Universidad Politécnica de Cartagena

Desarrollo de un Programa Piloto de Mejora Genética en Dorada (*Sparus aurata* L.): Efecto del Origen de los Reproductores y Estimación de Parámetros Genéticos para Caracteres de Crecimiento y de Calidad

Marta García Celdrán

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Universidad Politécnica de Cartagena
Departamento de Ciencia y Tecnología Agraria

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TESIS DOCTORAL:

DESARROLLO DE UN PROGRAMA PILOTO DE MEJORA GENETICA EN DORADA (*Sparus aurata* L.): EFECTO DEL ORIGEN DE LOS REPRODUCTORES Y ESTIMACION DE PARAMETROS GENETICOS PARA CARACTERES DE CRECIMIENTO Y DE CALIDAD

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CONFORMIDAD DE SOLICITUD DEAUTORIZACIÓN DE DEPÓSITO DE TESIS DOCTORAL POR EL/LA DIRECTOR/A DE LA TESIS

Dª. EVA ARMERO IBÁÑEZ Directora de la Tesis doctoral "Desarrollo de un programa piloto de mejora genética en dorada (*Sparus aurata* L): Efecto del origen de los reproductores y estimación de parámetros genéticos para caracteres de crecimiento y de calidad".

INFORMA:

Que la referida Tesis Doctoral, ha sido realizada por Dª MARTA GARCÍA CELDRÁN, dentro del programa de doctorado TÉCNICAS AVANZADAS EN INVESTIGACIÓN Y DESARROLLO AGRARIO Y ALIMENTARIO, dando mi conformidad para que sea presentada ante la Comisión de Doctorado para ser autorizado su depósito.

La rama de conocimiento en la que esta tesis ha sido desarrollada es:

- ☐ Ciencias
- ☐ Ciencias Sociales y Jurídicas
- X Ingeniería y Arquitectura

En Cartagena, a _27 de abril de 2015

EL/LA DIRECTOR/A DE LA TESIS

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COMISIÓN DE DOCTORADO

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CONFORMIDAD DE DEPÓSITO DE TESIS DOCTORAL POR LA COMISIÓN ACADÉMICA DEL PROGRAMA

D. FRANCISCO ARTÉS HERNÁNDEZ, Presidente de la Comisión Académica del Programa TÉCNICAS AVANZADAS EN INVESTIGACIÓN Y DESARROLLO AGRARIO Y ALIMENTARIO

INFORMA:

Que la Tesis Doctoral titulada, "Desarrollo de un programa piloto de mejora genética en dorada (*Sparus aurata* L): Efecto del origen de los reproductores y estimación de parámetros genéticos para caracteres de crecimiento y de calidad", ha sido realizada, dentro del mencionado programa de doctorado, por Dª. MARTA GARCÍA CELDRÁN, bajo la dirección y supervisión de la Dra. EVA ARMERO IBÁÑEZ y del Dr. GUILLERMO RAMIS VIDAL.

En reunión de la Comisión Académica de fecha 22/04/2015, visto que en la misma se acreditan los indicios de calidad correspondientes y la autorización del Director de la misma, se acordó dar la conformidad, con la finalidad de que sea autorizado su depósito por la Comisión de Doctorado.

La Rama de conocimiento por la que esta tesis ha sido desarrollada es:

☐ Ciencias
☐ Ciencias Sociales y Jurídicas
X Ingeniería y Arquitectura

En Cartagena, a 27 de abril de 2015

EL PRESIDENTE DE LA COMISIÓN ACADÉMICA DEL PROGRAMA

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COMISIÓN DE DOCTORADO

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INDICE

1. RESUMEN

La dorada (*Sparus aurata* L.) es una especie de una importante producción acuícola. Como consecuencia de la consolidación y crecimiento de esta industria existe un interés creciente en la mejora genética para maximizar las producciones. Sin embargo, y en parte debido a las características bilógicas de esta especie, son escasos los programas de mejora genética existentes en dorada así como el conocimiento de la estructuración de sus poblaciones. Además, es sabido que el diferente origen de los lotes de los reproductores puede causar variación en la calidad de dichas producciones.

Por todo ello, el objetivo principal de esta Tesis fue obtener información relevante para el desarrollo de un programa de mejora genética en dorada. Con este fin, se obtuvo una población de alevines de dorada a partir de tres lotes de reproductores de diferentes orígenes, tamaño y proporciones sexuales [Mar Cantábrico (CAN); n = 59, 2♂:1♀; Océano Atlántico (ATL); n = 98, 1♂:1♀ y Mar Mediterráneo (MED); n = 47; 1♂:1♀]. Los alevines fueron marcados para su identificación individual con *Passive Integrated Transponder* (PIT) y criados bajo las mismas condiciones industriales hasta la edad de sacrificio. Esta población fue analizada para desarrollar diversos objetivos concretos que se centraron en estudiar algunos aspectos escasamente tratados o desconocidos en dorada.

Inicialmente se estudió la variabilidad y la estructuración genética de esta población caracterizando genéticamente, mediante marcadores microsatélites, tanto reproductores como descendientes. Además, se establecieron sus relaciones de parentesco y se estudiaron las contribuciones a la puesta. Los resultados mostraron que los tres orígenes estudiados presentaron una alta variabilidad genética y fueron diferentes genéticamente entre sí, mostrando la progenie una mayor diferenciación.

Además, las contribuciones de los reproductores de los diferentes lotes fueron desiguales por lo que su tamaño efectivo disminuyó en la progenie. Se formaron un total de 201 familias de hermanos completos, más 21 familias de medios hermanos paternos y 9 familias de medios hermanos maternos.

Dadas las diferencias genéticas observadas entre los diferentes orígenes, seguidamente se analizó el efecto del origen de los reproductores sobre caracteres de interés comercial, lo que proporcionaría información fenotípica relevante para la adquisición de un stock. Así mismo, se estimaron parámetros genéticos (heredabilidades y correlaciones genéticas) y correlaciones fenotípicas para dichos caracteres con el fin de determinar posibles criterios de selección.

Dada su importancia económica para la industria, los caracteres crecimiento y presencia de malformaciones esqueléticas externas fueron analizados a diferentes edades (163, 368 y 516 días) así como a la edad de sacrificio (690 días). Se observó un efecto del origen de forma que los individuos del MED presentaron un mayor crecimiento a lo largo del experimento, mientras que aquellos del ATL mostraron un menor crecimiento y una mayor frecuencia de malformaciones vertebrales, presentado los del CAN un crecimiento intermedio y una menor frecuencia de dichas malformaciones. Las heredabilidades fueron estimadas a distintas edades (163 y 690 días) presentando valores medios y aumentando a la edad de sacrificio. Los valores estimados a esta edad fueron 0,25±0,06 para peso y 0,22±0,07 para talla siendo las correlaciones genéticas entre ambos caracteres altas y positivas a las dos edades estudiadas. En cuanto a las malformaciones, las heredabilidades fueron 0,56[0,17-0,69] para malformaciones vertebrales y 0,46[0,20-0,90] para malformaciones operculares, estimadas mediante métodos Bayesianos. Respecto a las correlaciones genéticas con crecimiento, inicialmente se observó una correlación genética positiva entre crecimiento

y malformaciones vertebrales, (83% de probabilidad de ser positiva para la correlación peso-malformaciones vertebrales; 81% para talla-malformaciones vertebrales). Sin embargo, estas correlaciones fueron negativas a la edad de sacrificio (94,2% de probabilidad de ser negativa para peso-malformaciones vertebrales; 80,6% para talla-malformaciones vertebrales). Esta correlación positiva al inicio podría explicarse por el hecho de que los individuos que presentaron un crecimiento más rápido fueron también más propensos a la aparición de malformaciones, resultando en individuos deformes y de menor crecimiento a la edad de sacrificio.

Como continuación de trabajo anterior, se planteó el estudio de anomalías morfológicas internas, de nuevo a la edad de 163 días, a partir de un análisis radiográfico. Los juveniles del CAN presentaron una menor frecuencia de malformaciones vertebrales así como de vejigas natatorias no inflamadas. Las heredabilidades fueron importantes para lordosis (0,53[0,25-0,77]), alteraciones de opérculo (0,37[0,01-0,81]) y no inflamación de la vejiga natatoria (0,36[0,12-0,72]), existiendo una correlación genética positiva entre lordosis y la presencia de vejiga no inflamada (0,48[0,07-0,97]).

Finalmente, puesto que los consumidores de pescado muestran un interés creciente por productos de calidad, se analizaron caracteres de calidad tanto de canal como de carne a la edad de sacrificio. De nuevo se observó un efecto del origen, presentando los individuos del ATL un menor porcentaje de grasa visceral pero también un menor rendimiento y peso de la canal. Con respecto a la calidad de la carne, los individuos de CAN mostraron mayores valores de firmeza y parámetros texturales derivados. Todos los caracteres de calidad de la canal analizados presentaron heredabilidades medias (0,17-0,24) y fueron estimados con precisión (errores estándar de 0,05 a 0,07) excepto el rendimiento canal y filete. En cuanto a la calidad de la carne,

se estimaron también heredabilidades medias para grasa muscular (0,31±0,08), humedad (0,24±0,07) y firmeza (0,21±0,06). Teniendo en cuenta las correlaciones genéticas de caracteres de canal y carne con aquellos de crecimiento, seleccionar por peso aumentaría el factor de condición (0,47±0,21), la indeseable grasa visceral (0,42±0,20) y la grasa muscular (0,29±0,14) pero disminuiría del rendimiento filete (-0,58±0,09) y la firmeza (-0,34±0,14). La selección por talla aumentaría el peso de la canal (0,87±0,07) y del filete (0,84±0,09). Además el factor de condición se mostró como un criterio de selección alternativo al ser no invasivo, de fácil medida y por mejorar indirectamente el contenido de grasa visceral (-0,46±0,16).

Las diferencias observadas entre los distintos orígenes sobre caracteres de interés ponen de manifiesto la importancia de la adquisición de un stock en dorada. Las estimaciones de heredabilidad para caracteres de crecimiento y para varias malformaciones a diferentes edades indican el potencial de mejora de estas características. En vista de los resultados, sería recomendable eliminar peces deformes y seleccionar por crecimiento, analizando la evolución de caracteres de calidad de canal y de carne en generaciones sucesivas.

2. ABSTRACT

Gilthead sea bream (*Sparus aurata* L.) is the most relevant marine species in Mediterranean aquaculture. As consequence of the growth and consolidation of the gilthead sea bream industry, there is an increasing interest for genetic improvement to maximize the efficiency of its production. However, efficient breeding programs for this species are scarce, partly due to the biology of this species, and very little it is known concerning their population structure. Moreover, growth rates and the overall quality of the end product can be affected by the use of different rearing systems as well as by the different genetic origin of the stocks.

Taking into account these circumstances, the main goal of this thesis was to obtain relevant information for the establishment of successful breeding programs in aquaculture of this species. For this purpose, a population of farmed gilthead sea bream sourced from three broodstock of different origins, breeders number and sex combinations [Cantabrian Sea (CAN), n = 59, 2♂:1♀; the Atlantic Ocean (ATL), n = 98, 1♂:1♀ and Mediterranean Sea (MED) n = 47; 1♂:1♀] was obtained. Fingerlings were individually tagged for individual identification with a *Passive Integrated Transporter* (PIT) and reared under the same intensive conditions until harvest size. This population was analysed to develop several specific objectives focused on study some aspects poorly studied or unknown in sea bream.

Initially, we study the genetic variability and the genetic structure of this population using a microsatellite multiplex to genetically characterize these broodstock and their progeny. Parental assignments and contributions were also analysed. Results showed the high genetic variability of the three studied origins and their genetic differentiation. Moreover, due to breeder unequal contributions effective sample sizes

were reduced in the progenies. A total of 201 full-sib families, 21 paternal half-sib families and 9 maternal half-sib families were represented.

Given the genetic differences observed among the studied origins, the effect of the origin of the broodstock on economically important traits was analysed which provide relevant phenotypic information for the acquisition of a stock in sea bream. Genetic parameters (heritabilities and genetic correlations) as well as phenotypic correlations for these traits were estimated in order to determine possible selection criteria.

Due to their economically importance, growth rate and the presence of external deformities were studied at different ages (163, 368 y 516 days) as well as the slaughter age (690 days). The origin had an effect on these traits. Fish from MED showed the fastest growth while those from ATL showed the slowest growth and the highest incidence of vertebral column deformities, showing those from CAN an intermediate growth and the lowest frequency of these deformities. Heritabilities were estimated at initial and final ages (163 and 690 days) showing medium values increased with age. Estimated heritabilities were 0.25±0.06 for weight and 0.22±0.07 for length, being the genetic correlations between these traits high and positive at both studied ages. Regarding malformations, the heritabilities were 0.56[0.17-0.69] for deformities in the vertebral column and 0.46[0.20-0.90] for deformities in the operculum, estimated under a Bayesian approach. At earlier age, positive genetic correlations between growth and deformities in the vertebral column were observed (83% probability of being positive for weight-vertebral column deformity; 81% for length-vertebral column deformity). However, these correlations were negative at slaughter (94.2% probability of being negative for weight-vertebral column; 80.6% for length-vertebral column). This initially

positive correlation could be explained by an increased aggravation of deformities in fast growing individuals, resulting later in deformed fish with slower growing rates.

Following the previous work, we decided to analyze internal abnormalities at 163 days from a radiographic analysis. In this case, juveniles from CAN showed the lowest frequency of skeletal deformities as well as the lowest frequency of uninflated swimbladder. Considerable heritabilities were estimated for lordosis (0.53[0.25-0.77]), lack of operculum (0.37[0.01-0.81]) and uninflated swimbladder (0.36[0.12-0.72]) with a positive genetic correlation between uninflated swimbladder and lordosis (0.48[0.07-0.97]).

Finally, since fish consumers show an increasing interest in quality products, carcass and raw flesh quality traits were analyzed at the slaughter age. An effect of the origin of the broodstock was observed. In this regard, fish from ATL showed the lowest visceral fat percentage, but at the same time, the lowest dressing weight and percentage. Regarding flesh quality traits, the highest values for hardness and derived textural parameters were observed in fish from CAN. All studied carcass traits showed medium heritabilities (ranging from 0.17 to 0.24) estimated with accuracy (standard errors from 0.05 to 0.07), except dressing and fillet percentages. Regarding flesh quality traits, heritabilities were medium as well for muscular fat (0.31±0.08**)**, moisture (0.24±0.07), and hardness (0.21±0.06). Due to the fact that growth is the most economically important objective in the majority of fish genetic selection programs, we studied its genetic correlations with carcass and flesh quality traits. According to their correlations, selection for harvest weight may lead to an increase in condition factor (0.47±0.21), the undesirable visceral fat (0.42±0.20) and in fillet fat percentage (0.29±0.14) and, at the same time, to a decrease in fillet yield (-0.58±0.09) and in the flesh hardness (-0.34±0.14). Selection on length could improve dressing (0.87±0.07) and fillet weight

(0.84±0.09). Condition factor was shown as an interesting alternative selection criterion since it is easy to measure, allows a non-invasive measurement in living candidates and could lead to a decrease in the undesirable visceral fat due to their genetic correlation (0.46±0.16).

The differences observed among the different studied origins on economically important traits proved the importance of the acquisition of a stock in sea bream. Heritability estimates for growth and for several deformities at different ages indicate the potential for improvement of these traits by selective breeding using a family-based selection program. In light of the results it can be recommended to eliminate deformed fish from a breeding nucleus and later, select on growth. Further studies in upcoming generations would clarify the evolution of other traits related to the quality of the carcass and the flesh.

3. INTRODUCCION GENERAL

3.1. Marco del proyecto

El presente trabajo se desarrolló en el marco de un proyecto de gran envergadura titulado "Desarrollo de un Programa Piloto de Mejora Genética en Dorada (*Sparus aurata* L.), PROGENSA®", financiado por el Ministerio de Medio Ambiente, Rural y Marino (MARM) a través de la Secretaría General del Mar y la Junta Nacional Asesora de Cultivos Marinos (JACUMAR) de España. En este proyecto participaron cuatro comunidades autónomas, a través de diferentes instituciones: la Universidad de las Palmas de Gran Canaria y el Instituto Canario de Ciencias Marinas; el Institut De Recerca I Tecnologia Agroalimentàries, de Cataluña; el Instituto de Investigación y Formación Agraria y Pesquera de El Toruño, de Andalucía, la Universidad de Murcia, la Universidad Politécnica de Cartagena y el Servicio de Pesca y Acuicultura de la Región de Murcia. El proyecto se llevó a cabo en colaboración con el sector, por lo que también participaron diferentes empresas de engorde de dorada en las diferentes CCAA, siendo la empresa Servicios Atuneros del Mediterráneo S.L. la que cedió sus instalaciones y servicios en el caso de la CA de la Región de Murcia.

3.2. Antecedentes biológicos de la dorada

3.2.1. Características morfológicas

La dorada se caracteriza por tener un cuerpo ovalado, alto y aplanado lateralmente. Su cabeza es grande con el perfil arqueado y los labios anchos. Coloración gris plateada con una mancha oscura en el inicio de la línea lateral y una pequeña banda escarlata en el borde superior del opérculo. Aleta caudal ahorquillada. Muestra una característica banda dorada entre los ojos.

Figura 1. Visión lateral de una dorada adulta (Fuente: Propia)

3.2.2. Hábitat y biología

La dorada es una especie marina muy común en fondos arenosos, fangosos rocosos, de algas y de *Posidonia oceánica*, en profundidades de hasta 60 metros. Habita principalmente en las aguas costeras y salobres del Océano Atlántico nororiental y el mar Mediterráneo.

Es un pez euritermo y erurihalino. De este modo, soporta temperaturas bastante elevadas, creciendo muy rápido a temperaturas de 25-26 ºC dejando de alimentarse si la temperatura baja de 12-13 ºC, siendo su mínimo letal del orden de 5-7 ºC. En cuanto a su carácter eurihalino, es capaz de capaz de vivir en condiciones de salinidad variables entre el 3 y el 70 ‰.

Es una especie hermafrodita proterándrica, primero madura como macho y partir del segundo o tercer año de vida se convierte a hembra. En la naturaleza, la época de puesta se extiende de forma general de noviembre a febrero. Se alimenta principalmente de moluscos, crustáceos y peces pequeños. Puede vivir más de diez años.

3.3. Ciclo de producción

3.3.1. Reproducción y obtención de alevines

Los centros de cría ("hatcheries") producen huevos a partir de individuos reproductores en condiciones muy controladas. Cuando se desean obtener puestas fuera de la época normal de reproducción, se emplean métodos artificiales de inducción a la puesta. De este modo, se han conseguido puestas en todas las épocas del año mediante la manipulación de las condiciones ambientales (temperatura y fotoperiodo) y la administración de hormonas. El porcentaje de fecundación es alrededor del 90 % y la tasa de eclosión oscila sobre el 70-80 %, de modo que cada hembra es capaz de aportar alrededor de 500.000 larvas. Las larvas de doradas recién eclosionadas miden unos 3 mm de largo y pesan entre 0,1 y 0,15 mg. Los tanques de cultivo larvario son de volúmenes grandes, oscilando entre 5.000 y 20.000 litros para cultivo intensivo y algo mayores, de 30.000- 50.000 litros si se usan técnicas de mesocosmos. Inicialmente las larvas se alimentan de organismos vivos como el rotífero *Brachionus plicatilis*, y el crustáceo *Artemia.* Después inician una coalimentación incorporando piensos microencapsulados, hasta que la metamorfosis a alevín se ha completado, y estos se alimentan a base de piensos fabricados con ingredientes naturales, principalmente marinos.

Cuando los alevines alcanzan los 1-2 gramos son enviados a las instalaciones de preengorde donde son mantenidos hasta que alcanzan los 15-20 gramos, momento en el que son más resistentes para soportar las condiciones de las instalaciones de engorde. Esta fase de preengorde puede durar entre 45 y 120 días, dependiendo de la temperatura del agua. A una temperatura óptima de 25-26 ºC, el crecimiento es bastante rápido, y las doradas alcanzan los 20 gramos en menos de 2 meses, ya que son capaces de duplicar su tamaño en unos 10 días. La alimentación se realiza con pienso seco y los índices de

conversión son bastante bajos, oscilando entre 1,2 y 1,4 con los nuevos piensos extruidos de elevado contenido energético. En la actualidad, se han ubicado en el litoral español varias instalaciones especializadas en preengorde que compran los alevines a los criaderos con 0,5-2 g y que los engordan hasta el peso demandado por las diferentes instalaciones de engorde. De hecho, el engorde de doradas es la principal piscicultura marina existente en España.

3.3.2. Sistemas de producción

En las instalaciones de engorde, las doradas alcanzan la talla comercial (350-400 gramos), proceso que puede durar entre 18 y 24 meses desde la eclosión del huevo, dependiendo sobre todo de la temperatura del agua. La talla comercial abarca desde los 250 gramos hasta más de 1.500 gramos En esta fase, el alimento es exclusivamente pienso seco comercial y los índices de conversión pueden oscilar entre 1,5 y 2.

Las doradas pueden ser engordadas en diferentes instalaciones y bajo diferentes sistemas de cultivo asociados. De este modo se engordan doradas en estanques, lagunas costeras y esteros, propios del sur de España, donde se realiza una acuicultura extensiva, o semiintensiva. La acuicultura extensiva consiste en sistemas de cultivo de baja densidad de cría (kg/m^3) y tecnología, en los que se aprovechan condiciones naturales favorables, con una mínima intervención física sobre el medio.

También se engordan doradas bajo sistemas de cultivos intensivos, es decir, más controlados, mayores densidades de cría y de mayor rendimiento, en los que el grado de tecnología e intervención es mucho mayor a los extensivos. Este tipo de cultivo se realiza en tanques, situados en instalaciones con base en tierra firme y que obtienen su agua mediante bombeo desde captaciones en el mar. Sin embargo, la producción de doradas se realiza sobre todo en jaulas flotantes constituidas por una red sumergida en la

masa de agua y sujeta a una corona de flotación y una barandilla superior. Tienen un diámetro comprendido entre los 16 y los 25 metros, y una profundidad de 10-12 metros de red. Esto supone que cada jaula tiene un volumen de 2.000-6.000 m^3 de capacidad. La máxima densidad asegurable en el caso de jaulas es 20 kg/m^3.

3.4. Situación actual del sector acuícola de dorada

La dorada (*Sparus aurata* L.) es una especie de una importante producción acuícola. Existe producción de dorada de acuicultura en 19 países siendo los principales productores Grecia, con aproximadamente 75.000 toneladas, lo que representa el 41,7% de la producción total, Turquía con 41.700 toneladas (23,2%) y España con 16.795 toneladas (9,3%).

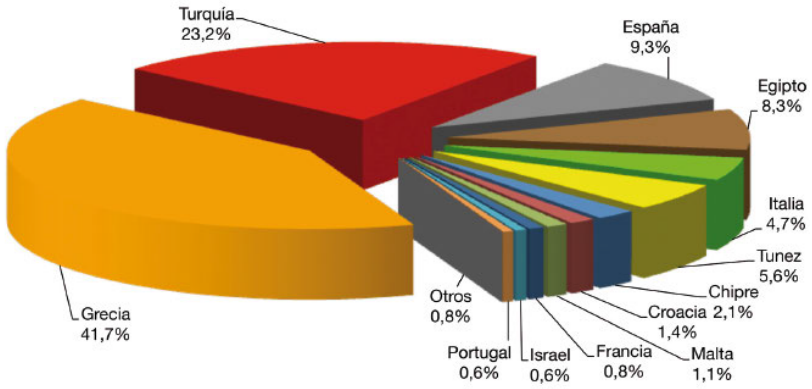

Figura 2. Distribución porcentual de la producción de acuicultura de dorada en el área mediterránea en 2013. (APROMAR, 2014)

A nivel nacional la producción de dorada se reparte en cinco comunidades autónomas. La comunidad Valenciana encabeza la producción con 6.974 toneladas (el 42% del total), seguida por Murcia (3.730 toneladas, 22%), Canarias (3.016 toneladas, 18%), Andalucía (1.786, 11%) y Cataluña (1.292, 8%).

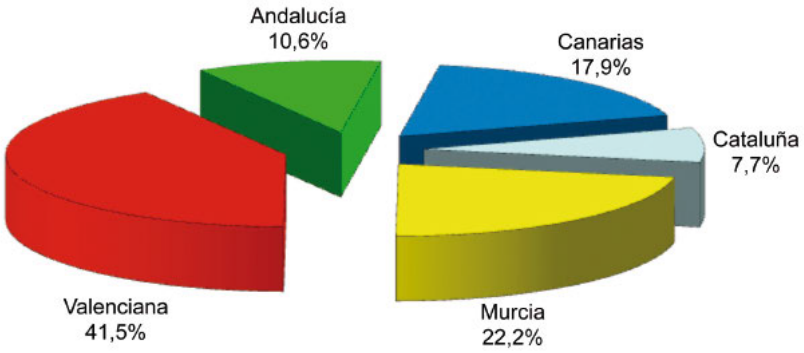

Figura 3. Distribución porcentual de las producciones de dorada en España por CC.AA. en 2013. (APROMAR, 2014)

El precio medio de primera venta de dorada en acuicultura producida en España en 2013 fue de 4,79 euros/kg mientras que el precio medio de venta al público fue 7,23 euros/kg.

