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RESEARCH ARTICLE

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Baseline genetic distinctiveness supports structured populations of thornback ray in the Mediterranean Sea

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Abstract

1. The thornback ray (*Raja clavata*) is the most important chondrichthyan in terms of landings in the Mediterranean Sea. Intense harvesting may induce negative genetic effects reducing the resilience of overfished species. For this reason, genetic diversity information should be considered in fisheries management and conservation policies.
2. Microsatellite markers were used to unravel the genetic features (variability, connectivity, sex-biased dispersal) of *R. clavata* populations, both at the small (around the coast of Sardinia, western Mediterranean Sea) and larger spatial scales (at the pan-Mediterranean level, and between the Atlantic Ocean and the Mediterranean Sea).
3. Individual clustering, multivariate and variance analyses rejected the hypothesis of genetic homogeneity, with significant genetic differences between Mediterranean and Atlantic rays, as well as within the Mediterranean Sea between its western and eastern basins. The data indicated that both the Strait of Gibraltar and the Sicilian Channel seem to be effective in limiting the dispersal of thornback ray individuals, but a further structuring was identified, with the significant genetic differentiation of the populations located in the Algero-Provençal and Tyrrhenian basins. Such a fine-scale arrangement suggests the occurrence of additional barriers to species dispersal. A lack of significant genetic differentiation, stable over the years, was measured at a local scale among *R. clavata* Sardinian samples.
4. Several possible mechanisms, both biological and abiotic (e.g. migratory behaviour, waterfronts and oceanographic discontinuities), are discussed. Overall, the genetic data presented, both at the local and regional level, could represent the baseline information for the temporal monitoring of populations and assessing the effects of present or future fisheries-related management actions.

Riccardo Melis and Laura Vacca share first authorship.

5. The data obtained are information of paramount importance for minimizing the gaps in our current knowledge of the genetic diversity of thornback rays and maximizing the information needed for the correct protection of *R. clavata* populations.

KEYWORDS

Atlantic Ocean, commercial species, elasmobranchs, genetic assessment, Mediterranean Sea, microsatellites, population structure, *Raja clavata*, reference baseline

1 | INTRODUCTION

Elasmobranchs are by far the most endangered group of fishes in the Mediterranean Sea, with a high percentage of species (40.7%) classified by the IUCN as threatened: Vulnerable (8.1%), Endangered (12.8%) and Critically Endangered (19.8%) (Ebert & Dando, 2021). During the last 50 years a decrease in Mediterranean shark and batoid populations has been recorded, largely due to the remarkable increase in fishing effort and the inherent low resilience of this taxon to harvesting (Serena et al., 2020 and references therein). Life-history traits such as slow growth, late maturity, low fecundity and productivity, long gestation periods, and a long life-span make elasmobranchs vulnerable and highly impacted by fisheries. Further factors (i.e. environmental degradation and habitat loss) have contributed to the decline in their abundance and diversity in the Mediterranean Sea (Peristeraki et al., 2020 and references therein), and urged the adoption of an action plan for their conservation (SPA/RAC–UN Environment/MAP, 2020). It is widely acknowledged that their management and conservation is a priority (Dulvy et al., 2014; Dulvy et al., 2017; Jorgensen et al., 2022); however, the design of effective management measures remains a challenge due to insufficient information on many species (Elliott et al., 2020; Dulvy et al., 2021). In the Mediterranean Sea, quite a large proportion of species (29.1%) are still classified as Data Deficient (18.6%) or Not Evaluated (10.5%) in the most recent IUCN Red List assessments (Ebert & Dando, 2021).

Intense harvesting and related population declines may lead to severe genetic consequences, such as the loss of genetic variation, inbreeding, genetic drift and selective genetic changes. These impacted and less diversified populations can have a compromised ability to cope with environmental changes and reduced chances of long-term persistence, limiting their overall resilience to overfishing (Domingues, Hilsdorf & Gadig, 2018 and references therein; Pacoureau et al., 2021). Therefore, genetic studies, describing the genetic diversity of shark and ray species, are urgently needed. It has been suggested that, apart from relying only on studies on basic biology, life history and population ecology, shark and ray fisheries management and conservation policies should also consider genetic diversity information, such as the effective population size, observed heterozygosity and allelic richness, because the long-term survival of a species is strongly dependent on the levels of genetic diversity within and between populations (Ovenden et al., 2015; Domingues, Hilsdorf & Gadig, 2018). Priority should be given to genetic studies on shark and ray species that are currently heavily exploited, conducting not only urgent analyses, but planning regular genetic monitoring to assess genetic variations over time. Such data series allow not only the temporal stability of the population structure to be assessed but also the loss of genetic diversity over time, and/or changes in population size (Domingues, Hilsdorf & Gadig, 2018; Jorgensen et al., 2022).

In this context, the present paper deals with the population genetic analysis of the thornback ray *Raja clavata* Linnaeus, 1758, an exploited elasmobranch widely spread and abundant in the Mediterranean and the adjacent eastern Atlantic Ocean (Cashion, Bailly & Pauly, 2019; Follesa et al., 2019).

Raja clavata is widely distributed in European waters; it is present in the Mediterranean Sea and the western Black Sea, the eastern Atlantic, including the North Sea, the Macaronesian archipelagos (the Azores, Madeira, Canaries), the Atlantic African coasts (from Morocco to Namibia), while its occurrence in South Africa and the south-west Indian Ocean is still debated (Pasolini et al., 2011). Thornback rays inhabit shelf and slope waters on mud-to-sand substrates at depths between 5 and 1,020 m but are usually found in shallow waters (Marongiu et al., 2017; Follesa et al., 2019). In the Mediterranean Sea, according to recent data, it is present in almost all General Fisheries Commission for the Mediterranean – Geographical Sub Areas (GSAs) with its highest frequency of occurrence around Corsica (GSA8) for the western basin and in the Ionian (GSA20) and Aegean Seas (GSA22) for the eastern basin (Follesa et al., 2019).

Thornback rays are frequently captured accidentally but are often retained as valuable by-catch of fisheries that focus on more productive teleost fish species (Follesa et al., 2019; Carpentieri et al., 2021). In particular, the thornback ray is the most important chondrichthyan in terms of landings, with a substantial increase in the last few years peaking in 2019 (Bellodi et al., 2022 and references therein). However, there is no evidence of significant population decline in *R. clavata*; on the contrary, stable or even increasing population trends are reported in some parts of their range, justifying their inclusion in the global IUCN Red List in the lower-risk categories as Near Threatened, (Ellis, 2016).

Using both mitochondrial and nuclear markers, a few studies have focused on phylogeography, connectivity and genetic population structure of *R. clavata*. In particular, weak but significant genetic

differences among sampling locations in the Atlantic Ocean (southern North Sea, the English Channel and the Irish Sea) and strong regional differentiations between the Mediterranean basin, the Azores and the European continental shelf were found using microsatellites (Chevolot et al., 2006a; Chevolot et al., 2006b). Additionally, Ferrari et al. (2018), using mitochondrial sequences, revealed that the phylogeographic structure identified in *R. clavata* was not consistent with the separation between western and eastern Mediterranean, as previously reported by Pasolini et al. (2011) using the amplified fragment length polymorphism (AFLP) technique.

The present study deals with the analysis of genetic diversity, population structure and connectivity of thornback skates within the Mediterranean Sea, and between the Mediterranean Sea and the Atlantic Ocean to better describe the genetic patterns and demographic connectivity of different groups of individuals as well as the distributions of genetic variation within and between populations. The main aim of the study is to complement and compare the genetic data obtained in previous studies with a very similar set of microsatellite loci (Chevolot et al., 2006a; Chevolot et al., 2006b), but implementing quite a different sampling scheme, with more intensive sampling in the Mediterranean Sea and a focus around Sardinia (central western Mediterranean).

Considering that the *R. clavata* life history traits (slow growth rate, late maturity, low fecundity, and no pelagic larval phase (Ellis, 2016 and references therein)) suggest limited dispersal capabilities, and the complex geomorphology of the Mediterranean Sea indicates the occurrence of a potential physical barrier to gene flow (Chevolot et al., 2006a; Chevolot et al., 2006b; Domingues, Hilsdorf & Gadig, 2018 and references therein), microsatellite variability data were used to test the hypothesis of genetic heterogeneity among populations, to evaluate the actual dispersal capabilities of the species in the area, to locate any potential physical barrier to gene flow, and to assess inter-annual and inter-generational variation using temporal replicates. The outcomes are discussed under an overview of the population structure of the species and the new genetic data offer a reference point for future monitoring.

