STUDY OF THE THIRD GENERATION OF GILTHEAD SEA BREAM *(Sparus aurata*, L.) FROM GENETIC SELECTION PROGRAM PROGENSA®. NEW INSIGHTS ON SELECTION TRAITS AND INDUSTRIAL APPLICATIONS

PhD thesis by SERGI LEÓN BERNABEU

PhD program in Sustainable Aquaculture and Marine Ecosystems (ACUISEMAR®)

Grupo de Investigación en Acuicultura (GIA®)

Institute of Sustainable Aquaculture and Marine Ecosystems (IU-ECOAQUA®)

Universidad de Las Palmas de Gran Canaria

Supervised by:

PROFESSOR JUAN MANUEL AFONSO LÓPEZ

PROFESSOR MANUEL MANCHADO CAMPAÑA

ACKNOWLEDGEMENTS

I want to thank Professor Afonso and Professor Manchado for creating the conditions for this work to be carried out, and Professor Zamorano for holding the fort in the meantime.

I also want to thank my colleagues in IU-ECOAQUA, specially Guacimara Alejandro and my fellow pHd student, Álvaro Lorenzo, for being always a helping hand and much more than lab mates.

I must not forget to thank AQUANARIA and Mr. Enrique Haro for letting things happen and creating the opportunity for me to get involved.

This work has been completed thanks to people that contributed with their inestimable work: Cathaysa Pérez, César Avendaño, César Burgos and Marta Servio. I also would like to mention Hyun Suk Shin and Islam Elalfy for their dedication in time to this work.

Lastly, I want to express my gratitude to all friends and relatives that have supported me unconditionally while carrying out this work.

And to Maria Matesanz for designing the cover of this manuscript.

Sergi León Bernabeu

FUNDING SOURCES

This work was funded by the national research project "IMPROVEMENT OF THE GILTHEAD SEA BREAM SECTOR COMPETITIVENESS THROUGH GENETIC SELECTION (PROGENSA-III), granted by the Spanish National Advisory Board Marine Culture (JACUMAR).

Private companies of the Spanish aquaculture industrial sector contributed with their own resources to this work: AQUANARIA, S.L. and Pesquerías Isla Mayor S.A. (PIMSA):

LIST OF CONTENTS

LIST OF ABBREVIATIONS

BW	Body Weight
cNiT trait	Carcass Non-Invasive Technological trait
CF	Condition Factor
CPH	Caudal Pedunculus Height
CW	Carcass Weight
DL	Days spawning, Large scale
dph	Days post-hatching
Dr%	Dressing percentage
EBV	Estimated Breeding Value
FCR	Food Conversion Ratio
FEc	Fish Eccentricity
FFM	Fish Fat Meter
FHA	Fish Equidistant Height A
FHB	Fish Equidistant Height B
FHC	Fish Equidistant Height C
FHD	Fish Equidistant Height D
FHE	Fish Equidistant Height E
Fi%	Filleting percentage
FilA	Fillet Area (square cm)
FilA%	Fillet Area (percentage)
FilML	Fillet Maximum Length
FMH	Fish Maximum Height
FoL	Fork Length
FW	Fillet Weight
HeEc	Head Eccentricity
HeH	Head Height
KETs	Key Enabling Technologies
mNiT trait	Morphological Non-Invasive technological trait
NiT	Non-invasive Technology
NIRS	Near InfraRed Spectroscopy
PIT	Passive Integrative Transponder

LIST OF ABBREVIATIONS

RAS	Recirculating Aquaculture System
RFID	Radio-Frequency Identification
SMAM	Sexual Maturity Age in Males
SL	Standard Length
TaEL	Tail Excluded Length
TLA	Total Lateral Area
TLL	Total Lateral Length
VF	Visceral Fat
WDef	Whole deformity

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Estimating genetic parameters is necessary both for determining the best traits to select for (higher heritability would result in more genetic gain in the next generation) and for determining the best candidates to breed among population (those with higher Estimated Breeding Value for the desired trait).

One widespread trend in the last years in breeding programs has been searching for alternative traits to perform indirect selection. Traits with higher heritability estimates and good genetic correlations with classic traits of interest for the industry. In industries where production stocks are comprised by a relatively high number of individuals, it becomes very relevant to use systems and methods that allow to record data in a fast and automated way. The present study and analysis have been conducted within the framework of PROGENSA-III, a Spanish national project which aims to optimise gilthead seabream genetic selection programs from multiple scopes, including development and application of Key Enabling Technologies (KETs).

Breeder contribution and family number were calculated in independent spawning batches (4DL model) and the mix of two different batches (2x4DL model), in order to evaluate the potential on genetic variability of mixing eggs from different spawning events during the spawning season, when obtaining offspring by mass spawning. Results showed that 2x4DL model was more effective in terms of increasing breeder contribution in the descendance.

Morphometric traits have hold interest as candidates for indirect selection due to their non-invasive assessment nature and their potential adaptability to automatization. In this study, 18 Non-invasive Technological traits (NiT traits) related with morphometry and carcass measured from images by using image analysis software IMAFISH_ML were evaluated. Height mNiT traits (morphometric Non-invasive Technological traits) around head (Fish Maximum Height, Head Height) showed high heritability estimates and genetic correlations between them and with growth traits, reflecting their potential as selection traits in gilthead seabream, and concluding that using image analysis and fast data recording systems such as IMAFISH_ML is highly recommendable in the selection processes of this species.

Heritability estimates for processed weights were higher than for entire body weight. Classically measured carcass traits and cNiT traits (carcass Non-invasibe Technological traits) are strongly correlated, excluding dressing %. NiT traits related to filleting measured through image analysis software, such as Fillet Maximum Length, Fillet Area and Total Lateral Area showed high and positive genetic correlation with growth, carcass weight, fillet weight and filleting %. Thus, indicating that, not only selecting for these traits would improve BW and carcass traits, but also that it could be achieved using non-invasive technologies.

Chapter 1. General introduction

Global human population has been drastically increasing during the last two decades. Since the tendency is for number of individuals to keep growing, food industry has been also developing accordingly, in order to fulfil population nutrition necessities. In this sense, aquaculture has played a very important role in the food production market, creating sustainable sources of high-quality lipids and proteins.

The term "Aquaculture" sums up all activities involved in the production, development and trading of aquatic organisms, animals or plants, in seawater or freshwater, etc. This means taking care of the organisms in every stage of their development, just like any other ranching industry. Aquaculture was the latest animal production industry to develop at high scale, and because of that, nowadays is the fastest growing food production sector (FAO report, 2016).

When talking about fish production, there are two typical kinds of companies: hatcheries and on-growing companies. Hatcheries breed adult fish and cultivate the progeny in an enclosed environment, eliminating the need to find the fish in the wild and even providing some species outside their natural season. Hatcheries rise fish until they are old enough to be transported and then they are sold to on-growing companies. On-growing companies take care of the fish and feed them until they reach commercial size and are ready to be consumed by the population. Sometimes, one company may carry out both functions.

Battling against weather conditions and optimising production model is an individual task of every company, however, there is a factor than can be "fixed" beforehand, and it is fish genetics.

Given that scenario, hatcheries want to ensure that fish produced by their broodstocks have the best performance potential in the on-growing phase in order to increase their turnover. On-growing companies, at the same time, search a type of fry that allows them to produce the best quality product in less time.

Breeding programs, or genetic programs, are an ancient tool whose main function is to adapt, in some extent, the animal that is produced to the production model.

1.1. Breeding and genetic selection programs

Chavanne *et al*. (2016) described the term 'selective breeding' (or artificial selection) as a combination of various methods by which humans obtain organisms with genetically based desirable traits through the mating of chosen parents.

Selective breeding was originated around 10,000 years ago, in the Neolithic age (Zeder and Smith 2009). Wild species began to be, unintentionally, transformed into domesticated crops and livestock. What served as methods for artificial selection back then was basically exposing them to unnatural conditions such as higher population density (and appearance of derived diseases), freedom deprivation, changes in feeds or absence of predators. Thus, animal genetic lines that could not be adapted to these conditions, would eventually die. Later, active selection would begin to be performed by, for instance, using largest animals for mating.

Much more later, it became a field of study and breeding turned into a type of science where genetic improvement could be calculated, predicted, and executed, and molecular bases of what humanity had been doing during centuries, were unveiled.

For aquatic species, however, domestication and culturing for nutrition purposes began just few decades ago. The complexity of breeding and rearing conditions for these species, when compared to cattle, and the tradition of consuming wild fish, kept genetic breeding programs for fish from appearing until certain conditions were met (technologies for controlling fish environment and the necessity of finding an alternative to extractive fishing for consuming fish).

1.2. Keys for genetic improvement: genetic parameters

The term "genetic improvement" is the main objective of breeding programs and refers to enhancing certain characteristics of the animals through changes in their DNA, which will eventually result in positive changes for the production model.

Quantitative genetics are essential for performing genetic selection efficiently, and more importantly, for ensuring progress. Quantitative genetic parameters are used to select individuals by its value for desired traits, or characteristics (phenotype), and its ability to transfer this value to the next generation.

In order to improve one desirable trait, it is necessary to obtain phenotypic variance for that trait within a population, this variation is affected by genetic variance and environmental variance.

Most important genetic parameters are (Falconer and Mackay, 1996):

- Heritability:
  Heritability is a statistic that estimates the probability of variation of a phenotypic trait in a population being due to genetic variation between individuals in that population, opposed to variation due to environmental factors (Wray *et al.*, 2008). Its value is

expressed as a proportion (0 to 1, where 0 would imply variation only due to environmental factors and 1 only to genetic factors).

- Estimated Breeding Value

  Estimated Breeding Value (EBV) is the estimation of the genetic talent of an animal for a particular trait. EBVs are expressed as the difference between one individual genetics and the genetic base to which the animal is compared to (for instance, the population composed by siblings and relatives descending from the same breeder population). EBVs are expressed in the units in which the measurements are taken (e.g. kilograms for weight, cm for length, etc.).

- Genetic correlation

  Genetic correlation is the estimation of the proportion of variance that two traits share due to genetic causes, or to put it in other words, the probability of inheriting talent for one trait when talent for a different specific trait is inherited. A genetic correlation of 0 implies independence of the genetic effects on two different traits, while a correlation of 1 implies identical influence of genetics in both traits.

Genetic parameters are not intrinsic of every trait, they are estimated from a given population under determined conditions.

Estimating genetic parameters is necessary both for determining the best traits to select for (higher heritability would result in more genetic gain in the next generation) and for determining the best candidates to breed from determined populations (those with higher EBV for the desired trait). However, there are other important factors that may influence the result, and need to be controlled, such as inbreeding and homozygosity increase, heterosis, etc (Kause *et al*., 2005; Sonesson *et al.*, 2012).

In programs where subject populations are obtained through unspecific mating (like mass spawning) within one initial population, it is common to observe elevated inbreeding after several generations. In order to prevent this, it is important to keep track of every animal pedigree. Pedigree is a record of the ancestry of an animal. Pedigree-based kinship coefficients, or co-ancestry coefficients, are expressed as the estimated proportion of shared genome between two individuals, and consequently, the probability of inheriting an identical unspecific allele by this pair descendants. Consanguinity coefficient of a single individual is ½ of the kinship coefficient between its parents. Thus, in diploid organisms, kinship coefficient of two siblings, descending from the two same parents, would be 0.25 ($(1/2)^2$) plus the parent individual consanguinity coefficient.

1.3. Industrial breeding programs

In general, industrial breeding programs follow a multi-tiered structure composed by nucleus, multiplier, and commercial populations. A scheme of a conventional breeding program is shown in **Figure 1**. Selection nucleus is constituted by individuals of high genetic value for one or more desired traits. Animals in the nucleus mate (either by natural mating and spawning or by artificial fertilization), thus producing high numbers of offspring from superior sires (mature males) and dams (mature females) (multiplier population). Broodstock composed by multiplier population will be later disseminating the genes of these superior animals widely in the production batches (commercial population), which are destined to consumption. When the EBV for desired traits of fish in the multiplier population is comparable with (or higher than) the breeding value in the nucleus population they can be included in the nucleus, as fish in the nucleus grow and lose reproductive potential over time. Following this scheme, genetic improvement through breeding programs allows the producers to take profit of the performed selection while simultaneously keep on improving the original line in a continuous way.

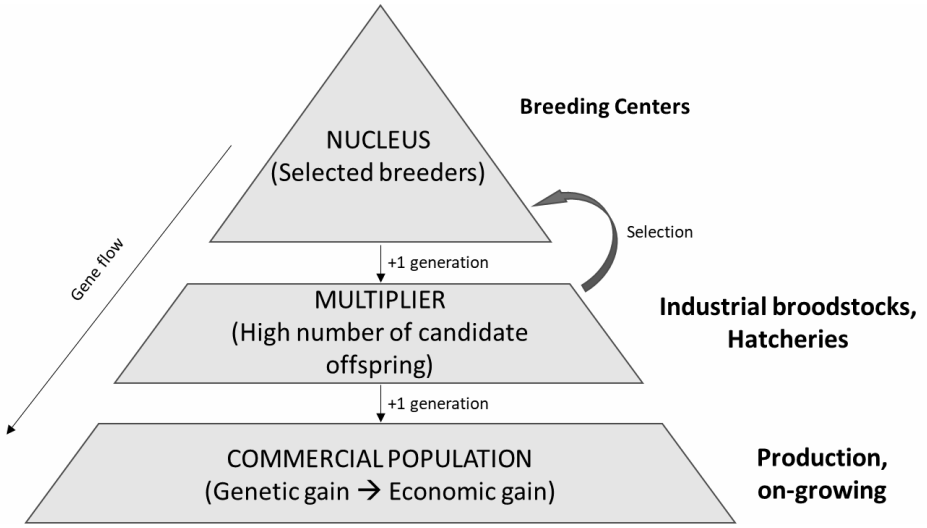

Figure 1. Scheme of a conventional breeding program with a multi-tiered structure.

As in any other industry, aquaculture sector is constantly challenged to improve profitability and promote a sustained growth. Companies may need to shorten the production cycles (and therefore the operational costs) or increase their product value or survival rate. One strategy extensively accepted, and highly consolidated in species such as salmon or tilapia, is the use of genetic breeding programs. Genetic selection offers a continuous, cumulative, and permanent improvement of the selected traits, extendable to the whole production chain (Falconer and Mackay, 1996). In Europe, more than 80% of aquaculture production uses animals obtained from breeding programs (Janssen *et al.*, 2017).

1.3.1. Novel traits and KETs

Selection traits are common in most of food industries: growth, disease resistance, stress resistance, product yield (carcass), flesh quality, etc. Other important traits are aesthetical (*e.g.* color and shape) and reproductive traits (such as fertility and progeny viability).

Genetic improvement by selection traits has its limitations. For example, phenotypic values of some traits may be notably affected by environmental factors, resulting in low heritability estimates. One widespread trend in the last years has been searching for alternative traits to perform indirect selection (Kause *et al.*, 2007; Fernandes *et al.*, 2015). These traits' phenotype would be more linked to family effects and with good genetic correlations with classic traits of interest for the industry.

At the same time, trait measurement and data obtaining techniques are also evolving. In industries where production stocks are comprised by a relatively high number of individuals, it becomes very relevant to use systems and methods that allow to record data in a fast and automated way. Methods that are able to perform measures without the necessity of killing the fish and without causing harm or stress are, as well, a priority for the sector, since animal welfare is becoming a major concern (Röcklinsberg, 2014). Newly developed Key Enabling Technologies (KETs) and derived tools serve as a good opportunity for this purpose.

The implementation of new image analysis technologies is rapidly improving the efficiency for fish trait evaluation (Pérez-Ruiz *et al.*, 2020), aligned with the advent of an industry 4.0 (Ferrari *et al*., 2021). Technologies based on image analysis have been successfully used in plants and livestock to optimize production systems and breeding programs (Osawa *et al*., 2008; Rius-Vilarrasa, *et al.*, 2009; Costa *et al*., 2011; Song *et al.*, 2018; De La Iglesia *et al.*, 2020). In such methodologies, several individual traits data acquisition is carried out, either lineal or dimensional, in a fast, repeatable, and reliable way, becoming very useful for genetic selection. In addition, this methodology is also non-invasive and, hence, can be used *in vivo,* reducing stress-related effects due to handling (Ruff *et al*., 1995). Navarro *et al.* (2016) reported a new method for measuring morphology in three species (gilthead seabream, red banded seabream and meagre), through an automatic image analysis software (IMAFISH_ML) for assessing Non-invasive Technological (NiT) traits, related to fish morphology (mNiT) and carcass (cNiT). This method, however, has not been yet evaluated in terms of additive genetic components and genetic relationships with regular industrial traits such as those related to growth.

In contraposition to those traits related with better growth performance, with direct benefits on production costs, there are also traits related with increasing product value, such as body composition traits, or product yield, such as carcass traits.

Body composition traits are directly related with flesh quality. Flesh quality is a complex concept that comprises several traits (freshness, appearance, smell, flavor, texture, taste, firmness, juiciness, etc.), most of them closely related to protein and muscular fatty acids content. Lipid profile and protein are two of the major strengths of the brand image of aquatic products destined to consumption due to their importance for human health and nutrition, however, it is not very clear how family effect modulates those traits (Nguyen *et al.*, 2015). Several laboratory techniques used for content measuring require processing and treating flesh samples (*e.g*. Soxhlet or Kjeldahl methods, for protein and lipid content, respectively), which often causes these traits to not be considered for selection programs.

1.4. Gilthead Sea Bream: main traits and particularities

1.4.1. Generalities

Gilthead sea bream (*Sparus aurata, L*) is one of the most cultured marine species in Mediterranean aquaculture. Its production is widespread along the Mediterranean coast and can also be found in peripheral areas like Madeira or Canary Islands. In 2019, overall production of gilthead sea bream in Europe and the Mediterranean countries was estimated to be 252,406 metric tons. It is the 61st most produced species in the world. Currently, consumption of this species is well consolidated, with Italy and North of Europe representing the largest markets. However, the gilthead sea bream industry is still far of being profitable mainly due to a limitation in fish performance and the low market prices (APROMAR, 2020).

Gilthead sea bream is a fish from the *Sparidae* family, and its complete taxonomic classification is as follows:

- Order: perciforms
- Class: *Actinopterygii*
- Phylum: chordata
- Kingdom: animalia

It is a pelagic euryhaline and eurythermal teleost fish and, therefore, it can be found wild in both marine and brackish waters. During its life cycle, it inhabits estuaries and coastal areas in the first stages, where, as a carnivore, it feeds on shellfish and shrimps until migrating to open sea, when spawning occurs in colder and more saline waters. Juveniles will close the cycle by migrating to warmer coastal waters (Stickney, 2000).

1.4.2. Morphologic and physiologic characteristics

Gilthead sea bream is a percoid fish with steep head and oval-shaped profile. Its head is convex and most of it is scale-less. Eyes are located on both sides, showing a golden line between them

which is a very distinctive feature of this species. Mouth is small and narrow, showing one row of canines and 2 rows of molars in both jaws. This type of mouth is suitable for cutting and crushing hard things like shells, not so for hunting and swallowing large preys. Gilthead seabream colour is silver-grey and has a peculiar big dark spot at the beginning of the lateral line that covers also the upper part of the operculum.

Gilthead sea breams reach 400g in body weight in 18 to 24 months post-hatching, depending on the temperature. Commercial weight ranges from 250g (ration product) to beyond 2kg (APROMAR 2018).

Gilhead sea breams are protandric hermaphrodites, which means that, by the time they reach sexual maturity, they develop male gametes and after more or less 2 years, they may switch to female.

1.4.3. Culturing and breeding conditions

Most common culturing systems used for industrial gilthead sea bream on-growing are floating cages (or net pens), inland tanks and estuaries.

Floating cages are used for maintaining fish in their natural environment as they grow (usually coastal waters but can also be off-shore), fish are kept in a space limited by nets and marine water permeate nets in and out. Fish can be fed on-site, manually or pumped from boats, or remotely through feeding barges. This system is considered intensive culturing, since it allows high population densities due to the natural water renewal inside production units (cages).

Inland tanks are used for farming fish outside their natural environment. Water is pumped from natural source and taken into controlled tanks through pipes. Inland tanks are isolated systems that can be controlled through water renewal, recirculation, and wastewater treatment. This system also allows to work with high population densities.

Estuaries are considered semi-intensive or extensive culturing systems. Estuaries are natural habitats located in coastal areas with at least one river flowing through and connected to sea. Farming sites located in estuaries take advantage of topography and hydrodynamics of the area and only small modifications are needed in order to control fish. As in a natural ecosystem, fish feed is composed (mostly) by live organisms, and population densities are low.

Gilthead sea bream is normally bred at industrial scale in hatcheries (nucleus and multiplier) through mass spawning, including organization of sex ratio in terms of biomass (Fernández-Palacios *et al*., 1990), in order to maximize spawn quality. This reproduction approach is widely extended in their breeding programs (Chavanne *et al.*, 2016) due to its cost-effectiveness. However, it may have negative effects in terms of maximum contribution of breeders when

compared to other techniques (Brown, 2003). On the other hand, egg stripping, followed by artificial fertilization, and paired matings have proven to be ineffective in producing enough eggs for meeting the needs of industrial production (Gorshkov *et al*., 1997).

Main traits on selection in gilthead sea bream are those related to growth performance and morphology, due to their impact on companies' costs and market prices (Chavanne *et al*., 2016). At the on-growing stage, a better growth performance reduces costs and risks at harvest whereas, at hatchery level, a high-quality morphology (lower deformity rates) enhances the commercial yield of fish fries (Afonso and Roo, 2007). However, deformity rates and growth traits are genetically correlated (García-Celdrán *et al.*, 2015; Lee-Montero *et al.*, 2015) and hence, they need to be precisely and accurately evaluated, with a high reproducibility without individual biases and in a cost-effective way (Gjedrem, 2000).

1.4.4. Market and yield of gilthead sea bream in Mediterranean Aquaculture

In 2018, aquaculture production exceeded fisheries production by 17,1 million tonnes. For the sixth consecutive year, aquaculture production led over extractive fishing. Gilthead sea bream is one representative of this pace change, since from total consumption of this species in the world, more than 96% was from aquaculture production (APROMAR, 2020).

World total production of gilthead sea bream in 2018 was 228.576 tn. 40% of that production (91.964 tn) came from European Union countries, and 14.930tn were produced in Spain, where gilthead sea bream was the third more produced fish species (APROMAR, 2020).

Currently, Turkey stands as the world's major producer of gilthead sea bream (**Figure 2**), after surpassing Greece (now 2nd major producer) few years ago. Several factors have contributed to the Turkish success, such as European economic crisis, Turkish government support and investment and the existing Turkish advantage in terms of production costs. This allowed Turkish producers to price their product well below their Greek counterpart, and to take positions in the markets consequently. As a result, and with the increasing offer from the European countries, market prices were affected downwards (FAO-GLOBEFISH, 2015).

In 2019, gilthead sea bream first sale average price in Spain was 4,11€/kg, 5,6% below 2018 value, and only 30% of the total sold product was produced domestically (in the country where it is consumed) (APROMAR, 2020).

Production of gilthead sea bream in the Mediterranean is conditioned mainly by fish feeds and genetics. For sustainability and cost reduction purposes, fish feeds formulae have been changed along the last years, by substituting fish meal and fish oil for ingredients coming from vegetable

sources. This may affect quality and Food Conversion Ratio (FCR) and lead to lower product quality (flesh quality) and longer production cycles.

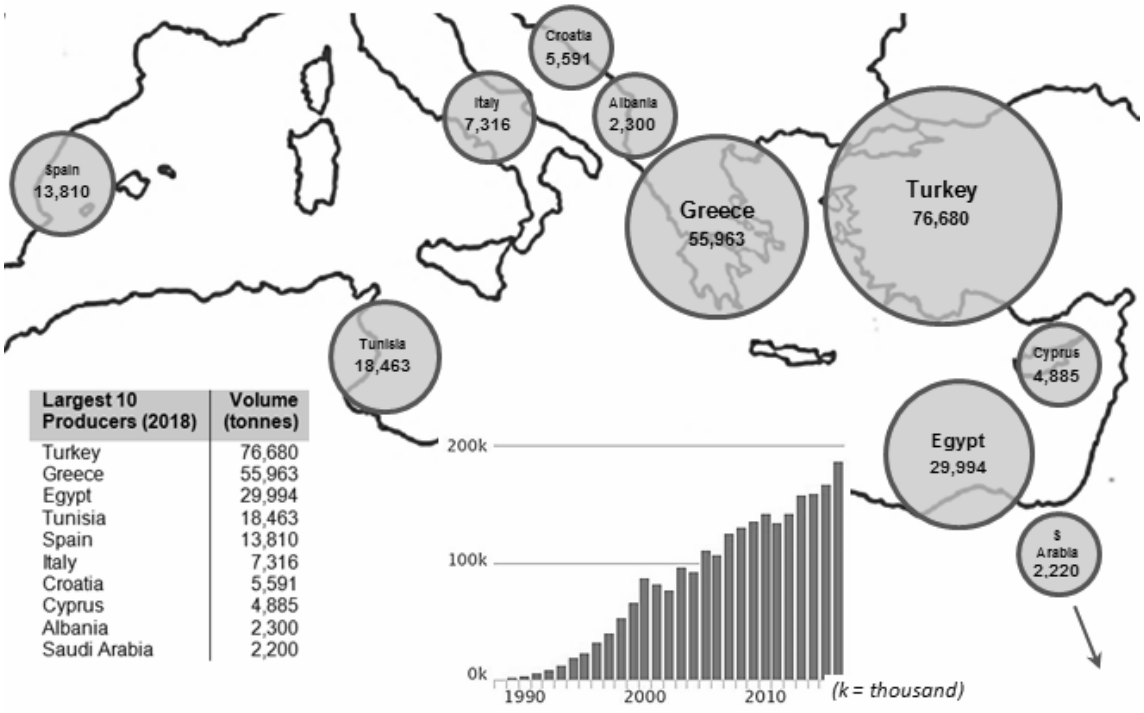

Figure 2. Aquaculture production in tonnes of gilthead sea bream in the Mediterranean during 2018. Graphic bar shows evolution of total gilthead sea bream production over the last years. (seafish.org).

