Lack of genetic structure in euryhaline *Chelon labrosus* from the estuaries under anthropic pressure in the Southern Bay of Biscay to the coastal waters of the Mediterranean Sea

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ABSTRACT

Over the last decade, xenoestrogenic effects have been reported in populations of thicklip grey mullet *Chelon labrosus* from contaminated estuaries in the Bay of Biscay, resulting in intersex condition. To understand the level of gene flow in individuals of different Basque estuaries microsatellite markers were used to evaluate the population structure and connectivity of *C. labrosus* from estuaries of the Basque coast. 46 microsatellites were tested and 10 validated for the analysis of 204 individuals collected from 5 selected Basque estuaries and 2 outgroups in the Bay of Cadiz and Thermaic Gulf. The polymorphic microsatellites revealed 74 total alleles, 2–19 alleles per locus. The mean observed heterozygosity (0.49 ± 0.02) was lower than the expected one (0.53 ± 0.01). There was no evidence of genetic differentiation ($F_{ST} = 0.0098$, $P = 0.0000$) among individuals or sites. Bayesian clustering analysis revealed a single population in all sampled locations. The results of this study indicate widespread genetic homogeneity and panmixia of *C. labrosus* across the current sampling areas spanning the Atlantic and Mediterranean basins. The hypothesis of panmixia could therefore be well supported so individuals inhabiting estuaries with high prevalence of intersex condition should be considered as members of the same single genetic group as those inhabiting adjacent estuaries without incidence of xenoestrogenicity.

1. Introduction

The thicklip grey mullet *Chelon labrosus* (Risso, 1827) is a member of the Mugilid family which consists of approximately 25 genera and 80 species (Crosetti and Blaber, 2016; Xia et al., 2016) widely distributed along the Northeast Atlantic Ocean and the Mediterranean and Black Seas (Crosetti and Blaber, 2016; Froese and Pauly, 2022). It is a euryhaline fish that exhibits a high degree of residency within coastal systems, using estuaries as nursery and foraging habitats, migrating up and down estuaries with the moving tide (Crosetti and Blaber, 2016; Froese and Pauly, 2022; Whitfield, 2020). Adults migrate and spawn offshore in marine waters during winter (December–February), with the pelagic planktonic eggs hatching within 3–4 days and the planktonic larval stage lasting approximately 4 weeks (Crosetti and Blaber, 2016).

Post-larvae juveniles of these marine reproducing mugilids form dense schools that migrate to inshore coastal waters and shallow estuaries in the first and second month of life where they remain until they reach the adult stage (Crosetti and Blaber, 2016; Crosetti and Cataudella, 1995; Mićković et al., 2010). *C. labrosus* individuals can survive in highly polluted aquatic environments where their benthic feeding habits make them particularly susceptible to bioaccumulation of contaminants. Therefore, the thicklip grey mullet is considered a good choice for use as an estuarine pollution sentinel species (Crosetti and Blaber, 2016; Ortiz-Zarragoitia et al., 2014).

In *C. labrosus*, xenoestrogenic effects have been reported in individuals from contaminated estuaries along the Basque coast (Bizarro et al., 2014; Diaz de Cerio et al., 2012; Ortiz-Zarragoitia et al., 2014; Puy-Azurmendi et al., 2013; Valencia et al., 2017). This has been mainly

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linked to exposure to alkylphenols, pesticides and other xenoestrogens in wastewater treatment plant (WWTP) effluents that are discharged into estuaries (Bizarro et al., 2014; Ortiz-Zarragoitia et al., 2014; Puy-Azurmendi et al., 2013). Intersex testes, characterised by the appearance of oocytes in testicular tissue (ovotestes) (Jobling et al., 2002; Tyler and Jobling, 2008; Bahamonde et al., 2013), have been identified in up to 83% of the males in estuaries like the one of Gernika, while males in other nearby estuaries have always shown normally developing testes (Ortiz-Zarragoitia et al., 2014). Thicklip grey mullets and mugilids in general are considered gonochoristic with separate sexes maintained throughout their 25-years lifespan (Devlin and Nagahama, 2002). Males and females reach sexual maturity between 2 and 3 years respectively (Sostoa, 1983), so early sex differentiation occurs in continental waters, and in polluted estuaries in close contact to xenoestrogens when they are bioavailable. Early exposure of *D. rerio* embryos to 17β-estradiol and 17α-methyltestosterone results in the generation of both female and male single-sex groups, proving that full feminisation can also occur during early sex differentiation (Rojo-Bartolomé et al., 2020). While feminised genetic males could develop testes after withdrawal from oestrogenic compounds (Baumann et al., 2014; Nash et al., 2004), exposure during gonad differentiation in the early life stages would generate intersex condition and could make the process irreversible (Rojo-Bartolomé et al., 2020).

The environmental factor most intimately related to fish sex differentiation temperature and normally out of unusual high temperatures can result in masculinization (Devlin and Nagahama, 2002). In most fish species tested, the offspring of sex reversed neomales show higher sensitivity to masculinizing temperatures (Piferrer and Anastasiadi, 2021). This suggest some kind of epigenetic mechanism such as DNA methylation of sex control genes that would render the offspring of these neomales more susceptible to sex reversal (Piferrer and Anastasiadi, 2021). With time DNA hypermethylation of some regions of the genome could lead to accumulation of mutations due to the propensity of methylated cytosines to spontaneously mutate into thymines. Whether this occurs in intersex individuals is not known, but it has multiple implications depending on whether the thicklip grey mullets maintain fidelity to their estuary of origin and their larvae too.

Based on the early life-history characteristics of *C. labrosus*, there appears to be a high potential for reproduction occurring between adults of different estuaries of provenance in the sea and for larval geographical dispersal (Pereira et al., 2023). The yearly oceanic reproductive migration in the species can generate different patterns of genetic structuring; from a total panmixia (genetic homogenization) to a moderate structuring due to isolation, either reproductive or geographical. While the most extreme catadromous species tend to show panmixia throughout their distribution area (Avise et al., 1986; Dannewitz et al., 2005), the possibility of genetic structure among the different groups of *C. labrosus* from estuary-to-estuary in the Basque coast can not be ruled out. In any case, the catadromous thinlip grey mullet *Chelon ramada*, with residence in freshwaters in contrast to the more estuarine *C. labrosus* (Crosetti and Blaber, 2016), has been recently identified through a microsatellite study of 457 individuals from 16 locations to form a panmictic population with high connectivity along its distribution from the Northeast Atlantic coast to the Mediterranean Sea (Pereira et al., 2023).

