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# Evidence of Genetic Segregation among Meagre (*Argyrosomus regius*) Atlantic Spawning Areas

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**Abstract:** The meagre *Argyrosomus regius*, one of the largest sciaenidae in the world, is a valuable resource for fisheries and aquaculture. Despite its socioeconomic relevance, knowledge about population dynamics and wild stocks is still scarce, and conservation risks are associated with overexploitation. Two genetic distinct groups, one in the North Atlantic Ocean and one in the eastern Mediterranean Sea, were identified by previous studies. However, little is known about the genetic structure of the Atlantic group, where four important spawning areas have been identified. To assess if each spawning area is an independent breeding unit, the genetic diversity, populational structure, and demographic history of *A. regius* along the North–East and Eastern Central Atlantic coast were analyzed, using 15 microsatellite loci. Results corroborate the hypothesis tested, suggesting four genetic groups: a first group encompassing individuals from the Gironde spawning area, a second group encompassing individuals from the Tagus spawning area, a third group corresponding to individuals captured in the Algarve region, and a forth group gathering individuals from Morocco and Mauritania. This study reveals the need for specific fisheries management plans considering genetic structure information, and highlights the need for international cooperation.

**Keywords:** Atlantic Ocean; spawning areas; demographic history; gene flow; population structure; stock management

## 1. Introduction

The meagre *Argyrosomus regius* (Asso 1801) is one of the largest sciaenidae in the world and a valuable resource for recreational and small-scale commercial fisheries along its distribution range. Annually, more than 5000 tons of *A. regius* are caught in the Atlantic and 1000 tons in the Mediterranean [1]. It is also an important candidate species for the diversification of European aquaculture [2,3]. However, the distribution and population dynamics of the wild stocks of *A. regius* remain scarcely known (see [4]), impairing a rational and sustainable management of the fishery.

*A. regius* is a marine migratory species with a wide distribution range encompassing the north-eastern (NE) and central-eastern (CE) Atlantic Ocean, the Mediterranean Sea, and the western Black Sea [2,5,6]. Much of the current knowledge on the population dynamics and distribution of this species results from studies of commercial and recreational fisheries catches [3,7]. In European waters, *A. regius* is mostly observed in estuaries and adjacent coastal areas. Adults congregate in specific estuaries during the spawning season from late spring to mid-summer, returning to offshore habitats for feeding and overwintering [3,7,8]. In the Atlantic coast, spawning aggregations are known in France, in the Gironde estuary; in Portugal, in the Tagus and Guadiana estuaries; in Spain, near Guadalquivir (Gulf of Cádiz); and in Mauritania, in the Lévrier Bay [5–7,9–11]. In the Mediterranean Sea, there are, at present, fewer spawning sites, the main one being the mouth of the River Nile, in Egypt [4]. Juveniles grow on the spawning grounds until autumn and are thought to migrate to near-shore waters, with occasional visits to the nursery sites in some locations, such as in the Guadalquivir estuary [7,8], or, in other cases, with a more recurrent use of the estuarine nursery area, such as in the Tagus [3].

Despite being listed as least concern (LC) by the IUCN [12], substantial conservation risks have been identified in European fisheries of *A. regius*, mostly related to the possible overexploitation of juveniles and adults in estuaries and adjacent coastal areas [2,3]. Additionally, the life-history characteristics of this species, namely, long longevity, large size and age at maturity, large variability in annual recruitment, and the formation of spawning aggregations in coastal waters and estuaries, pose significant management and conservation problems that means it ranks high among the world's most vulnerable marine fish [13]. In spite of conservation risks, the fisheries have remained largely outside the main management priorities, mostly because severe limited data and absence of the species from catches of large-scale industrial fisheries reduces awareness of their situation [14]. In addition, an overall lack of fishery-independent knowledge on *A. regius* stock structure, population size, and distribution renders management decisions difficult to sustain. Such paucity of data indicates a need for precautionary fisheries management but, at the same time, hampers the political implementation of more stringent measures such as catch and/or effort limitations or temporal area closures, rendering it difficult to reduce the conservation risks associated with exploitation [3].

A previous study using microsatellite loci identified the existence of two very distinct genetic groups, one in the Atlantic Ocean and one in the eastern Mediterranean Sea [4]. The level of differentiation observed was extremely high for a marine fish with potentially high dispersal capabilities, such as *A. regius*. This study indicates the southwest Iberian Peninsula as an intermediate area, but the populational structure along the Atlantic coast still remains uncertain.

Molecular biology tools such as microsatellites are commonly used in fisheries management to infer genetic populational trends and demographic dynamics, e.g., [15–17]. By unravelling the differences between populations, microsatellites analyses allow researchers to identify the species populational structure, genetic diversity, and connectivity patterns among populations [18]. Such information is essential to implement adjusted fisheries management targeting specific stocks, contributing to biodiversity conservation, relevant, among others, in the species' response capability to environmental changes [19].

The overarching aim of this study was to further evaluate the population genetic structure of *A. regius* in its North–East and Eastern Central Atlantic distribution range. To accomplish this, the working hypothesis that independent breeding units associated with the four known *A. regius* spawning aggregation areas in the region (i.e., Gironde, Tagus, Algarve—Gulf of Cádiz, and Mauritania–Morocco) constitute discrete genetic stocks was tested. A more detailed spatial analysis in terms of sampling locations was directed towards Portugal, considered a putative transition zone between two distinct genetic units (Atlantic Ocean and eastern Mediterranean Sea). Microsatellite loci were used to analyze the genetic differentiation, genetic diversity, and the demographic history of *A. regius* in proximity of the four known spawning areas. The information presented is considered

important to support the implementation of sustainable management of *A. regius* fisheries targeting Atlantic stocks.

