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Genetic parameter estimations of new traits of morphological quality on gilthead seabream (*Sparus aurata*) by using IMAFISH_ML software

Sergi León-Bernabeu [a], Hyun Suk Shin [a], Álvaro Lorenzo-Felipe [a], Cathaysa García-Pérez [a], Concepción Berbel [b], Islam Said Elalfy [a,c], Eva Armero [d], Jaume Pérez-Sánchez [e], Marta Arizcun [f], María Jesús Zamorano [a], Manuel Manchado [b,*], Juan Manuel Afonso [a]

[a] *Universidad de las Palmas de Gran Canaria (ULPGC), Institute of Sustainable Aquaculture and Marine Ecosystems (IU-ECOAQUA), Aquaculture Research Group (GIA) Muelle de Taliarte s/n, 35214 Telde, Las Palmas de Gran Canaria, Spain*
[b] *Investigación y Formación Agraria y Pesquera (IFAPA, Centro El Toruño, Junta de Andalucía, Camino Tiro Pichón s/n, 11500 El Puerto de Santa María, Cádiz, Spain*
[c] *University of Damanhour, 27 Galal Qoreitem Sq., Behind Damanhour Stadium, Damanhour City, Egypt*
[d] *Department of Agronomic Engineering, Technical University of Cartagena, Paseo Alfonso XIII 48, Cartagena, Spain*
[e] *Department of Biology, Culture and Pathology of Marine Species, Instituto Torre de la Sal IATS-CSIC, venta de San Antonio-Estación, Castellón, Spain*
[f] *Spanish Institute of Oceanography, Oceanographic of Murcia, carretera de la Azohía s/n, Puerto de Mazarrón, Murcia, Spain*

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ABSTRACT

In this study, a total of 18 novel productive traits, three related to carcass [cNiT] and fifteen related to morphometric [mNiT]), were measured in gilthead seabream (*Sparus aurata*) using Non-invasive Technologies (NiT) as implemented in IMAFISH_ML (MatLab script). Their potential to be used in industrial breeding programs were evaluated in 2348 offspring reared under different production systems (estuarine ponds, oceanic cage, inland tank) at harvest. All animals were photographed, and digitally measured and main genetic parameters were estimated. Heritability for growth traits was medium (0.25–0.37) whereas for NiT traits medium-high (0.24–0.61). In general, genetic correlations between mNiT, cNiT and growth and traits were high and positive. Image analysis artifacts such as fin unfold or shades, that may interfere in the precision of some digital measurements, were discarded as a major bias factor since heritability of NiT traits after correcting them were no significantly different from original ones. Indirect selection of growth traits through NiT traits produced a better predicted response than directly measuring *Body Weight* (13–23%), demonstrating that this methodological approach is highly cost-effective in terms of accuracy and data processing time.

1. Introduction

Gilthead seabream (*Sparus aurata*) is one of the most economically important marine species in Mediterranean aquaculture. In 2019, overall production in the Mediterranean basin and peripheral areas such as Madeira or Canary Islands was estimated to be 252,406 metric tons. Currently, consumption of this species is well consolidated with Italy and North of Europe representing the largest markets, however, the aquaculture industry is still far of being profitable mainly due to a reduction of fish performance and the low market prices (APROMAR, 2020).

To improve sector profitability and promote a sustained growth, industry needs to short the production cycles, and therefore the operational costs. One strategy extensively accepted, and highly consolidated in species such as salmon or tilapia, is the use of genetic breeding programs (Janssen et al., 2017). Genetic selection offers a continuous, cumulative, and permanent improvement of the selected traits, extendable to the whole production chain (Falconer and Mackay, 1996). Up to now, it is estimated that approximately 60% of gilthead seabream eggs produced in Europe come from breeding programs, reporting 5–29% genetic gain per generation on weight at harvest depending on the selection intensity (Brown, 2003; Knibb, 2000; Navarro et al., 2009a; Thorland et al., 2015b).

Main traits of selection in gilthead seabream 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

* Corresponding author.
E-mail address: manuel.manchado@juntadeandalucia.es (M. Manchado).

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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, 1997).

The implementation of new image analysis technologies is rapidly improving the efficiency for fish trait evaluation promoting the advent of an industry 4.0 (Pérez-Ruiz et al., 2020; 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, data acquisition is carried out for several individual traits, 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*, with minor 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. The main objective of this study is to provide new genetic estimates for novel morphological traits in gilthead seabream derived from automatic image analysis and their genetic relationship with other production traits. This novel approach should help companies to optimize time in selection operations without losing precision in the genetic parameters.