La producción de alevines de dorada en España se concentra en la Comunidad Valenciana, Islas Baleares, Cantabria y Andalucía y en 2013 fue de 51,4 millones de unidades. En cualquier caso, la producción española de dorada de talla comercial requiere de la importación de juveniles adicionales a los de producción propia. De este modo, se estima que en 2013 se importaron un total de 32,4 millones de alevines desde Grecia y Francia. Simultáneamente, se exportaron, aproximadamente, 2 millones de juveniles de dorada desde España a Portugal. El precio medio de venta de los alevines de dorada comercializados en España en 2012 fue de 0,21 euros (APROMAR, 2014).

3.5. Establecimiento de un programa de mejora genética en dorada

Como consecuencia de la consolidación y crecimiento de la acuicultura existe un interés creciente en la mejora genética para maximizar las producciones. Sin embargo, debido a las características bilógicas de la dorada, a la falta de fondos, infraestructura, y metodología, son escasos los programas de mejora genética existentes en esta especie.

3.5.1. Matriz de parentesco

La implementación de programas de mejora requiere el conocimiento del parentesco entre los individuos, imprescindible para poder estimar parámetros genéticos. A nivel industrial, las puestas masales constituyen la estrategia más extendida para llevar a cabo la reproducción. Dichas puestas se establecen a partir de lotes de reproductores constituidos por un elevado número de peces, entre 40 y 60 animales aproximadamente, en una ratio de dos machos por cada hembra. Desde el punto de vista genético, la puesta masal tiene la ventaja de minimizar las fuentes de parecido por ambiente común entre los miembros de la misma familia (Herbinger et al., 1999) pero tiene el inconveniente de que imposibilita reconocer la genealogía de los peces, tarea que se complica en el caso de la dorada dadas las características biológicas de esta especie. La dorada es una especie hermafrodita proterándrica, primero madura como macho y a partir del tercer año se convierte a hembra. Además, como hembra es capaz de poner un número variable de huevos que oscila entre 500.000 y 6.000.000 huevos/kg de hembra. Por otro lado, es una especie muy fecunda (la fecundidad es del orden de 800.000 huevos de media por kilogramo de hembra) de manera que un número reducido de reproductores podría usarse para llevar a cabo la reproducción y la obtención de juveniles, de forma que la empresa ahorraría costes y mano de obra. Es más, en ocasiones los mismos individuos son utilizados en la reproducción, actuando inicialmente como machos y posteriormente como hembras. Sin embargo, esta estrategia podría disminuir el tamaño efectivo de la población, aumentando el riesgo de consanguinidad, con la consiguiente disminución de los caracteres productivos. Además, las contribuciones familiares pueden ser desiguales bajo puestas masales. El uso simultáneo de sistemas de marcaje físico mediante *Passive Implant Transponder*, (PIT) y marcadores genéticos, como los microsatélites, permite trazar la matriz de

parentesco de los peces durante el proceso de cría y engorde de los mismos. Los microsatélites pueden ser combinados en PCRs múltiplex para reducir costes. De hecho, en el caso de la dorada, cada vez son más las múltiplex desarrolladas a partir de diferentes microsatélites. Sin embargo, el genotipar con distintos marcadores tiene como consecuencia no poder comparar estudios, además de crear la incertidumbre entre los investigadores y empresas a la hora de decidir qué marcadores o múltiplex utilizar. Ante esta necesidad, en el marco del PROGENSA® se desarrolló una Súper-Múltiplex (SMsa1) (Lee-Montero et al., 2013) eficaz, fiable y repetible, propuesta como como el primer panel de referencia de marcadores microsatélites en dorada y que fue puesta a punto con la colaboración de todas las CCAA participantes en el proyecto.

3.5.2. Población base

Para el desarrollo de un programa de mejora genética es importante conocer la variación genética de la población de partida, en la medida que la variación genética condiciona la respuesta a la selección a corto y largo plazo (Falconer y Mackay, 1996). Existen evidencias de la alta variabilidad genética de la dorada, poniendo de manifiesto la falta de presión de selección a la que ha sido sometida esta especie, lo que sería una de las razones de su éxito en la acuicultura.

Por otro lado, para establecer las directrices apropiadas para encontrar y mantener los stocks son necesarios datos a gran escala sobre la estructuración de las poblaciones (Alarcón et al., 2004). Sin embargo, la información disponible sobre la estructuración de las poblaciones en dorada es escasa y contradictoria (Arabaci et al., 2010) encontrándose diferentes grados de diferenciación genética entre estas poblaciones, inferidas por microsatélites.

3.5.3. Caracteres de interés

Antes de iniciar un programa de mejora genética se deben definir los objetivos u objetivo de mejora, es decir, aquellos caracteres de importancia económica que pueden variar según la especie e incluso la población (Gjedrem, 2000). La estimación de parámetros genéticos (heredabilidades y correlaciones genéticas) para dichos caracteres aportará información sobre cuales son susceptibles de ser mejorados lo que permitirá establecer criterios de selección (Falconer y McKay, 1996).

En dorada, como en otras especies criadas para el consumo humano, los caracteres de crecimiento son los más importantes económicamente ya que los costes de producción pueden reducirse significativamente mediante la reducción de la duración del ciclo de cría (Saillant et al., 2006). Por esta razón, el crecimiento es generalmente el primer objetivo en los programas de mejora de diferentes especies.

El siguiente carácter con repercusión en la producción es la calidad del pez, tanto a nivel de alevín como a talla de sacrificio, con respecto a la presencia de malformaciones morfológicas. En la industria de la acuicultura, las pérdidas debidas a la presencia de estas malformaciones ocurren en dos niveles; en los criaderos, reduciendo la tasa de supervivencia de las larvas y la eficiencia de crecimiento en peces malformados, y en las empresas de engorde, donde los peces deformes a la talla de comercialización tienen que ser descartados o vendidos a precios más bajos que los precios de mercado ya que dichas malformaciones son claramente evidentes, especialmente en aquellas especies como la dorada que se comercializan principalmente como pez entero. Por lo tanto, reduciendo la incidencia de larvas malformadas, disminuiría el coste de producción, tanto en los criaderos como en las empresas de engorde, a la vez de mejorar la calidad de las producciones (Fernández et al., 2008). Las

principales malformaciones en peces pueden agruparse en cinco categorías: forma, pigmentación, escamas, esqueléticas y vejiga natatoria (Divanach et al., 1996). De estas, las esqueléticas son las más importantes ya que afectan de manera severa la morfología de los peces.

En la actualidad, los consumidores de pescado muestran un interés creciente por productos de calidad a la vez que no están dispuestos a pagar un precio excesivo por ellos (Gjedrem, 1997). Esto, unido a la competitividad de las empresas, está haciendo cada vez más relevantes los caracteres de calidad. En este sentido la composición del músculo juega un papel principal a través de atributos de calidad de la carne como son el sabor, la jugosidad y la textura. Además, está aumentando el interés por caracteres de calidad relacionados con los rendimientos de la canal y del filete por parte de los consumidores y las empresas de transformación de pescado (Neira et al., 2004) ya que el mercado de dorada en filete está creciendo (Luna, 2006) abriendo nuevas posibilidades de comercialización.

Todos los caracteres comentados tienen interés comercial y deben ser incorporados en los objetivos de los programas de mejora. Sin embargo, son pocos los parámetros genéticos que han sido publicados para dichos caracteres en dorada, y menos aun los que se han estimado bajo condiciones industriales.

Por otro lado, en especies marianas se ha demostrado que el diferente origen genético de los lotes de los reproductores puede causar variación en los caracteres de interés comentados (Ayala et al., 2010). Sin embargo, no encontramos investigaciones anteriores en las que este hecho se haya estudiado en dorada cuando aportaría información fenotípica relevante a la hora de la elección del stock.

3.5.4. Interacción Genotipo-Ambiente

Finalmente, es importante comentar que las variaciones existentes en la localización de las instalaciones de dorada pueden dar lugar a resultados muy dispares en la respuesta a la selección para los diferentes caracteres de interés para los piscicultores. Las diferencias ambientales pueden producir una desviación ambiental en la varianza fenotípica. Si esta desviación no es independiente del genotipo sobre el que actúa, es porque existe una interacción genotipo-ambiente (Falconer y Mackay, 1996). Por esta razón, como parte de los objetivos globales del PROGENSA®, se estimó dicha interacción, engordando doradas de distintos orígenes (distintos genotipos) en las diferentes instalaciones de cada comunidad autónoma (jaulas insulares en Canarias, esteros en Andalucía y jaulas continentales en Cataluña y Murcia) y se llevó a cabo el estudio de caracteres de crecimiento y malformaciones (Lee-Montero et al., 2014). Las correlaciones genéticas fueron en general altas y positivas en todas las variables que presentaron heredabilidad distintas de cero, indicando la ausencia de interacción genotipo-ambiente entre sistemas de engorde y comunidades autónomas.

4. OBJETIVOS

Teniendo en cuenta todas estas circunstancias, y con el fin de obtener información relevante para el desarrollo de un programa de mejora genética en dorada, los objetivos específicos del presente trabajo fueron:

- Estudiar la variabilidad y la estructuración genética de esta especie, así como analizar las contribuciones familiares de diferentes lotes de reproductores.

- Analizar el efecto del origen de los reproductores sobre caracteres de interés comercial: Crecimiento (peso y talla), calidad de pez (malformaciones esqueléticas y anomalías morfológicas), calidad de la carne (composición muscular: colágeno, grasa, humedad y proteína, y parámetros texturales) y calidad de la canal (factor de condición, grasa visceral, peso canal, rendimiento canal, peso filete, rendimiento filete).

- Determinar parámetros genéticos (heredabilidades y correlaciones genéticas) así como correlaciones fenotípicas para dichos caracteres.

Teniendo en cuenta los objetivos mencionados, la presente tesis doctoral se ha extructurado en los siguientes cinco capítulos que se corresponden con cinco artículos científicos, tres de ellos ya publicados y dos en revisión. El capítulo I centrado en el estudio de la variabilidad genética de los diferentes lotes y estructura familiar, y los siguientes capítulos enfocados en la estima del efecto del origen de los reproductores y de los parámetros genéticos para caracteres de crecimiento y malformaciones externas

(capítulo II), anomalías morfológicas internas (capítulo III), calidad de canal (capítulo IV) y calidad de carne (capítulo V).

I. Genetic assessment of three gilthead sea bream (*Sparus aurata* L.) populations along the Spanish coast and of three broodstocks managements.

II. 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.

III. Genetic determination of skeletal deformities and uninflated swimbladder in a reared gilthead seabream (*Sparus aurata* L.) juvenile population sourced from broodstocks along the Spanish coasts.

IV. 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.

V. 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.

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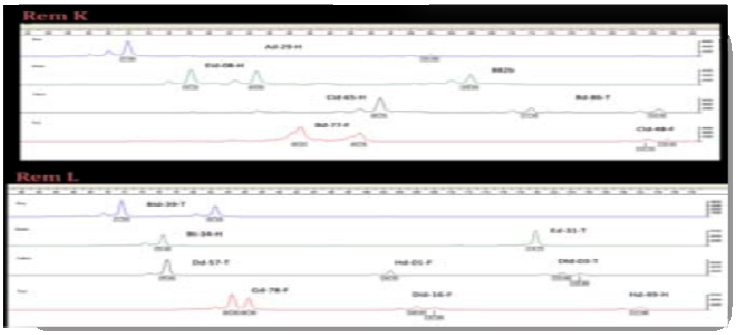

CAPÍTULO I

I. Genetic assessment of three gilthead sea bream (*Sparus aurata* L.) populations along the Spanish coast and of three broodstocks managements.

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Aquaculture International. En revisión.

Abstract

The gilthead sea bream (*Sparus aurata* L.) is one of the most important Sparid farmed in Europe, especially in the Mediterranean area. However, efficient breeding programs for this species are scarce and very little it is known concerning their population structure. The present study was mainly designed to genetically characterize, by microsatellite markers, three gilthead sea bream populations sampled along the Spanish coast (Cantabrian Sea, the Atlantic Ocean and Mediterranean Sea) and their progeny with the aim of studying the genetic variability and the genetic structure of this species. Moreover, we evaluated different strategies of broodstocks management (breeders' number, origin and sex combination) on parental contributions and on effective breeding numbers. That number of breeders in the stock was of great importance to the maximization of contribution since the larger broodstock contributed larger number of families. Due to breeder unequal contributions, effective sample sizes were reduced in the progenies and, consequently, the inbreeding rate increased. Our results highlight the high genetic variability of this species, as well as the existence of three genetically differentiated populations along the Spanish coast. These findings should be relevant for the establishment of successful breeding programs in aquaculture of the gilthead sea bream.

Introduction

The gilthead sea bream (*Sparus aurata* L.) inhabits mainly in the coastal and brackish waters of the north-eastern Atlantic Ocean and the Mediterranean Sea; being one of the most important Sparid farmed in Europe. Main producers of this species are Greece (40.9%), Turkey (22.7%) and Spain (11.0%). One hundred percent of the gilthead sea bream production in Spain is shared among five regions with Valencia (50.0%) and Murcia (20.0%) being the most important producers followed by Canary Island (14.1%), Catalonia (8.1%) and Andalucía (7.9%); (APROMAR 2013).

As consequence of the growth and consolidation of the gilthead sea bream industry, there is an increasing interest for genetic improvement to maximize the efficiency of its production. However, breeding programs are scarce due to the lack of funding and appropriate infrastructure and to the biology of this species. Gilthead sea bream is a prontandrous hermaphroditic, mass-spawing species in which individuals are males during the first two years of life and then gradually become females. Mass spawning prevents knowing the genealogy of fish which is essential to estimate genetic parameters and to implement breeding programs. Moreover, this Sparid is a highly fecund species therefore, only few broodstocks could be used in the production of a high number of juveniles in order to save money and labour in hatcheries. However, this strategy may result in lower effective population size increasing the risk of genetic drift, inbreeding and the related decrease in productive traits. The contribution of individual fish to the offspring is usually unknown in gilthead sea bream commercial farms and the only mating variables under control are the size and sex composition of the broodstocks. Nevertheless, in order to establish breeding programs in this kind of industrial production, and minimize the effects of inbreeding, fish progeny need to be traced back to their family groups. This matter can be solved by using both, physical tagging and

genetic identification of the exploited fish (Navarro et al. 2009). The use of molecular markers such as microsatellites allows pedigree reconstructions and thus, detection and avoidance of inbreeding. Moreover, these markers have great sensitivity in detecting genetic variability within and between populations so they are suitable for distinguishing slightly differentiated populations (Šegvić-Bubić et al. 2011) being the knowledge of all these parameters a prerequisite for fishery management. In addition, the development of multiplex PCRs allows geneticists to reduce the economic cost per reaction. Several multiplex PCRs have been described for gilthead sea bream; Batargias et al. (1999), Launey et al. (2003), Brown et al. (2005); and more recently Navarro et al. (2008), Porta et al. (2010) and Borrell et al. (2011). In our study we used the first reproducible, uniform and standardized gilthead sea bream panel (SMsa1; Super Multiplex *Sparus aurata*) which consisting of eleven high-quality and variability specific markers (Lee-Montero et al. 2013).

Large-scale data on the geographic structure of wild and cultivated sea bream populations are needed for setting up suitable guidelines for founding and maintaining cultivated stock (Alarcón et al. 2004). However, very little is known and conflicting data has been obtained concerning their population structure (Arabaci et al. 2010). Subdivision of this species needs to be clarified, particularly by investigating the cycle of life, ecology and demographics of wild populations, since key information on their biology is missing, in particular about spatial distribution.

Given the unresolved genetic status of populations of gilthead sea bream, as well as the lack of efficient breeding programs in this species, the aim of our study was to genetically characterize by microsatellite markers three populations sampled along the Spanish coast (Cantabrian Sea, the Atlantic Ocean and Mediterranean Sea) with the goals of: A) to study the genetic variability and genetic structure of this species. B) To

assess three broodstocks management (breeders' number, origin and sex combination) on parental contributions and on effective breeding numbers.

Materials and methods

Broodstock and offspring

In a first step, samples of adult sea bream were captured from wild populations from three geographically differentiated origins; Cantabrian Sea (CAN), the Atlantic Ocean (ATL), and Mediterranean Sea (MED), hence from the Northern, Southern, and Western Spanish coasts respectively. In a second step, broodstock from different origins were composed of different breeders number and sex combinations in different Spanish facilities: Instituto Canario de Ciencias Marinas, ICCM, Canarias (N = 59, 2♂:1♀); centro IFAPA, El Toruño, El Puerto de Santa María, Andalusia (N = 98, 1♂:1♀); and Instituto de Investigación y Tecnología Agroalimentaria, IRTA, Catalonia (N= 47, 1♂:1♀); respectively where they were not subjected to artificial selection. To induce spawning, broodstocks were cultivated under controlled photoperiod (6L:18D) and eggs were collected by buoyancy the same two consecutive days at each facility. Larvae obtained from these mass-spawnings were incubated for 48 h and later distributed in tanks and reared in the conditions described by Roo et al. (2009). Finally, a random sample of 1300 offspring (576, 363 and 361 from CAN, ATL and MED, respectively) was collected simultaneously at each facility.

PCR reaction and genotyping

Breeders and juveniles were genetically characterized. For this purpose, DNA was extracted from the caudal fin, previously preserved in absolute ethanol at room temperature, using the DNeasy kit (QIAGEN®) and then kept at 4°C following the

manufacturer's recommendations. DNA quality and quantity were determined using Nanodrop 2000 spectrophotometer v.3.7 (Thermo Fisher Scientific, Wilmington, U.S.A.). We used the SMsa1 (Super Multiplex *Sparus aurata*) as described in Lee-Montero et al. (2013). Electropherograms and genotypes were analysed using GeneMapper software v.3.7 (Life Technologies®).

Genetic variation and structuring within and among populations

The number of alleles at each locus (Na), the proportion of individual samples that were heterozygous (direct count heterozygosity, Ho) and the unbiased estimate of heterozygosity (He) for each population were assessed using GenAlex software v.6.0 (Peakall and Smouse 2006). To prevent the error originating in the comparison of the mean number of alleles (Na) between samples of different size, the allelic richness (Rs) per population was determined using the Fstat statistical package v.2.9.3 (Goudet 2001) based on the smallest sample size. A two-sided Tab sheet test implemented in Fstat, where *P* values and significance were evaluated using 1000 permutations, were carried out in order to determinate changes in the genetic variability (Ho, He and Rs) from broodstock to the progeny within origins, as well as to determine differences among populations from different origins in both generation groups. We also used this software to asses the total variation in gene frequencies (F_{IT}), partitioned into components of variation occurring within (F_{IS}) and among (F_{ST}) samples for each locus following Weir and Cockerman (1984). Significance levels of F_{IS} were assessed through randomization of alleles (1000 times) within samples for each population. Pairwise F_{ST} values between samples and *P* values were calculated using Fstat. For significance levels of F_{ST}, multi-locus genotypes were randomised between pairs of samples (1000 permutations) and then, the significance after Bonferroni correction was calculated (Goudet 2001).

Parentage assignments

Parental assignments between breeders and their descendents were determined using their genotypes in the exclusion method by Vitassing software v.8.2.1 (Vandeputte et al. 2006). The frequency of null alleles in each population was assessed using MicroChecker software v.2.2.3 (Van Oostherhout et al. 2004) which estimated allele frequency using four different algorithms. This frequency was regarded as significant when was greater than 0.05. Contingency tables were used to determinate the number of families and to assess their average size as well as variances of sire and dam contributions in each population.

Estimating effective breeding numbers (Nê)

We firstly used the classical formula $(4N_sN_d)/(N_s+N_d)$ to estimate Nê (Falconer 1989) where N_s is the number of sire breeders and N_d the number of dam breeders. This parameter is related to per-generation inbreeding rate (ΔF) as $\Delta F=1/2N\hat{e}$ (Falconer 1989). We also used an approach which takes into account the number of breeders who actually participate in the offspring: $N\hat{e} = 4(N-2)/((K_s+V_s/K_s)+(K_d+(V_d/K_d)-2))$ (Chevassus 1989) with N being the offspring sample size, K_s and K_d the mean numbers of offspring per sire and per dam, and V_s and V_d the variances of sire and dam family sizes. It is a useful approximation to the problem of estimating Nê when unequal breeder contributions to offspring occur.

Results

Genetic variation and genetic structuring among populations

All the breeders (table 1) and a total of 1280 juveniles (table 2) were successfully genotyped. The genetic characteristics of all the groups analyzed are presented in tables 1 (breeders) and 2 (offspring). The populations showed high levels of genetic variation (Ho≥ 0.70; Rs range 8.18 to 10.7). The comparative analysis did not reveal significant differences between populations in order of quantitative genetic variation (Ho, He, Rs; $P \geq 0.05$) and the genetic variability was maintained from the broodstock to the progeny within each origin ($P \geq 0.05$).

Table 1 Genetic variability parameters and effective breeding number (Nê) estimations in three breeder's populations of *Sparus aurata* L. from different origins along the Spanish coast after genetic analysis using eleven microsatellites included in the multiplex SMsa1.

	CAN	ATL	MED
N breeders	59	98	47
Na	9.36	10.9	9.64
Rs	9.02	9.32	9.63
He	0.75	0.73	0.75
Ho	0.77	0.73	0.73
F_{IS} (*p* values)	-0.010 n.s. (0.2833)	0.017 n.s. (0.1485)	0.029 n.s. (0.1121)
Breeders	38♂21♀	49♂49♀	22♂25♀
Nê	54.10	98	46.8
ΔF (%)	0.92	0.51	1.06

N: number of individuals per population. Na: number of alleles per locus. Rs: Allelic Richness. He: expected heterozygosity; Ho: observed heterozygosity. $F_{IS:}$ Degree of departure from expected Hardy-Weinberg proportions within groups. Breeders: all the possible among parents. Nê: Effective population size (Falconer, 1989) calculated for entire families. ΔF: rate of inbreeding (Falconer, 1989). n.s. = not significant.

Table 2 Genetic variability parameters and effective breeding number (Nê) estimations from three descendents populations *Sparus aurata* L. from different parental origins, after genetic analysis using eleven microsatellites included in the multiplex SMsa1.

	CAN	ATL	MED
N offspring	571	354	355
Na	9.27	10.18	8.36
Rs	9.05	10.72	8.18
He	0.72	0.72	0.71
Ho	0.72	0.72	0.74
F_{IS} (*p* values)	0.001 n.s. (0.4803)	0.008 n.s. (0.1848)	- 0.041***(0.0015)
Breeders	25♂19♀	35♂31♀	7♀7♂
Nê(1)	43.18	65.75	14
ΔF(1) (%)	1.15	0.76	3.57
Nê(2)	11.38	31.58	7.10
ΔF(2) (%)	4.39	1.58	7

N: number of individuals per population. N_a: number of alleles per locus. Rs: Allelic Richness. He: expected heterozygosity; Ho: observed heterozygosity. $F_{IS:}$ Degree of departure from expected Hardy-Weinberg proportions within groups. Breeders: breeders that left progenies after parental assignments using microsatellites in progenies. Nê (1): Effective population size (Falconer, 1989) calculated for entire families. ΔF (1): rate of inbreeding (Falconer, 1989). Nê (2): Effective population size (Chevassus, 1989) and ΔF (2): rate of inbreeding (Falconer, 1989). n.s. = not significant. ***P≤0.001

Breeder populations showed Hardy-Weinberg (HW) equilibrium (F_{IS} = 0.012, *P* = 0.126) and genetic differentiation (F_{ST} = 0.016, *P* = 0.001). When they were pairwise compared after Bonferroni correction in the pairwise F_{ST} test of differentiation (data not shown in tables) they were found to be genetically different among all of them: F_{ST} CAN-ATL = 0.013, *P* = 0.016; F_{ST} CAN-MED = 0.013, *P* = 0.016; F_{ST} ATL-MED = 0.020, *P* = 0.016. Regarding progeny, we found agreement with H-W expectations for populations from CAN and from ATL (non-significant F_{IS} values) but the population from MED showed a significant H-W disequilibrium due to an excess of heterozygotes (*P*≤ 0.001). Progenies were genetically different (F_{ST} = 0.057; *P* = 0.001) showing stronger differentiation than the breeders group. The pairwise F_{ST} test of differentiation (data not shown in tables) showed that the three studied populations were also genetically distinct with significant *P* values after Bonferroni corrections (F_{ST} CAN-ATL = 0.051, *P* = 0.016; F_{ST} CAN-MED = 0.054, *P* = 0.016; F_{ST} ATL-MED = 0.070, *P*

= 0.016), with the pairwise between populations from ATL and MED showing the highest F_{ST}.