2 | MATERIAL AND METHODS

2.1 | Sampling

In total, 294 *R. clavata* specimens were collected between 2004 and 2012, within scientific fishery bottom trawling campaigns (e.g. MEDITS; Spedicato et al., 2020) or by commercial fishermen, from 8 sites located in the north-eastern Atlantic Ocean and throughout the Mediterranean Sea (Figure 1, Table 1, Table S1).

Raja clavata temporal replicates were collected around Sardinia in the summer seasons of 2005 and 2012 from the same approximate three areas (Table S1). Laboratory procedures of tissue sampling and storage are described in the Supplementary Information (SI).

2.2 | DNA extraction, loci amplification and microsatellite data variability

DNA extraction protocols and PCR conditions with primer pairs are described in SI and Table S2, respectively. Individuals of *R. clavata* were genotyped at nine microsatellite loci (Chevolot et al., 2005; El Nagar et al., 2010) The microsatellites' allele size was scored using GENEMARKER v.1.8 (SoftGenetics Inc.) after setting the proper panels for binning analysis. Micro-Checker v.2.2.3 was used to check the data for genotyping errors and null alleles (Van Oosterhout et al., 2004) whose frequency for each locus and population was inspected with the program FreeNA (Chapuis & Estoup, 2007), using the algorithm by Dempster, Laird & Rubin (1977). Number of alleles (Na), observed (Ho) and unbiased expected heterozygosity (UHe) were calculated using GenAlEx v.6.5 (Peakall & Smouse, 2012). Total number of alleles per sample and allelic richness (Ar) were estimated using the R package PopGenReport v.3.0.4 (Adamack & Gruber, 2014). All loci and samples were tested for linkage disequilibrium (LD) and deviations from Hardy–Weinberg expectations (henceforth HWE, null hypothesis H1 = heterozygote deficiency) using exact tests in Genepop v.4.2 (Rousset, 2008) with default settings. The inbreeding coefficient F_{IS} was computed over all loci for each sample and for single locus over all samples with GenoDive v. 3.04 (Meirmans, 2020).

2.3 | Genetic differentiation and population structure

The software POWSIM v.4.1 (Ryman & Palm, 2006) was used to assess the statistical power to detect genetic population differences with the sets of microsatellite loci (SI).

Genetic differentiation among populations was investigated based on pairwise fixation indices calculated with 1,000 permutations using GenoDive. Pairwise F_{ST} values were also calculated using the ENA (excluding null alleles) method implemented in FreeNA to account for the possible effect of null alleles. All statistical tests with multiple pairwise comparisons were corrected, calculating the false discovery rate according to Benjamini & Hochberg (1995) as implemented in Myriads (Carvajal-Rodríguez, 2017).

A priori groupings of sampling sites were also tested with the Analysis of Molecular Variance (AMOVA) in Arlequin (Excoffier & Lischer, 2010; further details on the groups tested are provided in SI).

To corroborate the results, the occurrence of population structuring within the studied area was investigated using multiple approaches: (i) Bayesian clustering with STRUCTURE v.2.3.4 (Pritchard, Stephens & Donnelly, 2000) and BAPS v.6 (Bayesian Analysis of Population Structure) (Corander & Marttinen, 2006; Corander, Marttinen & Mäntyniemi, 2006; Corander et al., 2008); and (ii) discriminant analysis of principal components (DAPC) using the R package Adegenet v.2.0.1 (Jombart, Devillard & Balloux, 2010) implemented in R v.4.0.5 (R Core Team, 2020).

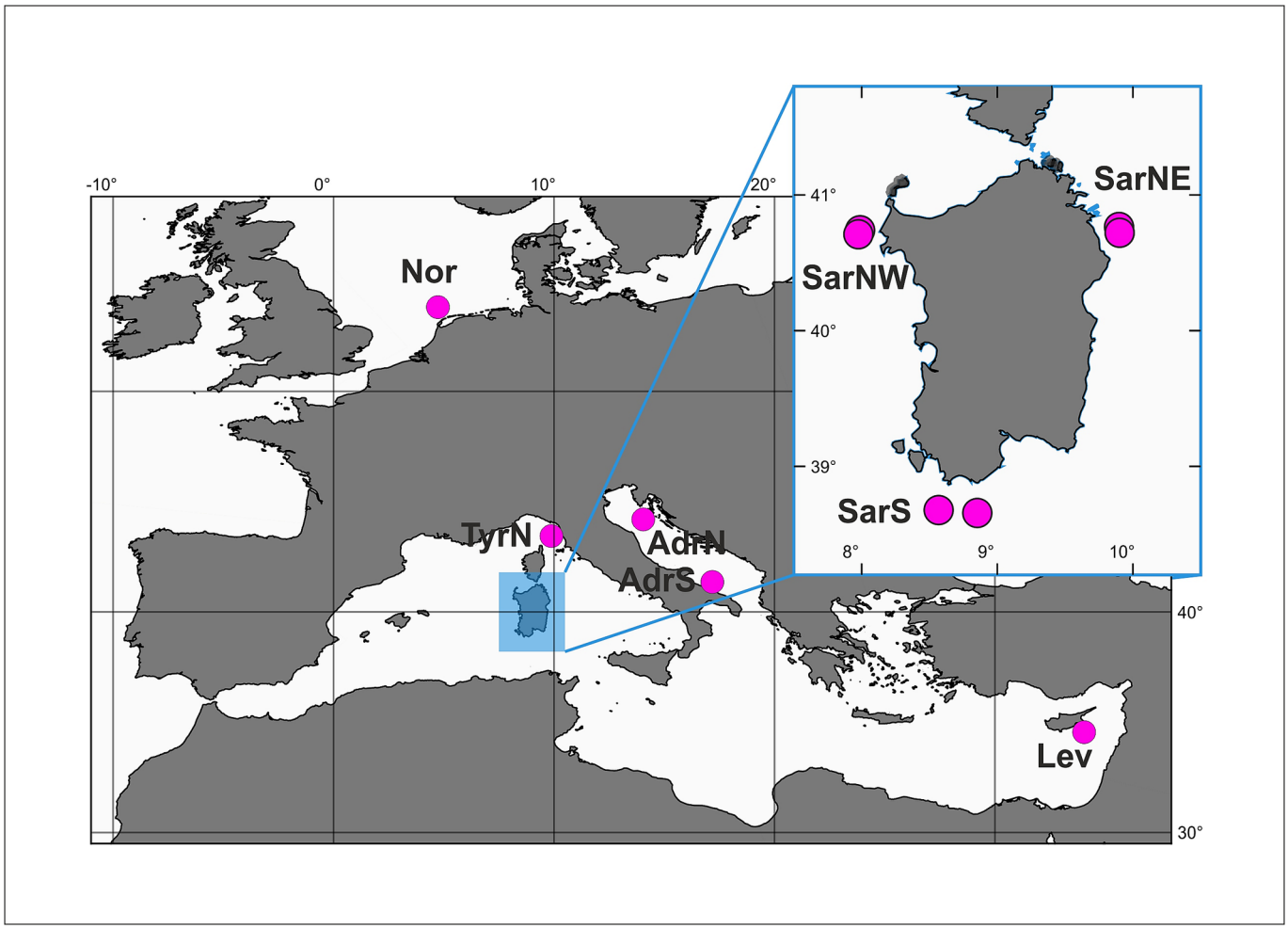

FIGURE 1 Sampling sites of *Raja clavata* population samples. Nor, North Sea; SarNW, Sardinia north west; SarNE, Sardinia north east; SarS, Sardinia south; TyrN, north Tyrrhenian (Livorno); AdrN, north Adriatic (Fano); AdrS, south Adriatic (Bari); Lev, Levantine Sea (Cyprus).

TABLE 1 Descriptive statistics of *Raja clavata* population samples: n, number of individuals; N, mean number of individual genotyped; Na, number of alleles; Ar, allelic richness; Ho, observed heterozygosity; uHe, unbiased expected heterozygosity; F_{IS}, inbreeding fixation index; HWE, probability for the multilocus Hardy–Weinberg test when H1, heterozygote deficit; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$. Significant values after false discovery rate correction for multiple tests are shown in bold. Abbreviations are in accordance with Figure 1.