1.4.5. Genetic improvement programs in gilthead seabream

Up to present day, it is estimated that approximately 60% of gilthead seabream eggs produced in Europe come from breeding programs, the most selected traits being growth performance and morphology (Janssen *et al.*, 2017).

Growth performance is a complex trait usually assessed through body weight at harvest (albeit it can also be assessed with other measures, such as length, or using growth indexes, such as Specific Growth Ratio). Body weight at harvest, which ranges between 400-600 gr in gilthead sea bream, has been estimated to have a heritability of 0.25-0.34 in other studies under industrial conditions (Knibb *et al.*, 1997; Navarro *et al.*, 2009a; Lee-Montero *et al.*, 2015; Elalfy *et al.*, 2021). Breeding programs selecting for this trait in gilthead sea bream have reported 5 - 29% genetic gain per generation, depending on the selection intensity (Brown, 2003; Janssen *et al.*, 2017).

Morphologic quality is usually assessed through presence or absence of deformities in the fish. This is a simplification because there are different types of deformities with different genetic origins or not affected by genetics at all, and determination of the presence/absence can be

affected by subjectivity, which, at times, may difficult its evaluation. García-Celdrán *et al.* (2015) estimated heritability for "deformity" trait in gilthead sea bream (under experimental conditions) ranging 0.03-0.9, depending on the type of deformity, and Lee-Montero *et al.* (2015) reported 0.16±0.04 for "whole deformity" trait. Whole deformity assesses presence or absence of malformations in the fish, regardless of their nature.

Other important traits, due to its direct influence in the rentability of the species production are carcass traits, such as dressing and filleting percentages, as well as gutted and fillet weight (at harvest). Usually, these traits are not used for selection directly, but the genetic correlation of processed weights (gutted and fillet) with "whole body weight at harvest" trait is very high, and the latter is easier to measure. Processing percentages, however, have been reported to present lower both heritability and genetic correlation with whole body weight at harvest (Navarro *et al.,* 2009a).

Chapter 2. Thesis objectives

This whole work aim was to study optimising options for gilthead sea bream genetic selection program: PROGENSA®, by using different approaches. Multiple aspects of breeding programs were considered and put under revision. Several experiments were carried out using third generation the breeding program.

The present study and analysis have been conducted within the framework of PROGENSA-III, a Spanish national project which aims to optimise gilthead seabream genetic selection programs from multiple scopes, including development and application of KETs.

PROGENSA® program originated with the project with the very same name (Afonso *et al*., 2012), being the first program in Spain dedicated to gilthead sea bream. The aim was to create a solid base for quality Spanish produced gilthead sea bream seed and to set the basis for techniques development and improvement, with the eyes set on the industry, which would be the ultimate beneficiary. Several years later, once program effectiveness in terms of production yield improvement was stated, other scopes were considered.

PROGENSA-III project, in which this work is framed, started in 2016. Some of the goals pursued with this project were:

- Genetic improvement of growth, morphology, product yield and disease resistance traits
- Study of genotype-environment interaction of these traits
- Development and validation of Key Enabling Technologies (KETs)
- Optimisation of industrial broodstock management

Since its birth, PROGENSA® program counted with the collaboration of companies from Spanish aquaculture sector. As an applied and integrative R&D project, industry partners provided their know-how as well as their facilities, which were essential for this project purpose. This thesis work was conducted in Canary Islands and in tight contact with Aquanaria S.L., a fish production company located in Gran Canaria island.

2.1. Specific objectives

This work was focused on 4 specific objectives:

- Evaluation of mass spawning management procedures in terms of optimising breeder contribution, applying 4DL strategy (gathering eggs from daily spawning during four consecutive days) in different moments of the spawning season.
  Increasing breeder contribution would produce more families and ultimately, improve genetic pool and variability of the offspring, providing better genetic selection results.

- Genetic evaluation of 3rd generation fish from breeding program based on industrial interest traits measured at harvest, from fish that had grown under different culturing systems.

  For genetic improvement to be exploited by the industry, it is important to assess genetic parameters from fish raised in different culturing systems.

  Trait categories that hold interest for the industry and that have been measured and evaluated in this work are:

  - Growth traits:
    - Body weight and growth rate

      Since economic market value of the fish is based on product weight, body weight at harvest (end of production cycle) is a very desired trait for producers. Growth rate improvement reduces production cycles, altogether causing increasement in profits.
    - Length at harvest and condition factor

      Length and its derived trait, condition factor, are also desired traits to track due to its important relationship with weight traits.
  - Carcass yield traits:
    - Carcass and fillet weight at harvest & dressing and filleting percentages

      Desired traits for fish processing industry. Most of processed products have higher value and yield traits are closely related to profit margin. Processed product weight traits and their relationship with whole fish weight are also commonly tracked.
    - Visceral fat weight

      Visceral fat may reduce processed product profit margin and may as well be used as an indicator of fish welfare and health.
  - Body composition traits:
    - Muscle protein and lipid content

      Protein and lipid are most relevant nutrients in fish. While protein content is relatively stable, lipid content is more variable, and its profile is also important. Even though these traits are highly affected by diet, it is important to know how they are genetically related with other selection traits and how to improve their assessment.
  - Deformity:
    - Absence of deformity is also a desirable trait due to its direct effect in the economic yield of the production.
- Evaluation of new traits derived from KETs as potential selection traits.

  Estimation of genetic parameters of technological traits, specifically NiT traits. Using computer image analysis software and other technologies to measure morphometric,

carcass and body composition traits, with the aim of taking one more step in integrating technologic and automatic tools into selection programs.

The main objective of this evaluation is to provide new genetic estimates for novel morphological traits in gilthead seabream, derived from this analysis, and their genetic relationship with other production traits. This novel approach should help companies to optimise time in selection operations without losing precision in the genetic parameters' estimation.

- Elaboration of a proposal for industrial genetic selection unit configuration.

Chapter 3. Materials and methods

3.1. Biologic material

Gilthead sea bream used in this work are part of F3 of PROGENSA® breeding program. When PROGENSA® program started, in 2009, wild gilthead sea breams were used to constitute F0. Resulting breeder populations were hosted in 3 different Spanish regions: Canary Islands, in ULPGC (University of Las Palmas de Gran Canaria); Catalonia, in IRTA (Institute of Agrifood Research and Technology) and Andalusia, in IFAPA (Andalusian Institute for Agricultural, Fishing, Food Research and Training, el Toruño center). From those breeder populations, descendants were shared between centres, in order to increase genetic pool variability. Selected fish from that F1 originated, eventually, F2, following the corresponding protocols with the work carried out in projects PROGENSA and INNOTECCS (INNOTECCS, 2014). Selected F2 animals were used as breeders for obtaining F3.

3.2. Broodstock management

Fish used in this study were obtained from two broodstock: Broodstock – Elite Andalucia (B_{AND}), located in Instituto IFAPA, Centro el Toruño Puerto de Santa María, Andalusia, Spain (IFAPA); Broodstock – Elite Canary Islands (B_{CAN}), located in IU-ECOAQUA, Parque Científico Tecnológico Marino, Puerto de Taliarte, Las Palmas, Spain (PCTM). Broodstock were established according to their Estimated Breeding Value (EBV) for harvest body weight trait and presence-absence of deformity. Also, consanguinity increase was minimized by selecting fish with high talent but far-related (low average predicted kinship coefficient between all possible pairs).

B_{AND} was constituted by 60 breeders (9 males and 51 females), whereas B_{CAN} was constituted by 30 breeders (5 males and 25 females). These asymmetric sex ratios were not deliberately set, populations were constituted when fish were 3 years old, but due to operational problems, spawning obtention had to be delayed one year and, by that time, some males had switched to females. B_{AND} was under controlled photoperiod (8L:16D), B_{CAN} was under natural photo/thermoperiod. Animals were fed ad libitum with Vitalis Cal (Skretting S.A., Cojóbar-Burgos, Spain), and egg production was monitored daily since spawning started (December 2015).

3.3. Spawning

Eggs were obtained by mass spawning. Along PROGENSA® program, different strategies for spawning management have been tested. Results showed that pooling eggs from consecutive days of spawning increased family number in offspring, since breeders had more opportunity to mate with different partners (Elalfy, 2016). When total egg production of both broodstock became high and stable, three egg batches per broodstock were established: one at the end of January 2016 (Batch-1; S_1), one at the beginning of February 2016 (Batch-2; S_2) and another at the beginning of March 2016 (Batch-3; S_3). In all cases, eggs from were collected and pooled during four consecutive days to maximize number of families, according to 4DL model (consisting in gathering eggs spawned during four consecutive days), as described by Elalfy (2016).

Egg incubation was carried out in cylinder conical tanks (1,000 L) at a density of 500-1000 eggs·L^{-1} for each batch. Water conditions were, for B_{AND}: temperature 19.0°C, salinity 34‰ and dissolved oxygen 6.4 mg·L^{-1}, and for B_{CAN}: temperature 22.1°C, salinity 37‰ and dissolved oxygen 5.61 mg·L^{-1}. No grading or scaling was performed.

3.4. Larval rearing

Larvae were reared under controlled conditions, as described in Roo *et al*. (2009). Larvae were weaned at 40 dph and then were fed commercial feed (Skretting S.A., Cojóbar-Burgos, Spain). Due to low survival of the populations, S_1 in IFAPA and S_2 in PCTM were discarded. At 110-130 dph (depending on the batch), 2,242 animals from IFAPA (from S_2) were transferred to PCTM facilities, and 786 animals from PCTM (from S_1) both facilities were transferred to IFAPA, in order to increase genetic variability of the on-growing groups by guaranteeing that offspring for both B_{AND} and B_{CAN} were present in the production populations.

3.5. Fingerling preparation

At fingerling stage (around 3gr average weight), fish were tagged with PIT (Passive Integrated Transponder) in abdominal cavity, as in protocol described by Navarro *et al.* (2006). At that point, a sample of caudal fin was taken from each fish and stored in absolute ethanol to eventually perform genotyping and parental assignment. Fish from S_1 and S_2 in PCTM and IFAPA were then mixed in both facilities, resulting in batch $S_{1/2}$.

Approximately two weeks later, when fish were recovered from tagging incision, fingerlings were transferred to different industrial farming sites with different culturing systems.

1,183 fish from $S_{1/2}$ and S_3 from IFAPA were sent to on-growing estuary farm site 1 (FS1), allocated near Guadalquivir river, in Doñana natural reserve. Later on, S_3 fish in FS1 were lost due to operational problems. All F3 fish transfers are shown in **Figure 3**.

4,400 fish from $S_{1/2}$ and S_3 from PCTM (Only $S_{1/2}$ containing fish from B_{AND} and B_{CAN}) were transferred to farm site 2 (FS2), where fish were kept in a cage, in Atlantic Ocean waters.

635 fish from S_3 (only from B_{AND}) were kept in inland tanks in IFAPA, simulating industrial conditions.

Three backup populations of 1,340, 1,580, and 5,300 were kept in tanks in IFAPA, PCTM and farm site 3 (FS3), respectively, in order to perform selection after fish had grown and had been genetically evaluated.

These populations had two purposes. Fish growing in companies and a sample of fish in IFAPA would be slaughtered, measured and sampled for multiple traits. The rest of the fish, kept in research facilities, would be genetically evaluated through their relatives' performance under industrial conditions and would be candidates to constitute next breeders' generation. Fish distribution according to their reproductive origin and spawning batch are shown in **Table 1**.

3.6. On-growing

On-growing period under industrial conditions lasted 13.3 months. No sorting processes were performed, and no fish were culled during that period. Each industrial group was treated by company workers as fish in company production stock. The description of the regional environmental characteristics and the rearing systems of the companies are described in **Table 2**.

3.7. Sampling and data gathering

3.7.1. Harvesting

Fish were slaughtered at age of 600-650 dph (depending on the batch). Fish were recovered from the culturing unit using company methodology, and slaughtered by chilling in seawater and ice mixture, following the same procedures as in Lee-Montero *et al.* (2015). Number of slaughtered fish in each group is shown in **Figure 3**.

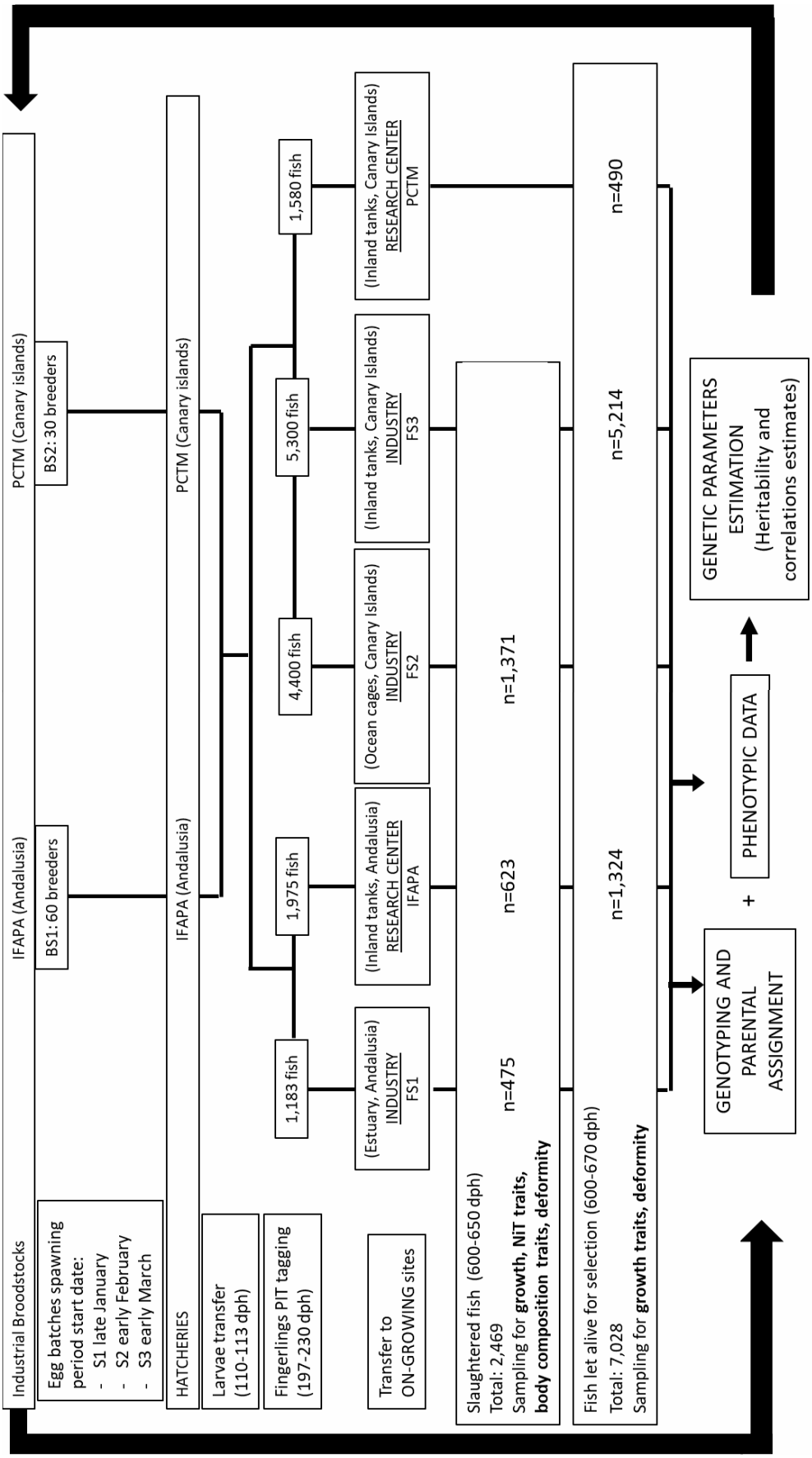

Figure 3. Diagram of the selection breeding program for gilthead seabream (PROGENSA-III). FS1 Farm site 1; IFAPA: Instituto de Investigación y Formación Agraria y Pesquera; FS2: Farm site 2; FS3: Farm site 3; PCTM: Parque Científico Tecnológico Marino (PCTM - ULPGC).

Table 1. Relationship between rearing facilities, spawning batches, original broodstock and offspring purpose.

Facility	Location	Spawning Batches	Contributing broodstocks	Offpsring purpose
Parque Científico Tecnológico Marino (PCTM)	Telde, Gran Canaria (Canary Islands)	$S_{1/2}$	B_{AND}, B_{CAN}	1
Farm site 1 (FS1)	Guadalquivir river, Seville (Andalusia)	$S_{1/2}$	B_{AND}, B_{CAN}	2
		S_3	-	2
Farm site 2 (FS2)	Telde, Gran Canaria (Canary Islands)	$S_{1/2}$	B_{AND}, B_{CAN}	1
		S_3	B_{CAN}	1
Farm site 3 (FS3)	San Bartolomé de Tirajana, Gran Canaria (Canary Islands)	$S_{1/2}$	B_{AND}, B_{CAN}	2
		S_3	B_{CAN}	2
Instituto de Formación Agraria y Pesquera de Andalucia (IFAPA)	Puerto de Santa María, Cádiz (Andalusia)	$S_{1/2}$	B_{AND}, B_{CAN}	1
		S_3	B_{AND}	2

1. Evaluation and breeding
2. Slaughtering and sampling

Table 2. Conditions of the facilities where fish were reared. Feeding systems and water conditions. Dissolved oxygen (DO) and salinity (S) are indicated.

Facility	Location	Rearing conditions	Water temperature (Annual mean)	Water conditions
Parque Científico Tecnológico Marino (PCTM)	Telde, Gran Canaria (Canary Islands)	Inland tanks, volume: 10 m^3 Density: 10 kg/m^3	21.8ºC	DO: 5.61 mg/l S: 37‰
Farm site 1 (FS1)	Guadalquivir river, Seville (Andalusia)	Estuary	18.2ºC	DO: 5.73 mg/l S: 6.9‰
Farm site 2 (FS2)	Telde, Gran Canaria (Canary Islands)	Oceanic cage, Volume:80m^3 Density: 15kg/m^3	21.8ºC	DO: 6.1 mg/l S: 36‰
Farm site 3 (FS3)	San Bartolomé de Tirajana, Gran Canaria (Canary Islands)	Inland tanks, volume: 15m^3 Density: 20kg/m^3	22.3ºC	DO: 6.6 mg/l S: 36‰
Instituto de Formación Agraria y Pesquera de Andalucia (IFAPA)	Puerto de Santa María, Cádiz (Andalusia)	Inland tanks, volume: 10m^3 Density: 20kg/m^3	19.0ºC	DO: 6.6 mg/l S: 36‰

3.7.2. Fish image acquisition

All slaughtered fish were photographed by using a digital camera (Olympus© FE-5035, Olympus, Shinjuku, Tokio, Japan), following the image capture protocol described by Navarro *et al.* (2016) for lateral images, in order to analyse NiT traits, including mNiT and cNiT traits, by using IMAFISH_ML.

3.7.3. Whole deformity assessment

Fish were visually evaluated to determine if they were affected by any deformity. Fish with deformation in column, operculum, mouth or fins, were classified as deform fish. Even different natures of deformity were identified, presence/absence of deformity was assessed considering only whether if fish showed a deformity or not, since at industrial level, any deform fish (regardless of its nature) is discarded.

3.7.4. Growth and carcass

Fish body weight, fork length and condition factor were measured according to Aqua-Excel-ATOL (AquaExcel Project, 2013) (ATOL: 0000351, ATOL: 0001658 and ATOL: 0001653, respectively). For an integrated and fast processing of the samples, fish were weighted and measured using Fishreader FR-200 workstation (Trovan Ltd.) which was also set for reading PIT tag simultaneously (**Figure 4**). All data obtained was transcribed in real time to a spreadsheet. Daily growth rate was estimated from body weight along on-growing period for each group.

Slaughtered fish were gutted (removal of all internal organs present in the abdominal cavity) and weighted again to obtain carcass weight (ATOL: 0001057). Visceral fat was also weighted (ATOL: 0000351). Lastly, fish were filleted, and both fillets were also weighted (without skin) and preserved in vacuum-sealed plastic bags (**Figure 5**). Bags were preserved in a freezer (-20ºC) until analysis.

3.7.5. Body composition

After measuring entire body weight, and before gutting fish, slaughtered fish from FS2 cages muscle fat was measured with Fish Fat Meter (FFM, Distell.com, West Lothian, Scotland), a device for assessing muscle fat content in a non-invasive way. Fat content measured through FFM is also considered as a NiT trait.

Defrosted entire fillets were mechanically homogenised through grinding (**Figure 5**). Flesh composition traits were measured through proximal composition analysis from fillet homogenisation sample using Near InfraRed Spectroscopy (NIRS) with FOODSCAN LAB (FOSS, Denmark). Muscle composition values for protein, fat, and moisture were determined.

3.7.6. Male Sexual maturity

Animals of the more genetically heterogeneous population (FS3-inland tanks, both $S_{1/2}$ and S_3), which was not slaughtered, were massaged in the abdominal area to determine if they were fluent. All fish were massaged since gender was unknown. The aim was to determine if age to reach sexual maturity was affected by family factors.

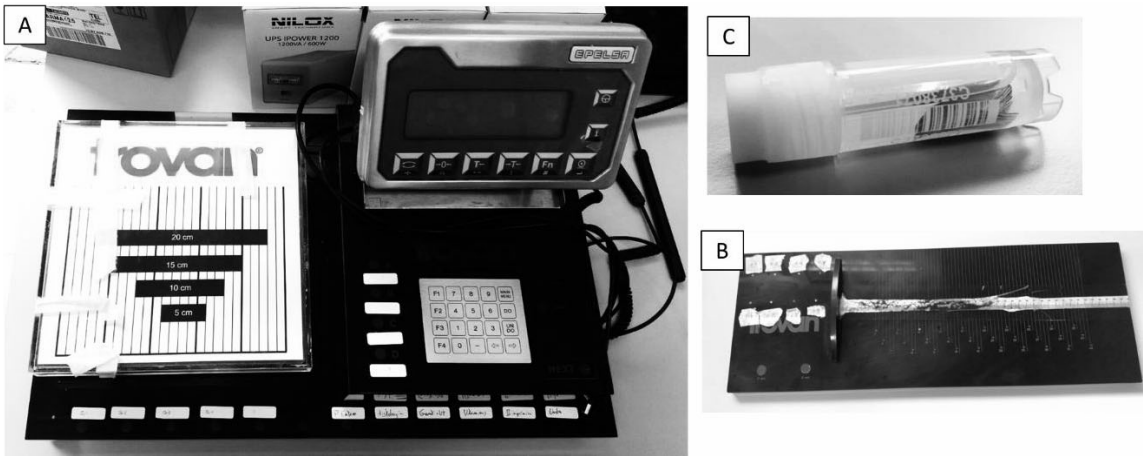

Figure 4. Sampling automated station and accessories. A. Fishreader FR-200 workstation (Trovan Ltd.) that includes scale and PIT reader and bar code reader; B. Ichthyometer with RFID PIT tags; C. 2ml tube with bar code for automatic inventory of fin samples.

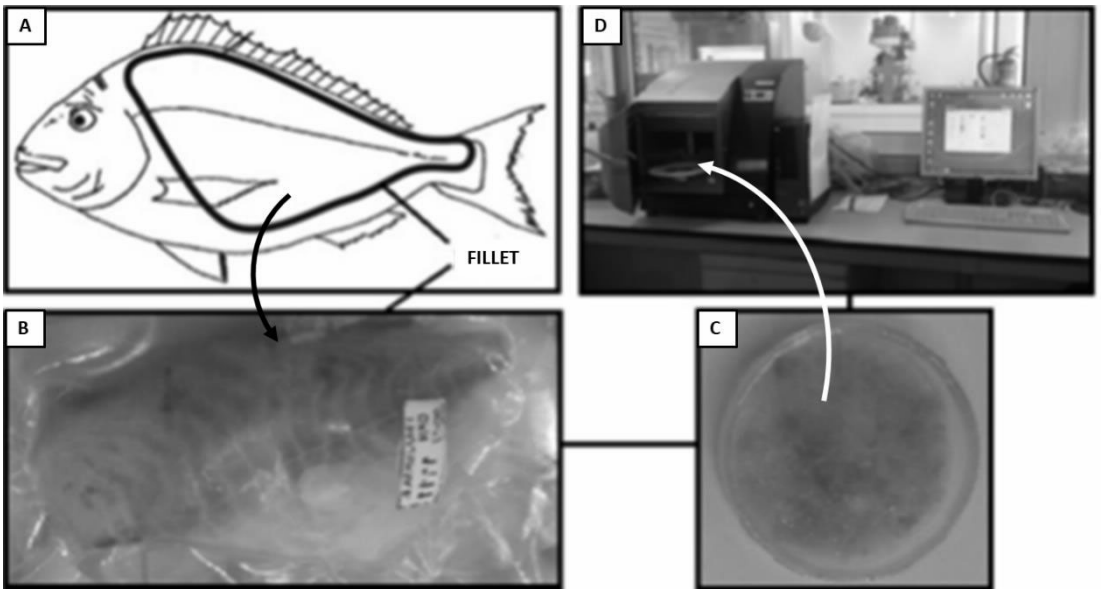

Figure 5. Fillet processing for body composition analysis. A. Gilthead sea bream fillet area; B. Fillets in vacuum-sealed plastic bags. C. Sample of flesh after homogenization by grinding; D. NIRS system setup.

3.8. Image analysis

3.8.1. IMAFISH_ML

All fish images were analysed using IMAFISH_ML, a computer vision algorithm programmed in MATLAB® v.7.5., described in Navarro *et al*. (2016). Once calibrated by setting an equivalence between pixels and real distance (by using a specific *script* for calibration), image analysis allows to identify successfully fish shape from image, and then, it automatically performs several measures (**Table 3**).

