinised syringe. Blood samples obtained for each experimental diet were analyzed for various hematological and biochemical parameters. Blood was collected from the caudal vessels using heparinized needles. Fish were lightly anesthetized to avoid the stress inherent in the handling, which leads to changes in certain plasma parameters. Then at day 86º, after individually weighted, total length measured and weight from liver registered, fish were sacrificed by over-anesthesia to collect the samples of liver and muscle, which were frozen and stored at −80 °C. The dead fish were removed daily and PIT, length and weight were taken.

In liver and muscle samples, moisture, ash, total lipids, lipid classes and fatty acids composition were determined (Task 1.6.)

3.2 Biochemical determinations

Moisture content was determined in 300–500 mg samples by thermal drying of samples in an oven at 110 °C until constant weight, according to the Official Method of Analysis of the Association of Official Analytical Chemists (AOAC, 1990). Total protein content will be calculated on the basis of the total nitrogen content of the sample following a modification of the original method of Kjeldahl (AOAC 2006).

Total lipid (TL) was extracted from the tissues and diet by homogenization in chloroform/methanol (2:1, v/v) according to the method of Folch *et al.*(1957). The organic solvent was evaporated under a stream of nitrogen and the lipid content was determined gravimetrically (Christie, 1982) and stored in chloroform/methanol (2:1), containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, under a N_2 atmosphere at -20 ºC. Diets were pre-hydrated overnight with 0.5 mL of distilled water per 100 mg of sample before lipid extraction.

Analysis of lipid class (LC) composition was performed by one-dimensional double development high-performance thin layer chromatography (HPTLC; 10 × 10 cm, Ø 0.15 mm) using methyl acetate/isopropanol/chloroform/methanol/0.25% (w/v) Kcl (5 : 5 : 5 : 2 : 1.8 / volume) as developing solvent system for the polar lipid classes (half plate), and isohexane/diethyl ether/acetic acid (22.5 : 2.5 : 0.25 / volume), as the developing solvent system for the neutral lipid separation (full development). Lipid classes were visualized by charring at 160 °C for 15 min after spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid, and quantified by scanning densitometry using a dual-wavelength

flying spot scanner Shimadzu CS-9001PC (Shimadzu, Duisburg, Germany) and individual lipid classes were confirmed by comparison to a well-characterized cod roe sample. (Olsen & Henderson 1989)

To determine the fatty acid profiles, TL extracts were subjected to acid-catalyzed transmethylation for 16 h at 50 °C, using 1 mL of toluene and 2 mL of 1% sulphuric acid (v/v) in methanol. The resultant fatty acid methyl esters (FAME) were purified by thin layer chromatography (TLC) and determined in reference to a standard visualized under spraying with 1% iodine in chloroform (Christie, 1982). During acid-catalyzed transmethylation, FAME is formed simultaneously with dimethyl acetals (DMA) which originate from the 1-alkenyl chain of plasmalogens. FAME and DMA were separated and quantified using a thermo scientific trace gc ultra gas chromatograph (GC) equipped with a flame ionization detector (250ºC) and a fused silica capillary column SupelcowaxTM 10 (30m x 0.32 mm I.D.). Helium was used as a carrier gas. The oven initial temperature was 180ºC for 10 min, followed by an increase at a rate of 2.5ºC/min to a final temperature of 215ºC. Individual FAME and DMA were identified by reference to authentic standards (PUFA nº 3) supplied by SUPELCO (Supelco PARK, Bellefonte, PA, USA) and to a well characterized fish oil. Prior to transmethylation, nonadecanoic acid (19:0) was added to the total lipid extract as an internal standard.

Plasma samples were separated after centrifugation at 700 g for 15 minutes at 4ºC and stored at -80°C until analysis. Plasma levels of triglycerides, cholesterol, plasma protein, glucose and lipase were measured by standard spectrophotometric assays using kits to determine the amounts.

3.3 Data Analysis

Data of growth, wet weight, survival, biomass, proximate biochemical, lipid classes and fatty acids contents were statistical analysed by SPSS package (version 20.0 for Windows) and results are reported as means ± SD (n=3). Non-detected fatty acids were considered as null for statistical analysis. Normal distribution was checked for all data with the one-sample Kolmogorov-Smirnoff test and homogeneity of the variances with the Levene test. When normal distribution of data failed or the data were expressed in percentage, arcsine square root transformation of data was performed. Data were submitted to one-way analysis of variance (ANOVA), and when normal distribution and/or homogeneity of the variances were not

achieved after transformation robust tests (Kruskal-Wallis) where done, followed by Tukey's post hoc test to test differences between different groups. In all statistical tests used, $P<0.05$ was considered significantly different (Pestana, D., Velosa, S. 2008).

4. Results

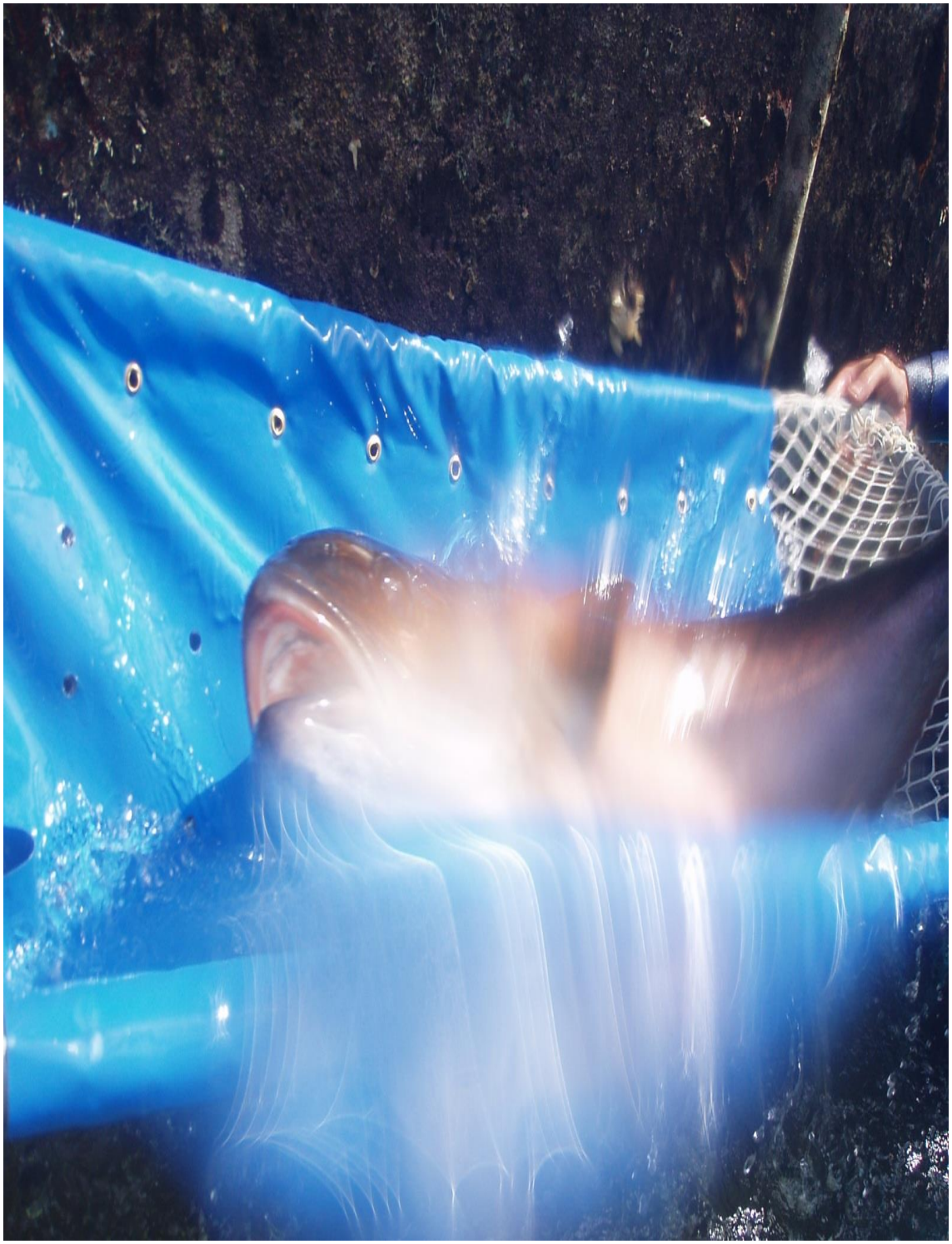

Figure 4 –*S. dumerili* adult being caught for sampling, Spanish Institute of Oceanography

4.1. Growth and Survival

The initial and final biomass of fish, did not show any significant difference ($p>0.05$) between the different diets, however with a weight dispersal, because of the big SD's displayed. Related to B%, fish fed with MA at LD (0.18 %) and SE at LD (0.41%) had the only positive B%, with this last one being the highest. Between the positive values, fish fed with MA at LD had the lowest amount of B% (0.18 %).

SGR showed significant differences ($P<0.05$) between group fed with SP at HD(0.0±0.1) and all the remaining groups, except for group fed with MA at HD (0.45±0.28) (Table 3). In one hand there was a tendency of stabilization of SGR between groups fed with SE at LD and MA at LD, while in the other hand, the same groups, but in HD, displayed an apposite tendency to increase their SGR through time, as displayed in Figure 6. The group fed with SP at HD was distinctively lower as displayed in Figure 6.

The Table 3 showed, concerning to CF, significant differences ($P<0.05$) between the group fed with MA at HD (2.25±0.19), which displayed one of the highest CF, and the group fed with SP at LD (1.62±0.11) and SE at LD (1.92±0.15). During the study, the fishes, as Figure 5 displayed showed less variation in CF, in the group fed with SE at LD.

Survival's highest value at the end of the study was showed by fish fed with SE at LD (60%; Table 3), even with the declive displayed in the Figure 4, in the first stage (0-34 fish), manage to stabilize until the end of the study. Fish fed with SP at LD had the lowest survival rate (20%;$P<0.05$). Fish, as displayed in Figure 7, showed a tendency for better survival rates at LD density as showed by group fed with SE (60%) and MA (53%) ($P<0.05$).

The highest FCR was presented by fish fed with MA at LD (6,01%) and HD (4,42%) and at SP at LD (4,54%). Considerably lower FCR were the fish fed with SE at LD (2,1%) and HD (1,46%) which displayed the lowest value.

As displayed at Table 3, fish fed on MA at LD presented the highest daily food ingestion, with 9.59±3.56 g/fish, and at HD 8.46±5.00 g/fish, followed by SE at LD 3.73±2.18 g/fish and at HD 3.42±2.27 g/fish, and as the lowest daily digestion, SP at LD 2.61±3.43 g/fish.