Parentage assignments

Parental assignments were determined allowing up to three errors. After revising genotypes most of the errors were identified as null alleles which differed depending on broodstocks. Other sources of error such as non-specific amplification and/or allele drop out were observed at much lower frequencies. Microchecker software revealed the existence of null alleles for the D4 marker in the offspring from CAN and ATL with a mean frequency of 0.112 and 0.065 respectively (Brookfield 1 estimator; data not shown) and non null alleles were found in the population from MED. Once identified, 97.9%, 85.6% and 95.22% of the offspring from CAN, ATL and MED respectively, were assigned to a single parent couple. These percentages of assignment increased if we included those descendents for which we could only assign one parental (98.6%, 98.31% and 98.03%, respectively). Due to the structure of the data (breeders nested within a single origin) very few individuals were found for which dam and sire assignment was not possible because of the different, and hence impossible, origins between progenitors and descendents.

The contribution in terms of number of families was different according to their broodstock (Table 3). Average family size was proportionally inversed to the number of spawner contribution to the progeny. The population from ATL showed the smallest average family size, according to its largest number of founders, while the population from MED showed the largest average family size and the smallest number of founders. The coefficients of variation, which were very high, showed the different breeder contributions to progenies.

Table 3 Pedigree tracing data in three populations *Sparus aurata* L. from different parental origins

	CAN	ATL	MED
Nº contributory families (parental couple)	68	111	22
Average family size	8.21	2.68	15.09
C.V (Average family size)	161.43	100.86	133.70
Nº contributory families (one parental)	71	131	28

C.V = Coefficient of variation (%)

Estimating effective breeding numbers (Nê) and differential parental contributions

We observed different breeder contributions to progenies. The Nê, taking into account the total number of males and females which participated in the mass-spawning is shown in table 1 as well as its related ΔF. Nevertheless, not all breeders contributed to offspring, and this implied reductions in the Nê estimates (table 2) and an increase in the ΔF according to their direct relationship. We observed an unequal contribution of breeders to progeny in the three studied populations which affected the Nê estimates. In the broodstock from CAN, 25.4% of the breeders did not produce progeny (15 breeders: 13 ♂ and 2 ♀) while in ATL 32.6% failed to contribute to offspring (32 breeders: 14 ♂ and 18 ♀) and 70.2% of the breeders in the broodstock from MED (33 breeders: 15 ♂ and 18 ♀). In fact, the effective breeding numbers calculated using Chevassus approach (table 2) revealed lower (Nê) values than expected with equal contributions. Therefore, the actual number of breeders detected in the present work in each population (N = 44 in CAN; N = 66 in ATL and N = 14 in MED) was reduced by about 74%, 52% and 49% respectively, when it was corrected for unequal number of males and females.

Discussion

Genetic variation and genetic structuring among populations

Almost all of the individuals were successfully genotyped at eleven microsatellite loci using the SMsa1 (Lee-Montero et al. 2013). Degraded tissues or low quality DNA could be the responsible of non-genotyped samples (Borrell et al. 2011). Genetic variability is an important attribute of the species under domestication, since those with higher levels of variation are most likely to present high additive genetic variance for productive traits (Alarcón et al. 2004). In this study the average Ho was 0.74 (breeders) and 0.72 (offspring) which are results similar to previous reports for Atlantic, Mediterranean (Alarcón et al. 2004; De Innocentiis et al. 2004) and for Adriatic (Šegvić-Bubić et al. 2011) sea bream populations. We did not observe reduction of genetic variability in the offspring populations ($P \geq 0.05$) compared to their reference broodstock. This high variability of the studied populations highlights the lack of selection pressure to which this species has been subjected, which might be one of the reasons for its success in aquaculture.

The genetic assessment of the broodstocks revealed H-W equilibrium for the populations under study. However, a significant excess in heterozygosity was found in the progeny from MED may be due to the heterozygotes advantage or non-random mating (Borrell et al. 2011).

The levels of genetic differentiation or similarity inferred by neutral molecular markers such as microsatellites represent a useful source of information for reconstructing the life history of a species and for inferring the actual situation in terms of gene flow (Franchini et al. 2012). Studied populations were genetically different in both, progeny ($F_{ST} = 0.016$) and offspring ($F_{ST} = 0.057$), showing heterogeneity among populations from different geographical origins and being the degree of differentiation

higher in the progenies. In the case of the breeders we obtained a global F_{ST} below average for a group of marine species (F_{ST} = 0.062; Ward 2006). In fish, negative correlation has been demonstrated between F_{ST} values and dispersal capability (Alarcón et al. 2004). According to this, sea bream should present high dispersal capability and, consequently, high exchange of migrants among subpopulations, allowing large effective subpopulation sizes and low structuring. Alarcón et al. (2004) and De Innocentiis et al. (2004) found values of F_{ST} = 0.036 and F_{ST} = 0.01 respectively, inferred by microsatellites when they compared Atlantic and Mediterranean sea bream populations and Šegvić-Bubić et al. (2011) obtained a global F_{ST} = 0.033 among Adriatic sea bream populations indicating slight degree of differentiation. These authors pointed out that in the absence of physical or ecological barriers sea bream would have free gene flow. However, Ben-Slimen et al. (2004) found a strong differentiation between western and eastern Mediterranean sea bream populations with a global F_{ST} = 0.0934. On the other hand, within small populations such as aquaculture stocks a restricted number of breeding individuals can lead to random drift in the gene frequencies between generations (Alarcon et al. 2004; Brown et al. 2005) and as a result populations of the same species tend to be genetically different. In fact, we observed a stronger differentiation in the progeny (higher F_{ST} values).

Our results revealed the existence of three genetically differentiated sea bream populations along the Spanish coast and suggest that the level of differentiation found in this study might increase over the time due to the small effective population sizes of our stocks.

Parentage assignments and the breeding aspect

The SMsa1 allowed highly accurate determination of parentage, which is necessary for correctly reconstructing fish genealogies and for estimating genetic parameters in breeding programs. Microsatellite-based traceability methods have demonstrated to be very useful for an accurate acquisition of pedigree information in *Sparus aurata* (Perez-Enriquez et al. 1999; Castro et al. 2007; Navarro et al. 2008; Borrell et al. 2011; Lee-Montero et al. 2013. In the present study even though there were a high number of possible parental pairs (number of dams x number of sires) in each population (798, 2401 and 550 in CAN, ATL and MED populations respectively) the majority of the progeny were successfully traced back. The polymorphism, null allele frequency and mutation rate of microsatellites are essential parameters on which the accuracy of parentage relationships inferred from these markers depends (Castro et al. 2007). According to Brookfield 1 estimator, one of the markers used in our multiplex showed null alleles. Nevertheless, the null-allele identification was easily performed due to the high power assignment from other markers (Navarro et al. 2008) and parental assignments were achieved successfully, so the presence of null alleles did not affect the multiplexes' efficacy of parentage assignment.

Results indicate that the number of breeders in the stock is of great importance to the maximization of contribution since the larger broodstock (CAN and ATL) contributed larger number of families. The protandric hermaphroditism shown by gilthead sea bream encourages farmers to use the same individuals to act initially as males and later as females. As a result, the effective population size of this species breeding stock would be smaller than those of non-hermaphrodite species (Alarcón et al. 2004). Moreover, sea bream is highly fecund and there is a temptation to spawn as few fish as possible in order to save expenses in hatcheries (Borrell et al. 2007). Our

result confirms that this strategy increases inbreeding as we observed that in the population with fewer numbers of founders (MED) there was a smaller number of contributory families and hence greater ΔF. Within a mass-spawning system, it is likely that there will be differences in mating success leading to a high degree of variation in contribution, perhaps associated with physiological factors, such as age or weight (Brown et al. 2005). Indeed, we observed significantly unequal breeder contributions to progeny. This outcome seriously affected the Nê estimates which decreased in the three studied populations when we applied the Chevassus approach taking into account the true variance in family size. Where this parameter departed from equality, the value of Nê was highly reduced and hence the ΔF was increased. Therefore, this reduction was more pronounced in the population from MED in which fewer breeders were involved in producing the progeny, reaching 7% rate of inbreeding which is in agreement with other studies; Vandeputte et al. (2004), Brown et al. (2005) and Borrell et al. (2011). As a general rule, there should be only a 0.5% of ΔF in breeding schemes (Sonesson et al. 2005). However, more than 5% of accumulated inbreeding was reached in our study in only one generation for the three studied populations. Therefore, if this progeny had to be used as breeders in upcoming culture cycles, more management effort would be required to diminish inbreeding.

This research provides an insight into the genetic variability and population structure of the gilthead sea bream and highlights the importance of a proper management of the broodstocks in hatcheries relative to inbreeding aspects due to breeder's unequal contributions. Our findings should be relevant for the establishment of successful breeding programs in aquaculture of this species.

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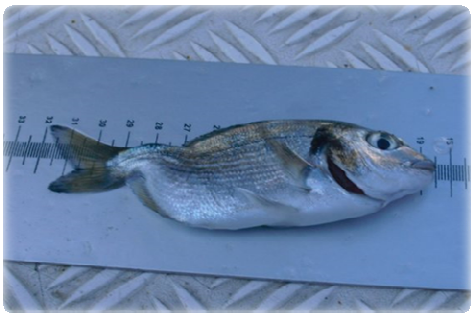

CAPÍTULO II

II. 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.

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ABSTRACT

Growth rates and the presence of deformities can be affected by the use of different rearing systems as well as by the different genetic origins of the stocks. At the same time, strategies that involve the development of selection schemes for these traits of economic interest are scarce. In this study the effect of the origin of the broodstock on growth traits and external deformities as well as genetic parameters (heritabilities and genetic correlations) for these traits were estimated at different ages (days post–hatching; dph). For this purpose, a population of farmed gilthead sea bream was obtained from three broodstock of different origins along the Spanish coasts [Cantabrian Sea (CAN), the Atlantic Ocean (ATL) and Mediterranean Sea (MED)] and reared under the same intensive conditions. Parental assignments between breeders and their offspring were carried out *a posteriori* using a microsatellite multiplex (SMsa1). Juveniles from MED showed the fastest growth while those from ATL showed the slowest growth and the highest incidence of vertebral column deformities. Differences among origins could be explained not only through their different genetic backgrounds but also by environmental conditions in the initial facilities, where different origins were reared separately, and by genotype x environment interactions. Growth traits showed low heritabilities at 163 dph (0.11±0.03) and medium at 690 dph (0.25±0.06 for weight; 0.22±0.07 for length) suggesting that selection at the later age would be more appropriate. Both traits were highly and positively correlated at both ages at the genetic and phenotypic level. External deformities in the vertebral column as well as in the operculum showed medium-high heritability at both studied ages with higher values at 690 dph (0.56 [0.17 - 0.69] and 0.46 [0.20 - 0.90], respectively). These results revealed that the ontogenesis of deformities exhibit a partial genetic basis. Nevertheless, for those in the rest of the head the heritability was close to zero. Initially, positive genetic

correlations between growth and deformities in the vertebral column were observed (83% probability of being positive for weight-vertebral column deformity; 81% for length-vertebral column deformity). However, these correlations seem to be negative at 690 dph (94.2% probability of being negative for weight-vertebral column; 80.6% for length-vertebral column). Results confirm that it could be recommended to eliminate deformed fish from a breeding nucleus and later, select on growth. All these findings should be relevant for the establishment of successful breeding programs in the aquaculture of this species.

1. Introduction

Gilthead sea bream (*Sparus aurata* L.) inhabits mainly in the coastal and brackish waters of the north-eastern Atlantic Ocean and the Mediterranean Sea. In fact, it is the most relevant marine species in Mediterranean aquaculture, which reached an annual production of 176,191 metric tons in 2012 (APROMAR, 2013). In this species, as in other species cultured for human consumption, growth traits are the most economically important as production costs can be significantly lowered by reducing the duration of the rearing cycle (Saillant et al., 2006). For this reason, growth rate is usually the first goal in breeding programs of different species. Gilthead sea bream is mainly commercialized at 350–500 g. Nevertheless, this weight is achieved at different ages, depending on factors related to nutrition and culture conditions, mainly on the isotherms during growth (Navarro et al., 2009).

The presence of deformities, which determine the overall fish quality, is the second most economically important trait for the industrial production of gilthead sea bream (Georgakopoulou et al., 2010). Despite the growth and consolidation of the sea bream industry the high level of skeletal deformities appearing in hatchery fish is an important problem for the development of this industry. Deformities reduce the physiological ability of fish for a correct development i.e. reduce their growth rate, increase their mortality rate and significantly affect the animal welfare (Andrades et al., 1996; Karahan et al., 2013). In the aquaculture industry, losses due to deformities occur at two levels; at hatcheries, reducing larval survival rate and growth efficiency in deformed fish, and on growing farms, where deformed fish at market size have to be discarded or sold at lower values than the market prices since they are clearly evident, especially in those species such as sea bream which are sold mainly as whole fish. Thus, reducing the incidence of larval deformities would reduce the cost of production, both in

the hatcheries and in the out-growing production sectors, and improve the quality of the products (Fernández et al., 2008). In gilthead sea bream the most common deformities are those that affect the opercular complex, neurocranium and vertebral column (Koumoundouros et al., 1997; Boglione et al., 2001; Roo et al., 2005). The presence of deformities in cultured fish is a widely studied but not fully understood and solved problem (Boglione et al., 2001).

Growth rates and the overall quality of the end product can be affected by the use of different rearing systems to produce commercial fish as well as by the different genetic origins of the stocks (Ayala et al., 2010). However, we did not find previous research in which the relationship between the origin of the broodstock and the growth as well as the presence of deformities has been studied in this species.

The optimization of selection program requires knowledge of genetic parameters of characters as optimal selection strategies depend primarily on heritability of individual characters and genetic correlations between characters (Falconer and McKay, 1996). In sea bream, some actions have been done in this respect (REPROSEL project; Acuicultura balear, S.A. CDETI Project).

In light of these circumstances, the main goals of this research were: A) To study the effect of the origin of the broodstock on growth traits and external deformities as well as to estimate their phenotypic correlation. B) To estimate genetic parameters (heritabilities and genetic correlations) for these traits in a population of gilthead sea bream sourced from broodstocks from three origins.

2. Materials and methods

2.1. Rearing conditions

Initially, samples of sea bream were captured from wild populations from three geographically differentiated origins; Cantabrian Sea (CAN), the Atlantic Ocean (ATL), and Mediterranean Sea (MED), hence from the Northern, Southern, and Western Spanish coasts, respectively (Fig. 1). From these samples, three broodstocks were established in different Spanish facilities shown in Figure. 1: Instituto Canario de Ciencias Marinas, ICCM, Canarias (n = 59, 2♂:1♀); centro IFAPA, El Toruño, El Puerto de Santa María, Andalusia (n = 98, 1♂:1♀); and Instituto de Investigación y Tecnología Agroalimentaria, IRTA, Catalonia (n = 47, 1♂:1♀); where they were genotyped as described in Lee-Montero et al. (2013) and maintained until their spawning season although not subjected to artificial selection.

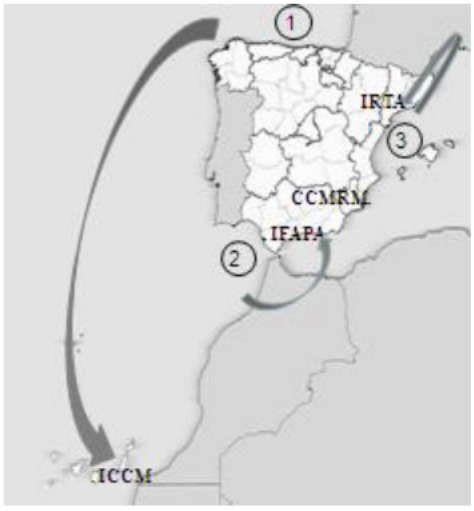

Fig. 1. Three geographical origins of Sea Bream samples from which broodstocks were established. Location of the Spanish research centers where larvae were obtained and where juveniles were reared. 1= Population from Cantabrian sea, 2= Population from the Atlantic Ocean 3= Population from Mediterranean Sea. ICCM = Instituto Canario de Ciencias Marinas, Canarias, IFAPA= Centro de Investigación y Formación Pesquera y Acuícola El Toruño, Andalusia, IRTA = Instituto de Investigación y Tecnología Agroalimentaria, Catalonia, CCMRM= Centro de cultivos Marinos de la Region de Murcia.

To induce spawning, broodstocks were reared under controlled photoperiod (6L:18D) and eggs were collected by buoyancy the same two consecutive days at each facility (Apr-09). Larvae obtained from these mass-spawnings were incubated for 48 h and later distributed in tanks and reared in the conditions described by Roo et al. (2009) in red porgy. At 84 days post–hatching (dph) (Jul-09) a random sample of 2500 individuals was taken to the on-growing facilities of the Centro de Cultivos Marinos de la Región de Murcia (San Pedro del Pinatar, CCMRM, Murcia). In this sample all original broodstock origins were represented as 845 descendants from CAN, 777 from ATL and 878 from MED which were sheltered and constituted the population under study. After an adaption period of 20-30 days, fingerlings were individually tagged in the abdominal cavity for individual identification with a Passive Integrated Transporter (PIT; Trovan Daimler–Benz) following the tagging protocol described by Navarro et al. (2006) and then randomly distributed (one third of each origin) in 12 tanks (850-l) in an open circuit with intake of water from the sea and reared under communal conditions. Food was provided manually using commercial fish feed (Skretting S.A., Cojóbar-Burgos, Spain). Once the pre-on-growing period was completed (325 dph) the majority of the fish (about 2000 specimens) were moved to the facilities of the company Servicios Atuneros del Mediterraneo S.L. (San Pedro del Pinatar, Murcia, Spain) where they were reared under intensive conditions to commercial size (≈300 g weight). The fish were raised in a cage of 11 m in diameter which is anchored in 38 m of depth in the Mediterranean sea (average water temperature = 18.2 ± 0.9ºC; Fig. 2, dissolved oxygen: 7.4 $mg \cdot l^{-1}$, data estimated from open sea condition; 100% oxygen-saturation, salinity: 37.9‰) and fed with commercial fish feed (39% protein, 21% fat 2% fiber; Dibaq S.A., Fuentepelayo-Segovia, Spain) following the feeding system specified by the company. At harvest size (690 dph) all the fish from the cage were slaughtered by immersion in

ice cold water (hypothermia). The remaining fish housed in CCMRM were distributed in to six tanks of 1500 l and maintained as a part of a breeding program (PROGENSA®, http://www.progensa.eu).

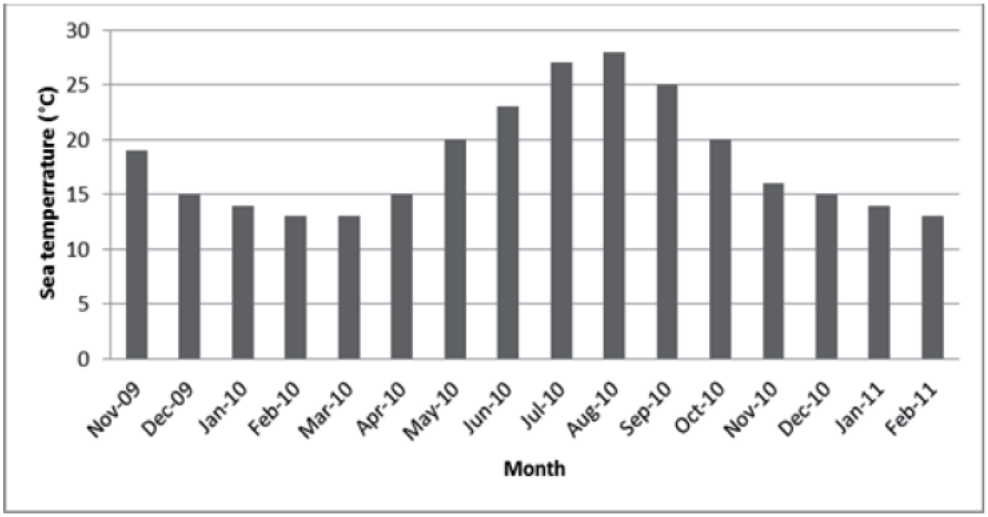

Fig. 2. Mean water sea temperature along the on-growing period.

2.2. Sampling

At 163 dph, all the fish on-grown at CCMRM were assessed and the weight and total length were recorded. Fish were also visually inspected in order to examine external deformities in the vertebral column (curvature), operculum (lack of operculum) and the rest of the head (cranium and jaw deformities). The presence of a minimum angle in the vertebral column and any fold at the operculum was enough to classify the fish as deformed. The following sampling took place before and after the hottest season, at 368 and 516 dph, respectively. Due to the difficulty of the sampling of individuals from the cage, a random sample of about 300 individuals was assessed and the same traits were recorded. Finally, at harvest size (690 dph) all the tagged fish from the cage were measured and analyzed. No fish were culled during larval rearing or in the on growing periods. Deformity examination was carried out by the same observer at different ages in order to avoid bias in the estimates.

2.3. PCR reaction and genotyping

Breeders and juveniles were genetically characterized. For this purpose, DNA was extracted from the caudal fin, previously preserved in absolute ethanol at room temperature, using the DNeasy kit (QIAGEN®) and then kept at 4°C following the manufacturer's recommendations. DNA quality and quantity were determined using a Nanodrop 2000 spectrophotometer v.3.7 (Thermo Fisher Scientific, Wilmington, U.S.A.). For individuals genotyping, the multiplex SMsa1 (Super Multiplex *Sparus aurata*) was used as described in Lee-Montero et al. (2013). Electropherograms and genotypes were analyzed using GeneMapper software v.3.7 (Life Technologies®).

Parental assignments between breeders of unknown gender and their descendants were determined using their genotypes in the exclusion method by VITASSING (v.8_2.1) software (Vandeputte et al., 2006). A total of 201 full-sib families (21 paternal half-sib families and 9 maternal half-sib families) were represented.

2.4. Data analysis

All growth data were tested for normality and homogeneity of variances using SPSS (v.19.0) (SPSS, Chicago, IL, USA) at each age and then analyzed by the General Linear Model:

$y_{ij} = \mu + \text{origin}_i + e_{ij}$

in which y_{ij} is an observation of an individual j from the origin i, μ is the overall mean, origin is the effect of the broodstock origin (i = CAN, ATL and MED), and e_{ij} is a random residual error.

Pearson correlation was carried out to determine phenotypic correlations among the analyzed parameters.

Regarding deformities (vertebral column, operculum and rest of the head), each fish was assigned as deformed or un-deformed and scored 1 or 0 for each of the three deformities. Moreover, each fish got an overall score. If fish had one or more kinds of deformities, they were assigned as deformed and scored 1 while un-deformed fish were scored 0. We calculated the frequency of individuals with deformities and the frequency of each deformity as a percentage of deformed individuals in total analyzed fish. Deformity scores were analyzed by logistic regression, using the SPSS® (v.19.0) (SPSS, Chicago, IL, USA) to detect the effects of the origin of the fish.

Genetic parameters were estimated at initial and final ages (163 and 690 dph) as more individuals could be included in the data matrix in order to obtain consistent results (Falconer and Mackay, 1996). Bivariate analyses were carried out using a Restricted Maximum Likelihood (REML) algorithm to obtain (co)variance components for growth traits using the following animal linear model:

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

where y is the recorded data on the studied traits, β includes fixed effect (broodstock origin), u is the random animal effect, e is the error and, X and Z are incidence matrices. Non-genetic maternal and/or paternal effects were not significant for the studied traits (growth and deformity) so they were removed from the model when analyzing deformity and growth traits. The model was resolved with the software package VCE (v 6.0) (Groeneveld et al., 2010). The magnitude of estimated heritability was established, following the classification of Cardellino 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 (Navarro et al., 2009).

Deformity was considered as a threshold trait and its genetic parameters (heritabilities and genetic correlations between deformities and between deformity and growth traits) were estimated in the underlying liability scale under a Bayesian approach by using a bivariate Gaussian mixed model. The estimates on the underlying liability scale assume that the susceptibility for the deformity is determined by an underlying liability that is distributed normally and inherited in a polygenic manner (Gjerde et al., 2005).

The analysis was performed using the thrgibbs1f90 developed by Misztal (2010). The model was the same than the one described previously for REML estimates. The analysis was carried out between two traits each time. The following multivariate normal distributions were assumed a priori for random effects:

$P(\beta) \sim k;$

$P(u|G) \sim (0, G \otimes A);$

$P(e|R) \sim (0, R \otimes A);$

Where A is the relationship matrix and k is a constant,

$$G = \begin{bmatrix} \sigma_{U1} & \sigma_{U1,U2} \\ \sigma_{U2,U1} & \sigma_{U2} \end{bmatrix},$$

$$R = \begin{bmatrix} \sigma_{e1} & \sigma_{e1,e2} \\ \sigma_{e2,e1} & \sigma_{e2} \end{bmatrix}.$$

Bounded uniform priors were assumed for the systematic effects and the (co)variance components (G, A). A single chain of 200,000 iterations was run. The first 50,000 iterations of each chain were discarded, and samples of the parameters of interest were saved every 5 iterations. Density plots to represent posterior marginal distribution of heritabilities, posterior means (PM) and the interval of 95% of the highest posterior density [HPD95%] were obtained through R project software (2008).

