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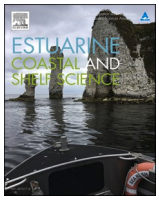

Culture of *Seriola dumerili* in a marine ecosystem: Insights from genetic and morphometric fish traits and implications of escape events

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ABSTRACT

Commercial net-pen mariculture of *Seriola dumerili* recently started in Mediterranean countries. While the introduction of new species allows for product diversification and strengthens the European seafood market, net-pen farming and escape events can lead to a number of socio-economic and ecological interactions in the coastal zone. To assist in the genetic management of broodstocks and mitigate the effects of escape events, the potential of morphological and genetic traits of wild, farmed and escaped *S. dumerili* from the Balearic and the Adriatic Sea were examined as a proxy of wild and farmed origin. Body shape, head profile and mouth position allowed correct classification between fish groups (82%). The global F_{ST} of 0.053 across all loci showed significant subdivision between wild and farmed fish, and allowed correct assignment of escapees. Integration of both genetic and morphometric data using the R package assignPOP improved the assignment results of the escaped group with unknown origin. Potential ecological and socioeconomic implications of escape events are discussed, along with guidelines promoting sustainable development of coastal net-pen aquaculture in a future to come.

1. Introduction

Aquaculture has proven to play a crucial role in global food security, leading the global production compared to capture fisheries since early 2010s (Tacon, 2020), and still growing at a rate of 3% per year (FAO, 2020). The European Union (EU) is the world's largest importer of fishery and aquaculture products (EUMOFA. European Commission, 2021). To date, the fin fish aquaculture sector in the EU, is focused mainly on a few species of fish such as Atlantic salmon (*Salmo salar*), European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) and rainbow trout (*Oncorhynchus mykiss*), accounting for only 20% of total EU seafood production. However, in the last decade, significant progress has been made in diversifying products and introducing new species with desirable production traits, such as improved growth performance, feed efficiency and product quality (Mylonas et al., 2019). Such development and diversification of marine products is a core aspect of the Blue Revolution, a paradigm in which aquaculture plays an important role in ensuring human nutrition and food security world-wide (see references in Ahmed and Thompson, 2019). However, such an increase in aquaculture production needs from a strategy to avoid negative interactions with coastal users that would jeopardise sustainable development of the industry (for a global overview, see Sanchez-Jerez et al., 2016). Due to the current global warming scenario, the latter approach is urgent, especially in the Mediterranean Sea, where the coastal zone will be relentlessly threatened by the increasing frequency and intensity of adverse meteorological events (Salat et al., 2019). Recently, the Storm Gloria (January 2020), with waves up to 14 m high ravaged most marine net-pen facilities and between 60 and 70% of Spanish finfish production (APROMAR, 2021; De Alfonso et al., 2021). Millions of fish died and/or escaped into the wild, with vast socioeconomic and ecological implications. Such implications of farmed fish escaping into the wild are well documented in the case of for Atlantic salmon, European seabass and gilthead seabream (Jensen et al., 2010; Arechavala-Lopez et al., 2018; Quinones et al., 2019). Fish escaping from marine net-pens *i)* can compete with other wild fish species and/or conspecifics for space and trophic resources, *ii)* represent a potential vector of diseases and parasites, and *iii)* impact the genetic diversity of local fish populations through hybridisation (Šegvić-Bubić

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et al., 2011; Arechavala-Lopez et al., 2013; Izquierdo-Gomez et al., 2016; Glover et al., 2017). Several tools, including scales analysis, genetics, fatty acids, otoliths, and morphometrics are available to identify escaped individuals of *S. salar* (Lund and Hansel, 1991; Skilbrei et al., 2015; Karlsson et al., 2016), *S. aurata* (Arechavala-Lopez et al., 2013; Šegvić-Bubić et al., 2014, 2020; Talijančić et al., 2021), *D. labrax* (Arechavala-Lopez et al., 2013; Šegvić-Bubić et al., 2017), and *Argyrosomus regius* (Arechavala-Lopez et al., 2021). Such tools can be used, among others, to monitor wild stocks or prevent economic fraud.

The European finfish industry put the focus on the production of greater amberjack *(Seriola dumerili)*, which will help diversifying the production within the Mediterranean boundaries. The greater amberjack is a cosmopolitan, pelagic and epibenthic teleost fish inhabiting both nearshore reef habitats and the open sea. Greater amberjack is a fast-growing species with excellent organoleptic features which make it suitable for industrial production and marketing purposes. Despite capture-based aquaculture of greater amberjack, i.e. farming based on captured juveniles from the wild, began some two decades ago in the Mediterranean Sea (Mazzola et al., 2000 and references therein), the fully controlled life cycle in captivity was not accomplished until few years ago, thus the species has a low domestication status (Teletchea, 2015). Moreover, the commercial farming of greater amberjack in the EU is also very low, with Greece and Spain as the main producers (<100 Tm). So far, broodstock reproductive dysfunctions, suboptimal feed formulations, disease outbreaks and/or fingerlings supply, have represented major bottlenecks for the development industrial production, all approached by scientific efforts at both European level (EU FP7 Diversify; https://www.diversifyfish.eu/) and national level, namely, the Greek project MAGIATIKO (https://magiatiko.weebly.com/) and the Spanish SERIOLA project. The authors of the study are aware of a low production of European fingerlings, with Spain supplying its own market, as well as other Mediterranean countries such as Croatia. However, information about the production of greater amberjack and origin of fingerlings farmed in other Mediterranean countries, such as Greece is scarce.

In a future to come, an increase of the commercial production of *S. dumerili* is forecasted, and so, the potential number of individuals escaping into the wild due to human error and other reasons (Jensen et al., 2010; Jackson et al., 2015). Farmed fish generally originate from a small number of parental individuals (broodstock) and therefore have a lower effective population size than the wild population. Thus, genetic introgression of escaped fish can affect the genetic diversity of local wild populations, reducing its overall fitness. This has already been documented for the main finfish species that are commercialized at an industrial scale in the EU, which escape frequently from net pens (Glover et al., 2012; Šegvić-Bubić et al., 2017; Žužul et al., 2019). Seemingly, wild populations of greater amberjack would be exposed to a potential genetic influence derived from the development of the industrial production and farmed counterparts escaping from net-pens. Due to fact of genetic structuring between the Atlantic and Mediterranean wild populations of greater amberjack, Šegvić-Bubić et al. (2016) recommended the use of locally derived broodstock when possible, to minimise potential disruption of locally adapted populations exposed to genetic introgression from escaped counterparts.

Since limited information is available on the genetic background of *S. dumerili* broodstocks currently used for fingerling production, scientifically sound information is needed to prevent and mitigate potential ecological impacts of escaped individuals on wild populations, and methods are needed to identify escaped individuals to prevent potential socioeconomic issues such as fraud selling points, or price decreasing due to the landing of large biomasses of the species, typically occurring after an escape event. To date, there has been a lack of studies focusing on exploring the morphological and/or genetic traits as proxies for origin discrimination of *S. dumerili* and evaluating the potential implications of escaped *S. dumerili* into the ecosystem. In order to fill these knowledge gaps, three techniques were used in this study: geometric morphometrics, microsatellite markers, and a combination of both in a supervised machine learning system. The objective was to investigate: i) the body shape characteristics of wild and farmed *S. dumerili* in the western and central Mediterranean, ii) its genetic variation and population structure at spatial and short-term temporal scales, and iii) the potential for discriminating the origin of the fish (i.e., wild and farmed) using integrated biomarkers data.