## 2. Materials and Methods

### 2.1. Sample Collection

Samples from 228 *A. regius* individuals were collected between 2019 and 2020 from landings by local fishermen and fish imports from eight locations of geographical areas in proximity of known spawning areas from the North–East and Eastern Central Atlantic: Gironde (1 location, France), Tagus (3 locations—offshore Vieira de Leiria, Tagus estuary, and Sado estuary—Portugal), Algarve (2 locations—offshore Olhão and Guadiana estuary—Portugal), and Mauritania–Morocco (2 locations, Morocco and Mauritania) (Figure 1 and Table 1). To simplify, hereafter, the geographical areas in the proximity of known spawning areas will be referred to as spawning areas. Fin clips were stored in 98% alcohol.

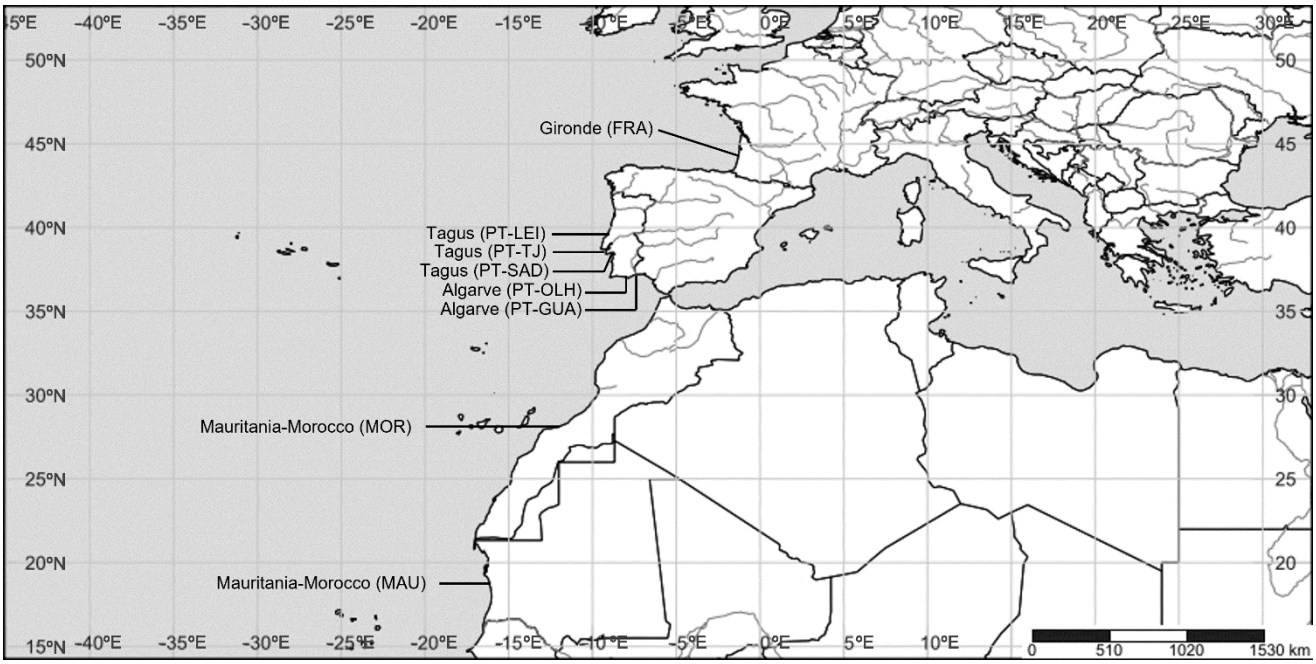


**Figure 1.** Locations of the *Argyrosomus regius* sampling sites and corresponding spawning areas. For location sites' acronyms, please check Table 1.

**Table 1.** Sampling data for *Argyrosomus regius* individuals analyzed in this study. Sampled localities and spawning areas are presented from north to south as in Figure 1.

| Spawning Area | Sampling Sites | Code | Country | Sampling Year | Environment | N |
|---|---|---|---|---|---|---|
| Gironde | France | FRA | France | 2020 | Inshore | 19 |
| Tagus | Vieira de Leiria | PT-LEI | Portugal | 2020 | Offshore | 35 *1 |
| Tagus | Tagus | PT-TJ | Portugal | 2019–2020 | Inshore | 38 |
| Tagus | Sado | PT-SAD | Portugal | 2019 | Inshore | 28 |
| Algarve | Guadiana | PT-GUA | Portugal | 2020 | Inshore | 35 |
| Algarve | Olhão | PT-OLH | Portugal | 2019 | Offshore | 35 |
| Mauritania–Morocco | Morocco | MOR | Morocco | 2019–2020 | Unknown | 17 *2 |
| Mauritania–Morocco | Mauritania | MAU | Mauritania | 2020 | Unknown | 21 *2 |

N—number of individuals; *1—samples obtained from the same fishing trip; *2—samples obtained from fish imports.

### 2.2. DNA Extraction and Genotyping

Total genomic DNA was extracted using Dneasy® Blood and Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA quality and quantity were evaluated using a Thermo Scientific NanoDrop™ 1000 Spectrophotometer (Waltham, MA, USA).

