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Degree in Agricultural Engineering and Biological Systems

Implementation of a gilthead seabream (Sparus aurata L.) breeding program in Murcia

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Resumen

La dorada (*Sparus aurata* L.) es un pez teleósteo de gran importancia en la acuicultura, que, junto a la lubina y el rodaballo, son las principales especies cultivadas en el mediterráneo. Los principales países productores de dorada en Europa son Turquía, Grecia y España, y dentro de España encontramos a la Región de Murcia como segundo productor con un 31,9 % del total. Debido a la alta demanda y su aumento en el coste de producción, es de principal prioridad los avances en la intervención genética.

El objetivo de este proyecto es conseguir información relevante para ser usada en la mejora genética de la dorada. Para ello, se estudió una población de doradas consistente en los progenitores y su descendencia. Después de las puestas masales, los alevines fueron criados con una metodología estándar hasta que pesaron al menos 20 gramos. Cada alevín fue marcado individualmente en la cavidad abdominal anterior mediante el sistema de marcaje físico *Passive Integrated Transponder*, y criado bajo condiciones intensivas en la Región de Murcia.

Debido a su gran importancia, evaluamos los principales parámetros fenotípicos como el peso y la longitud a tres diferentes edades (251, 762 y 980 días post eclosión), sin obviar las malformaciones, la calidad de la canal y carne junto a su pH y composición química, y el perfil de ácidos grasos.

Respecto al crecimiento, nuestras doradas alcanzaron un peso medio de 446,79 ± 2,7g y longitud media de 28,73 ± 0,05 cm a los 980 días post eclosión (dpe), con niveles de correlación a diferentes edades altos y positivos, sobre todo a los 251 días post eclosión. En el factor de condición, se observó que aumenta con la edad, siendo la diferencia mayor entre los pesos de 762 dpe y 980 dpe, que en las longitudes de las mismas fechas. El porcentaje de doradas que exhibían cualquier malformación a los 251 dpe fue del 2%, pero la alteración de opérculo disminuyó al 0.8% a los 980 dpe, manifestándose su recuperación.

La calidad de la canal y la carne, en el análisis de textura, mostró una dureza de 78,4 ± 1,12 N, una elasticidad de 6,58 ± 0,047 mm, un ratio de cohesividad de 0.704 ± 0.005, una gomosidad de 54.7 ± 0.76 N, y una masticabilidad de 359,7 ± 5,21 N mm. El pH analizado fue de 6.17 ± 0.014. En cuanto al rendimiento de nuestras doradas, estas tuvieron un 6,26 ± 0,078% de grasa visceral, esto conlleva a un rendimiento de la canal

de 88,3 ± 0,35% y un rendimiento del filete de 36,4 ± 0,33%. La composición química mostró parámetros normales, como un nivel de colágeno del 1,79 ± 0,055%, grasa intramuscular 4,64 ± 0,091%, proteína 21,9 ± 0,1% y humedad 73,1 ± 0,11%.

En el perfil de ácidos grasos de la dorada, se encontró la influencia de las dietas usadas, obteniendo un 28% de ácidos grasos saturados, un 47% de ácidos grasos monoinsaturados y un 25% de ácidos grasos poliinsaturados, siendo 13,5% la familia Omega-3 y 11,5% la familia Omega-6. Entre los ácidos encontrados, el más abundante es el ácido oleico con un 37,6%, el ácido palmítico con un 17,3%, y el ácido linolelaídico con un 8,93%, también se encuentran otros ácidos importantes en menores cantidades, así como el ácido eicosapentaenoico (EPA) en un 2,66%, el ácido docosahexaenoico (DHA) en un 6,32%, y el ácido heneicosanoico en un 1,98%.

Abstract

The gilthead sea bream (*Sparus aurata* L.) is a teleost fish of great relevance in aquaculture, which along with sea bass and turbot, are the main reared species in the mediterranean area. The main producer countries of gilthead sea bream in Europe are Turkey, Greece and Spain, and inside Spain we find the Region of Murcia as the second producer with 31.9% of the total production. Due to its high demand and rising production costs, is a main priority to produce strains of high genetic value.

The principal goal of this research is to obtain relevant information to be used in the genetic improvement of the gilthead sea bream. For this purpose, a gilthead sea bream broodstock consisting of breeders and their offspring were studied. After breeders' mass spawning, fingerlings were reared with a standard methodology until they weighed at least 20 grams. Each fingerling was individually tagged in the abdominal cavity by the system *Passive Integrated Transponder*, and reared under intensive conditions in the Region de Murcia.

Due to its great importance, the main phenotypic parameters like weight and length at three different ages (251, 762 and 980 days post-hatching), without omitting deformities, were evaluated. Quality tests were carried out to measure the flesh and fillet yield and pH, also a profile of fatty acids was elaborated.

Respecting to growth, our gilthead sea breams reached an average weight of 446.79 ± 2.7g and average length of 28.73 ± 0.05 cm at 980 days post-hatching (dph), with high and positive correlation levels at different ages, especially at 251 dph. We can observe that the condition factor increases with the age, being the higher difference between the weights at 762 dph and 980 dph, than the lengths at same dates. The deformity percentage that the gilthead sea breams showed at 251 dph for any deformity was 2%, but lack of operculum decreased with 0.8% frequency at 980 dph showing a recovery.

The flesh and fillet quality, in the texture analysis, showed a hardness of 78.4 ± 1.12 N, a springiness of 6.58 ± 0.047 mm, a cohesiveness ratio of 0.704 ± 0.005, a gumminess of 54.7 ± 0.76 N, and a chewiness of 359.7 ± 5.21 N mm. The analysed pH was from 6.17 ± 0.014. Regarding the efficiency of our gilthead sea breams, these had a 6.26 ± 0.078% of visceral fat, this implies a flesh yield of 88.3 ± 0.35% and a fillet yield of 36.4 ± 0.33%. The chemical composition showed normal parameters, like a collagen level of 1.79 ±

0.055%, intramuscular fat at 4.64 ± 0.091%, protein at 21.9 ± 0.2% and moisture at 73.1 ± 0.11%.

In the fatty acids profile of the gilthead sea breams, we found the influence of our diets, obtaining a 28% in saturated fatty acids, 47% in monounsaturated fatty acids and 25% in polyunsaturated fatty acids, being 13.5% from Omega-3 family and 11.5% from Omega-6 family. Among the found fatty acids, the most abundant acid is the oleic acid with 37.6%, the palmitic acid with 17.3%, and the linolelaidic acid with 8.93%, we also found others important fatty acids in minor quantities, like eicosapentaenoic acid (EPA) in 2.66%, docosahexaenoic acid (DHA) in 6.319% and the heneicosanoic acid in 1.98%.

1. INTRODUCTION

1.1. Production

1.1.1. World aquatic production

Aquaculture is the production of animals and plants in water by techniques aimed to improve its performance. This is not a complement from fishing, but its natural evolution. Aquaculture is also the stockbreeding with higher future projection since the necessary resources to produce a kilogram of capable aliment for consumption are less in water than in ground (APROMAR, 2018).

World aquaculture production comes from farms where they breed fishes, crustaceans, algae, molluscs and other invertebrates. These farms are playing a crucial function trying to eradicate famine and malnutrition, providing aliments rich in proteins, essential oils, vitamins and minerals, but worth to highlight, the contribution of long chain polyunsaturated fatty acids (omega 3) (APROMAR, 2018).

In 2016, the world aquatic production was from 202,2 million tonnes (aquaculture and fishing), 1.52% higher than in 2015. This production has been increasing since the three last decades 2.65% annually (Fig. 1)

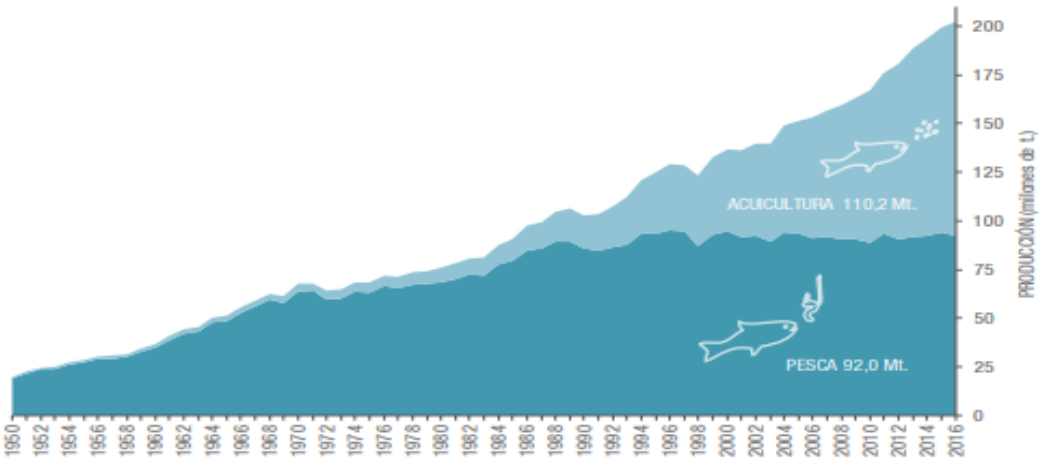

Figure 1. Evolution of world aquatic production (aquaculture and fishing) in 1950 - 2016 (APROMAR, 2018).

Aliments with an aquatic origin are one of the most important animal protein sources. In 2013, aquatic products have been the 17% of the world animal protein consume, and the 6.7% from the total consumed protein according to FAO. Besides offering high quality protein and being easily digestible, aquatic aliments contain essential omega3 fatty acids (EPA and DHA), vitamins like D, A and B, and minerals like calcium, iodine, zinc, iron and selenium) (APROMAR, 2018).

1.1.2. Production of gilthead sea bream in the European Union

European Union counts with 55.000 km of coastline, the second longest coastline of the world, behind Canada, offering appropriated environmental, physics and oceanographic conditions for aquaculture. On the business hand, companies have showed that they have the knowledge, experience and technical methods to sustain from the environmental point of view, economically profitable, offering safe, healthy and high quality aliments and stable jobs (APROMAR, 2018).

In 2016, 688.924 tonnes of aquaculture fish were cultured in the European Union, 4.1% higher regarding to 2015. The total volume of the 10 first fish species supposed 640.543 tonnes, 3.3% above 2015. The first breeding fish species produced in the European Union is the rainbow trout (*Oncorhynchus mykiss*), whose production in 2015 was of 185.400 tonnes, 5.5% higher than in the previous year and the second species is the Atlantic salmon (*Salmo salar*), with 181.030 tonnes, 2.7% lower than in 2015 (APROMAR, 2018).

As time goes by, more and more fishes are being bred due to its demand, this is the case of gilthead sea bream (*Sparus aurata*), which is the third main specie of cultured fish in the European Union (APROMAR, 2018), commonly found in the Mediterranean Sea (FAO, 2018). Gilthead sea bream have good properties that make it suitable for aquaculture, like its high survival rate and feeding habits.

The total aquaculture production of gilthead sea bream in Europe and other countries in 2017 is estimated in 207.167 t. These tonnes are practically similar to the production of 2016. In 2018, there will be an increase of 6% until reaching 220.500 t (APROMAR, 2018).

There are gilthead sea bream productions in 20 different countries, where through the years, the total production of gilthead sea bream has been increasing (Fig. 2), being Turkey the main producer with 72.000 t (34,8% of total production), Greece with 51.000 t (24,6 %), Egypt with 26.000 t (12,6%) and Spain with 13.642 t (6,6%) (Fig. 3).

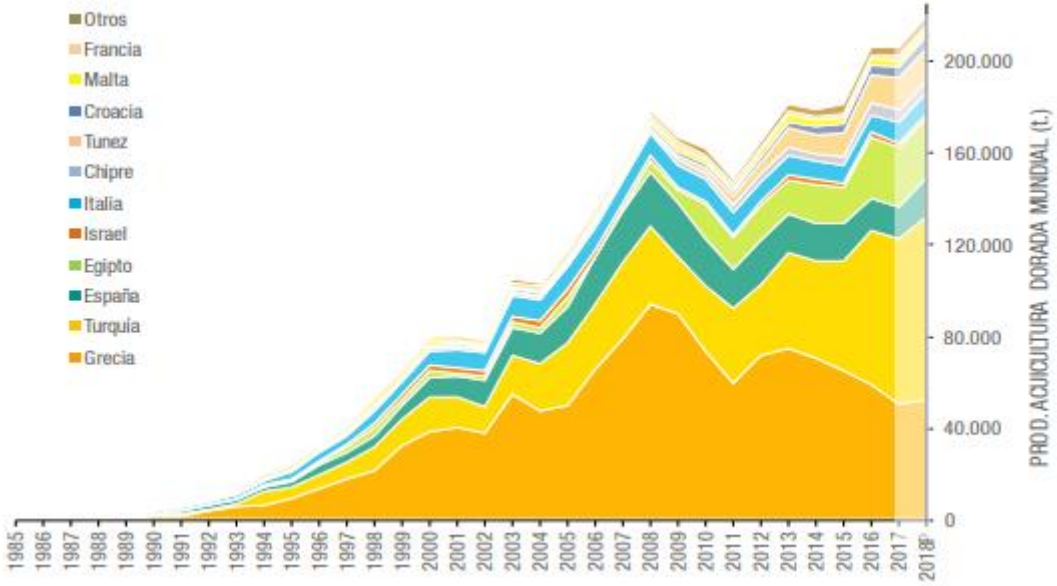

Figure 2. Evolution of world gilthead sea bream (*Sparus aurata*) production in different countries from 1985 to 2018 (APROMAR, 2018).