2. Material and methods

2.1. Fish sampling

The temporal and spatial sampling design consisted of wild and farmed individuals collected in the western and central Mediterranean Sea between 2013 and 2021 (Table 1, Fig. 1). Two sets of samples from the Balearic and the Adriatic Sea were included in the study: a historical dataset from 2013 to 2018, collected prior to the development of commercial aquaculture of *S. dumerili*, and a contemporary dataset from 2020 to 2021 from wild and farmed stocks. Samples of farmed fish were therefore represented only in the contemporary dataset and their collection coincides with the beginning of commercial farming in the Mediterranean as previous attempts were capture based. Wild fish were mainly collected from landings of the artisanal fishing fleet, including both juveniles (35 ± 3.1 cm total length) and adults (66.5 ± 13 cm total length). Fish collection in the Balearic Sea in 2020 was carried out after Storm Gloria, which caused substantial damage to fish farmers in mid-January 2020 (De Alfonso et al., 2021), freeing millions of fish into the wild, including farmed greater amberjack (*S. dumerili*). Therefore, when samples of wild fish were collected, those individuals that exhibited phenotypic characteristics associated with farmed fish, such as darker skin, eroded fins, and body wounds, were identified and visually assigned to the group of escaped fish. The farmed fish collected from both the Adriatic Sea and the Balearic Sea were produced in the same hatchery in the south of Spain (Futuna Blue España, Muelle Comercial, 0 S/N, 11500 El Puerto de Sta María, Cádiz, Spain) that holds domesticated broodstock from the Mediterranean Sea (Alboran Sea; pers. comm.). Three different generations of fish were included in the present study, i.e., juveniles stocked in marine net-pens in 2017 (Murcia, Spain) and those stocked in 2019 and 2020 (Zadar County, Croatia). A fin section was collected from each collected fish, both farmed or wild, and preserved in absolute ethanol for molecular analyses. In parallel, digital images of the fish were taken for subsequent morphometric analysis using a high-resolution camera (18 real MP) mounted on the carrier on which the fish were positioned laterally on the left size (for more detailed information on fish sampling procedure see Talijančić et al., 2019). The images included only contemporary samples, and excluded the farmed fish sampled in the Adriatic Sea in 2021.

2.2. Microsatellite genotyping and analyses

Total genomic DNA from fins was extracted by proteinase K digestion followed by a standard phenol-chloroform extraction protocol. Both the DNA quality and quantity were assessed by spectrophotometry (IMPLEN N50, Germany) and each sample was diluted to 10 ng μL^{-1} in DNAse/RNAse-free water. A total of 492 samples were successfully genotyped with 15 microsatellite markers developed for *S. dumerili* (Renshaw et al., 2006, 2007; Table S1), split into two PCR multiplexes and amplified using the Qiagen multiplex kit and standard primer dyes (FAM, NED, VIC and PET, Applied Biosystems). Amplification was carried in 12.5 mL reactions, with final concentrations of all primers set uniformly at 0.2 μM. PCR conditions included initial denaturation at 95 °C for 5 min, 25 cycles at 95 °C for 30 s, annealing at 57 °C for 90 s, and elongation at 72 °C for 30 s, with final elongation of 30 min at 60 °C. Fragments were separated on an ABI3130 automated sequencer (Applied Biosystems) using Macrogen (Macrogen Inc., Seoul, South Korea) services with an internal size standard (500 LIZ dye Size Standard, Applied Biosystems).

Table 1
Overview of sampling locations, number and origin of greater amberjack *Seriola dumerili* individuals used in the present study. Groups subjected to morphometric and/or genetic analysis with 15 neutral microsatellites markers (SSR) were indicated. For fish ontogenetic state, individuals of total length size ranging from 30 to 40 cm were categorized as juveniles while individuals with length sizes of 60–90 cm were categorized as adults. Cage stocking per each farmed group is included, together with the fish sampling year.

Population Origin and ID	Region/Country	Harbour/farm region	Age/Cage stocking	Longitude, Latitude	Sampling year	No. Samples	
						Morphometrics	SSR
Wild							
13BAW	Balearic Sea/Spain	Alicante	adults	38.18777, −0.55798	2013	–	54
20BAWj	Balearic Sea/Spain	Alicante	juveniles	38.18777, −0.55798	2020	47	48
20BAW	Balearic Sea/Spain	Alicante	adults	38.18777, −0.55798	2020	34	36
13ADW	Adriatic Sea/Croatia	Dubrovnik	adults	42.659371, 18.083330	2013	–	69
18ADW	Adriatic Sea/Croatia	Split	adults	43.503564, 16.435095	2018	–	20
20ADWj	Adriatic Sea/Croatia	Split	juveniles	43.503564, 16.435095	2020	63	69
Escapees							
20BAE	Balearic Sea/Spain	Alicante	adults	37.76775, −0.58588	2020	49	57
Farmed							
20BAF	Balearic Sea/Spain	Alicante	adults/2017	38.6164, 0.037131	2020	30	30
20ADFj	Adriatic Sea/Croatia	Zadar	juveniles/2019	44.027301, 15.215037	2020	30	48
21ADFj	Adriatic Sea/Croatia	Zadar	juveniles/2020	44.027301, 15.215037	2021	–	48
Overall							

The first two letters of the pop ID abbreviations denote the sampling year, the second two letters denote the geographic origin of samples (AD, Adriatic Sea; BA, Balearic Sea), while the last letters characterize the fish origin (W, wild; F, farmed; E, escapee) and ontogenetic stage (j, juvenile).

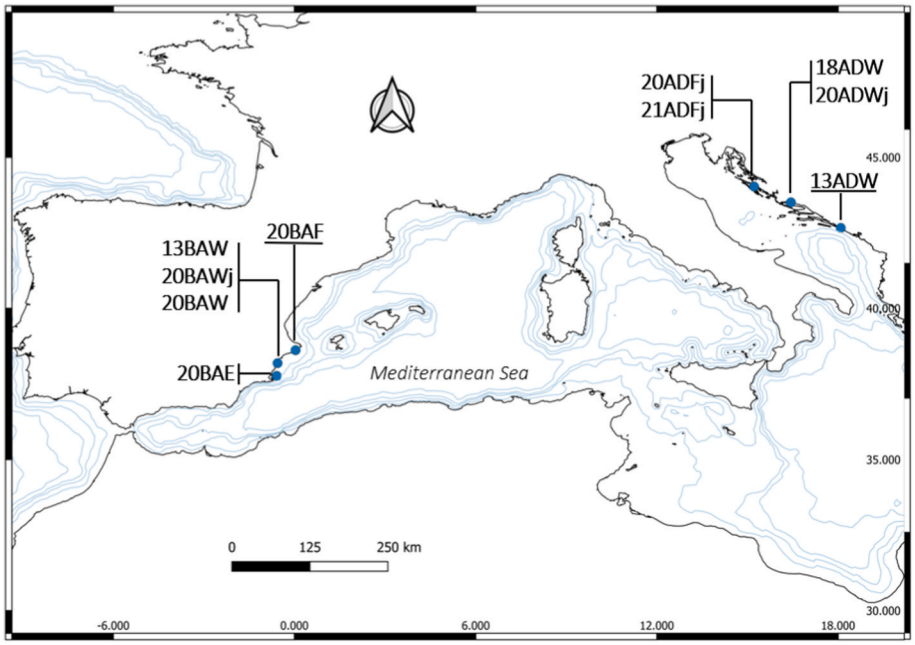

Fig. 1. Western and central Mediterranean Sea with sampling locations of *Seriola dumerili* where the first two numbers of location abbreviations denote sampling year (13, 2013; 18, 2018; 20, 2020; 21, 2021), the first two letters denote the geographic locations (AD, Adriatic Sea; BA, Balearic Sea) and the last letter denote the abbreviations for fish origins (W, wild; F, farmed; E, escapees). More information about fish groups included in the study are provided in Table 1.

Peak height values for each microsatellite allele were analysed using GeneMapper v.3.5 software by two different experts (Applied Biosystems).