Fifteen polymorphic microsatellite loci used previously in studies with *A. regius* were selected (Soc412, CA3, Soc423, GA17, Soc35, Soc432, Soc592, UBA42, UBA50, Soc44, UBA5, Soc140, Soc156, UBA54, and Soc11; [4,20–23]). The primer sets were grouped into three multiplex reactions and the reverse primers were labelled with a fluorescent dye (6-FAM, HEX, ATTO550, or ATTO565) at the 5′ end. The polymerase chain reactions (PCR) were set up in final volumes of 12 µL containing 1 µL of genomic DNA (40 ng/µL), 0.6 units of My Taq™ DNA polymerase (Bioline, London, UK), 1.2 µL of 5× My Taq Reaction Buffer (Bioline), 0.4 µM for each primer, and ultrapure water. The PCR reactions were run for 27–35 cycles on a Bio-Rad1 thermal cycler and included an initial activation step at 95 °C for 15 min, denaturation at 94 °C for 30 s, primer annealing at 60 °C for 90 s, extension at 72 °C for 60 s, and final extension at 72 °C for 10 min.

Samples were subsequently genotyped in an ABI PRISM® 310 Genetic Analyzer and fragments were sized with GeneScan™-500 LIZ™ Size Standard. Allele sizes were determined using the GeneMapper™ 3.7 software (Applied Biosystems, Waltham, MA, USA) (Supplementary Table S1).

### 2.3. Data Analyses

The presence of null alleles, stuttering, and allele dropout were tested for all loci using the MICRO-CHECKER v.2.2.3 [24], and the data were subsequently visually examined for correction. Departures from Hardy–Weinberg equilibrium and linkage equilibrium were assessed using ARLEQUIN v.3.5.2.2 [25] ($10^4$ permutations). The pairwise $F_{ST}$ values from the original data and the data with null alleles correction were obtained using FREENA [26] ($10^4$ permutations) and compared to verify the influence of the null alleles in the analyses.

Regarding genetic diversity, the mean number of alleles (Mna), the mean number of private alleles (MNp), the mean observed (Ho), expected (He), and unbiased expect (uHe) heterozygosities were calculated with GenAlEx v.6.5 [27]. The mean allelic richness (Ar) and the inbreeding coefficient ($F_{IS}$), with 95% confidence intervals ($10^4$ bootstrap replicates), were estimated with the diveRsity package in R v.4.1 [28].

To evaluate differentiation, pairwise $F_{ST}$ values were calculated ($10^4$ permutations) in ARLEQUIN v.3.5.2.2 [25]. To compare with pairwise $F_{ST}$ values, Jost's D and GST were also estimated using GenAlEx v.6.5 [27] ($10^4$ permutations). Patterns of differentiation between location sites were then visualized using principal coordinates analysis (PcoA), computed in GenAlEx v.6.5 [27]. The distribution of the genetic variation was evaluated among spawning areas, among location sites within spawning areas, and within location sites through a locus-by-locus analysis of molecular variance (AMOVA; $20^4$ permutations, $p$-value < 0.05) using the allelic frequencies as the genetic distance, performed in ARLEQUIN v.3.5.2.2 [25].

Population genetic structure was assessed through a Bayesian-model-based cluster analysis using STRUCTURE v.2.3.4 [29]. Runs were performed under the admixture model, with correlated allelic frequencies, and taking into account information on sampling location as spawning areas (LOCPRIOR model). The number of clusters (K) was set between 1–9 and for each K, 10 independent simulations were performed with an initial burn-in period of $10^5$, followed by $10^6$ Markov chain Monte Carlo (MCMC) algorithm iterations. The most likely value for K was determined from likelihood (lnP(D); [30]) and delta k (ΔK; [31]) values obtained with STRUCTURE HARVESTER [32]. Outputs were visualized in DISTRUCT v.1.1 [33].

Isolation by distance was analyzed through Mantel's test (9999 permutations) in GenAlEx v.6.5 [27]. This was performed by comparing the genetic variation (pairwise $F_{ST}$'s) and the geographic distances (in km) between location sites (considering the shortest marine route possible).

Contemporary effective population sizes (Ne) were calculated based on linkage disequilibrium (LDNe, single-sample approach) and heterozygote excess methods in NeEstimator v.2.01 [34], assuming random mating and a critical frequency threshold value of 0.05.

Additionally, to assess relative gene flow, the experimental divMigrate function of diveRsity package in R v.4.1 [28] was used, based on $F_{ST}$ measure.

## 3. Results

### 3.1. Genetic Diversity and Differentiation

Signs of null alleles, as a consequence of the excess of homozygotes, are detected in PT-SAD for UBA5, in MOR for Soc423, in PT-GUA for soc412, and in all location sites except MOR for UBA42. In addition to this, signs of stuttering are also detected in PT-LEI and PT-TJ for UBA42. However, no consistent departure from Hardy–Weinberg equilibrium is detected in any location site. Similar pairwise $F_{ST}$ values are obtained using either the original data or the data with null alleles correction (difference ranging between −0.0029 and 0.0037; Supplementary Table S2), indicating low influence of the null alleles. Therefore, all further analyses were performed using the original genotypic data for the fifteen microsatellite loci.

The number of alleles per locus ranges between 3 (Soc156) and 27 (Soc35). The genetic diversity appears to increase southward, with the mean number of alleles varying between 4.260 (FRA) and 6.467 (PT-GUA), and the allelic richness varying between 4.340 (FRA) and 5.190 (MOR) (Table 2). In total, 27 private alleles are detected, and the highest number of private alleles (8) is observed in the MOR location site.