Table 3. IMAFISH_ML fish measurements from fish images taken from lateral side, based on detected points depicted in **Figure 6**. Eccentricity traits, FEc and HeEc, indicate how ovalshaped the whole fish (tail excluded) and the fish head are, respectively. To calculate equidistant fish heights (FHA; FHB; FHC; FHD; FHE), total lateral length (TLL) is divided into six equal parts, then, heights of each one of these five points are measured. All measures are fully described in Navarro *et al.* (2016). **mNiT**: morphometric Non-invasive Technological traits. **cNiT**: carcass Non-invasive Technological traits.

Trait category	Acronym	Trait/measure	Calculation method from image
Area (**cNiT**)	TLA (cm^2)	*Total Lateral Area*	Area delimited by countour line
	FilA (cm^2)	*Fillet Area (square cm)*	Area from y_1 and y_3 axis
	FilA% (%)	*Fillet Area (percentage)*	Percentage FilA/TLA
Length (**mNiT**)	FoL (cm)	*Fork Length*	From x_1 to x_5 within the longitudinal axis
	FilML (cm)	*Fillet Maximum Length*	From x_2 to x_3 within the longitudinal axis
	SL (cm)	*Standard Length*	From x_1 to x_4 within the longitudinal axis
	TaEL (cm)	*Tail Excluded Length*	From x_1 to x_3 within the longitudinal axis
	TLL (cm)	*Total Lateral Length*	From x_1 to x_6 within the longitudinal axis
Height (**mNiT**)	HeH (cm)	*Head Height*	Axis y_1
	FMH (cm)	*Fish Maximum Height*	Axis y_2
	CPH (cm)	*Caudal Pedunculus Height*	Axis y_3
	FHA (cm)	*Fish Equidistant Height A*	Axis A_{12}
	FHB (cm)	*Fish Equidistant Height B*	Axis B_{12}
	FHC (cm)	*Fish Equidistant Height C*	Axis C_{12}
	FHD (cm)	*Fish Equidistant Height D*	Axis D_{12}
	FHA (cm)	*Fish Equidistant Height E*	Axis E_{12}
Shape (**mNiT**)	FEc (%)	*Fish Eccentricity*	It indicates how ovalshaped the fish head is. It comprehends the area between x1 and x_3
	HeEc (%)	*Head Eccentricity*	It indicates how ovalshaped the fish head is. It comprehends the area between x_1 and x_2

3.8.2. Image edition

One key step of IMAFISH_ML *script*, before image analysis, is image conversion into grayscale. After this process, some parts not belonging to the fish shape (such as shadows or fins) may be accidentally recognized by the program as part of the fish shape, this entailing errors in the subsequent measures. To avoid this error, all images were modified by using an image edition software (Adobe Photoshop CS. [2004], Berkeley, CA, Peachpit Press), as shown in **Figure 7**. Traits obtained from both edited and non-edited images of each fish were analysed.

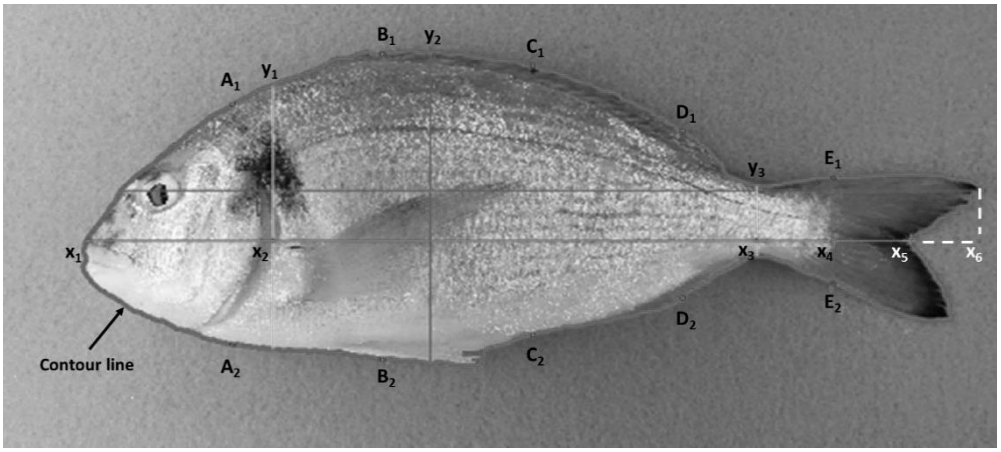

Figure 6. Automatically detected points in gilthead seabream lateral images using Matlab script for lateral images of IMAFISH_ML to determinate morphometric traits: points x_1, x_2, x_3, x_4, x_5, x_6 of the anteroposterior axis are used to determine longitudinal traits; y_1, y_2, y_3, y_4 and A_{12}, B_{12}, C_{12}, D_{12}, E_{12} are dorsoventral axis used to determine height traits; contour line determines the area occupied by the fish in pixels.

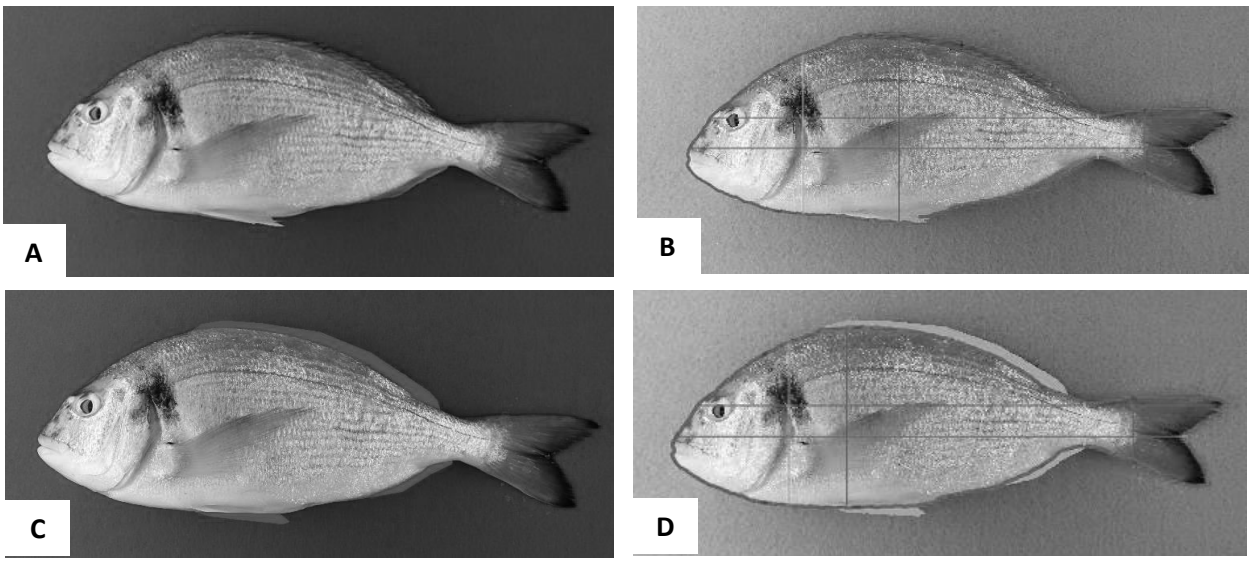

Figure 7. Image edition and software processing. A: Example of unedited image. B: IMAFISH_ML processing of unedited image original image A; C: Example of edited image A. D: IMAFISH_ML processing of edited image C.

3.9. Genetic analysis

DNA extraction of slaughtered and alive descendants (**Figure 3**) was performed from fin samples by using *BioSprint 96 DNA Blood Kit* (Qiagen), previously digested with proteinase K (Qiagen), following the protocol provided by the manufacturer. Genotyping was carried through Microsatellite Multiplex PCR Analysis (MMPA), by using a SuperMultiplex of 11 loci for *Sparus aurata* (SMsa1), followed by sequencing analysis, as described by Lee-Montero *et al.* (2013).

PCR fragments were separated by capillary electrophoresis on an ABI3130 Genetic Analyzer (Applied Biosystems). Electropherograms were analysed using GENEMAPPER (v.3.7) software (ThermoFisher scientific).

3.10. Parental assignment

Parental assignment was performed through exclusion method. This method consists in comparing genotypes from candidate parents and offspring and identifying incorrect parents by genotypes that violate the laws of Mendelian inheritance ("exclusion genotypes"). In this case, this was automatically carried out by using VITASSIGN program (Vandeputte *et al.*, 2006). Breeder gender was considered as unknown.

3.11. Genetic parameters estimation

All data were tested for normality and homogeneity of variance by using a General Linear Model analysis, by SPSS (v270) (IBM SPSS® Statistics). Variance components for all traits were estimated by Restricted Maximum Likelihood (REML) using the following mixed model:

$$y = X\beta + Zu + e$$

where y is the recorded data recorded on the studied traits, β the fixed effects (on-growing unit, on-growing facility, on-growing region, origin, age), u the random animal genetic effect, and e the residual error. Genetic correlation estimates for growth traits, measured in different environmental conditions (different culturing systems) were used to evaluate gene-environment interaction (G × E) (Falconer and Mackay, 1996). All models were resolved with the software package VCE (v 6.0) (Neumaier and Groeneveld, 1998; Groeneveld *et al.*, 2010). Two complementary programs to VCE were developed during this study, one named *VCE-Executer (v3.0)*, to help managing input data and automatise processes, and a second one, *VCE-analysis (v1.0)*, for processing output files.

The magnitude of estimated heritability was established, following the classification of Cardellino and Rovira (1987), as low (0.05–0.20), medium (0.20–0.45), high (0.45–0.65), and very high (>0.65). Correlations were classed as low (0–0.40), medium (0.45–0.55) and high (0.60–1), regardless of the sign, according to Navarro *et al.* (2009b).

Correlated response by indirect selection through secondary trait (Y), on the desired trait (X), was calculated according to Falconer and Mackay (1996) formula:

$$CR_X/R_X = i_Y h_Y r_A / i_X h_X$$

Where CR_X and R_X are the correlated and direct responses of the desired trait, respectively, i is the intensity of the selection, h is the square root of heritability and r_A is genetic correlation between both traits.

3.12. Proposal for industrial broodstock configuration

Candidates for broodstock configuration were determined after individual EBV and relatedness matrix had been estimated. It had to fulfil the following premises:

- Pool of more than 4,000 fish, 1 kg average body weight.
- 15m^3 tanks
- At least 3 tanks with RAS available

Broodstock unit objectives according to the company were:

- Several populations, in order to have spawning throughout the whole year
- Target density: 7 kg/m^3
- Low mean consanguinity value
- Highest average EBV

Members of the resulting breeder populations were selected among offspring using Optimal Contribution methodology, *i.e.* maximize average EBV for the selected trait and minimize relatedness relatedness between all possible matings within the future population, by using "SELECTION" software (supplied by Jesús Fernánez Martin). This program yields a tentative of candidates, considering origin population, desired number of populations and population size, individual EBV and consanguinity matrix.

Chapter 4. Results

4.1. Genotyping and parental assignment

Exclusion method provided an average parental assignment of 87% of the analysed descendants, which were assigned at least one known parent, accepting up to two mismatches. Parental assignment was different depending on the analysed population: 79% in IFAPA and FS1, 80% in PCTM, 92% in FS2 and 93% in FS3.

In cases when more than one parent pair was assigned and the pairs shown one common parent, descendants were included in the relationship matrix as 'just one known parental breeder'. After checking the genotypes, every error was identified as a null allele. The total number of individuals assigned was 5,449: B_{CAN}-S_1: 3,081; B_{CAN}-S_3: 633; B_{AND}-S_2: 1,376; B_{AND}-S_3: 360.

4.2. Evaluation of spawning management strategy

For each broodstock, 4DL method was applied three times during the spawning period: S_1, S_2, S_3. Due to survival problems, populations S_1 and S_2 were mixed in both research facilities, resulting in $S_{1/2}$ population. The strategy of taking different 4DL batches from the same breeder population altogether was considered as 2x4DL.

Total numbers of breeders, breeders contributing to offspring in each population and by sex, and the number of full-sibling (FS) and half-sibling (HS) families, are shown in **Table 4**. Statistical parameters are shown in **Table 5**.

The number of breeders that contributed to the two spawns in total, taking into account both facilities, was 82 (91.1% of total breeders in PROGENSA-III): 54 in B_{AND} (90% of total breeders in IFAPA) and 28 in B_{CAN} (93% of total breeders in PCTM). The total number of FS families was 217 (148 from B_{AND}, 69 from B_{CAN}) with a mean of 21.5 descendants per family, ranging between 1 and 193. HS families in B_{AND} offspring were 4 maternal and 4 paternal. HS families in B_{CAN} were 3 maternal and 3 paternal. Offspring distribution per family is shown in **Figure 8**.

Regarding B_{AND}, the number of breeders that contributed to S_2 and S_3 spawnings were 50 (83% of total) and 41 (68% of total), respectively. The number of FS families was 132 and 89, in S_2 and S_3, respectively, with a mean of 8.6 descendants per family. Among the 148 accounted FS families originated from this broodstock, 40.8% of them were only present in S_2 and not in S_3, whereas 10.9% were only present in S_3.

The number of breeders in B_{CAN} that contributed to S_1 and S_3 spawnings were 28 (93% of total) and 25 (83% of total, respectively. The number of FS families was 66 and 40, in S_1 and S_3, respectively, with a mean of 47.5 descendants per family. Among the 69 accounted FS families originated from this broodstock, 40.6% of them were only present in S_1 and not in S_3, whereas 4.3% were only present in S_3.

Number of families per breeder present in the broodstock (contributing or not) using 2x4DL model was greater than using 4DL. 4% and 11% higher in $S_{1/2}$ PCTM and IFAPA broodstock, respectively, and 78% and 67% in S_3 PCTM and IFAPA broodstock, respectively.

Table 4. Number of total breeders (tB) and contributing to the spawning breeders (cB) per broodstock, sex and spawning managing model. Number of Full-sib (FS) families are shown per broodstock and model, Half- sib (HS) families are shown per broodstock, sex and model.

Spawning managing model	Broodstock/ Population		Total Breeders (tB)	Contributing Breeders (cB)	Full-Sib Families (FS)	Half-Sib Families (HS)
2x4DL (Total)	PCTM (B_{CAN})	m♂	5	3	-	3
		f♀	25	25	-	3
		Sub-total	30	28	69	6
	IFAPA (B_{AND})	m♂	9	5	-	4
		f♀	51	49	-	4
		Sub-total	60	54	148	8
4DL ($S_{1/2}$)	PCTM (B_{CAN})	m♂	5	3	-	3
		f♀	25	25	-	3
		Sub-total	30	28	66	6
	IFAPA (B_{AND})	m♂	9	5	-	4
		f♀	51	45	-	4
		Sub-total	60	50	132	8
4DL (S_3)	PCTM (B_{CAN})	m♂	5	2	-	2
		f♀	25	23	-	0
		Sub-total	30	25	40	2
	IFAPA (B_{AND})	m♂	9	4	-	4
		f♀	51	37	-	0
		Sub-total	60	41	89	4

Table 5. Parameters calculated by broodstock and spawning model.

	4DL ($S_{1/2}$)		4DL (S_3)		2x4DL (Total)	
	PCTM (B_{CAN})	IFAPA (B_{AND})	PCTM (B_{CAN})	IFAPA (B_{AND})	PCTM (B_{CAN})	IFAPA (B_{AND})
% Breeders contributing to offspring	93%	83%	83%	68%	93%	90%
Nº of families per breeder	2.4	2.3	1.4	1.55	2.5	2.6
% of unique families	40.6%	40.8%	4.3%	10.9%	-	-

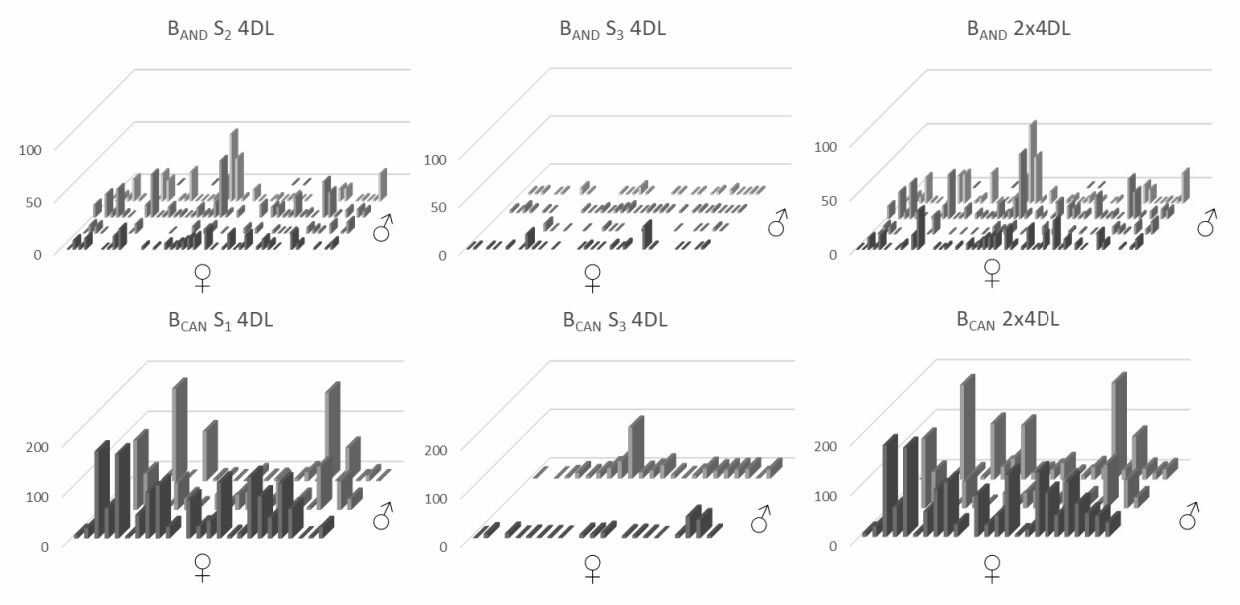

Figure 8. Family distribution of offspring analysed in PROGENSA-III. Left Y axis represents number of offspring per family.

4.3. Phenotypic data

4.3.1. Growth, carcass and body composition traits

Phenotypic results for growth traits (body weight, growth rate, fork length and condition factor), as well as flesh composition traits (protein, lipid and moisture % in muscle), carcass traits (VF: visceral fat weight, CW: carcass weight, FW: fillet weight Dr%: dressing percentage and Fi%: filleting percentage) and deformity in each facility, are shown in **Table 6**. Note that body composition and carcass traits values are only shown in the groups of fish that were slaughtered.

Regarding growth traits (excluding condition factor), fish reared in FS2 and FS3 (Atlantic oceanic cage and inland tanks) showed the highest values, whereas S_3 fish reared in IFAPA (Inland tanks), at the mainland, showed the lowest values. However, taking into account spawning and slaughtering dates, growth rate was lower in fish reared in FS1 and PCTM.

Concerning condition factor, fish reared in IFAPA showed the highest values and fish reared in PCTM the lowest ones.

Table 6. Phenotypic mean values of analysed growth, body composition and carcass traits and standard errors. Coefficient of variation is shown between parentheses. BW: body weight at harvest, FL: fork length, CF: condition factor. Significant differences between mean values for growth rate, processing percentages and body composition traits are represented with different letters as superscript (P<0.05).

Growth Traits	**FS1 (Estuary)**	**FS2 (Oceanic cage)**	**IFAPA (Inland tanks) - S_3**	**FS3 (Inland tanks)**	**PCTM (Inland tanks)**	**IFAPA (Inland tanks) - S_1**
BW (g)	491.32 ± 5.94 (0.20)	520.39 ± 3.57 (0.19)	357.8 ± 4.81 (0.26)	518.75 ± 3.24 (0.31)	478.92 ± 4.89 (0.17)	470.36 ± 3.26 (0.21)
growth rate (g/day)	0.75^{b} ± 0.01 (0.21)	0.82^{a} ± 0.005 (0.19)	0.59^{c} ± 0.01 (0.26)	0.81^{a} ± 0.00 (0.28)	0.54^{c} ± 0.01 (0.23)	0.74^{b} ± 0.01 (0.21)
FL (cm)	25.63 ± 0.1 (0.07)	28.25 ± 0.06 (0.06)	22.61 ± 0.11 (0.10)	28.35 ± 0.07 (0.12)	27.76 ± 0.10 (0.06)	25.30 ± 0.06 (0.7)
CF	2.88 ± 0.01 (0.07)	2.28 ± 0 (0.09)	3.03 ± 0.01 (0.08)	2.22 ± 0.01 (0.13)	2.18 ± 0.01 (0.07)	2.87 ± 0.01 (0.08)
deformity% (%)	1.87± 0.01 (0.13)	6.02 ± 0.01 (0.23)	13.06± 0.02 (0.30)	20.64 ± 0.01 (0.34)	6.50 ± 0.01 (0.23)	4.28± 0.01 (0.19)
Carcass						
carcass weight (g)	445.85 ± 5.42 (0.20)	474.36 ± 3.34 (0.19)	330.79 ± 4.40 (0.25)			
visceral fat (g)	14.4 ± 0.46 (0.52)	10.49 ± 0.17 (0.44)	7.81 ± 0.23 (0.55)			
fillet weight (g)	207.39 ± 2.81 (0.22)	182.74 ± 1.56 (0.23)	104.93 ± 1.73 (0.31)			
dressing% (%)	91 ± 0.00 (0.02)	91 ± 0.00 (0.03)	92 ± 0.00 (0.01)			
filleting% (%)	42^{b} ± 0.00 (0.06)	35^{a} ± 0.00 (0.1)	29^{c} ± 0.00 (0.12)			
Body Composition						
lipids (%)	5.08^{b} ± 0.11 (0.34)	6.57^{a} ± 0.07 (0.29)	7.73^{c} ± 0.12 (0.29)			
moisture (%)	71.06^{b} ± 0.09 (0.02)	71.94^{a} ± 0.07 (0.03)	68.64^{c} ± 0.10 (0.03)			
protein (%)	21.77^{b} ± 0.05 (0.03)	21.30^{a} ± 0.03 (0.4)	20.63^{c} ± 0.05 (0.05)			
FFM (%)	-	12.49^{a} ± 0.09 (0.20)	-			

Whole deformity rate was very different between groups, being the highest in FS3 inland tanks (20.64%), and lowest in FS1 (1.87%).

Fish cultured at IFAPA showed lowest carcass and fillet weight mean values. FS1 and FS2 groups showed highest mean values for fillet and carcass weight, respectively. FS1 group showed highest mean values concerning visceral fat, followed by FS2 and IFAPA. However, FS1 group reported lowest mean values for muscular lipid content, followed by IFAPA and FS2. Regarding protein content, FS1 group showed highest mean values, while IFAPA showed the lowest. Concerning moisture content, FS2 group showed the highest mean values and

IFAPA the lowest. All body composition traits mean values, showed significant differences between groups.

Regarding lipid % measured indirectly by non-invasive technology, such as FFM, only in fish from FS2, content was almost two time higher than measured with NIRS (invasive method).

Dressing% mean values were similar for every group, whereas filleting% mean values showed significant differences between three groups, FS1 group reporting the highest and IFAPA group the lowest.

4.3.2. Morphometric (m) and carcass (c) Non-invasive Technological traits (NiT traits)

The phenotypic results of mNiT and cNiT traits, as well as growth traits, for fish reared in FS1, FS2 and IFAPA at harvest size, are shown in **Table 7**.

NiT traits characterization by IMAFISH_ML required, for one lateral image, an average processing time of 1.34 seconds per fish photography. Editing one image took 1.5 min on average.

In concordance with growth traits, the mNiT and cNiT traits of fish were significantly different between on-growing facilities (FS1, FS2, IFAPA), showing FS2 the highest values in the majority of them, while fish reared in IFAPA showed the lowest ones. This superiority was maximum in cNiT traits (139.33 cm^2 and 96.19 cm^2 for FilA in FS2 and IFAPA, respectively), and minimum in shape mNiT traits (0.89 % and 0.88 % for FEc in FS2 and IFAPA, respectively). Mean values of height mNiT traits in FS1 group were significantly higher (8.56 cm and 7.14 cm for FHA in FS1 and IFAPA, respectively). In shape mNiT traits, fish from IFAPA reported higher mean value in HeEc than FS1 and FS2.

The comparison of the same NiT traits, from unedited and edited images, reported significant differences in all cNiT traits, all height mNiT traits except HeH and FHA, and only in FEc shape mNiT trait. There were not significant differences in all length mNiT traits, except in TaEL at the IFAPA facility.

In general, NiT traits (mNiT and cNiT) reported lower coefficient of variation than growth traits (body weight and daily growth rate), in all studied on-growing facilities. Fork length trait, manually measured and mNiT, showed the same coefficient of variation (7.56%), also in unedited and edited measures.

Table 7. Phenotypic mean values of analysed growth and NiT traits and standard errors. Coefficient of variation is shown between parentheses. BW: body weight, FL: fork length, CF: condition factor. Significant differences between means of the same trait measured, from edited and unedited images, are represented with different capital letters as superscript (P<0.05).