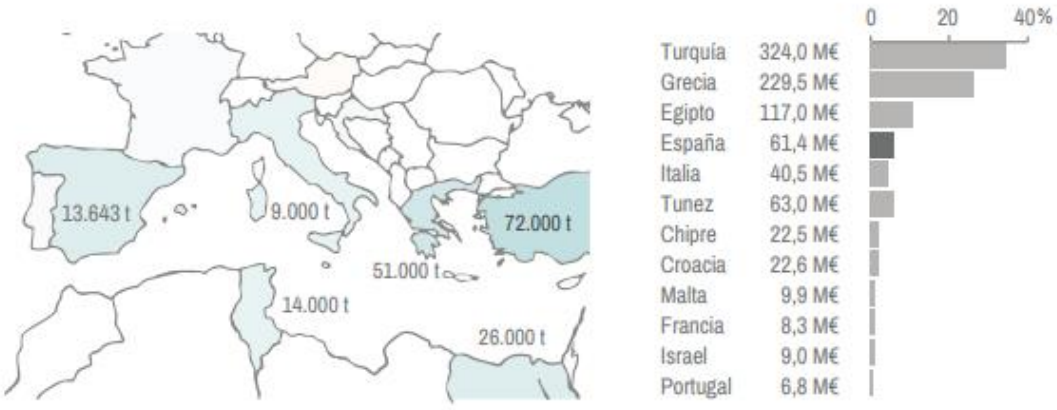

Figure 3. Distribution of gilthead sea bream production in the Mediterranean Area in 2017, volume and value (APROMAR, 2018).

1.1.3. Production of gilthead sea bream in Spain

The gilthead sea bream production in Spain was from 13.643 t in 2017, 0,7% lower than in the previous year. It is estimated for 2018 an increase of 21,6% until going over the 16.500 t (Fig. 4). The highest production of gilthead sea bream was from 23.930 t in 2008 (APROMAR, 2018)

In 2017, Valencian Community was ahead in the gilthead sea bream production of aquaculture in Spain with 5.590 t (41% of total production), followed by Murcia with 4.356 t (32%), Canary Islands with 2.063 t (15%), Andalusia with 980 t (7%) and Catalonia with 654 t (5%) (Fig. 5).

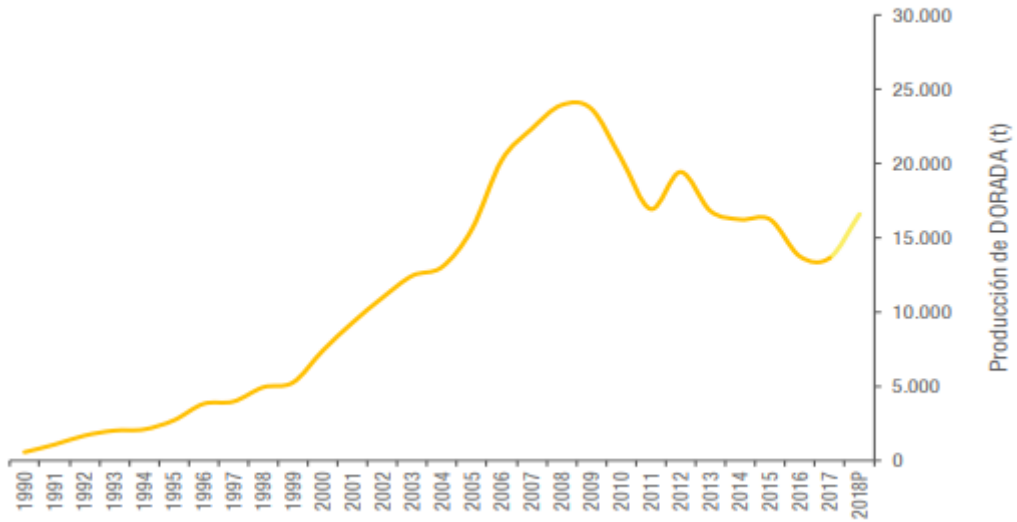

Figure 4. Evolution of gilthead sea bream (*Sparus aurata*) production in Spain (APROMAR, 2018).

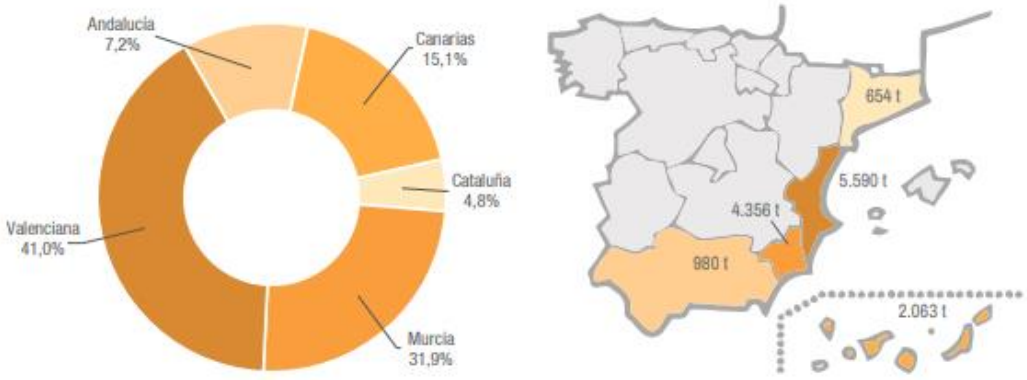

Figure 5. Distribution of the gilthead sea bream production in Spain by autonomous community (APROMAR, 2018).

Even though nowadays there are still a small quantity of wild gilthead sea bream that arrives to Spanish ports from capture fisheries (1.130 t in 2016), their volume holds relatively constant while breeding gilthead sea bream supposes the 91,6% from the total gilthead sea bream in the market (APROMAR, 2018).

1.2. Taxonomy

Scientific name: *Sparus aurata* Linnaeus, 1758 (Fig. 6)

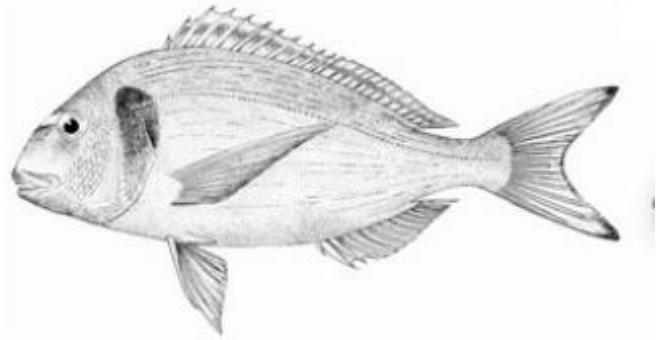

Figure 6. Gilthead sea bream (*Sparus aurata)* (FAO, 2018).

Synonym(s):

- *Aurata aurata* (Linnaeus, 1758)
- *Chrysophrys aurata* (Linnaeus, 1758)
- *Chrysophrys aurathus* (Linnaeus, 1758)
- *Chrysophrys auratus* (Linnaeus, 1758)
- *Chrysophrys crassirostris* (Valenciennes, 1830)
- *Pagrus auratus* (Linnaeus, 1758)
- *Sparus auratus* (Linnaeus, 1758)

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Sparidae

1.2.1. Morphologic characteristics

The gilthead sea bream has a tall and oval body, rather deep and compressed. It shows regularly a curved head profile (FAO, 2018), with a snout that is twice as long as the eye diameter (Muus and Nielsen, 1999). The eyes of the gilthead sea bream are small, and so is mouth, where there are thick lips and two types of teeth; four to 6 canine-like teeth anteriorly in each jaw, followed posteriorly by blunter teeth which become progressively molar-like and are arranged in 2 to 4 rows (teeth in the 2 outer rows stronger). Total gill rakes found on first arch are short, 11 to 13, with 7 or 8 lower and 5 (rarely 4) to 6 upper. The dorsal fin has 11 spines and 13 to 14 soft rays, and the anal fin has 3 spines and 11 or 12 soft rays. Counted along the lateral line and up to the caudal base, there are 73 to 85. Also the cheeks are scaly, but the preopercle is scaleless. The body is silvery grey, with a large black blotch at the beginning of the lateral line extending on upper margin of

opercle, where it is edged below by a reddish area. There is a golden frontal band between eyes edged by two dark areas (not well defined in young individuals), also dark longitudinal lines often present on sides of body, a dark band on dorsal fin and fork and tips of caudal fin edged with black (FAO, 2018). According to Bauchot and Hureau (1986), normally it reaches 30-35 cm of length, but it can reach a maximum of 70 cm (Muus and Nielsen, 1999; FAO, 2018) and a weight of 17.2 kg.

1.2.2. Distribution, habitat and biology

The gilthead seabream, *Sparus aurata*, is a subtropical Sparidae that occurs naturally in the Mediterranean and the Black Sea (rare), and in the Eastern Atlantic, from the British Isles, Strait of Gibraltar to Cape Verde and around the Canary Islands (Sola *et al*, 2006) (Fig. 7). Due to its euryhaline and eurythermal habits, the specie can tolerate rather high temperatures, growing very fast when temperature is 25-26ºC and ceasing to feed if temperature goes down to 12-13ºC (Aurelio Ortega, 2008), hence the gilthead seabream is found in both marine and brackishwater environments such as coastal lagoons and estuarine areas, in particular during the initial stages of its life cycle (FAO, 2018).

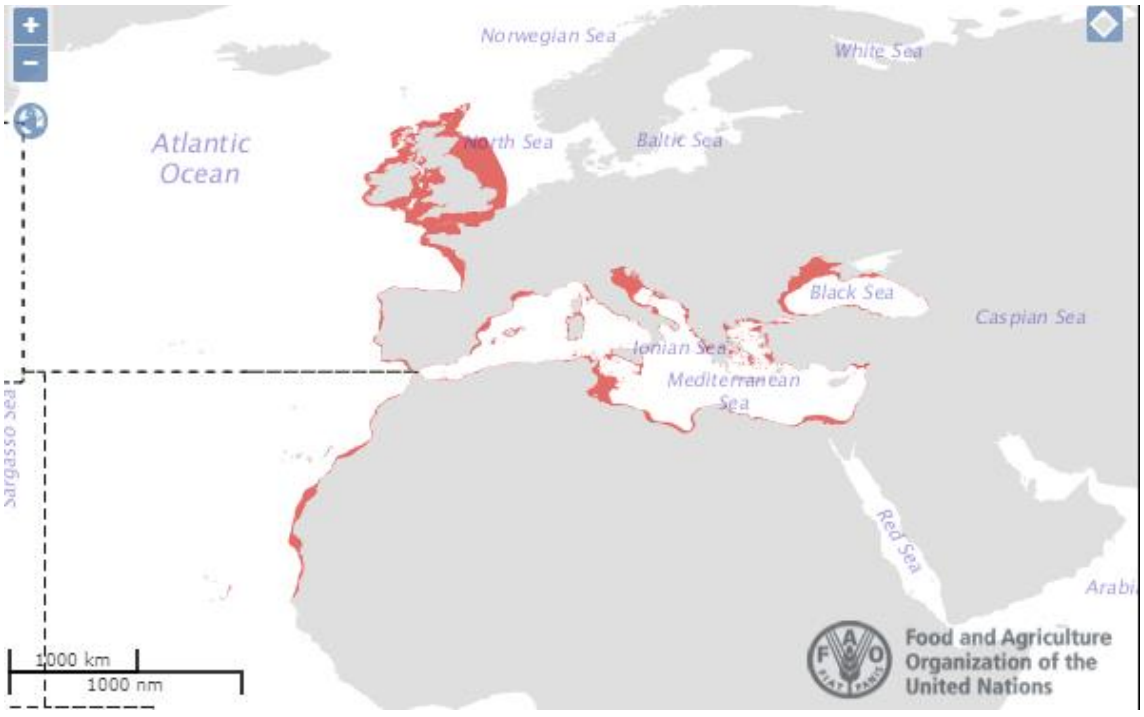

Figure 7. Geographical distribution of *Sparus aurata* (FAO, 2018)

Born in the open sea during October-December, juveniles typically migrate in early spring towards protected coastal waters, where they can find abundant trophic resources and milder temperatures. Very sensitive to low temperatures (lower lethal limit is 4 °C), in late autumn they return to the open sea, where the adult fish breed. In the open sea gilthead seabream are usually found on rocky and seagrass (*Posidonia oceanica*) meadows, but it is also frequently caught on sandy grounds. Young fish remain in relatively shallow areas (up to 30 m), whereas adults can reach deeper waters, generally not more than 50 m. (Sola *et al*., 2006; FAO, 2018).