2. Materials and methods

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

2.1. Biological material

Fish used in this study belonged to the third generation of PROGENSA®-III breeding program and came from two broodstock: Broodstock-1 (BS1), located at IFAPA, Centro el Toruño Puerto de Santa María, Andalusia, Spain (IFAPA); Broodstock-2 (BS2), located at IU-ECOAQUA, Parque Científico Tecnológico Marino, Puerto de Taliarte, Las Palmas, Spain (PCTM). Broodstock tanks were established according to a combined Estimated Breeding Value (EBV) that included weight at harvest and presence-absence of deformity while respecting a maximum of inbreeding rate (1%).

PROGENSA®-III selection scheme is shown in Fig. 1. BS1 was constituted by 60 breeders (9 males and 51 females), whereas BS2 by 30 animals (5 males and 25 females). These asymmetric sex ratios were not deliberately set. Broodstock were constituted when fish were three-years old, and they were kept together for one year before the trial. As these animals are hermaphrodites, some males become females producing this excess of females. BS1 was under controlled photoperiod (8L:16D), and BS2 was under natural photo/thermoperiod. Animals were fed ad libitum with Vitalis Cal (Skretting), and egg production was monitored daily since spawning started (December 2015). When total egg production became high and stable, three egg batches of each broodstock were set: one at the end of January (Batch-1; B1), one at the beginning of February (Batch-2; B2) and another at the beginning of March (Batch-3; B3). In all cases, eggs from the three batches were collected and pooled along four consecutive days to maximize family production according to the 4DL model contribution (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}. Water conditions were, for BS1: temperature 19.0 °C, salinity 34‰ and dissolved oxygen 6.4 mg L^{-1}, and for BS2: temperature 22.1 °C, salinity 37‰ and dissolved oxygen 5.61 mg L^{-1}. (Table 1, Fig. 2).

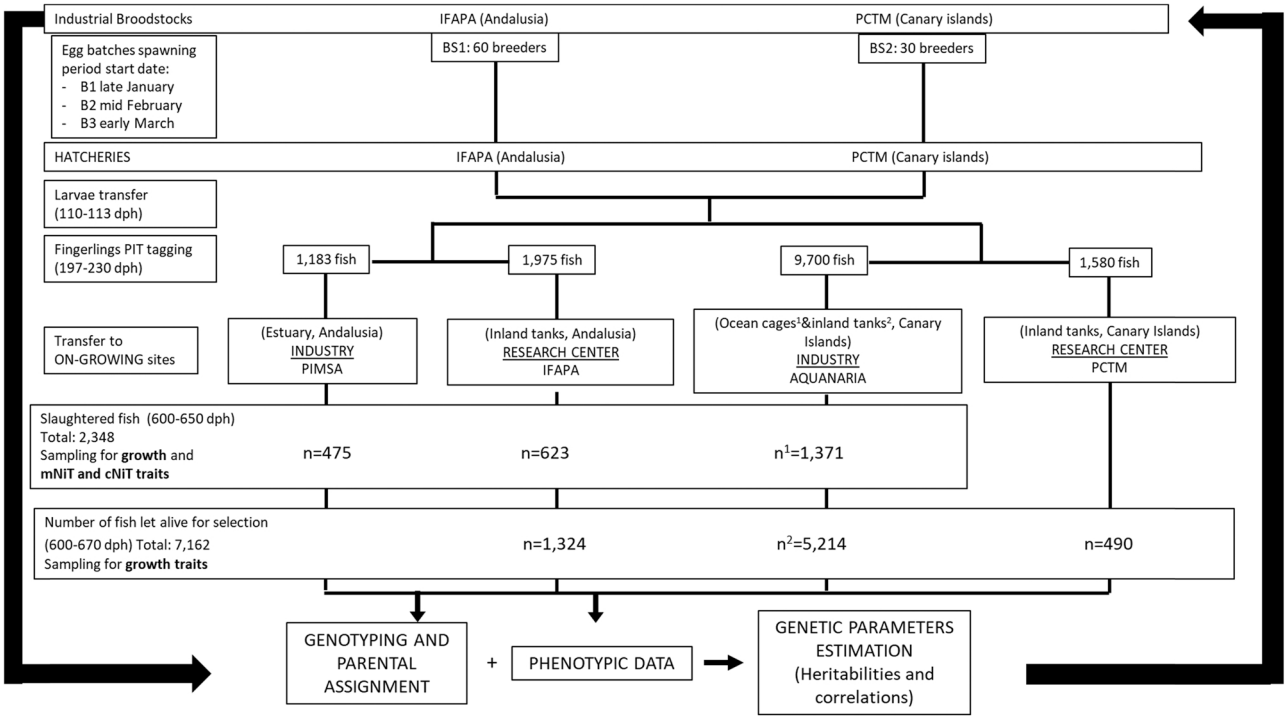

Fig. 1. Scheme of the selection breeding program for gilthead seabream (PROGENSA®-III); PIMSA: PIM. S.A.; IFAPA: Instituto de Investigación y Formación Agraria y Pesquera; AQUANARIA: Aquanaria S.L.; PCTM: Parque Científico Tecnológico Marino (PCTM-ULPGC).