Location	n	N	Na	Ar	Ho	uHe	F_{IS}	HWE
Atlantic Ocean								
Nor	26	25.500	6.000	5.480	0.471	0.599	0.218	***
Western Mediterranean Sea								
SarNW	49	48.125	5.500	4.636	0.450	0.539	0.168	***
SarNE	50	48.875	5.625	4.973	0.418	0.509	0.181	***
SarS	50	47.000	6.000	5.105	0.469	0.551	0.151	***
TyrN	30	29.875	6.125	5.399	0.482	0.510	0.056	***
Adriatic Sea								
AdrN	29	28.250	5.750	5.283	0.526	0.567	0.073	***
AdrS	30	29.625	6.125	5.477	0.513	0.532	0.037	***
Eastern Mediterranean Sea								
Lev	30	29.750	4.500	4.223	0.587	0.576	−0.019	ns

To determine whether genetic differentiation was driven by geographical distance creating a pattern of isolation by distance (IBD), linearized pairwise FST estimates (FST/1-FST) were correlated against log-transformed geographical distances between samples (Rousset, 1997) in GENODIVE using Mantel tests (standard and stratified; Meirmans, 2020). Given that null alleles can have important

consequences on inferences, the pairwise FST ENA values were used as recommended by Sere et al. (2017). Geographical distances were estimated as the minimum linear distance between pairs of locations by sea on Google Earth.

2.4 | Demography, population size and connectivity

Microsatellite data were checked for the occurrence of recent demographic changes, namely genetic bottlenecks and population growth using the software BOTTLENECK v.1.2.02 (Piry, Luikart & Cornuet, 1999).

NEESTIMATOR v.2.1 (Do et al., 2014) was used to calculate effective population size (Ne) and the number of effective breeders (Neb) for each sampling site. The LD method and the molecular co-ancestry method of Nomura (2008) were used to evaluate Ne and Neb, respectively.

To reconstruct source-sink population dynamics and the evolutionary processes leading to the present genetic diversity distribution, relative migration rates among all populations were estimated based on allele frequency data according to the method of Sundqvist et al. (2016) using the *divMigrate* function implemented in the R package diveRsity (Keenan et al., 2013).

2.5 | Sex-biased dispersal

Tests for sex-biased dispersal were conducted following Goudet, Perrin & Waser (2002). To test for possible effects of sex-biased dispersal on partitioning genetic variation, a corrected assignment index (AIc) (Paetkau et al., 1995) was computed in GenAlEx. The difference in AIc values between males and females was tested using the Wilcoxon's rank-sum test (Wilcoxon, 1945). Pairwise F_{ST} and F_{IS} values were calculated for both females and males to detect differences in their gene flow and inbreeding. Furthermore, relatedness (Re), the mean AIc and variance of the assignment index were tested for significance for each sex using 10,000 permutations in FSTAT v2.9.4 (Goudet, 2003).

3 | RESULTS

3.1 | Genetic diversity

Thornback rays from eight locations were initially genotyped at nine loci. However, Locus Leri21 was problematic: it was monomorphic in AdrN and failed to amplify in a large number of individuals, especially from the three temporal replicates collected around Sardinia in 2005 (SarNE_05, SarNW_05, SarS_05; Table S1), and hence it was removed. The final dataset for *R. clavata* consisted of 294 individuals genotyped at eight loci. After false discovery rate correction, two loci, (Leri24 and Leri63) were found to be in LD, but not in all population samples, therefore both loci were retained in the final analyses. Moreover, since temporal replicates from the Sardinian locations did not show differentiation (pairwise F_{ST} values not significant, Table S3), the subsequent analyses were computed by combining the two temporal replicates (henceforth SarNE_05 + SarNE_12 = SarNE, SarNW_05 + SarNW_12 = SarNW, SarS05_ + SarS_12 = SarS). The statistics of the microsatellite data for both population samples and loci are described in Table 1 and Table S4.

The microsatellite loci showed moderate polymorphism varying from six alleles at locus Leri24, Leri34, and Leri63 to 20 alleles at Leri44 (Table S4). The number of alleles (Na) per sample for all sites varied from 44 in SarNW to 49 in TyrN and AdrS, with the lowest value in Lev (36). Similarly, Ar reached the highest value in Nor and AdrS (5.480 and 5.477, respectively) and the lowest in Lev (4.223; Table 1). Significant deviations from HWE, measured as deficiency of heterozygotes, occurred at three of the eight loci and in all populations, except for Lev. In addition, many of the population-by-locus combination tests showed deviations from HWE. The deficit is usually generated by the presence of null alleles, population sub-structuring or inbreeding. In this study, the inbreeding coefficient F_{IS} ranged from 0.037 to 0.181, being significantly higher than zero in all sites except for TyrN, AdrS and Lev (Table 1). The proportion of null alleles (Fnu) ranged from 4.16% to 17.44%, with an average of 6.26%. According to Chapuis & Estoup (2007), this estimate can be classified as negligible (R < 0.05) to just moderate (0.05 < R < 0.20). Moderate proportions of Fnu can bias F_{ST} estimations (Chapuis & Estoup, 2007). However, no significant differences in the estimation of overall F_{ST} with and without the ENA correction for null alleles were detected (0.033 vs. 0.035, respectively, with overlapping 95% confidence interval, CI). Consequently, all the loci were adequate for the subsequent analyses and coherent with previous studies where loci with moderate null allele frequencies were successfully employed (Schwanck et al., 2020 and references therein).

3.2 | Genetic differentiation and population structure

The simulation obtained with POWSIM emphasized the high-resolution power of the dataset. Both Chi-square and Fisher's tests revealed genetic differentiation in most runs and were able to detect F_{ST} values as low as 0.01 in 100% of the runs in *R. clavata* (t_{60}: $\chi^2 = 1.000$, $F = 1.000$, $P_{value} < 0.05$).

The genetic relationships among the investigated locations are presented as a matrix of pairwise F_{ST} (Table 2). Pairwise values of both indices were low or moderately high, ranging from 0 to 0.163 (F_{ST}). Many comparisons were statistically significant even after correction for multiple testing, mainly those involving Lev and Nor. Low and not significant values for comparisons, especially involving Sardinian samples, highlighted the lack of significant genetic differentiation at a small local scale.

The Bayesian clustering analysis in STRUCTURE revealed the highest support for the presence of three to six genetic clusters (Figures 2, S1a, S1b). Delta K statistics, Likelihood and MaxMedK and MasMeanK Puechmaille's estimators peaked at K = 3, the three most prominent clusters coinciding with Lev, Nor and a third cluster containing the remaining locations. However, a secondary peak was evident at K = 6. BAPS analysis results indicated the best scenario at K = 2 (highest marginal likelihood value; log (ml) = −5635.596) grouping all the locations except Lev, immediately followed by K = 3 (log (ml) = −5654.254), corresponding to Lev, Nor and the remaining locations (Figure 2). Further substructures had lower statistical support. DAPC yielded overall congruent results with the occurrence of three clusters: Lev and Nor were clearly differentiated from all other populations

TABLE 2 Pairwise F_{ST} values (below the diagonal) and associated probabilities (above the diagonal). Acronyms are consistent with Figure 1. Significant values after false discovery rate correction for multiple tests are shown in bold. F_{ST} negative values are reported as zero. Abbreviations are in accordance with Figure 1.

	Nor	SarNW	SarNE	SarS	TyrN	AdrN	AdrS	Lev
Nor	--	**0.001**	**0.001**	**0.001**	**0.001**	**0.005**	**0.001**	**0.001**
SarNW	**0.033**	--	0.052	0.084	**0.006**	**0.003**	**0.004**	**0.001**
SarNE	**0.052**	0.009	--	0.514	0.114	**0.024**	0.282	**0.001**
SarS	**0.030**	0.007	0.000	--	**0.04**	0.118	0.096	**0.001**
TyrN	**0.044**	**0.021**	0.006	**0.014**	--	**0.018**	**0.006**	**0.001**
AdrN	**0.026**	**0.030**	**0.015**	0.008	**0.016**	--	0.299	**0.001**
AdrS	**0.051**	**0.023**	0.003	0.010	**0.021**	0.002	--	**0.001**
Lev	**0.103**	**0.157**	**0.139**	**0.120**	**0.124**	**0.105**	**0.148**	--