2.2.1. Genetic diversity

For each locus, the presence and frequency of null alleles was estimated using MICROCHECKER v.2.2.3 (Van Oosterhout et al., 2004) and FreeNA (Chapuis and Estoup, 2007). Deviation from Hardy-Weinberg equilibrium (HWE), observed and expected heterozygosity (H_o, H_e), fixation index (F_{IS}), and linkage disequilibrium (LD) were tested in GENEPOP 4.0 (Raymond and Rousset, 1995; Rousset, 2008). The mean number of alleles per locus (A) and the mean effective number of alleles across all loci (A_e) were calculated using POPGENE 1.32 (Yeh, 1999), whereas the polymorphism information content (PIC) of each locus was estimated using Cervus v3.0.3 (Kalinowski et al., 2007). Differences in allelic richness (A_r) and heterozygosity between fish origins (i.e., wild and farmed) were assessed using FSTAT 2.9.3 (Goudet, 2002). Sequential Bonferroni correction was used to account for multiple testing (Rice, 1989).

2.2.2. Effective population size and outlier tests

The NeEstimator V2 program (Do et al., 2014) was used to estimate contemporary effective population size (N_e) by the linkage disequilibrium method for populations with sample sizes greater than 30 individuals. A cut-off for alleles with low frequency ≤0.02 was applied to minimise potential bias caused by rare alleles. Recent declines in N_e over a relatively short period of time, e.g., several N_e generations, were tested using BOTTLENECK 1.2.02 (Piry et al., 1999) under the two-phase model (TPM), which includes 85% single-step mutations and 12% variance among multiple steps with 20,000 replications. As

recommended by Piry et al. (1999), significant heterozygote excess was tested for all loci using a one-tailed Wilcoxon signed-rank test. To identify potential non-neutral loci in the populations studied, the F_{dist} approach (Beaumont and Nichols, 1996) implemented in Arlequin 3.5 (Excoffier and Lischer, 2010) was run using a hierarchical island model with 100,000 simulations, three simulated groups, and 100 demes per group. Bayesian method implemented in BayeScan 2.0 (Foll and Gaggiotti, 2008) was also used to identify loci under selection, assuming a Dirichlet distribution of allele frequencies between populations. The program estimates a posterior probability for a model that includes selection where loci with a *q*-value (i.e. analog to a false discovery rate *p*-value) ≤ 0.05 were considered outliers. Testing was performed with the default parameters, including 10000 prior odds for the neutrality mode.

2.2.3. Differentiation and population structuring

Population differentiation was assessed with pair-wise and global F_{ST} values as a weighted average across 15 loci in ARLEQUIN v.3.5, and statistical significance was tested with 10,000 permutations. The D_{est} measure based on allele frequencies (Jost, 2008) was also estimated using GENODIVE (Meirmans and Van Tienderen, 2004). To assess the impact of null alleles at the population differentiation level and possible overestimation, FreeNA was used to calculate F_{ST} statistics with both exclusion and inclusion of the correction method (ENA, Excluding Null Alleles). A total of 50,000 bootstrap replicates were set to calculate the 95% confidence interval for the global F_{ST} values. Two approaches were used to evaluate population structuring, namely the Bayesian clustering program STRUCTURE 2.3 (Pritchard et al., 2000) and the non-model-based Discriminant Analysis of Principal Components (DAPC) method implemented in the Adegenet package (Jombart, 2008) for R (R Core Team, 2017). STRUCTURE parameters were set as follows: admixture ancestry model, correlated allele frequencies, a burn-in period of 50^3 iterations followed by 50^4 MCMC steps, and K values from 1 to the maximum number of groups sampled with 20 replicates each. The most likely number of clusters was assessed using ln P(D) and deltaK values estimated in Structure Harvester 0.6.93 (Earl and VonHoldt, 2012) and visualised using CLUMPP (Jakobsson and Rosenberg, 2007) and DISTRUCT (Rosenberg, 2004). When necessary, hierarchical population structuring was applied (e.g., Vähä et al., 2007) to examine additional clustering of genetic groups found in the first run. The DeltaK or lnP (D) method was applied until the lowest level of differentiation was found in all subgroups. DAPC was run with the *dapc* function using the sampling locations as a priori groups. The number of principal components (PCs) retained was optimised using the *xvalDapc* function (Jombart, 2008).

2.3. Geometric morphometrics

The shape of each fish was characterized with a total of 20 landmarks and semi-landmarks altogether (Fig. S1) and digitised using tpsDig Dig 2 software (Rohlf, 2010). The configurations of points extracted from each fish image (i.e., x,y coordinates) were subjected to a generalised Procrustes analysis (GPA) in tpsRelw software (Rohlf, 2010), removing the variability irrelevant to shape associated to the scale, orientation and position of the fish in the picture.

Semi-landmarks, as points that are not homologous but maintain a position correspondence, were allowed to slide along their tangent direction using the Procrustes distance criterion with the *ten iterations* setting. The allometric relationship of body shapes and log-transformed centroid size (CS) was examined using the *procD.lm* function of the R package geomorph (Adams and Castillo, 2013) to determine whether populations exhibit isometric or allometric growth by fitting an individual regression of size on shape for each population. Additionally, the homogeneity of slope (HOS) was tested using the *advanced.procD.lm* function in geomorph to calculate and display pairwise comparisons of slope angles (direction of shape change with size) and slope lengths (amount of shape change with size). Variation between and within sample populations was investigated using a set of complementary approaches: exploratory ordination and supervised machine learning (See further down section: Assignment of escaped labelled individuals to wild or farm origin).

Variation in body shape. Between-group principal component analysis (bgPCA) was performed to examine variation and to assess between-group structure and associated shape trajectories without distorting the shape space. A principal component analysis (PCA) was performed on the centroids of the groups and the entire sample, centred by the grand mean, was projected onto the resulting principal components (PC) using MorphoJ software (Klingenberg, 2011). In addition, cross-validated classification of individuals was based on the extracted bgPCs and performed with *classify* function of the R package Morpho (Schlager, 2017).

2.4. Assignment of escaped labelled individuals to wild or farm origin

The discriminative power of genetic and body shape approaches was investigated with the R package assignPOP (Chen et al., 2018) using morphometric, genetic and their combined datasets in a supervised machine learning framework. Prior to analysis, several steps were taken to ensure computational uniformity in the evaluation of the training dataset and assignment of escaped individuals: (i) alleles with low variance were removed from the dataset with *reduce.allele* function to more accurately determine the origin of the studied individuals; (ii) before performing PCA and cross-validation, the entire dataset was centred and scaled (mean and standard deviation of each feature were set to 0 and 1, respectively) because the integrated dataset contains different scales for genetic and morphometric traits; (iii) through the PCA dimensionality reduction procedure, the variables were transformed into PCs summarizing the mixed variations of the genetic and morphometric data, keeping those with an eigenvalue greater than 1; and (iv) the linear discriminant classification function (LDA) was used to build the predictive models for fish origin. Wild (20BAW, 20BAWj, 20ADWj, n = 144) and farmed fish (20BAF, 20ADFj; n = 60) were used as training data, while escaped fish (20BAE; n = 49) were considered unknown individuals and tested for their assignment to wild or farmed origin. Monte Carlo cross-validation (MC) was performed to estimate the predictive accuracy of the training data by reassigning individuals with known origins to determine statistical accuracy for the membership of escaped individuals. The most informative loci were determined using the *check.loci* function for all proportions of the training individuals by counting the frequency of occurrence of each locus in the MC analysis to evaluate whether a subset of loci could perform as well as when all loci were included. After evaluation, the datasets were used as input files for the *assign.X* function, in which a predictive model was built on the training data to generate predictions about the origin of the escaped individuals. After evaluation, individuals were assigned according to the highest probabilities of belonging to the most likely fish origin.