The gilthead sea bream is a protandrous hermaphrodite: it is a functional male in the first two years and at over 30 cm in length becomes female. Sexual maturity develops in males at 2 years of age (20-30 cm) and in females at 2-3 years (33-40 cm) (Sola *et al*., 2006; FAO, 2018; Bauchot *et al*., 1981; Buxton and Garrat, 1990). During the male phase, the bisexual gonad has functional testicular, with asynchronous spermatogenesis, and non-functional ovarian areas (Sola *et al*., 2006). Females are batch spawners that can lay 20,000-80,000 eggs every day for a period up to 3-4 months, ovarian development is also asynchronous. The eggs are spherical and pelagic, with a diameter slightly lower than 1 mm and a single large oil droplet. The planktonic larval stage can last about 50 days at 17-18° C (Sola *et al*., 2006). In captivity, sex reversal is conditioned by social and hormonal factors (FAO, 2018).

1.3. Improvement in aquaculture

1.3.1. Genetic improvement

Genetic improvement is a very important activity in the aquaculture sector, improving characteristics of zootechnical interest in farmed fish species, such as seabass, seabream, trout and sturgeon. These characteristics are for example growth rate, absence of deformities or disease resistance.

The realization of these objectives is substantially carried out through the control of artificial reproduction and rearing techniques and through individual labelling systems (microchip and genetic fingerprinting).

Furthermore, to do a posteriori parental assignment of the animals included in the programs, genetic characterisation of breeders and their descendants would be necessary, and to measure the level of genetic variability both of wild and reared populations. Genetic variability is an important attribute of the species under domestication, since those with higher levels of variation are most likely to present high additive genetic variance for productive traits (Alarcón et *al*., 2004). It is important to know the genetic variation of the starting population to develop a breeding program, to the extent that it affects the answer to short and long-term selection (Falconer and Mackay, 1996).

Regarding to breeding programs, notable technological progress has been done in the selection of economically significant fish species over the last years.

1.3.2. Breeding programs in the gilthead sea bream

Breeding programs have been applied principally of fish species belonging to Salmonids, a group of more than 25.000 species of whom only some are used in aquaculture (Crosetti et *al*., 2001). Since the 1990s some of these programs have been undertaken also on farmed gilthead sea breams to obtain an improvement in the quantitative traits, such as growth rate or to increase the presence of characteristics like resistance to stress or diseases, highly desired by farmers (Antonello, 2008).

Gilthead seabream is an important specie in European aquaculture, nevertheless the industrial production is not based on breeding strategies, through the development of selection schemes for economic traits. This fact is in part due to the high economic cost of the organization of the production with genetic criteria, the lack of methodology able to combine production and genetic variation, and the biological characteristics of gilthead seabream (PROGENSA®2009).

In gilthead sea bream, as in other species cultured for human consumption, growth traits are the most economically important as production cost can be significantly lowered by reducing the duration of the rearing cycle (Saillant et *al*., 2006). For this reason, growth rate is usually the first goal in breeding programs of different species (García-Celdrán et al., 2016). Gilthead sea bream is mainly commercialized at 350-500g. Nevertheless, this weight is achieved at different ages, depending of factors related to nutrition and rearing conditions, mainly on the isotherms during growth (Navarro et al., 2009).

The second most economically important trait for the industrial production of gilthead sea bream is the presence of deformities, determining the overall fish quality (Georgakopoulou et al., 2010). Despite the growth and consolidation of the sea bream industry, there is an important problem in the development of this industry, and it is the high level of skeletal deformities appearing in hatchery fish. Deformities reduce the physiological ability of fish for a correct development i.e. reduce their growth rate, increase their mortality rate and significantly affect the animal welfare (Andrades et al., 1996; Karahan et al., 2013).

In the aquaculture industry, losses due to deformities occur at two levels: at hatcheries, reducing larval survival rate and growth efficiency in deformed fish, and at on growing farms, where deformed fish at market size have to be discarded or sold at lower indexes than the market prices since they are clearly evident, especially in those species such as sea bream which are sold mainly as whole fish. Thus, reducing the incidence of larval deformities would reduce the cost of production, both in the hatcheries and in the out-growing production sectors, and improve the quality of the products (Fernández et al., 2008).

Opercula complex, neurocranium and vertebral column (lordosis and vertebral fusion) are the most common deformities found in gilthead sea bream (Koumoundouros et al., 1997; Boglione et al., 2001; Roo et al., 2005). Skeletal deformities might affect up to 30% of the production and several factors are believed to be the basis of them: nutritional, environmental, hydrodynamic conditions and genetic factors or their interaction (Andrades et al., 1996; Afonso et al, 2000; Castro et al., 2008; Fernández et al., 2008).

In the past decade, aquaculture has received help by geneticist by the implementation of selection and breeding programs with the aim of obtaining higher benefit in terms of productivity and sustainability in fish hatcheries. The use of molecular markers has significantly helped this goal (Borrel et *al.*, 2011)

1.4. Gilthead sea bream rearing system

One of the major concerns of the consumer of aquacultured products is quality, namely its safety, freshness and health value. Also, both consumers and producers are becoming increasingly aware of fish welfare issues. It is interesting to note that quality and welfare issues are intrinsically linked, as there is evidence that inadequate fish husbandry results in lower meat quality. Under farming conditions, fish quality is known to be influenced by extrinsic factors such as feeding strategies and diet composition (E.Matos et *al*., 2010).

1.4.1. Broodstock

Usually every hatchery has its own broodstock unit, where breeders of various age groups, from 1 year-old males to 5-year old females, are kept under long-term stocking conditions. Breeders can come either from a farm or from the wild. At the beginning of the spawning season selected batches of breeders are transferred from their long-term location to the spawning tanks. The control of the sex ratio in spawning tanks is a very important factor for gilthead seabream and precautions need to be taken because sex reversal is socially determined. The presence of young males at the end of the spawning period, for instance, increases the number of older fish that become females. On the other hand, the occurrence of older females reduces sex reversal in younger fish (FAO, 2018).

1.4.2. Out-of-season spawning

Gilthead seabream broodstock may be conditioned by environmental manipulation in order to extend or modify reproduction time. Fish are stocked in tanks equipped with a water heating/cooling system and computerized control of temperature and light intensity. Sexual maturation is obtained by exposing the broodstock to photoperiod and water temperature conditions that occur during the natural spawning period. Female spawning can be obtained by GnRH inoculation (5-20 mg/kg). There are two principal systems of gilthead seabream larval rearing called small-scale and large-scale. The small-scale ($<10m^3$) rearing system is characterized by maximum control of environmental parameters and is conceived in order to produce a large number of juveniles (150-

250/litre). The large-scale (~200 m³) technique simulates a natural ecosystem. This technique guarantees much better larval quality than the small-scale system, but produces far less juveniles (maximum 10/litre) (FAO, 2018).

Gilthead seabream larvae generally deplete their yolk sacs after 3-4 days of endogenous feeding. At this stage, the eyes are pigmented and the mouth developed, allowing the larvae to prey on larval organisms. In most rearing systems the first living organisms used for larval feeding are rotifers (e.g. *Brachionus plicatilis*); these are chosen due to the relative ease with which they can be culture on a large scale. After 10-11 days, rotifers are integrated with *Artemia salina* nauplii until the larvae accomplish metamorphosis (32-35 days post-hatching). Prior to being fed to the larvae, both rotifers and artemia are routinely enriched with commercial lipid preparations, to enhance their levels of certain essential fatty acids (EPA; DHA) and vitamins that are critically important for good growth, development and survival. In Mediterranean hatcheries microalgae (e.g. *Chlorella* sp., *Isochrysis galbana*, *Pavlova lutheri*, *Nannochloropsis oculata*, *N. gaditana, Dunaliella tertiolecta*) are used both for rotifer production and to improve the water quality in the larval tanks, creating the so-called 'green water' that is used during the initial rearing phases (FAO, 2018).

Weaning with a dry high-protein (50-60 percent) formulated diet takes place when fish reach a weight of 5-10 mg (FAO, 2018).

1.4.3. Nursery

Juveniles at about 45 days old are generally moved into a dedicated section of the hatchery equipped with larger round or rectangular tanks (10-25 m³), where weaning takes place. The weaning stage is a truly intensive rearing system. Initial fry density is generally 10-20/litre at a temperature of 18 °C and salinity of 35-37‰. Final density can reach 20 kg/m³ of 2-3 g fish. Feed is presented at 2-hour intervals from 08.00 to 20.00, using increasing percentages of artificial feeds composed of 150-300 µm particles. Dry feed should initially be presented at about 20 g/m³ (FAO, 2018).

1.4.4. Ongrowing techniques

Gilthead seabream can be farmed in various ways: in coastal ponds and lagoons, with extensive and semi intensive methods; or in land-based installations and in sea cages,

with intensive farming systems. These methods are very different, especially regarding fish farming density and food supply (FAO, 2018).

1.4.5. Semi-intensive systems

In this system human control of the farming environment is greater than in the extensive system. It may simply involve seeding lagoons with juveniles pre-fattened in an intensive system, to minimize mortality and shorten farming time. In this case it is also possible to fertilize the farming area in order to increase natural food availability (FAO, 2018).

Other types of semi-intensive farming involve more control, and comprise the provision of artificial feed and supplemental oxygen. This type of semi-intensive farming system is usually carried out in net enclosures within limited areas of the lagoons (FAO, 2018). The final production can vary widely, according to the size of the juveniles stocked and the amount of feed presented. The density in semi-intensive systems does not normally exceed 1 kg/m^3 and production ranges between 500-2 400 kg/ha/yr (FAO, 2018).

1.4.6. Extensive system

This system is based on the natural migration of euryhaline fish, when the fish may be caught, generally in typical fishing traps. Since this practice provides a very limited and unpredictable source of natural juveniles, many modern commercial extensive production units rely on both wild-caught and hatchery-reared juveniles. Generally, 2-3 g gilthead seabream are seeded into the lagoons in April-May (FAO, 2018).

Under these systems gilthead seabream reaches the first commercial size (350 g) in 20 months (Fig. 8) and are usually farmed together with mullets, eels and European seabass. In North Mediterranean lagoons, wintering in deep basins, with freshwater/seawater stratification, is needed in order to preserve one-year-old gilthead seabreams (FAO,2018).

The total production of this kind of polyculture ranges from 30-150 kg/ha/yr according to lagoon productivity. In north-eastern Italian lagoons the production of gilthead seabream represents 15-30 kg/ha/yr of the total. During the production cycle the fish feed on natural lagoon resources; no supplementary food is provided. In extensive fish farming

the fish density generally does not exceed 0.0025 kg/m³ (FAO, 2018).

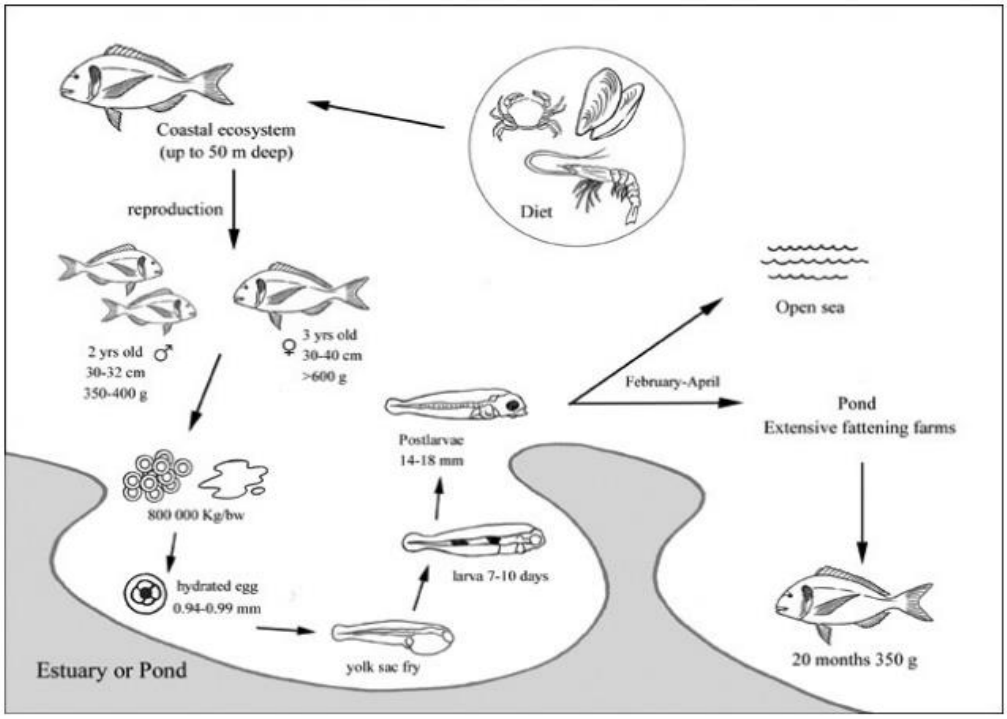

Figure 8. Production cycle of *Sparus aurata* - extensive system (FAO, 2018)

1.4.7. Intensive systems

Intensive grow-out normally follows other intensive farming phases, namely reproduction, larval rearing and pre-fattening (Fig. 9). Gilthead seabream intensive pre-fattening and grow-out phases may be carried out in land-based installations with rectangular concrete tanks that vary in size (200-3 000 m³) according to fish size and the demands of production. Grow-out may also occur in sea cages, either in sheltered or semi-exposed sites (floating cages) or totally exposed sites (semi-submersible or submersible cages).

Intensive systems may be stocked with juveniles purchased from separate hatcheries, but large production units normally rear their own. In intensive grow-out systems the FCR is usually very favourable (about 1.3:1).