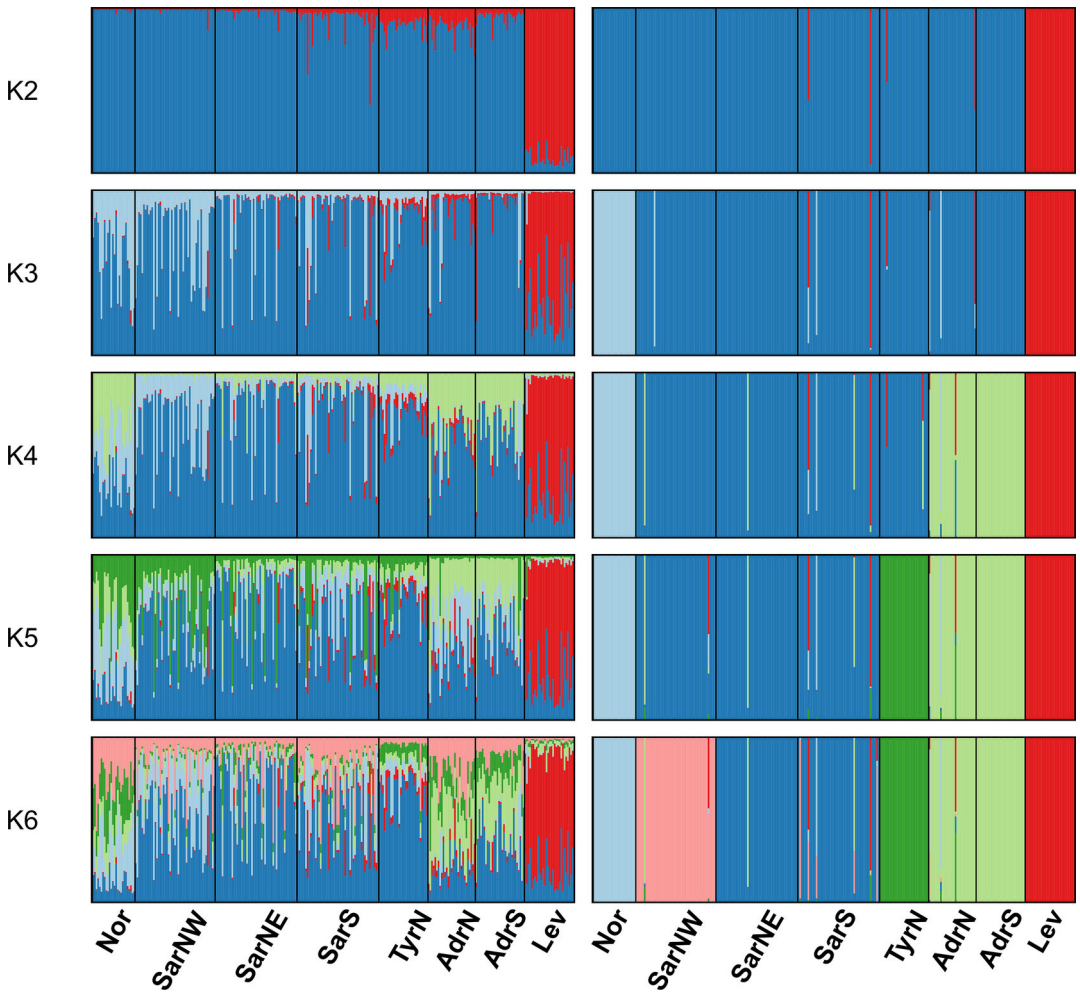

FIGURE 2 STRUCTURE (left) and BAPS (right) clustering barplots for *Raja clavata* samples. Membership probabilities (q) for each individual from K = 2 to K = 6 are shown, see Table 1 for geographical location codes. Abbreviations are in accordance with Figure 1.

(Figure 3). When the DAPC analysis was restricted to the Mediterranean locations, the eastern Mediterranean (Lev) and Adriatic (AdrS and AdrN) appear distinct from the western Mediterranean (Figure S2). Focusing only on the western Mediterranean, a further subdivision was observed separating TyrN from the south/north-eastern (SarS and SarNE) and north-western (SarNW) Sardinian samples (Figure S3).

Hierarchical AMOVA showed a global F_{ST} = 0.036 (P < 0.001), rejecting the hypothesis of genetic homogeneity. However, less than 3.59% of the total variance was among populations and 96.41% within populations. The AMOVA arranged based on the three clusters previously identified confirmed a significant level of structuring (F_{CT} = 0.067, P < 0.010), but additional structures were also supported, especially the six-group structure characterized by a significant differentiation among groups (F_{CT} = 0.037, P < 0.01) and the lowest differentiation within groups (F_{SC} = 0.001, P = NS; Table 3).

The Mantel test, performed to find out whether the population differentiation observed with the FST pairwise analyses was due to an IBD, showed that the variation observed could not be attributable to IBD (R^2 = 0.317, P = 0.082). However, due to the limitations of the Mantel test (Meirmans, 2012; Meirmans, 2015), a stratified Mantel tests within each of the above-mentioned identified clusters was performed and revealed significant IBD for K = 2 and K = 3, but not for K = 6.

3.3 | Demography, population size and connectivity

Bottleneck tests failed to show statistical evidence of a recent reduction in population size for *R. clavata* populations from different origins (Table S5).

The high estimate for the number of effective breeders within a cohort was recorded in the Nor sampling location, while the lowest were in the Adriatic samples (AdrN and AdrS; Table S6). High disparity of contemporaneous Ne with respect to the location was recorded, with the lowest values in Lev and the highest in Nor locations (Table S6). In general, wide CIs at many locations, often including infinite estimates for the upper limit of the CIs, suggest that very little inference can be drawn from those estimates (Table S6).

Directional relative migration networks based on G_{ST} estimates of *R. clavata* populations reconstructed with *divMigrate* show that gene flow exists among the studied localities (Figure S4). When applying arbitrary filter thresholds to retain the most informative connectivity values, the highest gene flow was detected among the neighbouring localities in the western Mediterranean basin (i.e. among Sardinian sites, and within TyrN), whereas migration to the most distant localities seems to be limited (AdrN, AdrS) or very scarce (Lev and Nor, Figure 4). Migration networks show that the predominance of gene flow is in general symmetrical, and no significant directional migration was observed.

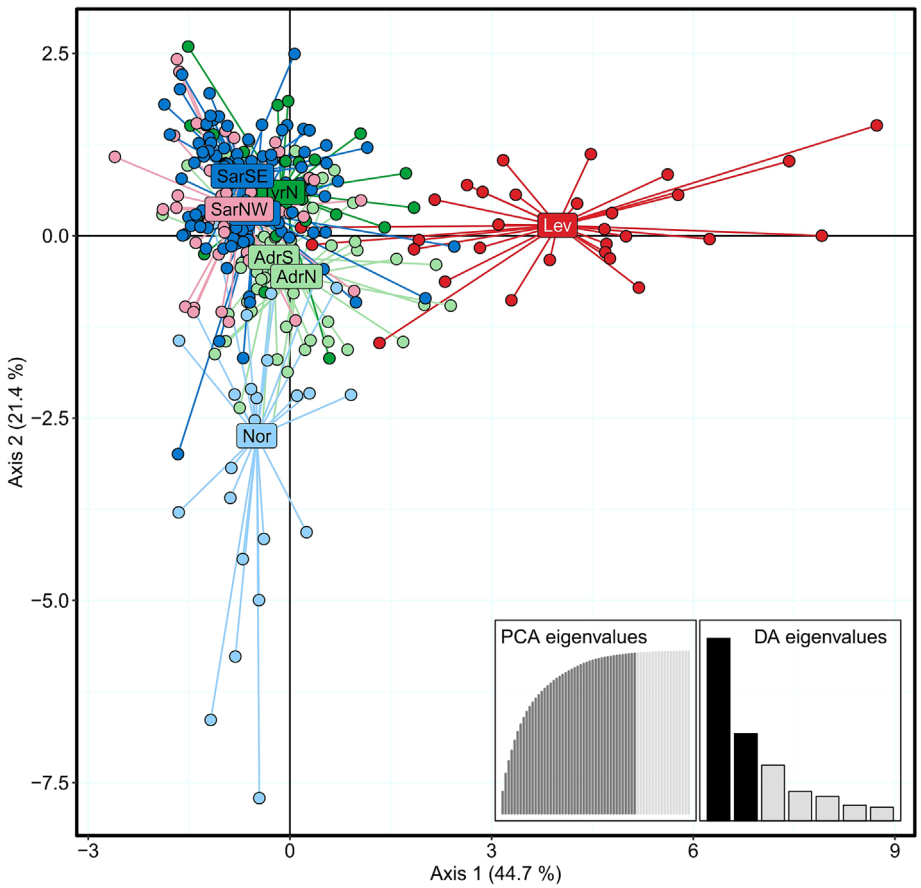

FIGURE 3 Discriminant analysis of principal components scatterplot for *Raja clavata* samples, coloured according to the BAPS plot in Figure 2. Abbreviations are in accordance with Figure 1.

TABLE 3 AMOVA results. Samples were grouped based on the results of previous analyses (i.e. STRUCTURE, BAPs, DAPC, K-clustering). See main text for details. Abbreviations are in accordance with Figure 1.