**Table 2.** *Argyrosomus regius* genetic diversity estimated using 15 microsatellite loci for Atlantic location sites: mean number of alleles (Mna); mean allelic richness (Ar), mean number of private alleles (MNp), observed heterozygosity (Ho), expected heterozygosity (He), mean unbiased estimate of expected heterozygosity (uHe), and inbreeding coefficient ($F_{IS}$), with lower and upper 95% confidence intervals ($F_{IS}$_Low and $F_{IS}$_High) determined with 1000 bootstrap replicates). For location sites' acronyms, please check Table 1.

| | FRA | PT-LEI | PT-TJ | PT-SAD | PT-GUA | PT-OLH | MOR | MAU |
|---|---|---|---|---|---|---|---|---|
| **Mna** | 4.867 | 5.867 | 6.000 | 5.600 | 6.400 | 6.467 | 6.267 | 6.400 |
| **Ar** | 4.260 | 4.610 | 4.620 | 4.590 | 4.850 | 4.860 | 5.190 | 5.160 |
| **MNp (Np)** | 0.133 (2) | 0.067 (1) | 0.200 (3) | 0.133 (2) | 0.267 (4) | 0.133 (2) | 0.533 (8) | 0.333 (5) |
| **Ho** | 0.530 | 0.599 | 0.584 | 0.566 | 0.538 | 0.536 | 0.602 | 0.567 |
| **He** | 0.569 | 0.589 | 0.589 | 0.578 | 0.584 | 0.573 | 0.591 | 0.575 |
| **uHe** | 0.585 | 0.598 | 0.597 | 0.589 | 0.592 | 0.581 | 0.609 | 0.590 |
| **$F_{IS}$** | 0.069 | −0.017 | 0.008 | 0.022 | 0.078 | 0.065 | −0.019 | 0.015 |
| **$F_{IS}$_Low** | −0.031 | −0.075 | −0.049 | −0.053 | 0.024 | −0.002 | −0.132 | −0.069 |
| **$F_{IS}$_High** | 0.162 | 0.038 | 0.065 | 0.092 | 0.129 | 0.132 | 0.080 | 0.091 |

Similar observed and unbiased expected heterozygosity values are found among location sites (Table 2). The inbreeding coefficient ($F_{IS}$) is close to zero for all sites, suggesting low levels of inbreeding. However, the PT-GUA $F_{IS}$ 95% confidence interval is above zero, rejecting the random mating hypothesis and suggesting some relatedness among individuals in this sample (Table 2).

Statistically significant genetic differences are obtained between FRA and all location sites except P-SAD and MOR; between PT-OLH and all location sites except PT-SAD, PT-GUA, and MOR; and between MAU and all location sites except MOR ($p$-value < 0.0008; Table 3). The $F_{ST}$ values obtained between sites are low, indicating little restriction in gene flow or extremely recent shared ancestry between them. The $F_{ST}$ values range between −0.002 (PT-GUA–PT-OLH) and 0.045 (PT-LEI–MAU) (Table 3). Nevertheless, when location sites are pooled by spawning areas, all comparisons are statistically different (Table 4), suggesting that each spawning area corresponds to a distinct genetic group.

**Table 3.** Pairwise estimates of $F_{ST}$ values among *Argyrosomus regius* Atlantic location sites (below diagonal) and corresponding *p*-values (above diagonal; * $p < 0.0008$ (Bonferroni correction)). For location sites' acronyms, please check Table 1.

| | FRA | PT-LEI | PT-TJ | PT-SAD | PT-GUA | PT-OLH | MOR | MAU |
|---|---|---|---|---|---|---|---|---|
| **FRA** | - | * | * | 0.001 | * | * | 0.01 | * |
| **PT-LEI** | 0.022 | - | 0.027 | 0.122 | 0.001 | * | 0.03 | * |
| **PT-TJ** | 0.033 | 0.007 | - | 0.232 | 0.009 | * | 0.152 | * |
| **PT-SAD** | 0.023 | 0.005 | 0.003 | - | 0.011 | 0.014 | 0.081 | * |
| **PT-GUA** | 0.031 | 0.015 | 0.011 | 0.012 | - | 0.83 | 0.136 | * |
| **PT-OLH** | 0.035 | 0.018 | 0.015 | 0.011 | −0.002 | - | 0.066 | * |
| **MOR** | 0.020 | 0.010 | 0.005 | 0.009 | 0.007 | 0.010 | - | 0.064 |
| **MAU** | 0.043 | 0.045 | 0.037 | 0.038 | 0.026 | 0.038 | 0.010 | - |

**Table 4.** Pairwise estimates of $F_{ST}$ values among *Argyrosomus regius* Atlantic spawning areas (below diagonal) and corresponding *p*-values (above diagonal; in bold * $p < 0.0008$ (Bonferroni correction)). For location sites' acronyms, please check Table 1.

| | Gironde | Tagus | Algarve | Mauritania–Morocco |
|---|---|---|---|---|
| **Gironde** | - | * | * | * |
| **Tagus** | **0.025** | - | * | * |
| **Algarve** | **0.034** | **0.013** | - | * |
| **Mauritania-Morocco** | **0.031** | **0.022** | **0.020** | - |

The GST and Jost's D values are similar to the $F_{ST}$ values, statistically significant for the same comparisons, and range from −0.0017 (PT-GUA–PT-OLH) to 0.0233 (PT-LEI–MAU), and from −0.0048 (PT-GUA–PT-OLH) to 0.0697 (PT-LEI–MAU), respectively (Supplementary Tables S3 and S4).