Mugilids can be candidate species for diversification of aquaculture in Europe and recently different protocols have been published for the induction of spawning, reproduction and culture of different representative species (García-Márquez et al., 2021; Ramos-Júdez et al., 2022; Vallainc et al., 2022). *C. labrosus* is in fact, a nominee fish species for diversification in Spain due to two of its most notorious biological traits, its omnivore and euryhaline nature (García-Márquez et al., 2021). However, significant knowledge gaps on the biology and ecology of the species remain. In this sense, nothing is known of the genetic structure of the population(s) in European waters which should be taken into account before allowing future intensive aquaculture initiatives across regional seas and basins.

Understanding the population structure of these migratory fish is also important in the face of site-specific anthropogenic pollution and habitat loss (Collins et al., 2013; Halpern et al., 2007; Hoegh-Guldberg and Bruno, 2010). In the context of our work, we would like to know the population structure of our pollution sentinel species to understand their history of exposure to xenoestrogens leading to the effects observed in some local estuaries in male individuals that develop intersex testes. The most adequate methodological approach to understand this would require physical tagging (implanted internal or external tags) of adults in winter to follow their reproductive migration and analyze whether they return to the same estuary of origin and whether at sea they encounter individuals from other estuaries (Pepping et al., 2020). The cliff dominated orography of the Basque coast does not recommend the use of internally implanted tags (recapture) or pop-up archival tags (washed into the shore) that would never be recovered, while pop-up satellite archival tags are normally suitable for larger species and may impact fish performance (Musyl et al., 2011). The utilization of PIT-tags (passive integrated transponders) or radio-telemetry would help us to spot episodes of exit and entry into estuaries where we could place permanent antenna installations (Cooke et al., 2022), but without informing us where the fish went for reproduction and who they reproduced with. At this stage, it seems relevant at least to understand whether we are facing a single genetic population or multiple populations each associated to one estuary without genetic flow among them.

To date, most genetic studies conducted on Mugilids that inhabit the North-East (NE) Atlantic coast of Europe have primarily used mitochondrial markers or microsatellites to provide information about the ancient history of populations and their evolutionary processes (Durand et al., 2013, 2012b; Xia et al., 2016; Pereira et al., 2023). To the best of our knowledge, this study is the first to investigate the population genetics of *C. labrosus*, employing microsatellite marker analysis to detect patterns of genetic structure. Microsatellite nuclear markers provide information on diversity and contemporary population processes due to their abundance in the genome and high degree of polymorphism (Durand et al., 2013; Pacheco-Almanzar et al., 2017; Pereira et al., 2023). This study aims to evaluate the population genetic structure of *C. labrosus* in the Bay of Biscay to understand the level of gene flow in the individuals in our estuaries and test the hypothesis of panmixia.

2. Materials and methods

2.1. Sampling details

In all, 204 *C. labrosus* individuals that had been collected in five estuaries along the Basque coast in the Southeast Bay of Biscay (December 2010–October 2015) and stored in the cryobank of the Biscay Bay Environmental Biospecimen Bank (BBEBB) were analysed. Additionally, individuals were sampled in two outgroup locations, one in the Bay of Cadiz (February 2019) and the other in the Mediterranean

Table 1

Provenance of the thicklip grey mullets (*C. labrosus*) analysed in this study. The names and abbreviations of the sampling locations are indicated (five in the Southeast Bay of Biscay, one in the South of Spain in the Bay of Cadiz, and one in the Mediterranean Sea, Greece) together with the sample size (*N*) and geographical coordinates (latitude and longitude) in decimal degrees.

Country	Location (ID)	N	Latitude	Longitude
Spain	Pasaia (PA)	28	43.321940	−1.931670
Spain	Ondarroa (ON)	30	43.320000	−2.431110
Spain	Gernika (GE)	30	43.323890	−2.673610
Spain	Plentzia (PL)	30	43.407220	−2.946110
Spain	Bilbao (BI)	29	43.336110	−3.016390
Spain	Cadiz (CA)	30	36.538063	−6.301807
Greece	Thermaic Gulf (TG)	27	40.634975	22.927675

Sea within Thermaic Gulf at Thessaloniki, Greece (February 2020) (Table 1; Fig. 1). The fish were obtained by angling with a rod (Basque coast) and from commercial captures (Cadiz and Thermaic Gulf). Brain, gonad and/or liver tissues of *C. labrosus* from the Bay of Biscay were collected in RNA later solution (Ambion™, ThermoFisher Scientific, Waltham, Massachusetts, USA) and frozen in liquid nitrogen until arrival at the laboratory where they were immediately stored in −80 °C. In mullets from Cadiz and Thessaloniki muscle and/or gills were dissected from fresh fish, preserved in 98% ethanol immediately after sampling and stored at −80 °C on arrival at the laboratory. Ethical approval from the Ethics Committee for Animal Experimentation (ECAE) of the University of the Basque Country (UPV/EHU) was not necessary, with all fishing activities and handling procedures done in accordance with the ECAE – UPV/EHU and regional authorities. According to the Access and Benefit Sharing Legislation in place in Spain, the present analysis to study population structure means no utilization of Spanish genetic resources.

3. DNA isolation and amplification of microsatellites

Total genomic DNA was extracted from 25 mg of tissue (brain, gonad, liver, muscle or gill) using DNeasy® Blood & Tissue Kit following the manufacturer's instructions (Cat. No.: 69504, QIAGEN, Hilden, Germany). The quality of the extracted DNA was assessed using gel electrophoresis (1% agarose gels), with clear high molecular weight bands on gels used as a selection criterion and subsequently stored at −20 °C until use.

A set of 46 microsatellite loci previously described for the *Mugil cephalus* complex (Miggiano et al., 2005; Shen et al., 2010; Xu et al., 2009, 2010) were screened on a representative sample of 30 individuals randomly selected from different locations under study to test the PCR amplification effectiveness on *C. labrosus* (Supplementary material, Table S1). All PCR analyses contained 10–100 ng DNA, 10X PCR reaction buffer, 20 μM of each primer, 100 mM MgCl$_2$, 10 mM dNTPs, 10 μg bovine serum albumin (BSA, REF: 10711454001, Roche Diagnostics GmbH, Mannheim, Germany) and 1.25 U BIOTAQ™ DNA polymerase (BIO-21040, Bioline GmbH, Luckenwalde, Germany). PCR profiles for amplification of microsatellites were conducted as follows: initial denaturation at 95 °C for 15 min, followed by 35 cycles at 94 °C for 30 s, annealing step (temperature for each primer set in Table 2) for 90 s and 72 °C for 60 s, and a final extension at 60 °C for 30 min (Bio-Rad C1000 Touch Thermal Cycler, Bio-Rad Laboratories Inc., Hercules, California, USA). The quality of the PCR products and the allele size range were evaluated using GelRed® stained 2% agarose gels (Biotium GelRed® 10, 000X in water, Catalogue No. 41003; Biotium Inc., Freemont, California, USA). A selection of useable loci was done, based on the presence of