Source of variation	Percentage of variation	Fixation Index	P_{value}
One group			
Among populations	3.59	$F_{ST} = 0.036$	***
Within populations	96.41		
Two groups (Lev/all others)			
Among groups	8.81	$F_{CT} = 0.088$	ns
Among populations	1.31	$F_{SC} = 0.014$	***
Within populations	89.88	$F_{ST} = 0.101$	***
Three groups (Lev/Nor/all others)			
Among groups	6.71	$F_{CT} = 0.067$	**
Among populations	0.74	$F_{SC} = 0.008$	**
Within populations	92.55	$F_{ST} = 0.074$	***
Four groups (Lev/Nor/AdrN+AdrS/all others)			
Among groups	4.32	$F_{CT} = 0.043$	**
Among populations	0.52	$F_{SC} = 0.005$	*
Within populations	95.17	$F_{ST} = 0.048$	***
Five groups (Lev/Nor/AdrN+AdrS/TyrN/all others)			
Among groups	3.78	$F_{CT} = 0.038$	**
Among populations	0.44	$F_{SC} = 0.004$	ns
Within populations	95.78	$F_{ST} = 0.042$	***
Six groups (Lev/Nor/AdrN+AdrS/TyrN/SarNW/SarS+SarNE)			
Among groups	3.66	$F_{CT} = 0.037$	**
Among populations	0.07	$F_{SC} = 0.001$	ns
Within populations	96.27	$F_{ST} = 0.037$	***

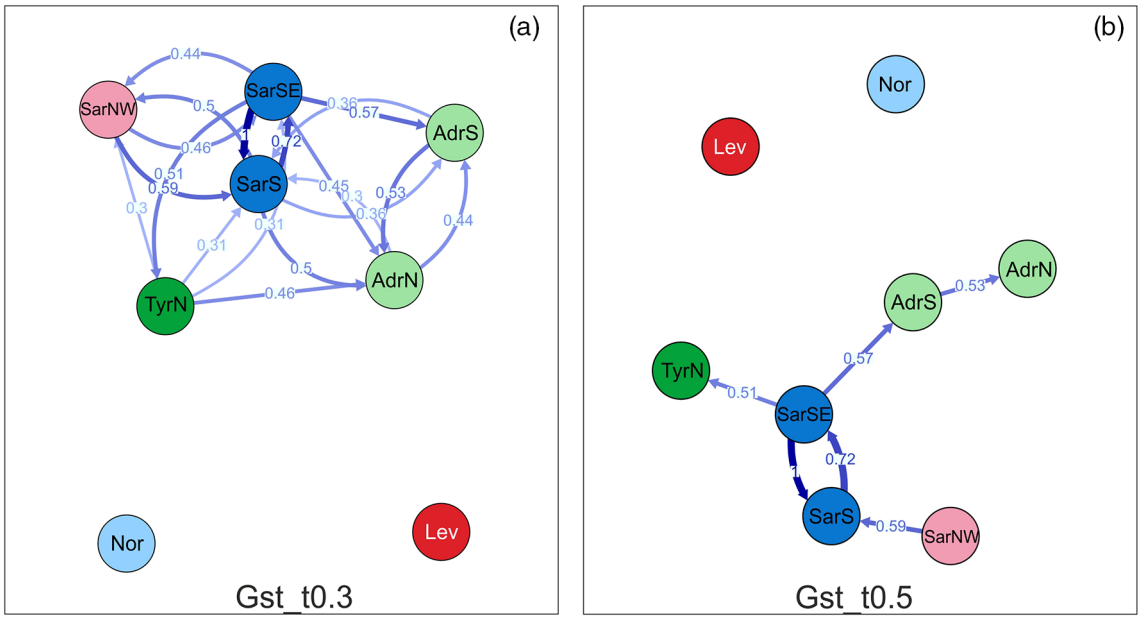

FIGURE 4 Migration network for *Raja clavata* populations based on the *Gst* distance, and two arbitrary thresholds: 0.3 and 0.5 in (a) and (b), respectively. Abbreviations are in accordance with Figure 1.

3.4 | Sex-biased dispersal

Analyses of sex-biased dispersal were conducted on a reduced dataset consisting only of individuals for which sex data and genotypes without missing data were available: 108 *R. clavata* (60 females and 48 males). According to Goudet, Perrin & Waser (2002) in general members of the most dispersing sex should display a higher F_{IS} and variance of the AIc values, along with lower F_{ST}, lower relatedness and lower mean AIc values than the resident sex. In the current study, patterns of molecular variation across sexes trended toward signals of male-biased dispersal in *R. clavata*; however, these were not statistically supported (Table S7).

4 | DISCUSSION

In this study, the population genetic structure of thornback ray was assessed at different spatial scales (within the Mediterranean Sea, and between the Mediterranean and the Atlantic Ocean, respectively) using polymorphic nuclear microsatellite loci.

The new data acquired in this study complement the information obtained in previous studies (Chevolot et al., 2006a; Chevolot et al., 2006b; Pasolini et al., 2011; Ferrari et al., 2018) but with different sampling coverage, especially focused on the western Mediterranean basin, around Sardinia. The results led to the rejection of the hypothesis of panmixia and confirm the occurrence of significant genetic differentiation between the investigated areas.

In *R. clavata*, weak to moderate but significant population differentiation was detected within the Mediterranean Sea and between the Mediterranean and the Atlantic Ocean. In general, the highest pairwise F_{ST} significant values involved the most distant locations (in the East Mediterranean (Levantine Sea) and Atlantic (North Sea)), while most of the lowest and non-significant values were obtained for the various Sardinian samples, highlighting a lack of significant genetic differentiation at a small local scale, that was stable over the years.

In the present study, a non-significant pattern of IBD was identified with the standard Mantel test of the whole dataset, contrary to a previous study where the pattern of IBD was detected when Atlantic and Mediterranean samples were analysed together, as well as within the Atlantic alone (Chevolot et al., 2006b). Although in the present study, a pattern of significant IBD only with stratified Mantel tests was detected (for K = 2: separating Lev from the other locations, and for K = 3 separating Lev and Nor). These results suggest that the genetic differentiation of the Levantine and North Atlantic samples is due to their distance, while in the rest of the Mediterranean (Adriatic and western basin) other factors such as oceanographic barriers impede the gene flow, playing a more prominent role than IBD.

The occurrence of three main genetic clusters of *R. clavata* was concordantly indicated by several analytical approaches (STRUCTURE, BAPS, DAPC), corresponding to the locations in the eastern Mediterranean (Levantine Sea), the Atlantic (North Sea), and a third cluster containing the remaining locations of the central and western Mediterranean Sea. Differently from pairwise comparisons of F_{ST}, a further subdivision within the central and western Mediterranean was statistically supported by individual-based approaches, highlighting a six-group structure, separating the samples located in different Mediterranean sub-basins (Adriatic Sea; northern Tyrrhenian Sea; eastern Tyrrhenian Sea together with the southern Algerian basin, and the Algero-Provençal basin).

Previous studies based on microsatellite data gave similar indications of genetic heterogeneity, however, their sampling coverage was quite different from the current study, and mainly focused on the Atlantic Ocean (Chevolot et al., 2005; Chevolot et al., 2006a). Investigating several sites in the southern North Sea, the English Channel, and the Irish Sea, Chevolot et al. (2006a) found a low but significant global genetic differentiation among these locations even though subsequent pairwise F_{ST} estimates showed no obvious correlations with oceanographic current systems, bottom topography, IBD or physical barrier. The authors claimed that the differentiation could be slightly underestimated at the pairwise level especially as two loci showed very high levels of polymorphism, thus decreasing the power to detect differentiation among samples due to size homoplasy. On the contrary, in the current study, based on a higher number of loci, the POWSIM simulations proved the dataset to have suitable resolution power to correctly detect low but significant F_{ST} values, as those empirically recorded in the present analysis, revealing a fine population structure.

On a larger scale, a previous phylogeographic study of *R. clavata* from European waters, using microsatellite loci and mitochondrial cytochrome b sequences, found strong regional differentiation between the Mediterranean basin, the Azores and the European continental shelf (Chevolot et al., 2006b). The results of AMOVA confirmed the significant differentiation among five groups of thornback rays located in British waters, southern Bay of Biscay, Portuguese waters, Azores and Mediterranean/Black Sea. Unfortunately, the individuals of non-Atlantic origin included in the analysis were only nine individuals from Corsica, 24 individuals from the Adriatic Sea and 35 individuals from the Black Sea. Moreover, they were analysed as a whole in AMOVA tests with no pairwise comparison or indication of sub-structuring within the Mediterranean basin. The Mediterranean samples showed a widespread, unique mitochondrial cytochrome b haplotype (Chevolot et al., 2006b), and low allelic microsatellite diversity, suggesting a strong bottleneck after the isolation of the basin from the Atlantic Ocean during the Last Glacial Maximum, and a continually restricted gene flow between the Atlantic and the Mediterranean areas. In particular, the lowest values of allelic and haplotype diversities were observed in the Black Sea population, signs of a probable strong bottleneck due to past and current isolation (Chevolot et al., 2006b).