Table 3 - Biometric parameters, B%, FCR, DFI and survival obtain between the distinct periods of the study (1 and 86 days)

		SP - LD	SP- HD	SE - LD	SE - HD	MA - LD	MA - HD
DAY 1	WW (g)	127.2 ± 50.4	109.2 ± 52.9	121.9 ± 74.6	118.1 ± 51.8	116.1 ± 51.5	118.3 ± 56.3
	Bi (g)	1908	2838	1829	2953	1974	2838
	Nº fish/tank	15	26	15	25	17	24
DAY 86	WW (g)	176.7 ± 36.9	0.0- ± 0.0	274.8 ± 149.6	319.4 ± 146.7	253.3 ± 100.3	283.0 ± 150.3
	Bf (g)	530	0.0	2473	2875	2280	1981
	Nº fish/tank	3	0	9	9	9	7
	CF	1.6 ± 0.1 a	0.0 ± 0.0	1.9 ± 0.2 ab	2 ± 0.2 bc	2.2 ± 0.3 bc	2.3 ± 0.2 c
	SGR (%)	0.0 ± 0.1 a	0.0 ± 0.0	0.6 ± 0.3 b	0.8 ± 0.4 b	0.6 ± 0.5 b	0.5 ± 0.3 ab
	S (%)	20	0	60	36	53	29
	HI (%)	0.9 ± 0.3	0 ± 0	0.8 ± 0.6	0.8 ± 0.6	0.7 ± 0.6	0.9 ± 0.5
	B (%)	-0.84	*	0.41	-0.03	0.18	-0.35
	FCR	4.54	*	2.10	1.46	6.01	4.42
	DFI (g/fish)	2.6 ± 3.4	*	3.7 ± 2.2	3.4 ± 2.3	9.6 ± 3.6	8.5 ± 5

Results are expressed as means ± SD; Different letters in superscript within the same row represent significant differences (P<0.05); Capital letters in the right column of data represent – **a**– statistical different from Alpha Biomar (P<0.05); **b**– statistical different from Skreting (P<0.05); **c**– statistical different from Mackerel (P<0.05); **Nº**- number of fish; **CF** - Condition Factor; **SGR** - Specic Growth Rate (percentage of body weight increase per unit time); **S** - Survival; H**I** - Hepatossomatic Index; **B** – Mean Biomass Relative Index ; **FCR**- Food Conversion Rate; **DFI** - Daily Food Ingest (g/fish) (*) There was no available data to perform calculatations

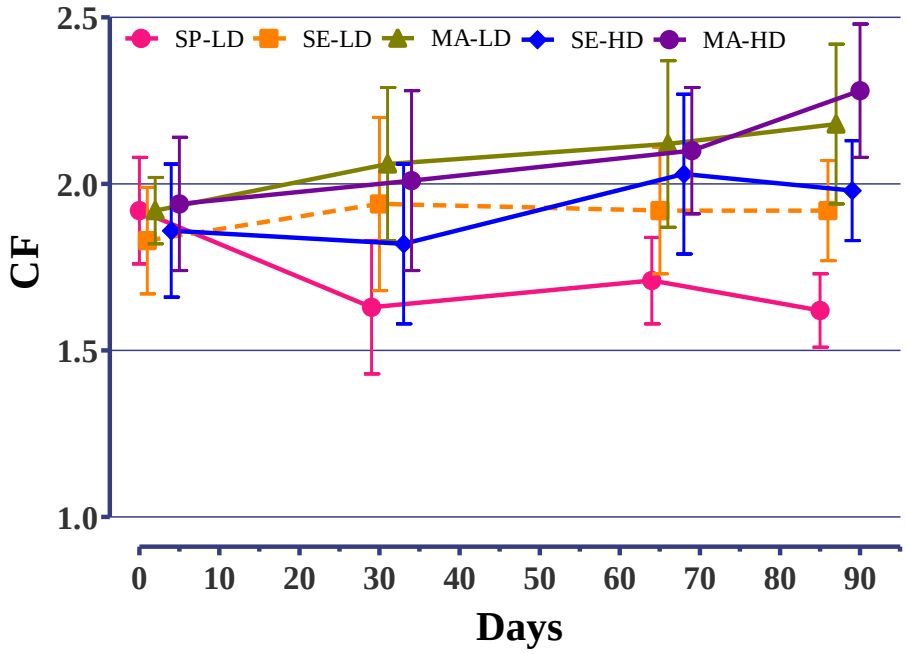

Figure 5 – Evolution of CF of the different groups during experimental period;

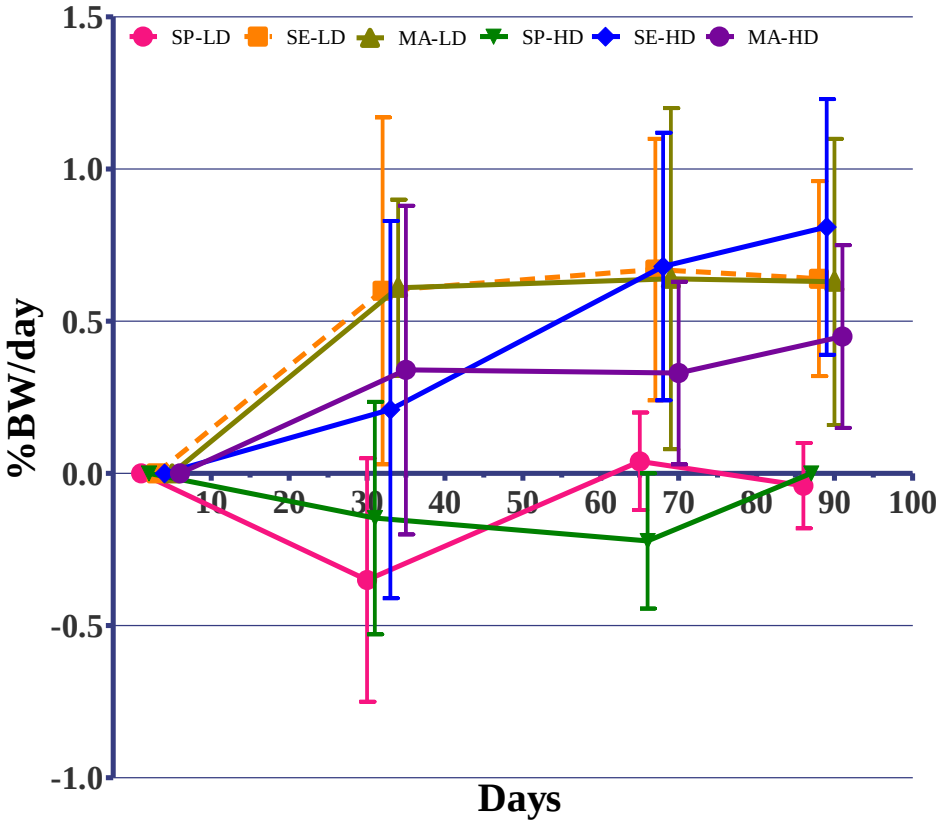

Figure 6 – Evolution of SGR (%) of the different groups during experimental period

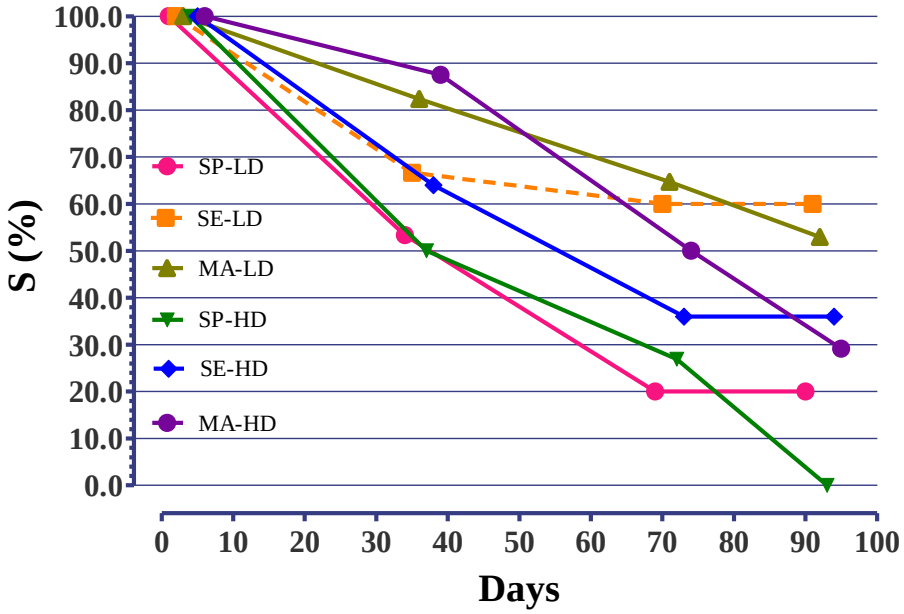

Figure 7 – Evolution of S (%) of the different groups during experimental period

4.2. Biochemical Determinations

The moisture content, total lipid and lipid classes of muscle and liver from *S. dumerili* fed with different diets are shown in Tables 4 and 5, respectively.

Moisture showed significant differences ($P<0.05$) between groups fed with SE and from all remaining groups in muscle and liver. In addition fish fed with SE presented the lowest content of moisture in muscle at LD (67.86±0.39 %) and at HD (68.93±0.41 %), and in liver (66.63±2.28 %) at LD and at HD (68.33±0.37) ($P<0.05$).

Total lipid also showed significant differences ($P<0.05$) between groups fed with SE and all remaining groups in muscle (Table 4), with the fish fed with SE, at either LD (20.49±6.37 %) or HD (18.37±5.34 %), showing an almost fourfold lipid content ($P<0.05$), when compared with others. while groups fed with MA, at LD (10.59±2.71 %) and HD (11.88±2.86 %), which displayed the lowest content, were significant different ($P<0.05$) from all remaining groups in liver (Table 5). Also in liver, fish fed with SE at LD displayed a higher lipid content (27.94±3.69 %; $P<0.05$) than IG (19.84±5.03 %).

In muscle tissue (Table 4), IG presented the highest percentage of SM (1.52±0.71 %) which was significantly different ($P<0.05$) from all the remaining groups, except for fish fed with MA at LD and SP at LD.

Table 4 – Muscle tissue moisture, total lipid and lipid classes of *S. dumerili* fed with three different diets under two different stocking densities, and IG (%)

	Muscle																							
	IG				SP				SE - LD				SE - HD				MA - LD				MA - HD			
Moisture	78.87	±	0.26	b	81.61	±	0.47	b	67.86	±	0.39	a	68.93	±	0.41	a	80.98	±	0.94	b	82.19	±	0.56	b
Total Lipid	4.01	±	1.07	a	3.37	±	2.51	a	20.49	±	6.37	b	18.37	±	5.34	b	3.77	±	0.93	a	3.38	±	1.35	a
LPC	0.00	±	0.00		0.00	±	0.00		0.00	±	0.00		0.00	±	0.00		0.00	±	0.00		0.00	±	0.00	
SM	1.52	±	0.71	b	0.92	±	0.33	ab	0.38	±	0.40	a	0.28	±	0.18	a	0.57	±	0.44	ab	0.30	±	0.23	a
PC	21.82	±	5.53		16.28	±	4.21		21.98	±	6.58		24.61	±	4.35		24.24	±	9.32		24.51	±	7.95	
PS	2.72	±	0.95		2.01	±	1.07		2.08	±	0.68		2.96	±	0.81		3.28	±	1.59		3.15	±	1.27	
PI	4.42	±	0.96		6.96	±	0.39		5.13	±	2.08		5.88	±	2.58		4.75	±	2.36		4.72	±	1.80	
PG	2.37	±	0.95		2.37	±	0.31		2.29	±	1.15		1.96	±	0.55		1.89	±	0.84		2.26	±	1.14	
PE	11.56	±	2.66		11.01	±	1.23		11.87	±	4.01		11.69	±	2.40		10.80	±	3.49		11.89	±	3.16	
DAG	0.87	±	0.47		0.00	±	0.00		1.13	±	0.84		0.72	±	0.21		1.12	±	0.27		1.01	±	0.45	
CHO	17.14	±	4.98		18.29	±	2.51		15.49	±	3.34		15.06	±	3.02		17.61	±	4.93		19.53	±	3.51	
FFA	2.16	±	0.95	c	1.68	±	0.66	bc	1.16	±	0.60	abc	0.47	±	0.23	a	0.57	±	0.35	a	0.63	±	0.39	ab
TAG	33.64	±	16.25		38.37	±	0.86		34.37	±	15.59		33.69	±	11.67		31.60	±	17.62		30.02	±	13.59	
SE	1.79	±	1.63		1.09	±	0.74		4.24	±	3.39		2.91	±	0.99		3.71	±	4.14		1.86	±	1.90	
TNL	55.60	±	10.57		59.43	±	4.77		56.39	±	13.71		52.84	±	8.87		54.61	±	16.55		53.06	±	14.20	
TPL	44.40	±	10.57		39.55	±	3.33		43.73	±	13.72		47.38	±	8.87		45.52	±	16.62		46.84	±	14.12	

Results are expressed as means ± SD; Different letters in superscript within the same row represent significant differences (P<0.05); Capital letters in the right column of data represent – **a** – statistical different from Alpha Biomar (P<0.05); **b** – statistical different from Skreting (P<0.05); **c** – statistical different from Mackerel (P<0.05); **LPC** - Lysohposphatidylcholine; **SM** - Sphingomyelin; **PC** -Phosphatidylcholine; **PS** - Phosphatidylserine; **PI** - Phosphatidylinositol; **PG** - Phosphatodylglycerol; **PE** - Phosphatidylethanolamine; **DAG** - Diacylglycerol; **CHO** - Cholesterol; **FFA** - Free Fatty Acid; **TAG** - Triacylglycerol; **SE** - Sterol Ester; **TNL** - Total Neutral Lipid;**TPL** - Total Polar Lipid.