polymorphism and good multiplexing PCRs capabilities (Table 2; Supplementary Table S1 bold and underlined). Forward primers for each selected locus were labelled with FAM, PET, VIC or NED fluorescent dyes. A total of 147 individuals from 5 locations along the Basque coast, 30 individuals from Cadiz, and 27 individuals from Thessaloniki were genotyped (Table 1). The following cycling conditions for amplification of microsatellites using multiplex PCR according to QIAGEN® Multiplex PCR Kit (Cat. No: 206143, QIAGEN, Hilden, Germany) were used: initial denaturation at 95 °C for 15 min, followed by 35 cycles at 94 °C for 30 s, annealing step of 57 °C for 90 s and extension of 72 °C for 60 s, and a final extension at 60 °C for 30 min (Bio-Rad C1000 Touch Thermal Cycler, Bio-Rad Laboratories Inc., Hercules, California, USA). PCR products were checked electrophoretically on GelRed® stained 2% agarose gels (Biotium Gel Red®; Biotium Inc., USA) before being sent for capillary electrophoresing (Macrogen SPAIN; Macrogen Inc., Seoul, South Korea). The microsatellite marker profiles were scored using Geneious Prime v.2019 software (Biomatters Ltd., Auckland, New Zealand), with LIZ-500 Size Standard (Applied Biosystems, Foster City, California, USA) to calibrate product size. Alleles were recorded as three-digit genotypes.

4. Data analyses

1. Loci assessment and genetic diversity

Calculations of allele frequency for each marker, the number of alleles (N_A), estimated heterozygosity (H_E), and observed heterozygosity (H_O) for each locus and locality were estimated using the GenAlEx v. 6.5 computer program (Peakall and Smouse, 2006, 2012). Deviations from the Hardy-Weinberg equilibrium (HWE) at each locus using Fisher's exact test, under Markov Chain Monte Carlo (MCMC) algorithms (Guo and Thompson, 1992), with 10,000 dememorizations, 10,000 batches (treatments per location), and 100,000 iterations per batch were calculated by GENEPOP 4.7.5 software (M. Raymond and Rousset, 1995). The same package was used to assess linkage disequilibrium (LD) between pairs of loci using Fisher's exact test according to (Pacheco-Almanzar et al., 2017) (10,000 dememorizations, 100 batches, 1,000 iterations per batch), with probability values for both tests (HWE & LD) corrected with Bonferroni multiple comparison test at p ≤ 0.05 (Rice, 1989). GENEPOP 4.7.5 was further used to calculate Wright's *F* statistics (Wright, 1965, 1978) using allele frequency-based correlations (F_{ST} & F_{IS}) at each locus according to (Weir and Cockerham, 1984). The program Micro-Checker v 2.2.3 (Van Oosterhout et al., 2004) was used to examine large allele dropout, stuttering, and null alleles as potential sources of error.
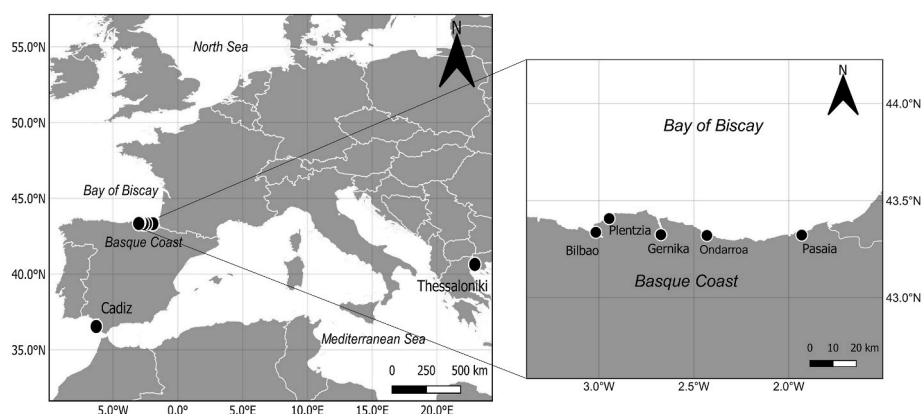

Fig. 1. Sampling locations of *C. labrosus* (black dots) across five locations along the Basque coast (inset, Southeast Bay of Biscay), one location in the Bay of Cadiz (South of the Iberian Peninsula, Spain) and one location in the Mediterranean Sea, Greece. This figure was produced with the GADM database (www.gadm.org), version 2.5, July 2015 and QGIS version 3.18.0-Zürich.

Table 2

Characteristics of 10 amplified microsatellite loci used to study the genetic diversity and population structure of the thicklip grey mullet (*C. labrosus*). Locus name (Locus), forward (F) and reverse(R) primer sequences, information of repeated Motif, used fluorescence label, how primers were multiplexed (the same number belongs to the same multiplexing group), multiplexing annealing PCR temperature T_A (°C), resulting allelic size length (bp) and the GenBank accession number of each selected locus. Loci Muce and its respective primers were reported by Xu et al. (2010), loci Muso and its respective primers were reported by Xu et al. (2009) and loci Mce and its respective primers were reported by Shen et al. (2010) (All of them were applied by the authors for *Mugil cephalus* and *Mugil so-iuy* and here were validated for *C. labrosus*). Five loci (in bold) were monomorphic in all studied individuals and were not used in further downstream analyses.