Growth Traits	**FS1 (Estuary)**		**FS2 (Oceanic cage)**		**IFAPA (Inland tanks)**	
BW (g)	491.32 ± 5.94 (0.20)		520.39 ± 3.57 (0.19)		357.8 ± 4.81 (0.26)	
Growth rate (g/day)	0.75 ± 0.01 (0.21)		0.82 ± 0.005 (0.19)		0.59 ± 0.01 (0.26)	
FL (cm)	25.63 ± 0.1 (0.07)		28.25 ± 0.06 (0.06)		22.61 ± 0.11 (0.10)	
CF	2.88 ± 0.01 (0.07)		2.28 ± 0 (0.09)		3.03 ± 0.01 (0.08)	
NiT Traits	**Unedited**	**Edited**	**Unedited**	**Edited**	**Unedited**	**Edited**
carcass						
Total Lateral Area (cm^2)	208.89^{A}±1.67 (0.13)	199.02^{B}±1.6 (0.13)	211.68^{A}±1.1 (0.14)	203.88^{B}±1.1 (0.14)	156.25^{A}±1.48 (0.18)	147.99^{B}±1.37 (0.18)
Fillet Area (cm^2)	143.97^{A}±1.22 (0.14)	134.47^{B}±1.15 (0.14)	146.21^{A}±0.81 (0.15)	139.33^{B}±0.77 (0.15)	104.67^{A}±1.09 (0.20)	96.19^{B}±0.98 (0.20)
Fillet Area %	0.68^{A}±0 (0.03)	0.67^{B}±0 (0.03)	0.68^{A}±0 (0.04)	0.68^{B}±0 (0.04)	0.66^{A}±0 (0.04)	0.64^{B}±0 (0.04)
morphometric						
Fillet Maximum Length (cm)	16.4±0.07 (0.07)	16.38±0.07 (0.07)	17.17± 0.05 (0.08)	17.15±0.05 (0.08)	13.86±0.08 (0.12)	13.56±0.08 (0.12)
Fork Length (cm)	27.87±0.11 (0.06)	27.86±0.11 (0.06)	30.31±0.07 (0.07)	30.19±0.07 (0.07)	24.06±0.11 (0.09)	24.03±0.11 (0.09)
Standard Length (cm)	25.41±0.10 (0.07)	25.4±0.10 (0.07)	26.33±0.06 (0.07)	26.28±0.06 (0.07)	22.03±0.11 (0.10)	21.97±0.11 (0.10)
Tail Esxcluded Length (cm)	22.78±0.09 (0.07)	22.75±0.09 (0.07)	23.64±0.06 (0.07)	23.64±0.06 (0.07)	19.87^{A}±0.1 (0.10)	19.52^{B}±0.1 (0.10)
Total Lateral Length (cm)	30.11±0.11 (0.06)	30.1±0.11 (0.06)	30.5±0.07 (0.07)	30.43±0.07 (0.07)	25.73±0.12 (0.09)	25.7±0.12 (0.09)
Fish Maximum Heigth (cm)	11.27^{A}±0.05 (0.09)	10.74^{B}±0.05 (0.08)	10.63^{A}±0.03 (0.08)	10.41^{B}±0.03 (0.09)	9.41^{A}±0.05 (0.11)	9.00^{B}±0.04 (0.10)
Head Height (cm)	9.56±0.04 (0.07)	9.56±0.04 (0.07)	9.38±0.02 (0.08)	9.36±0.02 (0.09)	8.18±0.03 (0.09)	8.16±0.03 (0.09)
Caudal Perdunculus Height (cm)	2.54^{A}±0.01 (0.10)	2.4^{B}±0.01 (0.09)	2.79^{A}±0 (0.09)	2.59^{B}±0.01 (0.11)	2.48^{A}±0.01 (0.14)	2.29^{B}±0.01 (0.11)
Fish Equidistant Height A (cm)	8.56±0.03 (0.07)	8.56±0.03 (0.07)	8.47±0.02 (0.08)	8.45±0.02 (0.08)	7.14±0.03 (0.10)	7.14±0.03 (0.10)
Fish Equidistant Height B (cm)	11.09^{A}±0.05 (0.08)	10.6^{B}±0.05 (0.08)	10.53^{A}±0.03 (0.09)	10.3^{B}±0.03 (0.08)	9.18^{A}±0.05 (0.11)	8.93^{B}±0.04 (0.10)
Fish Equidistant Height C (cm)	9.53^{A}±0.05 (0.09)	9.09^{B}±0.04 (0.09)	9.4^{A}±0.02 (0.09)	9.18^{B}±0.02 (0.09)	8.31^{A}±0.04 (0.10)	7.92^{B}±0.04 (0.10)
Fish Equidistant Height D (cm)	5.95^{A}±0.04 (0.13)	4.66^{B}±0.04 (0.15)	6.46^{A}±0.02 (0.11)	5.44^{B}±0.02 (0.12)	5.89^{A}±0.03 (0.12)	4.58^{B}±0.03 (0.13)
Fish Equidistant Height E (cm)	3.6^{A}±0.02 (0.09)	3.56^{B}±0.02 (0.09)	3.53^{A}±0.01 (0.08)	3.4^{B}±0.01 (0.09)	3.14^{A}±0.01 (0.09)	3.1^{B}±0.01 (0.09)
Head Eccentricity	0.66±0 (0.06)	0.66±0 (0.06)	0.69±0 (0.06)	0.69±0 (0.07)	0.73±0 (0.04)	0.73±0 (0.05)
Fish Eccentricity	0.87^{A}±0 (0.01)	0.87^{B}±0 (0.01)	0.89^{A}±0 (0.01)	0.89^{B}±0 (0.01)	0.88±0 (0.01)	0.88±0 (0.01)

4.4. Genotype-environment interaction of growth traits

Genetic correlations between production systems were studied for growth traits (**Table 8**). Estimations were high (0.83-1.00) concerning IFAPA with FS1 and between IFAPA and FS2 for all traits. Correlation estimates between FS1 and FS2 concerning CF was medium (0.42), and for body weight and fork length correlation was high (0.61).

Table 8. Genetic correlation (mean ± standard error) between growth traits (BW: body weight at harvest, FL: fork length at harvest, CF: condition factor) in different on-growing systems (estuarine ponds, oceanic cage, inland tanks) and facility.

	BW (g)		FL (cm)		CF	
	FS1 (Estuarine ponds)	IFAPA (Tanks)	FS1 (Estuarine ponds)	IFAPA (Tanks)	FS1 (Estuarine ponds)	IFAPA (Tanks)
FS2 (Oceanic cage)	0.61 ± 0.17	0.86 ± 0.08	0.61 ± 0.28	0.89 ± 0,08	0.42 ± 0,62	0.99 ± 0,02
FS1 (Estuarine ponds)		0.83 ± 0.16		0.91 ± 0,10		1.00 ± 0,01

4.5. Heritability and correlation estimations

4.5.1. Edited and unedited images

Heritability estimates of NiT traits calculated using IMAFISH_ML from unedited and edited images, and genetic correlation between them, are shown in **Table 9**. Heritability values from unedited images were slightly higher than edited ones, in all cases except for FHD (0.43 and 0.45, respectively) and TaEL(0.51 and 0.52, respectively). In any case, the estimates of heritability from different formats (unedited and edited) were very similar, and their genetic correlations were high and positive (> 0.89), in all traits.

4.5.2. Morphometric (m) and carcass (c) Non-invasive Technological traits (NiT traits)

Heritability estimates, as well as genetic and phenotypic correlations, for growth traits and analysed NiT traits (of unedited images): area traits (FilA%, FilA, TLA), length traits (FoL, FiML, SL, TaEL, TLL), height traits (FMH, HeH, CPH, FHA, FHB, FHC, FHD, FHD), and shape traits (HeEc, FEc) are shown in **Table 10**. NiT traits showed essentially high heritability estimates, showing shape mNiT traits the minimum and maximum values (0.24 for HeEc and 0.62 for FEc, respectively). Concerning area cNiT traits, heritability estimates were medium – high (0.25 for FilA% and 0.51 for TLA and FilA). Regarding length mNiT traits, heritability estimates were high, with minimum value for SL (0.46) and maximum value for FilML (0.52). For height mNiT traits, heritability estimates were mainly high, except for CPH and FHE, that presented medium values (0.38 and 0.34, respectively), being FHB the highest (0.59).

Table 9. Heritability estimates and standard error of every carcass and morphometric Non-Invasive Technological trait (cNiT and mNiT traits, respectively) calculated by IMAFISH_ML from edited and unedited images.

Trait Category	NiT trait	h^2 Unedited	h^2 Edited
Area (cNiT)	*Total Lateral Area*	0.51±0.10	0.50±0.10
	Fillet Area (cm^2)	0.51±0.10	0.50±0.10
	Fillet Area %	0.25±0.08	0.20±0.07
Length (mNiT)	*Fork Length*	0.47±0.11	0.46±0.11
	Fillet Maximum Length	0.52±0.12	0.49±0.11
	Standard Length	0.46±0.11	0.46±0.11
	Tail Esxcluded Length	0.51±0.11	0.52±0.11
	Total Lateral Length	0.46±0.11	0.45±0.11
Height (mNiT)	*Fish Maximum Heigth*	0.58±0.10	0.56±0.10
	Head Height	0.48±0.09	0.46±0.09
	Caudal Perdunculus Height	0.38±0.10	0.35±0.08
	Fish Equidistant Height A	0.54±0.10	0.53±0.10
	Fish Equidistant Height B	0.59±0.10	0.56±0.10
	Fish Equidistant Height C	0.58±0.09	0.55±0.09
	Fish Equidistant Height	0.43±0.08	0.45±0.09
	Fish Equidistant Height E	0.34±0.07	0.30±0.07
Shape (mNiT)	*Head Eccentricity*	0.24±0.06	0.19±0.05
	Fish Eccentricity	0.62±0.12	0.53±0.11

Genetic correlations between NiT traits of IMAFISH_ML were mostly high and positive (0.75-1), with some exceptions. Concerning height mNiT traits, CPH showed genetic correlations between 0.5 and 0.74 with area and length mNiT traits, and between 0.69 and 0.93 with the rest of height mNiT traits. FHE reported correlations ranging from 0.58, with FilA%, to 0.91, with CPH. Shape mNiT traits showed negative genetic correlations with the rest of NiT traits, HeEc being the most negative (-0.84 to -0.75). Genetic correlations of NiT traits with body weight were mostly strong and positive (0.80-1), except for FilA% (0.66) and shape traits (-0.63 and -0.39, for HeEc and FEc, respectively). Similarly, NiT traits genetic correlations with fork length (manual measurement) were mostly strong and positive (0.80-1) as well, except for FilA% (0.66), CPH (0.56), FHE (0.66) and shape traits (-0.45 and -0.25 for HeEc and FEc, respectively). Condition factor reported positive correlations with NiT traits ranged from 0.38 with TaEL to 0.85 with CPH. Shape mNiT traits showed medium and positive genetic correlation between them (0.52), and negative with the rest of NiT traits, ranging from -0.65 (with FoL) to -0.84 (with CPH and FHD) for HeEc, and from -0.06 (with FoL) to -0.72 (with CPH) for FEc.

4.5.3. Growth, carcass and body composition traits

Growth traits (body weight at harvest, fork length [manual measurement] and condition factor) showed medium additive genetic variation (0.25 for fork length and condition factor, and 0.37 for weight). Genetic correlation between weight and length was high (0.94), while correlation of both with CF was lower (0.55 and 0.25, respectively).

Heritability estimates, as well as genetic and phenotypic correlations, for carcass traits and body composition traits are shown in **Table 11**, together with growth traits estimates. In general, heritability values for processed weight traits (carcass and fillet weight, as well as visceral fat), were higher than percentage traits, carcass weight being the highest (0.54), and dressing % the lowest (0.08). Body composition heritability values were low, ranging from 0.13 ± 0.07 (moisture%) to 0.15 ± 0.07 (lipid%), excluding for lipid content measured with FFM, which was 0.44.

Genetic correlation values between carcass traits and growth traits were high and positive for BW and FL and processed weights (CW, FW and VF). Those weights showed medium positive correlations with CF. Dr% showed negative medium correlation with BW and Fl, and negative high with CF, whereas Fi% showed high positive correlation with BW and FL and medium positive correlation with CF.

Correlation between CW and FW could not be estimated, however both traits showed high and positive correlation with VF. Dr% showed negative medium correlation with both FW and CW and very high and negative correlation with VF. Fi% genetic correlations were positive with the rest of carcass traits and ranged from high (0.77) with CW and FW to low (0.14) with Dr%.

Body composition traits genetic correlations with growth traits were low and negative for protein and moisture and low-medium positive for lipids. Genetic correlation with carcass traits were low for muscle protein %, ranging from -0.02 with CW to 0.30 with VF. Muscle lipid % genetic correlations were positive and medium with CW and FW, positive and low with Fi% and VF, and negative and medium with Dr%.

Genetic correlations of lipid content with protein and moisture content were medium-high negative. Correlation between muscle protein content and moisture content was positive and medium.

Genetic and phenotypic correlations between carcass and body composition traits versus NiT traits is shown in **Table 12.1** and **Table 12.2**, respectively. Genetic correlations between processed weights and NiT traits were all high and positive, except for eccentricity traits. Correlations were negative high and low with HeEc and Fec, respectively. Concerning process yields correlations, Dr% showed medium negative correlation with all NiT traits except

eccentricity traits, which showed medium positive correlations. Fi% correlations with NiT traits were medium-high and positive for all traits except eccentricity traits, which were high and, with HeEc, and low and positive, with FEc.

As for genetic correlations of NiT traits with body composition traits, protein content correlations were low with all traits, correlations with moisture were low-medium and negative, and correlations with lipids were medium-high and positive. Eccentricity traits correlation with body composition traits differed again from the pattern of the rest of the traits. In this case, HeEc and FEc correlations with body composition traits were considered low and only FEc/protein % was considered medium and negative.

4.5.4. Whole deformity

Heritability estimate for whole deformity trait and correlations (genetic and phenotypic) with growth, carcass, body composition traits, as well as male sexual maturity are shown in **Table 11**. Genetic and phenotypic correlations with IMAFISH NiT traits are shown in **Table 12.1** and **Table 12.2**, respectively.

Heritability estimate for whole deformity trait was low (0.02). Genetic correlations with the rest of the traits were mostly low, except for those with CF and CPH that were medium and positive (0.49, 0.53, respectively) and medium and negative with Fi% and FEc (-0.54, -0.52, respectively).

4.5.5. Male sexual maturation age

Heritability estimate for Sexual Maturity Age in Males (SMAM) trait and correlations (genetic and phenotypic) with growth, carcass, body composition traits, as well as whole deformity are shown in **Table 11**. Genetic and phenotypic correlations with IMAFISH NiT traits are shown in **Table 12.1** and **Table 12.2**, respectively.

Heritability estimate for SMAM was medium (0.25). Genetic correlations with the rest of the traits were low and mostly positive, except with whole deformity, moisture %, dressing % and CF, which were negative.

Table 10. Heritability estimates (in bold at the diagonal, with ± standard error) for growth traits: body weight, fork length, condition factor and carcass and morphometric Non-invasive Technological (NiT) traits and their genetic correlations (above the diagonal, with ± standard error) and phenotypic correlations (below the diagonal) estimated from unedited images of gilthead seabream in PROGENSA-III. BW: Body Weight; FL: Fork Length; CF: Condition Factor; TLA: Total Lateral Area; FilA: Fillet Area (square cm); FilA%: Fillet Area (Percentage); FoL: Fork Length; FilML: Fillet Maximum Length; SL: Standard Length; TaEL: Tail Excluded Length; TLL: Total Lateral Length; HeH: Head Height; FMH: Fish Maximum Height; CPH: Caudal Pedunculus Height; FHA: Fish Equidistant Height A; FHB: Fish Equidistant Height B; FHC: Fish Equidistant Height C; FHD: Fish Equidistant Height D; FHE: Fish Equidistant Height E; HeEc: Head Eccentricity; FEc: Fish Eccentricity.

Measure method	**Regular traits**			**NiT Traits**																	
Trait Category	Growth			Carcass (cNiT)			Morphometric (mNiT)														
Trait	BW	FL	CF	TLA	FilA	FilA%	FoL	FilML	SL	TaEL	TLL	FMH	HeH	CPH	FHA	FHB	FHC	FHD	FHE	HeEc	FEc
BW	**0.37 ± 0.05**	0.94 ± 0.02	0.55 ± 0.11	1.00 ± 0.00	1.00 ± 0.00	0.66 ± 0.11	0.98 ± 0.01	0.96 ± 0.02	0.99 ± 0.01	0.97 ± 0.02	0.98 ± 0.02	0.97 ± 0.02	0.97 ± 0.01	0.84 ± 0.08	0.99 ± 0.01	0.97 ± 0.01	0.98 ± 0.01	0.98 ± 0.01	0.80 ± 0.07	-0.63 ± 0.11	-0.39 ± 0.16
FL	0.90	**0.25 ± 0.04**	0.25 ± 0.16	0.97 ± 0.01	0.96 ± 0.02	0.66 ± 0.13	1.00 ± 0.00	0.98 ± 0.01	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.90 ± 0.05	0.95 ± 0.02	0.56 ± 0.14	0.96 ± 0.02	0.91 ± 0.04	0.94 ± 0.03	0.95 ± 0.03	0.66 ± 0.07	-0.45 ± 0.15	-0.25 ± 0.19
CF	0.11	-0.28	**0.25 ± 0.05**	0.50 ± 0.20	0.55 ± 0.18	0.47 ± 0.20	0.38 ± 0.20	0.47 ± 0.21	0.41 ± 0.21	0.38 ± 0.22	0.38 ± 0.21	0.54 ± 0.18	0.49 ± 0.18	0.85 ± 0.10	0.46 ± 0.20	0.53 ± 0.19	0.50 ± 0.18	0.58 ± 0.19	0.72 ± 0.15	-0.74 ± 0.15	-0.58 ± 0.20
TLA	0.94	0.92	0.12	**0.51 ± 0.10**	1.00 ± 0.00	0.80 ± 0.09	0.98 ± 0.01	0.96 ± 0.01	0.97 ± 0.01	0.97 ± 0.01	0.97 ± 0.01	0.96 ± 0.02	0.98 ± 0.01	0.74 ± 0.13	1.00 ± 0.00	0.98 ± 0.01	0.98 ± 0.01	0.98 ± 0.01	0.77 ± 0.08	-0.71 ± 0.13	-0.26 ± 0.20
FilA	0.93	0.92	0.13	0.98	**0.51 ± 0.10**	0.86 ± 0.06	0.97 ± 0.01	0.97 ± 0.01	0.97 ± 0.01	0.97 ± 0.01	0.97 ± 0.01	0.96 ± 0.02	0.97 ± 0.01	0.71 ± 0.15	0.99 ± 0.00	0.98 ± 0.01	0.99 ± 0.01	0.98 ± 0.01	0.78 ± 0.08	-0.81 ± 0.10	-0.31 ± 0.20
FilA%	0.41	0.44	-0.01	0.37	0.55	**0.25 ± 0.08**	0.79 ± 0.10	0.90 ± 0.06	0.87 ± 0.07	0.87 ± 0.07	0.80 ± 0.09	0.79 ± 0.10	0.75 ± 0.10	0.59 ± 0.18	0.78 ± 0.09	0.80 ± 0.09	0.81 ± 0.09	0.72 ± 0.11	0.58 ± 0.15	-0.79 ± 0.09	-0.18 ± 0.24
FoL	0.90	0.94	-0.08	0.97	0.95	0.39	**0.47 ± 0.11**	0.99 ± 0.01	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.90 ± 0.04	0.95 ± 0.02	0.56 ± 0.18	0.98 ± 0.01	0.94 ± 0.02	0.95 ± 0.02	0.95 ± 0.03	0.69 ± 0.11	-0.65 ± 0.13	-0.06 ± 0.19
FilML	0.87	0.91	-0.03	0.91	0.96	0.66	0.93	**0.52 ± 0.12**	0.99 ± 0.00	0.99 ± 0.00	0.99 ± 0.01	0.91 ± 0.04	0.93 ± 0.03	0.59 ± 0.17	0.97 ± 0.02	0.93 ± 0.03	0.92 ± 0.04	0.94 ± 0.03	0.70 ± 0.10	-0.77 ± 0.11	-0.10 ± 0.21
SL	0.91	0.95	-0.03	0.97	0.95	0.40	0.99	0.94	**0.46 ± 0.11**	1.00 ± 0.00	1.00 ± 0.00	0.90 ± 0.04	0.95 ± 0.02	0.54 ± 0.16	0.98 ± 0.01	0.94 ± 0.03	0.95 ± 0.03	0.95 ± 0.03	0.69 ± 0.11	-0.70 ± 0.13	-0.07 ± 0.17
TaEL	0.91	0.95	-0.02	0.96	0.95	0.42	0.98	0.95	0.99	**0.51 ± 0.11**	0.99 ± 0.00	0.90 ± 0.04	0.95 ± 0.02	0.53 ± 0.16	0.97 ± 0.01	0.93 ± 0.03	0.94 ± 0.03	0.93 ± 0.03	0.63 ± 0.12	-0.70 ± 0.13	-0.14 ± 0.22
TLL	0.90	0.94	-0.04	0.96	0.94	0.40	0.99	0.93	0.99	0.98	**0.46 ± 0.11**	0.88 ± 0.05	0.95 ± 0.02	0.50 ± 0.13	0.98 ± 0.01	0.91 ± 0.04	0.95 ± 0.02	0.94 ± 0.03	0.68 ± 0.10	-0.70 ± 0.14	-0.08 ± 0.23
FMH	0.91	0.87	0.21	0.95	0.94	0.38	0.88	0.83	0.88	0.88	0.87	**0.58 ± 0.10**	0.96 ± 0.02	0.76 ± 0.10	0.92 ± 0.04	0.99 ± 0.00	0.99 ± 0.01	0.95 ± 0.03	0.72 ± 0.09	-0.77 ± 0.11	-0.51 ± 0.17
HeH	0.89	0.87	0.19	0.94	0.86	0.12	0.89	0.75	0.89	0.89	0.88	0.92	**0.48 ± 0.09**	0.69 ± 0.12	0.98 ± 0.01	0.98 ± 0.01	0.97 ± 0.01	0.95 ± 0.02	0.71 ± 0.09	-0.76 ± 0.12	-0.37 ± 0.18
CPH	0.74	0.65	0.37	0.71	0.69	0.24	0.62	0.59	0.64	0.63	0.61	0.70	0.68	**0.38 ± 0.10**	0.75 ± 0.13	0.75 ± 0.12	0.78 ± 0.10	0.93 ± 0.05	0.91 ± 0.05	-0.84 ± 0.11	-0.72 ± 0.14
FHA	0.93	0.91	0.15	0.97	0.95	0.41	0.94	0.90	0.93	0.93	0.94	0.91	0.93	0.69	**0.54 ± 0.10**	0.96 ± 0.02	0.97 ± 0.01	0.99 ± 0.01	0.79 ± 0.08	-0.81 ± 0.09	-0.17 ± 0.20
FHB	0.92	0.89	0.19	0.96	0.95	0.40	0.91	0.85	0.91	0.91	0.92	0.99	0.93	0.70	0.94	**0.59 ± 0.10**	0.99 ± 0.01	0.96 ± 0.02	0.73 ± 0.09	-0.77 ± 0.11	-0.43 ± 0.18
FHC	0.92	0.87	0.25	0.95	0.93	0.36	0.88	0.82	0.88	0.89	0.87	0.96	0.91	0.73	0.91	0.95	**0.58 ± 0.09**	0.95 ± 0.02	0.76 ± 0.08	-0.72 ± 0.12	-0.46 ± 0.17
FHD	0.82	0.77	0.27	0.83	0.83	0.32	0.74	0.72	0.77	0.77	0.72	0.81	0.78	0.69	0.77	0.80	0.87	**0.43 ± 0.08**	0.86 ± 0.07	-0.84 ± 0.09	-0.45 ± 0.18
FHE	0.70	0.64	0.28	0.72	0.68	0.17	0.66	0.59	0.64	0.61	0.67	0.67	0.68	0.74	0.71	0.68	0.66	0.55	**0.34 ± 0.07**	-0.80 ± 0.10	-0.42 ± 0.18
HeEc	-0.36	-0.28	-0.24	-0.30	-0.44	-0.76	-0.26	-0.47	-0.24	-0.24	-0.25	-0.33	-0.15	-0.27	-0.44	-0.35	-0.29	-0.20	-0.32	**0.24 ± 0.06**	0.52 ± 0.18
FEc	-0.23	-0.05	-0.54	-0.21	-0.22	-0.06	-0.03	0.01	-0.03	-0.02	-0.03	-0.46	-0.30	-0.32	-0.21	-0.40	-0.42	-0.30	-0.28	0.31	**0.62 ± 0.12**

Table 11. Heritability estimates (in bold at the diagonal, with ± standard error) for growth traits: body weight, fork length, condition factor and carcass and body composition traits and their genetic correlations (above the diagonal, with ± standard error) and phenotypic correlations (below the diagonal) estimated from gilthead seabream in PROGENSA-III. BW: Body Weight; FL: Fork Length; CF: Condition Factor; WDef: Whole deformity; FFM: Fish Fat Meter, CW: Carcass Weight, Dr%: Dressing %, VF: Visceral Fat, FW: Fillet Weight, Fi%: Filleting %; SMAM: Sexual Maturity Age in Males.