AMOVA results (Supplementary Table S5) indicate that almost all variation is explained within location sites (populations) (97.88%, *p*-value < 0.001), while only 0.35% (*p*-value = 0.061) of the variation is explained among location sites (populations) within spawning areas (groups) and 1.77% (*p*-value < 0.0014) of the variation is explained among spawning areas (groups). Despite the low percentage explained among spawning areas, the significant results corroborate the differentiation between these areas.

### 3.2. Population Structure

The STRUCTURE analysis indicates three clusters (Figure 2) based on ln P(D) and ΔK scores across spawning areas (Supplementary Figure S1). Despite the predominance of the third cluster (Table 5, yellow in Figure 2) in all location sites except MAU, location sites from Gironde and Tagus spawning areas present a larger admixture with the first cluster (Table 5; orange in Figure 2), while location sites from the Algarve present a larger admixture with the second cluster (Table 5; blue in Figure 2). The Mauritania–Morocco spawning area presents a balanced admixture of the second and third clusters. Contrary to the results obtained with the remaining analysis, which corroborate four genetic groups, these results suggest the existence of three populational groups of *A. regius* along its North–East and Central East Atlantic range: group I constituted by individuals from the Gironde and Tagus spawning areas; group II equivalent to the Algarve spawning area; and group III equivalent to the Mauritania–Morocco spawning area.

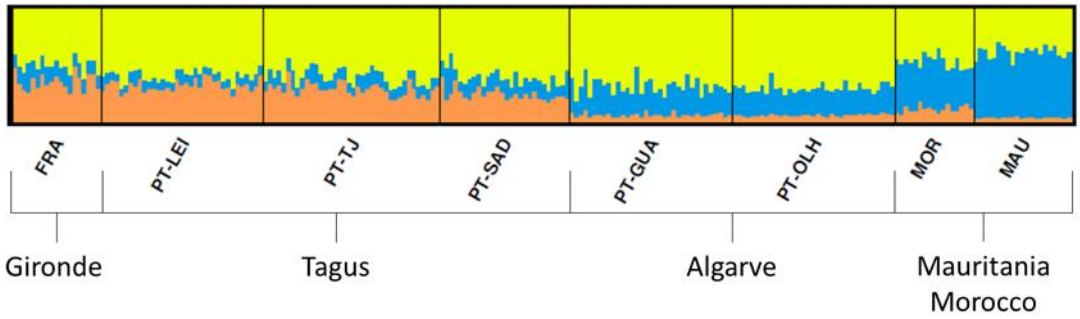


**Figure 2.** *Argyrosomus regius* most likely Atlantic population structure, computed under the admixture model with correlated allelic frequencies in STRUCTURE. Each individual is represented by a vertical bar broken into three different colors (orange, yellow, and blue) based on the assignment into inferred clusters (K = 3). Location site and spawning area of each group of individuals is identified in the figure. For location sites' acronyms, please check Table 1.

**Table 5.** Proportion of membership of each *Argyrosomus regius* location site in each of the three inferred clusters from STRUCTURE analysis. For location sites' acronyms, please check Table 1.

| Location Site | Inferred Clusters | | | Number of Individuals |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| **FRA** | 0.359 | 0.150 | 0.492 | 19 |
| **PT-LEI** | 0.326 | 0.089 | 0.585 | 35 |
| **PT-TJ** | 0.294 | 0.115 | 0.591 | 38 |
| **PT-SAD** | 0.246 | 0.154 | 0.600 | 28 |
| **PT-GUA** | 0.061 | 0.259 | 0.680 | 35 |
| **PT-OLH** | 0.060 | 0.244 | 0.696 | 35 |
| **MOR** | 0.116 | 0.422 | 0.461 | 17 |
| **MAU** | 0.037 | 0.590 | 0.373 | 21 |

The first axis of the principal coordinates analysis (PCoA, Figure 3), calculated using the pairwise $F_{ST}$ values, evidences a clear differentiation of the MAU from the remaining location sites, and the secondary axis evidences a clear differentiation of the FRA location site that is not so evident in the STRUCTURE analysis. The remaining location sites differentiation is also revealed by the first axis, corroborating the previous results.

The Mantel test reveals a positive correlation between genetic differentiation and geographical distances (R = 0.867; *p*-value = 0.001; Supplementary Figure S2).

### 3.3. Demographic History

Regarding the demographic history, different estimates are obtained with the linkage disequilibrium method and the heterozygote excess method. Low effective population sizes are estimated (<500 individuals) for PT-TJ, PT-SAD, PT-GUA, and MAU with the linkage disequilibrium method, while only low effective population sizes for PT-LEI are estimated with the heterozygote excess method (Table 6). Nonetheless, the majority of the 95% confidence intervals obtained with the two methods present a high range with low inferior limits (<500 individuals).