Table 5 – Liver tissue moisture, total lipid and lipid classes of *S. dumerili* fed with three different diets under two different stocking densities, and IG (%)

	Liver																							
	IG				SP - LD				SE - LD				SE - HD				MA - LD				MA -HD			
Moisture	75.13	±	0.00	b	75.45	±	0.17	b	66.63	±	2.28	a	68.33	±	0.37	a	75.94	±	0.19	b	77.72	±	0.51	b
Total Lipid	19.84	±	5.03	b	21.88	±	8.28	bc	27.94	±	3.69	c	24.46	±	2.28	bc	10.59	±	2.71	a	11.88	±	2.86	a
LPC	0.00	±	0.00		0.00	±	0.00		0.75	±	0.62		0.81	±	0.65		0.00	±	0.00		0.00	±	0.00	
SM	1.22	±	0.68		0.88	±	0.06		0.67	±	0.26		0.65	±	0.21		1.08	±	0.79		0.70	±	0.39	
PC	13.77	±	4.61		11.93	±	0.56		13.50	±	4.38		13.15	±	6.33		21.41	±	7.08		16.94	±	6.44	
PS	1.11	±	0.54		1.36	±	0.28		1.01	±	0.42		0.62	±	0.51		1.25	±	0.57		1.28	±	0.68	
PI	3.70	±	1.46		4.63	±	1.40		4.06	±	0.99		4.30	±	1.45		4.98	±	1.17		5.24	±	2.41	
PG	1.93	±	0.47		2.37	±	0.82		1.54	±	0.41		1.59	±	0.90		2.53	±	1.20		2.78	±	1.69	
PE	8.24	±	1.89		10.24	±	0.27		7.39	±	1.82		8.16	±	3.78		12.85	±	3.21		12.44	±	3.58	
DAG	2.25	±	1.03	ab	0.19	±	0.00	a	3.25	±	2.43	b	0.50	±	0.43	ab	0.85	±	1.67	ab	1.22	±	1.48	ab
CHO	11.80	±	4.18	a	20.31	±	5.27	b	11.27	±	3.01	a	14.00	±	1.35	ab	18.91	±	2.92	b	20.70	±	4.22	b
FFA	4.54	±	1.72		3.89	±	4.04		7.08	±	3.83		8.94	±	3.84		7.31	±	2.49		9.62	±	3.05	
TAG	44.66	±	14.47		39.36	±	0.44		45.11	±	11.16		44.10	±	14.03		21.76	±	9.47		20.82	±	14.73	
SE	6.35	±	2.52		3.12	±	0.35		3.72	±	6.03		2.61	±	1.32		2.96	±	1.09		3.40	±	1.27	
TNL	69.60	±	8.44		66.88	±	1.31		70.43	±	8.15		70.14	±	13.51		51.79	±	10.22		55.76	±	12.72	
TPL	29.96	±	8.40		31.40	±	1.61		28.92	±	8.29		29.28	±	13.14		44.11	±	11.15		39.38	±	12.90	

Results are expressed as means ± SD; Different letters in superscript within the same row represent significant differences (P<0.05); Capital letters in the right column of data represent – **a** – statistical different from Alpha Biomar (P<0.05); **b** – statistical different from Skreting (P<0.05); **c** – statistical different from Mackerel (P<0.05); **LPC** - Lysohposphatidylcholine; **SM** - Sphingomyelin; **PC** -Phosphatidylcholine; **PS** - Phosphatidylserine; **PI** - Phosphatidylinositol; **PG** - Phosphatodylglycerol; **PE** - Phosphatidylethanolamine; **DAG** - Diacylglycerol; **CHO** - Cholesterol; **FFA** - Free Fatty Acid; **TAG** - Triacylglycerol; **SE** - Sterol Ester; **TNL** - Total Neutral Lipid;**TPL** - Total Polar Lipid.

DAG showed significant differences (P<0.05) only in liver (Table 5), where the fish fed with SP at LD (0.19±0.00 %) was significantly different (P<0.05) from group fed with SE at LD (3.25±2.43 %). CHO only showed significant differences (P<0.05) in liver, where the IG group (11.80±4.18 %), and fish fed with SE diet at LD (11.27±3.01 %), were significantly different (P<0.05) from the groups fed with SP diet, SE diet at HD and MA, which presented the higher amounts.

FFA showed significant differences (P<0.05) only in muscle (Table 4) where the IG group presented the highest amount (2.16±0.95 %) and was significantly different (P<0.05) showing an almost threefold FFA amount from the remaining diets, except for the group fed with SP at LD.

TAG showed no significant differences (P<0.05) and was the lipid class that presented the highest percentage from all lipid classes percentages in this study in both tissues (Tables 4 and 5).

The TPL and TNL did not showed any significant differences (P<0.05) between different diets in both tissues (p>0.05), being TNL content higher than the TPL in all groups, in both tissues, and higher in liver than in the muscle. (Tables 4 and 5)

4.3. Fatty Acid Composition

The fatty acid composition of TL from muscle and liver of different groups is shown in Tables 6 and 7. Statistical analyses revealed important differences between fish fed with different types of food (P<0.05) but no difference between groups with different stocking density were detected (P>0.05). The fatty acids showing the highest content in both tissues were C16:0, C18:0, 18:1n-9, 18:2n-6, 20:5n-3 and 22:6n-3.

The fatty acid composition of tissues clearly reflected the composition of the diet supplied. The groups fed with MA presented the highest proportion of docosahexaenoic acid (DHA), arachidonic acid (ARA) and the lower amounts of eicosapentaenoic acid (EPA). In contrast, fish fed with SE presented the lower amounts of ARA and DHA and higher content in 18:2n-6 specifically in the liver. In consequence, fish fed with MA presented a higher DHA/EPA ratio and a lower EPA/AA ratio in both tissues while the opposite was observed in group SE. C16:0 content displayed significant differences (P<0.05) only in liver (Table 7), where MA groups at both LD and HD presented the lowest amounts (18.44±1.07

17.80±1.22 %; respectively; P<0.05), and were significantly different from all remaining groups except SP at LD group. The C16:1n-7 also only showed slight significant differences (P<0.05) in the liver tissue (Table 7)

The C18:0 showed significant differences (P<0.05) in both tissues (Table 6 and 7). In muscle, fish fed with MA at HD (8.77±0.49 %) was significantly different (P<0.05) from group SP at LD and IG. In liver the group fed with MA at HD (6.95±1.23 %) and LD (6.64±1.08 %) was significantly different (P<0.05) from fish fed with SP again and SE at LD.

Table 6 – Muscle tissue fatty acid composition of *S. dumerili* fed with three different diets under two different stocking densities, and IG (%)

	Muscle																							
	IG			a - LD			SE - LD			SE - HD			MA - LD			MA - HD								
C 14: 0	2.05	±	0.86	2.19	±	0.62	2.02	±	0.96	1.53	±	0.53	1.84	±	0.83	1.11	±	0.71						
C 16: 0 DMA	0.79	±	0.45	0.89	±	0.24	0.76	±	0.57	0.98	±	0.44	0.87	±	0.53	1.25	±	0.38						
C 16: 0	17.27	±	1.30	16.79	±	0.73	16.85	±	1.08	17.69	±	0.77	17.81	±	1.10	18.72	±	0.72						
C 16: 1 n-9	0.32	±	0.02	0.30	±	0.04	0.13	±	0.10	0.15	±	0.12	0.29	±	0.06	0.28	±	0.12						
C 16: 1 n-7	3.68	±	1.35	3.53	±	0.93	3.25	±	1.36	2.69	±	0.87	2.72	±	0.99	1.85	±	0.60						
C 18: 0 DMA	0.26	±	0.19	0.34	±	0.13	0.32	±	0.19	0.39	±	0.12	0.34	±	0.25	0.72	±	0.32						
C 18: 0	6.27	±	0.97	a	6.86	±	0.70	a	7.33	±	1.19	ab	7.94	±	0.65	ab	8.00	±	0.86	ab	8.77	±	0.49	b
C 18: 1 n-9 DMA	0.27	±	0.15	0.19	±	0.13	0.33	±	0.18	0.41	±	0.10	0.27	±	0.21	0.49	±	0.16						
C 18: 1 n-9	13.86	±	3.26	12.39	±	3.02	13.33	±	3.75	11.74	±	2.70	14.06	±	3.62	9.98	±	2.80						
C 18: 1 n-7 DMA	0.05	±	0.08	0.00	±	0.00	0.22	±	0.39	0.07	±	0.10	0.07	±	0.10	0.16	±	0.12						
C 18: 1 n-7	3.50	±	0.33	b	3.12	±	0.29	ab	3.27	±	0.29	ab	3.05	±	0.18	ab	2.90	±	0.19	ab	2.82	±	0.38	a
C 18: 2 n-6	11.33	±	1.76	a	11.46	±	1.93	a	14.43	±	1.94	a	13.07	±	0.54	a	6.91	±	2.34	b	5.55	±	1.96	b
C 18: 3 n-3	1.45	±	0.34	d	0.82	±	0.16	abc	1.34	±	0.49	cd	1.16	±	0.27	bcd	0.68	±	0.16	ab	0.44	±	0.14	a
C 20: 1 n-9	1.36	±	0.29	bc	0.89	±	0.21	abc	0.80	±	0.18	ab	0.73	±	0.11	a	1.45	±	0.43	c	0.98	±	0.42	abc
C 20: 4 n-3	0.61	±	0.11	c	0.51	±	0.07	bc	0.40	±	0.08	ab	0.41	±	0.07	ab	0.39	±	0.07	ab	0.29	±	0.04	a
C 20: 4 n-6	1.51	±	0.47	a	1.71	±	0.24	ab	1.36	±	0.51	a	1.58	±	0.39	a	2.06	±	0.49	ab	2.79	±	0.57	b
C 20: 5 n-3	8.40	±	0.70	b	8.95	±	0.64	b	8.00	±	0.83	b	8.19	±	0.58	b	4.36	±	0.57	a	4.06	±	0.36	a
C 22: 5 n-3	3.08	±	0.22	3.21	±	0.37	2.51	±	1.08	3.27	±	0.27	2.53	±	0.36	2.31	±	0.20						
C 22: 5 n-6	0.47	±	0.13	a	0.61	±	0.12	a	0.54	±	0.21	a	0.61	±	0.17	a	1.57	±	0.34	b	1.97	±	0.47	b
C 22: 6 n-3	17.57	±	4.70	a	18.28	±	3.22	a	17.41	±	5.94	a	20.06	±	3.99	ab	24.67	±	4.89	ab	30.07	±	5.53	b
C 24: 1 n-9	0.59	±	0.17	0.72	±	0.09	0.63	±	0.18	0.58	±	0.19	0.80	±	0.16	0.82	±	0.16						
UK.	1.07	±	0.16	b	0.44	±	0.07	a	0.93	±	0.27	b	0.79	±	0.13	b	1.01	±	0.20	b	1.19	±	0.24	b
SFA	25.59	±	1.39	25.85	±	0.89	26.20	±	1.21	27.16	±	0.91	27.66	±	1.04	28.61	±	0.91						
MUFA	23.63	±	5.19	21.13	±	5.16	21.96	±	5.87	19.42	±	3.92	22.56	±	5.59	17.38	±	4.37						
PUFA	44.42	±	4.46	45.54	±	1.68	45.98	±	5.23	48.36	±	4.32	43.18	±	4.89	47.47	±	4.45						
n-3 HUFA	29.65	±	4.75	30.95	±	3.44	28.31	±	6.65	31.93	±	4.26	31.96	±	4.71	36.73	±	5.39						
n-3	31.10	±	4.44	31.77	±	3.35	29.66	±	6.18	33.10	±	4.01	32.64	±	4.59	37.17	±	5.27						
n-6	13.32	±	2.06	ab	13.77	±	2.11	ab	16.33	±	1.59	a	15.26	±	0.65	a	10.54	±	2.62	b	10.30	±	1.57	b
n-9	16.41	±	3.56	14.48	±	3.87	15.22	±	3.87	13.61	±	2.91	16.87	±	4.43	12.55	±	3.50						
n-3/n-6	2.48	±	0.55	ab	2.49	±	0.55	ab	1.93	±	0.52	a	2.26	±	0.27	ab	3.95	±	1.22	bc	4.81	±	1.61	c
DHA/EPA	2.10	±	0.59	a	2.03	±	0.23	a	2.18	±	0.73	a	2.45	±	0.46	a	5.77	±	1.53	b	7.49	±	1.65	b
EPA/ARA	6.23	±	2.22	b	5.32	±	0.65	b	6.70	±	2.52	b	5.41	±	1.05	b	2.22	±	0.52	a	1.52	±	0.37	a
DHA/ARA	11.86	±	1.73	10.77	±	1.78	12.85	±	1.58	12.83	±	1.15	12.27	±	2.21	10.95	±	1.81						