Trait Category	**Growth**			**Morphologic quality**	**Carcass**					**Body composition**				**Reproductive**
Trait	BW	FL	CF	WDef	CW	Dr%	VF	FW	Fi%	Protein %	FFM	Lipid %	Moisture %	SMAM
BW	**0,37 ± 0,05**	0,94 ± 0,02	0,55 ± 0,11	-0,11 ± 0,23	1,00 ± 0,00	-0,43 ± 0,18	0,82 ± 0,06	1,00 ± 0,00	0,69 ± 0,13	-0,04 ± 0,20	0,48 ± 0,16	0,48 ± 0,17	-0,38 ± 0,16	0,07 ± 0,14
FL	0,9	**0,25 ± 0,04**	0,25 ± 0,16	-0,28 ± 0,24	0,93 ± 0,04	-0,29 ± 0,21	0,71 ± 0,09	0,96 ± 0,02	0,80 ± 0,11	-0,02 ± 0,19	0,46 ± 0,17	0,42 ± 0,20	-0,32 ± 0,17	0,10 ± 0,16
CF	0,11	-0,28	**0,25 ± 0,05**	0,49 ± 0,24	0,51 ± 0,19	-0,60 ± 0,20	0,52 ± 0,15	0,51 ± 0,17	0,21 ± 0,27	-0,15 ± 0,23	0,20 ± 0,17	0,28 ± 0,25	-0,32 ± 0,22	-0,13 ± 0,16
WDef	-0,28	-0,51	0,6	**0,02 ± 0,02**	0,09 ± 0,31	-0,19 ± 0,44	0,18 ± 0,39	0,03 ± 0,36	-0,54 ± 0,32	0,23 ± 0,45	0,07 ± 0,43	0,19 ± 0,42	-0,35 ± 0,34	-0,25 ± 0,23
CW	0,99	0,94	0,22	-0,19	**0,54 ± 0,09**	-0,58 ± 0,18	0,85 ± 0,05	-	0,77 ± 0,10	-0,02 ± 0,24	0,58 ± 0,14	0,60 ± 0,18	-0,47 ± 0,19	0,19 ± 0,17
Dr%	-0,09	-0,03	-0,18	-0,01	0,02	**0,08 ± 0,03**	-0,97 ± 0,09	-0,51 ± 0,21	0,14 ± 0,31	0,17 ± 0,30	-0,25 ± 0,27	-0,47 ± 0,26	0,08 ± 0,27	-0,12 ± 0,21
VF	0,66	0,6	0,2	-0,11	0,61	-0,19	**0,41 ± 0,07**	0,83 ± 0,06	0,35 ± 0,20	0,30 ± 0,14	0,53 ± 0,16	0,34 ± 0,21	-0,19 ± 0,20	0,26 ± 0,15
FW	0,94	0,89	0,21	-0,21	-	0	0,57	**0,49 ± 0,09**	0,77 ± 0,10	-0,01 ± 0,21	0,55 ± 0,15	0,58 ± 0,18	-0,52 ± 0,18	0,22 ± 0,14
Fi%	0,39	0,45	-0,05	-0,29	0,41	0,19	0,17	0,65	**0,18 ± 0,07**	0,15 ± 0,29	0,82 ± 0,16	0,35 ± 0,28	-0,49 ± 0,24	0,33 ± 0,22
Protein %	0,13	0,12	0,03	-0,05	0,1	0	0,11	0,06	-0,03	**0,14 ± 0,06**	-0,07 ± 0,21	-0,58 ± 0,21	0,58 ± 0,19	0,05 ± 0,21
FFM	0,41	0,37	0,26	-0,02	0,39	0,02	0,25	0,38	0,28	0,03	**0,44 ± 0,13**	0,84 ± 0,13	-0,88 ± 0,11	0,41 ± 0,19
Lipid %	0,21	0,2	0,08	-0,01	0,21	0,02	0,09	0,24	0,23	-0,7	0,39	**0,15 ± 0,07**	-0,95 ± 0,05	0,24 ± 0,22
Moisture %	-0,24	-0,23	-0,08	0,01	-0,22	-0,02	-0,1	-0,25	-0,23	0,56	-0,27	-0,77	**0,13 ± 0,05**	-0,38 ± 0,23
SMAM	-0,05	-0,03	0	-0,08	0,07	-0,02	0,07	0,08	0,05	0,01	0,13	0,04	-0,07	**0,25 ± 0,05**

Table 12.1. Genetic correlations, with ± standard error, between carcass and body composition traits and Non-invasive Technological traits, estimated from gilthead seabream in PROGENSA-III. BW: Body Weight; FL: Fork Length; CF: Condition Factor; TLA: Total Lateral Area; FilA: Fillet Area (square cm); FilA%: Fillet Area (Percentage); FoL: Fork Length; FilML: Fillet Maximum Length; SL: Standard Length; TaEL: Tail Excluded Length; TLL: Total Lateral Length; HeH: Head Height; FMH: Fish Maximum Height; CPH: Caudal Pedunculus Height; FHA: Fish Equidistant Height A; FHB: Fish Equidistant Height B; FHC: Fish Equidistant Height C; FHD: Fish Equidistant Height D; FHE: Fish Equidistant Height E; HeEc: Head Eccentricity; FEc: Fish Eccentricity, FFM: Fish Fat Meter, CW: Carcass Weight, Dr%: Dressing %, VF: Visceral Fat, FW: Fillet Weight, FY: Filleting %; WDef: Whole deformity; SMAM: Sexual Maturity Age in Males.

Trait category		**Carcass**					**Body composition**				**Morphologic quality**	**Reproductive**
	Trait	CW	Dr%	VF	FW	Fi%	Protein %	FFM	Lipid %	Moisture %	WDef	SMAM
Carcass NiT traits	TLA	1,00 ± 0,00	-0,54 ± 0,21	0,85 ± 0,06	1,00 ± 0,00	0,83 ± 0,08	-0,01 ± 0,23	0,65 ± 0,16	0,55 ± 0,18	-0,49 ± 0,21	-0,09 ± 0,29	0,24 ± 0,17
	FilA	1,00 ± 0,00	-0,56 ± 0,20	0,85 ± 0,06	1,00 ± 0,00	0,84 ± 0,08	-0,01 ± 0,25	0,66 ± 0,15	0,66 ± 0,15	-0,43 ± 0,22	0,11 ± 0,35	0,25 ± 0,18
	FilA%	0,78 ± 0,09	-0,49 ± 0,24	0,65 ± 0,12	0,76 ± 0,10	0,87 ± 0,12	-0,23 ± 0,28	0,60 ± 0,19	0,54 ± 0,19	-0,30 ± 0,26	0,25 ± 0,45	0,21 ± 0,17
Morphometric NiT trait	FoL	0,99 ± 0,01	-0,43 ± 0,26	0,83 ± 0,07	0,99 ± 0,01	0,93 ± 0,05	0,00 ± 0,25	0,67 ± 0,18	0,66 ± 0,17	-0,49 ± 0,22	-0,16 ± 0,35	0,30 ± 0,13
	FiML	0,97 ± 0,02	-0,44 ± 0,26	0,82 ± 0,07	0,97 ± 0,02	0,96 ± 0,04	-0,20 ± 0,29	0,78 ± 0,13	0,75 ± 0,13	-0,48 ± 0,24	-0,00 ± 0,37	0,33 ± 0,18
	SL	0,98 ± 0,01	-0,48 ± 0,24	0,80 ± 0,07	0,99 ± 0,01	0,89 ± 0,07	-0,12 ± 0,29	0,73 ± 0,15	0,71 ± 0,16	-0,52 ± 0,24	-0,06 ± 0,40	0,26 ± 0,19
	TaEL	0,98 ± 0,01	-0,38 ± 0,27	0,80 ± 0,08	0,99 ± 0,01	0,93 ± 0,04	-0,24 ± 0,27	0,80 ± 0,12	0,70 ± 0,17	-0,52 ± 0,22	-0,19 ± 0,34	0,31 ± 0,20
	TLL	0,98 ± 0,01	-0,35 ± 0,27	0,79 ± 0,08	0,99 ± 0,01	0,92 ± 0,05	0,04 ± 0,21	0,73 ± 0,18	0,74 ± 0,14	-0,49 ± 0,23	-0,07 ± 0,35	0,29 ± 0,19
	FMH	0,97 ± 0,01	-0,57 ± 0,19	0,88 ± 0,05	0,95 ± 0,03	0,77 ± 0,10	-0,02 ± 0,26	0,49 ± 0,20	0,61 ± 0,16	-0,36 ± 0,19	0,14 ± 0,33	0,22 ± 0,17
	HeH	0,98 ± 0,01	-0,52 ± 0,22	0,83 ± 0,07	0,97 ± 0,01	0,81 ± 0,09	0,12 ± 0,25	0,59 ± 0,15	0,57 ± 0,17	-0,38 ± 0,22	0,05 ± 0,33	0,22 ± 0,18
	CPH	0,81 ± 0,09	-0,55 ± 0,21	0,68 ± 0,11	0,84 ± 0,09	0,50 ± 0,21	-0,09 ± 0,25	0,63 ± 0,18	0,70 ± 0,16	-0,55 ± 0,20	0,53 ± 0,25	0,19 ± 0,12
	FHA	1,00 ± 0,00	-0,52 ± 0,20	0,86 ± 0,05	0,99 ± 0,01	0,85 ± 0,08	-0,02 ± 0,23	0,61 ± 0,14	0,61 ± 0,17	-0,43 ± 0,19	0,05 ± 0,33	0,21 ± 0,17
	FHB	0,98 ± 0,01	-0,54 ± 0,18	0,88 ± 0,05	0,97 ± 0,01	0,80 ± 0,09	-0,05 ± 0,25	0,53 ± 0,18	0,57 ± 0,19	-0,40 ± 0,20	0,00 ± 0,31	0,22 ± 0,17
	FHC	0,99 ± 0,01	-0,66 ± 0,18	0,86 ± 0,06	0,98 ± 0,01	0,74 ± 0,09	0,04 ± 0,24	0,52 ± 0,17	0,55 ± 0,18	-0,35 ± 0,20	-0,01 ± 0,20	0,14 ± 0,11
	FHD	0,98 ± 0,01	-0,50 ± 0,22	0,82 ± 0,07	0,98 ± 0,01	0,76 ± 0,11	-0,12 ± 0,18	0,66 ± 0,14	0,70 ± 0,14	-0,50 ± 0,18	0,20 ± 0,32	0,18 ± 0,17
	FHE	0,79 ± 0,07	-0,38 ± 0,22	0,70 ± 0,09	0,79 ± 0,08	0,55 ± 0,17	0,16 ± 0,19	0,46 ± 0,16	0,38 ± 0,21	-0,34 ± 0,20	0,43 ± 0,27	0,09 ± 0,14
	HeEc	-0,74 ± 0,11	0,53 ± 0,20	-0,70 ± 0,10	-0,72 ± 0,12	-0,55 ± 0,14	-0,13 ± 0,13	-0,41 ± 0,20	-0,34 ± 0,24	0,16 ± 0,22	-0,28 ± 0,31	0,05 ± 0,09
	FEc	-0,24 ± 0,19	0,61 ± 0,21	-0,52 ± 0,16	-0,24 ± 0,20	0,24 ± 0,23	-0,57 ± 0,16	0,27 ± 0,21	0,32 ± 0,23	-0,20 ± 0,21	-0,52 ± 0,36	0,11 ± 0,15

Table 12.2. Phenotypic correlations between carcass and body composition traits and Non-invasive Technological traits, estimated from gilthead seabream in PROGENSA-III. BW: Body Weight; FL: Fork Length; CF: Condition Factor; TLA: Total Lateral Area; FilA: Fillet Area (square cm); FilA%: Fillet Area (Percentage); FoL: Fork Length; FilML: Fillet Maximum Length; SL: Standard Length; TaEL: Tail Excluded Length; TLL: Total Lateral Length; HeH: Head Height; FMH: Fish Maximum Height; CPH: Caudal Pedunculus Height; FHA: Fish Equidistant Height A; FHB: Fish Equidistant Height B; FHC: Fish Equidistant Height C; FHD: Fish Equidistant Height D; FHE: Fish Equidistant Height E; HeEc: Head Eccentricity; FEc: Fish Eccentricity, FFM: Fish Fat Meter, CW: Carcass Weight, Dr%: Dressing %, VF: Visceral Fat, FW: Fillet Weight, Fi%: Filleting %; WDef: Whole deformity; SMAM: Sexual Maturity Age in Males.

Trait category		**Carcass traits**					**Body composition traits**				**Morphologic quality**	**Reproductive**
	Trait	CW	Dr%	VF	FW	Fi%	Protein %	FFM	Lipid %	Moisture %	WDef	SMAM
Carcass NiT traits	TLA	0,32	0,93	-0,05	0,57	0,87	0,36	0,12	0,18	-0,21	-0.26	0.08
	FilA	0,33	0,92	-0,06	0,57	0,86	0,37	0,13	0,19	-0,20	-0.26	0.09
	FilA%	0,27	0,40	-0,02	0,26	0,38	0,25	0,06	0,10	-0,10	-0.24	0.05
Morphometric NiT traits	FoL	0,30	0,89	-0,04	0,53	0,82	0,40	0,11	0,18	-0,21	-0.33	0.11
	FiML	0,38	0,85	-0,04	0,53	0,80	0,41	0,10	0,20	-0,20	-0.35	0.08
	SL	0,33	0,89	-0,05	0,53	0,83	0,41	0,10	0,19	-0,21	-0.34	0.09
	TaEL	0,37	0,89	-0,04	0,53	0,83	0,41	0,09	0,19	-0,21	-0.33	0.11
	TLL	0,31	0,88	-0,03	0,54	0,82	0,39	0,13	0,21	-0,21	-0.34	0.11
	FMH	0,32	0,89	-0,06	0,59	0,81	0,34	0,11	0,18	-0,18	-0.23	0.08
	HeH	0,28	0,87	-0,06	0,55	0,80	0,31	0,11	0,17	-0,19	-0.17	0.08
	CPH	0,31	0,71	-0,08	0,46	0,64	0,20	0,11	0,14	-0,17	-0.01	0.06
	FHA	0,33	0,92	-0,06	0,59	0,85	0,37	0,11	0,19	-0,22	-0.23	0.08
	FHB	0,32	0,91	-0,06	0,60	0,83	0,34	0,11	0,18	-0,20	-0.22	0.08
	FHC	0,33	0,90	-0,07	0,58	0,84	0,35	0,12	0,19	-0,21	-0.12	0.04
	FHD	0,29	0,80	-0,05	0,48	0,74	0,31	0,09	0,17	-0,19	-0.13	0.06
	FHE	0,19	0,65	-0,05	0,42	0,58	0,18	0,09	0,12	-0,15	-0.06	0.03
	HeEc	-0,22	-0,35	0,04	-0,28	-0,33	-0,15	-0,06	-0,09	0,07	0.04	0.01
	FEc	-0,03	-0,22	0,06	-0,27	-0,18	0,04	-0,11	0	0,02	-0.28	0.04

Chapter 5. Results discussion

5.1. Parental assignment

Robust tools for paternity test are essential for developing breeding programs (Navarro *et al.*, 2008). Lee-Montero *et al*. (2013) developed a SuperMultiplex of 11 microsatellite loci (SMsa1) for parental assignment in *Sparus aurata*. In this study, a parental assignment rate of 87% was obtained, supporting robust and reliable estimations of genetic parameters in concordance with Elalfy *et al*. (2021). Values up to 100% would require using the second SuperMultiplex containing additional 11 loci (SMsa2) as previously suggested Lee-Montero *et al.* (2013), or other works that also used two multiplex, such as Fernandes *et al*. (2017).

5.2. Evaluation of spawning management strategy

In this study, breeder contribution in F3 offspring was maximized by using several batches (S_1, S_2, S_3) from different moments along the spawning season, each one constituted by eggs from four consecutive days, 4DL contribution model (Elalfy, 2016). Thus, a total of 82 breeders out of 90 (91.1%) contributed by random mating to build a total of 217 families. This number is above the average number of families used in most gilthead sea bream breeding programs (Neira, 2010; Rye *et al*., 2010).

In this study, statistical results regarding breeders' contribution and family number for each independent batch (4DL model) and two separate batches (2x4DL model) were calculated, in order to evaluate the potential on genetic variability of mixing eggs from different spawning events during the spawning season, when obtaining offspring by mass spawning. Overall, contribution and family number of isolated batches were both lower, since both variables are highly correlated. In case of S_3 of both broodstock, contribution and therefore family number were notably lower. Albeit the important difference between the number of offspring from S_3 analysed of compared to other batches, which is evidently contributing to S_3 low numbers, this do not contradict the fact that 2x4DL still stands as a better option concerning total contribution and number of families (9.5% higher).

These results support usage of these contribution models and their application to other species like in Afonso *et al*. (2021) with *Litopenaeus vannamei*, where applying a similar model adapted to whiteleg shrimp culturing processes, improved the contribution in terms of number of families up to 43%, as average.

5.3. Data processing and phenotypic analysis

In the context of the fourth industrial revolution or Industry 4.0 (Frank *et al.*, 2019), innovation through technology in order to optimize SMEs business models (Dassisti *et al.*, 2019), and traceability by monitoring Key Performance Indicators (KPIs) (PerformFISH, 2017) become essential.

5.3.1. Image processing

For selection of industrial broodstock, it is very handy to have a system capable to generate and analyse data in a fast, reliable and cost-effective way. In this sense, tools like IMAFISH_ML software (Navarro *et al.*, 2016) can mark a difference as it notably reduces the time required to analyse multiple traits, allowing to produce large data matrix in short time. This system, in addition, allows collecting data from alive fish (previously anesthetized), and without causing them any damage, preserving fish welfare. In this study, 42,264 records were produced by IMAFISH_ML software, automatically, in only 52 minutes (18 novel technological traits from 2,348 fish).

5.3.2. Image edition

Within on-growing facility or location, a comparison between unedited and edited images was carried out for all NiT traits, reporting significant differences mainly in traits where IMAFISH_ML software did not exclude completely fins or shades from digitally inferred shape. A similar problem has been detected in other studies, such as in Fernandes *et al.* (2020), where they use a deep learning approach. Thus, cNiT, shape mNiT and height mNiT traits were more influenced mainly because dorsal and ventral fins affected their biometric measures. On the contrary, length NiT traits did not show significant differences. In any case, significant differences between NiT traits of different type of images (unedited and edited images) did not have influence on heritability estimates.

5.3.3. Growth traits

An on-growing facility effect was observed between locations and culturing systems; however, it was not the aim of this work to evaluate how optimal are different private specific protocols for growing fish, but to evaluate the methods used for gathering information and to evaluate traits. Fish reared in FS2 (Oceanic cage) showed a body weight at harvest 1.06 times higher than FS1 (Estuarine ponds) and 1.45 times higher than IFAPA (Inland tanks). Regarding populations for selection (not slaughtered), all of them were kept in inland tanks during the on-growing phase. These phenotypic BW and FL values could be explained by environmental

factors such as temperature (**Table 2**), age at harvest (652 dph FS1, 601 dph IFAPA, this lapse is due to distinct batches having different spawning and harvesting dates) and feeding protocols.

Growth rate was affected similarly to weight and length, significant differences between growth rate values were found mainly between different culturing systems. Thus, FS1 and FS2 fish showed middle and higher rates, respectively, what is consistent with their intensive and semi-intensive models, also respectively. Inland tanks in research facilities showing the lowest values can be explained due to the lack of intensive feeding protocols, more focused to populations upkeep. This agrees with previous selection generations of PROGENSA-I, where similar rearing conditions and locations were used (Lee-Montero *et al.*, 2015), and corrected in the mixed models as fixed factor.

In general, the variation coefficients were higher for growth traits than for NiT traits. Comparing the variation coefficient of fork length from manually (FL) and automatically measurements (FoL, unedited and edited), which were equal (7.6%), reports the utility of these novel non-invasive technological traits in breeding programs. Moreover, these NiT traits minimize errors and measuring time.

5.3.4. Whole deformity

Results concerning whole deformity rate show big differences between groups. In previous works, whole deformity rates in gilthead sea bream at similar age and between similar facilities, reached a maximum difference of around 50% (Lee-Montero *et al.*, 2015). However, in this study, difference between the highest and the lowest is more than 1000%. Differences could be originated by multiple factors. Roo *et al.* (2005) already reported effects of culturing conditions and larval nutrition on deformity development. Also, deform fish are less fit and either intensive or extensive culturing conditions could play a role in their survival, which would exclude them from the final sampling, increasing the proportion of fish without malformations. Lastly, although deformities are standardized and the personnel in charge of assessing them was well trained, presence/absence of malformation evaluation was carried out by different groups of people, which, even in a low extent, might have introduced some error in the final result.

5.4. Gene – environment interaction

Gene-environment interaction is to be expected in cases where same families are reared in different conditions (Cardellino and Rovira, 1987). In this study, the lowest genetic correlations between growth traits were found between estuarine ponds (FS1) and oceanic cage (FS2), and therefore, an interaction cannot be discarded. These results are in concordance with the work

carried out by Elalfy *et al.* (2021), who reported the lower genetic correlations for growth traits (*BW*, Growth rate) between these two on-growing production systems.

5.5. Genetic parameters and selection traits

5.5.1. Growth traits

Searching alternative traits to body weight, or other usual traits related to growth, in order to perform indirect selection in breeding programs, has been a recurrent field of study throughout the years (Kause *et al*., 2007). However, several factors should be considered such as rentability, relative gain, effects on animals, environmental effects, etc. Morphometric traits have hold interest as candidates for indirect selection due to their non-invasive assessment nature and their potential adaptability to automatization (Fernandes *et al.,* 2015; Vandeputte *et al*., 2020). In this study, 18 Non-invasive Technological traits related with morphometry and carcass measured from images by using image analysis software IMAFISH_ML (Navarro *et al*., 2016), were evaluated.

Selection response (direct or indirect) is one of the most important parameters for producers due to its effect on productive yields. Traits with a high heritability and genetic correlations, easily measurable, economic, and reproducible constitute one good criteria for genetic selection (Falconer and Mackay, 1996).

Genetic parameters were estimated for growth traits (body weight at harvest and fork length) showing medium heritability estimates with a high genetic correlation. It is in concordance with other estimates of gilthead seabream populations studied in similar locations (García-Celdrán *et al.*, 2015; Lee-Montero *et al.*, 2015). Concerning condition factor trait in this study, heritability estimate was 0.25 (600-670 days). It agrees with Navarro *et al.* (2009a, 2009b) who reported higher CF heritability value as harvest age increased (0.05 at 130 days versus 0.13 at 509 days).

5.5.2. Morphometric and carcass Non-invasive Technological traits

This study provided heritability and genetic correlations for 18 new Non-invasive Technological traits, from unedited and edited images of IMAFISH-ML software. Their results position these novel NiT traits as potential candidates for performing direct or indirect selection. Heritability estimates of NiT traits for unedited and edited images were similar (**Table 9**), and highly correlated from genetical point of view, concluding that unedited images are equally optimal for selection processes.

In general, heritability estimates of NiT traits from unedited images were high with high genetic correlations. Different NiT traits were evaluated according to their category; area or carcass traits (cNiT), and morphometric traits related to length, height, and shape (mNiT). Concerning

area category, estimated heritability for FilA and TLA was very similar and higher than BW at harvest (38% higher). Due to the high genetic correlations of both cNiT traits with body weight, indirect selection of body weight through these cNiT traits is 17.4% more efficient than directly estimated by BW itself. In a similar way, length mNiT traits, FilML and TaEL, showed high heritability estimates and high genetic correlations with body weight, allowing an indirect selection response 13.9% higher than directly by BW. With respect to height mNiT traits, FHB, FHC and FMH were the traits with the highest heritability estimates and reported robust genetic correlations with body weight. Thus, the indirect selection of body weight through FHB and FHC would be 22.5% and 22.7% more efficient than directly selecting by BW, respectively. FHE genetic correlations with morphometric traits were lower than others. Fish equidistant heights (A, B, C, D and E) are established upon total length of the fish. Due to that, FHA measures are calculated in the most anterior part of the fish, whereas FHE is calculated in the tail area. FHE low heritability when compared to other height traits is understandable because that depending on the length of the tail, measures are taken, most of the times, in different areas of the caudal pedunculus or in the middle of the caudal fin, thus becoming an important source of variation. In shape category, FEc showed the highest heritability, pointing that roundness of the fish is notably affected by family factors. However, since correlation with BW was scarce, eccentricity traits should not be considered for indirect selection.

In this study, fork length was measured by two methods: manually (FL) and through NiTs (FoL). The heritability of length by NiT was 88% higher than the manual estimate. This is due to a greater accuracy of the automatic measurement reducing the variability associated with data acquisition by the hand of different personnel, within and between facilities. This supports the use of the NiT methodology versus the manual methodology as it would improve response for the same selection intensity.

High heritability values for morphometric traits related to height have been previously reported in other non-sparidae fish species (Fernandes *et al.*, 2015). He *et al*. (2018) reported increasing heritability estimates for growth and morphological traits through fish development in Nile tilapia, as well as high genetic correlations between growth traits and morphology. In this study, height mNiT traits around head (FMH, HeH, FHA, FHB and FHC) showed high heritability estimates and genetic correlations, reflecting their potential as selection traits in gilthead seabream, and that using image analysis and fast data recording systems such as IMAFISH_ML is highly recommendable in the selection processes of this species.

5.5.3. Carcass and body composition traits

Carcass traits may hold more economic value for producers than growth traits (Rutten *et al*., 2004). Turra *et al.* (2012) reported good genetic correlation of carcass traits with body weight at harvest and a good correlated response to selection for carcass traits in Nile tilapia. At industrial level, nevertheless, carcass traits are still far from being major selection trait, in part because their assessment is too laborious for large number of fish.

In this study, heritability estimates for processed weights were higher than for entire body weight. This is different from the results of Navarro *et al.* (2009a) and García-Celdrán *et al.* (2015b), which reported lower heritability estimates for processed weight than for entire body weight. Despite that, genetic correlation of both traits with body weight at harvest was very high and positive, which is in concordance with the same work and others (Navarro *et al*., 2009a; Gulzari *et al*., 2022; García-Celdrán *et al.*, 2015b). One possible hypothesis is that, even if the possible variation introduced by the effect of the different personnel that performed gutting and filleting is affecting the estimation, removal of internal organs together with visceral fat, etc. may also be decreasing environmental variance that is affecting the measurement of entire body weight. However, visceral fat weight has also high genetic correlation with that trait and higher heritability estimate than body weight at harvest, which is similar to results obtained by Navarro *et al*. (2009b).

On the other hand, dressing and filleting percentages, which are the traits that are really related with product yield and economic value, showed low (Dr%) and medium (Fi%) heritability estimates. Dr% heritability estimate being notably lower than the estimate reported by Navarro *et al.* 2009a, but similar to García-Celdrán *et al.* (2015b), although all showed similar correlations. Among these two traits, only Fi% has high genetic correlation with weight traits (whole and processed). These results are in concordance with the ones reported by Gulzari *et al.* (2022).

Interestingly, classically measured carcass traits and cNiT traits are strongly correlated, excluding Dr%. Traits related to filleting such as FilML, FilA and TLA showed high and positive genetic correlation with growth, CW, FW and Fi%. Thus, indicating that, not only selecting for these traits would improve BW and carcass traits, but also that it could be achieved using non-invasive technologies. These results showed that selection according to FilML would be 63.2% and 13.8% more efficient than selecting for Fi% and BW, respectively, for improving these traits. Moreover, FilML and Fi% have a phenotypic correlation of 0.80.