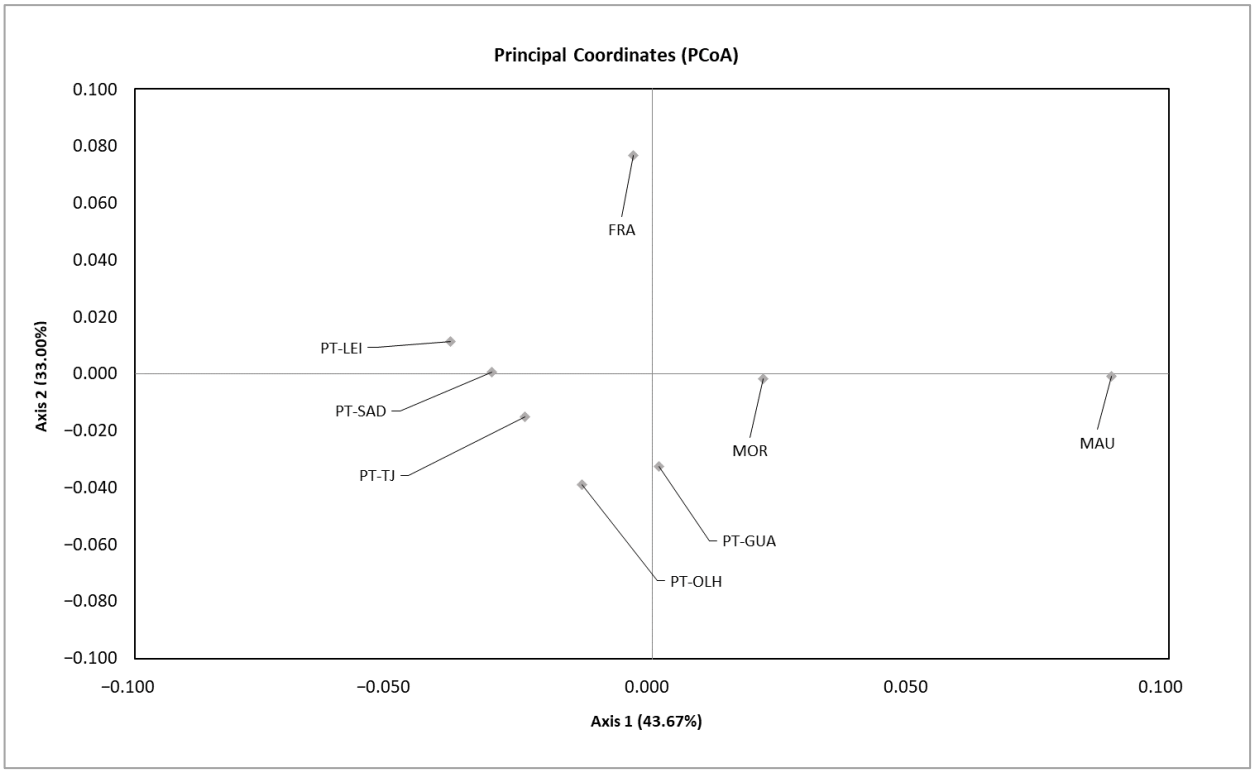


**Figure 3.** Principal coordinates analysis plot (PCoA) based on the pairwise estimates of $F_{ST}$ values among *Argyrosomus regius* Atlantic location sites. For location sites' acronyms, please check Table 1.

**Table 6.** *Argyrosomus regius* Atlantic effective population size (Ne) estimated for each location site with linkage disequilibrium (parametric and jackknife methods) and heterozygote excess method (parametric method). Ne values and respective upper and lower 95% confidence interval, at critical frequency threshold value of 0.05. For location sites' acronyms, please check Table 1.

| | Linkage Disequilibrium Method | | | | | Heterozygote Excess Method | | |
|---|---|---|---|---|---|---|---|---|
| | | Parametric Method | | Jackknife Method | | | Parametric Method | |
| **Location Site** | **Ne** | **Lower** | **Upper** | **Lower** | **Upper** | **Ne** | **Lower** | **Upper** |
| **FRA** | ∞ | 64.0 | ∞ | 63.1 | ∞ | ∞ | ∞ | ∞ |
| **PT-LEI** | ∞ | 128.5 | ∞ | 71.6 | ∞ | 214.2 | 7.9 | ∞ |
| **PT-TJ** | 107.1 | 57.1 | 441.5 | 27.1 | ∞ | ∞ | 21.3 | ∞ |
| **PT-SAD** | 203.2 | 59.3 | ∞ | 56.5 | ∞ | ∞ | 46.7 | ∞ |
| **PT-GUA** | 288.2 | 79.2 | ∞ | 56.7 | ∞ | ∞ | ∞ | ∞ |
| **PT-OLH** | ∞ | 177.4 | ∞ | 126.2 | ∞ | ∞ | ∞ | ∞ |
| **MOR** | ∞ | 61 | ∞ | 26.5 | ∞ | ∞ | 10.4 | ∞ |
| **MAU** | 77.6 | 32.9 | ∞ | 16.1 | ∞ | ∞ | 117.1 | ∞ |
| **All data set** | 1962.6 | 591.0 | ∞ | 380.9 | ∞ | ∞ | ∞ | ∞ |

The relative migration network resultant from the divMigrate analysis (Figure 4; Table 7) suggests a strong gene flow among location sites from the Tagus spawning area and among location sites from the Algarve. A weak gene flow is also suggested between the Tagus and Algarve location sites. In addition to that, the gene migration results suggest a higher gene flow from the southern to the northern location sites.

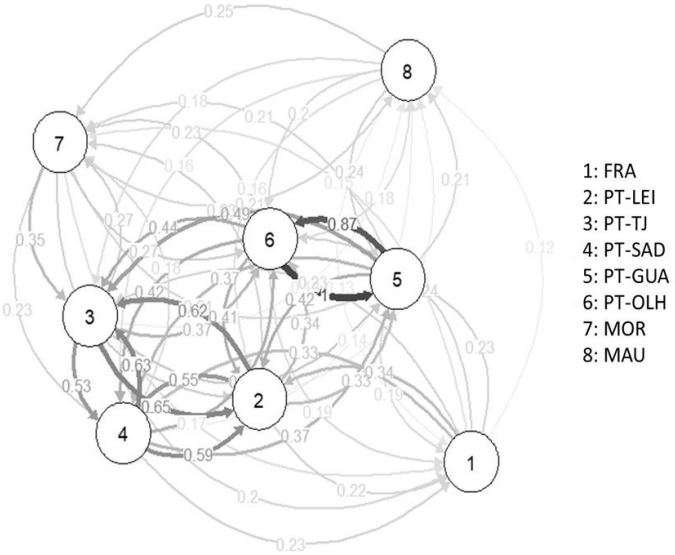


**Figure 4.** Relative migration network among *Argyrosomus regius* Atlantic location sites. Arrows indicate direction of gene flow and respective percentage of migration (Nm value). For location sites' acronyms, please check Table 1.