Data presented in mean percentage of total FA content ± SD; Different letters in superscript within the same row represent significant differences (P<0.05); Capital letters in the right column of data represent – **a** – statistical different from Alpha Biomar (P<0.05); **b** – statistical different from Skreting (P<0.05); **c** – statistical different from Mackerel (P<0.05); **d** – statistical different from Control (P<0.05); **SFA**: saturate fatty acids; **MUFA**: monounsaturated fatty acids; **PUFA**: polyunsaturated fatty acids; **n-3 HUFA**: sum of omega 3 highly unsaturated fatty acids; UK: Unknown; **n-3:** omega 3 fatty acids; **n-6**: omega 6 fatty acids; **n-9:** omega 9 fatty acids; **DHA:** 22:6 n-3; **EPA:** 20:5 n-3; **ARA:** 20:4 n-6

Table 7 – Liver tissue fatty acid composition of *S. dumerili* groups fed with three different diets under two different stocking densities, and IG (%)

Liver	IG			SP			SE - LD			SE - HD			MA - LD			MA - HD		
C 14: 0	1.62 ± 0.57	ab		1.75 ± 0.13	abc		2.79 ± 0.48	c		2.36 ± 0.42	bc		1.43 ± 0.30	ab		1.36 ± 0.50	a	
C 16: 0 DMA	0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00			0.10 ± 0.10			0.06 ± 0.09		
C 16: 0	16.97 ± 0.90	bc		16.65 ± 0.79	abc		14.87 ± 0.42	a		15.44 ± 0.82	ab		18.44 ± 1.07	c		17.80 ± 1.22	c	
C 16: 1 n-9	0.45 ± 0.06	b		0.48 ± 0.07	b		0.30 ± 0.02	a		0.30 ± 0.05	a		0.43 ± 0.05	b		0.43 ± 0.03	b	
C 16: 1 n-7	3.77 ± 1.01	ab		4.16 ± 0.27	ab		4.61 ± 0.75	b		4.60 ± 0.31	b		2.88 ± 0.98	ab		2.78 ± 1.23	a	
C 18: 0 DMA	0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00		
C 18: 0	4.91 ± 0.49	ab		4.38 ± 0.32	a		3.90 ± 1.25	a		5.14 ± 1.15	ab		6.64 ± 1.08	b		6.95 ± 1.23	b	
C 18: 1 n-9 DMA	0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00		
C 18: 1 n-9	17.12 ± 2.12	b		16.90 ± 3.03	b		18.28 ± 0.76	b		18.15 ± 0.43	b		12.63 ± 1.80	a		12.14 ± 2.01	a	
C 18: 1 n-7 DMA	0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00		
C 18: 1 n-7	4.22 ± 0.56			4.08 ± 0.05			3.39 ± 1.45			4.67 ± 1.23			3.38 ± 0.25			3.32 ± 0.26		
C 18: 2 n-6	12.80 ± 1.28	b		14.26 ± 1.40	b		21.84 ± 1.25	c		19.94 ± 1.23	c		4.12 ± 1.26	a		4.64 ± 1.76	a	
C 18: 3 n-3	1.44 ± 0.21	b		1.18 ± 0.02	b		2.37 ± 0.15	c		2.21 ± 0.17	c		0.59 ± 0.10	a		0.55 ± 0.13	a	
C 20: 1 n-9	1.15 ± 0.20	c		0.92 ± 0.21	ab		0.73 ± 0.04	a		0.78 ± 0.06	a		0.99 ± 0.11	ab		0.91 ± 0.20	ab	
C 20: 4 n-3	0.79 ± 0.17	c		0.67 ± 0.03	bc		0.66 ± 0.13	abc		0.81 ± 0.12	c		0.43 ± 0.08	a		0.44 ± 0.11	ab	
C 20: 4 n-6	1.93 ± 0.45	b		1.86 ± 0.39	b		1.12 ± 0.08	a		1.12 ± 0.11	a		4.42 ± 0.36	c		4.33 ± 0.61	c	
C 20: 5 n-3	8.57 ± 1.29	b		8.95 ± 1.77	b		8.47 ± 0.90	b		7.79 ± 0.68	b		5.13 ± 0.57	a		5.33 ± 0.76	a	
C 22: 5 n-3	3.44 ± 0.31	d		3.08 ± 0.13	cd		2.43 ± 0.56	bc		3.21 ± 0.72	cd		1.56 ± 0.23	a		1.71 ± 0.11	ab	
C 22: 5 n-6	0.41 ± 0.08	b		0.45 ± 0.03	b		0.25 ± 0.02	a		0.24 ± 0.02	a		1.67 ± 0.10	c		1.69 ± 0.20	c	
C 22: 6 n-3	14.31 ± 4.25	b		15.40 ± 2.00	b		7.06 ± 0.80	a		7.57 ± 0.62	a		27.64 ± 1.60	c		28.05 ± 2.91	c	
C 24: 1 n-9	0.40 ± 0.18	a		0.44 ± 0.06	ab		0.18 ± 0.09	a		0.26 ± 0.04	a		0.84 ± 0.20	b		0.88 ± 0.20	b	
UK.	1.87 ± 0.60	ab		1.22 ± 0.03	b		1.48 ± 0.39	a		1.61 ± 0.17	ab		1.78 ± 0.40	ab		1.96 ± 0.42	ab	
SFA	23.50 ± 1.18			22.78 ± 1.04			21.56 ± 0.98			22.93 ± 1.16			26.51 ± 1.92			26.10 ± 1.98		
MUFA	27.11 ± 3.36	a		26.98 ± 4.05	a		27.49 ± 2.11	a		28.75 ± 1.24	a		21.15 ± 2.76	b		20.46 ± 3.43	b	
PUFA	43.69 ± 3.18			45.85 ± 2.46			44.20 ± 1.60			42.89 ± 1.08			45.56 ± 2.10			46.75 ± 3.49		
n-3 HUFA	27.11 ± 3.61	b		28.10 ± 3.47	b		18.63 ± 1.08	a		19.38 ± 1.05	a		34.76 ± 1.53	c		35.54 ± 3.19	c	
n-3	28.55 ± 3.43	b		29.28 ± 3.49	b		20.99 ± 1.12	a		21.59 ± 1.05	a		35.35 ± 1.48	c		36.09 ± 3.10	c	
n-6	14.73 ± 0.88	b		16.12 ± 1.03	b		22.96 ± 1.28	c		21.05 ± 1.30	c		8.54 ± 1.33	a		8.97 ± 1.65	a	
n-9	19.12 ± 2.33	a		18.75 ± 4.06	a		19.48 ± 0.82	a		19.49 ± 0.46	a		14.89 ± 1.92	b		14.36 ± 2.21	B	
n-3/n-6	1.95 ± 0.31	b		1.84 ± 0.33	b		0.92 ± 0.08	a		1.03 ± 0.10	a		4.23 ± 0.64	c		4.18 ± 0.92	c	
DHA/EPA	1.75 ± 0.67	b		1.75 ± 0.20	b		0.84 ± 0.12	a		0.98 ± 0.14	ab		5.46 ± 0.75	c		5.34 ± 0.80	c	
EPA/ARA	4.65 ± 1.28	b		4.91 ± 0.76	b		7.56 ± 0.81	c		7.02 ± 0.78	c		1.17 ± 0.14	a		1.26 ± 0.25	a	
DHA/ARA	7.48 ± 2.07			8.46 ± 0.86			6.29 ± 0.66			6.83 ± 0.82			6.28 ± 0.47			6.52 ± 0.32		

Data presented in mean percentage of total FA content ± SD; Different letters in superscript within the same row represent significant differences (P<0.05); Capital letters in the right column of data represent – **a** – statistical different from Alpha Biomar (P<0.05); **b** – statistical different from Skreting (P<0.05); **c** – statistical different from Mackerel (P<0.05); **d** – statistical different from Control (P<0.05); **SFA**: saturate fatty acids; **MUFA**: monounsaturated fatty acids; **PUFA**: polyunsaturated fatty acids; **n-3 HUFA**: sum of omega 3 highly unsaturated fatty acids; UK: Unknown; **n-3:** omega 3 fatty acids; **n-6**: omega 6 fatty acids; **n-9:** omega 9 fatty acids; **DHA:** 22:6 n-3; **EPA:** 20:5 n-3; **ARA:** 20:4 n-6

Regarding C18:1n-9, this FA only showed significant differences ($P<0.05$) in the liver tissue (Table 6), where fish fed with MA at LD and HD displayed the lowest amount (12.63±1.80 % and 12.14±2.01 %, respectively; $P<0.05$), being significantly different ($P<0.05$) from all the remaining groups, including IG.

C16: 0 DMA, C18:0 DMA and C18:1n-7 DMA didn't show any significant differences ($P<0.05$) among groups in muscle tissue and neither the IG group.

The C18:1n-7 only showed significant differences ($P<0.05$) in the muscle tissue (Table 6), where the only significantly difference ($P<0.05$) was between the group fed with MA at HD (2.82±0.38 %) and the IG group (3.50±0.33 %).