Regarding flesh composition traits, genetic correlations of moisture, lipid and protein content with body weight at harvest were similar to the estimates reported by Elalfy *et al.* (2021) and García-Celdrán *et al.* (2015c), who used the same measuring method (NIRS). However,

heritability estimates obtained in this study were low compared to theirs, although their evaluation was performed at a later age. Heritability estimate of lipid content measured with FFM was medium, which is in range with the results obtained by Gulzari *et al*. (2022).

Heritability estimate for lipid content measured with FFM was 293% higher than the same trait measured with NIR and genetic correlation between them was high. Phenotypic correlation, however, was scarce, and there were also significative differences in phenotypic values, which could be linked to the settings and the measuring algorithm of one or both devices and should be revised prior performing further measures.

5.5.4. Whole deformity

Concerning whole deformity genetic parameters analysis, heritability estimate was low. This is in concordance with other studies (Lee-Montero *et al*., 2015) where "whole deformity" trait estimate was lower than other estimates for specific deformities. Kause *et al*. (2005) also reported low heritability estimates for deformity (0.02) in rainbow trout. Both studies reported a low but existing positive genetic correlation between growth traits and deformity. In this study, genetic correlation of WDef with FL and BW has been negative (although low), which points to a strong environmental component affecting observed deformities in this population. Negative correlations (also low) with shape NiT traits and positive with CF, indicate that fish roundness could be related with high deformity, which fits with the fact that most frequent deformities are column-related (Lee-Montero *et al*., 2015), this leading to fish shape "bending". Other works report strong genetic component affecting fish shape in *Solea spp*, with a computer vision approach (Blonk *et al*., 2010; Guerrero-Cortázar *et al.*, 2021)

5.5.5. Male Sexual maturation age

Sexual maturity age in cultivated fish is desirable to be delayed because of an associated decrease in growth performance during breeding season (Aksnes *et al.*, 1986; Kause *et al.*, 2003), and it has been reported to be affected by genetics in Atlantic salmon (Barson *et al*., 2015). Positive genetic correlations between sexual maturity at harvest and body weight at harvest have also been a concern in rainbow trout (Kause *et al.*, 2003).

Heritability estimate obtained in this study for SMAM was medium and genetic correlations with growth traits were low and positive, which is in concordance with estimates reported in rainbow trout (Kause *et al*., 2005) and Atlantic cod (Kolstad *et al*., 2006). Albeit genetic correlations with other traits in this study were mostly also low and positive, the highest estimates were related with lipid metabolism (VF, Lipid% and FFM) and fillet (Fi% and cNiT traits), which was reported to be related with sexual development in chinook salmon by Shearer *et al*. (2000).

Chapter 6. Results integration and application: industrial broodstock configuration

6.1. Candidate assessment

Distribution of EBV for body weight at harvest trait of F3 candidates is shown in **Figure 9**, accounting for the available genetic pool for creating breeder populations according to talent. Fish considered as candidates were F3 fish of canary region that were not slaughtered for sampling, that is, fish from PCTM and from FS3 (tanks). 3,000 fish in total from $S_{1/2}$ and S_3. Sorting was performed to discard fish with deform phenotype.

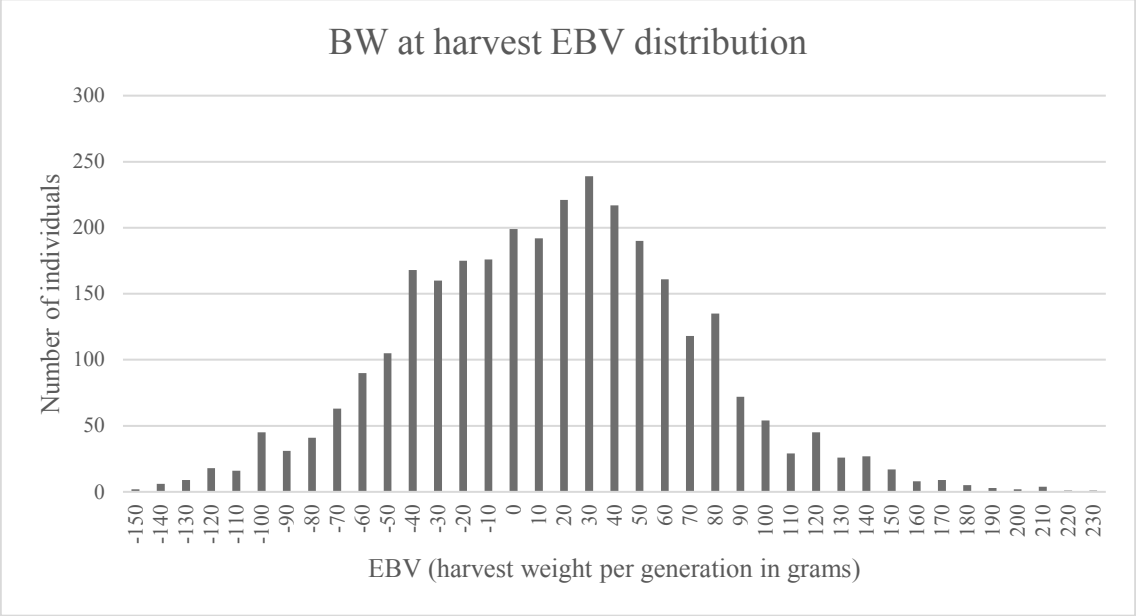

Figure 9. PROGENSA-III F3 ($S_{1/2}$ and S_3 from PCTM and FS3) EBV distribution for BW at harvest trait. BW: *body weight*.

6.2. Consanguinity control and inbreeding regulation criteria

Kinship coefficient is defined as the probability that two homologous alleles drawn from each of two individuals are identical by descent (IBD). In a population constituted by several individuals with known relationships between them, calculating the arithmetical mean of all kinship coefficients of all possible matings serves as a good indicator to infer differences in consanguinity level between populations.

Total number of families in this pool was 177. However, since genetic selection relies in family effect, when focusing on fish with higher EBV for the desired trait, number of families decreases drastically. In this case, top 300 individuals (top 10%), ranked by EBV of BW at harvest, come down to 51 families, and since male breeder number was relatively low, most of the descendants are also half-siblings. In order to boost growth improvement in the next generations but prevent recessive alleles homozygosis and other negative effects that underlie

inbreeding, populations needed to be optimized by maximizing average EBV and minimizing relatedness increase.

Finally, according with the company necessities and available units, four populations were constituted: H.1, H.2, SH.1 and SH.2. **Figure 10** shows distribution of these four final populations, based in their genetic gain and their average relatedness increase (with respect to the origin population). Also, top 300, 100 and 50 ranked fish population simulations were included for comparison.

These four populations, classified as High (H) and Super High (SH), as for their average genetic talent for growth traits, gave answer to company requests. In one hand, population size came down to 60-70 individuals, which is a suitable number for a breeder population that will breed under mass spawning, and yielded good population density according to company tank volumes. On the other hand, talent for BW at harvest was maximized under two criteria: H populations were aimed to be used for obtaining next generation for the breeding program (10% added to past generation BW at harvest genetic gain plus high genetic variability), and SH populations would be used for producing high growth fries for on growing. SH populations descendants are not meant to produce any other generation since they are destined to consumption, so members of the best grower families could be included, "trading" genetic variability for better average talent for growth.

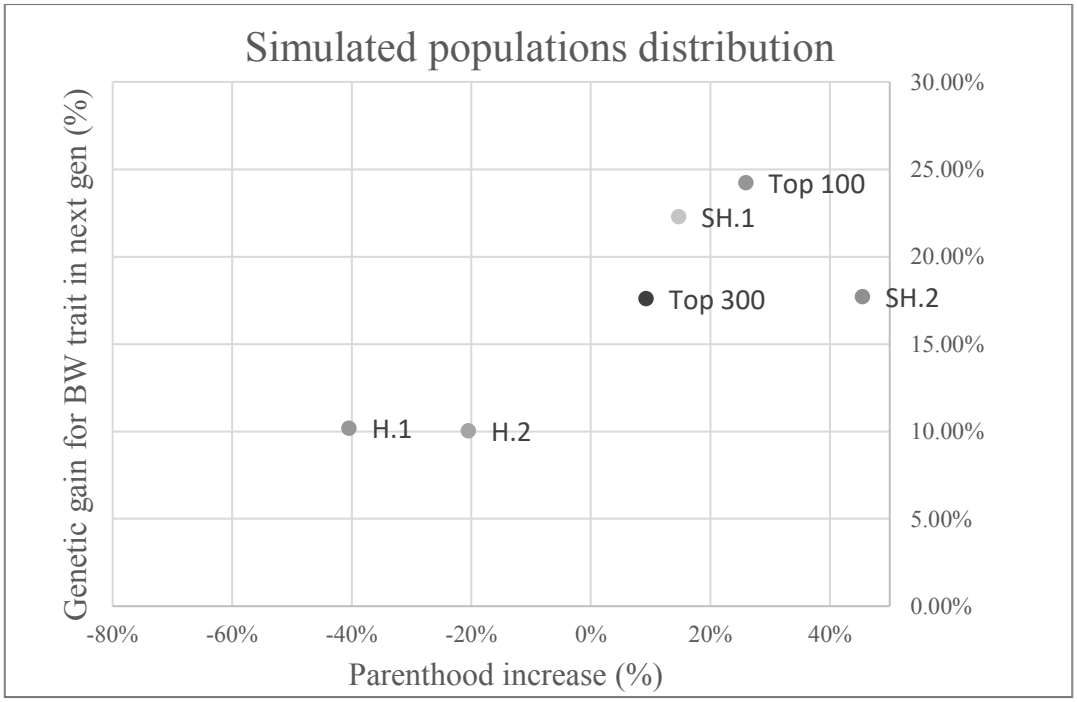

Figure 10. Distribution of simulated populations according to their genetic gain for BW at harvest trait and their average parenthood increase, obtained by using SELECTION program. H: high growth. SH: Super high growth

Chapter 7. Conclusions

1. 2x4DL strategy can increase number of families in breeding programs by culturing eggs from different times of the spawning period in gilthead sea bream. However, it is important to perform good husbandry and grading in order to improve survival and eventually mix both batches.

2. This study reports the additive genetic determination of novel Non-invasive Technological (NiT) traits, and their genetic relationships. These NiT traits were successfully assessed with IMAFISH_ML image analysis software in an automatic, fast and efficient way, without the need of any correction of the fish contour by image edition.

3. In general, NiT traits showed very high heritability estimates and robust genetic correlations between them and with respect to growth traits. Positioning those related to dorsal height and close to the head region as good candidates for genetic selection in gilthead seabream. Indirect selection of growth traits through these new NiT traits would allow a more efficient selection, up to 22.7% higher than selecting directly by body weight at harvest.

4. Carcass and fillet weights showed higher heritability estimates than body weight and a correlation of 1 with body weight at harvest. Correlations with morphometric traits were also high and positive, except with eccentricity traits.

5. Fillet yield can be improved through selecting by fillet area or fillet maximum length up to 63.2%, traits automatically measured by IMAFISH_ML, improving body weight at the same time.

6. Non-invasive technologies have provided higher heritability estimates that their classic method homologues for measuring fork length, muscular lipid content, and fillet.

7. Heritability estimate for whole deformity trait in this study was low, indicating an important effect of the environment in this malformation development.

8. Age of sexual maturation in males shows genetic additive component in gilthead sea bream and its heritability estimate in this study was medium.

References

Afonso J. M., Roo F. J., 2007. Anomalías morfológicas en peces cultivados: heredabilidad y selección. In: Genética y Genómica en Acuicultura. (Ed. by J. Espinosa; coord. by P. Martínez, A. Figueras.), pp. 215–240. Consejo Superior de Investigaciones Científicas, Madrid.

Afonso, J. M., Manchado. M., Estévez, A., Ramis, G., Lee-Montero, I., Ponce, M., Sánchez, J. A., Armero, E., Navarro, A., Puertas, M. A., Borrell, Y., García, M., Negrín-Báez, D., Crespo, A. M., Blanco, G., Mariadolores, E., Sánchez, J. J., Martín, N., Aguilera, C., Roo, F. J., Zamorano, M. J., Toro., M. A. 2012. PROGENSA®: development of a genetic improvement program in gilthead sea bream (*Sparus aurata* L.) between industry and research centers in Spain. Proceedings of AQUA 2012, September 1–5, Prague, Czech Republic.

Afonso, J. M., Shin, H. S., Martínez, M., Lorenzo-Felipe, A., Mujica, K., Viteri E., Hilderbrandt, S., Falcón, A., Peñate, A., Ramírez, J. S., Hernández, J. J., Évora, J., Hernández, F. M., Ferrer, M. A., Hernández, J. M., Lorenzo, J., León, C. J., Ginés, R., Zamorano, M. J., Fernández, J. Pachón, L. C., Yugcha, E., Tomalá, M, Franco, N., Forestieri, J., Escobar, M., Castillo, J. A., Reyes, E., Miranda, R., Torres, R., Suárez, H., Noboa, R., Harten, W., Lince, J. A., 2021. Programa de Mejora Genética para camarón (*Litopenaeus vanname*i) integrado en el modelo de negocio. AquaExpo 2021, Guayaquil, Ecuador.

Aksnes, A., Gjerde, B., Roald, S. O., 1986. Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, Salmo salar. Aquaculture 53, 7– 20.

APROMAR, 2018. La acuicultura en España. www.apromar.es

APROMAR, 2020. La acuicultura en España. www.apromar.es

AQUAEXCEL, 2013. Recovered from: http://archive.aquaexcel.eu/images/Deliverables_2013/aquaexcel_d3.2-validated.pdf

Barson, N. J., 2015. Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. Nature: International Weekly Journal of Science, 528(7582), 405–408.

Blonk, R. J. W., Komen, J., Tenghe, A., Kamstra, A., van Arendonk, J. A. M, 2010. Heritability of shape in common sole, *Solea solea*, estimated from image analysis data. Aquaculture, 307(1), 6–11.

Brown, R. C., 2003. Genetic Management and Selective Breeding in Farmed Populations of Gilthead Seabream (*Sparus aurata*). Universtiy of Stirling, pp. 197.

Cardelino R., Rovira J., 1987. Mejoramiento genético animal. Hemisferio Sur, Buenos Aires.

Chavanne, H., Janssen, K., Hofherr, J., Contini, F., Haffray, P., Komen, H., Nielsen, E., Bargelloni, L., 2016. A comprehensive survey on selective breeding programs and seed market in the European aquaculture fish industry. Aquaculture International. 24.

Costa, C., Antonucci, F., Pallottino, F., Aguzzi, J., Sun, D.W., Menesatti, P., 2011. Shape Analysis of Agricultural Products: A Review of Recent Research Advances and Potential Application to Computer Vision. Food Bioprocess Technol. 4.

Dassisti, M., Giovannini, A., Merla, P., Chimienti, M., Panetto, H., 2019. An approach to support Industry 4.0 adoption in SMEs using a core-metamodel. Annu. Rev. Contr. 47, 266-274.

De La Iglesia, D. H., González, G. V., García, M. V., Rivero, A. J. L., De Paz, J. F., 2020. Non-invasive automatic beef carcass classification based on sensor network and image analysis. Future Generation Computer Systems, 113, 318–328.

Elalfy, I. S., 2016. Estudio de la segunda generación de dorada (*Sparus aurata, L.*) del programa de mejora genética PROGENSA® para caracteres de interés comercial e implementación de nuevas tecnologías facilitadoras, bajo condiciones industriales de cultivo. University of Las Palmas de Gran Canaria, Canary islands, Spain.

Elalfy, I. S., Shin, H. S., Negrín-Báez, D., Navarro, A., Zamorano, M. J., Manchado, M., and Afonso. J. M., 2021. Genetic Parameters for Quality Traits by Non-Invasive Methods and Their G x E Interactions in Ocean Cages and Estuaries on Gilthead Seabream (*Sparus Aurata*)." Aquaculture 537, 736462.

FAO (Food and Agriculture Organization of the United Nations), 2015. European Seabass and Gilthead seabream.

FAO (Food and Agriculture Organization of the United Nations), 2016. The state of world fisheries and aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200pp.

Falconer D.S., Mackay T.F.C., 1996. Introducción a la Genética Cuantitativa. Acribia, Zaragoza.

Fernandes, A. F. A., Silva, M. de A., de Alvarenga, E. R., Teixeira, E. de A., da Silva Junior, A. F., Alves, G. F. de O., de Salles, S. C. M., de, Manduca, L. G., Turra, E. M., 2015. Morphometric traits as selection criteria for carcass yield and body weight in Nile tilapia (*Oreochromis niloticus L.*) at five ages. Aquaculture, 446, 303–309.

Fernandes, A. F. A., Turra, E. M., de Alvarenga, É. R., Passafaro, T. L., Lopes, F. B., Alves, G. F. O., Singh, V., Rosa, G. J. M., 2020. Deep Learning image segmentation for extraction of fish body measurements and prediction of body weight and carcass traits in Nile tilapia. Computers and Electronics in Agriculture, 170.

Fernandes, T., Herlin, M., Belluga, M., Ballón, G., Martinez, P., Toro, M., Fernández, J., 2017. Estimation of genetic parameters for growth traits in a hatchery population of gilthead sea bream (*Sparus aurata* L.). Aquaculture International, 25(1), 499–514.

Fernández-Palacios, H., Hernández, C. M., Fernández- Palacios, J. E., Vergara, J. M., Robaina, L., 1990. Influencia de distintas proporciones hembra: macho en la puesta de dorada (*Sparus aurata L.*). Ref. Actas III Congreso Nacional de Acuicultura. P 27-31. Editorial: Xunta de Galicia, A. Landín y A. Cerviño (eds.) (ISBN: 84-453-0149-7). Santiago de Compostela (La Coruña).

Ferrari, A. M., Volpi, L., Settembre-Blundo, D., García-Muiña, F. E., 2021. Dynamic life cycle assessment (LCA) integrating life cycle inventory (LCI) and Enterprise resource planning (ERP) in an industry 4.0 environment. Journal of Cleaner Production, 286.

Frank, A. G., Dalenogare, L. S., Ayala, N. F., 2019. Industry 4.0 technologies: implementation patterns in manufacturing companies. Int. J. Prod. Econ. 210, 15-26.

García-Celdrán, M., Ramis, G., Manchado, M., Estévez, A., Afonso. J. M., María-Dolores, E., Peñalver, J., Armero, E., 2015a. Estimates of heritabilities and genetic correlations of

growth and external skeletal deformities at different ages in a reared gilthead sea bream (*Sparus aurata L*.) population sourced from three broodstocks along the Spanish coasts. Aquaculture. 445, 33-41.

García-Celdrán, M., Ramis, G., Manchado, M., Estévez, A., Afonso, J. M., Armero, E., 2015b. Estimates of heritabilities and genetic correlations of carcass quality traits in a reared gilthead sea bream (*Sparus aurata* L.) population sourced from three broodstocks along the Spanish coasts. Aquaculture, 446, 175–180.

García-Celdrán, M., Ramis, G., Manchado, M., Estévez, A., Navarro, A., Armero, E., 2015c. Estimates of heritabilities and genetic correlations of raw flesh quality traits in a reared gilthead sea bream (*Sparus aurata* L.) population sourced from broodstocks along the Spanish coasts. Aquaculture, 446, 181–186.

Gorshkov, S., Gordin, H., Gorshkova, G., Knibb, W., 1997. Reproductive constraints for family selection of the gilthead sea-bream (*Sparus aurata*). The Israeli Journal of Aquaculture-Bamidghe, 49: 124-134.

Gjedrem, T., 2000. Genetic Improvement of Cold-Water Fish Species. Aquaculture Research 31 (1): 25–33.

Groeneveld E., Kovac M., Mielenz N., 2010. VCE User's Guide and Reference Manual Version 6.0. ftp://ftp.tzv.fal.de/pub/vce6/doc/vce6-manual-3.1-A4.pdf.

Guerrero-Cozar, I., Jimenez-Fernandez, E., Berbel, C., Espinosa, E., Claros, M. G., Zerolo, R., Manchado, M., 2021. Genetic Estimates for Growth and Shape-Related Traits in the Flatfish Senegalese Sole. ANIMALS, 11(5), 1206.

Gulzari, B., Komen, H., Nammula, V. R., Bastiaansen, J. W. M., 2022. Genetic parameters and genotype by environment interaction for production traits and organ weights of gilthead seabream (*Sparus aurata*) reared in sea cages. Aquaculture, 548(Part 1).

He, J., Zhao, Y., Zhao, J., Gao, J., Xu, P., Yang, R., 2018. Random regression analysis for body weights and main morphological traits in genetically improved farmed tilapia (*Oreochromis niloticus*). Journal of Applied Genetics. 59. 10.1007/s13353-018-0428-7.

INNOTECCS, 2014. INNOTECSS o Implementación de TECnologías INNOvadoras de mejora genética en lenguado senegalés (*Solea senegalensis*) y dorada (*Sparus aurata*) para la optimización de su producción industrial, http://giaqua.org/innotecss/

Janssen, K., Chavanne, H., Berentsen, P., Komen, H., 2017. Impact of selective breeding on European aquaculture. Aquaculture, 472 (Supplement 1), 8–16.

Kause, A., Ritola, O., Paananen, T., Mäntysaari, E., Eskelinen, U., 2003. Selection against early maturity in large rainbow trout *Oncorhynchus mykiss*: the quantitative genetics of sexual dimorphism and genotype-by-environment interactions. Aquaculture, 228(1), 53–68.

Kause, A., Ritola, O., Paananen, T., Wahlroos, H., Mäntysaari, E.A., 2005. Genetic trends in growth, sexual maturity and skeletal deformations, and rate of inbreeding in a breeding programme for rainbow trout (*Oncorhynchus mykiss*), Aquaculture, vol. 247, no. 1, 177–187.

Kause, A., Paananen, T., Ritola, O., Koskinen, H., 2007. Direct and indirect selection of visceral lipid weight, fillet weight, and fillet percentage in a rainbow trout breeding program. J. Anim. Sci. 85, 3218–3227.

Knibb, W., Gorshkova, G., Gorshkov, S., 1997. Selection for growth in the gilthead sea bream (Sparus aurata). The Israeli Journal of Aquaculture-Bamidghe, 49, 57-66.

Kolstad, K., Thorland, I., Refstie, T., and Gjerde, B. 2006. Body weight, sexual maturity, and spinal deformity in strains and families of Atlantic cod (Gadus morhua) at two years of age at different locations along the Norwegian coast. e ICES Journal of Marine Science, 63: 246-252.

Lee-Montero I., Navarro A., Borrell Y., García-Celdrán, M., Martín, N., Negrín-Báez, D., Blanco, G., Armero, E., Berbel, C., Zamorano, M. J., Sánchez, J. J., Estévez, A., Ramis, G., Manchado, M., Afonso, J. M., 2013. Development of the first standardised panel of two new microsatellites multiplex PCRs for gilthead seabream (*Sparus aurata L.*). Animal Genetics 44, 533–46.

Lee-Montero, I., Navarro, A., Negrín-Báez, D., Zamorano, M.J., Borrell, Y., Berbel, C., Sánchez, J.A., García-Celdran, M., Manchado, M., Estévez, A., Armero, E., Afonso, J.M., 2015. Genetic parameters and genotype-environment interactions for skeleton deformities and growth traits at different ages on gilthead seabream (*Sparus aurata L.*) in four Spanish regions. Anim. Genet. 46, 164–174.

Navarro A., Oliva V., Zamorano M. J., Gines R., Izquierdo M. S., Astorga N., Afonso J. M., 2006. Evaluation of PIT system as a method to tag fingerling of gilthead seabream (*Sparus auratus L.*): effects on growth, mortality and tag loss. Aquaculture 257, 309–15.

Navarro, A., Badilla, R., Zamorano, M. J., Pasamontes, V., Hildebrandt, S., Sánchez, J. J., Afonso, J. M., 2008. Development of two new microsatellite multiplex PCRs for three sparid species: Gilthead seabream (*Sparus auratus* L.), red porgy (*Pagrus pagrus* L.) and redbanded seabream (*P. auriga*, Valenciennes, 1843) and their application to paternity studies. Aquaculture, 285(1), 30–37

Navarro A., Zamorano M.J., Hildebrandt S., Gines R., Aguilera C., Afonso J.M., 2009a. Estimates of heritabilities and genetic correlations for growth and carcass traits in gilthead seabream (*Sparus auratus L.*), under industrial conditions. Aquaculture 289, 225–30.

Navarro A., Zamorano M.J., Hildebrandt S., Gines R., Aguilera C., Afonso J.M., 2009b. Estimates of heritabilities and genetic correlations for body composition traits and G×E interactions, in gilthead seabream (*Sparus auratus L.*). Aquaculture 295, 183–7.

Navarro, A., Lee-Montero, I., Santana, D., Henríquez, P., Ferrer, M.A., Morales, A., Soula, M., Badilla, R., Negrín-Baez, D., Zamorano, M.J., Afonso, J.M., 2016. IMAFISH_ML: A fully-automated image analysis software for assessing fish morphometric traits on gilthead seabream (*Sparus aurata L.*), meagre (*Argyrosomus regius*) and red porgy (*Pagrus pagrus*). Computers and Electronics in Agriculture. 121, 66-73.

Neira, R., 2010. Breeding in Aquaculture Species: Genetic Improvement Programs in Developing Countries. Proceedings of the 9th World Congress on Genetics Applied to Livestock Production. 8.

Neumaier, A., Groeneveld, E., 1998. Restricted maximum likelihood estimation of covariances in sparse linear models. Genetics Selection Evolution 1, 3–26.

Nguyen, N. H., Ponzoni, R. W., Hamzah, A., Yee, H.Y., Abu-Bakar, K., Khaw, H. L., 2010. Genetics of flesh quality in fish. Proceedings of the 9th World Congress on Genetics Applied to Livestock Production (WCGALP). 1-6.

Osawa, T., Kuchida, K., Hidaka, S., Kato, T., 2008. Genetic parameters for image analysis traits on M. longissimus thoracis and M. trapezius of carcass cross section in Japanese Black steers. J. Anim. Sci. 86, 40-46.