Locus	Primer Sequence (5′- 3′)	Repeat Motif	Fluorescent label	Multiplex Panel	T_A (°C)	Allelic sizes (bp)	GenBank Accession No.
Muce44	F: GCTTCGGAGGACCAAC R: CGACAGCCACTGTTATG	$(CA)_5(GA)_4$	FAM	I	57	285–311	HM060975
Mce6	F: GAGGAGGCTCGGAGGATT R: CGGGGCTTGTGACAGTTT	$(CA)_{15}$	PET	II	57	187–229	HM004328
Mce11	F: ATTAGCCAGGGCCACCAG R: CAGAAGCCAAAAGGACGG	$(TG)_{14}$	VIC	II	57	151–163	HM004332
Mce25	F: TCGGCATGTATATGAAAGCAC R: ACATAACTCTGCCACTGCTTG	$(TG)_{14}$	NED	II	57	123–125	HM004346
Muce38	F: GCACCAACATCTCACCTG R: CCTACCATTTACCCCTCT	$(GA)_5(GGAGA)_4$	FAM	III	57	231–251	HM060974
Mce10	F: CCACTGTAGGGCTGTATGC R: GGGAGGAGGATTTCTCAA	$(TG)_{12}$	VIC	III	57	124–140	HM004331
Muce26	F: TGCGGAGACAATGTAAAC R: AAATGAAACAATCCACCC	$(ACAA)_3$	FAM	IV	57	266–268	HM060972
Mce2	F: AGCCAAAGTTCCTGTAAGTACG R: TCAGATTAGGACCCGACCATA	$(GT)_{30}$	VIC	IV	57	134–168	HM004324
Muso32	F: GCAGTGCACATGGTAACAAA R: CGCCTACAGCATCAGACAAG	$(AC)_9$	PET	V	57	193–197	EU570307
Muso19	F: CACCACTATGGCATCCTTCA R: AACCCCTTTTCTTGCTCAAA	$(AC)_9$	VIC	V	57	142–162	EU570294
Muso25	**F: ATGAAAAGGGAGGGGCAATA** **R: CTGCTCACCTTGCGTTTACA**	**$(CA)_{11}$**	**PET**	**I**	**57**	**204**	**EU570300**
Muso36	**F: TCCTTTATGGGGAGACGATG** **R: CCCAATAGCCACAAATGTCC**	**$(GT)_3GA(GT)_6$**	**VIC**	**I**	**57**	**192**	**EU570311**
Mce27	**F: ACTGTGCACTTCTGGTTTCC** **R: ACATCTTTGAGGTTGCCC**	**$(CA)_{12}$**	**NED**	**I**	**57**	**126**	**HM004348**
Muso22	**F: TGATGAGAATGGTGGTGACG** **R: TTTTGGGCTGCTTGTCTCTC**	**$(GT)_{17}$**	**PET**	**III**	**57**	**189**	**EU570297**
Mce8	**F: AGGGATTGGGTTTAGGCG** **R: GTGCTCGACACTTTAGACTGAT**	**$(CA)_{20}$**	**PET**	**IV**	**57**	**175**	**HM004330**

2. Genetic structure analysis

F_{ST} values between pairs of locations calculated from Wright's *F* statistics (Weir and Cockerham, 1984) were used to calculate the genetic differentiation of the entire population with values of (Cavalli-Sforza and Edwards, 1967). We estimated null allele frequencies (Table 3) and F_{ST} (Table 4) using the FreeNA software (Chapuis and Estoup, 2007) with the number of replicates fixed at 25,000. We ran this analysis using the ENA correction method to efficiently correct for the positive bias induced by the presence of null alleles on F_{ST} estimation and, so, to provide an accurate estimation of F_{ST}. FreeNA provided F_{ST} values and confidence intervals with and without correction (excluding null alleles (ENA) correction), relative to the null alleles (Chapuis and Estoup, 2007) and a Chi-square test was performed to compare the values. If significant differences were observed in F_{ST} value comparisons, loci with null alleles present were to be discarded. The program ARLEQUIN version 3.5.2.2 was then used to perform a hierarchical analysis of molecular variance (AMOVA) to assess the presence of a differential genetic structure (Excoffier et al., 2005) across the total sample range and among putative regional groupings of samples. Genetic clusters in HW were determined using the program STRUCTURE v2.3.4 (Pritchard et al., 2000, 2009) using the admixture model. As the presence of null alleles introduces potential ambiguity around the true underlying genotype, we ran the program under two conditions; RECESSIVEALLELES set to 0 in which no ambiguity is assumed; and RECESSIVEALLELES set to 1 where missing data are assigned as recessive to better account for null alleles (Falush et al., 2007). To estimate the number of *K* populations, 20 independent runs were done for each *K* from $K = 1$ to $K = 10$, using a burn-in of 100,000 and 1,000,000 iterations of MCMC. The ΔK statistics (Evanno et al., 2005) were used to detect the uppermost hierarchical level of the population structure, based on the rate of change between successive K

values. The best estimate of K (ΔK) was calculated using the web-based STRUCTURE HARVESTER program (Earl and vonHoldt, 2012).

3 Estimation of genetic divergence

The GenAlEx 6.5 software (Peakall and Smouse, 2006, 2012) was used to perform a Principal Coordinate Analysis (PCoA) procedure to reveal patterns of genetic relationship, if any, among the 7 populations of *C. labrosus*. To test for Isolation-by-Distance (IBD), the same software was used to perform a Mantel test. The Mantel test investigates the relationship between the pairwise population F_{ST} values and geographic distance. The correlation between the geographic coordinates (longitude, latitude) and the genetic diversity parameters was studied.

5. Results

1. Microsatellite loci and genetic diversity

Of the 46 microsatellites selected from the literature previously used for the analysis of the dynamics of populations of *Mugil cephalus* (Shen et al., 2010; Xu et al., 2009, 2010), 18 loci amplified well in *C. labrosus* (Supplementary material, Table S1). 31 loci were excluded from further analysis either because of lack of allelic variations, poor amplification of alleles or poor multiplexing possibilities (Supplementary material, Table S1). Out of the remaining 15 loci, 5 were monomorphic and were not selected for the analyses (Table 2; Supplementary Table S1 bold and underlined).

Summary statistics for the 10 microsatellite loci in the seven populations from the South-East Bay of Biscay to the Mediterranean are shown in Table 3. A total of 74 alleles ranging from 123 to 311 bp in length were observed. The number of alleles per locus varied

Table 3
Population genetic parameters for 204 individuals of *C. labrosus* sampled across five locations along the Basque coast, one location in the Bay of Cadiz and one location in the Mediterranean Sea, as analysed by 10 microsatellite loci. N - Sample size; N_A - Allele number per locus; HWE - Hardy-Weinberg equilibrium after Bonferroni correction; H_O - Observed heterozygosity; H_E - Expected heterozygosity; F - fixation index (Weir and Cockerham, 1984). Hyphen - no values, Mean represents mean values across 10 polymorphic loci. Significant adjusted nominal level was 0.001.