3. Results

3.1. Genetic variation within populations

A total of 479 wild and farmed individuals of *Seriola dumerili* were genotyped at 15 microsatellite loci (Table 1). The proportion of missing data per locus and population varied from 0 to 5%, with an average of 0.5%. After applying a strict Bonferroni correction for multiple testing, all farmed populations showed significant deviation from Hardy-Weinberg equilibrium, with trends toward heterozygote excess at several loci (e.g., Sdu4, Sdu27, and Sdu46; Table S2). The presence of null alleles detected by MICROCHECKER was negligible, with only the loci Sdu7 and Sdu36 showing signs of null alleles at low frequencies <11% in one population. Estimation of F_{ST} with and without ENA correction method yielded comparable results: 0.0528 with vs. 0.0530

without ENA, with overlapping 95% CI (0.037–0.073). No consistent evidence of linkage disequilibrium was detected between pairs of loci. Therefore, all loci were retained, including the locus Sdu8, which was monomorphic in all but two populations (13BAW and 20ADWj; Table S2). All other loci were polymorphic (mean PIC 0.65) and the number of alleles per locus ranged from 2 to 27 (Table S2). Observed and expected heterozygosity varied among populations (0.65–0.76 for H_o; 0.65–0.73 for H_e), with farmed populations having significantly lower allelic richness (4.6 vs. 6.8) than wild populations (Table 2). On the other hand, the farmed populations had significantly higher observed heterozygosity (0.70 vs. 0.66) and lower inbreeding coefficient (F_{IS}) (−0.14 vs. −0.01) due to excess heterozygosity at several loci. A strong disparity in concurrent effective population sizes (N_e) was observed with respect to fish origin (Table 2). In general, very low N_e values were found in farmed and escaped populations with total estimates <15 individuals, while wild populations had total estimates of $N_e > 1000$ individuals. Consistent with the N_e estimates, the Bottleneck test and the use of Wilcoxon signed-rank test revealed significant heterozygote excess ($p < 0.05$) under the two-phase model in all farmed populations, providing evidence that farmed populations have recently experienced a reduction in population size. This is also confirmed by the results of the IAM and partially with SMM models (Table S3).

3.2. Among-population genetic structure

ARLEQUIN and BayeScan analyses revealed an outlier locus (Sdu39) in the full dataset (Table S4, Fig. S2). The influence of this candidate locus was tested by performing F_{ST} and STRUCTURE analyses with and without it (Table 3, Fig. S3). Because inclusion or exclusion of this locus had no effect on the resulting genetic pattern of structuring and limited effect on overall and pairwise F_{ST} values, Sdu39 was retained in all subsequent analyses. Assessment of statistical power for the dataset in POWSIM revealed that it was possible to detect genetic divergence up to $F_{ST} = 0.005$ with 100% confidence ($\chi 2$, Fisher's test) and up to $F_{ST} = 0.001$ with 76% confidence (Table S5).

Global genetic differentiation estimated using F_{ST} with the inclusion and exclusion of the Sdu39 locus was 0.053 and 0.045 ($p < 0.0001$), demonstrating a moderate level of differentiation among all populations. The high F_{ST} differentiation index of 0.114 was observed among farmed populations and was significantly different from the low index observed in the wild populations (0.008). After Bonferroni correction, 33 of 45 pairwise F_{ST} comparisons were statistically significant when permuted by Fisher's exact test (Table 3). Farmed samples from the Adriatic region (20ADFj and 21ADFj) showed high and significant pairwise differentiation in relation to all populations included in the dataset. A similar pattern was observed for the samples of farmed and escaped fish from the western Mediterranean (20BAE and 20BAF), but with a lower degree of genetic differentiation compared to the samples from the Adriatic Sea. Most non-significant comparisons were found among wild population pairs from both Mediterranean basins, with the exception of the wild fish group from the Adriatic Sea sampled in 2013, which showed significant interactions with fish groups from the Balearic Sea. Interestingly, all wild populations were found to be homogeneous when locus Sdu39 was excluded from the analysis (Table 3). The mean D_{est} value of 0.099 and the pairwise D_{est} values were larger in magnitude than the corresponding F_{ST} values (Table S6), but the pairwise matrices were highly correlated in all population comparisons (Mantel $r > 0.9$, $p < 0.001$).

The likelihood scores of the Bayesian clustering algorithm implemented in STRUCTURE and results of the delta K method were examined to infer relationships between populations. Delta K as a function of K peaked at K = 2, corresponding to the uppermost hierarchical level of structuring, and then peaked a second time at K = 5, indicating additional structure within the group (Fig. S4). Complementing this, the mean likelihood score (Ln(K)) reached a plateau at K = 5 (Fig. S4). For K = 2, the Adriatic farmed populations and the majority of escaped fish from the Balearic Sea were assigned to the first cluster (dark blue), while all wild populations were assigned to the second cluster (blue) together with the farmed fish from the Balearic Sea. Several individuals from the escaped fish group had a mixture proportion of membership to each of the two clusters (Fig. 2). At K = 5, all wild populations were grouped into one cluster (blue), while farmed populations were assigned to four different clusters, three of which were exclusively associated with the farmed fish origin (yellow, green, and dark blue). Only the farmed fish group ADFj showed an admixture pattern with individuals assigned to both the red and green clusters. As expected, the escaped fish showed heterogeneous cluster stratification with three clusters within the group. Some individuals were assigned to the farmed-origin clusters (red and dark blue), while other individuals were assigned to the wild-origin cluster (blue). More specifically, 10 of 57 individuals had a membership coefficient (q) above 0.8 in the wild-origin cluster. No further structure was detected within the wild populations (blue cluster).

The DAPC analysis supported the results of the STRUCTURE analysis (Fig. S5). The first two DAPC principal components (DA1 and DA2) clearly separated the Adriatic farmed populations from all other populations, while DA1 and DA3 additionally separated the Balearic farmed group from all wild groups, with escaped individuals scattered between wild and farmed populations.

Table 2
Summary statistics for genetic variation of Greater amberjack, showing the average number of alleles (A), effective number of alleles (A_e), allelic richness (A_r), expected (H_e) and observed (H_o) heterozygosity, fixation index (F_{IS}) and effective population size (N_e) for 15 microsatellite loci.

Population ID	A	A_e	A_r	H_o	H_e	F_{IS}	N_e
Wild							
13BAW	8.5 ± 4.9	4.7 ± 3.9	6.9 ± 3.7	0.65 ± 0.3	0.66 ± 0.3	0.019	315(164,2042)
20BAWj	9.0 ± 5.3	4.5 ± 4.1	6.6 ± 4.0	0.67 ± 0.2	0.69 ± 0.2	−0.031	∞(528,∞)
20BAW	8.7 ± 4.6	4.6 ± 3.7	7.0 ± 3.9	0.71 ± 0.2	0.73 ± 0.2	0.011	384(148,∞)
13ADW	9.4 ± 5.5	4.6 ± 4.1	6.6 ± 3.9	0.71 ± 0.2[a]	0.70 ± 0.2	−0.014	88(71,113)
18ADW	7.4 ± 3.2	4.1 ± 2.8	6.8 ± 3.5	0.72 ± 0.2	0.72 ± 0.2	0.010	
20ADWj	9.8 ± 5.3	4.5 ± 3.6	6.8 ± 3.7	0.65 ± 0.3	0.65 ± 0.3	−0.007	422(216,3488)
Overall	12.3 ± 6.3	4.8 ± 4.4	6.8[a]	0.66 ± 0.2[a]	0.66 ± 0.2	−0.014[a]	1078(685,2327)
Escapees							
20BAE	8.1 ± 3.3	4.2 ± 2.4	6.2 ± 2.6	0.74 ± 0.2[a]	0.72 ± 0.2	−0.026	13(12,15)
Farmed							
20BAF	4.7 ± 19	3.2 ± 1.5	4.3 ± 1.9	0.76 ± 0.2[a]	0.65 ± 0.2	−0.165	2(2,3)
20ADFj	5.4 ± 2.3	3.0 ± 1.4	4.5 ± 2.0	0.70 ± 0.3[a]	0.62 ± 0.2	−0.130	3(3,4)
21ADFj	5.7 ± 1.9	3.7 ± 1.8	4.9 ± 1.9	0.71 ± 0.3[a]	0.66 ± 0.3	−0.085	3(3,3)
Overall	7.7 ± 4.1	4.4 ± 2.7	4.6[b]	0.70 ± 0.3[b]	0.65 ± 0.3	−0.138[b]	9(8,10)

[a] HWE population deviation and F_{IS} at $p < 0.05$; superscript of different letters indicates statistical significance at $p < 0.05$ among population groups. Effective population size (N_e) of the populations with a sample size smaller than 30 individuals were not analysed.