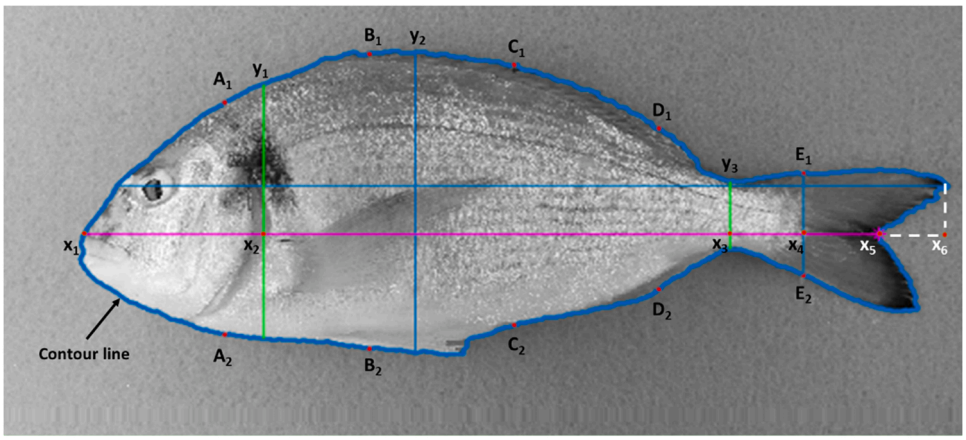

Fig. 2. Automatically detected points in gilthead seabream lateral-side images using script for lateral images of IMAFISH_ML to determinate morphometric traits: points x_1, x_2, x_3, x_4, x_5, x_6 of the anteroposterior axis are used to determine longitudinal traits; y_1, y_2, y_3, y_4 and A_{12}, B_{12}, 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.

Larvae were reared under controlled conditions (Roo et al., 2009). Not grading or scaling was performed. At 110–130 dph (depending on the egg batch), 2242 animals from IFAPA broodstock were transferred to PCTM facilities, and 786 animals from PCTM were transferred to IFAPA, to increase genetic variability and warrant that offspring from both BS-1 and BS-2 were present in the production populations. At fingerling stage, fish were intraperitoneally tagged with PIT (Passive Integrated Transponder) as described in Navarro et al. (2006). At that point, a sample of caudal fin was taken from each fish and stored in absolute ethanol to perform genotyping and parental assignment. Approximately two weeks later, fingerlings were transferred to two different industrial farming sites with different culturing systems (oceanic cage in Canary Islands, AQUANARIA; estuarine ponds in Andalusia, PIMSA), and three backup populations were kept in tanks (AQUANARIA, PCTM, IFAPA), to carry out the selection after fish evaluation.

The on-growing period spanned for 13.3 months under industrial conditions. No sorting processes were performed, and no fish were culled during that period. The description of the regional environmental characteristics and the rearing systems of the companies are described in Table 1.

2.2. Slaughtering, image capturing and sampling

Harvest was carried out at ages ranging between 600 and 650 dph (depending on the batch). Fish were slaughtered in slurring ice following the same procedures as described in Lee-Montero et al. (2015). Body weight, fork length and condition factor were measured according to Aqua-Excel-ATOL (AQUAEXCEL, 2013), (ATOL:0000351, ATOL:0001658 and ATOL:0001653, respectively, http://www.biowes.org/ontology/). Daily growth rate (DGR) was estimated from body weight along on-growing period. All fish were photographed by using a digital camera (Olympus© FE-5035, Olympus, Shinjuku, Tokyo, Japan), following the image capture protocol described by Navarro et al. (2016), for lateral images, in order to analyze 18 Non-invasive Technological (NiT) traits, including morphometric NiT (mNiT) and carcass NiT (cNiT) traits, by using IMAFISH_ML.

2.3. Image analysis

All fish images were analyzed 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 (there is also a specific *script* for calibration), the image analysis firstly identifies fish shape and thereafter it automatically performs a wide set 18 measures (Table 2).