**Table 7.** Estimates of migration percentage (Nm values, i.e., estimates of the gene flow from the location site in the rows to the location site in the columns; ranges between 0 and 1) among *Argyrosomus regius* Atlantic location sites. Values of Nm higher than 0.500 are displayed in bold. For location sites' acronyms, please check Table 1.

| From\To | FRA | PT-LEI | PT-TJ | PT-SAD | PT-GUA | PT-OLH | MOR | MAU |
|---|---|---|---|---|---|---|---|---|
| **FRA** | - | 0.340 | 0.228 | 0.331 | 0.230 | 0.236 | 0.153 | 0.125 |
| **PT-LEI** | 0.223 | - | **0.622** | **0.546** | 0.325 | 0.338 | 0.235 | 0.142 |
| **PT-TJ** | 0.205 | **0.647** | - | **0.534** | 0.281 | 0.368 | 0.266 | 0.127 |
| **PT-SAD** | 0.227 | **0.595** | **0.629** | - | 0.375 | 0.413 | 0.184 | 0.139 |
| **PT-GUA** | 0.186 | 0.419 | 0.490 | 0.370 | - | **0.875** | 0.207 | 0.210 |
| **PT-OLH** | 0.185 | 0.411 | 0.437 | 0.421 | **1.000** | - | 0.232 | 0.176 |
| **MOR** | 0.166 | 0.263 | 0.354 | 0.230 | 0.208 | 0.266 | - | 0.211 |
| **MAU** | 0.134 | 0.165 | 0.184 | 0.158 | 0.244 | 0.202 | 0.245 | - |

## 4. Discussion

This study suggests four genetic populational groups of *A. regius* along the North-East and Eastern Central Atlantic distribution range: one englobing the Gironde spawning area (group I); a second group encompassing location sites within the proximity of the Tagus spawning area (group II); a third group corresponding to the Algarve, possibly associated with the Gulf of Cádiz spawning aggregation area (group III); and a forth group associated with the Lévrier Bay spawning aggregation area covering Mauritania and Morocco (group IV). This scenario is supported by the genetic differentiation and migration network analyses and corroborates the null hypothesis tested in this study that independent breeding units associated with the four known *A. regius* spawning aggregation areas in the North–East and Eastern Central Atlantic region (i.e., Gironde, Tagus, Algarve—Gulf of Cádiz, and Mauritania–Morocco) constitute discrete genetic stocks. Structuring between spawning areas, despite significant, is probably masked by the high intrapopulation variability in the AMOVA analysis, resulting in a low percentage of variation explained among spawning areas. However, STRUCTURE does not recover four genetic groups but only three, which may be a consequence of suboptimal performance of the analysis due to the low obtained $F_{ST}$ results (<0.05), possibly related to an high dispersal capacity of the species [35].

Haffray et al. [4] suggested the existence of two genetically differentiated groups of populations: one in the Atlantic Ocean and another in the eastern Mediterranean Sea. The

present study, that focuses on the Atlantic *A. regius* distribution range, further elaborates this hypothesis by suggesting four groups within this specific region, contributing to the knowledge about a data-limited species, highly dependent on fisheries data. Isolation by distance may explain, in part, the observed genetic patterns, where most samples exchange migrants according to their geographic separation. However, the discontinuity of *A. regius* spawning areas along the Atlantic coast, and the existence of some level of fidelity of the species to spawning areas such as the Tagus estuary, as observed in preliminary tracking data of adult meagre tagged (acoustic transmitters) inside the Tagus estuary that return in the following spawning season [36], can also be factors that explain the observed structure. Genetic structuring has been also observed in other sciaenidae with potentially high dispersal capabilities, such as the mulloway (*Argyrosomus japonicus* Temminck and Schlegel 1843), the red drum (*Sciaenops ocellatus* Linnaeus 1766), and the white croaker (*Genyonemus lineatus* Ayres 1855) [37–39].