Pérez-Ruiz, M., Tarrat-Martín, D., Sánchez-Guerrero, M. J., Valera, M., 2020. Advances in horse morphometric measurements using LiDAR. Computers and Electronics in Agriculture, 174.

PerformFISH, 2017. Integrating Innovative Approaches for Competitive and Sustainable Performance across the Mediterranean Aquaculture Value Chain. Recovered from: http://performfish.eu/.

Rius-Vilarrasa, E., Bünger, L., Maltin, C., Matthews, K.R., Roehe, R., 2009. Evaluation of Video Image Analysis (VIA) technology to predict meat yield of sheep carcasses on-line under UK abattoir conditions. Meat Sci. 82, 94–100.

Röcklinsberg, H., 2014. Fish Consumption: Choices in the Intersection of Public Concern, Fish Welfare, Food Security, Human Health and Climate Change. Journal of Agricultural & Environmental Ethics, 28(3), 533–551.

Roo F. J., Hernandez-Cruz C. M., Fernandez-Palacios H., Izquierdo M. S., 2005 .Development of skeletal deformities in gilthead sea bream (*Sparus aurata*) reared under different larval culture and dietary conditions. In: Larvi '05 – Fish & Shellfish Larviculture Symposium (ed. by C. Hendry, G. Van Stappen, M. Wille & P. Sorgeloos), pp. 441–4. Special Publication 36. European Aquaculture Society, Oostende.

Roo F. J., Hernandez-Cruz C. M., Socorro J. A., Fernández-Palacios H., Montero, D., Izquierdo M. S., 2009. Effect of DHA content in rotifers on the occurrence of skeletal deformities in red porgy *Pagrus pagrus* (Linnaeus, 1758). Aquaculture, 287, 84–93.

Ruff, B. P., Marchant, J. A., Frost, A. R., 1995. Fish sizing and monitoring using a stereo image analysis system applied to fish farming. Aquacultural Engineering, 14, 155-173.

Rutten, M. J. M., Bovenhui, H., Komen, H., 2004. Modeling fillet traits based on body measurements in three Nile tilapia strains (*Oreochromis niloticus* L.). Aquaculture 231, 113–122.

Rye, M., Gjerde, B., Gjedrem, T., 2010. Genetic improvement programs for aquaculture species in developed countries. Proceedings of the 9th World Congress on Genetics Applied to Livestock Production. 8.

Shearer, K. D., Swanson, P., 2000. The effect of whole body lipid on early sexual maturation of 1+ age male chinook salmon (Oncorhynchus tshawytscha). Aquaculture,Volume 190, Issues 3-4, Pages 343-367.

Sonesson A.K., Woolliams, J.A., Meuwissen, T.H.E., 2012. Genomic selection requires genomic control of inbreeding. Genetics Selection Evolution.; 44(1) :27. doi:10.1186/1297-9686-44-27

Song, X., Bokkers, E. A. M., van der Tol, P. P. J., Groot Koerkamp, P. W. G., van Mourik, S., 2018. Automated body weight prediction of dairy cows using 3-dimensional vision. Journal of Dairy Science, 101(5), 4448–4459.

Stickney, R. R., 2000. Encyclopedia of Aquaculture. John Wiley & Sons, Inc., Toronto.

Turra, E. M., Oliveira, D. A. A., Valente, B. D., Teixeira, E. A., Prado, S. A., Alvarenga, E. R., Melo, D. C., Felipe, V. P. S., Fernandes, A. F. A., Silva, M. A., 2012. Longitudinal

genetic analyses of fillet traits in Nile tilapia *Oreochromis niloticus*. Aquaculture 356, 381–390.

Vandeputte, M., Mauger, S., Dupont-Nivet, M., 2006. An evaluation of allowing for mismatches as a way to manage genotyping errors in parentage assignment by exclusion. Molecular Ecology Notes, 6(1), 265–267.

Vandeputte, M., Fraslin, C., Haffray, P., Bestin, A., Allal, F., Kocour, M., Prchal, M., Dupont-Nivet, M., 2020. How to genetically increase fillet yield in fish: Relevant genetic parameters and methods to predict genetic gain. Aquaculture, 519.

Wray, N., Visscher, P., 2008. Estimating trait heritability. Nature education, 1(1), 29.

Zeder, M.A., Smith, B.D., 2009. A conversation on agricultural origins. Current Anthropology 50:681–69

Anexo:
Resumen en español

Capítulo 1. Introducción

Los programas de mejora en la acuicultura actual

La población mundial ha incrementado drásticamente durante las dos últimas décadas y la previsión es que lo siga haciendo en los años venideros. Ante este escenario, es necesario que la industria alimentaria se desarrolle de manera acorde para abastecer la creciente demanda. En este sentido, la acuicultura ha jugado un importante papel en el mercado de la producción alimentaria, creando fuentes sostenibles de proteínas y lípidos de calidad.

La producción de pescado de acuicultura es de los sectores que cuenta con más diversidad de especies y con la mayor producción (en toneladas) a nivel mundial. En 2017, cerca del 50% de las toneladas de alimento provenientes de la producción acuícola anual fueron de especies de peces.

Generalmente, las empresas productoras se diferencian en criaderos y "empresas de engorde". Los criaderos se centran en optimizar las condiciones de los especímenes reproductores y primeras etapas de la vida de los peces obtenidos de la puesta. De esta manera, se elimina la necesidad de obtener peces del medio natural y a su vez, proporcionan a las "empresas de engorde" una materia prima inicial de calidad. Las "empresas de engorde", por su parte alimentan y cuidan a los peces hasta que alcanzan el tamaño adecuado para su comercialización y consumo. En algunos casos, algunas empresas ejercen ambos roles con el fin de controlar el ciclo entero.

Como todas las industrias de producción alimentaria, a la hora de optimizar el modelo de producción, es importante considerar las características genéticas del organismo a producir y sus sinergias con el sistema de producción. Por ejemplo, los criaderos estarán interesados en obtener el mejor rendimiento de la puesta de sus reproductores, y que sus alevines tengan el mejor potencial de crecimiento en la fase de engorde, para añadir valor a su producto. Las "empresas de engorde", a su vez, valorarán un alevín que les permita obtener un producto de calidad en el menor tiempo posible.

Claves para la mejora genética: parámetros genéticos

La genética cuantitativa es esencial para llevar a cabo sección genética de manera eficaz. Los parámetros genéticos son utilizados para seleccionar individuos según su valor respecto a un carácter o característica determinados, así como su habilidad para transferir ese valor a la siguiente generación. Los parámetros genéticos más importantes son:

- Heredabilidad

  La heredabilidad es un estadístico que estima la probabilidad de variación en el fenotipo de un carácter en una población debido a la variación genética entre individuos de la población, en contraposición a la variación debido a factores ambientales.

- Valor Genético Aditivo

  El Valor Genético Aditivo (VGA) es la estima del talento genético de un animal para un carácter particular. Los VGA se expresan como la diferencia entre el valor individual y la población base a la que se compara el animal.

- Correlación genética

  La correlación genética estima la proporción de varianza que comparten dos caracteres debido a causas genéticas. Una correlación de 0 significaría independencia de los efectos genéticos sobre los dos caracteres, mientras que, si fuera de 1, la genética tendría una influencia igual en los dos caracteres, es decir, la probabilidad de heredar el talento para un carácter sería la misma que la de heredar el talento para el otro carácter.

Los parámetros genéticos no son intrínsecos de cada carácter, se estiman a partir de una población específica en unas condiciones específicas. La estima de parámetros genéticos es necesaria tanto para determinar los mejores caracteres para hacer selección (una alta heredabilidad resultaría en mayor ganancia genética en la siguiente generación) como para determinar los mejores candidatos para criar entre los individuos de una población (esos con mayor VGA para el carácter de interés). La consanguineidad, el incremento de homocigosidad, la heterosis o vigor híbrido… son otros factores que pueden influenciar la descendencia.

En programas donde los individuos se entrecruzan de manera aleatoria (como la puesta masal), es común que la consanguinidad aumente tras varias generaciones. Para prevenir esto, es importante tener en cuenta el *pedigree* de cada animal. El *pedigree* registra la ascendencia de cada animal. Los coeficientes de parentesco basados en el *pedrigree* se expresan como la proporción estimada de genoma compartido entre dos individuos. El coeficiente de parentesco de un individuo es ½ del coeficiente entre sus progenitores. De esta manera, en organismos diploides, el coeficiente de parentesco entre dos hermanos descendientes del mismo par de progenitores sería 0.25 ($(1/2)^2$) más el coeficiente de consanguinidad individual.

Programas de mejora industriales

En general, los programas de mejora industriales siguen una estructura multinivel compuesta por: núcleo de selección, multiplicadora y poblaciones comerciales. La **Figura 1** muestra la estructura de un programa de mejora estándar. El núcleo de selección está constituido por individuos de alto valor genético para uno o más caracteres de interés. Los animales del núcleo se aparean (ya sea por apareamiento natural o fertilización artificial) y producen un alto número de descendientes, que eventualmente pasan a conformar las poblaciones de reproductores en los criaderos (multiplicadora). De esas poblaciones se obtienen según le convenga a la empresa, los peces que se van a engordar (población comercial). En ocasiones donde los peces de la multiplicadora presentan un VGA para los caracteres de interés comparable (o mayor) al VGA de individuos del núcleo, pueden pasar a formar parte de éste. Teniendo en cuenta que los peces del núcleo envejecen y pierden potencial reproductivo a lo largo del tiempo, de esta manera se consigue que los productores puedan sacar beneficio de la selección a la vez que siguen mejorando la línea de manera ininterrumpida.

Como cualquier otra industria, el sector de la acuicultura enfrenta desafíos constantemente para incrementar su rentabilidad y poder crecer de manera estable. La reducción de los ciclos de producción, la mejora de la tasa de supervivencia o la mejora de la calidad del producto, pueden ser unos ejemplos. Los programas de mejora genética ya son una herramienta consolidada en la industria de la tilapia o el salmón para acercarse a estos objetivos, pues la mejora genética ofrece una mejora continua, acumulativa y permanente de los caracteres de selección (Falconer y Macay, 1996). En Europa, más del 80% de la producción en acuicultura usa animales obtenidos a partir de programas de selección (Janssen *et al*., 2007)

Nuevos caracteres de selección

Los caracteres de selección de interés suelen ser comunes en los diferentes sectores que conforman la industria alimentaria: crecimiento, resistencia a enfermedades, resistencia al estrés, rendimiento de producto, calidad de la carne, etc. Otros caracteres importantes pueden ser estéticos (como el color o la morfología) o reproductivos (como la fertilidad o la viabilidad de la progenie).

La mejora genética a través de la selección de caracteres de interés tiene sus limitaciones. Por ejemplo, el fenotipo de algunos caracteres puede verse notablemente afectado por factores ambientales, resultando en bajas estimas de heredabilidad. Un recurso común en los últimos años es la determinación de caracteres alternativos para hacer selección indirecta (Kause *et al.*, 2007; Fernandes *et al.*, 2015). Idealmente, el fenotipo de estos caracteres estaría más afectado

por la familia y tendrían una buena correlación genética con los caracteres clásicos de interés para la industria.

Al mismo tiempo, la medida de caracteres y las técnicas para la obtención de distintos tipos de datos, también se encuentran en evolución. En industrias donde los *stocks* de producción se comprenden de un elevado número de individuos, se vuelve muy relevante el uso de sistemas y métodos que permitan recoger datos de manera rápida y automática. Métodos que sean capaces de hacer medidas sin la necesidad de matar los peces y sin causarles daño o estrés son también una prioridad para el sector, dada la importancia que está cobrando en los últimos años el bienestar animal (Röcklinsberg, 2014). El desarrollo de los KET (Tecnologías Clave Facilitadoras) y las herramientas derivadas de ella, sirven para este propósito.

La implementación de novedosas tecnologías de análisis de imagen está mejorando rápidamente la eficiencia en la evaluación de caracteres de selección en peces (Pérez-Ruiz *et al*., 2020), lo que acerca al sector a la llamada "industria 4.0" (Ferrari *et al*., 2021). Las tecnologías basadas en el análisis de imagen han sido usadas con éxito en plantas y ganado para optimizar los sistemas de producción y los programas de mejora (Osawa *et al*., 2008; Rius-Vilarrasa, *et al.*, 2009; Costa *et al*., 2011; Song *et al.*, 2018; De La Iglesia *et al.*, 2020). La obtención de distintos datos de manera simultánea, rápida, repetible y fiable supone una gran ventaja para la estimación efectiva de parámetros genéticos. Estas tecnologías, además, son de carácter no invasivo y se pueden usar in vivo, reduciendo el estrés por manejo (Ruff *et al*., 1995).

Navarro *et al.* (2016) describió un método para realizar medidas de morfología en tres especies de peces (dorada, pargo y corvina), a través de un software de análisis de imagen (IMAFISH_ML), que mide caracteres tecnológicos no invasivos (*NiT*), relacionados con la morfología (*mNiT*) y la canal (*cNiT*) del pez. Este método no ha sido evaluado en cuanto a su utilidad para la determinación de componentes de genética aditiva y de las relaciones genéticas con otros caracteres de interés industrial, como los relacionados con el crecimiento.

Los caracteres de composición de la carne están estrechamente ligados a su calidad. La calidad de la carne es un concepto complejo que se compone de varios caracteres (frescura, apariencia, olor, textura, sabor, firmeza, jugosidad, etc.), la mayoría estrechamente relacionados con el contenido y la calidad de la proteína y los ácidos grasos en músculo. Estos caracteres afectan a la imagen del producto, dada su importancia para la salud y la nutrición humana, sin embargo, no está muy claro que efectos tiene la genética sobre estos caracteres (Nguyen *et al.*, 2015). Varias técnicas de laboratorio usadas para medir la composición del musculo requieren procesar y tratar las muestras de carne previamente (e.g. métodos Soxhlet o Kjeldahl, para contenido de proteína y de lípidos, respectivamente), lo que causa que estas técnicas no se consideren para los programas de selección, dada su laboriosidad.

La dorada: características principales y particularidades

La dorada (*Sparus aurata, L*) es una de las especies marinas más cultivadas en la acuicultura mediterránea. Su producción se extiende por toda la costa mediterránea, llegándose a encontrar también en áreas periféricas como Madeira o Canarias. En 2019, la producción total de dorada en Europa y el Mediterráneo se estimó en 252,406 toneladas. Es la sesentaiunava especie más producida en el mundo. Actualmente, su consumo está bien consolidado, con Italia y el Norte de Europa representando sus mayores mercados. Sin embargo, el margen de beneficio de la industria dedicada a la dorada es estrecho, principalmente debido a reducciones en el desempeño del cultivo y bajos precios de mercado (APROMAR, 2020).

Como pez teleósteo eurihalino y euritermo, puede encontrase tanto en aguas marinas como salobres en estado salvaje. Durante sus primeras etapas de vida, habita en estuarios y zonas costeras donde, como carnívoro, se alimenta de invertebrados hasta que migra a mar abierto, donde se aparea, en aguas más frías y salinas. Los juveniles cierran el ciclo al migrar de nuevo a zonas costeras más cálidas (FAO 2020).

Morfología y fisiología

La dorada es un pércido con un perfil ovalado y una cabeza alargada y prácticamente sin escamas. Los ojos se encuentran a ambos lados de la cabeza, con una línea dorada que recorre su cabeza entre ellos, de ahí su nombre. La boca es pequeña y estrecha, con una fila de caninos y dos de molares en ambas mandíbulas. Este tipo de boca es adecuada para cortar y aplastar organismos duros con cáscara, pero no para cazar o engullir grandes presas. La dorada es de color gris-plateado y tiene un punto negro al principio de la línea lateral, que cubre también la parte superior del opérculo.

Las doradas son protándricas hermafroditas, lo que significa que, cuando alcanzan la madurez sexual, desarrollan gametos masculinos y después de 2 años, o en ocasiones menos, pueden desarrollar gametos femeninos.

Condiciones de cría y cultivo

Los sistemas de cultivo más comunes utilizados para el engorde industrial de dorada son los viveros flotantes y los estuarios.

Los viveros flotantes tienen la finalidad de mantener los peces en su entorno natural durante su crecimiento (normalmente aguas costeras), limitados por redes que dejan pasar agua al interior, pero no dejan salir a los peces. Los peces pueden ser alimentados in situ -manualmente o con cañón- o de manera remota desde una plataforma de alimentación. Este sistema se considera

cultivo intensivo, ya que permite trabajar con altas densidades debido a la renovación natural del agua dentro de las unidades de producción (viveros).

Los estuarios están considerados como sistemas de cultivo extensivos o semi-intensivos. Los estuarios son hábitats naturales localizados en zonas costeras, con al menos un río que conecte con el mar. Las granjas situadas en estuarios aprovechan la topografía e hidrodinámicas de la zona y solo se necesitan pequeñas modificaciones para controlar los peces. Al ser un ecosistema natural, la dieta de los peces está compuesta (mayormente) por organismos vivos y las densidades de población son relativamente bajas.

La reproducción de la dorada a nivel industrial es llevada a cabo en criaderos (núcleo y multiplicadora) por mediante de puesta masal, organizando la ratio de sexos en términos de biomasa (Fernández-Palacios *et al*., 1990), con el fin de maximizar la calidad de la puesta. Este enfoque reproductivo es ampliamente utilizado en los programas de mejora de la especie por su coste-efectividad. Sin embargo, esto puede tener efectos negativos en cuanto a la contribución máxima de los reproductores si se compara con otras técnicas (Brown, 2003). Por otra parte, la fertilización artificial y los cruces específicos no producen huevos suficientes para satisfacer las necesidades de la producción industrial (Gorshkov *et al.*, 1997).

Los principales caracteres usados en los programas de selección genética de dorada son los relacionados con el crecimiento y la morfología, dado su impacto en los costes de producción y los precios de mercado (Chavanne *et al*., 2016). En la etapa de engorde, un crecimiento más rápido reduce costes y riesgos en la cosecha, mientras que, en los criaderos, una morfología de calidad (baja tasa de deformidad), mejora el rendimiento comercial de los alevines (Afonso y Roo, 2007). La tasa de deformidad y el crecimiento, sin embargo, están correlacionadas genéticamente (García-Celdrán *et al.*, 2015; Lee-Montero *et al.,* 2015) y, por lo tanto, la evaluación genética tiene que ser precisa y sin sesgos (Gjedrem, 2000).

Mercado y rentabilidad de la dorada en la acuicultura Mediterránea

En 2018, la producción acuícola excedió a la de pesquerías con 17,1 millones de toneladas de diferencia, siendo el sexto año consecutivo en que la acuicultura superó a la pesca extractiva en términos de tonelaje. El cultivo de dorada ha formado parte de este cambio, ya que, del consumo total de esta especie en el mundo, más del 96% fueron doradas cultivadas (APROMAR, 2020).

La producción mundial de dorada en 2018 fue de 228.576 toneladas, siendo 91.964 tn (40%) provenientes de países de la Unión Europea, 14.930 de las cuales fueron producidas en España, donde la dorada es la tercera especie más producida.

En 2015, Turquía ya era el país con más producción de dorada del mundo (**Figura 2**), después de superar a Grecia unos años antes. Varios factores contribuyeron al éxito de turco, como por ejemplo la crisis económica que azotó Europa, la competidora directa, o el apoyo e inversión por parte de la administración pública turca, junto con el la ya existente ventaja de Turquía en cuanto a costes de producción. Eso permitió a las productoras turcas poner su pescado a la venta notablemente por debajo de sus competidores griegos y tomar parte de sus posiciones de mercado. Como resultado, los precios fueron afectados a la baja (GLOBEFISH, 2015).

En 2019, el precio medio de primera venta de dorada en España fue de 4,11 €/kg, 5,6% por debajo del de 2018, y solo el 30% del total de dorada vendida en España, fue producto nacional (APROMAR, 2020).

Programas de mejora genética en dorada

En 2017, se estimó que aproximadamente el 60% de los huevos de dorada producidos en Europa provenían de programas de mejora, siendo los caracteres de selección más utilizados el crecimiento y la morfología (Janssen *et al*., 2017).

El crecimiento es un carácter complejo normalmente cuantificado a partir del peso en el momento de la cosecha (otras medidas para la cuantificación podrían ser la longitud o índices de crecimiento, como el Índice de Crecimiento Específico SGR)). Los programas de selección que se han centrado en este carácter han reportado una mejora de entre el 5 y el 29% por generación, dependiendo de la intensidad de selección (Brown, 2003; Janssen *et al.*, 2017).

La calidad morfológica es cuantificada normalmente a presencia o ausencia de deformidades en los peces. Se trata de una simplificación porque existen diferentes tipos de deformidad, con diferentes orígenes genéticos o no afectados por la genética, y a veces la determinación de la presencia/ausencia puede verse afectada por la subjetividad, lo que puede dificultar su evaluación. García-Celdrán *et al*. (2015) estimó la heredabilidad en dorada para distintos tipos de deformidad, desarrolladas bajo condiciones experimentales, con un coeficiente de entre 0.03 y 0.9, dependiendo del tipo de deformidad. Lee-Montero *et al.* (2015) reportó una estima de 0.16±0.04 para el carácter "deformidad total", en el que se contemplaba si el pez presentaba alguna deformación o no, sin diferenciar su naturaleza.

Capítulo 2: Objetivos de la tesis

El propósito de este trabajo era estudiar opciones para la optimización de los programas de mejora genética de dorada, usando distintos enfoques. Se llevaron a cabo varios experimentos usando la tercera generación del programa de mejora español de dorada PROGENSA®.

El programa PROGENSA® se originó con el proyecto que lleva su mismo nombre (Afonso *et al*., 2012), siendo el primer programa dedicado a la dorada en España. El objetivo del proyecto era sentar una base sólida para la producción de semilla de dorada de calidad en España y explorar técnicas para el desarrollo y la mejora de procesos, siempre desde el punto de vista industrial. Años más tarde, una vez el programa demostró su eficacia en términos de mejora del rendimiento productivo, se empezaron a considerar otros enfoques.

El proyecto PROGENSA-III, en el cual se enmarca esta tesis, dio comienzo en 2016. Algunas de las metas que perseguía el proyecto eran:

- La mejora genética de caracteres de crecimiento, morfológicos, de rendimiento y de resistencia a enfermedades.
- El estudio de la interacción genotipo – ambiente de esos caracteres
- El desarrollo y validación de tecnologías facilitadoras clave (KET)
- La optimización del manejo de reproductores a nivel industrial

Desde su inicio, PROGENSA® contó con la colaboración de empresas del sector acuícola español. Como un proyecto de I+D aplicado e integrativo, las empresas socias aportaron su *know-how*, así como sus mismas instalaciones, las cuales fueron esenciales para el propósito del proyecto. Esta tesis fue desarrollada en las Islas Canarias.

Objetivos específicos

Este trabajo se ha centrado en 4 objetivos específicos:

- Evaluación de la gestión de la puesta masal en términos de optimizar la contribución de los reproductores de dorada., aplicando la estrategia 4DL (recoger la puesta durante cuatro días consecutivos) en diferentes momentos de la temporada de puesta.
  Aumentar la contribución de los reproductores produciría más familias y en última 1instancia mejoraría el *pool* genético y la variabilidad en la descendencia, aportando mejor resultados en la selección genética.
- Evaluación genética de la tercera generación del programa de mejora, basada en caracteres de interés para la industria medidos al momento de la cosecha en peces criados bajo diferentes sistemas de cultivo.
  Los caracteres de interés escogidos son:

- Caracteres de crecimiento:
  - Peso corporal a la cosecha y tasa de crecimiento

    Dado que el producto de acuicultura se precia generalmente en base a su peso, la optimización del peso corporal a la cosecha (final del ciclo productivo) es un carácter muy importante para las productoras. La mejora de la tasa de crecimiento reduce la duración de los ciclos de producción, ya que se alcanza antes el peso para la cosecha. Todo esto revierte positivamente en el margen de beneficio.
  - Longitud y factor de condición a la cosecha

    Monitorear la longitud y su carácter derivado (a partir del peso), el factor de condición, es también importante, dada su relación genética con los caracteres de peso.
- Caracteres rendimiento canal y de producto:
  - Pesos canal y de filete y rendimientos de procesado

    Caracteres de interés para la industria del procesado. La mayoría de los productos procesado tienen un mayor rendimiento económico.
  - Peso de la grasa visceral

    La grasa visceral puede reducir el rendimiento de los productos procesados y el margen de beneficio, al ser una parte del pez que no se aprovecha. También puede servir como indicador de la condición de salud y bienestar del pez.
- Caracteres de la composición de la carne
  - Las proteínas y los lípidos son los nutrientes más relevantes presentes en la carne de pescado. Aunque estos caracteres están altamente afectados por la dieta que se les da a los peces, es importante saber como están relacionados genéticamente con otros caracteres de selección, así como mejorar los métodos de medición.
- Deformidad
  - La ausencia de deformidad también es un carácter de interés por su efecto directo en el rendimiento económico de la producción.

- Evaluación de nuevos caracteres derivados de KET como potenciales caracteres de selección.

  La estimación de parámetros genéticos de caracteres tecnológicos, especialmente caracteres NiT. Por medio del uso de programas informáticos de análisis de imagen y otras tecnologías para medir caracteres morfométricos, de rendimiento de producto y de composición de la carne, con el fin de dar un paso más en la integración de herramientas tecnológicas automáticas en los programas de selección genética.
- Elaboración de una propuesta para la configuración de un stock de reproductores industrial.

Capítulo 3. Materiales y métodos

Material biológico

Las doradas usadas en este estudio son parte de la tercera generación (F3) del programa de mejora genética PROGENSA®. Cuando el programa empezó, en 2009, se usaron doradas salvajes para conformar la F0. Estas poblaciones se mantuvieron en 3 localizaciones distintas de la geografía española: Canarias, en la ULPGC (Universidad de Las Palmas de Gran Canaria); Cataluña, en IRTA (Instituto de Investigación de Tecnologías Agroalimentarias) y Andalucía, en IFAPA (Instituto de Investigación y Formación Agraria y Pesquera de Andalucía, centro el Toruño). Los descendientes, de estos primeros reproductores de cada centro, se distribuyeron a los demás centros, con el fin de aumentar la variabilidad el *pool* genético. De esa F1 se obtuvo eventualmente una F2, siguiendo los protocolos correspondientes desarrollados en los proyectos de mejora PROGENSA e INNOTECS. La F3 se obtuvo a partir de reproductores seleccionados de la F2.