One key step of IMAFISH_ML *script* before analysis is the conversion of all images into grayscale. This process is critical since software can accidentally recognize some non-specific fish body areas such as shadows or fins. To avoid this error, all images were carefully revised and modified if required by using an image edition software (Adobe Photoshop CS. [2004], Berkeley, CA, Peachpit Press), as shown in Fig. 3. Traits obtained from both edited and non-edited images were further analyzed to set the impact of these errors on genetic estimates.

2.4. Genotyping

DNA extraction of slaughtered and alive descendants (Fig. 1) was performed from fin samples by using *BioSprint 96 DNA Blood Kit* (Qiagen), previously digested with proteinase K (Qiagen), following the manufacturer's protocol. Genotyping was assessed by Microsatellite

Table 1
Main features and physico-chemical parameters for on-growing facilities. Feeding systems and water conditions (temperature, 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	21.8 °C	DO: 5.61 mg/l S: 37‰
Aquanaria, S.L. (*Tanks)*	Telde, Gran Canaria (Canary Islands)	Inland tanks, volume: 15 m^3 Density: 20 kg/m^3	22.3 °C	DO: 6.6 mg/l S: 36‰
Aquanaria, S.L. (*Ocean cages)*	San Bartolomé de Tirajana, Gran Canaria (Canary Islands)	Oceanic cage, Volume:80 m^3 Density: 15 kg/m^3	21.8 °C	DO: 6.1 mg/l S: 36‰
PIMSA	Guadalquivir estuary, Seville (Andalusia)	Earthen pond	18.2 °C	DO: 5.73 mg/l S: 6.9‰
Instituto de Formación Agraria y Pesquera de Andalucia (IFAPA)	Puerto de Santa María, Cádiz (Andalusia)	Inland tanks, volume: 10 m^3 Density: 20 kg/m^3	19.0 °C	DO: 6.6 mg/l S: 36‰

Table 2
IMAFISH_ML fish measurements from lateral-side fish images based on detected points depicted in Fig. 2. Eccentricity traits, FEc and HeEc, indicate how oval-shaped the whole body (caudal fin excluded) and the head are, respectively. To calculate equidistant fish heights (FHA; FHB; FHC; FHD; FHE), total lateral length (TLL) is divided into six equal parts and separating heights are determined. 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	Calculation method from image
Area (cNiT)	TLA (cm^2)	*Total lateral area*	Area delimited by contour 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 oval-shaped is fish head. It comprises the area between x_1 and x_3
	HeEc (%)	*Head eccentricity*	It indicates how oval-shaped is fish head. It comprises the area between x_1 and x_2

Multiplex PCR Analysis (MMPA), using a SuperMultiplex of 11 *loci* (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 analyzed using *GENEMAPPER* (v.3.7) software (Applied Biosystems). Finally, parental assignment was performed through exclusion method, carried out by using *VITASSIGN* program (Vandeputte et al., 2006). Breeder gender was considered as unknown.

2.5. Statistic data analysis

All data were tested for normality and homogeneity of variance by using a General Linear Model analysis, by SPSS (v270) (ᵻIBM 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 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 using 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 automatize processes, and a second one, *VCE-analysis (v1.0)*, for processing output files.

The magnitude of estimated heritability was established, following the classification of Cardelino and Rovira (1987), as low (0.05–0.15), medium (0.20–0.40), high (0.45–0.60), 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: $CRx/Rx = i_Y h_Y r_A / i_X h_X$. Where CRx and Rx 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.