In contrast, in the current study the Mediterranean thornback rays were found to be differentiated; the easternmost population sample from the Levantine Sea differed from the others, characterized

by the lowest values in Na and Ar, the highest pairwise F_{ST} values, as well as complete isolation in the migration networks, indicating a clear differentiation of the easternmost part of the Mediterranean Basin. The AMOVA based on microsatellite data confirmed further significant structuring among the eastern Mediterranean, the Adriatic samples and within the western Mediterranean. In contrast, previous studies based on mitochondrial sequences and nuclear markers (AFLP) reported significant genetic differences among Atlantic and Mediterranean samples, and within the Mediterranean Sea. Nevertheless, they failed to detect a significant differentiation between western and eastern population samples (Pasolini et al., 2011; Ferrari et al., 2018). In particular, the mitochondrial control region sequence marker only separated the easternmost Mediterranean sample, but did not effectively differentiate the Adriatic from the western Mediterranean, while the nuclear marker (AFLP) revealed an unexpected genetic similarity between the eastern Mediterranean and Algerian (western) samples, the last one clustering separately from the other two undifferentiated samples from the western Mediterranean (Algero-Provençal and Tyrrhenian basins) (Pasolini et al., 2011). However, Pasolini et al. (2011) pointed out the difficulty in locating the precise geographic origin of the Algerian sample, obtained from fishery landings, and hence were potentially collected out of the Algerian national waters (i.e. further east beyond the Sicilian Channel).

In summary, several studies, using both mitochondrial and nuclear markers (Chevolot et al., 2006b; Pasolini et al., 2011; Ferrari et al., 2018) and the present study concordantly inferred genetic divergence between Atlantic and Mediterranean *R. clavata* populations. Large areas of unsuitable deep sea, long coastal distances, along with physical barriers such as the Gibraltar Strait could have limited the gene flow and allowed significant levels of genetic differentiation to develop among populations between the Atlantic and Mediterranean, as described in several other elasmobranchs (Hirschfeld et al., 2021 and references therein).

The role of the Strait of Sicily as an effective barrier in limiting the gene flow between the eastern and western Mediterranean basins remains unresolved with contradictory results obtained for the Mediterranean *R. clavata* using different markers. A weak, but tangible level of genetic differentiation within the Mediterranean was recorded only in the present study, using a larger set of microsatellite loci and wider sampling than that used by Chevolot et al. (2006b). Similarly, our microsatellite data outperformed AFLP markers and mitochondrial sequences in identifying fine scale differentiation among Mediterranean samples (Chevolot et al., 2006b; Pasolini et al., 2011; Ferrari et al., 2018).

Patterns of dispersal are not easy to describe in marine animals, and they can be the result of a combined effect of ecology, behaviour, life history of the species under study, and the occurrence of biogeographic physical barriers limiting gene flow. Both 'hard' (e.g. bathymetry, current systems, land bridges and/or straits) and 'soft' (e.g. distance) oceanographic barriers can be invoked to explain the population structuring of the thornback ray (Phillips et al., 2021).

Directional relative migration networks showed the highest gene flow among the neighbouring localities in the western Mediterranean basin, whereas migration to the most distant localities seems to be limited. As described for other elasmobranchs, the genetic structuring in the thornback rays, at different spatial scales, is presumably maintained by the reduced dispersal capabilities related to the limited movements of adult individuals and to the pronounced benthic ecology of the eggs and hatchlings (Pasolini et al., 2011 and references therein). Our data indicate that both the Strait of Gibraltar and the Sicilian channel seem to be effective in limiting the dispersal of thornback ray individuals, but the finer structuring measured, with the significant differentiation of the populations located in the Algero-Provençal and Tyrrhenian basins, suggests the occurrence of additional barriers to dispersal.

Asymmetrical dispersal by sex is frequently reported in elasmobranchs to explain differentiation, with many studies pointing to male-biased dispersal (MBD) as the commonest behaviour (Phillips et al., 2021 and references therein). In the present analysis, patterns of molecular variation across sexes trended toward signals of male-biased dispersal in *R. clavata* populations around Sardinia, the only individuals for which sex data were available. Signs of limited female gene flow with possible MBD have been already reported for *R. clavata* within the north-east Atlantic (Chevolot et al., 2006a; Chevolot et al., 2006b). Based on the disproportionate energetic investment of females and males into their offspring, female philopatry and MBD are expected to be widespread in elasmobranchs. In particular, the common reproductive philopatric behaviour in female elasmobranchs suggests a strong fitness advantage to this strategy especially in species whose nursery habitats are fragmented or patchy in distribution, where females tend to stay close to these spots, while males maintain genetic connectivity along contiguous coastlines (Phillips et al., 2021).

Additionally, apart from Lev, the genetic diversity (Na, uHe) and Ar were relatively uniform among the *R. clavata* samples, with no apparent evidence of reduced genetic variation. Bottleneck tests failed to show statistical evidence that *R. clavata* populations had undergone a recent reduction in population size. Therefore, the results indicate that *R. clavata* has demographically stable populations, with no sign of recent reduced genetic variability despite the remarkable fishing pressure they are experiencing, and they appear to be resilient to the ever-growing threat represented by overfishing. Considering ages at 50% maturity for *R. clavata* of 7–8 years (Ellis, 2016), temporal genetic stability over one generation (from 2005 to 2012) has been assessed for the Sardinian populations, similar to the results obtained for the Atlantic populations by Chevolot et al. (2006a) who did not observe significant differences between 2 years or two seasonal sampling periods, and Chevolot et al. (2008) who recorded no significant loss of genetic diversity over a 40-year timeframe in the Bay of Biscay (1965 and 2003–2004). Nevertheless, even if the absence of bottleneck signs or genetic erosion indicate a relatively good status of thornback rays, the reliability of genetic bottleneck tests for detecting recent

population declines has been questioned in long-lived species such as *Scyliorhinus canicula* (Lippé, Dumont & Bernatchez, 2006; Peery et al., 2012; Bradke et al., 2021), and hence these results are to be interpreted cautiously.

The Ne values obtained for thornback ray populations in the Mediterranean were lower than those recorded in the Atlantic (present study and Chevolot et al., 2008; Marandel et al., 2018). Caution is needed in interpreting Ne results based on different methods and markers; the 'infinite' Ne values and the wide CI at many locations could indicate the lack of a strong genetic signature (Table S8). However, the general indication is that Mediterranean populations, less large and variable than their Atlantic counterparts, can be more vulnerable to exploitation and thus deserve careful monitoring over the years to direct management efforts at both local and global scales.

4.1 | Final conclusions and management implications

In this research, sampling and experimental analyses were designed to primarily acquire useful information, describing the genetic features (variability, connectivity, sex-biased dispersal) of thornback ray populations, both at the small (i.e. around the coast of Sardinia, western Mediterranean Sea) and at a larger spatial scales (i.e. at the pan-Mediterranean level, and between the Atlantic and the Mediterranean Sea).

Different analytical approaches (individual clustering, multivariate and variance analyses) rejected the hypothesis of genetic homogeneity with significant genetic differences between the Mediterranean and Atlantic rays, as well as within the Mediterranean between the western and e basins.

The data indicated that both the Strait of Gibraltar and the Sicilian Channel seem to be effective in limiting the dispersal of thornback individuals, but a finer structuring was identified, with the significant genetic differentiation of the populations located in the Algero-Provençal and Tyrrhenian basins, suggesting the occurrence of additional barriers to dispersal, and recommending special attention and close monitoring of these populations also due to the fishing pressure regularly exerted on them.

In conclusion, the present study shows how the genetic data can be helpful, complementing the biological data, in identifying differentiation useful to delineate populations units or stocks, and in quantifying potential genetic erosion in them (reduction of genetic variability) that would require the adoption of specific management measures (e.g. stricter fisheries regulations or spatial/temporal restrictions). In the future, special effort should be directed to gathering information to investigate the occurrence of discrete groups of populations or vulnerable habitats for the species (e.g. nursery grounds, fidelity sites, parturition sites, feeding grounds, aggregation sites) in areas not covered or underrepresented in the present and previous studies; these data are the basic information needed to implement sound conservation actions.