When gilthead seabreams are reared in tanks very high densities are used, ranging from 15-45 kg/m³ and massive oxygen injection is needed to ensure fish survival. Under

excellent conditions (18-26 °C), pre-fattened small gilthead seabreams (5 g) reach first commercial size (350-400 g) in about one year.

Ongrowing in sea cages is simple and economical; it is the fattening system normally used in the Mediterranean basin. Although densities (10-15 kg/m^3) are lower than in tanks, there are great advantages that make cages farming more profitable. For example, there are no energy costs for pumping, aeration, or post-rearing water treatment. However, it is not possible to control temperature in cage rearing, resulting in a longer rearing period to market size, or the necessity to stock larger juveniles. On average, larger pre-fattened gilthead seabream (10 g) reach first commercial size (350-400 g) in about one year, while smaller juveniles (5 g) reach the same size in about 16 months (FAO, 2018).

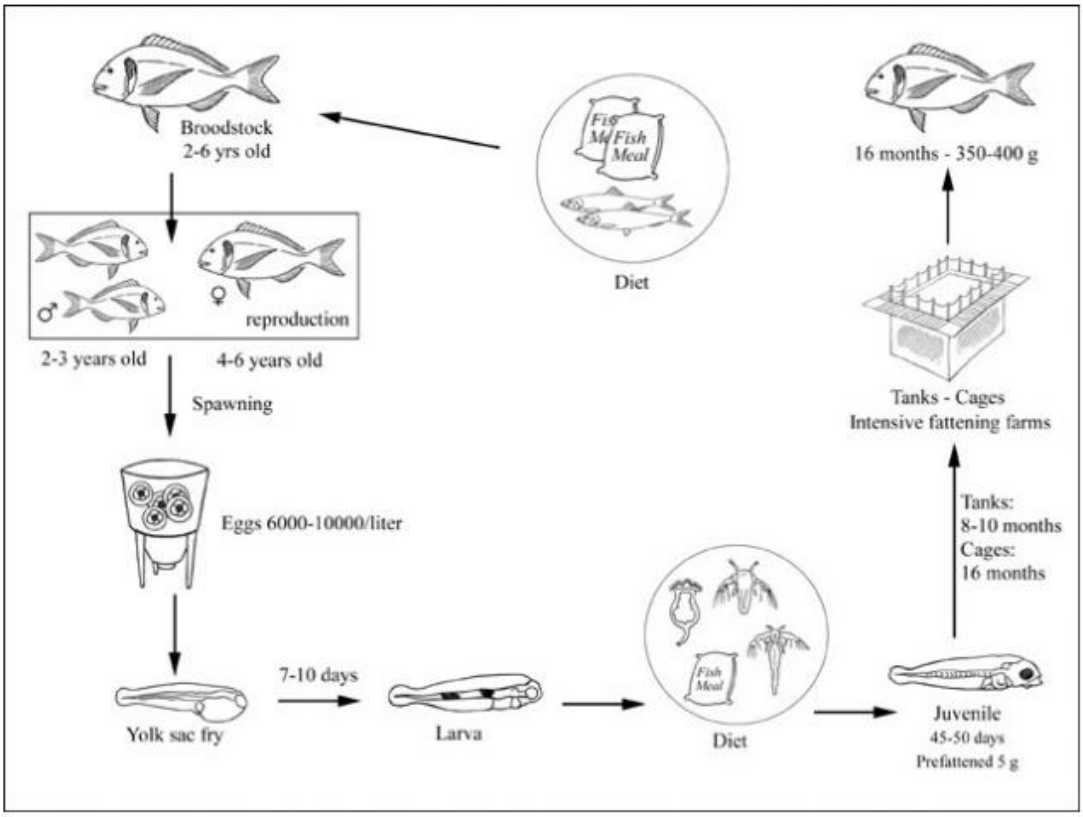

Figure 9. Production cycle of *Sparus aurata* - intensive system (FAO, 2018)

1.5. Feeding

The maximun portion that involves a maximun growth depends on factors related with frequency and quantity of aliment: duration of eating (time of satiated), quantity of individual food (stomach capacity), time among meals (frequency of feeding), and the interaction of all of them (Brett, 1979). Also factors as consequences of the influence of biotic and abiotic factors, in which temperature and weight are the most important.

All of it always providing the diet is the most adequate for the species concerned. The aliment demand comes from the basic requirements of the fish. Additional demand is dictated for its potential growth capacity (influenced by the growth hormone). The time of satiated is very variable according the species and it can depend on the weight or not. However, the quantity of aliment needed to use, is pretty influenced by the weight: small fishes consume bigger quantities; and by the temperature: the digestion rate increases with the temperature, therefore the daily ingestion too (Elliot, 1975). This premise is applied in the elaboration of the artificial feeding tables on the part of composed feed manufacturer.

Possibly, the most significant and simplest way to indicate that a diet or portion rate is the adequate for an organism, is the capacity of convert the aliment into flesh: the alimentary efficiency index (AEI = G/R), where G is the index of growth in weight and R the daily portion of aliment, can be expressed in terms of wet weight, dry weight or caloric value (Brett & Groves, 1979), or multiplying by 100, as percentage. Here we also add the average daily profit, which is the capacity of the individual of converting the grams of aliment into grams of weight, which will give to us a prediction of the pre-fattening cost of the animal.

The most used index to measure the efficiency of the feed are:

Food Conversion Ratio

FCR = ingested aliment (Kg) / weight increment (Kg)

Protein efficiency index:

PEI = weight increment (Kg) / ingested protein (Kg)

Others useful factors:

CF (condition factor) = 1000 × weight/length^3, where weight (g) and length (cm).

ADC (apparent digestibility coefficient) = 100 × D/I = 100 × (I - F)/I, where D (quantity of digested aliment), I (ingested aliment), F (feces produced).

1.6. Growth and development

According to Schreck and Moyle, growth is defined as any change in size or amount of body material, regardless of whether that change is positive or negative, temporary or long-lasting. Depending on the level of organization, it can be measured in terms of number, linear dimension, weight, volume, energy content, or the amount of a specific component such as protein. The major influences on fish growth are temperature and food consumption rate, but growth can be affected from many other characteristics (Schreck and Moyle, 1990)

The massive increase in the girth of the fish that occurs between hatching and maturity takes place by the hypertrophy of muscle fibres involving among other things the synthesis of contractile filaments (Ian A. Johnston, 1999).

1.6.1. Length

Measurements of body length give direct evidence for growth or lack of growth. Increases in length generally are retained, though a fish might shrink somewhat during starvation. Body length can be measured in many ways, although total length, fork length, and standard length are used most commonly for fish. Length can be easily and inexpensively measured in the field or laboratory, on live or preserved fish. Changes in length commonly occur with preservation but these changes are quantifiable and cease with time (Schreck and Moyle, 1990).

1.6.2. Weight

Frequently, length and weight of a fish are both measured. One may be calculated from the other, though with statistical error. Change in weight (mass) is probably the most commonly used assessment of whole-body growth of fish as well as of suborganismal growth. Weight also is the traditional measure for estimates of production-the elaboration of a group's or population's biomass-which are of interest to ecologists and resource

managers. Weight is relatively easy to measure and whole animals can be weighed without killing them. Scales and balances for weighing quantities in excess of I g generally are neither sophisticated nor expensive. Electronic balances that can weigh very small quantities or that can be used in an unsteady environment such as on a boat are costly by comparison (Schreck and Moyle, 1990).

1.6.3. Wet weight

The simplest way to assess changes in weight is to periodically weigh the animals of interest; the individuals may represent a sample from a large population. If variations in individual weights are not of interest, the sampled fish can be weighed in mass and an average individual weight can be computed. These values are compared with weights determined previously to calculate growth or growth rates. Obvious sources of error in measurements of wet weight include retention of excess water on the surface or in the buccal cavity of live fish and dehydration of dead fish (Schreck and Moyle, 1990).

1.6.4. Dry weight

When a whole fish or tissue sample is dried under reasonably benign conditions, it reaches an asymptotic or "constant" weight: the dry weight. The procedure removes internal water, which is a transient material, and eliminates the errors due to excess external water or dehydration that plague wet-weight determinations. In most cases, dry weights can be accepted if changes in weight between successive weighing are less than 0.1% (Schreck and Moyle, 1990).

1.6.5. Muscle growth

Muscle growth in fish differs from that of mammals in that muscle recruitment continues throughout much of the life cycle. In mammals, post-natal muscle growth only involves the hypertrophy of the fibres formed prior to birth (Ian A. Johnston, 1999).

The phenotype of all the different muscle fibre types changes during ontogeny as the fish matures. Muscle fibres in embryos and yolk-sac larvae are more aerobic in character than subsequent stages containing high volume densities of mitochondria and possessing a relatively low capacity for anaerobic glycolysis (Ian A. Johnston, 1999).

1.6.6. Gonadal development

Gilthead seabream begin gonadal development during September in preparation for winter spawning which starts around late December to early January in the eastern Mediterranean region. Spawning or gamete release occurs over a 3–4-month period, during which females can spawn 0.5–2 times their body weight in eggs through multiple spawning. The majority of nutrients required to produce this large volume of eggs are derived from the food consumed supplemented by nutrients from body reserves, as indicated by changes in the body composition. Reproduction of seabream causes a loss in body weight, which takes a number of months to replenish after spawning ceases. The net result is a 3–4-month spawning period, during which females lose weight and males undergo a reduced weight gain while continuing to feed (G.Wm. Kissil et al., 2001).

1.7. Quality of flesh

For the study of the quality of flesh in the gilthead sea bream, as every other fish, we apply the criteria traditionally used in other species. This would be the best system to adapt aquaculture products to a methodology and systematic of excessively efficiency, and that can help to take the leap from the traditional concept of commercial quality. Here we include: chemical composition of the flesh, instrumental and sensorial characteristics of flesh.

1.7.1. Chemical composition of flesh

In fishes, ashes represent a 13%. The fat content increases at the expense of quantity of water, while the level of proteins keep constant around 17%. Regarding the proteins, these have a high biological value.

Its nutritional value is a combination of quantity and quality.

According to the fat content, we can differentiate three kind of fishes:

- White fish as gadids with a 0.5% of fat.

- Semi fatty fish as pleuronectidae family, with 2.5% of fat, where the gilthead sea bream would be included.
- Fatty fish, as tunids or clupeids with more than 10% of fat.

This parameter can vary according to:

- Age: At higher age, the content of fat increases.
- Genetic variation: Genotype is a factor that affects to composition, especially lipids, although it depends more on the nutritional value than genetic.
- Feeding: This is the most important, an increase of the alimentary rate is reflected as an increment of the fat and protein percentage.
- Corporal region: Fats accumulate in different areas where lipid metabolism occurs.

1.7.2. Fat quality

Fat is a fundamental factor in the sensorial and technological properties of the fish flesh and its conservation due to its absolute quantity and chemical composition.

Unsaturated fatty acids predominate in marine fishes, and saturated fatty acids in fresh water fishes. The fat from fatty fish is rich in triglycerides, while the white fish is fundamentally formed of phospholipids, rich in unsaturated fatty acids and of long chain although this can vary depending on the year season. The amount of lipidic unsaturation increases with artificial feeding.

1.7.3. Instrumental quality of flesh

a) pH: pH from fishes is nearly neutrality, around 6-6.5 and it is influenced by the species.

b) Water retention capacity (WRT): flesh capacity to hold the flow that it contains during the application of external forces like cuts, pressure, grinding, etc. It is important for technological processes: conservation, filleting, cooking and

transformation. Water is the most abundant component of the muscle (60-85%) and the most variable.

c) Colour: since the physical point of view, the colour is determined by three components: the light, the object and the observer, who introduces subjective and psychological aspects.

d) Texture: this concept includes a series of mechanical properties of the flesh of big importance like technological aspects and appreciation of the flesh quality at the moment of consumption. The texture of the flesh is related to the muscular fibre in terms of contraction grade and posterior resolution from rigor mortis and the quantity and nature of the conjunctive tissue. The proteins from the conjunctive tissue are characterized by its water insolubility.

1.8. Fatty acids

Lipids may be very transient body materials, but they are an important source of potential chemical energy, and their presence or absence reflects the physiological capacity offish. Lipids are readily separated from proteins, carbohydrates, and other cellular compounds by their solubility in nonpolar solvents such as ethyl ether, chloroform, methanol, and methylene chloride. Total lipid content may be determined gravimetrically following extraction and evaporation of the solvent. 'Total lipids" also have been estimated by colorimetric procedures. Lipid classes such as triglycerides and long-chain fatty acids can be assayed by enzymatic or colorimetric methods and kits for these types of analyse are commercially available. Fatty acids may be determined specifically by chromatography or generally by oxidative and other chemical procedures. In addition, lipid class analysis can be accomplished relatively easily with a system involving thin-layer chromatography and a flame ionization detector. Proper sample storage is important to avoid decomposition and oxidation. Samples should not be dried with heat and are better stored frozen without exposure to oxygen (I.A. Johnston, 1999)

Lipids, and especially fatty acids, have long been used as biological markers and general indicators of diet in marine ecology. Fatty acids are the primary constituent of most lipids and in marine organisms they are most commonly composed of chains of 14 to 24 carbon

atoms of varying degrees of unsaturation (i.e. containing one or more double bonds). These fatty acids generally remain intact through digestion, absorption and transport in the bloodstream, and are also taken up by tissues in their intact state. Thus, fatty acids can be deposited in animal tissue with minimal modification from diet and in a predictable way. Additionally, animals can biosynthesize a relatively limited number of fatty acids. These biochemical restrictions, coupled with the fact that fatty acids in the marine food web are exceptionally complex and diverse, provide the opportunity to use fatty acids to understand trophic interactions in marine ecosystems. Although a number of 'indicator' fatty acids exist which may be used as biological markers, it is likely that the quantitative pattern of all fatty acids in a species or individual will be most informative at higher trophic levels. It has been demonstrated that tissue fatty acids can be valuable in studying bottom-up trophic dynamics among and within fish and invertebrate species. Additionally, once fatty acid patterns are characterized in prey or diet items, these patterns can be used to study the diets of higher trophic level predators. To use fatty acids to understand trophic interactions both among forage species and also near the top of the food web in PWS and other areas of the GOA, it is necessary to first characterize fatty acid patterns and their variation in the prey species assemblage (Iverson et *al*., 2002).