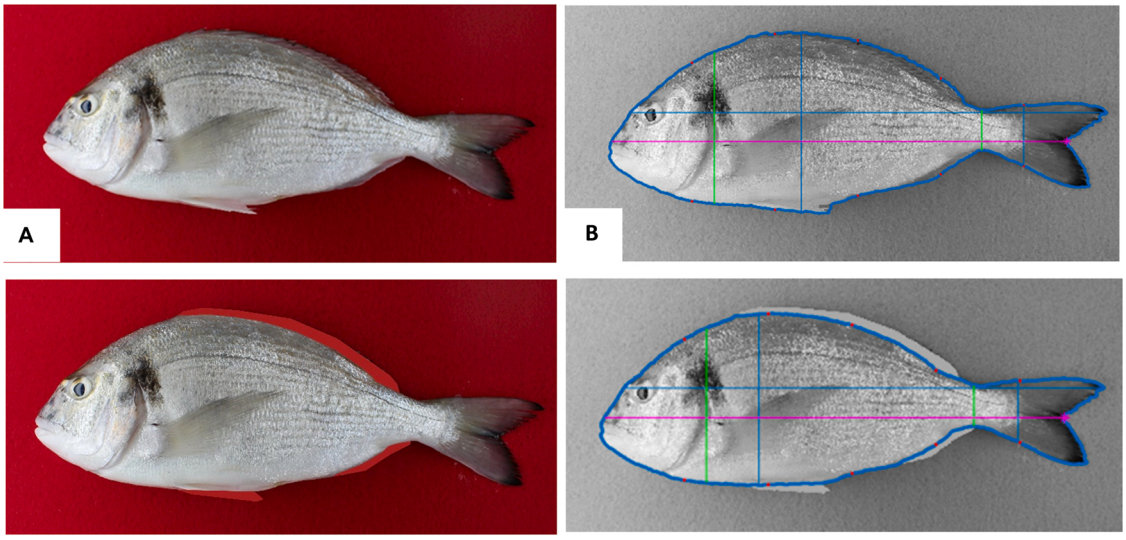

Fig. 3. Image edition and software processing. A: example of unedited image. B: IMAFISH_ML processing of unedited image original image A; C: example of edited image A. D: IMAFISH_ML processing of edited image C.

Table 3
Phenotypic data. Mean±standard error is indicated. Coefficient of variation is shown between parentheses. BW: *body weight*, FL: *fork length*, CF: *condition factor*. Significant differences between traits measured in edited and unedited images, are represented with different capital letters as superscript (P<0.05).

Growth Traits	AQUANARIA (Oceanic cage)		PIMSA (Estuarine ponds)		IFAPA (Inland tanks)	
BW (g)	520.39±3.57 (0.19)		491.32±5.94 (0.20)		357.8±4.81 (0.26)	
Growth rate (g day^{-1})	0.82±0.005 (0.19)		0.75±0.01 (0.21)		0.59±0.01 (0.26)	
FL (cm)	28.25±0.06 (0.06)		25.63±0.1 (0.07)		22.61±0.11 (0.10)	
CF	2.28±0 (0.09)		2.88±0.01 (0.07)		3.03±0.01 (0.08)	
NiT Traits	Unedited	Edited	Unedited	Edited	Unedited	Edited
Carcass						
Total lateral area (cm^2)	211.68^A±1.1 (0.14)	203.88^B±1.1 (0.14)	208.89^A±1.67 (0.13)	199.02^B±1.6 (0.13)	156.25^A±1.48 (0.18)	147.99^B±1.37 (0.18)
Fillet area (cm^2)	146.21^A±0.81 (0.15)	139.33^B±0.77 (0.15)	143.97^A±1.22 (0.14)	134.47^B±1.15 (0.14)	104.67^A±1.09 (0.20)	96.19^B±0.98 (0.20)
Fillet area %	0.69^A±0 (0.04)	0.68^B±0 (0.05)	0.68^A±0 (0.03)	0.67^B±0 (0.03)	0.66^A±0 (0.04)	0.64^B±0 (0.04)
morphometric						
Fillet maximum length (cm)	17.17±0.05 (0.08)	17.15±0.05 (0.08)	16.4±0.07 (0.07)	16.38±0.07 (0.07)	13.86±0.08 (0.12)	13.56±0.08 (0.12)
Fork length (cm)	30.31±0.07 (0.07)	30.19±0.07 (0.07)	27.87±0.11 (0.06)	27.86±0.11 (0.06)	24.06±0.11 (0.09)	24.03±0.11 (0.09)
Standard length (cm)	26.33±0.06 (0.07)	26.28±0.06 (0.07)	25.41±0.10 (0.07)	25.4±0.10 (0.07)	22.03±0.11 (0.10)	21.97±0.11 (0.10)
Tail excluded length (cm)	23.64±0.06 (0.07)	23.64±0.06 (0.07)	22.78±0.09 (0.07)	22.75±0.09 (0.07)	19.87^A±0.1 (0.10)	19.52^B±0.1 (0.10)
Total lateral length (cm)	30.5±0.07 (0.07)	30.43±0.07 (0.07)	30.11±0.11 (0.06)	30.1±0.11 (0.06)	25.73±0.12 (0.09)	25.7±0.12 (0.09)
Fish maximum heigth (cm)	10.63^A±0.03 (0.08)	10.41^B±0.03 (0.09)	11.27^A±0.05 (0.09)	10.74^B±0.05 (0.08)	9.41^A±0.05 (0.11)	9.00^B±0.04 (0.10)
Head height (cm)	9.38±0.02 (0.08)	9.36±0.02 (0.09)	9.56±0.04 (0.07)	9.56±0.04 (0.07)	8.18±0.03 (0.09)	8.16±0.03 (0.09)
Caudal pedunculus height (cm)	2.79^A±0 (0.09)	2.59^B±0.01 (0.11)	2.54^A±0.01 (0.10)	2.4^B±0.01 (0.09)	2.48^A±0.01 (0.14)	2.29^B±0.01 (0.11)
Fish equidistant height A (cm)	8.47±0.02 (0.08)	8.45±0.02 (0.08)	8.56±0.03 (0.07)	8.56±0.03 (0.07)	7.14±0.03 (0.10)	7.14±0.03 (0.10)
Fish equidistant height B (cm)	10.53^A±0.03 (0.09)	10.3^B±0.03 (0.08)	11.09^A±0.05 (0.08)	10.6^B±0.05 (0.08)	9.18^A±0.05 (0.11)	8.93^B±0.04 (0.10)
Fish equidistant height C (cm)	9. 4^A±0.02 (0.09)	9.18^B±0.02 (0.09)	9.53^A±0.05 (0.09)	9.09^B±0.04 (0.09)	8.31^A±0.04 (0.10)	7.92^B±0.04 (0.10)
Fish equidistant height D (cm)	6.46^A±0.02 (0.11)	5.44^B±0.02 (0.12)	5.95^A±0.04 (0.13)	4.66^B±0.04 (0.15)	5.89^A±0.03 (0.12)	4.58^B±0.03 (0.13)
Fish equidistant height E (cm)	3.53^A±0.01 (0.08)	3.4^B±0.01 (0.09)	3.6^A±0.02 (0.09)	3.56^B±0.02 (0.09)	3.14^A±0.01 (0.09)	3.1^B±0.01 (0.09)
Head eccentricity	0.69±0 (0.06)	0.69±0 (0.07)	0.66±0 (0.06)	0.66±0 (0.06)	0.73±0 (0.04)	0.73±0 (0.05)
Fish eccentricity	0.89^A±0 (0.01)	0.89^B±0 (0.01)	0.87^A±0 (0.01)	0.87^B±0 (0.01)	0.88±0 (0.01)	0.88±0 (0.01)