3. Results and discussion

3.1. Phenotypic results

Phenotypic results for growth traits and external deformities at different ages for a sea bream population sourced from broodstocks of different origins are shown in Table 1. Regarding growth traits, fish from MED showed the fastest growth (body weight and total length) at all studied ages followed by CAN and ATL, respectively reared under the same conditions, pointing out an effect of the origin of the broodstocks on these traits. Fish from that origin showed significant differences ($P \leq 0.05$) for growth at all ages except at 368 dph (not significant differences with CAN) which could be due to the lower number of individuals measured. All origins were different from each other at 690 dph for body weight and length (Table 1). Glover et al. (2009) compared salmon of farmed, wild and hybrid origin in a simulated aquaculture production cycle. At slaughter, they found that the farmed salmon were over twice the size of wild salmon, whilst hybrids were intermediate. However, we did not find other researches in which the effect of the origin of the broodstocks on growth traits has been studied in sea bream.

Animal growth is the result of a series of physiological and ethological processes, from food intake to substance deposition (Brett, 1979). This growth depends on many factors related to nutrition and on many abiotic factors related to fish culture conditions, being known that growth rate strongly correlates with temperature fluctuations (Ginés et al., 2004, Karahan et al., 2013). In our study, we observed an increase of the growth with the temperature (Fig. 2). The duration of the rearing cycle to obtain an average final weight of 231 g was 527 days. However, it was not possible to obtain a commercial size of 300 g at slaughter due to the requirements of the project (PROGENSA®, http://www.progensa.eu). Average growth parameters at harvest size

(690 dph) in this study were lower than those published previously in gilthead sea bream. This fact could be explained partially due to temperature fluctuations present in our region (Fig. 2). In the Canary Island, which is a region where low temperature fluctuations are registered, Navarro et al. (2009) observed 485.6 g of body weight and 27.7 cm of fork length at 509 dph for individuals reared in a cage. Ginés et al. (2004) obtained 400 g of weight at 345 dph. Borrell et al. (2011) studied growth in a progeny reared in tanks and divided into fast- and slow-growth groups at 520 dph obtaining results for body weight in the slow group similar to those obtained in the present research at comparable ages.

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

Regarding deformities in the vertebral column, fish from ATL showed the highest percentage of this deformity ($P \leq 0.05$) at all studied ages (Table 1) ranging from 1.2% at 163 dph to 10.9% at harvest (690 dph). However, higher incidences have been observed for this deformity in other studies. Castro et al. (2008) found 5.6% of externally lordotic individuals in three-month-old sea breams. Karahan et al. (2013) found 54% of deformed fish when they studied external deformations in the spine in European sea bass 800 g mean weight in different farms. Moreover, an increase in the

incidence of deformities in the vertebral column was observed along the experiment in all origins may be due to the fact that these anomalies are more difficult to detect by external examination of small fish at a hatchery level. Another explanation could be an increase of these deformities over time as fish grow, as was observed by Bardon et al. (2009) in European sea bass at different ages. This raise was not observed at the intermediate age of 516 dph which may be due to the lower random number of sampled individuals.

Regarding the lack of operculum (Table 1), fish from MED exhibited a high frequency of this deformity reaching a frequency of 32% at 163 dph in agreement with previous research (Verhaegen et al., 2007). These authors observed opercular complex as the most frequent deformity during larval rearing of gilthead sea bream affecting up to 80% of the juveniles. In addition, we observed that this deformity was gradually diminishing during the growth of the fish and regardless of the broodstock origins and at 690 dph no significant differences were observed for this deformity among origins. This decrease was not observed at intermediate age of 516 dph may be due to the lower random number of sampled individuals. These results are in concordance with previous research in which a deformity recovery was suggested (De Wolf et al., 2004; Beraldo and Canavese, 2011). In fact, in the present study gilthead sea bream operculum deformity prevalence for all studied broodstock origins at 163 dph was lower than those obtained by other authors at earlier ages (Galeotti et al., 2000; Verhaegen et al., 2007) who observed 31.9% prevalence in 80 days-old fish and 28.5% prevalence in 69 days-old larvae, respectively.

The incidence of deformities in the rest of the head was very low not observing significant differences ($P \geq 0.05$) during fish development or across broodstock origin (Table 1).

The highest frequency of deformed fish was observed in fish from MED at 163 dph with a 32.7% of individuals showing any kind of deformity (Table 1) but mainly due to high incidence of lack of operculum. Georgakopoulou et al. (2010) cited deformities affecting 7-20% of sea bream juveniles. At harvest (690 dph) the frequency of deformed fish reached 12.5% in fish from ATL and there were no significant differences between CAN and ATL due to the operculum recovery mentioned above. However, this prevalence can reach higher values as described by Dupont-Nivet et al. (2008) in European sea bass with values of deformed fish ranging from 58 to 83% at commercial size (average 400 g) in different farms.

Table 1: Phenotypic results (least square means ± standard error) for body weight and total length and frequency (%) of deformities in a population of gilthead sea bream at different ages sourced from broodstocks from three origins

Broodstock origin[1]	N	Weight (g)	Length(cm)	Column	Operculum	Head	Deformed
At 163dph (Sep-09)							
CAN	845	11.9^{a}±0.2	9.13^{a}±0.04	0.1^{a}	7.2^{a}	0.3^{a}	7.6^{a}
ATL	777	11.7^{a}±0.2	8.99^{a}±0.05	1.2^{b}	4.9^{a}	0.0^{a}	6.1^{a}
MED	878	14.8^{b}±0.2	9.69^{b}±0.04	0.7^{a}	32^{b}	0.0^{a}	32.7^{b}
At 368dph (Apr-10)							
CAN	137	48.2^{b}±1.6	15.58^{b}±0.15	1.5^{a}	5.1^{a}	2.2^{a}	8.8^{a}
ATL	82	38.9^{a}±2.0	14.45^{a}±0.20	12.2^{b}	1.2^{a}	1.2^{a}	14.6^{ab}
MED	46	54.2^{b}±2.8	15.22^{b}±0.27	4.5^{ab}	15.9^{b}	0.1^{a}	20.5^{b}
At 516dph (Sep-10)							
CAN	160	149^{b}±3.9	22.47^{b}±0.23	0.1^{a}	1.3^{a}	1.9^{a}	3.3^{a}
ATL	56	122^{a}±2.2	20.27^{a}±0.13	5.8^{b}	1.9^{ab}	0.0^{a}	7.7^{ab}
MED	84	171^{c}±3.1	22.89^{b}±0.18	2.4^{ab}	10.7^{b}	1.4^{a}	14.5^{b}
At 690dph (Feb-11)							
CAN	438	237^{b}±2.1	24.71^{b}±0.07	1.8^{a}	2.4^{a}	0.2^{a}	4.4^{a}
ATL	252	187^{a}±2.7	22.51^{a}±0.10	10.9^{b}	1.2^{a}	0.4^{a}	12.5^{b}
MED	227	269^{c}±2.9	25.01^{c}±0.10	2.6^{a}	7.3^{a}	0.0^{a}	9.9^{b}

[1]Broodstock origin: CAN = Cantabrian Sea, ATL = The Atlantic Ocean, MED = Mediterranean Sea

[abc]Different superscripted letters within each row indicate significant differences among origins

Due to the fact that in our study all fish were held in the same station the majority of their life where they were raised under the same conditions, differences between them seem to be the result of differences in their genetic backgrounds. Nevertheless, larvae from the different origins were reared in different facilities until 84 dph. In spite of the fact that we tried to reduce environmental effects to a minimum in this initial phase standardizing the rearing protocol, all environmental variations cannot be controlled. Genetic sensitivity to these environmental stressors could exist (Karahan et al., 2013), which may then be expressed as genotype by environment interactions. In fact, environmental variations have shown to impact the expression of deformities especially during larval and juvenile stages (Bardon et al., 2009; Karahan et al., 2013). Moreover, the same could happen after mixing of the origins in 12 tanks during the pre-on-growing period since the tank effect cannot be analyzed.

The economic consequences of the incidence of vertebral deformities are relevant as they reduce the fish weight at harvest, and more importantly, they reduce the commercial value of slaughtered fish per kilogram (Gjerde et al., 2005). We have not found a significant effect of the studied deformities on growth traits but it could be due to low incidence of deformities in some cases that makes these estimates inaccurate. Bardon et al. (2009) pointed out that spine deformities could have a negative impact on growth, as they mechanically shorten the fish, and probably reduce their access to food by preventing correct swimming. In our study, the highest incidence of deformities in the vertebral column in fish from ATL could partly explain their lowest growth, but future researches are needed to clarify this issue.

3.2. Heritability and correlations of growth traits

Weight and length depend on factors related to nutrition and culture conditions. However, in general, they usually have a strong genetic determinant in all animals (Cardellino and Rovira, 1987). Some heritability estimates for growth traits exist for fish, but mainly for species other than sea bream such as Atlantic salmon (Gjerde et al., 1994), turbot (Gjerde et al., 1997), Atlantic cod (Gjerde et al., 2004; Kolstad et al., 2006; Kettunen and Fjalestad, 2007), black bream (Doupé and Lymbery, 2005), tilapia (Gall and Bakar, 2002), rainbow trout (Kause et al., 2005) and gilthead sea bass (Saillant et al., 2006; Dupont- Nivet et al., 2008).

In the preset research, heritabilities and their corresponding standard errors ($h^2 \pm$ SE) of growth traits at 163 dph (Table 2) were estimated in 1250 individuals correctly assigned to a unique parental couple using the SMsa1. At this age, body weight and total length showed low heritabilities (0.11±0.03). However, medium heritabilities for body weight (0.25±0.06) and for total length (0.22±0.06) were estimated at harvest size (690 dph) estimated in 902 individuals correctly assigned (Table 2). These results are in concordance with those previously reported by Navarro et al. (2009), Saillant et al. (2006) in sea bass, Elvingson and Johansson, (1993) in rainbow trout and Kettunen and Fjalestad, (2007) in Atlantic cod who pointed out that heritability estimates for growth traits increased with age. These results could be due to a lower ratio of weight or length measure errors to the individual measure in bigger fish resulting in an increased accuracy of the measurements in older fish (Saillant et al., 2006). These values suggest that selection at more advanced ages would be more appropriate.

Body weight and total length were highly and positively correlated at the genetic and phenotypic level and at both studied ages (Table 2). In fact, weight and length have

been reported as genetically and phenotypically correlated traits in sea bream (Navarro et al., 2009) as well as in other marine species (Elvingson and Johansson, 1993; Winkelman and Peterson, 1994; Vandeputte et al., 2004, 2008).

Table 2: Heritabilities ± standard errors (in bold at the diagonal), genetic correlations ± standard errors (above the diagonal) and phenotypic correlations ± standard errors (below the diagonal) of growth traits estimated from 1.250 gilthead sea bream at 163 days post-hatching and from 902 gilthead sea bream at 690 days post-hatching

	$Weight_{163dph}$	$Length_{163dph}$	$Weight_{690dph}$	$Length_{690dph}$
$Weight_{163dph}$	**0.11±0.03**	0.96±0.03		
$Length_{163dph}$	0.91**±0.01	**0.11±0.03**		
$Weight_{690dph}$			**0.25±0.06**	0.86±0.05
$Length_{690dph}$			0.82**±0.02	**0.22±0.07**

**Significant Pearson correlations between growth traits ($P \leq 0.01$).

3.3. Heritability and correlations of external deformities

Different results were obtained depending on the studied deformity. The posterior mean (PM) and the interval of 95% of the highest posterior density [HPD95%] of heritability for deformities in the vertebral column was 0.38 [0.10 - 0.76] at 163 dph (Fig. 3) and 0.56 [0.17 - 0.69] at 690 dph (Fig. 5). Castro et al. (2008) did not observe evidence of genetic component (heritabilities adjusting a linear animal model) for lordosis in gilthead sea bream under experimental conditions. However, an increasing number of studies suggest that at least some deformities in fish are determined by an additive genetic component. Andrades et al. (1996) found a 27% incidence of spinal deformities in larvae at hatching against a 5% incidence in adults; thus they argue that the skeletal deformities of adults probably have congenital causes, either genetic or environmental, during embryonic development. Afonso et al. (2000) suggested that a triple column abnormality in sea bream consisting of a consecutive repetition of lordosis–scoliosis kyphosis from head to tail had a polygenic origin. Estimated

heritabilities for spinal deformities on the liability scale revealed heritable component in the European sea bass (Bardon et al., 2009; Karahan et al., 2013), Atlantic salmon (McKay and Gjerde, 1986; Gjerde et al., 2005;) and in Atlantic cod (Kolstad et al., 2006) using a linear animal model.

Regarding deformities in the operculum, heritabilities showed values of 0.43 [0.20 - 0.76] (Fig. 3) and 0.46 [0.20 - 0.90] (Fig. 5) at 163 dph and 690 dph respectively. However, conflicting results about the genetic basis of this deformity have been found. Beraldo et al. (2003) pointed out that the random and independent position of opercular irregularities (on one or both sides), the varying incidence in hatcheries, the extreme rarity of bone agenesis in the operculum, and the variety of forms of defect patterns suggest that the defect is nonheritable. Castro et al. (2008) found no evidence of genetic component as other authors pointed out in tilapia (Handwerker and Tave, 1994; Tave and Handwerker, 1994).

With regard to deformity in the rest of the head, the estimated heritabilities were close to zero along the experiment; PM = 0.08 at 163 dph (Fig. 3) and 0.03 at 690 dph (Fig. 5) as was previously described in common carp (Kocour et al., 2006) suggesting an absence of additive genetic variation. Simulation studies (van Vleck, 1972) and experimental data (Kause et al., 2007) demonstrated that heritabilities for threshold characters could be downbiased when the proportion of abnormal fish is below 5% which is the case for head deformities in this study.

Despite the wide range of the estimates, external deformities in the vertebral column and in the operculum seem to have a heritable component at different ages in sea bream. Therefore, it can be recommended to eliminate deformed fish from a breeding nucleus. Moreover, culling of deformed fish ensures an ethical production (Kause et al., 2007) since skeletal deformities reduce animal welfare. Finally, to clearly

demonstrate the genetic basis of such deformations, we would recommend estimating breeding values of fish for deformities through their relatives and including it in a breeding program to finally obtain the selection response.

Most of the genetic correlations among deformities at 163 dph (Fig. 3) and at 690 dph (Fig. 5) were very imprecise and it was not possible to draw any conclusion about them in agreement with previous results reported by Kocour et al. (2006) who pointed out imprecise genetic correlations among mouth, anal and caudal deformities in common carp. The genetic correlation between deformities in the vertebral column and in the operculum at 690 dph (Fig. 5) seems to be negative (-0.82 [-1 - 0.55]).

3.4. Correlations between growth traits and external deformities

Although it was not possible to estimate genetic correlation with high accuracy, initially (163 dph), genetic correlations among growth traits and deformities in the vertebral column (Fig. 4) showed high probability of being positive (83% for weight-deformity in the vertebral column and 81% for length-deformity in the vertebral column correlations). However, at harvest (690 dph) these correlations tended to be negative (94.2% probability for weight-vertebral column and 80.6% for length-vertebral column correlations) (Fig. 6). This initially positive correlation could be explained by an increased aggravation of deformities in fast growing individuals (Karahan et al., 2013) resulting later in deformed fish with slower growing rates (negative correlation at 690 dph). In this regard, conflicting results have been found across the studies in fish of advanced age. Karahan et al. (2013) did not found genetic correlation between external deformations in the vertebral column and growth traits (weight and length) although daily growth coefficient had a genetic impact on internal (r_g = 0.34) and external (r_g = 0.28) deformities in sea bass. In other species such as Atlantic salmon (Gjerde et al.,

2005) and rainbow trout (Kause et al., 2005) low and negative correlations were obtained for vertebral deformities and growth. In European sea bass Bardon et al. (2009) observed that scoliosis was positively correlated with growth traits while lordosis was weakly and negatively correlated with these traits. In Atlantic cod medium and positive results were obtained between spinal deformity and weight (Kolstad et al., 2006).

Genetic correlation between the lack of operculum and length tended to be positive at 690 dph (Fig. 6) with a probability of 83.2%. However, genetic correlations between weight and operculum were close to zero at both studied ages. Those genetic correlations for deformities in the rest of the head and growth traits were imprecise along the experiment (Fig. 4 and 6).

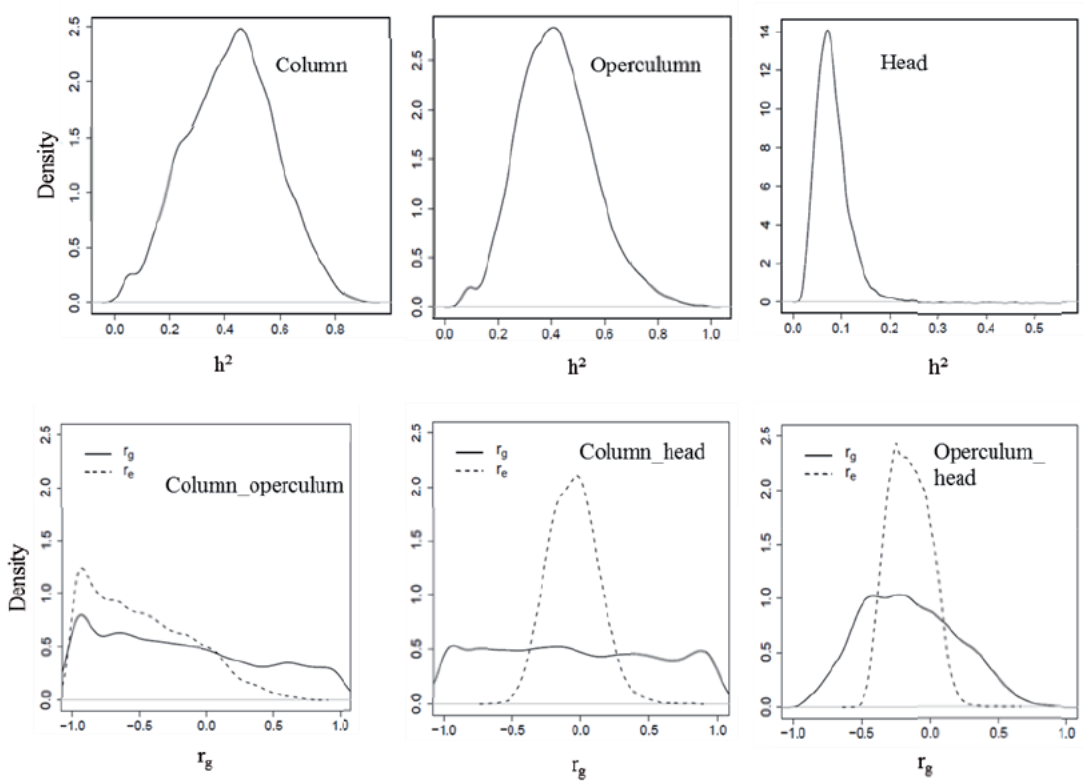

Fig. 3. Posterior marginal distribution of heritabilities and genetic correlations of deformation traits estimated from 1250 gilthead sea bream at 163 days post-hatching
h^2= heritability; r_g = genetic correlation; r_e= residual correlation

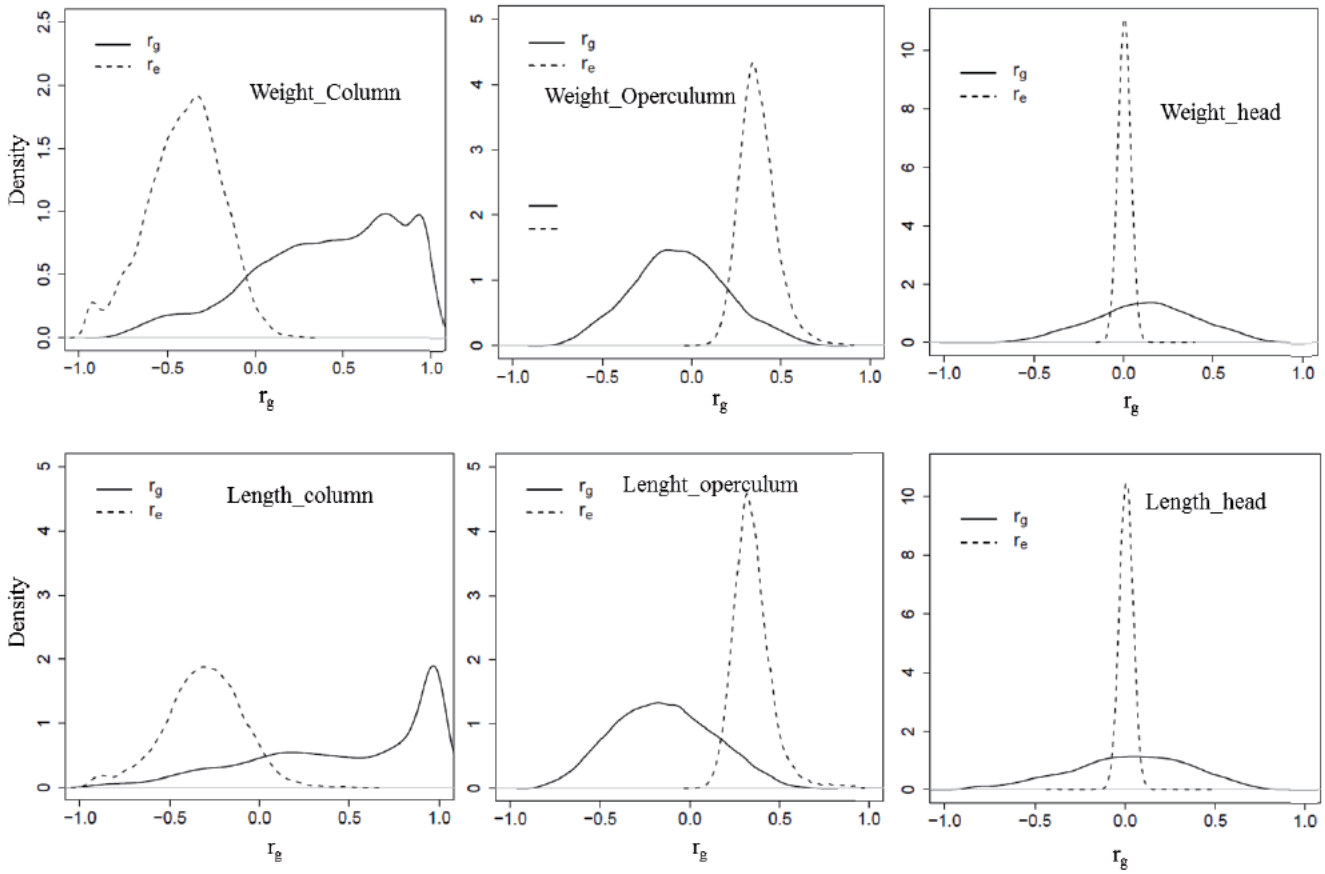

Fig. 4. Posterior marginal distribution of genetic correlations between growth and deformation traits estimated from 1250 gilthead sea bream at 163 days post-hatching. r_g = genetic correlation; r_e = residual correlation

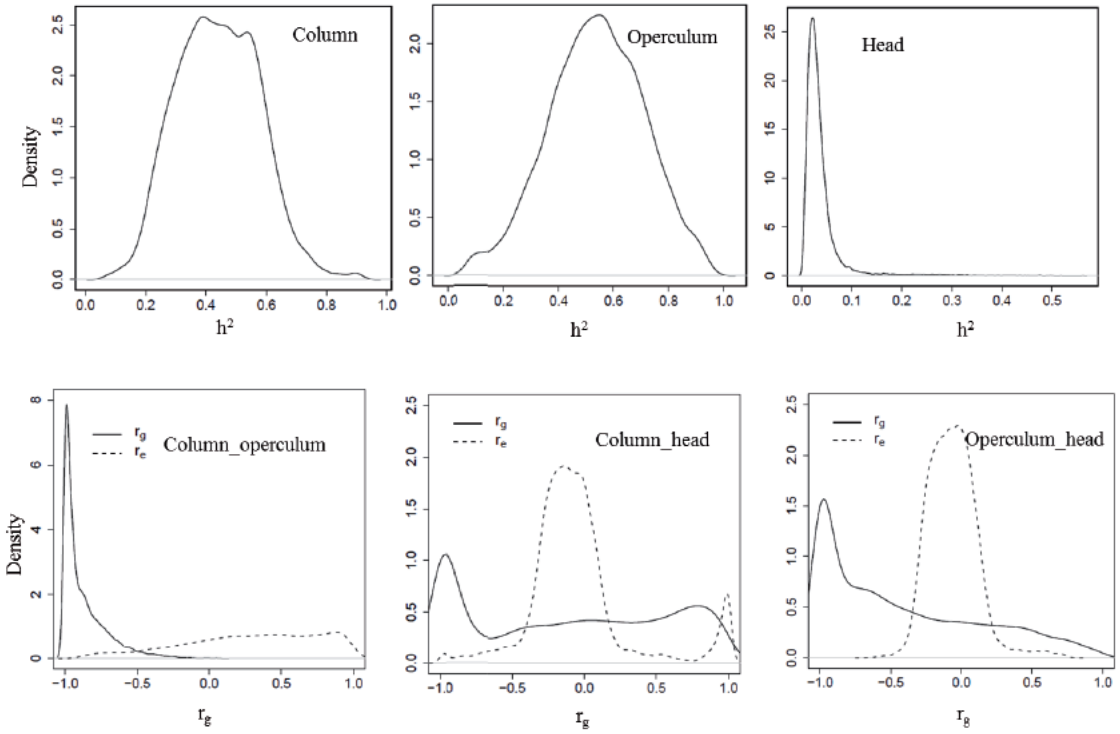

Fig. 5. Posterior marginal distribution of heritabilities and genetic correlations of deformation traits estimated from 902 gilthead sea bream at 690 days post-hatching.
h^2 = heritability; r_g = genetic correlation; r_e = residual correlation