Location		Microsatellite Loci										Mean
		Muce44	Mce11	Mce25	Mce6	Muce38	Mce10	Muce26	Mce2	Muso19	Muso32	
Pasaia	N	26	28	28	28	24	28	26	27	28	26	26.9
	N_A	5	5	2	12	3	6	2	9	1	2	4.7
	HWE	0.00	0.16	0.02	0.00	0.11	0.66	0.06	0.38	–	0.64	0.23
	H_O	0.42	0.54	0.00	0.68	0.25	0.79	0.04	0.74	–	0.50	0.44
	H_E	0.51	0.61	0.07	0.85	0.34	0.75	0.11	0.76	–	0.49	0.50
	F	0.19	0.14	1.00	0.21	0.29	−0.03	0.66	0.04	–	0.01	0.28
Ondarroa	N	30	30	30	30	29	29	30	30	30	28	29.6
	N_A	5	5	1	12	4	6	2	8	1	2	4.6
	HWE	0.84	0.75	–	0.19	0.40	0.56	0.00	0.66	–	0.88	0.54
	H_O	0.63	0.67	–	0.73	0.34	0.76	0.03	0.73	–	0.57	0.56
	H_E	0.56	0.63	–	0.83	0.35	0.71	0.21	0.70	–	0.49	0.56
	F	−0.12	−0.05	–	0.14	0.04	−0.05	0.84	−0.04	–	−0.15	0.08
Gernika	N	30	30	30	30	29	30	28	27	30	30	29.4
	N_A	7	5	1	13	3	6	2	7	1	2	4.7
	HWE	0.94	0.46	–	0.60	0.00	0.41	0.18	0.12	–	0.17	0.36
	H_O	0.60	0.67	–	0.80	0.21	0.73	0.11	0.63	–	0.37	0.51
	H_E	0.53	0.66	–	0.76	0.41	0.70	0.16	0.69	–	0.47	0.55
	F	−0.13	0.00	–	−0.04	0.51	−0.04	0.36	0.10	–	0.24	0.13
Plentzia	N	30	30	30	30	28	30	29	30	30	30	29.7
	N_A	7	4	1	13	3	8	2	8	1	2	4.9
	HWE	0.40	0.81	–	0.01	0.01	0.00	0.00	0.48	–	0.38	0.26
	H_O	0.50	0.60	–	0.73	0.07	0.63	0.03	0.73	–	0.43	0.47
	H_E	0.56	0.54	–	0.86	0.19	0.75	0.21	0.77	–	0.49	0.55
	F	0.12	−0.10	–	0.17	0.64	0.18	0.84	0.07	–	0.13	0.26
Bilbao	N	29	29	29	29	25	29	29	29	29	29	28.6
	N_A	7	5	1	11	4	6	2	8	1	3	4.8
	HWE	0.58	0.43	–	0.86	0.00	0.94	0.05	0.05	–	0.42	0.42
	H_O	0.66	0.55	–	0.90	0.24	0.76	0.03	0.76	–	0.34	0.53
	H_E	0.64	0.58	–	0.83	0.48	0.68	0.10	0.75	–	0.37	0.55
	F	0.00	0.06	–	−0.06	0.51	−0.01	0.66	0.00	–	0.10	0.16
Cadiz	N	30	30	30	30	30	30	30	30	30	30	30
	N_A	7	6	2	15	4	5	2	12	3	2	5.8
	HWE	0.01	0.01	0.05	0.14	0.07	0.59	0.03	0.31	0.01	1.00	0.22
	H_O	0.57	0.53	0.03	0.87	0.23	0.70	0.10	0.83	0.03	0.70	0.46
	H_E	0.63	0.64	0.10	0.86	0.31	0.65	0.21	0.78	0.13	0.50	0.48
	F	0.12	0.18	0.66	0.01	0.27	−0.05	0.53	−0.04	0.74	−0.40	0.20
Thermaic Gulf	N	27	27	27	27	13	27	27	27	27	27	25.6
	N_A	7	5	1	12	5	6	2	9	3	3	5.3
	HWE	0.01	0.17	–	0.14	0.00	0.23	0.01	0.72	0.02	0.82	0.23
	H_O	0.41	0.44	–	0.67	0.23	0.74	0.04	0.78	0.04	0.59	0.44
	H_E	0.60	0.48	–	0.79	0.62	0.74	0.17	0.74	0.07	0.52	0.53
	F	0.34	0.09	–	0.18	0.65	−0.02	0.79	−0.04	0.50	−0.13	0.26

Table 4
Null allele frequency estimates using EM algorithm (Dempster et al., 1977) with FreeNA software (25,000 replicates).

	PA	ON	GE	PL	BI	CA	TG	Mean
Muce44	0.097	0.000	0.000	0.000	0.000	0.067	0.112	0.039
Mce11	0.043	0.000	0.000	0.000	0.000	0.076	0.029	0.021
Mce25	0.128	0.001	0.001	0.001	0.001	0.110	0.001	0.035
Mce6	0.094	0.019	0.000	0.071	0.000	0.015	0.037	0.034
Muce38	0.088	0.015	0.167	0.149	0.179	0.083	0.242	0.132
Mce10	0.000	0.000	0.000	0.085	0.000	0.000	0.016	0.015
Muce26	0.116	0.188	0.083	0.191	0.111	0.126	0.162	0.139
Mce2	0.000	0.000	0.043	0.000	0.025	0.000	0.000	0.010
Muso19	0.001	0.001	0.001	0.001	0.001	0.117	0.001	0.018
Muso32	0.000	0.000	0.074	0.036	0.025	0.000	0.000	0.019
Mean	**0.057**	**0.022**	**0.037**	**0.053**	**0.034**	**0.059**	**0.060**	**0.046**
Frequency estimates (%)	**6**	**2**	**4**	**5**	**3**	**6**	**6**	**5**

considerably from 2 in loci Mce25, Muce26, and Muso32 to 19 in locus Mce6, depending on the location. The highest level of polymorphism was observed in loci Muce44, Mce11, Mce6, Muce38, Mce10 and Mce 2 (Table 3). The number of alleles per population varied slightly, with 46 (in mullets from Ondarroa-ON) to 49 (Plentzia-PL) alleles for populations from the Basque coast and 53 to 58 alleles for mullets from

Cadiz (CA) and Thessaloniki (TG) respectively. There was no evidence of large allele dropout at any loci. Stuttering was identified as a potential issue at two loci (Mce6 and Mce25). Significant LD (Fisher's method; $P < 0.05$) was found for six different combinations of loci (Supplementary material, Tables S2 and S3, $P < 0.05$). Loci showing potential stuttering or LD were retained in the dataset as genuine stuttering and LD are

expected to affect all sites equally. After adjusting for multiple comparisons, none of the mullet populations deviated from HWE at any loci after Bonferroni correction (Table 3). The average H_O per population (mean ± standard error) ranged from 0.44 ± 0.09 (Pasaia-PA and TG) to 0.56 ± 0.08 (ON), while the average H_E by location ranged from 0.48 ± 0.08 (CA) to 0.56 ± 0.08 (ON). The fixation index (F) averaged across all loci ranged from 0.08 (ON) to 0.28 (PA) (Table 3). In most sampling locations, there were more homozygous genotypes than expected (F positive), but there was no significant evidence of an excess of homozygotes at any location. Significant and high F_{IS} values were observed for 3 loci, indicating a heterozygotic deficiency (loci Muce 38, Muce 26 and Muso 19) (Table 3). In terms of null allele frequencies, the overall frequency estimate was 5%, and ranged from 2% (ON), to 6% (PA, CA and TG) (Table 4).