3. Results

3.1. Genotyping and parental assignment

Average parental assignment to at least one known parent using the multiplex SMsa1 PCR under the exclusion method was 87%. Some differences in assignment rates between populations were found: 79% in IFAPA and PIMSA, 80% in PCTM, and 93% in AQUANARIA. In cases when more than one parent pair was assigned and the pairs shared a parent in common, descendants were included in the relationship matrix as 'just one known parental breeder'. After checking the genotypes, all errors were identified as a null allele.

The number of breeders that contributed to the two spawns was 82 (91.1% of total breeders in PROGENSA®-III), 54 in BS1 (90% of total breeders in IFAPA) and 28 in BS2 (93% of total breeders in PCTM). The total number of full-sibling families was 217 with a mean of 21.5 descendants per family ranging between 1 and 193.

3.2. Phenotyping

The phenotypic results for growth, mNiT and cNiT traits of fish reared in the three facilities (AQUANARIA, PIMSA and IFAPA) at harvest size are shown in Table 3. For growth traits (weight, length, daily growth rate), the highest values were found in fish reared at AQUANARIA (at the Atlantic oceanic cage) whereas the fish reared at IFAPA (at inland tanks) had the lowest ones. Regarding condition factor, it was the opposite, with fish reared at IFAPA having the highest values and fish reared in AQUANARIA the lowest ones.

NiT traits characterization by IMAFISH_ML required an average processing time, for one lateral image of 1.34 s per fish photography. Required time for manually editing images using image edition software was 1.5 min on average per fish.

In concordance with growth traits, mNiT and cNiT traits of fish were significantly different between on-growing facilities (AQUANARIA, PIMSA, IFAPA). AQUANARIA had the highest and IFAPA the lowest values. This difference was maximum in cNiT traits (139.33 cm^2 and 96.19 cm^2 for FilA in AQUANARIA and IFAPA, respectively), and minimum in shape mNiT traits (0.89% and 0.88% for FEc in AQUANARIA and IFAPA, respectively). For height mNiT traits, mean values of PIMSA (estuarine ponds) were significantly higher (8.56 cm and 7.14 cm for FHA in PIMSA and IFAPA, respectively). In shape mNiT traits, fish from IFAPA had a higher mean value in HeEc than AQUANARIA and PIMSA.

The comparison of unedited and edited images for the same NiT traits indicated 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 length mNiT traits, except in TaEL at the IFAPA facility (Table 3).