The C18:2n-6 showed significant differences ($P<0.05$) in both tissues (Table 6 and 7). In the muscle tissue (Table 6), the groups fed with MA at LD and at HD had the lowest amounts (6.91±2.34 % and 5.55±1.96 %, respectively; $P<0.05$), and were significantly different ($P<0.05$) from all the remaining groups and IG group (11.33±1.76 %). In the liver tissue (Table 6), the groups fed with SE at LD (21.84±1.25 %) and at HD (19.94±1.23 %) were the highest amounts ($P<0.05$), and significantly different ($P<0.05$) from all the other groups, especially to the groups fed with MA, having almost fivefold their amount. All the other groups, except for SP at LD, were significantly different ($P<0.05$) from the IG group (12.80±1.28 %).

The C20:4n-6 (ARA) showed significant differences ($P<0.05$) in both tissues (Table 6 and 7) and in low amounts. In the muscle tissue (Table 6), the group fed with MA diet at HD (2.79±0.57 %), that had one of the highest amounts ($P<0.05$), was significantly different ($P<0.05$) from the group fed with SE at LD (1.36±0.51 %), SE at HD (1.58±0.39 %). The only significant difference between IG group (1.51±0.47 %) and the rest of the groups was with the group fed with MA at HD. In the liver tissue (Table 7), the groups fed with MA at LD and at HD had the higher amounts (4.42±0.36 % and 4.33±0.61 %, respectively; $P<0.05$), and were significantly different ($P<0.05$) from all the remaining groups. The same groups fed with SE at LD and at HD had the lower amounts (1.12±0.08 % and 1.12±0.11 %, respectively; $P<0.05$), and were even significantly different ($P<0.05$) from all the remaining groups. All groups were significantly different ($P<0.05$) from the IG group (1.93±0.45 %)

The C20:5n-3 (EPA) showed significant differences ($P<0.05$) in both tissues (Table 6 and 7). In the muscle (Table 6), the groups fed with MA at LD and at HD had the lower amounts (4.36±0.57 % and 4.06±0.36 %, respectively; $P<0.05$), and were significantly different ($P<0.05$) from all the remaining groups and the only being significantly different ($P<0.05$) from the IG group (8.40±0.70 %).

As in liver (Table 7), the groups fed with MA at LD were again the lowest amounts (5.13±0.57 % and 5.33±0.76 %, respectively; P<0.05), and significantly different (P<0.05) from all the remaining groups and again the only being significantly different (P<0.05) from the IG group (8.57±1.29 %)

The C22:6n-3 (DHA) showed significant differences in both tissues (Table 6 and 7). In the muscle tissue (Table 6), the group fed with MA diet at HD (30.07±5.53 %), one of the highest amounts, was significantly different (P<0.05) from groups fed SE at LD (17.41±5.94 %), SP at LD (18.28±3.22 %), and the only being significantly different (P<0.05) from IG group (17.57±4.70 %). In the liver (Table 7), the groups fed with MA diet at LD and HD were the highest amounts (27.64 ±1.60 % 28.05±2.91 %; respectively; P<0.05), and were significantly different (P<0.05) from all the remaining groups, especially from the groups fed with SE, having almost fourfold their amount, and the IG group (14.31±4.25 %). The same groups fed with SE at LD and at HD had the lower amounts (7.06±0.80 % and 7.57±0.62 %, respectively; P<0.05), and were significantly different (P<0.05) from the IG group (14.31±4.25 %) and the group fed with SP at LD (15.40±2.00 %). The IG group (14.31±4.25 %) was significantly different (P<0.05) from all the remaining groups, except for the group fed with SP at LD.

The MUFA only showed significant differences (P<0.05) in the liver tissue (Table 7), where fish fed with MA at LD and HD displayed the lowest amount (21.15±2.76 % and 20.46±3.43 %, respectively; P<0.05), being significantly different (P<0.05) from all the remaining groups, including IG.

The n-3 HUFA, showed no significant differences (P>0.05) between the different groups and groups with IG group in the muscle, only in the liver tissue (Table 7), where the groups fed with MA at LD and HD were the higher amounts (34.76 ±1.53 % 35.54±3.19 %; respectively; P<0.05), and were significantly different (P<0.05) from all the remaining groups, specially the groups fed with SE, having almost the double of their amount. The IG group (27.11±3.61 %) was significantly different (P<0.05) from all the remaining groups, except for the group fed with SP at LD. The groups fed with SE at LD (18.63±1.08 %) and at HD (19.38±1.05 %), were significantly different (P<0.05) from the IG group (27.11±3.61 %) and the group fed with SP at LD (28.10±3.47 %).

The n-3 total amount, showed significant differences (P<0.05) only in the liver tissue (Table 7), where the groups fed with MA at LD (35.35±1.48 %) and HD (36.09±3.10 %), again the higher amounts (P<0.05), were significantly different (P<0.05) from all the remaining groups. Table 7 also displayed that the groups fed with SE diet at LD

(20.99±1.12 %) and at HD (21.59±1.05 %), the lower amounts, also were significantly different ($P<0.05$) from all other groups, including the IG group (28.55±3.43 %). The IG group was significantly different ($P<0.05$) from all the remaining groups, except for the group fed with SP at LD.

The n-6 total amount showed significant differences ($P<0.05$) in both tissues (Table 6 and 7). As shown by Table 6, in the muscle the groups fed with MA at LD and HD displayed the lower amounts (10.54±2.62 % 10.30±1.57 %; respectively; $P<0.05$), and were significantly different ($P<0.05$) from all the remaining groups and IG group (13.32±2.06 %). In the liver (Table 7), as in the muscle before, the groups fed with MA at LD and HD displayed once again the lower amounts (8.54±1.33 % 8.97±1.65 %, respectively; $P<0.05$), and were significantly different ($P<0.05$) from all the remaining groups. The groups fed with SE diet at LD and HD displayed the higher amounts (22.96±1.28 % and 21.05±1.30 %; respectively; $P<0.05$), and also were significantly different ($P<0.05$) from all other groups. The IG group (14.73±0.88 %) was significantly different ($P<0.05$) from all the remaining groups, except for the group fed with SP at LD.

The n-9 total amount showed significant differences ($P<0.05$) in liver tissue (Table 6 and 7). In the liver tissue (Table 7), the groups fed with MA diet at LD and HD, showed the lowest amounts (14.89±1.92 % and 14.36±2.21 %, respectively; $P<0.05$), were significantly different ($P<0.05$) from all remaining groups, and the only significantly different ($P<0.05$) from IG group (19.12±2.33 %).

The n-3/n-6 ratio showed significant differences ($P<0.05$) in both tissues (Table 6 and 7). In the muscle tissue (Table 6), the groups fed with MA at LD (3.26±0.92 %) and HD (3.73±1.02 %) were the higher amounts and significantly different ($P<0.05$) from all the remaining groups, and the only MA at HD was significantly different ($P<0.05$) from IG group (2.48±0.55 %). In the liver tissue (Table 7), the groups fed with MA diet at LD and HD were the higher amounts (4.23±0.64 % and 4.18±0.92 %, respectively; $P<0.05$) and significantly different ($P<0.05$) from all the remaining groups, having almost threefold their amount. The group fed with SE diet at LD and HD were the lower amounts (0.92±0.08 % and 1.03±0.10 %, respectively; $P<0.05$), and were also significantly different ($P<0.05$) from all the remaining groups. The IG group (1.95±0.31 %) was significantly different ($P<0.05$) from all the remaining groups, except for the group fed with SP at LD.

The DHA/EPA showed significant differences ($P<0.05$) between different diets in both tissues (Table 6 and 7). In the muscle (Table 6), the groups fed with MA at LD and HD had the higher amounts (5.77±1.53 % and 7.49±1.65 %, respectively; $P<0.05$), and were

significantly different (P<0.05) from all the remaining groups, having almost fivefold more, and the IG group (2.10±0.59 %). In the liver (Table 7), the same groups fed with MA diet at LD and HD were again the higher amounts having almost fourfold more (5.46±0.75 % and 5.34±0.80 %, respectively; P<0.05), and were significantly different (P<0.05) from all the remaining groups. The group fed with SE diet at LD (0.92±0.08 %), one of the lower amounts (P<0.05), was also significantly different (P<0.05) from all other groups, except for the group fed with SE at HD. The IG group (1.75±0.67 %) was significantly different (P<0.05) from all the remaining groups, except for the group fed with SP at LD and SE at HD.

The EPA/ARA showed significant differences (P<0.05) in both tissues (Table 6 and 7). In the muscle (Table 6), the groups fed with MA diet at LD and at HD had the lower amounts (2.22±0.52 % and 1.52±0.37 %, respectively; P<0.05), and were significantly different (P<0.05) from all the remaining groups. In the liver (Table 7), the same groups fed with MA diet at LD and at HD had again the lower amounts having almost sevenfold less than other groups (1.17±0.14% and 1.26±0.25 %, respectively; P<0.05), and were significantly different (P<0.05) from all the remaining groups. The groups fed with Skreting diet at LD and HD had the higher amounts (7.56±0.81 % and 7.02±0.78 %, respectively; P<0.05), and were also significantly different (P<0.05) from all other remaining groups. The IG group (4.65±1.28 %) was significantly different (P<0.05) from all the remaining groups, except for the group fed with SP at LD.

The DHA/ARA showed no significant differences (P<0.05) between different groups in both tissues (Table 6 and 7)

4.4. Plasma Biochemical Parameters

Table 8 resumes the values of cholesterol, glucose, lipase, total protein and triglycerides found in the plasma of fish fed different diets under different densities. Cholesterol, glucose and triglycerides showed no significant differences between different diets and densities ($p>0.05$). Only total protein in plasma increased significantly during the study, and with the exception of the SP at LD, all groups were significantly different ($p>0.05$) from IG, which displayed one of the lowest content (33.74±2.81 g/l).

Table 8 - Cholesterol (mg/dL), glucose (mg/dL), total protein (mg/dL) and triglycerides (mg/dL) present in plasma of cultured *S. dumerili*

	Cholesterol (mg/dl)	Glucose (mg/dl)	Total protein (g/l)		Tryglicerides (mg/dl)
IG	265,84 ± 33,37	118,78 ± 53,76	33,74 ± 2,81	a	747,92 ± 323,63
SP	295,20 ± 38,01	100,47 ± 6,50	58,75 ± 3,27	ab	442,21 ± 7,31
SE - LD	317,12 ± 16,40	126,86 ± 5,23	74,22 ± 5,30	bc	309,27 ± 42,86
SE - HD	364,97 ± 57,71	149,64 ± 12,16	90,08 ± 7,32	c	400,39 ± 83,55
MA - LD	346,70 ± 35,71	132,38 ± 14,90	79,69 ± 8,97	bc	481,85 ± 38,61
MA - HD	350,00 ± 86,70	138,25 ± 24,52	83,23 ± 14,76	bc	335,14 ± 90,88

Results are expressed as means ± SD (n= x); Different letters in superscript within the same row represent significant differences ($P<0.05$); Capital letters in the right column of data represent – **A** – statistical different from Aplha Biomar ($P<0.05$); **B** – statistical different from Skreting ($P<0.05$); **C** – statistical different from Mackerel ($P<0.05$); **D** – statistical different from Control ($P<0.05$);

5. Discussion

There was no significant differences between the groups fed with SE and MA relatively to SGR. These results were not predicted, taking in consideration the considerably higher DFI values in groups fed with MA (9.5±3.6 g/fish at LD and 8.5±5 g/fish), comparing with the groups fed with SE (3.7±2.2 g/fish at LD and 3.4±2.3 g/fish at HD), and plus that according to Papadakis et al (2008), it is normal to verify a higher SGR in individuals fed with higher moist content instead of dry, which should had been the fish fed with the MA diet.