In this context, considering that the Action Plan for the Conservation of Cartilaginous Fishes (Chondrichtyans) in the Mediterranean Sea (SPA/RAC–UN Environment/MAP, 2020) recognizes the urgency of implementing a permanent monitoring of fisheries especially where chondrichthyans are impacted by human activities, genetic data can assist in the timely detection of any decline of populations. The genetic monitoring of exploited populations is highly recommended and should be 'easily' realized through routine tissue sampling undertaken during the regular fisheries monitoring programmes (e.g. during surveys at sea, landing-site observations, etc.).

The outcomes obtained from the genetic monitoring could be shared with various specialized working groups to contribute to place-based conservation initiatives like for example the Important Sharks and Rays Areas approach recently developed by the IUCN Species Survival Commission Shark Specialist Group (Hyde et al., 2022).

Moreover, the genetic monitoring of exploited populations can constitute a valuable practice to continuously assess the species along with their habitat use. Using these standardized data, conservation measures could be established according to each species' ecology and reach visible results (e.g. management of sustainable fisheries for commercially viable species and establishment of no-take protected areas) that could extend to a larger ecosystem scale since elasmobranchs play a key role in maintaining a healthy ecosystem, thus making them priority targets for conservation.

Finally, the use of multiple data, combining genetic with morphological and statistical approaches, may enable the possibility to reconstruct past fluctuations of populations and predict the effects of anthropic pressures and assess the effectiveness of present/future conservation actions.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest associated with this work.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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REFERENCES

Adamack, A.T. & Gruber, B. (2014). PopGenReport: simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution*, 5(4), 384–387. https://doi.org/10.1111/2041-210x.12158

Bellodi, A., Massaro, A., Zupa, W., Donnaloia, M., Follesa, M.C., Ligas, A. et al. (2022). Assessing thornback ray growth pattern in different areas of Western-Central Mediterranean Sea through a multi-model inference analysis. *Journal of Sea Research*, 179, 102–141. https://doi.org/10.1016/j.seares.2021.102141

Benjamini, Y. & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B Methodological*, 57(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bradke, D.R., Altobelli, J.T., Russell, A.L., Jaeger, C.P. & Moore, J.A. (2021). Low bottleneck detection in long-lived species despite lost genetic diversity: a case study of tuatara and eastern Massasauga rattlesnakes. *Journal of Heredity*, 112(4), 346–356. https://doi.org/10.1093/jhered/esab025

Carpentieri, P., Nastasi, A., Sessa, M. & Srour, A. (Eds.) (2021). *Incidental catch of vulnerable species in Mediterranean and Black Sea fisheries – a review. General fisheries Commission for the Mediterranean. Studies and reviews*, No. 101. Rome: FAO. https://doi.org/10.4060/cb5405en

Carvajal-Rodríguez, A. (2017). Myriads: P-value-based multiple testing correction. *Bioinformatics*, 34(6), 1043–1045. https://doi.org/10.1093/bioinformatics/btx746

Cashion, M.S., Bailly, N. & Pauly, D. (2019). Official catch data underrepresent shark and ray taxa caught in Mediterranean and Black Sea fisheries. *Marine Policy*, 105, 1–9. https://doi.org/10.1016/j.marpol.2019.02.041

Chapuis, M.P. & Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, 24(3), 621–631. https://doi.org/10.1093/molbev/msl191

Chevolot, M., Ellis, J.R., Hoarau, G., Rijnsdorp, A.D., Stain, W.T. & Olsen, J.L. (2006a). Population structure of the thornback ray (*Raja clavata* L.) in British waters. *Journal of Sea Research*, 56(4), 305–316. https://doi.org/10.1016/j.seares.2006.05.005

Chevolot, M., Ellis, J.R., Rijnsdorp, A.D., Stam, W.T. & Olsen, J.L. (2008). Temporal changes in allele frequencies but stable genetic diversity over the past 40 years in the Irish Sea population of thornback ray, *Raja clavata*. *Heredity*, 101(2), 120–126. https://doi.org/10.1038/hdy.2008.36

Chevolot, M., Hoarau, G., Rijnsdorp, A.D., Stam, W.T. & Olsen, J.L. (2006b). Phylogeography and population structure of thornback rays (*Raja clavata* L., Rajidae). *Molecular Ecology*, 15(12), 3693–3705. https://doi.org/10.1111/j.1365-294X.2006.03043.x

Chevolot, M., Reusch, T.B.H., Boele-Bos, S., Stam, W.T. & Olsen, J. L. (2005). Characterization and isolation of DNA microsatellite primers in *Raja clavata* L. (thornback ray, Rajidae). *Molecular Ecology Notes*, 5(2), 427–429. https://doi.org/10.1111/j.1471-8286.2005.00951.x

Corander, J. & Marttinen, P. (2006). Bayesian identification of admixture events using multilocus molecular markers. *Molecular Ecology*, 15(10), 2833–2843. https://doi.org/10.1111/j.1365-294X.2006.02994.x

Corander, J., Marttinen, P. & Mäntyniemi, S. (2006). A Bayesian method for identification of stock mixtures from molecular marker data. *Fishery Bulletin*, 104(4), 550–558.

Corander, J., Marttinen, P., Sirén, J. & Tang, J. (2008). Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *BMC Bioinformatics*, 9(1), 539. https://doi.org/10.1186/1471-2105-9-539

Dempster, A.P., Laird, N.M. & Rubin, D.B. (1977). Maximum likelihood from incomplete data via the EM algorithm. *Journal of the Royal Statistical Society: Series B Methodological*, 39(1), 1–22. https://doi.org/10.1111/j.2517-6161.1977.tb01600.x

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J. & Ovenden, J.R. (2014). NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (ne) from genetic data. *Molecular Ecology Resources*, 14(1), 209–214. https://doi.org/10.1111/1755-0998.12157

Domingues, R.R., Hilsdorf, A.W.S. & Gadig, O.B.F. (2018). The importance of considering genetic diversity in shark and ray conservation policies. *Conservation Genetics*, 19(3), 501–525. https://doi.org/10.1007/s10592-017-1038-3

Dulvy, N.K., Fowler, S.L., Musick, J.A., Cavanagh, R.D., Kyne, P.M., Harrison, L.R. et al. (2014). Extinction risk and conservation of the world's sharks and rays. *eLife*, 3, e00590. https://doi.org/10.7554/eLife.00590

Dulvy, N.K., Pacoureau, N., Rigby, C.L., Pollom, R.A., Jabado, R.W., Ebert, D.A. et al. (2021). Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. *Current Biology*, 31(21), 4773–4787 e4778. https://doi.org/10.1016/j.cub.2021.08.062

Dulvy, N.K., Simpfendorfer, C.A., Davidson, L.N.K., Fordham, S.V., Brautigam, A., Sant, G. et al. (2017). Challenges and priorities in shark and ray conservation. *Current Biology*, 27(11), R565–R572. https://doi.org/10.1016/j.cub.2017.04.038

Ebert, D.A. & Dando, M. (2021). *Field guide to sharks, rays and chimeras of Europe and the Mediterranean*. Princeton University Press. ISBN 9780691205984

El Nagar, A., McHugh, M., Rapp, T., Sims, D.W. & Genner, M.J. (2010). Characterisation of polymorphic microsatellite markers for skates (Elasmobranchii: Rajidae) from expressed sequence tags. *Conservation Genetics*, 11(3), 1203–1206. https://doi.org/10.1007/s10592-009-9919-8

Elliott, S.A.M., Carpentier, A., Feunteun, E. & Trancart, T. (2020). Distribution and life history trait models indicate vulnerability of skates. *Progress in Oceanography*, 181, 102256. https://doi.org/10.1016/j.pocean.2019.102256

Ellis, J. (2016). *Raja clavata*. The IUCN red list of threatened species 2016. *The IUCN Red List of Threatened Species*. https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T39399A103110667.en

Excoffier, L. & Lischer, H.E.L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and windows. *Molecular Ecology Resources*, 10(3), 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Ferrari, A., Tinti, F., Bertucci Maresca, V., Velona, A., Cannas, R., Thasitis, I. et al. (2018). Natural history and molecular evolution of demersal Mediterranean sharks and skates inferred by comparative phylogeographic and demographic analyses. *PeerJ*, 6, e5560. https://doi.org/10.7717/peerj.5560

Follesa, M.C., Marongiu, M.F., Zupa, W., Bellodi, A., Cau, A., Cannas, R. et al. (2019). Spatial variability of Chondrichthyes in the northern Mediterranean. *Scientia Marina*, 83(S1), 81–100. https://doi.org/10.3989/scimar.04998.23A