Manejo de reproductores

Los peces usados en esta evaluación se obtuvieron de dos poblaciones de reproductores: Elite Andalucia (B_{AND}), mantenida en IFAPA, centro el Toruño en Puerto de Santa Maria y Elite Canarias (B_{CAN}), en IU-ECOAQUA, Parque Científico Tecnológico Marino, Puerto de Taliarte, Las Palmas (PCTM). Las poblaciones se configuraron en base a su VGA, con el fin de optimizar el máximo valor medio para el carácter de peso a la cosecha, y el mínimo para la presencia de deformidad. Además de minimizar el el potencial incremento de parentesco medio entre la descendencia.

B_{AND} se conformó de 60 reproductores (9 machos y 51 hembras), mientras que B_{CAN} fue constituido por 30 reproductores (5 machos y 25 hembras). Estas proporciones tan asimétricas entre ambos sexos no fueron a propósito, las poblaciones se configuraron cuando los peces tenían 3 año de edad, pero debido a problemas operativos, la puesta se tuvo que retrasar por un año. Para entonces, muchos machos habían cambiado a hembras. B_{AND} se mantuvo bajo condiciones de fotoperiodo controlado (8 Luz:16 Oscuridad), B_{CAN} estuvo bajo condiciones de luz naturales. La temperatura del agua fue la natural. Los animales fueron alimentados con Vitalis Cal (Skretting S.A., Cojóbar-Burgos, España) y la producción de huevos fue monitoreada diariamente desde que dio comienzo la puesta (diciembre de 2015).

Puesta

Los huevos se obtuvieron por mediado de puestas masales. Se establecieron tres lotes de huevos por población de reproductores en los momentos del periodo de puesta en que la producción fue estable y de calidad: finales de enero 2016 (Lote S1), principios de febrero 2016 (Lote S2) y principios de marzo 2016 (Lote S3). En todos los casos, los huevos fueron recolectados y juntados durante 4 días consecutivos, con el fin de maximizar el número de familias de la población resultante, siguiendo el modelo 4DL, como fue descrito en Elalfy (2016).

Cultivo larvario

Las larvas fueron criadas siguiendo el protocolo descrito por Roo *et al*. (2009). El destete fue a los 40 post-eclosión y posteriormente fueron alimentados con dieta comercial (Skretting S.A., Cojóbar-Burgos, España). Por razones de baja supervivencia, los lotes S_1 en IFAPA y S_2 en PCTM fueron descartados. A los 110-130 días post-eclosión (dependiendo del lote), 2,242 animales de IFAPA (Lote S_2) fueron transferidos a PCTM y 786 de PCTM (Lote S_1) fueron transferidos a IFAPA, con la finalidad de incrementar la variabilidad genética de los grupos de engorde garantizando que la descendencia de ambos lotes de reproductores estuviera presente en todos los sistemas de producción.

Manejo de alevines

Alrededor de los 3 gramos de peso medio, los peces fueron marcados con PIT (Transpondedor Pasivo Integrado) *tag* en la cavidad abdominal, siguiendo el protocolo descrito por Navarro *et al*. (2006). En este punto, se tomó una muestra de aleta caudal de cada e y se preservó en etanol en tubos individuales, para más adelante llevar a cabo genotipado y asignación de parentesco. Los peces de S_1 y S_2 de IFAPA y PCTM se juntaron formando el lote $S_{1/2}$.

Aproximadamente dos semanas después, cuando los peces se recuperaron de la incisión del marcaje, los alevines fueron transferidos a distintas instalaciones industriales para su engorde. Peces de IFAPA y PCTM se enviaron a Explotación 1 (empresa dedicada al engorde en estuarios, en adelante FS1) y Explotación 2 (empresa dedicada al engorde en viveros flotantes, en adelante FS2), respectivamente. Las instalaciones de IFAPA se utilizaron para simular condiciones industriales de engorde en tanques en tierra. Todas las transferencias de la F3 a las instalaciones de engorde se muestran en la **Figura 3**.

Tres poblaciones se mantuvieron en condiciones de engorde en PCTM, IFAPA y Explotación 3 (en adelante FS3) (todas en tanques en tierra) con el propósito de servir de *backup* y servir más adelante para hacer selección en base al desempeño de sus hermanos. En la **Tabla 1** se muestra cómo se distribuyeron las poblaciones según su origen y su propósito (candidatos a reproductores seleccionados o sacrificio y toma de datos).

Muestreo y obtención de datos

Engorde y cosecha

Durante un periodo de 13.3 meses, los peces estuvieron bajo condiciones industriales de engorde. No se cribó ni desdobló las poblaciones. La descripción de las condiciones ambientales de cada granja se muestra en la **Tabla 2**. Los peces fueron cosechados a la edad de 600-650 post-eclosión (dependiendo del lote). El despesque desde sus unidades de cultivo se hizo siguiendo la metodología de cada empresa y el sacrificio se hizo por shock térmico en mezcla de agua hielo. El número final de peces sacrificados se muestra en la **Figura 3**.

Obtención de imágenes y evaluación de la deformidad

Todos los peces sacrificados fueron fotografiados usando una cámara digital (Olympus© FE-5035, Olympus, Shinjuku, Tokio, Japón), siguiendo el protocolo de captura de imágenes descrito por Navarro *et al.* (2016) para imágenes laterales, con el fin de analizar los caracteres NiT con el programa IMAFISH_ML.

Los peces también se evaluaron visualmente en busca de deformidades. Los peces con malformaciones en columna, opérculo, boca o aletas se clasificaron como peces deformes. Los peces solo se identificaron como deformes o no, independientemente de la naturaleza de la deformidad y el número de malformaciones, ya que, a nivel industrial, todo pez deforme es usualmente descartado.

Caracteres de crecimiento y rendimiento de producto

El peso corporal, la longitud furcal y el factor de condición de todos los peces fueron medidos según lo descrito en Aqua-Excel-ATOL (AquaExcel Project, 2013). La tasa de crecimiento diaria fue calculada a partir del peso a la cosecha y la edad. Los peces sacrificados fueron eviscerados (eliminación de todos los órganos internos presentes en la cavidad abdominal) y pesados de nuevo para obtener el peso canal. La grasa visceral también fue pesada. Finalmente, los peces se filetearon y ambos filetes (sin piel) se pesaron. Posteriormente, los filetes se conservaron individualmente en bolsas selladas al vacío (**Figura 5**) y congeladas a -20ºC.

Composición de la carne

Antes del eviscerado, los peces del grupo FS2, fueron analizados con Fish Fat Meter (FFM, Distell.com, West Lothian, Escocia) para determinar los lípidos en músculo de manera no invasiva. Posteriormente, los filetes congelados durante el muestreo se trituraron y homogenizaron. El contenido en lípidos, proteínas y humedad del filete de cada pez se analizó usando el sistema por NIR FOODSCAN LAB (FOSS, Dinamarca).

Madurez sexual en machos

A los animales del grupo más heterogéneo (FS3), se les practicó un masaje abdominal para determinar si eran fluentes en esperma. Todos los peces fueron masajeados dado que no se conocía el sexo. El objetivo era incluir este dato en la evaluación para determinar si la edad de madurez sexual tenía algún componente familiar.

Análisis de imágenes

IMAFISH_ML

Todas las imágenes tomadas de los peces fueron analizadas corriendo el algoritmo de IMAFISH_ML en el programa MATLAB (MATLAB® v.7.5.), que fue desarrollado en el trabajo de Navarro *et al*. (2016). Una vez hechas las calibraciones, el programa lleva a cabo distintas mediciones a partir de la imagen del pez (**Tabla 3**).

Edición de imágenes

Un paso clave en el algoritmo de IMAFISH_ML, antes del análisis de imagen, es la conversión de la fotografía en color a escala de grises. En este proceso, el programa identificaba, en algunos casos, aletas y sombras como parte del cuerpo del pez, ocasionando errores en las medidas. Para evitar este error las imágenes se fueron editadas con un programa (Adobe Photoshop CS. [2004], Berkeley, CA, Peachpit Press) tal y como se muestra en la **Figura 7**. Ambos tipos de fotografía, editadas y no editadas, se analizaron por igual.

Análisis genético y asignación de parentesco

El genotipado se obtuvo por medio de marcadores de microsatélites. Se llevó a cabo una supermultiplex PCR para *Sparus aurata* (SMsa1), de 11 loci, tal y como se describe en Lee-Montero *et al.* (2013).

La asignación de parentesco se hizo por medio del método de exclusión, por el cual se comparan los genotipos de los reproductores y la descendencia y se excluyen las asignaciones no posibles. Se resolvió de forma automática con el programa VIATSSIGN (Vandeputte *et al.*, 2006). El género de los reproductores fue considerado como desconocido en todos los casos.

Estimación de parámetros genéticos

Se testó la normalidad y homogeneidad de varianzas para todos los datos. Los componentes de la varianza para todos los caracteres fueron estimados por el método de máxima verosimilitud restringida (MVR) usando el siguiente modelo mixto:

$$y = X\beta + Zu + e$$

donde y es la medida observada, β los efectos fijos (unidad de engorde, tipo de instalación de engorde, región, origen, edad), u el efecto aleatorio del animal y e el error residual. Las estimas para las correlaciones genéticas entre caracteres de crecimiento medidos en distintos sistemas de cultivo sirvieron para evaluar la interacción genotipo-ambiente (Falconey and Macay, 1996). Todos los modelos fueron resueltos con el programa VCE (v 6.0) (Neumaier and Groeneveld 1998; Groeneveld *et al.*, 2010).

La magnitud de las heredabilidades estimadas fue establecida, según la clasificación de Cardellino y Rovira (1987), como baja (0.00-0.20), media (0.20-0.45), alta (0.45-0.65) y muy alta (>0.65). Las correlaciones se clasificaron como bajas (0-0.40), medias (0.40-0.60), altas (0.60-1), obviando el signo.

La respuesta correlacionada por selección indirecta de un carácter secundario (Y), sobre un carácter deseado (X), fue calculada según la fórmula de Falconer y Mackay (1996):

$$CR_X/R_X = i_Y h_Y r_A / i_X h_X$$

Donde CRx y Rx son la respuesta correlacionada y la directa, respectivamente, según el carácter de interés, i es la intensidad de selección, h es la raíz cuadrada de la heredabilidad y r_A es la correlación genética entre los dos caracteres.

Capítulo 4: resultados y discusión

Asignación de parentesco

La SuperMultiplex de 11 loci puesta a punto por Lee-Montero *et al*. (2013) se utilizó en este estudio con un 87% de éxito en la asignación, respaldando las estimaciones de parámetros genéticos que han derivado de la matriz de parentesco y en concordancia con Elalfy *et al*. (2021).

Evaluación del protocolo 2x4DL para la puesta masal

La contribución de las poblaciones de reproductores en la constitución de la F3 fue maximizada al englobar distintos lotes obtenidos de la misma población en distintos momentos del periodo de puesta. Cada uno de estos lotes fue conformado inicialmente por huevos que se recogieron durante 4 días seguidos, 4DL (Elalfy, 2016). Tal y como se muestra en la **Tabla 4**, 82 reproductores de un total de 90 entre ambas poblaciones B_{CAN} y B_{AND} contribuyeron apareándose aleatoriamente para formar un total de 217 familias. Este número se sitúa por encima de la media en cuanto a número de familias usado en programas de mejora de dorada (Neira, 2010; Rye *et al*., 2010).

En la **Tabla 5** se muestran las estadísticas en cuanto a las distintas poblaciones cuando se contemplan como poblaciones distintas (varias aplicaciones del modelo 4DL) o como una sola población (modelo 2x4DL). La distribución por cada lote de las familias en cuanto a padres, madres y número de descendientes se muestra en la **Figura 8**. En general, la contribución y el número de familias de las poblaciones aisladas fueron ambas más bajas, ya que ambas variables están muy relacionadas. Demás, tanto en $S_{1/2}$ como en S_3, aparecieron familias únicas que no estaban presentes en el otro lote. En el caso de la S3 de ambas poblaciones de reproductores, la contribución y número de familias es notablemente menor que en S_1 y S_2. Si bien los bajos valores de la S_3 se deben a un número menor de integrantes incluidos en la evaluación final, los resultados indican que la estrategia 2x4DL es una mejor opción que 4DL en términos de aumentar la contribución de reproductores y el número de familias.

Procesado de datos y análisis fenotípico

Procesado de imágenes

Las herramientas computarizadas que permiten generar y gestionar datos de manera rápida, fiable y rentable pueden marcar la diferencia en ambientes donde se trabaja con un alto número de muestras y datos. El procesado automático de imágenes mediante IMAFISH_ML (Navarro *et al.*, 2016) ha generado un total de 42,264 medidas de 18 variables en 52 minutos, de un total de 2,348 fotografías.

Caracteres NiT morfométricos y de rendimiento de la canal

Los resultados fenotípicos del análisis de mNiT y cNiT medidos por IMAFISH_ML en las distintas estaciones se muestran en la **Tabla 7**. En general, los CV (coeficientes de variación) fueron más altos para los caracteres de crecimiento (medidos por el método clásico), que para lol caracteres NiT. El CV de la longitud furcal medida manual y automáticamente (IMAFISH) fueron iguales (7.6%). Salvando las diferencias en la medida, esto apoya el uso de las tecnologías de análisis de imagen como una buena alternativa a los métodos de medida clásicos, ya que con IMAFISH se reducen los errores y el tiempo dedicado.

Parámetros genéticos y caracteres de selección

Edición de imágenes

La **Tabla 9** muestra que, en general, las heredabilidades de los mismos caracteres medidos desde fotos editadas y sin editar, fueron bastante similares. Las correlaciones genéticas fueron altas y positivas entre editadas y sin editar. Esto indicaría que ambos tipos de fotografía serían adecuados para la evaluación, y teniendo en cuenta el tiempo invertido en editar una imagen (alrededor de 1.5 minutos), se podría descartar la necesidad de editarlas, aunque se perdiera precisión en la medida en algunas imágenes.

Caracteres de crecimiento

En la **Tabla 10** se muestran las estimas de heredabilidad de los caracteres de crecimiento y NiT (imágenes no editadas), así como las de las correlaciones entre ellos. Las estimas de heredabilidad para los caracteres de crecimiento fueron medias (0.37 para peso corporal (BW) y 0.25 para longitud furcal (FL) y factor de condición (CF)). García-Celdrán *et al.* (2015) y Lee-Montero *et al*. (2015) obtuvieron estimas similares en poblaciones de dorada estudiadas en condiciones similares. Las correlaciones genéticas fueron altas y positivas entre BW y FL, mientras que la de ambas con CF fue media-baja.

Caracteres NiT morfométricos y de rendimiento de la canal

En general, las estimas de heredabilidad de los caracteres NiT, medidos de imágenes no editadas, fueron altas y con altas correlaciones positivas entre ellos. En cuanto a los caracteres cNiT, las estimas de FilA y TLA fueron un 38% más altas que para BW. Teniendo en cuenta as correlaciones genéticas de estos caracteres NiT con BW, usar FilA o TLA como carácter de selección indirecta, haría la selección un 17.4% más eficiente que seleccionando por BW. DE manera similar, los caracteres mNiT de longitud FilML and TaEL, sus estimas de heredabilidad y correlaciones con BW indican que la selección en base a estos caracteres sería un 13.9% más eficiente que seleccionando por BW en cuanto a ganancia genética. Con respecto a los

caracteres mNiT de altura, FHB, FHC y FMH fueron los caracteres con las estimas de heredabilidad más altas, todas con correlaciones altas y positivas con BW. Por lo tanto, seleccionar por FHB y FHC resultaría en una respuesta indirecta de BW de 22.5% y 22.7%, respectivamente. La estima de heredabilidad de FHE fue notablemente más baja, así como sus correlaciones con el resto de caracteres NiT. Al dividir la longitud total en equidistantes, la medida de FHE (la más posterior), depende de la morfología y longitud de la cola, lo cual puede suponer una fuente de variación. En la categoría de caracteres NiT de excentricidad, FEc mostró la mayor estima de heredabilidad comparado al resto de caracteres, indicando que la redondez del pez se ve influida de manera importante por el componente genético. Sin embargo, las correlaciones con los caracteres de crecimiento fueron bajas.

La longitud furcal fue medida de dos maneras distintas en este estudio: manual (FL) y por IMAFISH_ML (FoL). La estima de heredabilidad de FoL fue un 88% más alta que la de FL. Esto puede deberse a una mayor precisión en la medida por parte del sistema automático, que reduce la variabilidad asociada a la obtención de datos por parte del distinto personal a cargo de esa tarea. Esto respalda el uso de la metodología no invasiva en comparación a la manual, dado que la respuesta a la selección se vería incrementada aplicando la misma intensidad de selección.

Fernandes *et al.* (2015) reportó estimas de heredabilidad altas para los caracteres morfométricos relacionados con la altura en tilapia. En este estudio, las estimas de heredabilidad de los mNiT de altura medidos cerca de la cabeza (*FMH, HeH, FHA, FHB and FHC*) fueron altas, reflejando el potencial como caracteres de selección en dorada e indicando que el uso de sistemas de obtención de datos automatizado como IMAFISH_ML en programas de selección es altamente recomendable.

Caracteres de procesado y composición de la carne

En la **Tabla 11** se muestran las estimas de heredabilidad de los caracteres de crecimiento, de la canal y de composición de la carne, así como las de las correlaciones entre ellos. En las **Tablas 12.1** y **12.2** se muestran las correlaciones genéticas y fenotípicas, respectivamente, entre caracteres NiT (IMAFISH) y los caracteres de la canal y de composición.

Los caracteres de procesado o de la canal están más relacionados con la eficacia del modelo productivo y el beneficio económico que los caracteres de crecimiento (Rutten *et al*., 2004). Turra *et al.* (2012) reportó buena correlación genética entre los caracteres de crecimiento y los de la canal, así como una buena respuesta correlacionada a la selección por caracteres de la canal respecto al peso corporal en *Oreochromis niloticus*. A nivel industrial, sin embargo, no son tan priorizados en programas de selección, al ser su medida y evaluación más laboriosa.

En este estudio, las estimas de heredabilidad de los caracteres de pesos procesados (canal y filete) a la cosecha fueron más altos que para el peso corporal entero, lo cual difiere de los resultados de Navarro *et al.* (2009a) and García-Celdrán *et al.* (2015b), que reportaron estimas de heredabilidad más bajas para los pesos procesados. Por otro lado, las correlaciones entre pesos procesados y entero fueron altas y positivas, lo que concuerda con esos trabajos y con Gulzari *et al*. (2022). Una posible hipótesis para explicar esto es que, pese a que la posible variación debido al distinto personal que llevó a cabo los eviscerados y fileteado pueda estar afectando la estimación, la eliminación del paquete visceral, etc. puede estar disminuyendo la varianza ambiental que sí estaría afectando al peso entero.

Los porcentajes de eviscerado y fileteado, que están directamente relacionados con el rendimiento de producto y económico, mostraron estimas de heredabilidad bajas (Dr%) y medias (Fi%). La estima de Fi% fue notablemente más baja que la obtenida por Navarro *et al.* 2009a, pero similar a la de García-Celdrán *et al.*, (2015b), aunque todas mostraron correlaciones similares. Solo el porcentaje de fileteado mostró correlaciones altas con los caracteres de peso (entero y procesado), lo que concuerda con los resultados de Gulzari *et al.* (2022).

Los resultados mostraron una alta correlación entre caracteres de la canal medidos por métodos clásicos y por cNiT, con la excepción de Dr%. Se estimó una fuerte correlación entre caracteres relacionados con el fileteado, como FilML, FilA y TLA, y los caracteres de crecimiento y la canal. Esto indicaría, no solo que seleccionar por estos caracteres mejoraría BW a la cosecha y el rendimiento del procesado, sino que además se conseguiría usando únicamente métodos no invasivos. La selección indirecta en base a FilML sería un 63.2% y un 13.8% más eficaz que seleccionar por Fi% y BW, respetivamente, para mejorar estos dos caracteres. Además, la correlación fenotípica entre FilMl y Fi% es de 0.80.

Con respecto a los caracteres de composición de la carne, las correlaciones genéticas del contenido en humedad, grasa y proteína con los caracteres de BW a la cosecha son similares a las estimas obtenidas por Elalfy *et al.* (2021) y García-Celdrán *et al.* (2015b), donde se usaron los mismos métodos de medida (NIRS). La estima de heredabilidad para el contenido de grasa medido con FFM fue media, de un valor similar al obtenido por Gulzari *et al*. (2022). Este valor fue un 293% mayor que el valor de la estima para el contenido de grasa medido con NIRS y la correlación genética entre ambos caracteres fue alta. Sin embargo, la correlación fenotípica fue escasa y sus medias fenotípicas fueron distintas, lo que podría estar relacionado con la configuración de uno o ambos dispositivos y su algoritmo de medida y deberían revisarse antes de efectuar futuras medidas.

Deformidad total

En la **Tabla 11** se muestran la estima de heredabilidad para el carácter de deformidad total, así como las correlaciones (genéticas y fenotípicas) con los caracteres de crecimiento, caracteres de la canal y de composición de la carne. Las correlaciones genéticas y fenotípicas con los caracteres NiT de IMAFISH, se muestran en las **Tablas 12.1** y **12.2**, respectivamente.

La estima de heredabilidad para el carácter de deformidad total fue bajo (0.02). Englobar todas las deformidades en un solo carácter "global", causa que su origen genético quede diluido, ya que intervienen distintos factores según la naturaleza de la deformidad (Lee-Montero *et al*., 2015). Kause *et al.* (2005) también obtuvo una estima baja (0.02) para este carácter en trucha arcoíris. Ambos estudios también encontraron una baja pero existente correlación genética entre los caracteres de crecimiento y de deformidad. En el presunte estudio, la correlación estimada de WDef con FL y BW fue negativa (aunque baja), lo que apunta a un mayor componente ambiental incidiendo en el desarrollo de las deformidades observadas en esta población. Correlaciones negativas (también bajas) con caracteres NiT de excentricidad y positivas con CF indican que la "redondez" del pez podría estar relacionada con una alta deformidad, que encajaría con el hecho de que las deformidades más frecuentes son las relacionadas con la columna (Lee-Montero *et al*., 2015), lo que lleva a la forma del pez a doblarse y verse menos fusiforme. Otros trabajos reportan un relevante componente genético afectando a la forma del pez en lenguado (Blonk *et al*., 2010; Guerrero-Cortázar *et al.,* 2021).

Maduración sexual en machos

La madurez sexual en los peces de cultivo está asociada con una disminución de la eficiencia de la alimentación y el crecimiento en músculo durante la época de maduración, es por eso que es deseable retrasarla (Kause *et al.*, 2003; Aksnes *et al.*, 1986). La edad de maduración está afectada por la genética en salmón Atlántico (Barson *et al*., 2015). En trucha arcoíris se han observado correlaciones genéticas con el peso a la cosecha (Kause *et al.,* 2003).

La estima de heredabilidad para el carácter SAM obtenida en este estudio fue media (0.25), y las correlaciones genéticas con caracteres de crecimiento bajas y positivas, lo cual está en línea con lo observado en trucha arcoíris (Kause *et al*., 2005) y en bacalao Atlántico (Kolstad *et al*., 2006). Aunque las correlaciones con los demás caracteres fueron mayormente bajas y positivas, las más altas son las relacionadas con el metabolismo lipídico (VF, Lipid%, FFM) y el filete (Fi%, caracteres cNiT), que fueron relacionados con el desarrollo sexual en *Oncorhynchus tshawytscha* por Shearer *et al*. (2000).

Capítulo 5: Conclusiones

1. La estrategia 2x4DL incrementa el número de familias en los programas de selección de dorada respecto a la estrategia 4DL. Incluyendo en la siguiente generación individuos provenientes de puestas de dos momentos distintos del periodo de puesta. Es importante un buen manejo de las dos poblaciones para incrementar la supervivencia y poder unir ambas en un solo lote heterogéneo.

2. Es estudio muestra las estimas de parámetros genéticos para caracteres de Tecnologías No-invasivas (NiT). Los caracteres se midieron con éxito por medio del programa de análisis de imagen IMAFISH_ML de forma rápida y automática, sin necesidad de editar previamente las imágenes.

3. En general, los caracteres NiT mostraron estimas de heredabilidad altas y correlaciones robustas entre ellos y con los caracteres de crecimiento. Se determinó que los caracteres relacionados con la altura alrededor de la cabeza de la dorada fueron los que aparentaron mayor potencial como candidatos para selección genética. La selección indirecta por estos caracteres supondría una eficiencia 22.7% mayor respecto al peso corporal a la cosecha que seleccionando directamente por ese carácter.

4. Las estimas de heredabilidad de los pesos canal y eviscerado fueron más alas que la del peso entero a la cosecha y las correlaciones de ambos con este último de 1. Las correlaciones con los caracteres morfométricos también fueron altas y positivas, con excepción de los de excentricidad.

5. El rendimiento del fileteado puede mejorarse seleccionando por los caracteres de área filete o máxima longitud de filete (hasta un 63.2% de respuesta correlacionada), caracteres medidos automáticamente por IMAFISH_ML, mejorando a su vez el peso a la cosecha (en un 13.8%).

6. Las tecnologías no invasivas proporcionaron mejores estimas de heredabilidad que los métodos de medición clásicos en los mismos caracteres: filete, longitud furcal y porcentaje de grasa muscular.

7. La estima de heredabilidad para el carácter de deformidad total en este estudio fue bajo, indicando un importante efecto de factores ambientales en el desarrollo de las deformidades observadas.

8. La edad de maduración sexual en los machos de dorada se ve afectada por la genética y su estima de heredabilidad en este estudio fue 0.25.