Indeed, highly moisturized diets increase food consumption (Ruohonen et al 1997), because of the better and fast adaptability to such diets (frozen fish or semi-moisted pellets), which had been already verified in other studies (Skaramura et al., 2001). These facts explain why groups fed with MA had such higher DFI values. The fact that this increase of food consumtion is not converted to growth efficiently, might had been due to the fact this feeding behavior requires less time for maximum hydration in the stomach (Ruohonen et al 1997), achieving ad libidum quicker than any other groups fed with extruded pellet, not converting that in growth, and for that showed a similar SGR evolution as the other groups in the study (Figure 6).

FCR is the ratio of the mass of food provided to biomass gained. Thus, the lower the value, the more efficiently the food is turned into fish muscle (Lu et al 2012). The higher DFI in groups fed with MA, with no increment of biomass associated, explains the lower FCR of the groups fed with SE (2.1 at LD and 1.46 at HD), and the higher FCR of the groups fed with MA (6.01 at LD and 4.42 at HD).

To summarise, these results showed the lack of efficiency of the diet MA in comparition to SE diet.

Regarding the two different tested densities in this study, differences were detected in S (%), Mean Weight and B%. Also, Ellis (2002) recorded an adverse effect of increasing density, with a reduction in the efficiency of food conversion (i.e., an increase in FCR), but our results were opposite with and increasing of FCR in the LD groups within the same diet, SE and MA.

High stocking density has been reported to cause decreased growth in salmonids (Vijayna and Leatherland 1988) due to different factors such decreased in food consumption and social interactions (Wedemeyer 1997). In this present study, even though the groups fed with MA at HD had a low S% (29%), the results showed a high DFI (8.5±5), so a decrease in food consumption was not considered as a valid explanation for the S% results.

Social interactions, in the other hand, are known to influence the growth variation of many fish species by leading to the formation of feeding hierarchies and thus decreasing the growth of the low ranking individuals (Koebele, 1985).

Aggressive behaviour expressed as social hierarchy or territoriality has often been postulated as a mechanism of competition for food and space, which benefits the dominants and therefore resulting in growth dispensation (Magnuson, 1962), low growth to the subordinated fish, and consequently, death. In the present study, the SD values for all groups were almost half of the value of the average Mean Weight (Table 3), meaning by this that there was a heterogeneous growth, that may have led to the origination of a social hierarchy, competition for food and aggressive behaviour that had as a consequence the increased mortality in the groups.

Previous study, S.-Z. Dou et al., (2004), had the same theory, where the large flounder could survive well regardless of size hierarchies, but the small flounder would suffer a high mortality of 37% in the presence of large flounder, in contrast with 9% when cultured alone. Considering S% (Table 3), even though the HD stocking groups fed with SE (36%) and MA (29%) were the one more affected, this also affected the LD stocking groups with S% between 60%-53%, making believe there was something else affecting.

Well, according to Kadri *et al*., (1996) the mode of food distribution over time and space is an important factor affecting social interactions and thus size variability like in this study, due to the high SD in the diferente groups, showed by Table 3. For example, when food is distributed evenly over the water surface of a tank, all individuals will have equal access to food and thus growth benefits of dominance would be reduced (McCarthy *et al*., 1999). However when the food supplied is unevenly distributed in time and space, dominant individuals will have easier access to the food excluding other size groups, and this might be what happen in our study.

According to Ellis (2002), in rainbow trout studies, CF is considered to be an important stress indicator, which will reflect ingestion efficiency and better conditions of culture. The CF values of the different groups in this study, are adequate and in agree with the previous research values with the same diets (Jerez et al Barcelona 2011).

The S (%) values in this study were in line with the previous research, with the same diets (Jerez et al Barcelona 2011), and with the S% values being higher in the groups at LD in all different diets.

Regarding fish fed with SP, the S (%) values were the lowest in the study, with only 20% in the LD group and no survival at HD, which lead to the conclusion that this diet was not good in any density.

According to the present study results, it can be deduce that SP diet is not a good diet, regardless the density, because had total mortality in the HD group, and in the LD group, had a negative biomass increment (-0.84), the lower S% (20) and the worst SGR% (0.00±0.14) in all study. This may be due to the quality and palatability of the pellet or the not acceptance by the fishes. Kissil et al. (2000) demonstrated that presence of some differences in diets that might affect the palatability, might not allow fish to consume the same amount of diet. On the other hand it can be deduce that SE diet is the most efficient diet, with one of the higher SGR%, higher S% and specially higher B% and lower FCR in the study, because fish feeding is one of the most important factor in commercial fish farming because feeding regime may have consequences on both growth efficiency and feed wastage (Azzaydi et al., 2000), and the higher B% induces to an efficient use of the food ingested.

There is also an obvious lipid accumulation in the muscle tissue in group fed with SE diet, which had the highest amounts (20.49±6.37) at HD (18.37±5.34), that were almost fivefold higher than the others. Previous studies like Bell et al. (1994) and Sargent et al. (1995) let it well established that the deposition of fatty acids in tissue lipids was strongly affected by dietary fatty acid. This lead us to think that there was an incorporation efficiency by the fishes, as in Castell et al., 1994 were the elevated levels of specific fatty acids in larval tissue compared to dietary amounts have been refered to that. However is is difficult to differentiate whether this elevation is due to preferential retention/utilization.

In the TPL class, the PC and PE were the main classes detected in muscle and liver tissues of the fishes. The phospholipids (PL) are considered to be a structural lipid that is constituent of cell membranes, therefore dietary PL are believed to be extremely crucial, for example, on larval development in fish (Cahu et al. 2003). Previous study demonstrated that dietary PL fractions, such as phosphatidylcholine (PC), could enhance the acceptability of diet (O.Uyan et al 2009) suggesting that PC stimulates feeding by acting as a feed attractant in gilthead sea bream (*Sparus aurata*) larvae owing to the trimethyl group of the nitrogen moiety (Koven et al.1994). In the present study, the most abundant PL was PC in each diet, density

and tissue, and there were not significant differences (P<0.05) between them, therefore mentioned, showing a correct available amount by the diets tested in this study.

Among the total neutral lipids (TNL) class, TAG was the most abundant lipid class, which is generally the major lipid class in the diet of marine fish (Tocher et al 2003). Body lipid reserves, particularly neutral lipid in adult female muscle and liver of marine fish, are an important energy store which is used, for example, during the reproductive process (Sargent et al., 2002). There was no significant differences (P<0.05) between the different groups, but according to Rodriguez-Barreto et al., 2012, the higher TAG accumulation detected in muscle and liver from cultured fish, could be related both to the supply of high energy commercial pellets and to reduced locomotor activity, as described for several species as *Dicentrarchus labrax* (Alasavar et al., 2002), *Sparus aurata* (Grigorakis, 2007), *Diplodus sargus* (Cejas et al., 2004), *Pagellus bogaraveo* (Alvarez et al., 2009) and *Anguilla japonica* (Oku et al., 2009). TAG accumulation in the groups fed with MA diet, a non-commercial pellet could be related to the amount of lipid of the MA himself, which converge with the 31.8% of crude fat described in the proximal composition of this diet (Table1), that is almost two times the content of the other diets.

The most economical and inexpensive source of energy in fish diets is CHO (Craig. S. and Louis A. Helfrich et al 2002), and it is also an essential component of biomembranes systems in all eukaryotic species. CHO has an important structural function interacting closely with phospholipids moderating the membrane fluidity (Sargent et al 2002). Unsaturated fatty acids play an important role in the transportation of other lipids. It has been repeatedly shown that feeding PUFA will lower the cholesterol (CHO) levels in animals (FAO). But results of this study were different, because the PUFA levels accumulated in muscle and liver tissue of the fishes were no significantly different (P<0.05) in all groups, unlike the CHO. Minor changes were measured in CHO levels after overcrowding of the sea bass (Di Marco et al 2008) as already reported in other species in response to acute stressors (Ruane et al., 2001; Biswas et al., 2006), but in this study, the different densities didn't had the influence to cause any significant difference (P<0.05) between the groups with different densities and same diet also.

The content of TNL, even though not presenting any significantly different (P<0.05) from TPL, was higher in all groups. It can be deduce that it is due to the higher amount of triglycerides in all diets, as in all densities and in both tissues, even though the decrease of polar lipid content of amberjack is considered to be a normal phenomenon (Yakamoto et al 2008).

The fact that the majority of the FFA are in correlation in the tissue of the fishes (Table 6 and 7), as in the pellets (Table 2), reflects the influence of the composition of the diets in the fatty acid composition of the fish tissues.

Regarding 18:1n-9, fish fed with MA were the only significantly different ($P<0.05$), and displayed the lowest amounts in this study (Table 5). Taking in consideration that, in the wild, *S. dumerili* diet is composed mainly by several finfish rich in 18:1n-9 (Karakoltsidis, et al., 1995 and Matallanas et al. 1995) and that the amount of 18:1n-9 is between 14-17% (Sun Young Lin et al 2012), we can deduce that or there was a degradation of the quality of the MA diet that affected directly the quality and quantity of the 18:1n-9 provided.

The amount of 18:2n-6 is higher in the groups fed with SE, so it is suggested that 18:2n-6 is in excess in SE diet composition, considering that this fatty cid is responsible for the most detrimental modifications to the fatty acid composition of cultured fish (Turchini et al., 2009) and it is accumulated, unchanged, in the lipids of the fish due to his reduced capacity of denaturation and elongation in the chain (Owen et al 1975; Yamada et al 1980

The fact that fish fed with MA presented the highest amounts of ARA and the lower amounts of EPA, were reflected in the lower EPA/ARA ratio by the groups fed with MA, meaning there was a low quantity of EPA in MA composition.

Castell et al. (1994) found that an increase of ARA levels resulted in reduced fat in whole body and liver of juvenile turbot. The results of the present study indicated a strong influence of ARA levels in tissue fatty acids, since the amount of fatty acids in the MA diet was almost three fold the amount of the others (Table 2), and the results showed that the fish fed with MA diet had the lower amounts in the study (Table 4 and 5).

Although ARA is the chief precursor of eicosanoids, which actions are determined by the EPA/ARA ratio that depend on the dietary content of these fatty acids provided by diets. EPA competitively interferes with ARA in the production of prostaglandins PGE3 and PGF3, inhibiting the conversion of ARA to the biologically more potent PGE2 and PGE2α, being EPA derivates less biologically active than those produced from ARA (Tocher et al 2003; Henrotte et al., 2010, 2011; Sorbera et al., 2001). So for the mentioned reasons, the lower amount of EPA and lower EPA/ARA ratio found in groups fed with MA, could positively affect certain physiological functions. A number of more recent studies have been conducted on dietary ARA and ARA/EPA ratios in larval gilthead sea bream which have confirmed that elevated ARA can improve growth and survival (Bessonart et al., 1999) and resistance to handling stress (Koven et al., 2001a).

Moreover, the DHA/EPA ratio was higher in the groups fed with MA due to their higher amount of DHA in the liver, that was almost threefold the amount in groups fed with SE (Table 5) and the for mentioned lower amount of EPA. Although due to their important role in maintaining cell membrane structure and function, marine fish require mainly n-3 HUFA and, in particular, EPA and DHA for optimum performance (Sargent et al. 1989), and both fatty acids have competitive interactions for their incorporation into phospholipids (Sargent et al., 2002), thus this ratio in the tissues could have a positive effect on certain physiological functions.