1.8.1. Evolution of fatty acids consumption

On the basis of estimates from studies in Paleolithic nutrition and modern-day hunter-gatherer populations, it appears that human beings evolved consuming a diet that was much lower in saturated fatty acids than is today's diet. Furthermore, the diet contained small and roughly equal amounts of n26 and n23 PUFAs (ratio of 1–2:1) and much lower amounts of trans fatty acids than does today's diet. The current Western diet is very high in n26 fatty acids (the ratio of n26 to n23 fatty acids is 20–30:1) because of the indiscriminate recommendation to substitute n26 fatty acids for saturated fats to lower serum cholesterol concentrations. Intake of n23 fatty acids is much lower today because of the decrease in fish consumption and the industrial production of animal feeds rich in grains containing n26 fatty acids, leading to production of meat rich in n26 and poor in n23 fatty acids. The same is true for cultured fish and. Even cultivated vegetables contain fewer n23 fatty acids than do plants in the. In summary, modern agriculture, with its

emphasis on production, has decreased the n23 fatty acid content in many foods: green leafy vegetables, animal meats, eggs, and even fish (Artemis P. Simopoulos, 1999).

1.8.2. Biological effect and importance of n-6 and n-3 fatty acids

Linoleic acid (LA; 18:2n26) and ALA (18:3n23) and their long-chain derivatives are important components of animal and plant cell membranes. When humans ingest fish or fish oil, the ingested eicosapentaenoic acid (EPA; 20:5n23) and docosahexaenoic acid (DHA; 22:6n23) partially replace the n26 fatty acids [especially arachidonic acid (AA; 20:4n26)] in cell membranes, especially those of platelets, erythrocytes, neutrophils, monocytes and liver cells. As a result, ingestion of EPA and DHA from fish or fish oil leads to 1) decreased production of prostaglandin E2 metabolites; 2) decreased concentrations of thromboxane A2, a potent platelet aggregator and vasoconstrictor; 3) decreased formation of leukotriene B4, an inducer of inflammation and a powerful inducer of leukocyte chemotaxis and adherence; 4) increased concentrations of thromboxane A3, a weak platelet aggregator and vasoconstrictor; 5) increased concentrations of prostacyclin PGI3, leading to an overall increase in total prostacyclin by increasing PGI3 without decreasing PGI2 (both PGI2 and PGI3 are active vasodilators and inhibitors of platelet aggregation); and 6) increased concentrations of leukotriene B5, a weak inducer of inflammation and chemotactic (Artemis P. Simopoulos, 1999).

The hypolipidemic, antithrombotic, and anti-inflammatory effects of n23 fatty acids have been studied extensively in animal models, tissue cultures, and cells (Table 1). The effects of fatty acids on gene expression have been investigated and this focus of interest has led to studies at the molecular level (Table 2)

Table 1. Effects of n23 fatty acids on factors involved in the pathophysiology of atherosclerosis and inflammation (Artemis P. Simopoulos, 1999).

Factor	Function	Effect of n−3 fatty acid on factor concentrations
Arachidonic acid	Eicosanoid precursor, aggregates platelets, and stimulates white blood cells	↑
Thromboxane A_2	Platelet aggregation, vasoconstriction, increases intracellular Ca^{2+}	↓
Prostacyclin	Prevents platelet aggregation, vasodilator, increases cyclic AMP	↑
Leukotriene B_4	Neutrophil chemoattractant increases intracellular Ca^{2+}	↓
Tissue plasminogen activator	Increases endogenous fibrinolysis	↑
Fibrinogen	Blood clotting factor	↓
Red blood cell deformability	Decreases tendency to thrombosis and improves oxygen delivery to tissues	↑
Platelet activating factor	Activates platelets and white blood cells	↓
Platelet-derived growth factor	Chemoattractant and mitogen for smooth muscles and macrophages	↓
Oxygen free radicals	Causes cellular damage, enhances LDL uptake via the scavenger pathway, stimulates arachidonic acid metabolism	↓
Lipid hydroperoxides	Stimulates eicosanoid formation	↓
Interleukin 1 and tumor necrosis factor	Stimulate neutrophil oxygen free radical formation, lymphocyte proliferation, and platelet activating factor; expresses intercellular adhesion molecule 1 on endothelial cells; and inhibits plasminogen activator and thus is procoagulant	↓
Endothelial-derived relaxation factor	Reduces arterial vasoconstrictor response	↑
VLDL	Related to LDL and HDL concentrations	↓
HDL	Decreases the risk of coronary heart disease	↑
Lipoprotein (a)	Atherogenic and thrombogenic	↓
Triacylglycerols and chylomicrons	Contribute to postprandial lipemia	↓

[1] Data from Weber and Leaf (34). ↑, increases; ↓, decreases.

Table 2. Effects of polyunsaturated fatty acids on several genes encoding enzyme proteins involved in lipogenesis, glycolysis, and glucose transport (Artemis P. Simopoulos, 1999).

Function, gene, and reference	Linoleic acid	α-Linolenic acid	Arachidonic acid	Eicosapentaenoic acid	Docosahexaenoic acid
Hepatic cells					
Lipogenesis					
FAS (35–38)	↓	↓	↓	↓	↓
S14 (35–38)	↓	↓	↓	↓	↓
SCD1 (39)	↓	↓	↓	↓	↓
SCD2 (40)	↓	↓	↓	↓	↓
ACC (38)	↓	↓	↓	↓	↓
ME (38)	↓	↓	↓	↓	↓
Glycolysis					
G6PD (41)	↓				
GK (41)	↓	↓	↓	↓	↓
PK (42)	—	↓	↓	↓	↓
Mature adiposites					
Glucose transport					
GLUT4 (43)	—	—	↓	↓	—
GLUT1 (43)	—	—	↑	↑	—

[1] FAS, fatty acid synthase; SCD, steroyl-CoA desaturase; ACC, acetyl CoA carboxylase; ME, malic enzyme; G6PD, glucose-6-phosphate dehydrogenase; GK, glucokinase; PK, pyruvate kinase; GLUT, glucose transporter; ↓, suppresses or decreases; ↑, induces or increases.

1.8.3. Fatty acids in fish

It has been reported that, a high dietary consumption of marine n 3 fatty acids may prevent the development of atherosclerosis and thrombosis. Considering the high benefits of consumption of marine oils in human health, the Nutrition Committee of the American Heart Association, recommends consuming fish two or three times a week (A. Mnari et al., 2007), since it can exert suppressive effects on cardiovascular, cancer, inflammatory and autoimmune diseases. In comparison to red meat and poultry, the health benefit of fish consumption is based on levels of n-3 fatty acids (FA) and in particular a high ratio of n-3/n-6 FAs and high levels of eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA). Muscle metabolic profile and the ratio of n-3/n-6 FA of wild and farmed fish vary according to their species, genetic profile, habitat, season, and nutrition. The lipid composition of aquaculture feed can influence FA content of farmed fish flesh resulting in lower ratios of n3/n6 FA compared to wild fish. Recently, aquaculture feed has been re-formulated to increase this ratio in favour of n-3 FA (Lenas *et al.*, 2011).

2. PURPOSES OF THIS STUDY

The framework of this research is a local and national project in Spain title "Desarrollo de un Programa Piloto de Mejora genetica en Dorada (*Sparus aurata L.*), PROGENSA®", focused in the improvement of growth characters and integral quality of fish (regarding to malformation problems). The objective of this work is to evaluate the phenotypical characterization of the gilthead sea bream, focused on:

- Growth parameters: Weight and length evolution.
- Flesh quality: Malformations, condition factor, visceral fat, flesh and fillet yield and chemical composition.
- Fillet quality: Texture, pH, % of fat and fatty acid profile.

3. MATERIALS AND METHODS

3.1. Rearing conditions of the broodstock

The broodstock (n = 140, 50-male and 90-female), was reared and established in Planta Experimental de Centro de Cultivos Marinos del Centro Oceanográfico de la region de Murcia (CCMRM; Insituto Español de Oceanografía; San Pedro del Pinatar, Murcia), as breeders that had not been subjected to artificial selection. All the breeders were identified with Passive Integrated Transporter (PIT; Trovan Daimler-Benz) (Fig. 10) for having a total traceability.

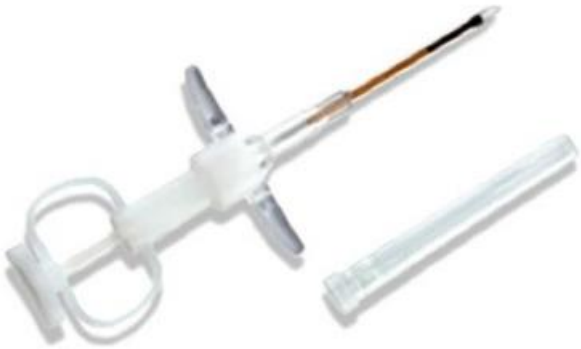

Figure 10. ID-100KB Animal Transponder with Butterfly Pusher (TROVAN).

The broodstock was maintained under natural photoperiod until their spawning season in tanks at 19-20ºC. The eggs were collected during two consectutive days, and were born on February 26th, 2016, with a fertilization rate of 80% and a hatching rate of 94%.

The larval rearing was conducted in a 5m3 cilindric tank with the standard methodology: initial Density of 100 larvae/L, Temperature = 19ºC, Salinity: 38%, Photoperiod: 16:8 (L:D). The larvae were reared with the "green water" method adding 100L/tank/day of phytoplankton (*Nannochloropsis sp*.) for the first 20 days. During this step, the water renewal was from 2% per day. Afterwards the tank was put on a flow-through system (30%/hour) with light airing in order to maintain the dissolved oxygen indexes around 6mg/L. Light intensity was of 1000 lux on the water surface and the photoperiod 16:8 (L:D). The larvae were fed with the rotifers (10-20/ml) from the 6th until the 25th day post-hatching (dph), *Artemia* nauplii (1/ml) from the 17th until the 27th dph, *Artemia*

enriched metanauplii (3-5/ml) from the 20th until the 55th dph, and commercial feed from the 40th dph onwards. Food was provided manually using commercial fish feed (Skretting S.A., Cojóbar-Burgos, Spain).

At 251 days post-hatching (dph) a random sample of 2.500 individuals were individually tagged in the abdominal cavity for individual identification with a PIT, following the tagging protocol described by Navarro et al. (2006), total weight and length were measured. At 251 dph, all the fish on-grown at CCMRM were assessed and the weight and total length were recorded. Fish were also visually inspected in order to examine external deformities in the vertebral column (curvature), operculum (lack of operculum) and rest of the head (cranium and jaw deformities). The presence of a minimum angle in the vertebral column and any fold at operculum was enough to classify the fish as deformed. A sample of caudal fin was collected and preserved in absolute ethanol at room temperature and then the individuals were randomly distributed in 2 tanks until they got over.

Ten days later, fish were moved to the facilities of the company Servicios Atuneros del Mediterraneo S.L. (San Pedro del Pinatar, Murcia, Spain), where they were reared in a cage in the Mediterranean sea under intensive conditions: cage of 11 meters in diameter which is anchored in 38 meters of depth in the Mediterranean sea (Fig. 11) (average water temperature = 18,2 ± 0,9 ºC, dissolved oxygen: 7,4 mg/l, 100% oxygen-saturation, salinity: 37,9%; data estimated from open sea condition) and fed with commercial fish feed (39% protein, 21% fat, 2% fibre; Dibaq S.A, Fuentepelayo-Segovia, Spain) following the feeding system specified by the company.