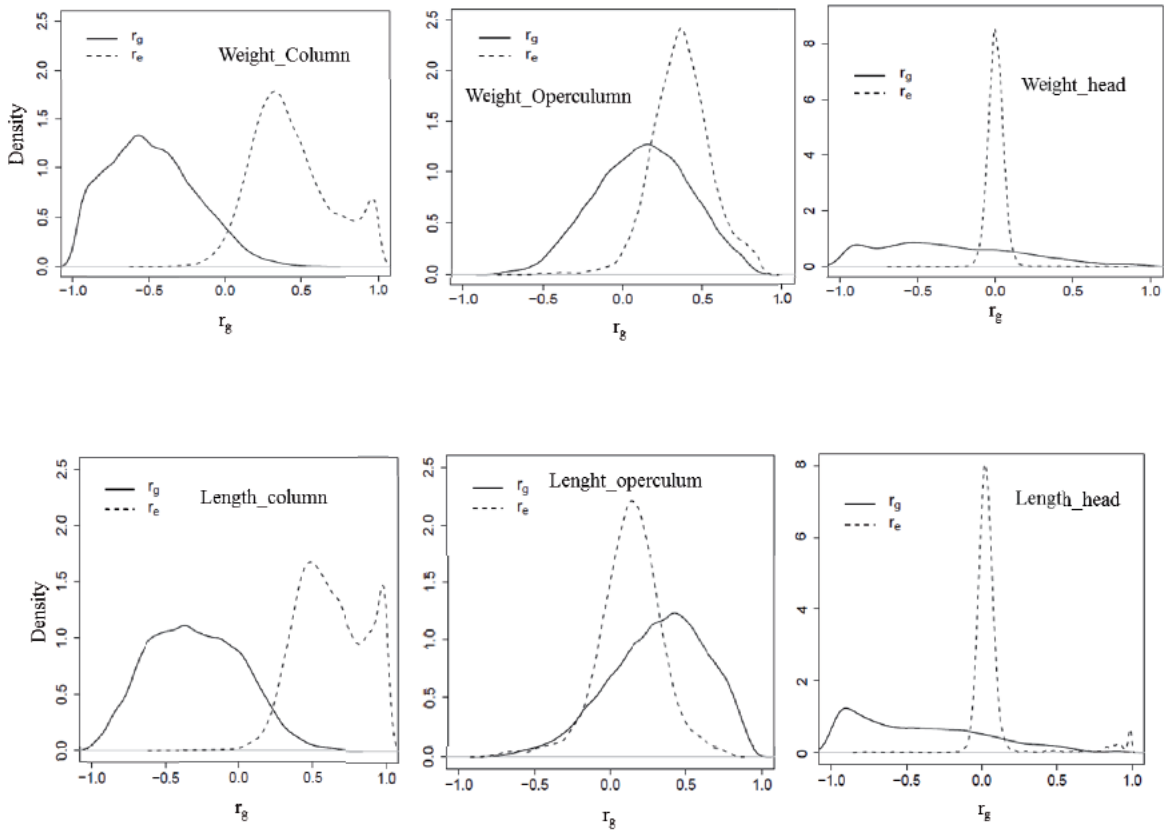

Fig. 6. Posterior marginal distribution of genetic correlations between growth and deformation traits estimated from 902 gilthead sea bream at 690 days post-hatching.
r_g = genetic correlation; r_e= residual correlation

4. Conclusions

Juveniles from MED broodstock were the best performers since fingerlings from that origin showed the fastest growth rate while those from ATL showed the slowest growth rate and at the same time the highest incidence of vertebral column deformities. Therefore, these results proved the importance of the acquisition of a stock in sea bream. However, it is relevant to consider that differences among origins could be explained through their different genetic backgrounds but also by environmental conditions in the initial facilities and by genotype x environment interactions.

Heritability estimates for growth and deformities in the vertebral column and in the operculum indicate the potential for improvement of all these traits by selective

breeding using a family-based selection program taking into account that final recordings at the end of the growing period will provide better information. At harvest, genetic correlation between growth and vertebral column deformity tended to be negative, although between length and operculum tended to be positive suggesting that weight could be a better criterion for selection. Results confirm that it can be recommended, by precaution, to eliminate deformed fish from a breeding nucleus and later, select on growth which is the most economically important objective in the majority of fish genetic selection programs. Nevertheless, other traits related to the quality of the meat should be considered as well as possible criteria to be included in a breeding program.

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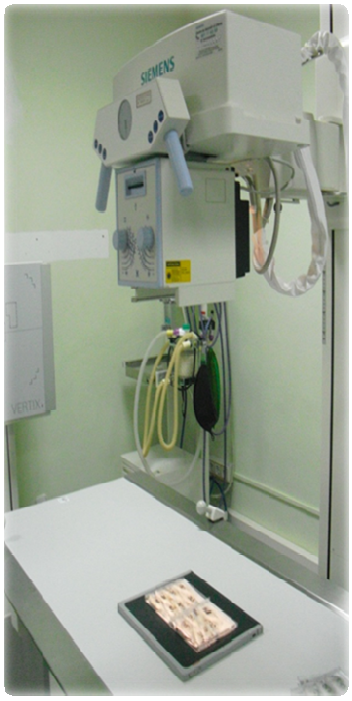

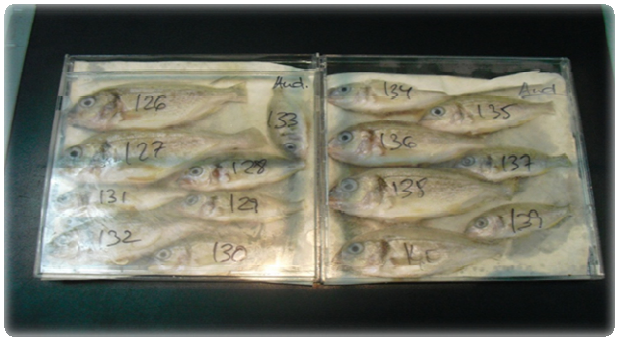

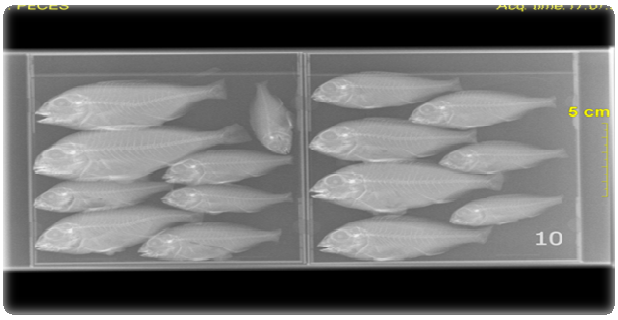

CAPÍTULO III

III. Genetic determination of skeletal deformities and uninflated swimbladder in a reared gilthead seabream (*Sparus aurata* L.) juvenile population sourced from broodstocks along the Spanish coast.

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Animal Genetics. En revisión.

Summary

Lordosis, lack of operculum and failure to inflate the swimbladder constitute a major problem for the gilthead seabream aquaculture industry. In this study, the effect of the broodstocks origin on these major anomalies in juvenile seabream was analyzed. A population of farmed seabream (n=909) obtained by industrial mass-spawning from broodstock from three different origins and reared under common conditions was analyzed from X-ray photograph for skeletal deformities and for uninflated swimbladder. Lordosis and lack of operculum were the most common deformities. The origin had an effect on skeletal deformities as well as on uninflated swimbladder. Differences among origins could be partly explained through their different genetic background, but also environmental conditions in the initial facilities and genotype x environment interaction should be considered. A *posteriori* reconstruction of pedigree was carried out using a microsatellites multiplex (SMsa1) to estimate genetic parameters for these traits. Considerable heritabilities were estimated for lordosis (0.53[0.25-0.77]), lack of operculum (0.37[0.01-0.81]) and uninflated swimbladder (0.36[0.12-0.72]) with a positive genetic correlation between uninflated swimbladder and lordosis (0.48[0.07-0.97]). All these findings should be relevant for the establishment of successful breeding programs in aquaculture of this species.

Introduction

The gilthead seabream (*Sparus aurata* L.) is the most relevant marine species in Mediterranean aquaculture, which reached an annual production of 176 191 metric tons in 2012 (APROMAR 2013). Despite the development and consolidation of the seabream industry, the high level of body anomalies occurring in hatchery-produced fish is an important problem that causes significant losses to the industry. This is often associated with growth depression, leading to high mortality rates (Andrades *et al.* 1996) and also having important implications on the animal welfare (Karahan *et al.* 2013). Their presence downgrades the biological performance and the marketing image, affecting the production cost and the commercial value of the reared fish (Koumoundouros *et al.* 1997). Thus, reducing the incidence of larval deformities would reduce the cost of production and improve the quality of the products (Fernández *et al.* 2008).

The main morphological anomalies in fish can be grouped depending on the aspect involved: shape, pigmentation, scales, skeleton, and swimbladder (Divanach *et al.* 1996). In gilthead seabream the most common deformities are those that affect the opercular complex, neurocranium and vertebral column (lordosis and vertebral fusion) (Koumoundouros *et al.* 1997; Boglione *et al.* 2001; Roo *et al.* 2005). Skeletal deformities might affect up to 30% of the production and several factors are believed to be the basis of them: Nutritional, environmental, hydrodynamic conditions and genetic factors or their interaction (Andrades *et al.* 1996; Afonso *et al.* 2000; Castro *et al*. 2008; Fernández *et al.* 2008). Different degrees of genetic differentiation between sea bream populations inferred by microsatellites have been found (Alarcón *et al.* 2004; De Innocentiis *et al.* 2004; Ben-Slimen *et al.* 2004; Šegvić-Bubić *et al.* 2011). However, we did not find previous researches in which the relationship between the origin of the

broodstock and the presence of anomalies has been studied. Regarding genetic parameters, few studies have reported heritability estimates in gilthead seabream (Castro *et al.* 2008) or in other marine species (McKay & Gjerde 1986; Gjerde *et al*. 2005; Kolstad *et al.* 2006; Bardon *et al*. 2009; Karahan *et al.* 2013) and conflicting results have been found.

The swimbladder (SB) of fish is a hydrostatic, buoyancy-regulating organ which plays a role in the perception and production of sounds as well as in respiratory processes. Failure to inflate the SB has been regarded as a major obstacle in the rearing of important commercial species. Fish lacking functional SB have been reported to show skeletal deformities in seabream and seabass (Andrades *et al.* 1996; Chatain 1994; Divanach *et al.* 1996) as well as in other marine species (Kitajima *et al.* 1994; Trotter *et al.* 2001; Jacquemond 2004). Culture conditions are commonly suggested as main contributory factors to its aetiology. However, little information is available on whether SB inflation also has a genetic basis. Few researches have been found about its heritability in other species (Harrell *et al.* 2002) and none in gilthead seabream.

Therefore, the goals of this research were: A) To study the effect of origin of the broodstocks on the major anomalies present in gilthead seabream: skeletal deformities (vertebral and cranial deformities) and uninflated swimbladder. B) To study the incidence of skeletal deformities along the vertebral column. C) To estimate genetic parameters (heritabilities and genetic correlations) for these anomalies.

Materials and methods

Biological material

Initially, samples of seabream were captured from wild populations from three geographically differentiated origins; Cantabrian Sea (CAN), the Atlantic Ocean (ATL),

and Mediterranean Sea (MED), hence from the Northern, Southern, and Western Spanish coasts respectively. From these samples, three broodstocks were established as is described in Lee-Montero *et al.* (2013). Larvae obtained from mass-spawnings were reared in the conditions described by Roo *et al.* (2009). At 84 days post–hatching (dph) a random sample of 2500 descendants (845 from CAN, 777 from ATL and 878 from MED) was taken to the on-growing facilities of the Centro de Cultivos Marinos de la Región de Murcia (CCRM, San Pedro del Pinatar, Murcia) and constituted the population under study. After an adaption period of 20-30 days, fry were individually tagged in the abdominal cavity for individual identification with a Passive Integrated Transporter (PIT; Trovan Daimler–Benz), according to Navarro *et al.* (2006) and then randomly distributed (one third of each origin) in 12 tank in an open circuit with intake water from the sea and reared under communal conditions. Later, at 163 dph a random sample of about 300 juveniles from each origin was slaughtered by immersion in ice cold water (hypothermia). In that way, almost 1000 samples (304 from CAN, 287 from ATL and 323 from MED) were obtained which were placed on their left side in PVC boxes (12.2 x 12.2 cm) and frozen at -20ºC until anomalies determination. The remaining fish housed in CCRM were conserved for further studies as a part of a selective breeding program (PROGENSA® 2009).

Anomalies

Fish were radiographed using a medical X-ray system (Siemens Multix Swing). The X-ray photographs were used for the skeleton and SB examination (Fig. 1) which was carried out for just one observer in order to avoid bias in the estimates. Vertebral deformities were identified and classified according to a deformities catalog (PROGENSA® 2009). Depending on the region in which lordosis was detected this

deformity was classified as pre-haemal (3rd–10th vertebra) and haemal (11th–21st vertebra) according to Boglione *et al.* (2001). The degree of lordosis was evaluated by measuring the angle between the line from the first vertebra in the head region to the curvature point and the line from the curvature point to the last vertebra in the tail region (Andrades *et al.* 1996). The number of affected vertebrae by skeletal deformities was also recorded to study the incidence of skeletal deformities along the vertebral column. Cranial deformities (upper and lower jaws) were classified following Fernández *et al.* (2008) while the lack of left operculum was evaluated by visual inspection, considering as anomalous those fish with any fold at operculum, which deviated from the normal condition. The uninflated SB was determined following Andrades *et al.* (1996).

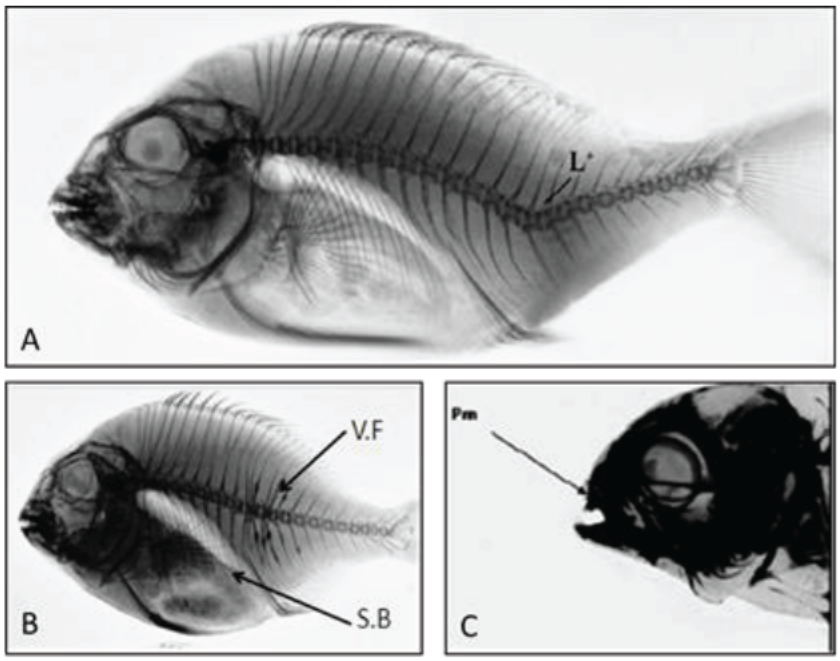

Figure 1 Gilthead seabream showing A) Lordosis. B) Vertebral fusion and uninflated swimbladder. C) Premaxillar deformity

Parental assignments

PCR reaction, genotyping and parental assignments between breeders and their descendants were determined as described in Lee-Montero *et al.* (2013). A total of 225 full-sib families, 5 paternal half-sib families and 7 maternal half-sib families were represented.

Data analysis

In order to calculate the frequency of deformed individuals (showing skeletal deformities) each fish was assigned as deformed or un-deformed and scored 1 or 0, respectively. Moreover, all kinds of skeletal deformities and the uninflated swimbaldder were also assigned and scored (0 or 1) separately for each fish. The frequency of individuals with skeletal deformities and the frequency of each deformity were calculated per broodstock origin as a percentage of deformed individuals in total analyzed fish. Anomalies scores were analyzed by logistic regression, using the SPSS® (v.19.0) (SPSS, Chicago, IL, USA) to detect the effect of the broodstocks origin ($P<0.05$).

Anomalies were considered as threshold traits and their genetic parameters (heritabilities and genetic correlations) were estimated in the underlying liability scale under a Bayesian approach by using a bivariate Gaussian mixed model. The estimates on the underlying liability scale assume that the susceptibility for the anomaly is determined by an underlying liability that is distributed normally and inherited in a polygenic manner (Gjerde *et al.* 2005). The analysis was performed using the thrgibbs1f90 developed by Misztal (2010) using the following animal linear model:

$y = X\beta + Zu + e$

where y is the recorded data on the studied traits, β includes fixed effect (broodstock origin), u the random animal effect, e the error and, X and Z are incidence matrices. The analysis was carried out between two traits each time. The following multivariate normal distributions were assumed a priori for random effects:

$P(\beta) \sim k;$

$P(u|G) \sim (0, G \otimes A);$

$P(e|R) \sim (0, R \otimes A);$

Where A is the relationship matrix, k is a constant,

$$G = \begin{bmatrix} \sigma_{U1} & \sigma_{U1,U2} \\ \sigma_{U2,U1} & \sigma_{U2} \end{bmatrix},$$

$$R = \begin{bmatrix} \sigma_{e1} & \sigma_{e1,e2} \\ \sigma_{e2,e1} & \sigma_{e2} \end{bmatrix}.$$

Bounded uniform priors were assumed for the systematic effects and the (co)variance components (G, A). A single chain of 200 000 iterations was run. The first 50 000 iterations of each chain were discarded, and samples of the parameters of interest were saved every 5 iterations. Density plots to represent posterior marginal distribution of heritabilities, posterior means (PM) and the interval of 95% of the highest posterior density [HPD95%] were obtained through R statistical software (2008).

Results

Phenotypic results

Frequencies of deformed fish, vertebral and cranial skeletal deformities, and uninflated SB are given in Table 1. The frequency of deformed fish was lower in fish

from CAN in which a 23.4% of examined individuals had at least one or more kinds of deformities while this frequency was almost 40% for ATL and MED origins.

Lordosis was the most common vertebral deformity, with frequencies around 20% in two of the studied origins (ATL and MED), followed by vertebral shortening. Fish from CAN showed the lowest incidence of these deformities. Lordosis haemal was more common than lordosis pre-haemal. Other minor skeletal deformities such as vertebral fusion and kyphosis were also detected although no effect of the broodstock origin was found for them and their incidence was very low (<3%).

Regardless of the origin, the pre-haemal and haemal regions were the area most affected by skeletal deformities since the majority of them were recorder between the vertebrae 8 and 14 (Fig. 2). The highest incidences of affected vertebrae were recorded in fish from MED. Vertebrae were specially affected by lordosis with a maximum at vertebrae 10 and 12. The major lordotic curvature was 59.5° found between vertebrae 10-24 in fish from MED (data not shown in tables). The minor curvature was found in individuals from CAN with a value of 2.8° between vertebrae 10-11. Kyphosis was distributed along the vertebral column with a maximum value at vertebra 10. For vertebral fusion, the maximum value was registered in vertebrae 10 and 11.

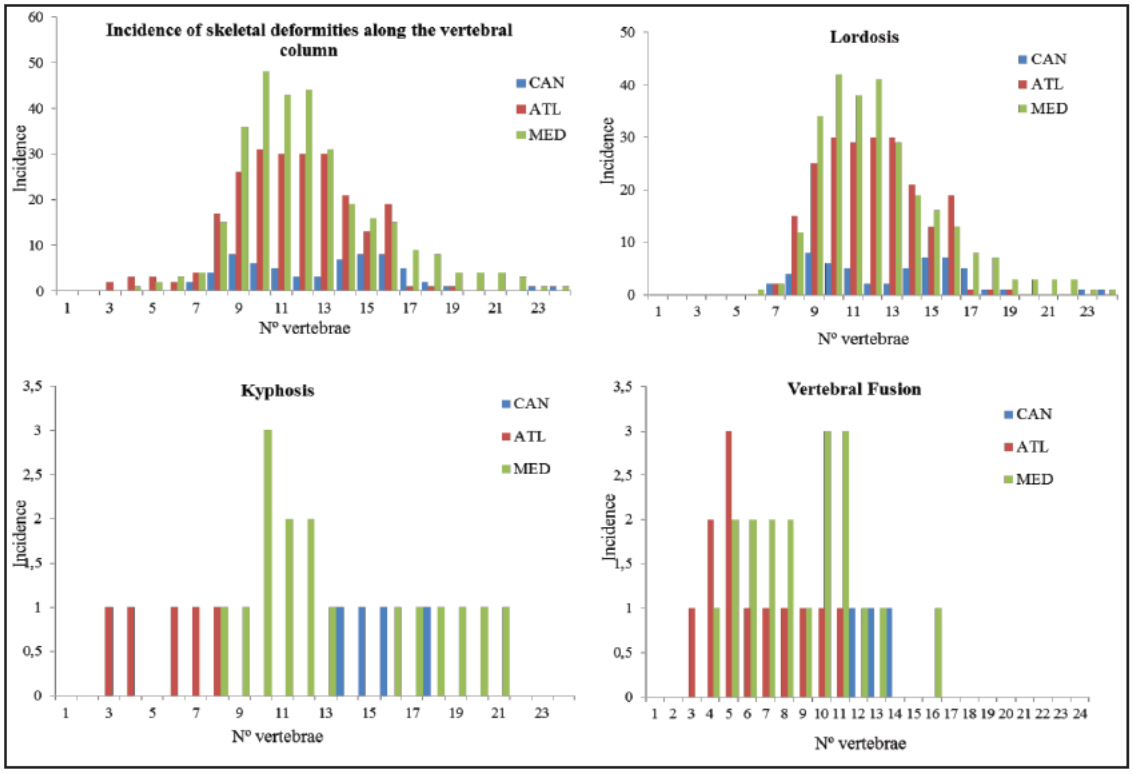

Figure 2 Incidence of skeletal deformities along the vertebral column in gilthead seabream sourced from broodstocks from three origins. Broodstocks origins: CAN = Cantabrian Sea, ATL = The Atlantic Ocean, MED = Mediterranean Sea.

Lack of operculum was the most frequent cranial deformity in all origins although no statistical differences between them were detected. The premaxillar was the only one cranial deformity observed in the jaw. Individuals from CAN and MED showed higher frequencies than those from ATL in which deformities in the jaw were not observed.

Fish from ATL showed the highest frequency (>70%) of uninflated SB coinciding with those that exhibit the highest frequency of lordotic fish.

Table 1 Frequencies (%) of deformed fish (skeletal deformities), vertebral deformities, cranial deformities and uninflated swimbladder (SB) in gilthead seabream sourced from broodstocks from three origins

Broodstock origin[1]	CAN	ATL	MED
N	304	282	323
Deformed	23.4[a]	37.9[b]	38.7[b]
Lordosis	6.6[a]	19.2[b]	21.6[b]
L.Pre-haemal	2.3[a]	5.3[b]	8.2[b]
L.Haemal	4.3[a]	13.9[b]	13.5[b]
Vertebral shortening	3.6[a]	7.8[b]	10.5[b]
Vertebral Fusion	1.3[a]	1.4[a]	1.5[a]
Kyphosis	0.7[a]	0.7[a]	2.5[a]
Lack of operculum	8.2[a]	9.2[a]	5.9[a]
Premaxillar	3.9[b]	0[a]	1.8[b]
Uninflated SB	6.9[a]	70.9[c]	32.5[b]

[1]Broodstocks origin: CAN = Cantabrian Sea, ATL = The Atlantic Ocean, MED = Mediterranean Sea

[abc]Different superscripts within each row indicate significant differences among origins

Heritabilities and genetic correlations between anomalies

Heritabilities estimates (h^2) and genetic correlations (r_g) between anomalies on the liability scale were estimated in a sub-sample of 612 individuals which were correctly assigned to a unique parental couple using the SMsa1. Heritability distributions for vertebral deformities are shown in Fig. 3. Posterior mean (PM) and interval of 95% of the highest posterior density [HPD95%] were 0.53[0.25-0.77] for lordosis, 0.23[0.01-0.68] for vertebral shortening, 0.42[0.05-0.76] for vertebral fusion and 0.36[0.01-0.73] for kyphosis. Genetic correlations between vertebral deformities were imprecise in almost all the cases and only those that were estimated with major

accuracy are shown. Lordosis was negatively correlated with vertebral shortening (-0.64[-1-0.03]) (Fig. 3) with a probability of being negative of 90.2%.