As mentioned before, the excessive accumulation of EPA could be prejudicial, and it had probably affected the production of DHA because of their competition in the groups fed with SE. Moreoever, the groups fed with SE had the lower amounts of ARA and DHA, inducing to a higher EPA/ARA ratio, suggesting that the ratio is unbalanced and there is too much EPA accumulation, to insure ARA production. Copeman L. et al (2002), presented 1.9±0.1 of DHA/EPA ratio with a 11% EPA enrichment diet in a study with yellowtail flounder, *Limanda ferruginea*, which goes along with the 2.18±0.73 (at LD) and 2.45±0.46 (at HD) of DHA/EPA ratio found in our study with the groups fed with SE. This fact, plus the fact that in the same study the amount of EPA had and average of 4.0, that is half of the amount found in te groups fed with SE (8.00±0.83 at LD and 8.19±0.58 at HD), indicates that EPA is most probably, not needed at the concentration provided by the SE diet (4.05±0.24) showing signs to be accumulating in the tissues

This study suggests that the fatty acid profile of the different groups, reflects the fatty acid composition of the diets (Table 2), namely the amounts of 18:1n-9, 18:2n-6 and 20:5n-3, 20:4n-6 and 22:6n-3 (Table 2) that had effects in the ratios of DHA, EPA and ARA, affecting the balance between them, which will directly affect growth, as shown in Rodriguez-Barreto et al (2012).

Regarding the plasma analysis, total protein was the only displaying statistical differences between diets and densities. According to Gras et al (1983) and Adham et al (1997), stress and malnutrition are the main reasons for the decrease of the protein level in plasma. The fact that fish fed with SE and MA showed higher protein content in the plasma, could be related to the fact those diets have higher protein levels (Table 1). However, different densities and schooling behavior associated with lack of aggressive actions might have been involved (Papoutsoglou et al. 1998). This assumption is supported by the fish final weightened that could explain the higher plasma protein amounts found in fish fed with SE at HD. Many studies have shown that density may influence the stress level under culture

conditions. For instance, the sea bass (*Dicentrarchus labrax* L.) shows higher stress levels at high densities (100 kg/m^{-3}), as indicated by high levels of cortisol (Gornati et al., 2004). Likewise, high stocking densities (40.8 kg/m^{-3}) in juvenile gilthead sea bream (*S. aurata* L.) also promote chronic stress (Montero et al., 1999). Some authors have suggested that the increase of glucose could be used as an index of stress (Benfey and Biron 2000), because high glucose levels in teleosts are generally associated with higher levels of cortisol resulting from great stress (Kubokawa et al 1999). In the present study, there was no significant differences ($P>0.05$) of glucose and triglyceride levels in plasma samples of different groups (Table 8), therefore none of the diets and densities being tested surpassed the levels displayed by the control, and this might be due to the fact that both LD (1.9 kg/m^{-3}) and HD (2.8 kg/m^{-3}) are too low compared with the stocking densities of the previous studies, not origining any perturbation in the stress indicators.

6. Final Considerations

Considering S%, mortality was higher in all groups of this study, especially in HD groups, making believe there was something else affecting. More research is needed to better understand the social hierarchies in cultured *S.dumerili*, and in further studies, size grading can be a solution to be count on, with the assumption that small fish will grow better when their larger contemporaries are removed.

Higher survival rate was observed in fish held at low stocking density, where the SE diet proved to be the most efficient diet in all study when it comes to stocking densities.

Regarding stress, as an important factor in the growth and survival of the fish, in further studies, cortisol plasma analyses should be done, because is widely used as a general indicator of stressful situations in fish, and will probably give us more information about the fish health status. The fact that there were no trace of stress signs in the different densities tested in this study, may be due to the fact that the densities were too low, comparing with other studies, so higher densities should be tried in further ones.

As previous, this study suggests that the fatty acid profile of the different groups, reflects the fatty acid composition of the diets, namely the amounts of 18:1n-9, 18:2n-6 and 20:5n-3, 20:4n-6 and 22:6n-3, that had effects in the ratios of DHA, EPA and ARA, affecting the balance between them, which will directly affect growth, and consenquently survival.

The amount of 18:2n-6 is higher in the groups fed with SE, so as mentioned before, it is suggested to be in excess in SE diet composition, considering that this fatty cid is responsible for the most detrimental modifications to the fatty acid composition of cultured fish. Fish fed with SE and SP diets showed lower DHA content and higher 18:2n-6 proportion, reflecting the fatty acid composition of these diets.

These preliminary results indicate that SE diet promotes a better performance than the SP diet in terms of growth and survival rates, which are comparable to fish fed with MA. Although, SE diet presented a higher lipid deposition in tissues due to increased lipid content in pellets.

The higher DFI, and also the higher FCR, of the MA diet, showed that having the higher ingested food, doesn’t directly mean growth.

7. References

Acerete, L., Balasch, J.C., Espinosa, E., Josa, A., Tort, L., 2004. Physiological responses in Eurasian perch (*Perca fluviatilis*. L.) subjected to stress by transport and handling. Aquaculture 237, 167-178

Ackefors, H., Wilkins, N. (Eds.), Aquaculture: A Biotechnology in progress. European Aquaculture Society, Belgium. 209-213 pp.

Adham, K., Khairalla, A. Abu-Shabana, M., Abdel-Maguid, N. And Abdel-Moneim, A. 1997. Environmental stress in lake Maryut and physiological response of Tilapia zilli. J. Environ. Sci. Health, PT A: Environ. Sci. Eng. Toxic Hazard. Subst. Control 2A: 9–10.

Alasalvar, C., Taylor, K.D.A., Zubcov, E., Sha-hidi, F., Alexis, M., (2002). Differentiation of cultured and wild sea bass (Dicentrarchus labrax): total lipid content, fatty acid and trace mineral composition. Food Chemistry, 79: 145-150.

Alvarez, V., Medina, I., Prego, R., Auburg, S.P., 2009. Lipid and mineral distribution
In different zones of farmed and wild blackspot seabream (Pagellus bogaraveo). European Journal of Lipid Science and Technology 111, 957–966.

Andaloro, F., Potoschi, A., Porrello, S., 1992. Contribution to the knowledge of growth of greater amberjack, *Seriola dumerili* (Cuv., 1817) in the Sicilian Channel (Mediterranean Sea). Rapp. Comm. Intern. Mer. Méditer. 33, 282. Finfish Species Divenification, vol. 16. CIHEAM. Zaragoza.

A.O.A.C. Association of Official Analytical Chemist, 1995. Official Methods of Analysis, 16th ed. AOAC International, Washington, DC

Ashley, P., 2007. Fish welfare: Current issues in aquaculture. Applied animal Behaviour Sciente 104: 1-37

Azzaydi, M.; Martinéz, F.J..; Zamora, A.; Sánchez-Vázquez, F.J.; Madrid, J.A. Effect of meal size modulation on growth performance and feeding Barton, B.A., Iwama, I.W., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteriods. A. Rev. Fish Dis. 1, 3–26.

Badalamenti, F., D'Anna, G., Lopiano, L., Scilipoti, D. And Mazzola, A. (1995). Feeding habits of young-of-the-year greater amberjack *Seriola dumerili* (Risso, 1810) along the N/W Sicilian coast. *Sci.Mar.* 59(3-4): 317-323.

Barton, B. A. and G. K. Iwama. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases,* 1:3-26

Benfey, J., Biron, M., 2000 Acute stress response in triploid rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*)Original Research Article *Aquaculture, Volume 184, Issues 1–2, 3 April 2000, Pages 167-176*

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., Sargent, J.R., 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. J. Nutr. 131, 1535– 1543.

Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R., Porter, A., Smullen, R.P., Sargent, J.R., 2002. Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid compositions and hepatic fatty acid metabolism. J. Nutr. 132, 222– 230.

Bessonart, M., Izquierdo, M.S., Salhi, M., Hernandez-Cruz, C.M., Gonzalez, M.M., Fernandez - Palacios, H., 1999. Effect of dietary arachidonic acid levels on growth and fatty acid composition of gilthead sea bream (*Sparus aurata L*.) larvae. Aquaculture 179, 265– 275.

Biswas, A.K., Seoka, M., Tanaka, Y., Takii, K., Kumai, H., 2006. Effect of photoperiod manipulation on the growth performance and stress response of juvenile red sea bream (Pagrus major). Aquaculture 258, 350–356

Buddington RK, Krogdahl A, Bakke-mckellep AM (1997) The intestines of carnivorous fish: structure and functions and relations with diet. Acta Physiol Scand 161:67–80

Buss, K., Graff, D.R., Miller, E.R., 1970. Trout culture in vertical units. Prog. Fish-Cult. 32, 187–191.

Bromley PJ (1980) The effect of dietary water content and feeding rate on the growth and food conversion

Cahu, C.L., Jambonino Infante, J.L. & Barbosa, V. (2003) Effect of dietary phospholipids level and phospholipids:neutral lipid value on the development of sea bass (Dicentrarchus labrax) larvae fed a compound diet. Br. J. Nutr., 90, 21–28.

Castell, J.D., Bell, J.G., Tocher, D.R., Sargent, J.R., 1994. Effects of purified diets containing different combinations of arachidonic and docosahexaenoic acid on survival, growth and fatty acid composition of juvenile turbot (*Scophthalmus maximus*). Aquaculture 128, 315– 333.

Cejas, J.R., Almansa, E., Jerez, S., Bolaños, A., Samper, M., Lorenzo, A., 2004. Lipid and Fatty acid composition of muscle and liver from wild and captive mature female Broodstocks of white seabream, Diplodus sargus. Comparative Biochemistry and Physiology Part B 138, 31–102.

Christie, W.W., 1982. Lipid Analysis. Pergamon, Oxford, UK.

Di Marco, P.et al. / Aquaculture 275 (2008) 319–328 327 Wedemeyer, G.A., 1997. Effects of rearing conditions on the health and physiological quality of fish in intensive culture. In: Iwama, G.K., Pickering,

Ellis, T., North, B., Scott, A.P., Bromage, N.R., Porter, M., Gadd, D., 2002. The relationship between stocking density and welfare in farmed rainbow trout. J. Fish Biol. 61, 493–531.

Ellis. T., Hijran Yavuzcan Yildiz, Jose Lópe-Olmeda, Maria Teresa Spedicato, Lluis Tort, Øyvind Øverli & Catarina I.M.Martins., 2012 Fish Physiology and Biochemistry

Eurostat (2011). Agriculture, fishery and forestry statistics, Main results – 2010-11; Eurostat Pocketbooks

FAO (2012). The State of World Aquaculture. FAO Fisheries Department;. Food and Agriculture Organization of the United Nations, Rome, Italy

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

García, A., Díaz, M.V., 1995. Culture of *Seriola dumerilii*. Cahiers Options Méditerranéennes. Marine Aquaculture Finfish Species Diversification, vol. 16. CIHEAM, Zaragoza, Spain, pp. 103 114

Ghioni, C., Tocher, D.R., Bell, M.V., Dick, J.R., Sargent, J.R., 1999. Low C_{18} to C_{20} fatty acid elongase activity and limited conversion of stearidonic acid, 18:4n-3, to eicosapentaenoic acid, 20:5n-3, in a cell line from turbot, *Scophthalmus maximus*. Biochim. Biophys. Acta 1437, 170– 181.

Gornati, R., Terova, G., Yigetti, D., Prati, M., Saroglia, M., Bernardini, G., 2004. Effects of population density on seabass (Dicentrarchus labrax, L.) Gene expression. Aquaculture. 230: 229-239. Rhythms in European sea bass (*Dicentrarchus labrax*, L.).Aquaculture, v.170, p.253-266, 1999

Gras, J. 1983. Proteínas plasmáticas. Fisicoquímica. Metabolismo. Fisiopatología y clínica de las proteínas extracelulares. 4 edn. Edited by Editorial JIMS, Barcelona.