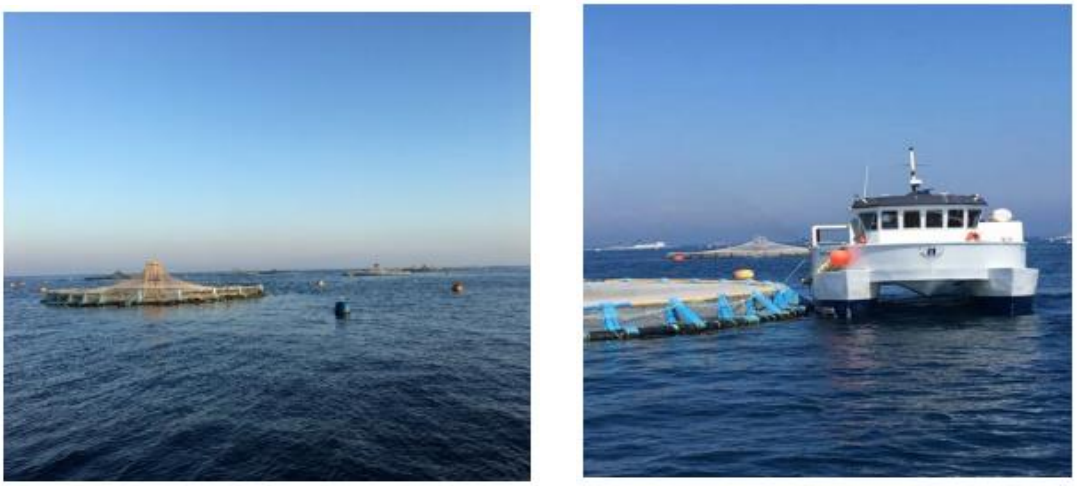

Figure 11. Cages of the Servicios Atuneros del Mediterraneo S.L (San Pedro del Pinatar, Murcia)

The following sampling took place on the 29th of March, 2017, at 762 dph, and the same traits were recorded, weights, length and malformations. The 3rd of November of 2017, 980 dph, the last sampling was realized, 100 samples of the higher weight were selected to be moved to the IEO in order to be genetically evaluated subsequently. The rest were sacrificed in ice.

3.2. Feeding

Respecting to the nutrition characteristics, we have two different diets of comercial feeds, in the first period it was used D4 for 15 months (Table 3) where the content in protein and digestible is higher (Table 4), until they weigh around 220 g, then they are fed with D6 until the sacrifice.

Table 3. Monthly distribution of the different diets

Month	***Feeding days***	***Kg. Feed***	***Diet***
December	18	138	D4
January	21	78	D4
February	22	66	D4
March	25	75	D4
April	25	75	D4
May	26	78	D4
June	29	105	D4
July	29	140	D4
August	30	161	D4
September	29	333	D4
October	28	266	D4
November	22	180	D4
December	20	100	D4
January	22	110	D4
February	20	100	D4
March	18	225	D4
April	18	225	D4
May	26	130	D4
June	28	155	D6
July	31	155	D6
August	31	150	D6
September	29	145	D6
October	31	155	D6
November	1	5	D6

Table 4. Diet composition.

Diet	***Protein***	***Fat***	***Ashes***	***Cellulose***	***Digestible Energy***
D4	46.5%	19%	7%	2.75%	17.9 MJ/kg
D6	44%	20%	7.175	3.075%	17.625 MJ/kg

3.2.1. Fatty acids profiles in the different diets

The table 5 includes the fatty acid profiles of the two different diets that were used for feeding the gilthead sea bream.

Table 5. Comparison of fatty acids profile of the different diets.

Nº of C	D4 Diet	D6 Diet
Saturated fatty acids (SFA)		
C 14:0	2,916	1,891
C 16:0	15,508	11,474
C 18:0	5,158	4,310
C 22:0	0,834	0,000
TOTAL SFA	**24,416**	**17,675**
Monounsaturated fatty acids (MUFA)		
C 16:1	4,056	2,660
C 18:1 n9 c	29,931	42,983
C 20:1	3,034	2,389
C 22:1	2,054	1,175
TOTAL MUFA	**39,075**	**49,207**
Polyunsaturated fatty acids (PUFA)		
Omega-3 family		
C 18:3 (n9,12,15)	4,614	6,056
C 20:5	4,581	2,813
C 22:6 n3	6,190	4,017
Omega-6 family		
C 18:2 n6 t	3,118	3,587
C 18:2 n6 c	15,840	16,645
C 20:2	0,978	0,000
C 20:4	1,186	0,000
TOTAL PUFA	**36,508**	**33,118**

3.3. Analysis of flesh and fillet quality

After the sacrifice, gilthead sea breams are taken into a frigorific chamber at 4ºC until the measure of flesh quality and extraction of fillets. The first measurement carried out is the final weight at sacrifice by a scale model Scout Pro APU 601 with Max= 500g and d= 0.1g, the second measured is the length, by a ictiometer of 50cm. With the length already measured, malformation is the next parameter, gilthead sea breams are observed carefully to gather enough information about the different malformations (like lordosis or alterated operculum) to calculate the percentage of malformations.

Then the texture is measured with a texturometer, carrying out a profile analysis test with a compression aluminium plate of 100 mm diameter and 0.8 mm/s speed, by double compression per sample (Fig 12). The variables that are measured are: hardness (maximum strength of the first compression cycle, in N), cohesiveness (relation of the area of positive strength during the second compression in comparison with the one of the second, without units), elasticity (height that the food recovers during the time within the two cycles of compression, in mm), springiness (hardness multiplied by cohesiveness, in N) and chewiness (toughness multiplied by cohesiveness and elasticity, in N*mm), calculated according to Bourne (1978).

Figure 12. Measurement of texture.

With the use of a pH meter HI-98230 (HANNA^{R}) equipped with a metallic electrode ISFET (Ion sensitive Field Effect Transistor) the pH was measured 3 hours post-mortem in the dorsal area.

For the evisceration, the visceral fat, gills and ventricle are separated from the fillet and then weighed with a scale model SARTORIUS AG GOTTINGEN BL600 Germany with Max= 600g and d=0.1g. With the obtained data, the percentage of these parts regarding the final weight at sacrifice and the fillet yield (ratio between the fillet weight and the final weight at sacrifice) is calculated.

Figure 13. Removal of chip and evisceration.

Figure 14. Filleting of the gilthead sea bream.

Once they have been slaughtered and the fillets extracted, these fillets are conserved in -20ºC to subsequently carry out the calculation of different quality parameters of flesh like: Total collagen of the muscle, and chemical components of the muscle like fat, moisture and protein.

For the measurement of these parameters, it is considered the different ISO standards:

- Total collagen of the muscle: determined by the analysis of hydroxyproline (ISO 3496).
- Chemical composition of the muscle: percentage of moisture (ISO R1442), protein (ISO R-397) and fat (ISO 1443).

3.4. Fatty acids profile

The fatty acids profile of the gilthead sea bream is determined through three steps:

1. Grinding and lyophilization of gilthead sea bream filet at -80ºC.
2. Separation and extraction of fatty acids by Direct FAME Synthesis (Fatty Acid Methyl Ester; O´Fallon et al. 2007).
3. Identification of types and proportions of fatty acids by gas chromatography

Grinding and lyophilization of gilthead sea bream filet at -80ºC

After grinding the gilthead sea bream filet previously frozen at -80ºC, they are frozen until they are taken into the lyophilizator, then they are kept in vacuum conditions at -40ºC for 24h.

Separation and extraction of fatty acids by *Direct FAME Synthesis*

Esters from fatty acids (FAME) showed in **Table 7** are quantified. To carry out this quantification, it is necessary the use of an internal standard (C17:0), to fix the extraction process in each sample. The fatty acid standard Supelco 37 Component FAME Mix (Sigma-Aldrich) is used for the calculation of the response factor of each fatty acid.

The protocol of direct FAME synthesis for each sample consists in:

- Measure 40 µL of fatty phase and put it in a Pyrex culture tube and add 0.7 mL of 10 N KOH in water (for the saponification of fatty acids), 5.3 mL of MeOH (for the methylation of fatty acids) and 1 mL of the C17:0 internal standard (0.5 mg/mL of MeOH) (for the control of the process).
- Incubate in a 55ºC water bath for 1.5h shaking with vortex every 20 min to properly permeate, dissolve, and hydrolyze the sample.
- Cooling below room temperature with the water tap and add 0.58 mL of 24 N H2SO4.
- Mix the content of the tube by inversion and incubate again in a 55ºC water bath for 1.5 h shaking with vortex every 20min.
- Cooling with tap water and add 3 mL of hexane 95% HPLC in extraction hood.
- Centrifuge the tubes at 3000 rpm for 5 min.
- Place the hexane layer, containing the FAME, into a GC vial with a pipette for gas chromatography (Fig 15).

Figure 15. Centrifuged samples of fatty acids (Hexane is the transparent layer).

Identification of types and proportions of fatty acids by gas chromatography

- Poke 0.5 µL in the gas chromatography under the indicated conditions in the following table:

Table 6. Working conditions in gas chromatography.

Column	**Supelco SP-2560 (100m x 0.25mm x 0.2 µL)**
Carrier gas	Helium at lineal speed of 20 cm/s
Split ratio	100:1
Tº of injection and detector	260 ºC
Tº oven	Starting at 100ºC for 5min, then increase 4ºC each min until reaching 240ºC and hold for 30min.
Sample	Sigmal Aldrich 47885-U

Table 7. List of Fatty acids determined by gas chromatohraphy.

Systematic nomenclature	Common name	Nº of C
Saturated fatty acids (SFA)		
Tetradecanoic acid	Myristic acid	C 14:0
Pentadecanoic acid	Pentadecylic acid	C 15:0
Hexadecanoic acid	Palmitic acid	C 16:0
Heptadecanoic acid	Margaric acid	C 17:0
Octadecanoic acid	Stearic acid	C 18:0
Nonadecanoic acid	Nonadecylic acid	C 19:0
Eicosanoic acid	Arachidic acid	C 20:0
Heneicosanoic acid	-	C 21:0
Docosanoic acid	Behenic acid	C 22:0
Monounsaturated fatty acids (MUFA)		
cis-9-tetradecenoic acid	Myristoleic acid	C 14:1
cis-10-pentadecenoic acid	-	C 15:1
cis-9-hexadecenoic acid	Palmitoleic acid	C 16:1
cis-10-heptadecenoic acid	Margaroleic acid	C 17:1
trans-9-octadecenoic acid	Elaidic acid	C 18:1 n9 t
cis-9-octadecenoic acid	Oleic acid	C 18:1 n9 c
cis-11-eicosenoic acid	Gadoleic acid	C 20:1
cis-13-docosenoic acid	Erucic acid	C 22:1
-	Lignoceric acid	C 24:1
cis-15-tetrasenoic acid	Nervonic acid	C 24:1 n9
Polyunsaturated fatty acids (PUFA)		
Omega-3 family		
cis-9,12,15-octadecatrienoic acid	Alpha-linolenic acid	C 18:3 (n9,12,15)
cis-6,9,12,15-octadecatetraenoic acid	Stearidonic acid	C 18:4
cis-11,14,17-eicosatrienoic acid	Eicosatrienoic acid	C 20:3 n3
cis-5,8,11,14,17-eicosapentaenoic acid	Timnodonic acid	C 20:5
cis-4,7,10,13,16,19-docosahexaenoic acid	Cervonic acid	C 22:6 n3
Omega-6 family		
trans-9,12-octadecanoic acid	Linolelaidic acid	C 18:2 n6 t
cis-9,12-octadecanoic acid	Linoleic acid	C 18:2 n6 c
cis-6,9,12-octadecatrienoic acid	Gamma-linolenic acid	C 18:3 n6
cis-11,14-eicosadienoic acid	Eicosadienoic acid	C 20:2
cis-8,11,14-eicosatrienoic acid	Dihomo-gamma-linolenic acid	C 20:3
cis-5,8,11,14-eicosatetraenoic acid	Arachidonic acid	C 20:4
cis-7,10,13,16-docosatetraenoico	Adrenic acid	C 22:4

Finally, the percentage of each fatty acid is obtained considering it equal to its percentage of methyl ester about the total through the expression:

$$\%_{a.g} = (RF*Response_{ag}/Response_{ag})/\Sigma(FR*Response_{ag}/Response_{ag})$$

Where:

- $\%_{a.g}$ is the percentage of fatty acid
- RF is the response factor of each fatty acid methyl ester
- $Response_{a.g}$ is the response area of the methyl of each fatty acid
- $Response_{ref}$ is the response area of the methyl of the reference fatty acids, in this case is the heptadecanoic acid.

3.5. Statistical analysis

The statistical analysis is realized with the software SPSS® (SPSS, Chicago, IL, USA), which includes a descriptive analysis of the variables (mean, standard error, maximum, minimum, quartiles) and Pearson correlation among the analysed parameters, that is declared as significant when $P < 0.05$. All growth data were tested for normality and homogeneity of variances.

4. RESULTS AND DISCUSSIONS

4.1. Growth traits

Phenotypic results for growth traits at three different ages (251 dph – November 2016, 762 dph – March and 980 – November 2017) for 563 gilthead sea bream descendant's population are shown in the following figures 16,17 and 18.

Regarding to weight, at 251 dph individuals presented an average weight of 44.32 ± 0.7g, standard deviation of 18.99g, variation coefficient equal to 42.85% (Fig. 16a). At 762 dph they presented an average weight of 313.6 ± 2g, standard deviation of 51.254g, variation coefficient equal to 16.34% (Fig. 16b). And finally at 980 dph they presented an average weight of 446.79 ± 2.7 g, standard deviation of 73.31 g, variation coefficient equal to 16.41% (Fig. 16c). With these results we can say that the weight is more asymmetrical at the beginning (251 dph), there are more dispersed data than in 762 and 980 dph, this means that at the beginning there is more variability, and as the fish grows up, the data tends to normalize, so the distribution is more symmetrical, with the data closer to the mean than the extremes values.