2. Genetic structure

As indicated by F_{ST} values, very low but significant genetic differences were found at nuclear loci between Pasaia (PA) and Bilbao (BI), Gernika (GE) and BI + Thermaic Gulf (TG), and BI and Cadiz (CA) + TG populations (Table 5). However, estimates of the global F_{ST} values both with ($F_{ST} = 0.011250$) and without ($F_{ST} = 0.007908$) correction relative to null alleles showed little genetic differentiation for all loci. The overall AMOVA results showed that only 0.98% of the variation was distributed between the origin locales, while 99.02% was allocated within locations ($F_{ST} = 0.00976$, P = 0.0000) (Table 6). This was further supported by global pairwise F_{ST} comparisons of *C. labrosus* populations extracted from AMOVA, indicating that the mullets from each location are not genetically different from each other.

In STRUCTURE, there were no appreciable differences in optimum values of *K* or assignment of individuals to each cluster when RECESSIVEALLELES was set to 0 or 1, therefore we only report results as per RECESSIVEALLELES set to 0. No loci were dropped due to the presence of null alleles to minimise power loss, as when dealing with a low number of loci, it is generally preferable to account for null alleles rather than exclude loci (Wagner et al., 2006). Although the application of the Evanno test to the results of Bayesian clustering analysis of the entire data set conducted in STRUCTURE revealed a peak at $K = 2$ in the plot of estimated Delta *K* versus *K*, the Delta *K* value was very low (Supplementary Material, Fig. S1), an indication that the strength of the signal detected in STRUCTURE is weak (Evanno et al., 2005); hence the non-existence of different population clusters in *C. labrosus* samples. Assignment tests in STRUCTURE revealed that we have one main population ($K = 1$) across all the sampled locations (Fig. 2).

6. Genetic divergence and isolation-by-distance (IBD)

The PCoA indicated that there may be some form of genetic structure and the possibility of IBD, with 77.43 of variation being explained by the first 2 axes. The Mantel test of the correlation coefficient showed a low

Table 5

Pairwise population F_{ST} values (below diagonal) (Weir and Cockerham, 1984) and their corresponding p-values (above diagonal) for comparisons of *C. labrosus* populations among the seven sampling locations based on 10 polymorphic microsatellite loci. Values in **bold** indicate significant comparisons ($P < 0.05$).

Location	PA	ON	GE	PL	BI	CA	TG
Pasaia (PA)	–	0.703	0.072	0.982	**0.027**	0.279	0.081
Ondarroa (ON)	0.002	–	0.757	0.496	0.135	0.486	0.072
Gernika (GE)	0.009	0.005	–	0.063	**0.027**	0.252	**0.018**
Plentzia (PL)	0.010	0.001	0.011	–	0.063	0.189	0.126
Bilbao (BI)	**0.015**	0.006	**0.013**	0.010	–	**0.000**	**0.000**
Cadiz (CA)	0.004	0.000	0.004	0.006	**0.020**	–	0.324
Thermaic Gulf (TG)	0.010	0.011	**0.018**	0.010	**0.027**	0.004	–

Table 6

Results of the global analysis of molecular variance (AMOVA) as a weighted average of 10 polymorphic loci (AMOVA. $F_{ST} = 0.00976$. P = 0.0000. n = 204).

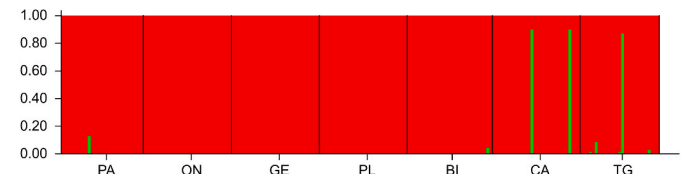| Source of variation	Sum of squares	Variance of components	Percentage variation
Among populations	21.359	0.023	0.976
Within populations	901.691	2.292	99.024
Total	923.050	2.315	

Fig. 2. STRUCTURE plots from microsatellite data for *C. labrosus* using an LOCPRIOR admixture model. Presence of a single group is represented by a single red colour, with the proportion of each bar assigned to a single colour representing the probability that an individual belongs to that group (Supplementary Material Fig. S2). See Table 1 for additional information on sample names.

and not significant correlation between geographic distance and pairwise F_{ST} values (R = −0.051, P = 0.590; $R^2 = 0.0026$). There was no pattern of IBD since there was no indication of the existence of a distinct geographical structure in the studied locations, nor was a longitudinal trend of genetic variation detected.

7. Discussion

The study evaluated the genetic diversity and population structure of genetic connectivity as a consequence of reproductive migration patterns in thicklip grey mullet *Chelon labrosus* from estuaries of the Basque coast in the Southern Bay of Biscay (SBB). 46 microsatellite markers were tested and 15 validated for the analysis of 204 individuals from 5 estuaries in the SBB and 2 outgroups in the Bay of Cadiz (CA) and Thermaic Gulf (TG). The results of this study indicate widespread genetic homogeneity and panmixia of *C. labrosus* across the current sampling areas spanning the Atlantic and Mediterranean basins. There was no evidence of genetic structure for *C. labrosus* across ~5000 km of coastline, despite the use of ten highly variable microsatellite loci. F_{ST} values indicate that the thicklip grey mullets from each location are not genetically different from each other. Furthermore, Bayesian clustering analysis of the microsatellite data supported the single population group findings, suggesting the absence of genetic differentiation between sampling locations. Since there was no evidence of genetic differentiation, these populations should be grouped as belonging to a single genetic group.