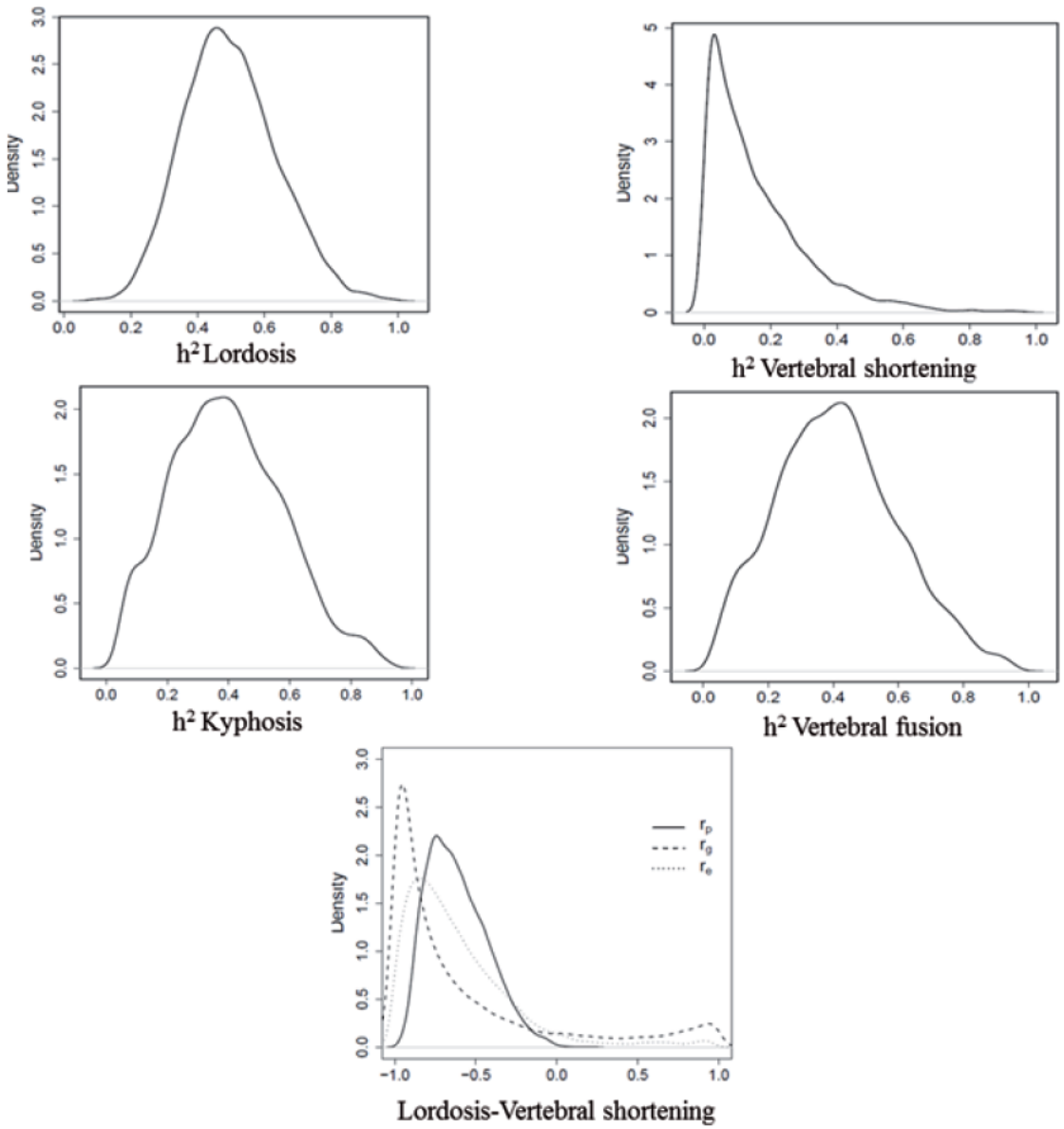

Figure 3 Posterior marginal distributions of heritabilities (h^2), genetic correlations (r_g), phenotypic correlation (r_p) and residual correlation (r_e) between vertebral skeletal deformities estimated from 612 gilthead seabream at 163 days post-hatching.

Cranial deformities showed heritabilities of 0.37[0.01-0.81] and 0.43[0.12-0.78] for lack of operculum and premaxillar deformity respectively (Fig. 4). Both deformities were negatively correlated (Fig. 4) with a probability of 89%.

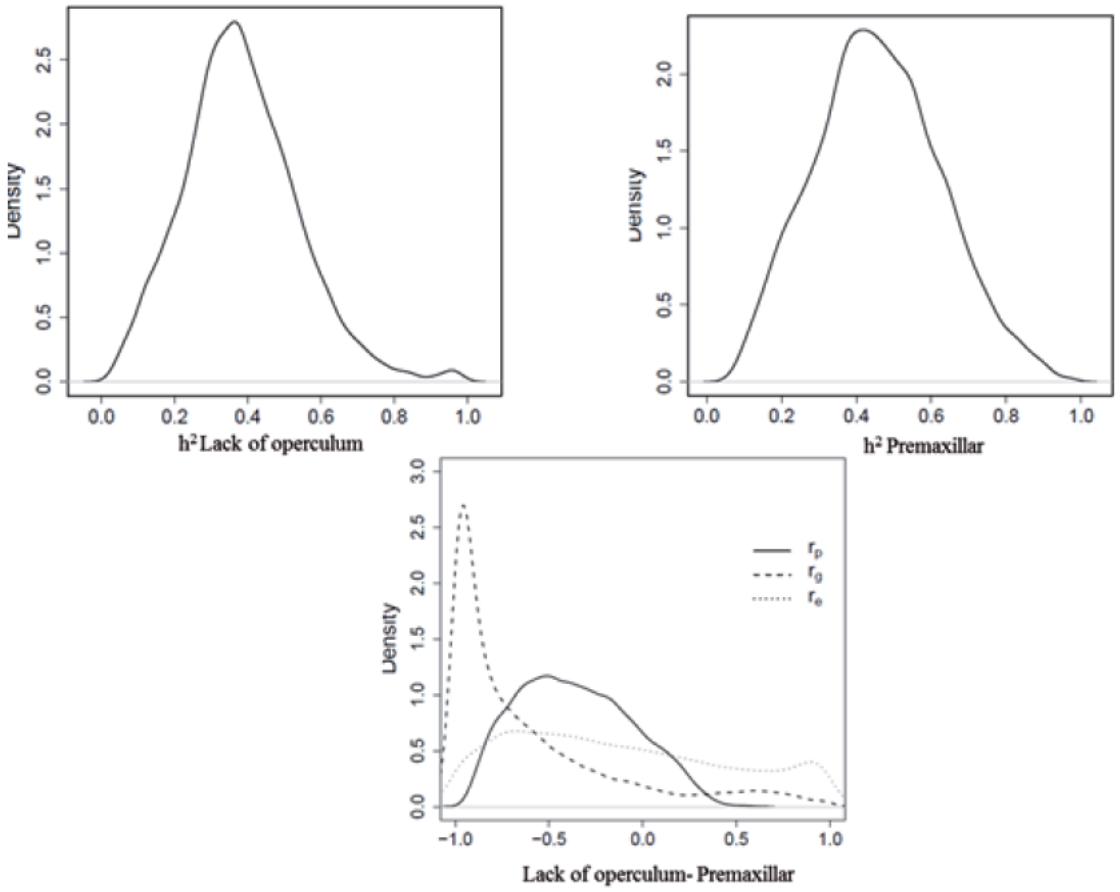

Figure 4 Posterior marginal distributions of heritabilities (h^2), genetic correlation (r_g), phenotypic correlation (r_p) and residual correlation (r_e) of cranial skeletal deformities estimated from 612 gilthead seabream at 163 days post-hatching.

The uninflated SB showed a heritability of 0.36[0.12-0.72] and was positively correlated with lordosis (0.48[0.07-0.97]; Fig. 5) with a high probability (93%).

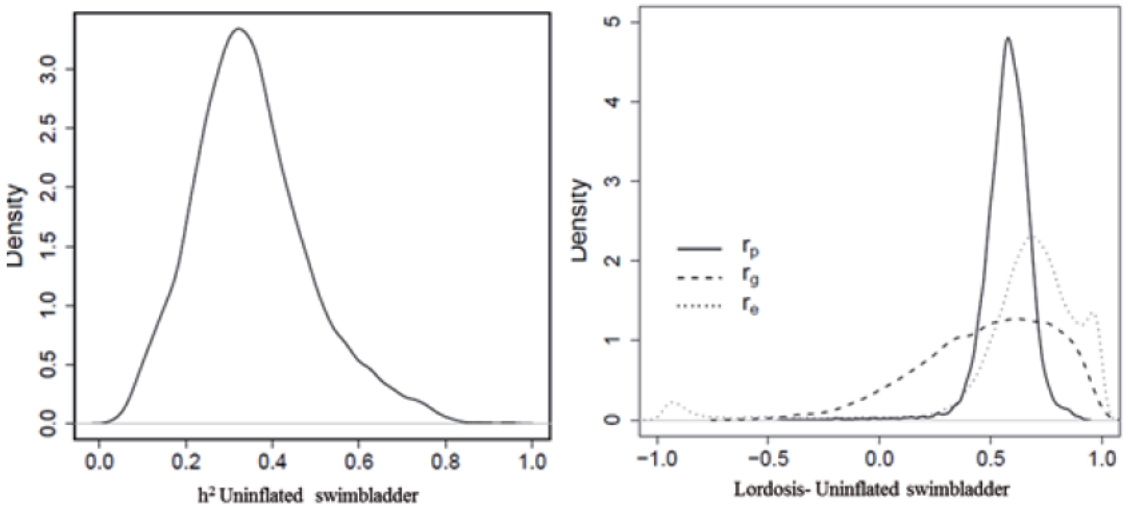

Figure 5 Posterior marginal distributions of heritabilities (h^2) for uninflated swimbladder, genetic correlation (r_g), phenotypic correlation (r_p) and residual correlation (r_e) between uninflated swimbladder and lordosis estimated from 612 gilthead seabream at 163 days post-hatching.

Discussion

Phenotypic results

The presence of deformities, as a measure of fish quality, is the second most economically important trait for the industrial production of gilthead seabream (Georgakopoulou *et al.* 2010). Lordosis and lack of operculum can reach more than 80% individuals in some reared populations (Beraldo *et al.* 2003; Verhaegen *et al.* 2007) seriously compromising both fish morphology and biological performance. In the present study the most frequent deformity for all origins was lordosis followed by lack of operculum except for individuals from MED in which lack of operculum was the third more frequent deformity after vertebral shortening. Vertebral fusion and kyphosis were also observed in the sample but their incidence was low in all origins in agreement with Karahan *et al.* (2013).

Several factors have been associated with skeletal deformities such as temperature (Sfakianakis *et al.* 2006; Georgakopoulou *et al.* 2010), water current (Kihara *et al.* 2002; Karahan *et al.* 2013), intense swimming (Bardon *et al.* 2009), diet composition (Fernández *et al.* 2008), uninflated SB (Chatain 1994), and intensive rearing conditions (Andrades *et al.* 1996; Koumoundouros *et al.* 1997; Boglione *et al.* 2001; Belardo *et al.* 2003; Roo *et al.* 2005). Moreover, an effect of the broodstock origin on lordosis, vertebral shortening and premaxillar deformity was observed in this research. The premaxillar was the only cranial structure affected in the jaw resulting in specimens with compressed snout. This kind of deformity has been reported quite common in reared seabream (Andrades *et al.* 1996; Boglione *et al*. 2001; Fernández *et al.* 2008).

Differences among origins seem to be the results of differences in their genetic backgrounds. Nevertheless, larvae from each origin were reared in different facilities until 84 dph. Despite we tried to reduce environmental effects to a minimum in this initial phase standardizing the rearing protocol, some environmental perturbations could happen. In fact, environmental variations have shown to impact the expression of deformities especially during larval and juvenile stages (Andrades *et al*. 1996; Bardon *et al*. 2009; Karahan *et al.* 2013). In addition, genetic sensitivity to these environmental stressors could exists (Karahan *et al.* 2013), which may then be expressed as genotype by environment interactions.

Vertebral deformities were distributed along the vertebral column and a variable number of vertebrae were affected. Regardless of the origin, the pre-haemal and haemal regions were the areas most affected coinciding with previous research (Andrades *et al.* 1996; Fernández *et al.* 2008; Boglione *et al.* 2001). Regarding the lordotic curvature the observed changes ranged from minor deviations in form, to severe lordosis affecting

almost all the vertebral column. The major degree of lordosis was found in fish from MED coinciding with the highest frequency of lordotic individuals. Andrades *et al.* (1996) found a major lordotic curvature of 45° between vertebrae 10 and 13. Therefore, it seems that skeletal deformities tend to be more frequent and severe in the middle of the vertebral column being the pre-haemal and haemal regions the most affected commonly.

Culture conditions have been suggested as main contributory factors in the presence of uninflated SB. An effect of the broodstock origin was found in the present study as well. However, we cannot rule out the effect of the environmental conditions in the initial facilities as we mentioned above. Slightly lower rates of uninflated SB than those obtained in our study have been reported in seabass larvae (40 dph) previously (García de León *et al.* 1998; Saillant *et al.* 2002; Peruzzi *et al.* 2007).

The X-ray examination carried out here seems to be a useful method to study body anomalies in breeding programmes since it could be performed without killing the fish which is effective for individual selection. Moreover, vertebral deformities may be seen in fish that externally look normal (Gjerde *et al.* 2005; Bardon *et al.* 2009; Karahan *et al*. 2013), uninflated SB could be easily perceived even in individuals of small size and images can be stored for later use, e.g. for data checking or to serve as reference for later generations.

Heritabilities and genetic correlations between anomalies

No evidence of genetic component (heritabilities adjusting an animal model) has been found for lordosis in gilthead seabream (Castro *et al.* 2008). However, an increasing number of studies suggest that some fish deformities are determined by a relevant additive genetic component that would allow the improvement of these traits

through selective breeding. According to this, we found considerable heritability for lordosis which is one of the most relevant deformities in gilthead seabream (Belardo *et al.* 2003). Estimated heritabilities for vertebral deformities on the liability scale revealed heritable component in the European seabass (Bardon *et al.* 2009; Karahan *et al.* 2013), Atlantic salmon (McKay & Gjerde 1986; Gjerde *et al.* 2005), and in Atlantic cod (Kolstad *et al.* 2006) using a linear animal model.

We found substantial heritabilities for lack of operculum and deformities in the jaw. Nevertheless, other authors found no evidence of genetic component for cranial deformities in seabream (Castro *et al.* 2008), tilapia (Handwerker & Tave 1994; Tave & Handwerker 1994) and in common carp (Kocour *et al.* 2006). This suggests the absence of additive genetic variation, although it could also be a consequence of the difficulty in correctly identifying head deformities.

The genetic basis of phenomena of hypertrophy of the SB has been little investigated despite being known to cause considerable losses in some species. We estimated the heritability for uninflated SB which is the first result reported in gilthead seabream. Conflicting results about the genetic basis of this anomaly have been reported. In seabass, Peruzzi *et al.* (2007) suggest that paternally and maternally inhered factors may contribute to the expression of SB anomalies. However, in the same species García de León *et al.* (1998) reported no significant parental effect for such anomaly although using a lower number of families. In striped bass, Harrell *et al.* (2002) estimated moderate genetic value (0.35) for full-sibling families, and a low value (0.04) for half-sib dam families. Therefore, further studies are necessary to clarify this fact.

Regarding correlations, we found negative genetic correlations between skeletal deformities. Nevertheless, Afonso *et al.* (2000) suggested that a column abnormality in seabream consisting of a consecutive repetition of lordosis–scoliosis– kyphosis from

head to tail had polygenic origin. In seabass Bardom *et al.* (2009) found a high genetic correlation (0.69) between lordosis and scoliosis.

We were unable to estimate genetic correlations between vertebral and cranial skeletal deformities with accuracy. In this regard, Kocour *et al.* (2006) pointed out imprecise genetic correlations among mouth, anal and caudal deformities.

The uninflated SB has been reported to show skeletal deformities at the phenotypic level in seabream and seabass (Chatain 1994; Andrades *et al.* 1996; Divanach *et al.* 1996) as well as in other species (Kitajima *et al.* 1994; Trotter *et al.* 2001; Jacquemond 2004). Moreover, we found a positive genetic correlation between lordosis and uninflated SB, first reported in this species.

Conclusions

Juveniles from CAN showed the lowest frequency of skeletal deformities as well as the lowest frequency of uninflated SB which constitutes relevant information for the establishment of the broodstock to produce commercial fish.

Vertebral and cranial skeletal deformities and uninflated SB showed considerable heritabilities being the genetic correlation between lordosis and uninflated SB positive. Therefore, it could be recommended, by precaution, to eliminate deformed fish from a breeding nucleus although it would also be relevant to study the genetic correlation between deformities and growth which is the most economically important objective in the majority of fish genetic selection programs.

All these findings should be relevant for the establishment of successful breeding programs in aquaculture of this species.

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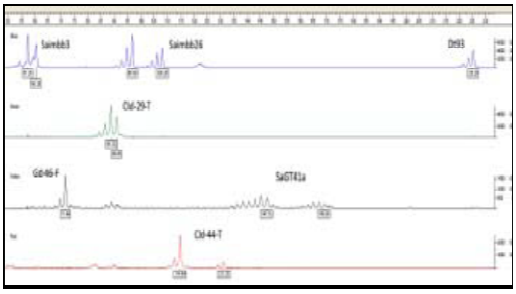

CAPÍTULO IV

IV. 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.

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ABSTRACT

Carcass quality traits such as visceral fat and fish morphology have a direct influence on final product and consumer preferences, especially in species as sea bream that are sold as whole fish. Nevertheless, strategies that involve the development of selection schemes for these traits of economic interest in gilthead sea bream are limited. In this study the effect of the origin of the broodstock on carcass quality traits was analyzed in harvest size (690 days post_hatching) gilthead sea bream for the first time. For this purpose, a population (n = 890) of farmed gilthead sea bream obtained by industrial mass-spawnings from broodstocks from three different origins [Cantabrian Sea (CAN), the Atlantic Ocean (ATL) and Mediterranean Sea (MED)] was analyzed for condition factor, visceral fat content, dressing weight, dressing percentage, fillet weight and fillet percentage. Moreover, with the goal of estimating genetic parameters (heritabilities and genetic correlations) for carcass quality traits as well as their correlations with growth (harvest weight and length), a reconstruction of pedigree was carried out *a posteriori*. The origin had an effect on several carcass traits. Fish from ATL showed the lowest visceral fat percentage, dressing weight and percentage and those form CAN the lowest condition factor. These differences among origins can be explained through their different genetic backgrounds but also by environmental conditions in the initial facilities, where each origin was reared, and the derivate genotype x environment interactions. All carcass traits showed medium heritabilities (ranging from 0.17 to 0.24) and were estimated with accuracy (standard errors from 0.05 to 0.07) except dressing (0.07±0.05) and fillet (0.11±0.05) percentage. Due to their genetic correlations, selection on weight could lead to an increase in condition factor

(0.47±0.21) but, at the same, to an undesirable increase in visceral fat (0.42±0.20) and a decrease in fillet yield (-0.58±0.09). However, selection on length could improve dressing (0.87±0.07) and fillet weight (0.84±0.09). Alternatively, visceral fat content could be decreased by selection through condition factor (-0.46±0.16). All findings reported in this study should be relevant for the establishment of successful breeding programs in aquaculture of this species.

1. Introduction

Gilthead sea bream (*Sparus aurata* L.) is a member of the family *Sparidae* and one of the most important farmed fish in Europe, especially in the Mediterranean area. The main producer countries are Greece, Turkey and Spain that accounted for the 40.9%, 22.7% and 11.0% of total production, respectively. In Spain, this species is cultivated along the Mediterranean and Atlantic coasts although the 70% of total production is concentrated in the East coasts (APROMAR, 2013).

Although industry has focused their efforts mainly in the improvement of growth, nutrition and morphology parameters, quality traits are crucial for the achievement of high-quality products demanded by consumers. The knowledge about the genetic correlations between growth and carcass traits is important in order to make decisions in genetic selection processes. Therefore, these parameters should be incorporated into the goals of commercial breeding programs although the genetic information available for these types of traits in sea bream is limited. Consumers will not buy low-quality products, even at reduced prices. However, most consumers are usually unwilling to pay too high a price for superior quality (Gjedrem, 1997). In this regard, carcass quality traits directly influence yield of final product and consumer preferences (Neira et al., 2004). One important quality factor is the fat that is concentrated inside the visceral cavity which has a negative effect on carcass quality traits for several reasons. Visceral fat affects carcass yield (Rye and Gjerde, 1996; Kause et al., 2002; Neira et al., 2004; Haffray et al., 2007) and the visual impression received by consumers, especially in species as gilthead sea bream which are mainly marketed as whole fish. Moreover, visceral fat could give a strong and unpleasant smell that often emanates from well-fed aquaculture fish (Grigorakis, 2007). Finally, it

represents lost energy since the visceral fat is thrown away together with the intestines when the fish is gutted.

Other important carcass quality traits are dressing and fillet percentages since a high percentage means that a large part of the carcass is edible (Gjedrem, 2005) and because one of the aims of farmed fish production is to convert feed into muscle and decrease slaughter waste. Hence, the genetic improvement of fillet should be of high priority for breeders, although this is challenging in practice (Kause et al., 2011).

On the other hand, the morphology of the fish, which is reflected in the condition factor, is also an important trait influencing the final decision by the consumer and the price of species that are sold as whole fish (Navarro et al., 2009a). The morphology affects the consumer impression i.e. it plays a role in the visual sense.

Strategies for the implementation of selection schemes for economically important traits in gilthead sea bream are still scarce due to the biological characteristic of this species. Gilthead sea bream is a mass-spawing species in which individuals are males during the first two years of life and then gradually become females. Molecular markers such as microsatellites are useful tools to assign parentage and to estimate genetic parameters (Castro et al., 2008; Lee-Montero et al., 2013).

The use of different rearing systems and broodstocks to produce commercial fish usually causes great variability of the production i.e. in the growth rates and the overall quality of the end product (Ayala et al., 2010). Different degrees of genetic differentiation between sea bream populations inferred by microsatellites have been found (Alarcón et al. 2004; Ben-Slimen et al. 2004; De Innocentiis et al. 2004; Šegvić-Bubić et al. 2011). However, we did not find works in which the relationship between the origin of the broodstock and carcass quality traits had been studied in this species.

Considering all these circumstances, the aims of this research were: A) To study the effect of the origin of the broodstock on carcass quality traits (condition factor, visceral fat content, dressing weight, dressing percentage, fillet weight and fillet percentage) as well as to estimate the phenotypic correlations between them and with growth traits (harvest weight and length). B) To estimate genetic parameters (heritabilities and genetic correlations) for growth and these carcass quality traits in a population of gilthead sea bream sourced from broodstock from three origins.

2. Materials and methods

2.1. Broodstocks and offspring. Rearing conditions

Initially, samples of sea bream were captured from wild populations from three geographically differentiated origins along the Spanish coast; Cantabrian Sea (CAN), the Atlantic Ocean (ATL), and Mediterranean Sea (MED). From these samples, three broodstocks were established in different Spanish facilities where fingerlings were obtained and reared in the same conditions. At 84 days post_hatching (dph) a random sample of 2500 individuals, in which all origins were represented, was taken to the on-growing facilities of the Centro de Cultivos Marinos de la Región de Murcia.

Fingerlings were individually tagged in the abdominal cavity for individual identification and then randomly distributed in 12 tanks and reared under communal conditions. At 325 dph the majority of the fish (about 2000 specimens) were moved to the facilities of the company Servicios Atuneros del Mediterraneo S.L. (San Pedro del Pinatar, Murcia, Spain) where they were reared in a cage in the Mediterranean sea under intensive conditions. At harvest size (690 dph), the fish were slaughtered and transported to the laboratory for assays. Specimens were kept on ice during the study at 4ºC refrigeration until the moment of their analyses.

The establishment of broodstocks, the conditions of spawn, the rearing conditions and the slaughter process are explained in more detail in García-Celdrán et al. (2015).

2.2. Analyzed traits

Body weight and fork length were measured and condition factor was determined (100 × body weight × fork length^{-3}). All individuals were inspected and all of them were sexual immature. Visceral fat deposits were manually removed, weighed and expressed as a percentage of the body weight; then gutted body weight was recorded (dressing weight) and also expressed as a percentage of the body weight (dressing percentage). Fish were manually skinned and filleted and both fillets were weighed together (fillet weight) and also expressed as a percentage of the body weight (fillet percentage).