Table 3
Pairwise F_{ST} values based on 15 microsatellite loci (below the diagonal) and 14 microsatellite loci (above the diagonal; Sdu39 locus excluded) among 10 populations of greater amberjack *Seriola dumerili* sampled in the Adriatic and Balearic Seas, including wild, farmed and escaped populations. Significant F_{ST} values at $p < 0.001$ in bold (Bonferroni correction). Population codes are explained in Table 1.

	13BAW	20BAWj	20BAW	13ADW	18ADW	20ADWj	20BAE	20BAF	20ADFj	21ADFj
13BAW		0.001	0.005	0.000	0.001	0.001	**0.033**	**0.042**	**0.088**	**0.068**
20BAWj	0.006		0.002	0.001	−0.001	0.003	**0.030**	**0.043**	**0.100**	**0.081**
20BAW	0.004	0.004		0.003	−0.003	0.005	**0.018**	**0.042**	**0.098**	**0.070**
13ADW	**0.006**	**0.019**	**0.010**		−0.001	0.000	**0.036**	**0.047**	**0.093**	**0.082**
18ADW	0.000	0.003	−0.003	0.005		0.004	**0.018**	**0.048**	**0.097**	**0.076**
20ADWj	**0.006**	**0.020**	**0.012**	0.000	0.010		**0.038**	**0.044**	**0.099**	**0.072**
20BAE	**0.033**	**0.035**	**0.020**	**0.048**	**0.018**	**0.050**		**0.069**	**0.098**	**0.061**
20BAF	**0.039**	**0.050**	**0.040**	**0.048**	**0.045**	**0.046**	**0.068**		**0.123**	**0.096**
20ADFj	**0.096**	**0.110**	**0.108**	**0.122**	**0.106**	**0.127**	**0.098**	**0.134**		**0.107**
21ADFj	**0.071**	**0.085**	**0.075**	**0.097**	**0.076**	**0.090**	**0.059**	**0.101**	**0.106**	

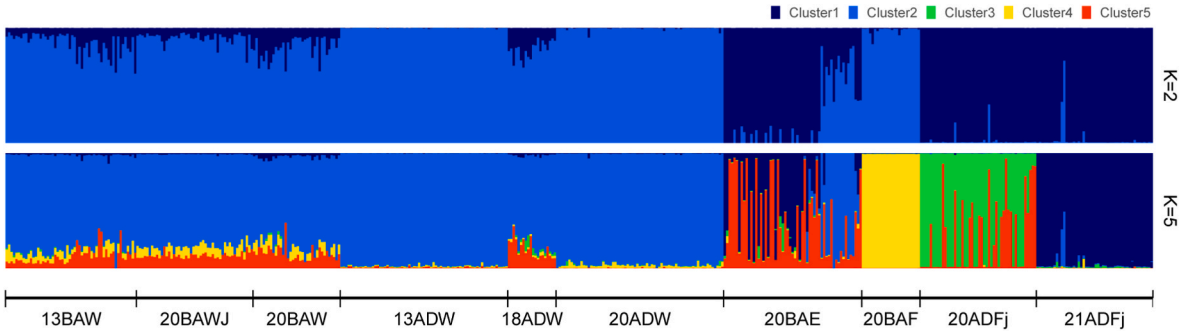

Fig. 2. Bayesian STRUCTURE clustering of the greater amberjack *Seriola dumerili* from the Adriatic and Balearic Seas, including wild, farmed, and escaped origins, assuming two and five inferred clusters (K = 2; K = 5) based on 15 microsatellite loci. Abbreviations for populations are indicated in Table 1.

3.3. Geometric morphometrics

In total, 253 photos of individual fish were analysed. Significant allometries were determined using the HOS test, with a small but significant (5%; $p < 0.001$) proportion of shape variation related to size. Pairwise comparisons of slope angles, however, revealed antiparallel allometries only for 20BAE vs. 20ADFj ($p = 0.04$) and 20BAE vs. 20BAW ($p = 0.03$), while the others showed the same allometric pattern (Table S7). A significant proportion of the variation in body shape was attributed to the population effect (32%, $p < 0.001$), with slopes of parallel allometric trajectories showing persistent shape differences among populations along the size axis (Fig. S6). Although the effect of size on shape was small overall, no fit to a common regression line or to a common model for size correction of the shape variables was performed for the subsequent shape analysis.

The first two bgPCAs explained 80.86% of the shape variation associated with the predefined populations and showed discernible separation trends where origin and ontogenetic stages formed distinct morphospaces, despite some overlap among individuals (Fig. 3). Most notably, 20BAE and 20BAF individuals separated from the rest along the bgPCA1 axis (54.17%), whereas clustered juveniles were separated from adults along the bgPCA 2 axis (26.51%). All pairwise population differences in body shape were significant ($p < 0.05$), with an overall classification accuracy of 82.21%. Farmed great amberjack (20ADFj, 20BAF) were 100% correctly classified, while escapees (20BAE) were the most frequently misclassified group, with 18.37% of individuals assigned to wild and 10.2% to farmed populations (Table 4). The general body shape differentiation was mainly determined by mouth position and head profile shape along bgPC1 and body height along bgPC2 (Fig. 4). Body shape of adult escapees (20BAE) appeared to be more similar to Balearic farmed fish (20BAF) as they mostly occurred along the positive values of the bgPCA1 axis, corresponding to traits such as a supraterminal mouth with a lower head nape. In contrast, wild adult amberjack (20BAW) presented a more terminally positioned mouth, as

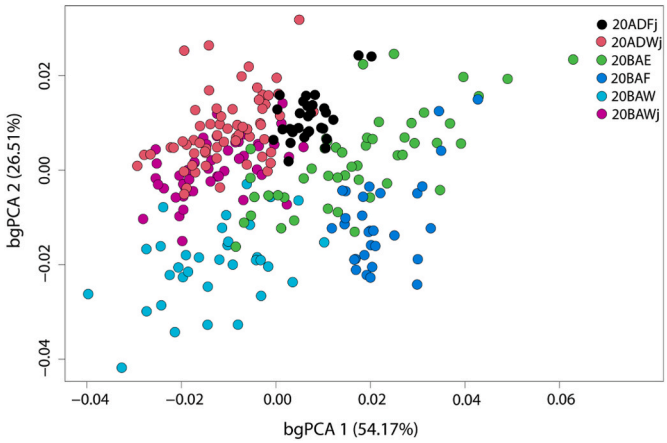

Fig. 3. Scatterplot for the first two principal components (bgPC1 and bgPC2) of between-group principal component analysis.

well as an elongated and fusiform body shape. Juveniles of all populations clustered in positive values along the bgPCA2 axis and exhibited a stockier body shape compared to adults.

3.4. Assignment of escaped fish

Prior to the MC test, Sdu8 was removed as a low variance locus and the remaining 14 loci were used along with 40 Procrustes coordinates as an integrated dataset for evaluating individuals from known populations, as the basis for assigning escaped amberjack to wild or farmed origin. Overall assignment accuracy was approximately 95% (Fig. 5A), with a smaller proportion of the training loci (0.25 and 0.5) with the highest F_{ST} values yielding similar overall assignment accuracy as when all loci were included (Table S8). Consequently, seven loci (Sdu46,

Table 4
Between-group PCA jackknife (leave-one-out) cross-validated classification result using 253 individuals of the greater amberjack *Seriola dumerili*, with an overall classification accuracy of 82.21%.