Goudet, J. (2003). FSTAT (version 2.9.4), a program (for Windows 95 and above) to estimate and test population genetics parameters. http://www.unil.ch/izea/softwares/fstat.html

Goudet, J., Perrin, N. & Waser, P. (2002). Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Molecular Ecology*, 11(6), 1103–1114. https://doi.org/10.1046/j.1365-294x.2002.01496.x

Hirschfeld, M., Dudgeon, C., Sheaves, M. & Barnett, A. (2021). Barriers in a sea of elasmobranchs: from fishing for populations to testing hypotheses in population genetics. *Global Ecology and Biogeography*, 30(11), 2147–2163. https://doi.org/10.1111/geb.13379

Hyde, C.A., Notabartolo di Sciara, G., Sorrentino, L., Boyd, C., Finucci, B., Fowler, S.L. et al. (2022). Putting sharks on the map: a global standard for improving shark area-based conservation. *Frontiers in Marine Science*, 9, 968853. https://doi.org/10.3389/fmars.2022.968853

Jombart, T., Devillard, S. & Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, 11(1), 94. https://doi.org/10.1186/1471-2156-11-94

Jorgensen, S.J., Micheli, F., White, T.D., Van Houtan, K.S., Alfaro-Shigueto, J., Andrzejaczek, S. et al. (2022). Emergent research and priorities for shark and ray conservation. *Endangered Species Research*, 47, 171–203. https://doi.org/10.3354/esr01169

Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W. & Prodöhl, P.A. (2013). DiveRsity: an R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, 4(8), 782–788. https://doi.org/10.1111/2041-210X.12067

Lippé, C., Dumont, P. & Bernatchez, L. (2006). High genetic diversity and no inbreeding in the endangered copper redhorse, *Moxostoma hubbsi* (Catostomidae, Pisces): the positive sides of a long generation time. *Molecular Ecology*, 15(7), 1769–1780. https://doi.org/10.1111/j.1365-294X.2006.02902.x

Marandel, F., Lorance, P., Andrello, M., Charrier, G., Le Cam, S., Lehuta, S. et al. (2018). Insights from genetic and demographic connectivity for the management of rays and skates. *Canadian Journal of Fisheries and Aquatic Sciences*, 75(8), 1291–1302. https://doi.org/10.1139/cjfas-2017-0291

Marongiu, M.F., Porcu, C., Bellodi, A., Cannas, R., Cau, A., Cuccu, D. et al. (2017). Temporal dynamics of demersal chondrichthyan species in the central western Mediterranean Sea: the case study in Sardinia Island. *Fisheries Research*, 193, 81–94. https://doi.org/10.1016/j.fishres.2017.04.001

Meirmans, P.G. (2012). The trouble with isolation by distance. *Molecular Ecology*, 21(12), 2839–2846. https://doi.org/10.1111/j.1365-294X.2012.05578.x

Meirmans, P.G. (2015). Seven common mistakes in population genetics and how to avoid them. *Molecular Ecology*, 24(13), 3223–3231. https://doi.org/10.1111/mec.13243

Meirmans, P.G. (2020). Genodive version 3.0: easy-to-use software for the analysis of genetic data of diploids and polyploids. *Molecular Ecology Resources*, 20(4), 1126–1131. https://doi.org/10.1111/1755-0998.13145

Nomura, T. (2008). Estimation of effective number of breeders from molecular coancestry of single cohort sample. *Evolutionary Applications*, 1(3), 462–474. https://doi.org/10.1111/j.1752-4571.2008.00015.x

Ovenden, J.R., Berry, O., Welch, D.J., Buckworth, R.C. & Dichmont, C.M. (2015). Ocean's eleven: a critical evaluation of the role of population, evolutionary and molecular genetics in the management of wild fisheries. *Fish and Fisheries*, 16(1), 125–159. https://doi.org/10.1111/faf.12052

Pacoureau, N., Rigby, C.L., Kyne, P.M., Sherley, R.B., Winker, H., Carlson, J.K. et al. (2021). Half a century of global decline in oceanic sharks and rays. *Nature*, 589(7843), 567–571. https://doi.org/10.1038/s41586-020-03173-9

Paetkau, D., Calvert, W., Stirling, I. & Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, 4(3), 347–354. https://doi.org/10.1111/j.1365-294x.1995.tb00227.x

Pasolini, P., Ragazzini, C., Zaccaro, Z., Cariani, A., Ferrara, G., Gonzalez, E.G. et al. (2011). Quaternary geographical sibling speciation and population structuring in the eastern Atlantic skates (suborder Rajoidea) *Raja clavata* and *R. straeleni*. *Marine Biology*, 158(10), 2173–2186. https://doi.org/10.1007/s00227-011-1722-7

Peakall, R. & Smouse, P.E. (2012). GenAlEx 6.5: genetic analysis in excel. Population genetic software for teaching and research-an update. *Bioinformatics (Oxford, England)*, 28(19), 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Peery, M.Z., Kirby, R., Reid, B.N., Stoelting, R., Doucet-Bëer, E., Robinson, S. et al. (2012). Reliability of genetic bottleneck tests for detecting recent population declines. *Molecular Ecology*, 21(14), 3403–3418. https://doi.org/10.1111/j.1365-294X.2012.05635.x

Peristeraki, P., Tserpes, G., Kavadas, S., Kallianiotis, A. & Stergiou, K.I. (2020). The effect of bottom trawl fishery on biomass variations of demersal chondrichthyes in the eastern Mediterranean. *Fisheries Research*, 221, 105367. https://doi.org/10.1016/j.fishres.2019.105367

Phillips, N.M., Devloo-Delva, F., McCall, C. & Daly-Engel, T.S. (2021). Reviewing the genetic evidence for sex-biased dispersal in elasmobranchs. *Reviews in Fish Biology and Fisheries*, 31(4), 821–841. https://doi.org/10.1007/s11160-021-09673-9

Piry, S., Luikart, G. & Cornuet, J.M. (1999). Computer note. BOTTLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. *Journal of Heredity*, 90(4), 502–503. https://doi.org/10.1093/jhered/90.4.502

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155(2), 945–959. https://doi.org/10.1093/genetics/155.2.945

R Core Team. (2020). R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/

Rousset, F. (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, 145(4), 1219–1228. https://doi.org/10.1093/genetics/145.4.1219

Rousset, F. (2008). Genepop'007: a complete re-implementation of the genepop software for windows and Linux. *Molecular Ecology Resources*, 8(1), 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Ryman, N. & Palm, S. (2006). POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology Notes*, 6(3), 600–602. https://doi.org/10.1111/j.1471-8286.2006.01378.x

Schwanck, T.N., Schweinsberg, M., Lampert, K.P., Guttridge, T.L., Tollrian, R. & O'Shea, O. (2020). Linking local movement and molecular analysis to explore philopatry and population connectivity of the southern stingray Hypanus americanus. *Journal of Fish Biology*, 96(6), 1475–1488. https://doi.org/10.1111/jfb.14325

Sere, M., Thevenon, S., Belem, A.M.G. & De Meeus, T. (2017). Comparison of different genetic distances to test isolation by distance between populations. *Heredity*, 119(2), 55–63. https://doi.org/10.1038/hdy.2017.26

Serena, F., Abella, A.J., Bargnesi, F., Barone, M., Colloca, F., Ferretti, F. et al. (2020). Species diversity, taxonomy and distribution of Chondrichthyes in the Mediterranean and Black Sea. *European Zoological Journal*, 87(1), 497–536. https://doi.org/10.1080/24750263.2020.1805518

SPA/RAC–UN Environment/MAP. (2020). Action Plan for the Conservation of Cartilaginous Fishes (Chondrichtyans) in the Mediterranean Sea; by: Bradai, M N., Ed SPA/RAC. Tunis, 18 pp.

Spedicato, M.T., Massuti, E., Merigot, B., Tserpes, G., Jadaud, A. & Relini, G. (2020). The MEDITS trawl survey specifications in an ecosystem approach to fishery management (vol 84, pg 309, 2020).

Scientia Marina, 84(3), 309–309. https://doi.org/10.3989/scimar.04915.11X

Sundqvist, L., Keenan, K., Zackrisson, M., Prodöhl, P. & Kleinhans, D. (2016). Directional genetic differentiation and relative migration. *Ecology and Evolution*, 6(11), 3461–3475. https://doi.org/10.1002/ece3.2096

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. (2004). Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4(3), 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Wilcoxon, F. (1945). Individual comparisons by ranking methods. *Biometrics Bulletin*, 1(6), 80–83. https://doi.org/10.2307/3001968

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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