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. For fork length trait, manual assessed and mNiT showed the same coefficient of variation (7.56%), also in unedited and edited measures.

Table 4
Heritability estimates and standard errors of every carcass (cNiT) and morphometric non-invasive technological traits (mNiT) 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 excluded length	0.51±0.11	0.52±0.11
	Total lateral length	0.46±0.11	0.45±0.11
Height (mNiT)	*Fish maximum height*	0.58±0.10	0.56±0.10
	Head height	0.48±0.09	0.46±0.09
	Caudal pedunculus 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 D	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

Table 5
Heritabilities (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 (in italics 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*; 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*.

Trait category	Growth traits			Carcass traits			Morphometric traits		
	BW	FL	CF	TLA	FilA	FilA%	FoL	FilML	SL
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
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
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
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
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
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
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
FilML	0.87	0.91	-0.03	0.91	0.96	0.66	0.93	**0.52±0.12**	0.99±0.00
SL	0.91	0.95	-0.03	0.97	0.95	0.40	0.99	0.94	**0.46±0.11**
TaEL	0.91	0.95	-0.02	0.96	0.95	0.42	0.98	0.95	0.99
TLL	0.90	0.94	-0.04	0.96	0.94	0.40	0.99	0.93	0.99
FMH	0.91	0.87	0.21	0.95	0.94	0.38	0.88	0.83	0.88
HeH	0.89	0.87	0.19	0.94	0.86	0.12	0.89	0.75	0.89
CPH	0.74	0.65	0.37	0.71	0.69	0.24	0.62	0.59	0.64
FHA	0.93	0.91	0.15	0.97	0.95	0.41	0.94	0.90	0.93
FHB	0.92	0.89	0.19	0.96	0.95	0.40	0.91	0.85	0.91
FHC	0.92	0.87	0.25	0.95	0.93	0.36	0.88	0.82	0.88
FHD	0.82	0.77	0.27	0.83	0.83	0.32	0.74	0.72	0.77
FHE	0.70	0.64	0.28	0.72	0.68	0.17	0.66	0.59	0.64
HeEc	-0.36	-0.28	-0.24	-0.30	-0.44	-0.76	-0.26	-0.47	-0.24
FEc	-0.23	-0.05	-0.54	-0.21	-0.22	-0.06	-0.03	0.01	-0.03

3.3. Heritability and correlations

Heritabilities and genetic correlations for NiT traits calculated using IMAFISH_ML from unedited and edited images are shown in Table 4. Heritability values from unedited images were slightly higher than edited ones, in all cases except for *Fish Equidistant Height D* (0.43 and 0.45, respectively) and *Tail Excluded Length* (0.51 and 0.52, respectively). In any case, heritability estimates for unedited and edited images were very similar, and their genetic correlations were high and positive (>0.89) for all traits.

Heritabilities and genetic and phenotypic correlations for growth and analyzed NiT traits using 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 5. As a whole, heritabilities for NiT traits were considered high showing shape mNiT traits the minimum and maximum values (0.24 for HeEc and 0.62 for FEc, respectively). Heritabilities for area cNiT traits were medium-high (0.25 for FilA% and 0.51 for TLA and FilA, respectively), high for length mNiT traits, with minimum value for SL (0.46) and maximum value for FilML (0.52) and high for height mNiT traits except for CPH and FHE that presented medium values (0.38 and 0.34, respectively). Growth traits (body weight and fork length [manual measurement], condition factor) showed medium additive genetic variation (0.25 for fork length and condition factor, and 0.37 for weight).

Genetic correlations between production systems were studied for growth traits (Table 6). Estimations were high (0.83–1.00) between IFAPA and PIMSA and between IFAPA and AQUANARIA for all traits. For condition factor trait, correlation was medium (0.42) between AQUANARIA and PIMSA, and for body weight and fork length correlation was high (0.61) between AQUANARIA and PIMSA and between AQUANARIA and IFAPA.

Genetic correlation between body weight and fork length was very high (0.94), while correlation these two traits with CF was lower (0.55 and 0.25, respectively). Genetic correlations between NiT traits of IMAFISH_ML were mostly high and positive (0.75–1). Concerning height mNiT traits, CPH showed genetic correlations between 0.50 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 correlations (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. Discussion

Searching for alternative traits to body weight 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 profitability, 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 IMAFISH_ML analysis software (Navarro et al., 2016) were evaluated.