The microsatellite primers tested were developed for *Mugil cephalus* and *Mugil so-iuy*; however, of the 18 primers that amplified well, only 15 allowed for multiplexing in PCR, from which 10 were used for further analyses of genetic structure and diversity of *C. labrosus* (Supplementary material, Table S1). These markers amplified well due to the close phylogenetic relationship between these mugilid species (Durand et al., 2012a, 2012b; Xia et al., 2016). Although microsatellite diversity levels in *C. labrosus* differed depending on the loci compared, a lower number of alleles were observed in the sample analysed when compared to the populations of *M. cephalus* and *M. so-iuy* (Supplementary material, Table S2) (Shen et al., 2010; Xu et al., 2009, 2010), except for loci Mce6, Mce10 and Muce44. For example, the loci Mce8, Mce27, Muso22, Muso25 and Muso36 which are monomorphic in *C. labrosus* display 4–8 alleles and 5–12 alleles for loci Mce8 and Mce27 in samples of

M. cephalus from Taiwan, Peru, East Australia, and Spain (Shen et al., 2010), and four alleles each for loci Muso22, Muso25, and Muso36 in samples of *M. so-iuy* from China (Xu et al., 2009). At polymorphic loci Muce26 and Muce38, two and five alleles were obtained respectively in *C. labrosus* compared to 3 and 6 alleles in samples of both *M. so-iuy* and *M. cephalus* (Shen et al., 2010). To illustrate this difference between species, when comparing *Chelon ramada* and *Mugil liza*, three alleles were observed at the Mce27 locus of *C. ramada* from South Africa (Shen et al., 2010) but 10–11 alleles in samples of *M. liza* from Brazil and Argentina (Mai et al., 2014). Comparatively, more alleles were observed in *C. labrosus* samples on loci Mce6 (19 alleles), Mce10 (9 alleles) and Muce44 (9 alleles) (Table 2) compared to the 4–9 alleles at Mce6 and 4–6 alleles at Mce10 described in *M. cephalus*, and the 5 alleles at Muce44 described in both *M. cephalus* and *M so-iuy* (Shen et al., 2010). At the same time different allele numbers were found in *C. labrosus* vs *C. ramada* sampled in the same distribution range (Pereira et al., 2023) for Mce27 (1 allele vs 11 alleles), Mce10 (10 vs 17), Muso19 (3 vs 4) and Mce12 (13 vs 7) (Pereira et al., 2023). In samples of *M. curema* from the Gulf of Mexico, loci Mce6 and Mce10 only displayed 2–5 alleles each (Pacheco-Almanzar et al., 2017).

The average heterozygosity observed (0.49 ± 0.02) was lower than the one declared for 12 marine fish species (0.77 ± 0.19) reported by (DeWoody and Avise, 2000) in a review study which compared the genetic diversity in fishes occupying different habitats. Compared to other mugilid species, the average mean values of observed heterozygosity (H_O) in *C. labrosus* (0.437–0.559) (Table 2) were within the range observed for *M. curema* (0.372–0.530) (Pacheco-Almanzar et al., 2017), for *M. cephalus* (0.588–0.760 and 0.425–0.975) (Colín et al., 2020; Shen et al., 2010), for *C. ramada* (averaged 0.547–0.655 and 0.033–0.900) (Pereira et al., 2023 and Shen et al., 2010), for *Chelon auratus* (averaged 0.033–0.743) (Behrouz et al., 2018) and for *M. liza* (0.654–0.716) (Mai et al., 2014). The same trend was observed with the average values of expected heterozygosity (H_E). Furthermore, the observed heterozygosity values in *C. labrosus* were not only comparable to other mugilid species, but were within the range reported in anadromous salmonids and brackish water fish, and fell between the higher and lower genetically diverse marine and freshwater fish, respectively (DeWoody and Avise, 2000; Martinez et al., 2018). In all locations except for Ondarroa, the H_O in *C. labrosus* was lower than the expected H_E. Generally, with lower H_O than H_E, reduction in allelic variation and the existence of low-frequency alleles may be due to changes in the marine ecosystems associated with anthropogenic climate change, pollution, habitat loss, and over-exploitation (Collins et al., 2013; Halpern et al., 2007; Hoegh-Guldberg and Bruno, 2010). Such changes result in a reduction of the species' reproductive potential. However, this does not seem to be the case for *C. labrosus* in the SBB, a species that remains unexploited and thrives in polluted aquatic environments.

The presently validated molecular markers (ten heterozygous microsatellite loci) studied on 204 individuals did not find any significant genetic variation among individuals from the 7 different geographical origins studied. Five weak and marginally significant pairwise comparisons were recorded: BI – PA ($F_{ST} = 0.0147$, P = 0.0027), BI – GE ($F_{ST} = 0.0128$, P = 0.0027), CA – BI ($F_{ST} = 0.0120$, P = 0.0000), TG – GE ($F_{ST} = 0.0176$, P = 0.0181) and TG – BI ($F_{ST} = 0.0269$, P = 0.0000). These significant and small F_{ST} comparisons may be a product of temporal instability in gene frequencies (Dannewitz et al., 2005). In any case, the pairwise F_{ST} comparisons are very close to 0 and suggest that no genetic distinctions can be drawn between any two compared localities. More precisely, a lack of spatial genetic differentiation was observed in an AMOVA analysis ($F_{ST} = 0.0098$, P = 0.0000), where most of the variance was attributed from within the populations rather than from among populations (Table 6). The $K = 1$ gene pools depicted with the Bayesian clustering approach supported the single population group findings, reinforcing the lack of genetic differentiation between sampling locations (Fig. 2).

This absence of genetic structuring in *C. labrosus* has been reported in many marine species that share similar biology. In *M. cephalus*, a lack of genetic and phylogeographic structure was reported in populations from the Gulf of Mexico and Atlantic Coasts (Rocha-Olivares et al., 2000) and along ~550 km of the Queensland coastline in Australia (Huey et al., 2013), despite using mtDNA and six highly polymorphic microsatellite loci, respectively. Typical panmictic populations show similar results. This is the case of *Anguilla anguilla* from southern and northern Europe (6 microsatellite loci and mtDNA sequences) (Palm et al., 2009; Ragauskas et al., 2014), *Anguilla japonicus* from East Asia (8 microsatellite loci and SNPs) (Gong et al., 2019; Yu et al., 2020), *Acanthopagrus australis* from Australia (6 microsatellite loci) (Roberts and Ayre, 2010), *Balaenoptera physalus* from the NE Atlantic (10 microsatellite loci) (Quintela et al., 2014) and most significantly in the related mugilid, catadromous and geographically close species *C. ramada* (11 microsatellite loci) (Pereira et al., 2023). In the Iberian Peninsula and the Mediterranean, analysis of the genetic structure of 3 *Trachurus* species using restriction fragment length polymorphisms (RFLPs) on individuals from Atlantic and Mediterranean samples did not find any genetic variation among samples within *T. picturatus* and *T. mediterraneus* or European samples of *T. picturatus* (Comesaña et al., 2008; Karaiskou et al., 2004). These results were consistent with recent evaluations of *T. picturatus* population genetic structure in the same area when mtDNA and 10 polymorphic microsatellite loci were applied (Moreira et al., 2019, 2020). Furthermore, in two species belonging to the family Sparidae, mtDNA displayed little to no genetic differentiation in *Pagellus bogaraveo* and *Pagrus pagrus* when comparing Atlantic and Mediterranean individuals (Bargelloni et al., 2003). Such absence in genetic structuring in various marine fishes is commonly associated with two factors, *i.e.* a large effective population size that narrows the impact of genetic drift and life history traits that promote dispersal and high gene flow among populations either through passive dispersal of eggs and planktonic larvae or active migration of adults and or juveniles (Carvalho and Hauser, 1998; DeWoody and Avise, 2000; Martinez et al., 2018; Moreira et al., 2020; Pereira et al., 2023). *C. labrosus* exhibits those life history traits, synchronising spawning during winter (December–February) when spawning- or reproductive migrations involve the movement of maturing individuals from foraging areas in freshwater and estuarine environments into marine waters where they will spawn (Ortiz-Zarragoitia et al., 2014). The life-history and dispersal strategy along the Iberian coast suggests a metapopulation scenario, with a high season-related gene flow among the sampled locations preventing any stable genetic drift (Moreira et al., 2020; Pereira et al., 2023).