Respecting the length, at 251 dph individuals presented an average length of 13.89 ± 0.06cm, standard deviation of 1.73 cm, variation coefficient equal to 12.43 % (Fig. 17a). At 762 dph they presented an average length of 26.82 ± 0.06cm, standard deviation of 1.45 cm, variation coefficient equal to 5.4 % (Fig. 17b). And finally at 980 dph they presented an average length of 28.73 ± 0.05cm, standard deviation of 1.67 cm, variation coefficient equal to 5.82 % (Fig. 17c). As we can see, the length is more symmetrical at the beginning (251 dph) than the weight, there are more data closer to the mean than the extremes values. In the different measures, it is observed that from 251 to 762 dph, there is more difference of length than from 762 to 980 dph, where it barely increases.

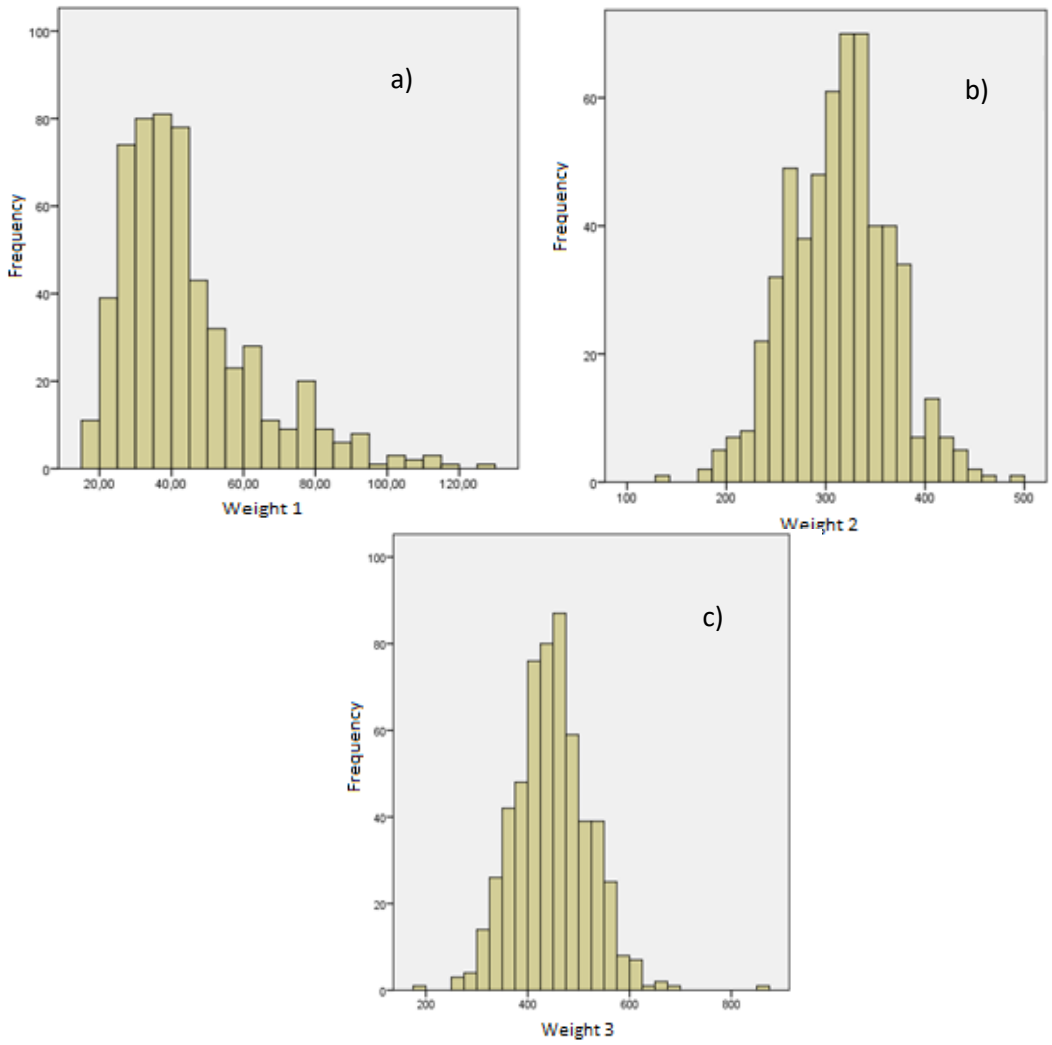

Figure 16. Weight frequency distribution at a) 251, b) 762 and c) 980 days post-hatching.

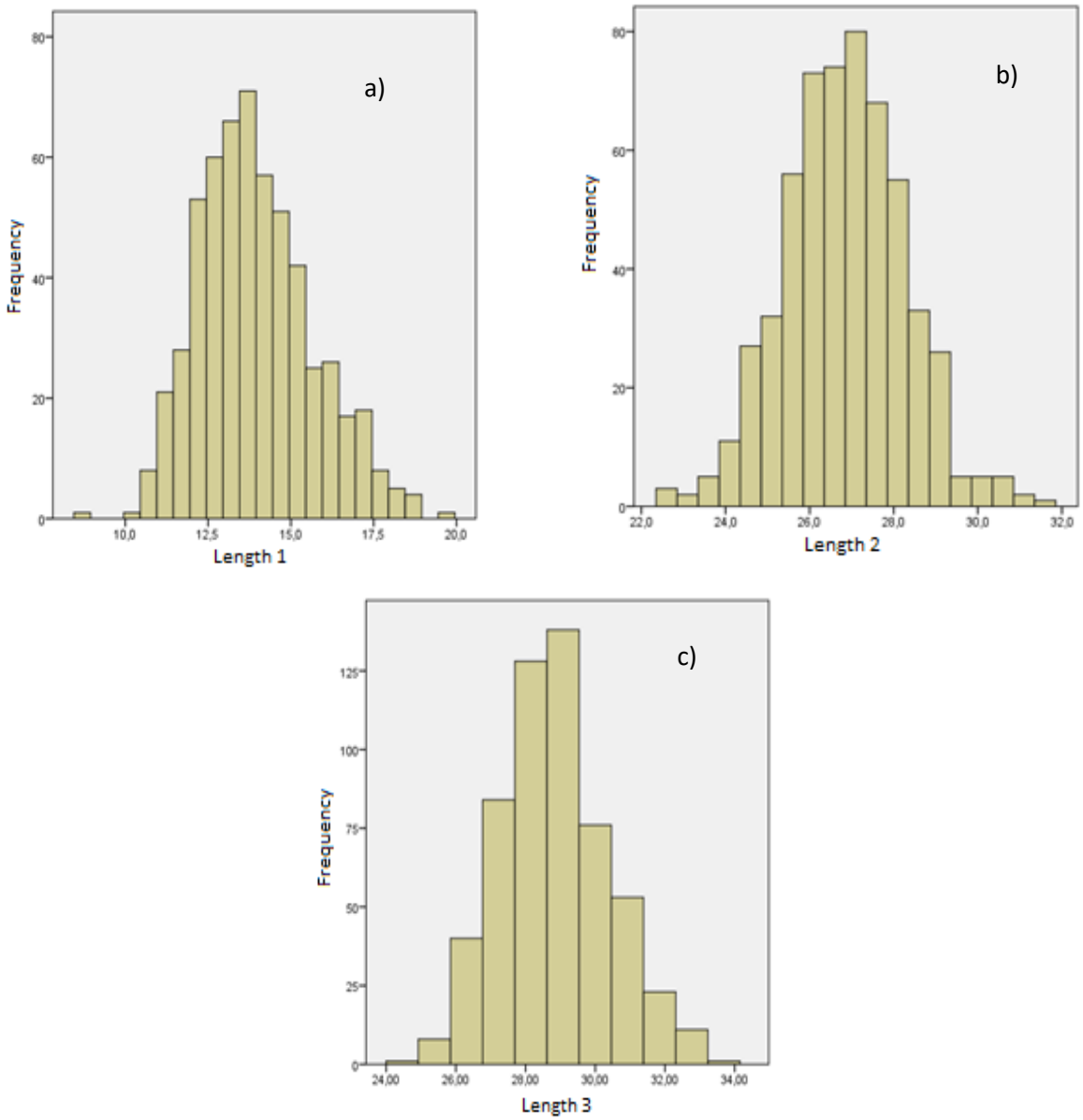

Figure 17. Length frequency distribution at a) 251, b) 762 and c) 980 days post-hatching.

This growth depends on many factors related to nutrition and on many abiotic factors related to fish rearing conditions, being known that growth rate strongly correlates with temperature fluctuations (Ginés *et al*., 2004; Karahan *et al*., 2013). In comparison to Ginés *et al*., 2004, our average growth parameters at 980 dph were lower than his study at 48 weeks. This fact could be explained partially due to temperature fluctuations present in the region of Murcia. If we compare to Navarro *et al* (2009), they observed 485.6g of body weight and 27.7 cm of fork length at 509 dph in the Canary Islands, which is a

region where low temperature fluctuations are registered, while we observed 313.56g and 26.82 cm at 980 dph. Regarding to phenotypic correlations, our data was very precise in almost all the cases due to the large number of samples analysed, body weight and total length at 251 dph, showed a correlation of ,956 in these fishes. Weight and length have been reported as phenotypically correlated traits in sea bream (Navarro *et al*., 2009a) as well as in other marine species (Elvingson and Johansson, 1993; Winkelman and Peterson, 1994; Vandeputte *et al*., 2004, 2008). The correlation between weight and length drops slightly (0.685) at 762 dph but increase again (0.749) in 980 dph.

Table 8. Weight and length correlation at different ages

		Weight 1	Length 1	Weight 2	Length 2	Weight 3	Length 3
Weight 1 251 dph	Pearson Correlation	1					
	Sig. (bilateral)						
	N	739					
Length 1 251 dph	Pearson Correlation	,956**	1				
	Sig. (bilateral)	,000					
	N	739	739	738			
Weight 2 762 dph	Pearson Correlation	,173**	,155**	1			
	Sig. (bilateral)	,000	,000				
	N	738	738	815	815		
Length 2 762 dph	Pearson Correlation	,147**	,161**	,685**	1		
	Sig. (bilateral)	,000	,000	,000			
	N	739	739	815	816	588	
Weight 3 980 dph	Pearson Correlation	,035	,059	,671**	,608**	1	
	Sig. (bilateral)	,410	,164	,000	,000		
	N	564	564	587	588	588	
Length 3 980 dph	Pearson Correlation	,018	,068	,621**	,666**	,749**	1
	Sig. (bilateral)	,666	,105	,000	,000	,000	
	N	564	564	587	588	588	588

**. The correlation is significant at level 0.01 (bilateral).

However, the correlations within weight or length at early ages were surprisingly very low. Thus, the correlation between weight at 251dph and 762 dph was 0,173, and between length at 251dph and 762 dph was 0.161. Therefore, selection at early ages can led to bad results, since the biggest fish at early ages were not the biggest ones at the end. After, the correlations at late age were higher ($\rho_{\text{weight 762-980dph}} = 0.671$, $\rho_{\text{length 762-980dph}} = 0.666$). Thus, fish can be selected at 762 dph according to their weight, because we are going to obtain good response to improve their growth through generations.

Regarding to the condition factor, at 251 dph individuals presented an average condition factor of 1.59 ± 0.01 g cm^{-3}, standard deviation of 0.148 g cm^{-3}, variation coefficient equal to 9.31 % (Fig. 18a). At 762 dph they presented an average condition factor of 1.78 ± 0.01 g cm^{-3}, standard deviation of 0.197 g cm^{-3}, variation coefficient equal to 11.07 % (Fig. 18b). And finally at 980 dph they presented an average condition factor of 1.91 ± 0.01g cm^{-3}, standard deviation of 0.218 g cm^{-3}, variation coefficient equal to 11.41 % (Fig. 18c).

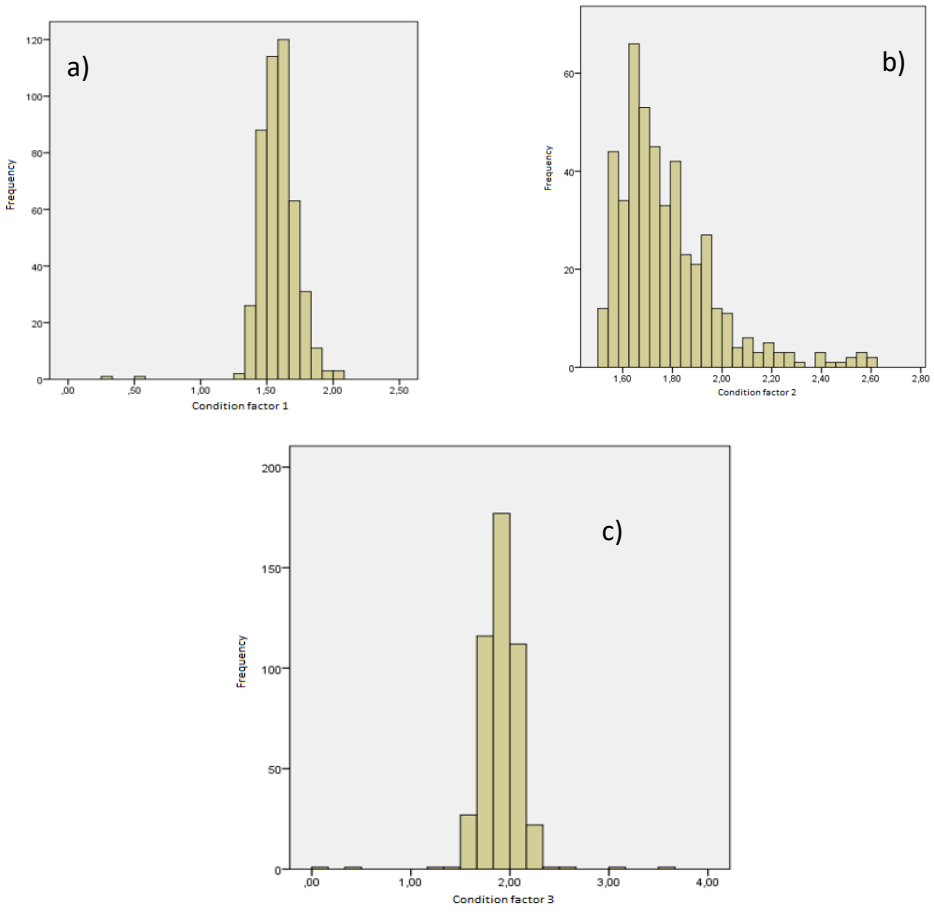

Figure 18. Condition factor frequency distribution at a) 251, b) 762 and c) 980 days post-hatching.