Grigorakis, K., 2007. Compositional and organoleptic quality of farmed and wild Gilthead sea bream (Sparus aurata) and sea bass (Dicentrarchus labrax) and factors Affecting it: a review. Aquaculture 272, 55–75.

Henrotte, E., Mandiki, S.N.M., Agbohessi, T.P., Vandecan, M., Melard, C., Kestemont, P.,2010. Egg and larval quality and egg fatty acid composition of Eurasian perch breeders (Perca fluviatilis) fed different dietary DHA/EPA/ARA ratios. Aquaculture Research 41, e53–e61.

Henrotte, E., Milla, S., Mandiki, S.N.M., Kestemont, P., 2011. Arachidonic acid induces production of 17,20b-Dihydroxy-4-pregnen-3-one (DHP) via a putative PGE2 receptor in fish follicles from the eurasian perch. Lipids 46, 179–187.

Hughes, SG., Barrows FT (1990) Measurements of the abilities of cultured fishes to moisturize their digesta.

Jerez, S., Cejas, J. Santamaría, F. J., Villamandos, J. E., Samper, M., Felipe, B. C., 2003. Cultivo larvario, preengorde y engorde de medregales (*Seriola* sp.) Nacidos en cautividad. Proceedings of the IX Congreso Nacional de Acuicultura, 12-16 May, 2003 Cádiz, Spain, pp. 390-391.

Jerez,S., Samper,M., Santamaría, F.J., Villamandos, J.E., Cejas, J.R., Felipe, B.C., 2006. Natural spawning of greater amberjack (*Seriola dumerili*) kept in captivity in the Canary Islands. Aquaculture 252, 199-207 Cahiers Options Méditenanéennes, Marine aquaculnre Spain, pp. 103- 114.

Karakoltsidis, P.A., Zotos, A., Constantinides, S.M., 1995. Composition of the commercially important Mediterranean finfish, crustaceans and molluscs. Journal of Food Composition and Analysis 8, 258–273

Kissil, G.Wm., Lupatsch, I., Higgs, D.A. & Hardy, R.W. (2000) Dietary substitution soy and rapeseed protein concentrates for fish meal, and their effects on growth and nutrient utilization in gilthead seabream, Sparus aurata L. Aquacult. Res., 31, 595–601.

Koven, W.M., Henderson, R.J., Sargent, J.R., 1994. Lipid digestion in turbot (Scophtalmus maximus): I. Lipid class and fatty acid composition of digesta from different segments of the digestive tract. Fish Physiol. Biochem. 13, 69–79.

Koven, W.M.; Barry, Y.; Lutsky, S.; Ben-Atia, I; Weiss, R.; Harel, M.; Beherns, P.; Tandler, A. The effect of dietary arachidonic acid (20:4n-6) on growth, survival and resistance to handling stress in gilthead seabream (*Sparus aurata*) larvae. Aquaculture, v.193, p.107, 122, 2001.

Kozul, V., Skaramuca, B., Glamuzina, B., Glavic, N., Tutman, P., 2001. Comparative gonadogenesis and hormonal induction of spawning of cultured and wild mediterranean amberjack (*Seriola dumerili*, Risso 1810). Sci. Mar., 65 (3): 215-220.

Laidley CW, Leatherland JF (1988) Cohort sampling, anaesthesia and stocking-density effects on plasma cortisol, thyroid hormone, metabolite and ion levels in rainbow trout, Salmo gairdneri Richardson. J Fish Biol 33:73–88

Lazzari, A., Barbera, G., 1989. Farming the Mediterranean yellowtail, *Seriola dumerilii* (Risso, 1810) in concrete ponds: results and perspectives. In: De Pauw, N., Jaspers, E.

Lee SM, Hwang UG, Cho SH (2000) Effects of feeding frequency and dietary moisture content on growth Lee SM, Jeon IG, Kim KS (1997) Effects of extruded-floating, slow-sinking, fast-sinking or moist pellet

Lemaire, P., Drai, P., Mathieu, A., Lemaire, S., Carriere, S., Giudicelli, J., Lafaurie, M., 1991. Changes with different diets in plasma enzymes (GOT, GPT, LDH, ALP) and plasma lipids (cholesterol, triglycerides) of sea-bass (Dicentrarchus labrax). Aquaculture. 93: 63-75.

Masumoto T (2002) Yellowtail, *Seriola* quinqueradiata. In: Webster CD, Lim C (eds) Nutrient requirements and feeding of finfish for aquaculture. CAB Int, Wallingford, pp 131–146

Matallanas, J., Casadevall, M., Carrasson, M., Bolx, J., Fernandez, V., 1995. The Food of *Seriola dumerili* (Pisces: Carangidae) in the Catalan Sea (Western Mediterranean). Journal of the Marine Biological Association of the UK 75, 257–260.

Marino, G., Mandich, A., Massari, A., Andaloro, F., Porrello, S., 1995. Aspects of reproductive biology of the Mediterranean amberjack (*Seriola dumerilii* Risso, 1810): Gonadal developement. In: Basurco, B. (Ed.), Cahiers Options Méditerranéennes. Marine Aquaculture Finfish Species Diversification, vol. 16. CIHEAM, Zaragoza, Spain, pp. 115-124.

Mazeaud, M.M., Mazeaud, F., Donaldson, E.M., 1977. Primary and secondary effects of stress in fish: some new data with a general review. T. Am. Fish. Soc. 106: 201-212.

Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in Teleosts: dynamics, mechanisms of action, and metabolic Regulation. Rev. Fish Biol. Fish. 9, 211 –268.

Montero, D., Izquierdo, M.S., Tort, L., Robaina, L., Vergara, J.M., 1999. High stocking density producescrowding stress altering some physiological and biochemical parameters in gilthead seabream, Sparus aurata, juveniles. Fish Physiol. Biochem. 20: 53-60.

Micale, V., Maricchiolo, G., Genovese, L., 1999. The reproductive biology of the amberjack, *Seriola dumerili*i (Risso, 1810): I Oocyte development in captivity. Aquac. Res. 30, 349-355

Mylonas,C.C., Papandroulakis,N., Smboukis,A., Papadaki,M., Divanach, P., 2004. Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using gnrha implants. Aquaculture 237, 141-154.

Marino, G., Mandich, A., Massari, A., Andaloro, F., Porrello, S., 1995. Aspects of reproductive biology of the Mediterranean amberjack (*Seriola dumerili*i Risso) during spawning period. J. Appl. Ichthyol. 11, 9-24.

Mazzola, A., Favaloro, E., Sara, G. (2000) Cultivation of the Mediterranean amberjack, *Seriola dumerili* (Riso,1810), in submerged cages in the Western Mediterranean Sea. Aquaculture 181:257–268

Olsen, R.E., Henderson, R.J., 1989. The rapid analysis of neutral and polar marine lipids Using double-development HPTLC and scanning densitometry. Journal of Experimental Marine Biology and Ecology 129, 189–197.

Oku, T. Sugawara, A., Choudhury, M., Komatsu, M., Yamada, S., Ando, S., (2009) Lipid and fatty acid compositions differentiate be-tween wild and cultured Japanese eel (*Anguilla japonica*), *Food Chemistry,* 115: 436-440

Owen, J. M., Adron, J. W., Middleton, C., & Cowey, C. B. (1975). Elongation and desaturation of dietary fatty acids in turbot Scophthalmus Maximus L., and rainbow trout Salmo gairdnerii rich. Lipids, 10, 528–531.

Papadakis, I.E., S. Chatzifotis, P. Divanach, M. Kentouri, 2008. Weaning of greater amberjack (*Seriola dumerili*i Risso 1810) juveniles from moist to dry pellet. Aquaculture International 16 (1): 13-25

Papoutsoglou S.E., Tziha G., Vrettos X. & Athanasiou A. (1998) Effects of stocking density on behavior and growth rate of European sea bass (Dicentrarchus labrax) juveniles reared in a closed circulated system. Aquacultural Engineering18,135^ 144

Pavlidis, M., Berry, M., Divanach, P., Kentouri, M., 1997. Diel pattern of haematocrit, serum metabolites, osmotic pressure, electrolytes and thyroid hormones in sea bass and sea bream. Aquac. Int. 5, 237– 247.

Pestana, D., Velosa, S. (2008) Introdução à Probabilidade e Estatística. 4º edição. Fundação Calouste Gulbenkian

Pfeffer, E., 1995. Carbohydrate utilization and its determination. J. Appl. Ichthyol. 11: 175-182. Poston HA (1974) Effect of feeding brown trout (Salmo trutta) a diet pelleted in dry and moist forms. J Fish

Rodríguez-Barreto, D., Jerez,S., Cejas, J.R., Martin, M.V., Acosta, N.G., Bolaňos, A., Lorenzo, A. 2012 Comparative study of lipid and fatty acod composition in different tissues of wild and cultured female broodstock of greater amberjack (*Seriola dumerili*)

Ruane, N.M., Huisman, E.A., Komen, J., 2001. Plasma cortisol and metabolite level profiles in two isogenic strains of common carp during confinement. J. Fish Biol. 59, 1–12.

Ruohonen K, Grove DJ, Mc Ilroy JT (1997) The amount of food ingested in a single meal by rainbow trout offered chopped herring, dry and wet diets. J Fish Biol 51:93–105 Comp Biochem Physiol 96A:109–111

S.J.S. Johansen & M.Jobling. 1998. The influence of feeding regime on growth and slaughter traits of cage-reared Atlantic salmon, Aquac. Int. 6, 1-17.

Sargent, J.R., Henderson, R.J., Tocher, D.R., 1989. The lipids. In: Halver, J.E. (Ed.), Fish Nutrition, 2nd ed.Academic Press, San Diego, CA, pp. 153‑218.

Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition, 3rd ed. Elsevier, USA, pp. 181‑ 257.

Skaramuca, B., V. Kozul, Teskeredzic, J. Bolotin, V. Onofri, 2001. Growth rate of tank-reared Mediterranean amberjack, *Seriola dumerili* (Riso 1810) fed on three different diets. *J. Appl. Ichthyol. 17: 130-133*.

Thompson, B.A., Beasly, M., Wilson, C.A., 1999. Age distribution and growth of greater amberjack Seriola *dumerili*, from the north-central Gulf of Mexico. Fish. Bull. 97, 362-371.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107-184.

Torstensen, B.E., Lie, O., Froyland, L., 2000. Lipid metabolism and tissue composition in Atlantic salmon (Salmo salar L.). Effects of capelin oil, palm oil and oleic acid-enriched sunflower oil as dietary lipid sources. Lipids 35, 653–664.

Torstensen, B.E., Froyland, L. & Lie, O. (2004) Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil effects on Atlantic salmon (Salmo salar L.) Tissue and lipoprotein lipid composition and lipogenic enzyme activities. Aquac. Nutr., 10, 175–192.

Turchini, G.M., Torstensen, B.E., Ng, W.K., 2009. Fish oil replacement in finfish nutrition. Reviews in Aquaculture 1, 10–57.

Uyan, O., Koshio, O., Ishikawa, M., Yokoyama, S., Uyan, S., Ren, Hernández, L.H.H., 2009. The influence of dietary phospholipid level on the performances of juvenile amberjack, Seriola *dumerili*, fed non-fishmeal diets Aquaculture Nutrition. Volume 15, Issue 5, Pages 550 - 557 2009.

Yamada, K., Kobayashi, K., & Yone, Y. (1980). Conversion of linolenic Acid to o-3- highly unsaturated fatty acids in marine fishes and Rainbow trout. Bulletin of the Japanese Society of Scientific Fisheries, 46, 1231–1233.