	20ADFj	20ADWj	20BAE	20BAF	20BAW	20BAWj	Total
20ADFj	**30 (100%)**	0	0	0	0	0	30
20ADWj	2 (3.17%)	**50 (79.37%)**	0	0	0	11 (17.46%)	63
20BAE	1 (2.04%)	0	**35 (71.43%)**	4 (8.16%)	6 (12.24%)	3 (6.12%)	49
20BAF	0	0	0	**30 (100%)**	0	0	30
20BAW	1 (2.94%)	0	2 (5.88%)	1 (2.94%)	**27 (79.41%)**	3 (8.82%)	34
20BAWj	1 (2.13%)	8 (17.02%)	1 (2.13%)	0	1 (2.13%)	**36 (76.6%)**	47

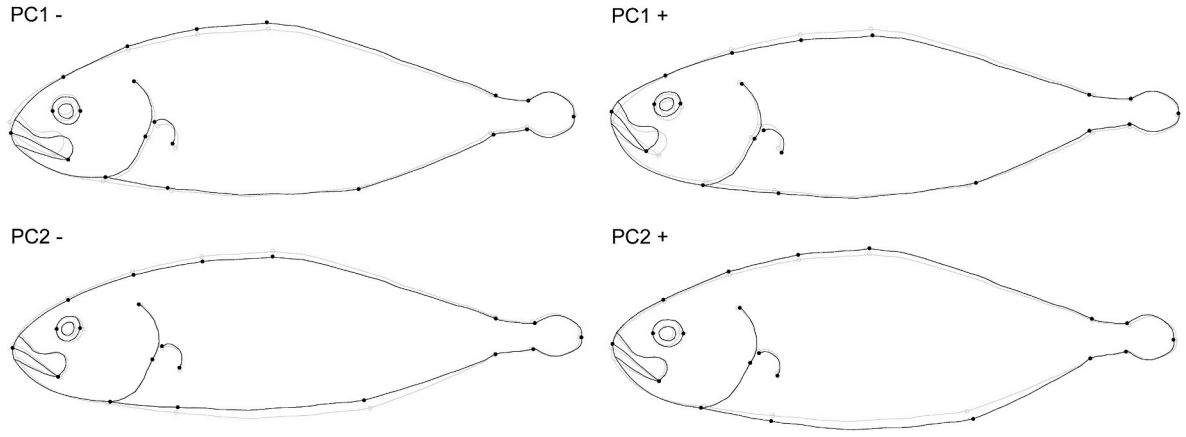

Fig. 4. Shape changes associated with the bgPCA 1 and 2 axes. For each bgPCA, the shapes corresponding to the observed extremes in the positive and negative directions are shown as an outline warped body shape of a greater amberjack.

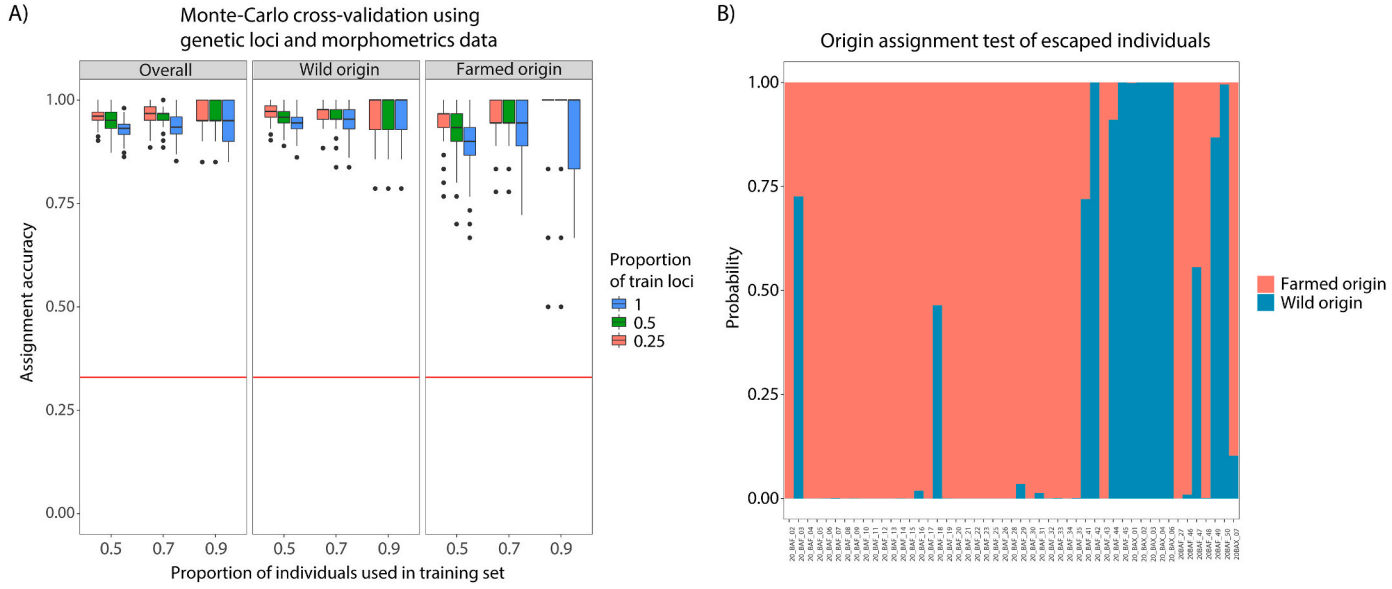

Fig. 5. A) Assignment accuracies estimated via Monte-Carlo cross-validation and linear discriminant analysis methods, with sampling of three subsets of high F_{ST} loci plus geometric morphometrics data, crossed by three levels of training individuals, with 99 iterations. Box plot details: the line within the box is the median, top and bottom edges of the box are the 25th and 75th percentiles; the ends of the whiskers are the minimum and maximum of non-outliers; outliers are shown as dots. Horizontal red lines indicate the null origin assignment rate, which was 33%. B) Escapee origin assignment with all morphometric data and 7 loci used for the test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Sdu44, Sdu39, Sdu21, Sdu5, Sdu27, Sdu7) were identified as most informative in the MC assignment test and were used for subsequent assignment of escapees. Of the total 49 escapees, 36 (73.5%) were assigned to farmed and 13 (26.5%) to wild origin (Fig. 5B; Table S9). When comparing these wild-assigned individuals with those identified by Bayesian cluster analysis in the group, both analyses depicted the same misclassified individuals, although the AssignPOP analysis detected three additional wild-like individuals based on the combined morphometric and genetic datasets (Table S9).

4. Discussion

To the extent of our knowledge, this article provides the first morphological and genetic description of both wild and farmed *Seriola*

dumerili populations from the western and central Mediterranean. Due to the complex interplay between aquaculture sites, wild and farmed fish, descriptors are needed to reliably identify the origin of fish. Thus, three different approaches, e.g., geometric morphometrics, microsatellite markers, and a combination of both in a supervised machine learning framework, were applied *i)* to quantify genetic and body shape population differences and *ii)* to detect possible fish escapees, comparing 479 sampled individuals grouped by fish origin (wild *vs* farmed) and ontogenetic state (juveniles *vs* adults).

4.1. Genetic characteristics of wild and reared fish

The panel of two microsatellite multiplex PCRs for *S. dumerili* designed in this study using 15 markers developed by Renshaw et al. (2006, 2007) proved robust and effective in identifying the origin of *S. dumerili* without prior knowledge of pedigree and characterisation of populations. Moderate genetic variability was observed in all populations with overall expected heterozygosity of >65% for both wild and farmed fish, which is slightly higher than previously reported indices for wild *S. dumerili* populations sampled from a wider geographical area in the Mediterranean (Šegvić-Bubić et al., 2016), where only seven loci were used. All farmed populations, as well as the escaped group, deviated from HWE due to excess heterozygosity at several loci. Excess heterozygosity usually occurs when a population undergoes a recent reduction in its effective size, and it may persist for a certain number of generations until a new equilibrium is established (Cornuet and Luikart, 1996). In the present study, farmed populations were characterised by very low effective population sizes ($N_e < 10$ individuals), in contrast to the overall estimates of $N_e > 1000$ individuals for wild populations. This discrepancy in N_e was also associated with a lower-than-average number of alleles per locus, significantly lower allelic richness, and lower heterozygosity in cultured versus wild populations. Such bias in diversity indices has also been observed in several species with a much longer history of farming and generations under familial selection, such as gilthead seabream (Šegvić-Bubić et al., 2011, 2014; Žužul et al., 2019), European seabass (Šegvić-Bubić et al., 2017), and Atlantic salmon (Skaala et al., 2004). The low N_e value observed in the escaped fish group (<15 individuals) can be further explained by the mixed age structure and genetic substructuring in this group (see further Discussion), which tends to shift N_e values downward (Waples and England, 2011; Robinson and Moyer, 2013; Holleley et al., 2014).