Several studies have shown that along the Basque coastline, individuals of *C. labrosus* inhabiting different contaminated estuaries exhibit varying levels of xenoestrogenicity (Bizarro et al., 2014; Ortiz-Zarragoitia et al., 2014; Puy-Azurmendi et al., 2013; Valencia et al., 2017). These individuals are geographically separated and are differentially exposed to xenoestrogens in their estuaries of residence. Prevalence of intersex (ovotestis) has been found all along the annual reproductive cycle, with percentages ranging from 3 to 83 of analysed males. Although no intersex has been recorded in individuals from Plentzia, the Pasaia and Gernika sites have been defined as xenoestrogenic pollution "hotspots" with an intersex prevalence of up to 56 and 83 respectively (Ortiz-Zarragoitia et al., 2014). Xenoestrogen concentrations measured in bile from individuals from Gernika have been as high as 7,942 ng g^{-1} nonylphenol and 95 ng g^{-1} octylphenol (Puy-Azurmendi et al., 2013), and on average, 177.5 ng mL^{-1} bisphenol-A (BPA) and 1,508 ng mL^{-1} β-hexachlorocyclohexane (β-HCH) (Ros et al., 2015). In addition, intersex males at varying levels have been reported in other estuaries such as those of Bilbao, Ondarroa and Deba (Bizarro et al., 2014; Diaz De Cerio et al., 2012; Puy-Azurmendi et al., 2013; Rojo-Bartolomé et al., 2017; Valencia et al., 2017) while intersex has not been reported in individuals for other locations including BOC and TGM. Although biomonitoring studies showed that different geographical population units were under different pressures the current microsatellite data does not support the existence of any discrete units.

F_{ST} and Bayesian clustering analysis show the population dynamics of *C. labrosus* in the SBB has been in synchrony, suggesting the contribution of gene flow to the lack of genetic partitioning of these populations. If free-swimming planktonic *Chelon* larvae are transported by oceanic currents in spring-summer from open waters to the coast randomly, if adults returning to estuaries after reproduction do not return to their estuary of origin and if reproduction occurs among adults of different estuaries, then a long-distance dispersal and gene flow over a wide geographic scale will be promoted (Whitfield, 2020; Whitfield et al., 2012). This is evidenced by the limited genetic structure observed (Teodósio et al., 2016; Pereira et al., 2023). Experimental evidence on such synergy is lacking for *C. labrosus*, though several coastal species have congeners or populations with this kind of distribution (Gandra et al., 2021; Huey et al., 2013; Liao et al., 2021; Moreira et al., 2020; Rocha-Olivares et al., 2000; Pereira et al., 2023). Regardless of the forces determining connectivity among *C. labrosus* in the Iberian Peninsula, the current microsatellites show that restrictions to gene flow seem to be unlikely. The lack of genetic differentiation found between the Atlantic Ocean and the Mediterranean Sea for other marine species (Comesaña et al., 2008; Debes et al., 2008; Karaiskou et al., 2004; Pereira et al., 2023) suggests that active barriers to connectivity seem to be non-functional on *C. labrosus*, confirming that the transition from the Atlantic to the Mediterranean might not be an efficient phylogeographic break for all species. The hypothesis of panmixia could therefore be well supported by these observations although spawning or breeding grounds for the species remains unknown.

Fish population genetic structure is often analysed using a variety of different molecular and statistical methods including RFLPs, mtDNA sequences, genotyping DNA microsatellites, amplified fragment length polymorphisms (AFLPs) and SNPs, and such an approach banking on a battery of methodologies could help to improve our understanding of *Chelon labrosus* populations. However, we need to adopt the use of other non-genetic population markers such as those based on the analysis of otolith shape and chemical signatures. Otoliths may inform about the life history of the fish within each estuary in the absence of genetic differences (Moreira et al., 2019, 2020).

8. Conclusions

In conclusion, our data set of ten microsatellites revealed a lack of spatial genetic structuring in 204 individuals of *C. labrosus* sampled at five locations along the SBB and two outgroup locations in the Bay of Cadiz and the Mediterranean Sea. The microsatellite results suggest that *C. labrosus* comprises a single panmictic population. None the less, it is important to highlight that greater geographical coverage and sampling size would be beneficial to support the existence of a unique genetic stock. However, panmixia does not rule out the possibility of adults always returning to their estuary of origin after reproductive migration to sea nor the probable existence of local population units. Genetic markers are more conservative at broad spatiotemporal scales than markers that are based on environmentally-dependent phenotypic traits (Moreira et al., 2020). Otolith elemental composition can be used to distinguish between groups of fishes that have experienced long-term separation because of varied environmental conditions such as water temperature, salinity, habitat and feeding conditions (Correia et al., 2011, 2014) and to tell us whether mullets migrating for reproduction show site-fidelity to their estuary of origin.

CRediT authorship contribution statement

Anthony Nzioka: Writing – original draft, preparation, Investigation, Methodology, Formal analysis, Writing – review & editing. **María José Madeira:** Investigation, Methodology, Formal analysis, Writing – review & editing. **Lambros Kokokiris:** Investigation, Writing – review & editing. **Maren Ortiz-Zarrogoitia:** Investigation. **Oihane Diaz de Cerio:** Investigation, Methodology, Writing – review & editing, Conceptualization, Coordination, Supervision. **Ibon Cancio:** Investigation, Methodology, Writing – review & editing, Funding acquisition, Conceptualization, Coordination, Supervision, All authors have read and agreed to the published version of the manuscript.

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.marenvres.2023.106058.

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