Genetic diversity appears to increase southward, suggesting older and/or larger populations in the southern range of the species. The values of genetic diversity here observed for *A. regius* location sites differ from the ones previously reported by Haffray et al. [4] for Atlantic populations. Despite a slightly higher mean number of alleles values obtained for the Tagus location sites, the allelic richness values are slightly lower than the ones reported by Haffray et al. [4] (allelic richness values ranging between 5.33 and 8.72). These differences may be explained by several methodological distinctions between studies (e.g., sample size, number of loci, selected loci), but the possibility that there was an actual reduction of genetic diversity since 2012 cannot be excluded. Genetic diversity is also lower than that observed for other overfished Sciaenidae species, such as the dusky kob (*Argyrosomus coronus* Griffiths and Heemstra, 1995), the mulloway, and the silver kob (*Argyrosomus inodorus* Griffiths and Heemstra, 1995) [39–41]. Furthermore, low effective population size (<500 individuals, as defined by Franklin and Frankham [42]) were estimated for some location sites from Tagus, Algarve, and Morocco–Mauritania. The infinite Ne estimated through the heterozygote excess method could have been inflated by a low number of loci or samples per location site [43]. Thus, it is not possible to rule out the long-term viability of the species, mostly considering that low inferior confidence interval of effective population size was obtained for all location sites, through the linkage disequilibrium method, as indicated also by Haffray et al. [4]. As mentioned, *A. regius* is among the world's most vulnerable marine fish due to its inherent biological characteristics [13]. Considering their large size, spatial and temporal predictability of spawning, and juvenile aggregations in coastal and transitional waters, it is clear why overfishing is the major threat to the species. The consequences of this vulnerability are evidenced by the fisheries data that suggest a clear reduction in *A. regius* stocks, particularly in the Mediterranean. For instance, in Egypt, past *A. regius* stocks oscillations are thought to be linked with the species exploration and hydraulic regime changes resulting from the construction of the Aswan Dam [44,45]. Off the Balearic Islands, the species was, at one point, considered as extinct, which originated a reintroduction program based on releasing hatchery-reared fish [46]. In Portugal, a 20 year time series data of *A. regius* commercial landings inside the Tagus estuary and adjacent coastal area [47] presented some evidence of a recent decrease, both in absolute (total yield) and relative (proportion caught in the estuary) terms. This was also confirmed by Mota [48], who conducted a survey focused on analyzing the perception of commercial fishermen targeting *A. regius* inside the Tagus estuary.

Recent studies indicated that the genetic effective population size in marine fishes is usually, at least, two times smaller than the results of a population size census [18]. Thus, considering the vulnerability of the species in terms of conservation and the low inferior limits of effective population size (95% confidence intervals) suggested by this study, it is critical to promote the implementation of management plans for *A. regius* fisheries. Understanding the affinity between populations resulting from the prevalence of gene flow among them is essential to promote a sustainable exploitation of fish stocks [18]. For the North–East and Central East Atlantic distribution range, the four populational

groups suggested by this study should be considered when delineating future *A. regius* fisheries management plans, contemplating specific conservation measures for each group, which should include international articulation to maintain the natural gene flow between different spawning areas.

As for the Mauritania–Morocco spawning area, no clear scenario is possible to delineate with the present data: while genetic divergence of Morocco's population is concordant with the isolation by distance scenario, the results point to a clear distinction of Mauritania from the remaining location sites. The low number of location sites sampled in this area, and the lack of detailed knowledge about the spawning areas of the region, do not allow conclusions to be drawn about *A. regius* genetic structure and stocks in these countries.

The Tagus estuary and adjacent coastal areas represent the bulk (60–70%) of *A. regius* landings in Portugal [47], with this estuary being considered the most important nursery and spawning area in the country [3,49]. Considering its central localization in the Atlantic *A. regius* distribution range, and the connectivity observed with adjacent spawning areas, special attention should be paid to Tagus fisheries management. This is particularly important since a populational reduction could result in a higher structuration of the species in its Atlantic distribution range.

Studies using distinct methodologies that include additional biomarkers, such as otolith microchemistry and sound produce by adult *A. regius* associated with spawning behavior, together with artificial tags (acoustic biotelemetry and pressure–temperature archival tags) are currently underway. Combined, such studies will allow better understanding, at different spatial and temporal scales, of the patterns of use of the Tagus estuary by *A. regius* adults and juveniles, and the connectivity between Tagus and adjacent spawning areas. According to the preliminary work of Mota [48], Tagus fishermen seem to be open to adjusting their activity in order to increase the sustainability of the fisheries directed at this species, a possibility relevant for the implementation of management plans focusing on the sustainable exploitation of *A. regius*.

Furthermore, considering the increasingly importance of the species for aquaculture exploration, and the fact that *A. regius* aquaculture escapees have been addressed for the western Mediterranean e.g., [50], further studies should focus on unraveling its impact in the wild populations, namely, in what concerns its genetic structure, since escapees could be related to one of the highest impacts of aquaculture on biodiversity, genetic alterations of wild stocks [51].

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse10121843/s1, Figure S1: (a) Likelihood and (b) Delta K scores for different numbers of k (1 to 9) obtained in structure analyses with *Argyrosomus regius* Atlantic location sites; Figure S2: Mantel test comparing the genetic variation (pairwise $F_{ST}$'s) and the geographic distances (in km) between the corresponding *Argyrosomus regius* Atlantic location sites (considering the shortest marine route possible); Table S1: Data of the 15 microsatellite loci assayed for each of the 228 samples from 8 *Argyrosomus regius* location sites. ID—sample identification; SITE—*Argyrosomus regius* Atlantic location site; Table S2: Pairwise estimates of $F_{ST}$ values among *Argyrosomus regius* Atlantic location sites using the original data (below diagonal) and the data with null alleles correction (above diagonal); Table S3: Pairwise estimates of GST values among *Argyrosomus regius* Atlantic location sites (below diagonal) and corresponding *p*-values (above diagonal; in bold $p < 0.0008$ (Bonferroni correction); Table S4: Pairwise estimates of Jost's D values among *Argyrosomus regius* Atlantic location sites (below diagonal) and corresponding *p*-values (above diagonal; in bold $p < 0.0008$ (Bonferroni correction); Table S5: Locus-by-locus analysis of molecular variance (AMOVA) of *Argyrosomus regius* Atlantic spawning areas, considering location sites as populations and spawning areas as groups.

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