The extent to which diversity is lost in hatchery-produced fish depends on hatchery protocols, including the number, size, and source of broodstock, as well as spawning methods. Limited broodstock size can promote both the occurrence of the founder effect and genetic drift due to lower Ne, which in turn can lead to a loss of genetic diversity (Waples et al., 2012). This situation poses a serious threat to wild populations as more farmed fish escape to the wild, so that despite an increase in the fish census size in the wild, a reduction in effective size (i.e., the Ryman-Laikre effect) can be expected in a combined system of farmed and wild populations. Due to the overrepresentation of farmed fish that originate from small numbers of captive parents that exhibit high variance in reproductive success, genetic exchange between captive and wild populations can lead to a reduction in overall effective population size and diversity in the subsequent generation (Ryman and Laikre, 1991). Some detailed studies of genetic variability in farmed populations of *S. dumerili* are generally lacking, which is not surprising given the infancy of commercial sea cage farming practice. Rodriguez-Barreto et al. (2013) used molecular markers to reconstruct the pedigree of the greater amberjack and reported unequal contribution of breeders during joint mass spawning, which could lead to significant inbreeding across generations when breeder numbers are low. Their offspring also showed excess heterozygosity at most loci studied. In addition, reproductive dysfunctions exist for both captive-reared females and males that further compromise the contribution of breeders to a mass spawning event. Insufficient release of luteinising hormone from the pituitary gland at the end of oogenesis due to dysfunctional release of GnRH from the hypothalamus has been attributed to cage- or tank-reared females (Mylonas et al., 2010; Zohar, 2021), while males suffer from poor sperm quality (Fakriadis and Mylonas, 2021). However, two decades of research on reproductive control and hormone treatment in greater amberjack have identified husbandry conditions, GnRHa hormone doses, and timing of treatment that can ensure adequate egg production for commercial purposes (Corriero et al., 2021 and references within).

Indices of genetic diversity for both current and historical wild populations, including juveniles and adults, were comparable, indicating temporal stability of genetic variability despite intensive fishing effort within the regions. The Bottleneck test under the TPM model showed no signal of genetic erosion in any of the wild populations, while the opposite was found in the farmed populations that have recently experienced a reduction in population size.

Although the greater amberjack is an important recreational and commercial species for Mediterranean countries and the United States, data on population structure and genetic diversity throughout the species' range are still lacking. Applying mitochondrial and nuclear markers to populations from the Mediterranean and eastern Atlantic, Šegvić-Bubić et al. (2017) reported two distinct coexisting mtDNA lineages in both basins that likely evolved outside the Mediterranean due to paleogeographic or climatic events. On the other hand, microsatellites revealed a lack of genetic subdivision within the Mediterranean populations and a strong break between the Atlantic and the Mediterranean due to the predominant oceanographic features in the area of the Strait of Gibraltar. The temporally and spatially consistent pattern of genetic connectivity of wild populations within the Mediterranean was also confirmed in the present study, with an overall F_{ST} value of 0.008. It appears that the reproductive characteristics of *S. dumerili*, i.e., multiple spawners with a spawning period of several months (Harris et al., 2007), together with the long pelagic larval stage of ~30 days (Hasegawa et al., 2020) and passive dispersal of juveniles associated with floating objects (FAD effect, Hasegawa et al., 2017), supported by ocean currents, synergistically promote gene flow over a geographically wide range.

In the present study, the main source of genetic differentiation was found within farmed populations ($F_{ST} = 0.114$) and between farmed, escaped and wild populations. Inter- and intra-farmed strain divergence has been detected in many other farmed species (Glover et al., 2012, 2013; Šegvić-Bubić et al., 2017; Žužul et al., 2019) and arises due to founder effects, genetic drift, and captive domestication selection (Waters et al., 2015). The geographic origin of the *S. dumerili* broodstock whose progeny were genotyped here is not known, but the use of different lineages may accelerate the differentiation of breeding strains. Consistent with the above, the uppermost level of population structuring segregated the present data set into two clusters based on the origin of the fish (wild and farmed). Only farmed fish from the Balearic Sea (20BAF) were assigned to the wild fish cluster, which could indicate the use of broodstock sourced from the Mediterranean Sea for the production of juveniles and would explain similarity of genotypes between groups. This does not seem to be the case for the broodstocks of the other two farmed populations sampled in the Adriatic Sea. Examination of the additional structure signal at $K = 5$ revealed that all farmed populations were assigned to a separate cluster. Only farmed fish from the Adriatic Sea (2020) showed a diverse genetic composition, with individuals assigned to two clusters, suggesting the use of broodstock originating from different lineages, e.g., Atlantic and Mediterranean, for management reasons. However, such a conclusion should be supported by additional fish sampling. In the escaped fish group, only several individuals were assigned to the wild origin cluster with an assignment score higher than 80%.

4.2. Morphology and feralisation

Wild and farmed greater amberjack differed in body shape, particularly in head profile and mouth position, while escaped individuals

exhibited a notable convergence leading from farmed to wild body shape traits. These morphologies allowed for a high overall inter-group correct classification score (82.21%), with adult farmed amberjack (20BAF) and farmed juveniles (20ADFj) being 100% correctly classified. The lowest correct classification (71.43%) shown by escaped fish (20BAE) could indicate the presence of different fish origins or a gradual feralisation process towards the wild phenotype, i.e. the adaptation process of an escaped fish to the wild environment, where phenotypic traits change over time once the fish is no longer exposed to farming conditions (Arechavala-Lopez et al., 2013; Toledo-Guedes et al., 2021). The latter is less likely due to the short time period between the massive escape event caused by Storm Gloria and fish sampling.

The extent of differences between feral and wild fish depends on time spent in captivity, in particular, the number of generations without exchange with wild conspecifics (Teletchea, 2017). Although the greater amberjack only recently reached the level of domestication in aquaculture, where the entire life cycle is controlled in captivity, without an established selective breeding programme (Teletchea, 2015; Mylonas et al., 2016; Corriero et al., 2021), the farm footprint on body shape was clearly noted in the present study. Namely, farmed adults had a smaller head profile and a smaller, supraterminal mouth compared to the elongated and fusiform body shape of the adult amberjack with terminally positioned mouth. Such divergence patterns are consistent with previous findings for gilthead seabream (Arechavala-Lopez et al., 2012; Šegvić-Bubić et al., 2014; Talijančić et al., 2019, 2021) and European seabass (Arechavala-Lopez et al., 2012). Farming conditions such as low habitat complexity, stable and abundant non-elusive food, uniform water velocity, and high fish density appear to elicit similar phenotypic responses in the anterior part of the body regardless of the marine fish species cultured (Wringe et al., 2016). A smaller head profile and upward positioning of the mouth represent common directional changes in morphological traits that occur not only in farmed fish but also in wild fish near fish farms in the Mediterranean Sea (Abaad et al., 2016; Talijančić et al., 2021). Dietary and behavioural changes, particularly the shift in foraging from wild trophic resources to artificial feed, may have triggered morphological-plastic responses in the feeding apparatus of greater amberjack, as observed in gilthead seabream populations in the eastern Adriatic (Talijančić et al., 2019, 2021). In addition, rearing temperatures also induce changes in morphology, with increasing temperatures promoting an elongation of body shape, particularly the head (Fernandez-Montero et al., 2018). The latter suggests that the environment has a greater influence on the phenotype or on regulating the extent of plasticity than the underlying species-specific genetic architecture. Such a fish response impacts the successful recognition of individuals that have escaped from aquaculture facilities, as plasticity allows escapees to revert to a wild-type phenotype, as recently observed in European seabass (Toledo-Guedes et al., 2021).