2.3. PCR reaction and genotyping

The genetic characterization of breeders and juveniles and the parental assignments between them were conducted according to García-Celdrán et al. (2015).

2.4. Analysis data

All data were tested for normality and homogeneity of variances using SPSS® (v.19.0) (SPSS, Chicago, IL, USA) and then analyzed by the General Liner Model:

$y_{ij} = \mu + origin_i + \beta * body\ weight_j + e_{ij}$

in which y is the data recorded for the analyzed variable, μ is the overall mean, origin is the effect of the broodstock origin (i = CAN, ATL and MED), β is the regression

coefficient between the analyzed variable and the covariate body weight and e_{ij} is a random residual error.

Pearson correlation was carried out to determine phenotypic correlations among the analyzed parameters. The level of significant difference was set at $P<0.05$.

Bivariate analyses were carried out using a Restricted Maximum Likelihood (REML) algorithm to obtain (co)variance components through the following animal linear model, with body weight as a covariate for carcass quality traits:

$y = X\beta + Zu + e$

where y is the recorded data on the studied traits, β the fixed broodstock origin effect, u the random animal effect and e the error. Non-genetic maternal and/or paternal effects were not significant for the studied traits so they were removed from the model when analyzing carcass traits. The model was resolved with the software package VCE (v 6.0) (Groeneveld et al., 2010). The magnitude of estimated heritability was established, following the classification of Cardellino and Rovira (1987), as low (0.05–0.15), medium (0.20–0.40), high (0.45–0.60) and very high (N>0.65). Correlations were classed as low (0–0.40), medium (0.45–0.55) and high (0.60–1), regardless of the sign (Navarro et al., 2009a, b).

3. Results and discussion

3.1. Phenotypic results

The effect of the broodstock origin of the fingerlings on carcass quality traits was assessed in gilthead sea bream for the first time in this study. Due to the wide range of values observed for weight, discussed in detail in García-Celdrán et al. (2015), phenotypic results for carcass traits (Table 1) were adjusted to a constant final weight (232.5 g) to be compared. However, due to the requirements of the project

(PROGENSA®, http://www.progensa.eu) it was not possible to obtain a commercial size of 300 g at slaughter. All studied traits showed significant differences ($P \leq 0.05$) among origins except fillet weight and percentage ($P = 0.08$). The covariate fish weight was positive and highly significant ($P \leq 0.01$) for all studied traits. Thus, when the final weight increased 10 grams, condition factor increased 0.01, visceral fat 0.06%, dressing weight 7.7 grams, dressing percentage 0.3%, fillet weight 3.8 grams and fillet percentage 0.1%. Other authors (Ginés et al., 2004; Grigorakis and Alexis, 2005) have also reported positive correlation between fish weight and the amount of visceral fat.

Table 1: Phenotypic results (least square means ± standard error) for weight, condition factor and carcass traits for gilthead sea bream from three broodstock origins at harvest age (690 days post-hatching) adjusted to a final fish weight of 232.53 grams.

Broodstock origin[1]	CAN	ATL	MED	Covariate[2]
N	424	245	221	890
Weight (g)	$238^{b} \pm 2.0$	$188^{a} \pm 2.8$	$271^{c} \pm 2.9$	
Condition Factor ($g\ cm^{-3}$)	$1.56^{a} \pm 0.010$	$1.71^{b} \pm 0.015$	$1.67^{b} \pm 0.015$	$0.001^{**} \pm 0.000$
Visceral fat (%)	$6.24^{a} \pm 0.052$	$5.89^{b} \pm 0.077$	$6.26^{a} \pm 0.078$	$0.006^{**} \pm 0.001$
Dressing weight (g)	$204.4^{a} \pm 0.71$	$199.2^{b} \pm 1.06$	$205.3^{a} \pm 1.08$	$0.772^{**} \pm 0.011$
Dressing percentage (%)	$88.0^{a} \pm 0.23$	$86.5^{b} \pm 0.35$	$88.3^{a} \pm 0.35$	$0.034^{**} \pm 0.004$
Fillet weight (g)	85.5 ± 0.50	83.8 ± 0.75	85.5 ± 0.76	$0.380^{**} \pm 0.008$
Fillet percentage (%)	36.8 ± 0.22	36.0 ± 0.32	36.4 ± 0.33	$0.009^{**} \pm 0.003$

[1]Broodstock origin: CAN = Cantabrian Sea, ATL = The Atlantic Ocean, MED = Mediterranean Sea

[2]Regression coefficient for the covariate fish weight, units are the unit of each trait per gram of fish weight, and standard error (**P <0.01)

[abc]Different superscripts within each row indicate significant differences among origins (P<0.05)

Regarding condition factor, Glover et al. (2009) compared salmon of farmed, wild and hybrid origin in a simulated aquaculture production cycle. At slaughter, they found that this trait was considerably higher in farmed contra wild salmon, with hybrids

displaying intermediate or similar values. Vandeputte et al. (2014) compared five wild populations of sea bass founding differences in shape. In the present research, fish from CAN showed a lower value for condition factor than those from ATL and MED pointing out an effect of the origin of the broodstocks on this trait. Other factors have shown an effect on this parameter as well. An increase of condition factor with intensification of culture has been observed for gilthead sea bream (Francescon et al., 1988; Sañudo et al., 1993; Flos et al., 2002; Navarro et al., 2009b), while condition factor reduces during food deprivation (Grigorakis and Alexis, 2005).

The broodstock origin had an effect on visceral fat percentage in the present study with fish from ATL showing the lowest percentage (5.7% lower than the overall average). Visceral fat content is influenced by a variety of factors related to diet (Santinha et al., 1999; Grigorakis and Alexis, 2005), season (Grigorakis et al., 2002), production system (Navarro et al., 2009b) and sex (Kause et al., 2002; Neira et al., 2004). In the case of gilthead sea bream reported values for this trait ranged from 0.79% to 2.98% (Navarro et al., 2009b; Santinha et al., 1999; Grigorakis et al., 2002; Grigorakis and Alexis, 2005). Our results showed a high visceral fat deposition (about 6% in all origins) and were in the upper range of values reported for the former authors probably due to the fact that they were older (690 dph) than those of previous studies. As a general rule, young animals use their energy for muscle growth and then deposit more fat when they get older (Gjedrem, 1997).

Dressing and fillet percentages are characteristics highly appreciated by consumers and fish processing companies (Neira et al., 2004) and filleted sea bream is growing (Luna, 2006) opening new possibilities for sea bream commercialization. In the present study, an effect of the broodstock origin was observed on dressing weight and dressing percentage with fish from ATL showing the lowest values in spite of the

lowest percentage of visceral fat observed in fish from that origin but these differences were lower (2.6% for dressing weight and 1.7% for dressing percentage lower than the overall average) than for visceral fat percentage. However, no significant differences among origins for fillet weight and percentage were observed in any case. In this regard, Vandeputte et al. (2014) found that populations of sea bass were different in carcass yield, but no in fillet yield. Skeletal muscle is the largest organ system in fish and corresponds actually to the edible part of it. Expressed as fillet yield in gilthead sea bream of commercial sizes the skeletal muscle represents the 34.3–48% of the total body weight (Grigorakis, 2007). This is in agreement with the results obtained in the present research as well as with those obtained by other authors in the same species (Navarro et al., 2009b).

In the present study, fish were held in the same station the majority of their life raised under the same conditions. Moreover, they were slaughtered and stored the same way. Differences observed in them seem to be the results of differences in their genetic backgrounds. However, larvae from each origin were reared in different facilities until 84 dph. Despite that the rearing protocol was standardized some environmental perturbations could happen which may be expressed as genotype x environment interaction. The same could happen after mixing of the origins in 12 tanks during the pre-on-growing period since the tank effect cannot be analyzed.

3.2. Heritability of carcass quality traits

Heritabilities and their corresponding standard errors ($h^2 \pm SE$) of carcass traits (Table 2) were estimated in 890 individuals correctly assigned to a unique parental couple using the SMsa1. All carcass traits, except dressing and fillet percentages, showed medium heritabilities that could allow genetic improvements through

implementation of selection programs. Although few researches have been found regarding the genetic basis of carcass quality trait for sea bream, we found references for other species.

The estimate of heritability for condition factor was 0.18±0.07 and was in the range of estimates reported in sea bream (Navarro et al., 2009a) and sea bass (Dupont-Nivet et al., 2008; Saillant et al., 2009). However, higher genetic variance has been observed for this trait in other species including Atlantic salmon (Rye and Gjerde, 1996), common carp (Vandeputte et al., 2004), rainbow trout (Kause et al., 2002) and European whitefish (Kause et al., 2011) with values ranging from 0.31 to 0.49 respectively. The lower heritabilities of condition factor in comparison with other species can be explained because gilthead sea bream presents, proportionally, a greater growth in height than in length (Navarro et al., 2009a).

Visceral fat showed medium heritability (0.20±0.06). Higher values has been found for this trait in gilthead sea bream (Navarro et al., 2009b) as well as in other species with heritabilities ranging from 0.30 to 0.68; Rye and Gjerde (1996) in Atlantic salmon, Gjerde and Schaeffer (1989) in rainbow trout and Saillant et al. (2009) in sea bass, highlight the genetic basis for this trait so that selection for decreased fat content is feasible.

Within livestock breeding programs the yield of the final product is a highly desirable trait, and is commonly a target for selection (Powell et al., 2008). In the present research, medium heritability was observed for dressing weight (0.24±0.06), for the first time reported in sea bream, while heritability for dressing percentage was low (0.07±0.05) in accordance with previous results in salmon. In this regard, Powell et al. (2008) found high genetic variation for carcass weight (0.51) but low for carcass yield (0.02) and Neira et al. (2004) observed medium heritability for carcass weight.

However, higher genetic variance for dressing percentage than those estimated in the present research has been reported with considerable variation among studies ranging from medium heritabilities (0.20; Rye and Gjerde, 1996; 0.23-0.33; Neira et al., 2004; 0.31; Navarro et al., 2009a, b; 0.36; Gjerde and Schaeffer, 1989) to high (0.45; Kause et al., 2002) and very high (0.58 to 0.74; Haffray et al., 2007) estimates.

Regarding fillet traits, we observed medium heritability for fillet weight (0.17±0.05) in agreement with results obtained by Kause et al. (2002, 2007), while for fillet percentage the estimated heritability was lower (0.11±0.05). In this regard, medium genetic variance for fillet yield have been found in black sea bream (0.30; Doupe and Lymbery, 2005), common carp (0.21; Kocour et al., 2007) and in rainbow trout (0.33; Kause et al., 2002). The higher heritability for fillet weight than for fillet percentage reported here is in accordance with those obtained in sea bream (Navarro et al., 2009a) as well as in other species (Neira et al., 2004; Rutten et al., 2005; Powell et al., 2008; Saillant et al., 2009; Kause et al., 2011).

The moderate genetic variance for carcass and fillet yield observed in our study suggests that response to selection for increasing yields would be slow. This is probably due to a low degree of repeatability of the processes of gutting and filleting without skin leading to variations in these data (Kocour et al., 2007). Variability in genetic parameters (heritabilities and genetic correlations) among studies may also result from genotype by environment interactions. Such variability highlights the fact that these parameters are population and environment specific (Kause et al., 2002). In this regard, there was considerable variation among the estimated correlations between pairs of traits among species.

3.3. Correlations between growth traits and carcass quality traits

Due to the fact that growth is the most economically important objective in the majority of fish genetic selection programs (Saillant et al., 2006) it is important to study its genetic correlations with carcass traits in order to make decisions in selection processes. Moreover, the evaluation of carcass traits, which are laborious to record, requires technical skills and the slaughter of the fish, whereas growth traits can easily be measured in the field and allows the evaluation of a high number of fish per time unit (Kause et al., 2007).

Body weight was phenotypically positively correlated with all carcass traits (P<0.01) except with dressing weight and fillet weight.

Regarding genetic correlations, weight was positively correlated with condition factor in concordance with previous results (Rye and Gjerde 1996; Kause et al. 2002; Saillant et al., 2009; Kause et al., 2011). However, the correlation between length and condition factor was close to zero although imprecise due to the high standard error in line with results obtained previously in sea bream (Navarro et al., 2009a) and sea bass (Dupont-Nivet et al., 2008) suggesting that improvements in growth would produce no change in fish conformation.

The genetic correlation between weight and visceral fat was in the upper range of those previously reported in the same species (Navarro et al., 2009b) indicating an undesirable increase of fat with selection for growth in the population under study in agreement with Rye and Gjerde (1996) and Saillant et al. (2009). The correlation between length and visceral fat was imprecise and it was not possible to draw any conclusion about them.

Due to convergence problems, the genetic correlation between harvest weight and dressing weight could not be estimated. However, dressing weight and length were

positively correlated in line with results reported by Powell et al. (2008) showing that dressing weight increase as length increases. In this regard, strong genetic correlations between body weight and carcass weight have been obtained in other species (Neira et al., 2004; Doupé and Lymbery, 2005).

The genetic correlations between growth traits and dressing percentage were close to zero in accordance with those obtained by Navarro et al. (2009b) questioning the use of yield traits in selection criteria. In fact, the results are inconsistent among species ranging from medium and negative (Neira et al., 2004; Kause et al., 2011) to high and positive (Rye and Gjerde 1996) correlations.

Positive and high phenotypic and genetic correlations between growth and fillet weight have been found in sea bream (Navarro et al., 2009a) as well as in other species (Kause et al., 2002; Rutten et al., 2005; Kause et al., 2007, 2011; Powell et al., 2008) indicating the potential for using easily recorded weight as a selection criterion for improved meat weight of fish at harvest. We were unable to estimate the genetic correlation between harvest weight and fillet weight due to convergence problems. However, fillet weight was strongly genetically correlated to length suggesting that fillet weight could be controlled in fish with improved length and therefore, its inclusion in breeding programmes might be recommended.

Regarding growth and fillet percentage, negative genetic correlation were obtained in the present study in agreement with Navarro et al. (2009a) but with low consistency in the case of length due to the high standard error. The results are not consistent among species ranging from genetic correlations close to zero (Kause et al., 2007, 2011) to medium (Kause et al., 2002; Saillant et al., 2009) to high (Neira et al., 2004; Doupé and Lymbery, 2005; Rutten et al., 2005; Kocour et al., 2007).

Weight and length showed medium heritabilities at harvest and both traits were phenotypically and genetically correlated suggesting that both traits could be useful criterion for selection. In fact, Navarro et al. (2009a) and Vandeputte et al. (2008) proposed using length instead of weight since it is easy to measure in the field and more repeatable.

3.4. Correlations between carcass quality traits

Genetic correlations between carcass quality traits were imprecise in many cases, especially those between dressing and fillet percentages and the rest of analyzed traits as was indicated by their large standard errors (Table 2) probably due to the low heritability of these traits (Kause et al., 2011). However, phenotypic correlations were very precise in all cases due to the large number of samples analyzed.

Regarding condition factor, Kause et al. (2002), Grigorakis and Alexis (2005), Navarro et al. (2009b) and Saillant et al. (2009) reported positive phenotypic correlations with visceral fat. We found that both traits were highly and significantly correlated at the phenotypic level as well, being our results much higher. Previous researches have reported high and positive genetic correlations between these both traits (Rye and Gjerde, 1996; Navarro et al., 2009b; Saillant et al., 2009) while we found this correlation medium and negative in agreement with Kause et al. (2002). Therefore, due to the medium heritability shown for both traits and due to their genetic correlation, it seems that condition factor could be useful as a non-invasive index for predicting the response of fish fat deposit content in a breeding programme. Moreover, this parameter could easily be measured on the live breeding candidates which is effective in the case of individual selection. However, because the evaluation of visceral fat is lethal for breeding candidates, this implies that selection through fat should be done on the basis

of slaughtering traits of their relatives. This practice would require identification of families, either by separate rearing or by genotyping (Kocour et al., 2007).

We found some conflicting result in the present research. Condition factor was negatively correlated with visceral fat and positively correlated with weight. Nevertheless, we observed a negative correlation between weight and visceral fat, as we said before. Therefore, further studies are necessary to draw more precise conclusions about these correlations.

The genetic correlation between condition factor and dressing weight was high and negative so selection for increasing condition factor may also lead to a decrease in dressing weight in the population under study. The rest of the genetic correlations for condition factor and carcass traits were found to be inconsistent although the phenotypic correlation between condition factor and dressing and fillet percentages were found to be high and significant (P <0.01).

Regarding the genetic correlations between visceral fat with dressing and fillet weight, high and medium values were obtained respectively. In spite of their high phenotypic correlation, the genetic correlations between visceral fat with dressing and fillet percentages were inconsistent in accordance with Kause et al. (2002) and Saillant et al. (2009). However, Haffray et al. (2007) and Navarro et al. (2009a) reported very high and negative genetic correlation between visceral fat and dressing yield.

Concerning carcass weight, it was highly and positively correlated with dressing percentage at the phenotypic level although its genetic correlations with dressing percentage and the rest of carcass trait analyzed were imprecise as was denoted by their large standard errors.

Dressing and fillet percentage showed a strong phenotypic correlation while the genetic correlations among dressing yield and the rest of carcass traits studied were imprecise as well as the genetic correlation between fillet weight and percentage.

Table 2: Genetic correlations ± standard error (above the diagonal), phenotypic correlations ± standard error (below the diagonal) and heritabilities ± standard errors (in bold at the diagonal) of condition factor, carcass and growth traits estimated from 890 gilthead sea bream at harvest age (690 days post-hatching)

	Condition Factor	Visceral fat	Dressing weight	Dressing (%)	Fillet weight	Fillet (%)	Weight	Lenght
Condition Factor	**0.18±0.07**	-0.46±0.16	-0.62±0.16	-0.10±0.84	-0.15±0.23	0.23±0.92	0.47±0.21	0.01±0.18
Visceral fat	0.76**±0.02	**0.20±0.06**	0.62±0.15	0.50±0.25	0.53±0.17	-0.23±0.95	0.42±0.20	0.33±0.23
Dressing weight	0.47±0.03	0.47±0.03	**0.24±0.06**	0.82±0.99	0.01±0.99	0.01±0.99	-	0.87±0.07
Dressing (%)	0.94**±0.01	0.79**±0.03	0.62**±0.03	**0.07±0.05**	0.99±0.98	0.96±0.99	0.01±0.11	0.01±0.12
Fillet weight	-0.02±0.03	0.12±0.03	0.26±0.02	0.15±0.03	**0.17±0.05**	0.99±0.98	-	0.84±0.09
Fillet (%)	0.92**±0.01	0.75**±0.02	0.54±0.03	0.93**±0.02	0.38±0.03	**0.11±0.05**	-0.58±0.09	-0.59±0.50
Weight	0.99*±0.01	0.76**±0.02	0.47±0.03	0.94**±0.01	-0.02±0.01	0.92**±0.01	**0.25±0.07**	0.86±0.05
Lenght	0.82**±0.02	0.60**±0.03	-0.03±0.02	0.77**±0.02	-0.02±0.02	0.75**±0.02	0.82**+0.02	**0.22±0.07**

Dressing (%) = Dressing percentage
Fillet (%) = Fillet percentage
** Significant Pearson correlations between carcass quality traits (P <0.01).
- Denotes the correlation that we were unable to estimate due to convergence problems

4. Conclusions

Our results highlight that the acquisition of a stock is an important aspect of broodstock management in sea bream since the different origins presented in the studied population had an effect on important carcass traits. Regarding these traits, juveniles from ATL broodstock were the best performers since fingerlings from that origin showed the highest condition factor and the lowest visceral fat percentage. These differences among origins may be explained through their different genetic backgrounds. However, the effect of the origin could be affected by environmental conditions in the initial facilities and by genotype x environment interactions.

All carcass traits, except dressing and fillet percentages, showed medium heritability and could be included in a selection breeding program. Growth traits are the traits assigned the highest importance in selection programs. As a result of their genetic correlations, selection on weight could lead to an increase in condition factor but, at the same, to an undesirable increase in visceral fat and a decrease in fillet yield so these traits need to be controlled. However, other carcass traits such as dressing and fillet weigh could be improved through direct selection of length.

The evaluation of carcass traits requires the slaughter of the fish and technical skills. Therefore, condition factor is shown as an interesting alternative trait to be included in a breeding programme since it is easy to measure and allows a non-invasive measurement in living candidates. Selection for this trait could lead to a decrease in the undesirable visceral fat and to an increase in weight, due to the genetic correlations between these traits. All these findings should be relevant for the establishment of successful breeding programs in aquaculture of this species.

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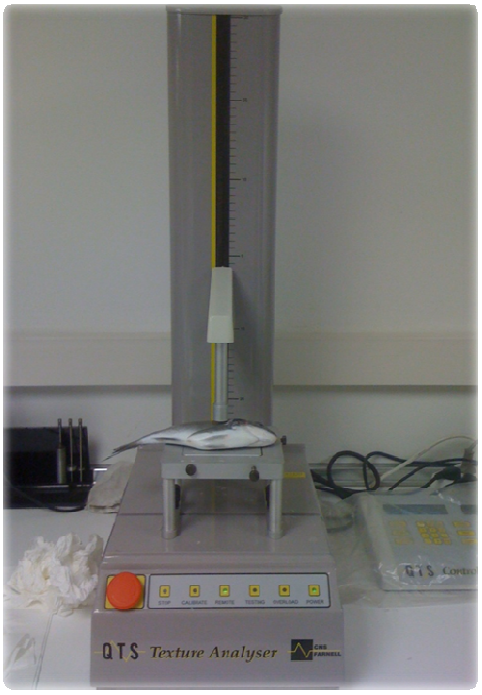

CAPÍTULO V

V. 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.

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ABSTRACT

In gilthead sea bream, flesh quality traits such as body composition and texture directly influence yield of final product and consumer preferences so they should be considered in the breeding goal. However, strategies that involve the development of selection schemes for these traits of economic interest are scarce. Taking into account these circumstances, in this study the effect of the origin of the broodstock on the major flesh quality traits was analyzed at harvest size (690 days post-hatching) and genetic parameters (heritabilities and genetic correlations) were estimated as well as their correlations with harvest weight. For this purpose, a population of farmed gilthead sea bream was obtained from three broodstock of different geographical origins along the Spanish coast [Cantabrian Sea (CAN), the Atlantic Ocean (ATL) and Mediterranean Sea (MED)]. Parental assignments between breeders and their offspring were carried out *a posteriori* using a microsatellite multiplex (SMsa1). In the offspring, raw flesh composition (muscular collagen, fat, moisture and protein contents) were determined (n = 700). Textural parameters (hardness, cohesiveness and derived traits) were measured in an industry relevant number of offspring (n = 890) for the first time in this species. The origin had an effect on muscular fat content as well as on hardness and derived textural parameters. Fish from MED showed the lowest fat percentage and those from CAN the highest values for textural parameters. Differences among origins could be explained through their different genetic backgrounds. However, the effect of the origin could be affected by environmental conditions in the initial facilities, where each origin was reared separately, and by genotype x environment interactions. Heritabilities were medium for muscular fat (0.31±0.08**)** and moisture (0.24±0.07). The genetic correlation between them was very high and negative (-0.99±0.02). Selection for harvest weight may lead to an increase in fillet fat percentage due to the genetic correlation between the

two traits (0.29±0.14). Hardness showed a medium heritability (0.21±0.06) and an unfavourable negative genetic correlation with harvest weight (-0.34±0.14). All findings reported in this study should be relevant for the establishment of successful breeding programs in aquaculture of this species.

1. Introduction

The gilthead sea bream (*Sparus aurata* L.) is one of the most important farmed fish in Europe, especially in the Mediterranean area. Total worldwide production reached 176,191 metric Tonnes (mT) with Greece (40.9%), Turkey (22.7%) and Spain (11.0%) as the major producers. In our country four regions share 100% of the gilthead sea bream production with Murcia being the second producer (APROMAR, 2013).

Due to consumer demands, quality traits have become an important parameter for the gilthead sea bream industries and therefore they should be considered in the breeding goal. Body composition and texture of flesh directly influence yield of final product and consumer preferences (Neira et al., 2004). In this regard, muscle composition plays an important role in aspects related to flesh quality such as flavour, juiciness, texture and appearance, and changes in these parameters may have consequences for the market. Specifically, flavour and juiciness are highly and positively correlated with fat content in the muscle (Grigorakis, 2007). On the other hand, between 3% and 10% of the protein content is collagen, which constitutes the connective tissue among cells and is related to fillet texture. Fillets with lower collagen content are tender since collagen has a positive and significant correlation with the firmness of raw flesh (Hatae et al., 1986). Flesh composition in gilthead sea bream is influenced by a variety of environmental factors such as abiotic (Ginés et al., 2004), nutrition (Izquierdo et al., 2005; Suárez et al., 2010; Nasopoulou et al., 2011), or factors related to the farming system (Grigorakis et al., 2002; Grigorakis and Alexis, 2005). However, genetic determination for flesh quality traits has been poorly studied in this species (Navarro et al., 2009).