4.1. Genotyping and parental assignment

Gilthead seabream is normally reproduced at industrial scale in hatchery (nucleus and multiplier) by mass spawning to maximize the spawn quality, including organization of sex ratio in terms of biomass (Fernández-Palacios et al., 1990). At the same time, this reproduction

Morphometric traits											
TaEL	TLL	FMH	HeH	CPH	FHA	FHB	FHC	FHD	FHE	HeEc	FEc
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
-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
-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**

approach is widely extended in their breeding programs (Chavanne et al., 2016) due to its cost-effectiveness. However, it has negative effects in terms of maximum contribution of breeders and consequently more difficulty for inbreeding control (Brown, 2003). In this study, the breeder contribution was maximized by using several batches (B1, B2, B3) from different moments along the spawning season, each one constituted by eggs from four consecutive days (Elalfy, 2016). Thus, a total of 82 breeders out of 90 (91.1%) contributed by random mating to build a total of 217 families, which is highly recommended in breeding programs (Neira, 2010; Rye et al., 2010).

Robust tools for paternity testing are essential for developing breeding programs (Navarro et al., 2008). Lee-Montero et al. (2013) developed a SuperMultiplex of 11 microsatellite *loci* (SMsa1) for parentage assignment *in S. 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).

4.2. Phenotypic data from image processing

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

For genetic selection of industrial broodstock, it is very handy to have a technology capable to generate and analyze data in a fast, reliable and cost-effective way. In this sense, tools like IMAFISH_ML software (Navarro et al., 2016) can be extremely useful as it notably reduces the time required to analyze 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, 42264 records were automatically produced by IMAFISH_ML software in only 52 min (18 novel technological traits from 2348 fish).

An on-growing facility effect was observed between locations and culturing systems; however, this experiment design did not include the evaluation of different private specific protocols. Fish reared in AQUANARIA (Oceanic cage) showed a harvest weight 1.06 times higher than PIMSA (Estuarine ponds) and 1.45 times higher than IFAPA (Inland tanks). These phenotypic values could be explained by environmental factors such as temperature (Table 1) and age at harvest (652 dph PIMSA, 601 dph IFAPA). 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.

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 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 inflated the biometric measures. On the contrary, length NiT traits did not show significant differences (Table 3 and Fig. 3). In any case, significant differences between NiT traits of different type of images (unedited and edited images) had no influence on heritability estimates.

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.

4.3. Genetic parameters

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).

Table 6
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	
	PIMSA (estuarine ponds)	IFAPA (inland tanks)	PIMSA (estuarine ponds)	IFAPA (inland tanks)	PIMSA (estuarine ponds)	IFAPA (inland tanks)
AQUANARIA (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
PIMSA (estuary)		0.83±0.16		0.91±0,10		1.00±0,01

Genetic parameters were estimated for growth traits (body weight and fork length) showing medium heritabilities with a high genetic correlation. It is in concordance with other estimates of gilthead seabream populations studied in the same localities (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 agreed 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).

Gene-environment interaction is to be expected in cases where same families are reared in different conditions (Cardelino and Rovira, 1987). In this study, the lowest genetic correlations between growth traits were found between estuarine ponds (PIMSA) and oceanic cage (AQUANARIA), 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.

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. Heritabilities of NiT traits for unedited and edited images were similar (Table 4), 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, inferred heritabilities for FilA and TLA were very similar and higher than body weight (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 body weight itself. In a similar way, length mNiT traits, FilML and TaEL, showed high heritabilities and high genetic correlations with body weight, allowing an indirect selection response 13% higher than directly by body weight. With respect to height mNiT traits, FHB, FHC and FMH were the traits with the highest heritabilities 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 body weight, respectively. FHE genetic correlations with morphometric traits were lower than others. Fish equidistant heights (A–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, 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 body weight 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 of multiple users 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 heritabilities 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) reported high heritabilities and genetic correlations, reflecting their potential as selection traits in gilthead seabream using image analysis technologies indicating that fast data recording systems such as IMAFISH_ML are highly recommendable to be used in the selection processes of this species.

5. Concluding remarks

This study reports the additive genetic determination of novel Non-invasive Technological (NiT) traits, and the genetic relationships. These 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.

In general, NiT traits showed very high heritability estimates and robust genetic correlations between them and with respect to growth traits. NiT traits that showed the highest potential were those related to dorsal height and close to the head region, positioning them 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*). These results also pave the road for studying indirect selection of other traits of interest aside of growth and morphology such as product yield, flesh composition, fecundity (Chavanne et al., 2016) by using non-invasive and automated methods. In this sense, Elalfy et al. (2021), already studied genetic interaction between growth and NiT traits with body composition.

Declaration of Competing Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Lorenzo (IUSIANI) and Alvaro Lorenzo (IU-ECOAQUA), respectively.

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