4.3. A combined approach to fish origin assessment

The use of multiple integrated biomarkers to distinguish and assign individuals to a population has been successfully used in fisheries conservation and management (Nikolić et al., 2020; TinHan et al., 2020). Indeed, each marker has discriminatory power that may vary depending on species, farming intensity, or the methods used, i.e., the assignment accuracy of genetic markers may be affected by high gene flow in fish or by farmed introgression while morphometric traits are affected by fish plasticity and feralisation in the case of older escapees. In the present study, integration of both genetic and morphometric data using the R package assignPOP (Chen et al., 2018) improved assignment results of the escaped group with unknown origin. The baseline source populations used for training were independent of the test population, and a high assignment accuracy (95%) was achieved with a proportion of 0.25 and 0.5 of the training loci. Assignment of individuals to a source population with a threshold above 80% can be considered reliable since genetic introgression of farmed fish and the presence of hybrids in the wild are not yet expected due to the relatively recent onset of the marine net-pen production of the species. Still, further fish sampling and genetic studies at a larger spatial scale are recommended to allow sufficient discriminatory power of the genetic dataset to predict the source population(s) of individuals prior to an increase in greater amberjack production and likely widespread genetic introgression of escaped fish in the wild.

4.4. Ecological and socioeconomic implications of escape events

In the current study, a relatively small massive escape event affected to a maximum of two marine net-pens (pers. com), holding 100,000 to 150,000 *S. dumerili* per net-pen. Although the potential negative effects of escaped fish on the Mediterranean environment have been hypothesised and locally described (for a review see Arechavala-Lopez et al., 2018), the effects of escapees at a larger ecosystem scale remain unexplored. Apart from the genetic impacts of escaped fish on wild populations, there are other important socioecological or economic consequences of escaped fish that should be addressed. There may be a potential increase of competition for food and habitat resources between escaped and wild fish since escapees prey on natural resources (Arechavala-Lopez et al., 2012; Toledo-Guedes et al., 2014; Valero-Rodriguez et al., 2015), become feral and attain the old age classes within the wild population (Toledo-Guedes et al., 2009; Izquierdo-Gómez et al., 2017). This is especially relevant for a predatory species with a high trophic index such as *S. dumerilli* (i.e., 4.5; Froese and Pauly, 2022) that preys mainly on cephalopods and clupeid fish, and to a lesser extent on European hake (*Merluccius merluccius*) and crustaceans, all of which have high commercial value for fisheries (Badalamenti et al., 1995; Matallanas et al., 1995; Andaloro and Pipitone, 1997). Hence, escaped individuals of *S. dumerili* might influence ecosystem dynamics, especially when hundreds of thousands of fish escape after massive escape events (Toledo-Guedes et al., 2014; Izquierdo-Gómez et al., 2016). The spatial dispersion of fish from the escape location varies among traditionally farmed species in the Mediterranean Sea (i.e., *S. aurata*, *D. labrax* and *A. regius*) where fish can remain near the farm for weeks allowing the time frame for recapture (Izquierdo-Gomez et al., 2016; Izquierdo-Gomez and Sanchez-Jerez, 2016). However, *S. dumerili* is a highly migratory and circumglobal species and thus it might disperse far from fish farms encompassing larger areas of the coastal ecosystem than European seabass, Gilthead seabream or meagre. Although further tagging experiments are needed to assess the spatial distribution of *S. dumerili* escaping from marine net-pens, fishermen claimed escapees were recaptured tens of kilometres from the escape location within the first week after Storm Gloria. Given the relatively low domestication level of *S. dumerili* (Teletchea and Fontaine, 2014), escaped individuals are expected to adapt more quickly and successfully to wild environments, since their behavioural instincts are more intact than in traditionally net-caged species that have been domesticated and selected for several decades. Such scenarios further highlight the negative impacts that *S. dumerili* escaping from marine net-pens may have on the environment.

In a globalised world, seafood is one of the most internationally traded commodities; however, complex supply chains make it highly vulnerable to fraud, as mislabelling of products is ubiquitous (Kroetz et al., 2020). In the case of the escaped *S. dumerili* analysed in this study, escapees were sold to retailers as wild fish, which could have potential implications. Although it seems not to be the case for *Seriola dumerili* in Spain (*pers.obs*: both wild and escaped individuals were sold at 15–20€/kg), selling escaped fish as wild fish might result in economic fraud/losses for retailers/consumers, since wild fish is generally more expensive than farmed fish. Mislabelling might also lead to potential health risks for consumers due to the broader array of potential contaminants, including antibiotics. The nutrient profile also differs between farmed and wild fish, particularly in terms of lipid content and fatty acid composition (Grigorakis, 2017), which might result in a in

lower quality of the final product. Furthermore, there is no information on how fishers perceive the capture of escapees and, though it seems that an increase of landed fish should generate a higher income for them, there are no studies exploring this aspect. The evaluation of these implications might be more complex, as they depend on the recapture dynamics of escapees, which vary depending on the escaped biomass (Izquierdo-Gomez et al., 2016).

4.5. Future guidelines

The development of adaptive contingency plans to prevent, mitigate and control the potential negative effects of escape events in the wild are strongly recommended, since to the knowledge of the authors, no Mediterranean countries have as yet enforced any such plans in their national aquaculture regulations. The use of genetic monitoring and local breeders in hatcheries would help mitigate potential genetic hybridisation with wild conspecifics, which in turn leads to a reduction in effective population size and genetic diversity due to the Ryman-Laikre effect (Waples et al., 2016), including fitness consequences for wild populations. Therefore, continuous monitoring that allows detection of genetic introgression and ecological/life-history changes in wild populations following introgression is recommended.

In the present study, the conjunction of only seven microsatellite loci and body shape data proved to be a successful tool for estimates of within-population genetic diversity, phenotypic variation, and finally fish origin discrimination and as such can become an integral part of the management system for escaped *S.dumerili* in Mediterranean countries. The proposed markers, together with the development of cost-effective and rapid scale-based discrimination techniques, could ensure the control of fish origin throughout the entire commercial chain, especially at landing ports to avoid fraud. Contingency plans to mitigate the negative effects of escapes should be supported by effective recapture measures. Therefore, a combination of acoustic telemetry and tagging and recapture studies is strongly recommended to unveil the dispersal behaviour of escaped *S.dumerili*. Trophic studies would help elucidate the feralisation process of escaped *S. dumerili*, as this is key to understanding the potential impact of escapees on the trophic chain, wild populations, and/or fisheries resources. To reduce the frequency and magnitude of escapes from aquaculture farms, there is a need to develop better quality standards for fish farms in the Mediterranean (e.g., Norwegian standard: NS 9415; Jensen et al., 2010) and meteorological models that can predict the risk of escapes due to storms, strong winds, water currents, and/or waves.

From a broodstock management perspective, it is recommended to establish baseline information on genetic and demographic traits of natural populations and aquaculture stocks. To minimise the effects of interbreeding (1–2% per generation) that may later affect wild fish through hybridisation, the breeding population should be large enough (>30–50 individuals per generation; Waples et al., 2012). However, to counteract unequal sex ratios and biassed reproductive success, which in the case of *S. dumerilli* is additionally triggered by reproductive dysfunctions in captivity, the number of breeders should be much higher to keep inbreeding levels below 1–2%, with most Mediterranean hatcheries using broodstock of 100–200 individuals for their breeding programmes (Cossu et al., 2019).

In summary, sustainable development of industrial aquaculture in marine net-pens can only be achieved if it is grounded on science-based knowledge and orchestrated through an adaptive participatory approach involving all actors present in the coastal zone, including farmers, commercial fishers, authorities and other stakeholders.

CRediT authorship contribution statement

Tanja Šegvić-Bubić: Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Igor Talijančić:** Writing – original draft, Methodology, Formal analysis, Data curation. **Iva Žužul:** Visualization, Formal analysis, Data curation. **Luka Žuvić:** Formal analysis, Data curation. **Leon Grubišić:** Funding acquisition, Data curation. **David Izquierdo-Gomez:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2022.108115.

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