Texture is also an important trait related to flesh quality in fish (Gjedrem, 2005; Ayala et al., 2010). The use of different systems of handling postharved (refrigeration,

freezing, etc.) influences significantly on the post-mortem degradation and hence, on the flesh quality (Ayala et al., 2010). These changes in the texture can be monitored by instrumental techniques, using a texturometer. A widely used test is the Texture Profile Analysis (TPA), which rely on compression. This test consists of two identical cycles which reproduce the process of chewing food in the mouth, obtaining a curve (force *vs.* time), from which properties are calculated as chewiness, gumminess, adhesiveness, hardness, and elasticity (Friedman et al., 1963; Szczesniak et al., 1963).

The use of different rearing systems and broodstocks to produce commercial fish usually causes great variability of the production i.e. in the growth rates and the overall quality of the end product (Ayala et al., 2010). However, we did not find works in which the relationship between the origin of the broodstock and flesh quality traits had been studied in this species.

Growth rate is usually the first goal in breeding programs of different species. Nevertheless, the effect of selecting for increased body weight on other commercially important traits such as body composition and flesh needs to be considered (Powell et al., 2008). Strategies that involve the development of selection schemes for these traits of economic interest in gilthead sea bream are still scarce due to the biological characteristic of this species. Gilthead sea bream is a mass-spawning species in which individuals are males during the first two years of life and then gradually become females. Mass spawning prevents knowing the genealogy of fish under culture conditions which is essential to estimate genetic parameters and to introduce selective programs. The use of molecular markers such as microsatellites is a useful tool for addressing these matters (Castro et al., 2008).

Taking into account all the circumstances mentioned above, the main goals of this research were: A) To study the effect of that origin of the broodstock on major raw

flesh quality traits (muscle composition and textural parameters) as well as to estimate the phenotypic correlation between them and harvest weight. B) To estimate genetic parameters (heritabilities and genetic correlations) for major flesh quality traits and for harvest weight in a population of gilthead sea bream sourced from broodstocks from three origins.

2. Materials and methods

2.1. Rearing conditions

Initially, samples of sea bream were captured from wild populations from three geographically differentiated origins along the Spanish coast; Cantabrian Sea (CAN), the Atlantic Ocean (ATL), and Mediterranean Sea (MED). From these samples, three broodstocks were established in different Spanish facilities where fingerlings were obtained and reared in the same conditions. At 84 days post-hatching (dph) a random sample of 2500 individuals, in which all origins were represented, was taken to the on-growing facilities of the Centro de Cultivos Marinos de la Región de Murcia. Fingerlings were individually tagged in the abdominal cavity for individual identification and then randomly distributed in 12 tanks and reared under communal conditions. At 325 dph the majority of the fish (about 2000 specimens) were moved to the facilities of the company Servicios Atuneros del Mediterraneo S.L. (San Pedro del Pinatar, Murcia, Spain) where they were reared in a cage in the Mediterranean sea under intensive conditions. At harvest size (690 dph), the fish were slaughtered and transported to the laboratory for assays. Specimens were kept on ice during the study at 4ºC refrigeration until the moment of their analyses.

The establishment of broodstocks, the conditions of spawn, the rearing conditions and the slaughter process are explained in depth in García-Celdrán et al. (2015).

2.2. Analyzed traits

Texture profile analysis (TPA) was measured on the back of the whole fish (Fig. 1) as described in Ginés et al. (2002) for two post-mortem storage periods of two days (n = 890). For this purpose we used a texture analyser QTS-25 (Brookfield CNS Farnell, Borehamwood, Hertfordshire, England) equipped with a load cell of 25 kg and Texture Pro V.2.1 software. The samples were compressed perpendicular to the muscle fibres with a 25 mm diameter cylindrical probe. The testing conditions were: 20ºC room temperature; two consecutive cycles of 50% compression; cross-head moved at a constant speed of 5 mm/s and a trigger point of 0.5 N. Texture variables, hardness (peak force of the first compression cycle; expressed as N), cohesiveness (ratio of positive force area during the second compression compared to that during the first compression; no units), springiness (height that the food recovers during the time that elapses between the two compression cycles expressed as mm), gumminess (hardness multiplied by cohesiveness; N) and chewiness (hardness multiplied by cohesiveness multiplied by springiness; N mm), were calculated as described by Bourne (1978).

Body harvest weight was also measured. Fish were then manually skinned and filleted (Fig. 1). Thereafter these raw fillets were vacuum-packed using a packaging INELVI VISC 500 (Industrial eléctrica Vilar S.L, Barcelona, España) and frozen (-20ºC) prior to compositional analysis. Flesh composition (muscular collagen, fat, moisture and protein contents) was determined (n = 700) by the indirect method Near Infrared Spectroscopy, NIR, using the FoodScan ^{TM} equipment at IRTA.

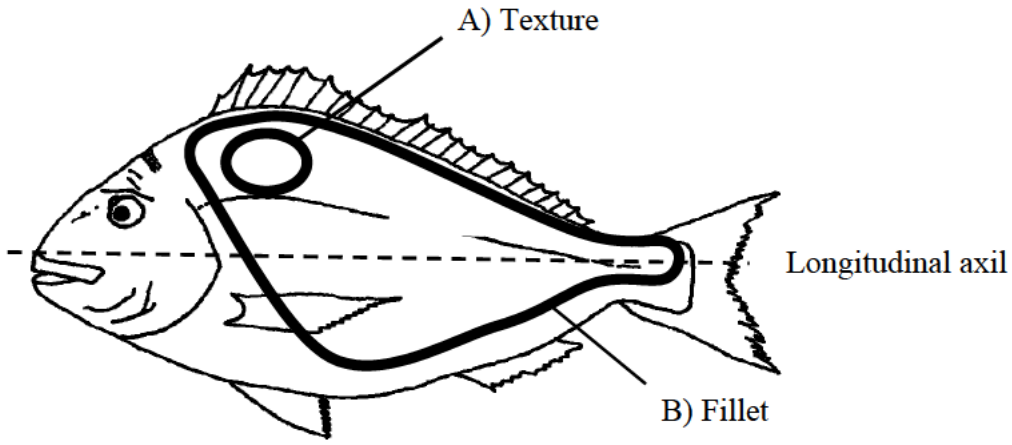

Fig. 1. Measurements made in gilthead sea bream as whole fish. A) The circle shows the position of the cylindrical probe in the measurement of maximum hardness with a texture analyser; B) The black line shows the outline of the area obtained in each fish fillet

2.3. PCR reaction and genotyping

The genetic characterization of breeders and juveniles and the parental assignments between them were conducted according to García-Celdrán et al. (2015).

2.4. Data analysis

All data were tested for normality and homogeneity of variances using SPSS® (v.19.0) (SPSS, Chicago, IL, USA) and then analyzed using the next General Liner Model:

$y_{ijk} = \mu + \text{origin}_j + \text{time of storage}_k + \beta * \text{body weight} + e_{ijk}$

in which y_{ijk} is an observation of an individual i^{th} from the origin j^{th} and with time of storage k^{th}, μ is the overall mean, origin is the effect of the broodstock origin of the fingerlings (j = CAN, ATL and MED), time of storage was included only for texture variables (j =1 day or 2 days post-mortem), β is the regression coefficient between the

analyzed variable and the covariate body weight and e_{ijk} is a random residual error. The level of significant difference was set at $P<0.05$.

Bivariate analyses were carried out using a Restricted Maximum Likelihood (REML) algorithm to obtain (co)variance components for all traits using the following animal linear model, with body weight as a covariate for flesh quality traits:

$y = X\beta + Zu + e$

where y is the recorded data on the studied traits, β includes fixed effect (broodstock origin and time of storage, the latter one only for texture variable), u the random animal effect, e the error and, X and Z are incidence matrices. Non-genetic maternal and/or paternal effects were not significant for the studied traits so they were removed from the model when analyzing flesh quality traits. The model was resolved with the software package VCE (v 6.0) (Groeneveld et al., 2010). The magnitude of estimated heritability was established, following the classification of Cardellino 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 (Navarro et al., 2009). Pearson correlation was carried out to determine phenotypic correlations among the analyzed parameters.

3. Results and discussion

3.1. Phenotypic results

The effect of the broodstocks origin of the fingerlings on chemical flesh composition was assessed in gilthead sea bream for the first time in this study. Due to the wide range of values observed for weight, discussed in detail in García-Celdrán et al. (2015), phenotypic results for quality traits (Table 1) were adjusted to a constant final weight (234.8 g) to be compared. Not significant differences ($P\geq0.05$) among

origins for flesh components (collagen, moisture and protein) were observed. However, an effect of the origin was found on muscular fat. Fish from MED showed a lower fat percentage than those from CAN and ATL. In this regard, Vandeputte et al. (2014) compared five wild populations of sea bass founding differences in muscular fat content.

The covariate was highly significant (P<0.01) and positive for fat and negative for moisture. Therefore, per ten grams of weight increase in the fish, the muscular fat would increase 0.1% and moisture would decrease 0.08%, in agreement with Ginés et al. (2004) and Grigorakis and Alexis (2005). Fat percentage has been reported as highly variable in cultured gilthead sea bream, ranging from 2% to 11%, and inversely proportional to moisture, ranging from 68% to 74% (Robaina et al., 1997; Flos et al., 2002; Ginés et al., 2004; Grigorakis and Alexis, 2005; Grigorakis, 2007). In the present study, fat percentage was near to 5% in agreement with previous research not only for the mean values but also for the inverse relationship between fat and moisture. This fact was indicated by the significant phenotypic correlations (Table 3) between both traits (P<0.01). Protein is considered to increase with fish size, remaining stable after a certain size of fish is reached (Grigorakis et al., 2002). In fact, we did not find an effect of the fish weight covariate on the protein amount (Table 1) but it could be due to the range of weight that we studied.

Other studies have observed that chemical flesh composition of sea bream can be modified by environmental factors (water temperature, salinity), feed (diet composition, feeding ratio, etc.), harvesting and post-harvesting procedures (Grigorakis, 2007). In this regard, Navarro et al. (2009) found significant differences between facilities (cages *vs*. tanks) for flesh composition traits (muscular fat, moisture, ash) except for collagen. However, Sato et al. (1986), reported collagen values for 24 fish

species that varied between 3.4 and 21.9 mg/g depending on the species, muscular area, the slaughter season, nutritional conditions and the age. Peso-Echarri et al. (2012) found an effect of the dietary alginate on fat and ash but not on protein and moisture.

Table 1: Phenotypic results (least square means ± standard error) for harvest weight and flesh composition traits in a population of gilthead sea bream at harvest age (690 days post-hatching) sourced from broodstocks from three origins adjusted to a final fish weight of 234.84 grams

Broodstocks origin[1]	CAN	ATL	MED	Covariate[2]
N	334	175	191	700
Harvest weight (g)	238^{b}±2.4	188^{a}±3.3	271^{c}±3.1	
Collagen (%)	1.77 ± 0.038	1.86 ± 0.059	1.79 ± 0.055	0.000 ± 0.001
Muscular fat (%)	4.94^{a} ± 0.064	4.96^{a} ± 0.099	4.64^{b} ± 0.091	0.010** ± 0.01
Moisture (%)	72.9 ± 0.07	72.9 ± 0.12	73.1 ± 0.11	-0.008** ± 0.001
Protein (%)	21.9 ± 0.07	22.0 ± 0.11	21.9 ± 0.10	0.000 ± 0.01

[1]Broodstock origin: CAN = Cantabrian Sea, ATL = The Atlantic Ocean, MED = Mediterranean Sea
[2]Regression coefficient for the covariate fish weight and standard error (**P <0.01)
[abc]Different superscripts within each row indicate significant differences among origins

The instrumental texture analysis of muscle for gilthead sea bream from different origins adjusted to final weight (234.8 g) is shown in Table 2. Fish muscle texture depends on many intrinsic biological factors that are related to muscle fibres and densities involving fat content and collagen (Navarro et al., 2009; Li et al., 2011; Peso-Echarri et al., 2012). Moreover, an effect of the origin was found in our study, since there were significant differences (P<0.05) among origins for all studied textural parameters (hardness, cohesiveness, gumminess, chewiness) except for springiness. The highest values of texture parameters were observed in fish from CAN. An effect of the origin on texture have been reported in species such as salmon (Johnston et al., 2006; Glover et al., 2009; Bahuaud et al., 2010), rainbow trout (Salem et al., 2005) and for the first time in gilthead sea bream in the present research.

Estimates of flesh texture are scarce, due to the difficulty of measuring it in a large number of fresh fish (Gjedrem, 1997). In spite of this fact, we measured TPA in an industry relevant number of offspring (n = 890) for the first time in sea bream in order to obtain consistent results. The huge number of samples to process made necessary to measure them in two consecutive days. Textural parameters showed higher values than those obtained in other studies (Ayala et al., 2010, 2011; Peso-Echarri et al., 2012) although in previous research these parameters were measured in fillets. We measured texture in whole fish since it is the primary way in which sea bream is commercialized and it is far faster than measurement in fillets when a large number of samples are to be analyzed. Hardness was negatively influenced by the weight as was denoted by the covariate. Therefore, larger fish showed softer muscles.

Table 2: Harvest weight and textural parameters (least square means ± standard error) in a population of gilthead sea bream at harvest age (690 days post-hatching) sourced from broodstocks from three origins adjusted to a final fish weight of 234.84 grams

Broodstocks origin[1]	CAN	ATL	MED	Covariate[2]
N	424	243	222	890
Harvest weight (g)	238^{b}±2.4	188^{a}±3.3	271^{c}±3.1	
Hardness (N)	87.0 ^{a} ± 0.74	80.5 ^{b} ± 1.11	78.4 ^{b} ± 1.12	-0.032** ± 0.012
Springiness (mm)	6.56 ± 0.031	6.53 ± 0.047	6.58 ± 0.047	0.000 ± 0.000
Cohesiveness (ratio)	0.683 ^{a} ± 0.003	0.693 ^{b} ± 0.005	0.704 ^{b} ± 0.005	0.000** ± 0.000
Gumminess (N)	59.0^{a} ± 0.50	55.6 ^{b} ± 0.75	54.7 ^{b} ± 0.76	-0.005 ± 0.008
Chewiness (N mm)	386.0 ^{a} ± 3.44	362.2 ^{b} ± 5.17	359.7 ^{b} ± 5.21	-0.034 ± 0.055

[1]Broodstock origin: CAN = Cantabrian Sea, ATL = The Atlantic Ocean, MED = Mediterranean Sea

[2]Regression coefficient for the covariate fish weight and standard error (**P <0.01)

[abc]Different superscripts within each row indicate significant differences among origins

Due to the fact that in our study all fish were held in the same station the majority of their life where they were raised under the same conditions, slaughtered and stored the same way, differences in them seem to be the results of differences in their genetic backgrounds. However, the origin effect could be affected by the environment in each initial facility. In spite of the fact that the rearing protocol was standardized, larvae were reared in different facilities until 84 dph. At that point, some environmental perturbations could happen which may be expressed as genotype x environment interaction. The same could happen after mixing of the origins in 12 tanks during the pre-on-growing period since the tank effect cannot be analyzed.

3.2. Heritability of raw flesh quality traits

Heritabilities and their corresponding standard errors (h^2 ± SE) of flesh composition traits, texture and harvest weight (Table 3) were estimated in 700-890 individuals correctly assigned to a unique parental couple using the SMsa1 multiplex. Heritabilities were very low for collagen (0.03±0.02) and protein (0.03±0.03) and medium for muscular fat (0.31±0.08**)** and moisture (0.24±0.07). Few references have been found regarding the genetic basis for flesh quality traits of sea bream or other species. Navarro et al. (2009) found low heritabilities for flesh composition traits determined by chemical methods; 0.05±0.03 for fat and 0.09±0.03 for moisture in gilthead sea bream. However, higher heritabilities have been observed; 0.58±0.09 in common carp (Kocour et al., 2007) estimated using Torry Fish Fatmeter and 0.77±0.07 in European sea bass (Haffray et al., 2007) using microwaves. In this study the results were intermediate to those previous since we found a medium heritability for muscular fat and moisture determined by NIR, therefore variation among studies could be partly related to the accuracy of the recording technique. Moreover, this variability of

heritability values may result from genetic differences among the studied populations, genotype-by-environment interactions and differences in the degree of environmental variation among populations (Kause et al., 2002). Regarding collagen, our results are in line with those previously recorded by Navarro et al. (2009) whom estimated a very low heritability (0.02±0.01). The very low heritability for protein, for the first time reported in sea bream, has been found in other species as well; 0.04 in European whitefish (Kause et al., 2009, 2011) and 0.03 in Rainbow trout (Gjerde and Schaeffer, 1989). One of the aims of farmed fish production is to convert feed into muscle and ultimately protein growth rather than excess lipid. Hence, the genetic improvement of body protein percentage should be of high priority for breeders, but this is challenging in practice (Kause et al., 2011) and according to our results.

3.3. Correlations between growth traits and raw flesh quality traits

We assessed whether muscle components, laborious traits to record, could be genetically improved by indirect selection on weight, the most important trait for selection (Saillant et al., 2006) and an inexpensive easily measured trait in live candidates.

In this study, the genetic correlation between muscular fat and growth seems to be positive (0.29±0.14) in agreement with Navarro et al. (2009) which suggest that selection for increased harvest weight leads to increased fillet fat percentage. In gilthead sea bream this positive genetic correlation would indirectly improve flesh quality (juiciness and flavour) through growth. However, fatter fish are also expect to have a poorer feed conversion ratio as energy goes into fat not protein growth. Positive genetic and phenotypic correlations have been found between muscular fat and growth in sea bass (Haffray et al., 2007), salmon (Rye and Gjerde, 1996; Neira et al., 2004; Powell et

al., 2008), common carp (Kocour et al., 2007) and European whitefish (Kause et al., 2011). An alternative for measuring fillet fat in living candidates is the Fish Fat Meter which is a cost effective, portable and reliable indicator for this trait which could be useful in a breeding program since desirable phenotypes could be achieved via directional individual selection of this trait (Gjedrem, 1997; Powell et al., 2008; Saillant et al., 2009).

The genetic correlations between harvest weight and the rest of muscular components (collagen, moisture and protein) were found to be imprecise and it was not possible to draw any conclusion about them. Phenotypic correlations between harvest weight and muscular components were low in all cases.

3.4. Correlations between muscle components traits

Genetic correlations between flesh components were high although the correlations between protein and the rest of the muscle components analyzed were medium and imprecise. Collagen was positively correlated with muscular fat and negatively with moisture. The negative genetic and phenotypic correlations between moisture and muscular fat found in this study are in agreement with those described previously in sea bream (Navarro et al., 2009) and rainbow trout (Kause et al., 2002). Phenotypic correlations among muscle components were precise for all analyzed traits, due to the large number of samples analyzed, being high and positive for protein and collagen (P <0.01) while those between moisture with muscular fat and with protein were negative.

3.5. Heritability and correlations for texture

Texture was measured in whole fish and a medium heritability (0.21±0.06) for hardness was found. Heritabilities for the rest of textural parameters were not estimated since they are derived traits of the former. In this regard, low heritabilities have been found in salmon (Neira et al., 2004; Larsson et al., 2012) who found low (0.06-0.09) and low-medium (0.16) heritabilities respectively. However, Kause et al. (2011) found higher heritability (0.30±0.09) in European whitefish although the measurement method was different in both studies (whole fish *vs*. fillet). We did not find studies about the genetic basis of textural parameters in gilthead sea bream but in light of these circumstances it seems that heritability of texture can be easily influenced by the accuracy of the recording technique, potentially generating variation between studies. As texture necessarily require the sacrifice of the individual, it is not possible to measure this trait on breeding candidates themselves, rather, siblings are used for this purpose. Thus, the attempt to develop fast methods to test texture in a large number of breeding candidates themselves would be highly beneficial (Ginés et al., 2002; Larsson et al., 2012). We measured textural parameters in the first and the second day post-mortem, which corresponds to the usual period for fish to reach market (Ginés et al., 2002). In addition, to improve efficiency of selection is necessary to measure a large number of individuals as soon as possible, in order to save money and labour (Saillant et al., 2009; Blonk et al., 2010).

We found unfavorable negative genetic and phenotypic correlation between hardness and weight in agreement with previous studies in European whitefish (Kause et al., 2011) which may indicate that selection for increasing harvest weight leads to softer fillets. However, Neira et al. (2004) described a high and positive genetic correlation between meat texture and weight. Texture can also be indirectly assessed

through its relationship with collagen content which has a positive and significant correlation with the firmness of raw flesh (Hatae et al., 1986). Moreover, the texture of the cooked fish is affected by the muscular collagen-derived gelatin formed during the cooking process (Sato et al., 1986). Nevertheless, we found a high but unexpected negative genetic correlation between collagen and hardness so further studies could be needed to clarified this issue as well as the genetic correlation between raw and cooked product. The rest of genetic and phenotypic correlations between hardness and flesh components were close to zero in all cases.

Finally, to improve growth rate selection criteria is usually the highest weight for the same age, and consequently adult weight will be increased unintentionally. Thus, for the same harvest weight fish would tend to be more immature and it could provoke changes in their flesh quality traits. Therefore, these results should be checked through selection response in future generations.

Table 3: Genetic correlations ± standard error (above the diagonal), phenotypic correlations ± standard error (below the diagonal) and heritabilities ± standard errors (in bold at the diagonal) of flesh composition traits, textural parameter and harvest weight estimated from 700-890 gilthead sea bream at harvest age (690 days post-hatching)

	Collagen	Muscular fat	Moisture	Protein	Hardness	Harvest weight
Collagen	**0.03±0.02**	0.94±0.17	-0.99±0.02	0.55±0.47	-0.99±0.01	-0.23±0.33
Muscular fat	0.05±0.04	**0.31±0.08**	-0.89±0.08	0.21±0.50	-0.24±0.20	0.29±0.14
Moisture	-0.34**±0.04	-0.73**±0.03	**0.24±0.07**	-0.54±0.41	0.03±0.22	0.18±0.28
Protein	0.70**±0.03	-0.12±0.04	-0.40**±0.03	**0.03**±0.03	0.01±0.58	-0.38±0.30
Hardness	0.01±0.04	-0.09±0.04	-0.01±0.03	0.03±0.03	**0.21±0.06**	-0.34±0.14
Harvest weight	0.04±0.03	-0.01±0.04	0.04±0.03	0.03±0.04	-0.04±0.03	**0.25±0.07**

** Significant Pearson correlations between flesh quality traits (P <0.01).

4. Conclusions

The different broodstock origins presented in the studied population had an effect on raw traits of interest such as fat content and textural parameters. Juveniles from CAN broodstock were the best performers since fingerlings from that origin showed the highest percentage of muscular fat and the hardest muscle. Differences among origins could be explained through their different genetic backgrounds but also by environmental conditions in the initial facilities and by genotype x environment interactions.

Harvest weight, muscular fat and moisture showed medium heritability. Selection for harvest weight may lead to an increase in fillet fat percentage, and therefore in flavor and juiciness, due to the genetic correlation between these traits.

Phenotypic determinations and genetic parameters for texture under industrial conditions are reported in the present research for the first time in gilthead sea bream. Selection for faster growth could lead to undesirable softer fillet due to the genetic correlation between weight and hardness and due to the medium heritabilities showed for both parameters.

All these findings should be relevant for the establishment of successful breeding programs in aquaculture of this species.

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6. CONCLUSIONES GENERALES.

A partir de los resultados obtenidos en cada uno de los capítulos de esta Tesis podemos extraer las siguientes conclusiones:

La alta variabilidad genética estimada mediante marcadores microsatélites en doradas de diferentes orígenes indica la falta de procesos de selección a los que ha sido sometida esta especie. La dorada se muestra con potencialidad genética para establecer programas de mejora exitosos.

Las contribuciones de los reproductores de los diferentes lotes estudiados fueron desiguales dando lugar a la consiguiente disminución de su tamaño efectivo. Este hecho pone de manifiesto la importancia del manejo de los reproductores con el fin de maximizar las contribuciones y controlar, en la medida de lo posible, incrementos de consanguinidad generacional.

El origen tuvo un efecto en caracteres de interés comercial, como son caracteres de crecimiento (peso y talla), calidad de pez (malformaciones esqueléticas y vejiga natatoria), calidad de canal (factor de condición y grasa visceral) y calidad de carne (grasa muscular y parámetros texturales), demostrando la importancia en la elección de los reproductores como población fundadora en un programa de mejora genética.

Los caracteres de interés anteriormente mencionados presentaron heredabilidades considerables indicando el potencial de los mismos para ser mejorados genéticamente. Además, destacaron por su relevancia las correlaciones genéticas positivas del peso con el factor de condición, porcentaje de grasa visceral y del filete; y negativas con malformaciones en la columna vertebral.

En vista de los resultados, para el establecimiento de un programa de mejora genética en esta especie se propone en primer lugar eliminar peces deformes y posteriormente seleccionar por crecimiento dada la importancia económica para el sector de ambos caracteres. Sin embargo, esta propuesta inicial debe ser analizada tanto para dichos caracteres, como para aquellos de calidad de canal y de carne en generaciones sucesivas.

Todos los resultados obtenidos aportan información relevante para el establecimiento de un programa de mejora genética en dorada.