If we compare our condition factor obtained data with the study of García-Celdrán *et al*. (2015), they obtained a condition factor of 1.62 g cm^{-3} at 163 dph, and 1.72 g cm^{-3} at 690 dph while we obtained 1.59 g cm^{-3} at 251 dph and 1.91 g cm^{-3} at 762 dph. We can observe that in both studies, the condition factor increases along the time, this could be the reason why length decrease its growing with the age.

Deformities

Several factors may cause skeleton deformities in fish in natural and aquaculture conditions; the temperature (Sfakianakis *et al.*, 2006; Georgakopoulou *et al.*, 2010), the water current (Kihara *et al*., 2002; Karahan *et al.*, 2013), intensive swimming of fish during pre-growing (Bardon *et al*., 2009), the diet composition (Fernández *et al*., 2008), the non-inflation of the swimbladder (Chatain, 1994), and intensive rearing conditions (Andrades *et al*., 1996; Koumoundouros *et al*., 1997; Boglione *et al*., 2001; Belardo *et al.*, 2003; Roo *et al.*, 2005).

Regarding deformities, our samples did not show any deformity in the vertebral column, while in the research of Lee-Montero *et al.* (2015), they showed a frequency from 1.2% to 10.9%. However, the lack of operculum was a deformity that appeared with a frequency of 0.8%, but according to the deformity recovery (De Wolf *et al*., 2004; Beraldo and Canavese, 2011; Lee-Montero *et al*., 2015), we observed that the deformity was not present in some fishes in the third measurement (980 dph).

With a 0.5% frequency, an asymmetry of the caudal fin was observed at 251 dph in the study, but a completely recovery was observed at 762 dph.

Our samples showed the highest deformity frequency at 251 dph with 2%, with any kind of deformity, although mainly the lack of operculum was detected. At 762 dph, the frequency of deformed fish reached 1.8%, in comparison to the fishes of Lee-Montero *et al.* (2015), our fishes were better performers since they had 32.7% and a 12.5% of deformed individuals at 163 dph and 690 dph.

Carcass and flesh quality

The following table (Table 9) shows the different parameters studied regarding to texture and pH fixed by final weight.

Table 9. Average and standard error of the measures of texture and pH fixed by final weight.

		Covariate
	Average ± se	**Final weight**
Hardness (N)	78.4 ± 1.12	-0.032** ± 0.012
Springiness (mm)	6.58 ± 0.047	0.000 ± 0.000
Cohesiveness (ratio)	0.704 ± 0.005	0.000** ± 0.000
Gumminess (N)	54.7 ± 0.76	-0.005 ± 0.008
Chewiness (N mm)	359.7 ± 5.21	-0.034 ± 0.055
pH	6.17 ± 0.014	0.000 ± 0.000

s.e.: standard error

If we compare our results with the study realized by Ayala et al. (2010), in which the textural parameters and pH of gilthead sea breams were analysed during the post-mortem storage on ice, we can observe that the obtained data from our samples in some cases is almost three times more than in the study, we obtained a hardness of 78.4 N while in the study 29.91 N, the same happened with springiness, where we obtained 6.58 mm comparing to 3.13 mm measured in the study. However, we got similar results respecting the cohesiveness and pH, 0.704 from us and 0.46 from Ayala et al. referring to cohesiveness, and 6.17 from our samples comparing to 6.51. According to the results of Ayala, they obtained a gumminess of 13.67 N while our results show 54.7 N, significantly higher. Chewiness is the parameter that more differs from the study of Ayala, whose measurement was from 359.7 N mm for us while was 44.58 N mm for Ayala. This differences could be partly due to the sacrifice weight since in Ayala the gilthead sea breams had an average sacrifice weight of 414g.

Table 10. Average and standard error of the measures from the carcass quality fixed by final weight.

		Covariate
	Average ± se	Final weight
Visceral fat (%)	6.26 ± 0.078	0.006** ± 0.001
Carcass yield (%)	88.3 ± 0.35	0.034** ± 0.004
Fillet yield (%)	36.4 ± 0.33	0.009** ± 0.003

s.e. : standard error

If we compare our results with the research realized by Navarro et al. (2009), in which the gilthead sea breams were reared in tanks and cages, it was observed the fillet and flesh yield for different rearing places. According to Navarro et al. (2009), they obtained fillet yield of 35.1% and carcass yield of 93% in gilthead sea breams reared in tanks, regarding to the cages, they obtained 35.7% and 90.4% respectively. In comparison to our obtained data, both results are similar, although our carcass yield was around 2-3% lower and the fillet yield 1-2% higher.

In another research, Navarro et al. (2009), the same gilthead sea breams from the previous research were also analysed in terms of visceral fat and they obtained 2.6% in the gilthead sea breams from cages and 2.0% in the others from tanks. We can observe that our visceral fat data is significant higher 6.26% than the others, therefore, this could be why our samples showed a lower carcass yield. Also, these differences could be explained because Navarro sacrificed the gilthead sea breams at 509 days of age, with an average weight of 365g.

Table 11. Average and standard error of the chemical composition fixed by final weight.

		Covariate
	Average ± se	Final weight
Collagen (%)	1.79 ± 0.055	0.000 ± 0.001
Intramuscular fat (%)	4.64 ± 0.091	0.010** ± 0.01
Moisture (%)	73.1 ± 0.11	-0.008** ± 0.001
Protein (%)	21.9 ± 0.10	0.000 ± 0.01

s.e. : standard error

As we can see in this table (Table 11), the parameters more influenced by the covariate are the intramuscular fat and the moisture, it is observed for the fat that for every gram increased in the weight, a 0.01% is increased in the fat. For example, if we increase a fish by 50 grams, this fish would increase its fat around 0.5 grams. However, in the case of the moisture it happens the opposite, for every gram increased in the weight, a 0.01% is decreased in its moisture.

The flesh quality parameters are becoming more and more important; the muscle composition plays an important role in these parameters. In the gilthead sea bream the obtained results regarding the fat in fillets are very variable, from 2% to 11%, values that are inversely proportional to moisture, from 68% to 74%. These results coincide with our study, not only for the average values, but the inverse relation between fat and moisture (Navarro et al., 2009).

In the study carried out by Ginés et al. (2004), they studied quality parameters like fat, protein and moisture content in gilthead sea breams reared in tanks. They presented higher results in intramuscular fat (6.0% respecting to ours 4.64%) in comparison to our study, similar results in proteins, but a lower content in moisture (70.5% comparing to ours 73.1%). Respecting the collagen content, it depends on the species, in the previously said research, Navarro et al. (2009), they obtained 0.4 % and 0.7 % for gilthead sea breams reared in cages and tanks, we could observe that we obtained a higher value in comparison to them (1.79 %).

4.2. Fatty acids profile in the gilthead sea bream

In the following table (Table 12), we can find the fatty acid profile of the gilthead sea bream.

Table 12. Fatty acids profile of the gilthead sea bream.

Fatty acids	Mean	Standard error
Saturated fatty acids (SFA)		
C 14:0	3,086	0,028
C 15:0	0,329	0,005
C 16:0	17,260	0,133
C 18:0	4,537	0,046
C 19:0	0,338	0,070
C 20:0	0,341	0,007
C 21:0	1,975	0,042
C 22:0	0,147	0,013
TOTAL SFA	**28,014**	**0,344**
Monounsaturated fatty acids (MUFA)		
C 14:1	0,024	0,003
C 15:1	1,586	0,518
C 16:1	0,296	0,006
C 17:1	0,448	0,021
C 18:1 n9 t	3,463	0,185
C 18:1 n9 c	37,560	0,532
C 20:1	2,328	0,030
C 22:1	0,866	0,014
C 24:1	0,016	0,002
C 24:1 n9	0,413	0,017
TOTAL MUFA	**47,000**	**1,329**
Polyunsaturated fatty acids (PUFA)		
Omega-3 family		
C 18:3 (n9,12,15)	3,598	0,062
C 18:4	0,564	0,018
C 20:3 n3	0,368	0,009
C 20:5	2,661	0,054
C 22:6 n3	6,319	0,129
Omega-6 family		
C 18:2 n6 t	8,930	0,496
C 18:2 n6 c	0,207	0,011
C 18:3 n6	0,244	0,020
C 20:2	0,775	0,012
C 20:3	0,371	0,009
C 20:4	0,698	0,013
C 22:4	0,252	0,009
TOTAL PUFA	**24,986**	**0,842**

The results show the following answer about the fatty acids profile: we find as predominant fatty acids the oleic acid (37.6 ± 0.53 %), the palmitic acid (17.26 ± 0.13%) and the linoleaidic acid (8.93 ± 0.5%).

The fatty acids profile of the gilthead sea bream presents in higher percentage monounsaturated fatty acids, where the oleic acid is found, then the saturated fatty acids where the palmitic acid belongs to, and then the polyunsaturated acids, with the omega3 and omega6 families of acids.

Respecting our diets, D4 diet has a higher content in SFA (24%) and PUFA (36.5%) than D6 (17.68% and 33.12%), while D6 is higher in MUFA (49%). The main difference between both diets was found in oleic acid, in which the diet provided at the end of the trial (D6) showed higher content (42.98% comparing to 29.95%). According to P. Di Marco (2017) in his study about conventional feed and organic feed in gilthead sea breams, we were able to see how the fatty acids found in the gilthead sea bream changed in quantity regarding the kind of diet Di Marco used, He observed that the most significant different between both diets, was found in the fillet fatty acid composition which is mainly related to feed ingredients. The fillets of organic sea bream were actually characterized by higher PUFA, particularly linoleic acid, and lower n-3 PUFA levels than those of conventional fish, which is consistent with the analogous compositional differences shown by the feeds eaten.

In our diets, the most abundant fatty acids were the palmitic acid, oleic acid and linoleic acid. Although others important fatty acids were found in smaller quantities like myristic acid, stearic acid, behenic acid, palmitoleic acid, gadoleic acid, erucic acid, alpha-linolenic acid, timnodonic acid (EPA), cervonic acid (DHA), linolelaidic acid, arachidonic acid and eicosadienoic acid.

If we compare the fatty acids profile obtained from the gilthead sea breams with our diets and with conventional feed (P. Di Marco, 2017), we can find the following differences:

- The total SFA was higher in the gilthead sea breams fed with our diets (28%) comparing to conventional feed (23%), where in both cases the palmitic acid was the predominant acid, although it was slightly higher in our samples (17%) than in Di Marco's (16%).

- The total MUFA was also higher in our gilthead sea breams (47%) while in conventional feed (34%), here we can say that our samples showed a truly higher content in oleic acid (37%) than in conventional feed (20%).
- Finally, in PUFA we obtained a lower percentage in our samples (25%) while in conventional feed (38%), in this group we find the timnodonic acid (EPA) and cervonic acid (DHA), whose percentage was lower in our fishes (2.66 and 6.3%) than in the study of Di Marco (2.68 and 8.54%)

5. CONCLUSIONS

The obtained conclusions through the different parts of our study are:

- Fish from this study were very good performers since they showed a faster growth rate and a lower incidence of deformities and some of them even presented a deformity recovery, if compared with previous studies. They showed a high weight and well correlated with the length, especially at the beginning, but we cannot select an individual by its initial weight or length, since the correlation between weight and length at different ages was low.
- Regarding the flesh and fillet quality, we found that our samples showed a higher amount of visceral fat than other studies, this could influence in the carcass yield. However, our efficiency data were very similar to the average *Sparus aurata* L. researches. We can assume there is an inverse relation between fat a moisture, since moisture shows a negative covariate with final weight.
- Respecting the fatty acids profile, gilt head sea bream showed a healthy profile, with high percentage of oleic acid, EPA and DHA. We observed how the fatty acids found in the diets, were also found in the gilthead sea breams at similar quantities, especially the oleic and the palmitic acids whose content were higher, but this happened as well with the acids in minor proportion. We can say the diets we used influenced over the fatty acids profile of the gilthead sea breams.

  In future researches the genetic component for these traits will be investigated, especially for fatty acid profile

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