



LJMU Research Online

Leon, A, Urso, I, Damalas, D, Martinsohn, J, Zanzi, A, Mariani, S, Sperone, E, Micarelli, P, Garibaldi, F, Megalofonou, P, Bargelloni, L, Franch, R, Macias, D, Prodohl, P, Fitzpatrick, S, Stagioni, M, Tinti, F and Cariani, A

Genetic differentiation and phylogeography of Mediterranean-North Eastern Atlantic blue shark (*Prionace glauca*, L. 1758) using mitochondrial DNA: panmixia or complex stock structure?

<http://researchonline.ljmu.ac.uk/id/eprint/11577/>

Article

Citation (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

Leon, A, Urso, I, Damalas, D, Martinsohn, J, Zanzi, A, Mariani, S, Sperone, E, Micarelli, P, Garibaldi, F, Megalofonou, P, Bargelloni, L, Franch, R, Macias, D, Prodohl, P, Fitzpatrick, S, Stagioni, M, Tinti, F and Cariani, A (2017) Genetic differentiation and phylogeography of Mediterranean-North Eastern Atlantic

LJMU has developed **LJMU Research Online** for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact researchonline@ljmu.ac.uk

<http://researchonline.ljmu.ac.uk/>



Genetic differentiation and phylogeography of Mediterranean-North Eastern Atlantic blue shark (*Prionace glauca*, L. 1758) using mitochondrial DNA: panmixia or complex stock structure?

Agostino Leone^{1,*}, Ilenia Urso^{1,*}, Dimitrios Damalas^{2,12}, Jann Martinsohn², Antonella Zanzi², Stefano Mariani³, Emilio Sperone⁴, Primo Micarelli⁵, Fulvio Garibaldi⁶, Persefoni Megalofonou⁷, Luca Bargelloni⁸, Rafaella Franch⁸, David Macias⁹, Paulo Prodöhl¹⁰, Séan Fitzpatrick¹⁰, Marco Stagoni¹¹, Fausto Tinti¹ and Alessia Cariani¹

¹ Department of Biological, Geological and Environmental Sciences (BiGeA), Laboratory of Genetics & Genomics of Marine Resources and Environment (GenoDREAM), University of Bologna, Ravenna, Italy

² Joint Research Centre (JRC), Directorate D-Sustainable Resources, Unit D2-Water and Marine Resources, European Commission, Ispra, Italy

³ School of Environment & Life Sciences, University of Salford, Salford, Greater Manchester, United Kingdom

⁴ Department of Biology, Ecology and Earth Science (DiBEST), University of Calabria, Arcavacata di Rende, Italy

⁵ Sharks Research Center, Aquarium Mondo Marino, Massa Marittima, Italy

⁶ Department of Earth, Environment and Life Sciences, University of Genova, Genova, Italy

⁷ Department of Biology, National and Kapodistrian University of Athens, Ilissia, Greece

⁸ Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro, Italy

⁹ Centro Oceanográfico de Málaga, Instituto Español de Oceanografía, Málaga, Spain

¹⁰ School of Biological Sciences, Queen's University, Institute for Global Food Security, Belfast, United Kingdom

¹¹ Department of Biological, Geological and Environmental Sciences, Marine Biology and Fisheries Laboratory, University of Bologna, Fano, Italy

¹² Current affiliation: Hellenic Centre for Marine Research, Athinon, Anavyssos, Greece

* These authors contributed equally to this work.

Submitted 21 August 2017
Accepted 9 November 2017
Published 6 December 2017

Corresponding author
Agostino Leone,
agostino.leone2@unibo.it

Academic editor
Rita Castillo

Additional Information and
Declarations can be found on
page 14

DOI 10.7717/peerj.4112

© Copyright
2017 Leone et al.

Distributed under
Creative Commons CC-BY 4.0

ABSTRACT

Background. The blue shark (*Prionace glauca*, Linnaeus 1758) is one of the most abundant epipelagic shark inhabiting all the oceans except the poles, including the Mediterranean Sea, but its genetic structure has not been confirmed at basin and interoceanic distances. Past tagging programs in the Atlantic Ocean failed to find evidence of migration of blue sharks between the Mediterranean and the adjacent Atlantic, despite the extreme vagility of the species. Although the high rate of by-catch in the Mediterranean basin, to date no genetic study on Mediterranean blue shark was carried out, which constitutes a significant knowledge gap, considering that this population is classified as “Critically Endangered”, unlike its open-ocean counterpart. **Methods.** Blue shark phylogeography and demography in the Mediterranean Sea and North-Eastern Atlantic Ocean were inferred using two mitochondrial genes (Cytb and

OPEN ACCESS

control region) amplified from 207 and 170 individuals respectively, collected from six localities across the Mediterranean and two from the North-Eastern Atlantic.

Results. Although no obvious pattern of geographical differentiation was apparent from the haplotype network, Φ_{st} analyses indicated significant genetic structure among four geographical groups. Demographic analyses suggest that these populations have experienced a constant population expansion in the last 0.4–0.1 million of years.

Discussion. The weak, but significant, differences in Mediterranean and adjacent North-eastern Atlantic blue sharks revealed a complex phylogeographic structure, which appears to reject the assumption of panmixia across the study area, but also supports a certain degree of population connectivity across the Strait of Gibraltar, despite the lack of evidence of migratory movements observed by tagging data. Analyses of spatial genetic structure in relation to sex-ratio and size could indicate some level of sex/stage biased migratory behaviour.

Subjects Aquaculture, Fisheries and Fish Science, Biogeography, Evolutionary Studies, Genetics, Marine Biology

Keywords mtDNA, Population expansion, Blue shark, Phylogeography, Geographical breaks, Mediterranean stocks, Sharks

INTRODUCTION

The blue shark (*Prionace glauca*, Linnaeus 1758; BS henceforth) is one of the most abundant epipelagic sharks that is found in all oceans from 60°N to 50°S (Compagno, 1984). Blue sharks are rarely targeted by commercial fishing, but feature prominently as by-catch of fisheries targeting large pelagic fish, especially swordfish and tuna longlines (Fowler et al., 2005). BS populations trend data are available only for a part of the geographic range and stock assessments are highly uncertain (Dulvy et al., 2014; Coelho et al., 2017); due to the huge amount of by-caught BS (approx. 20 million per annum, Stevens, 2009), the species has been categorized worldwide as “Near Threatened” in the IUCN Red List (Stevens, 2009). Based on recent assessment (ICCAT, 2015), the North Atlantic stock is unlikely to be currently overfished. The Mediterranean BS, on the other hand, is estimated to have undergone a 90% decline over three generations, primarily due to overfishing (Ferretti et al., 2008), and is now categorized as “Critically Endangered” (Sims et al., 2016). Given the vast amount of poorly reported by-catch, the increasing commercial value of the species (Megalofonou, Damalas & Yannopolous, 2005) and the persistent issue of the global trade in shark fin products, of which BS is the main component (Clarke et al., 2006), a more explicit management is needed for this species, which should be underpinned by robust knowledge of its population structure.

In the Atlantic, BS is distributed from Canada to Argentina, on the western side, and from Norway to South Africa on the eastern side, including the Mediterranean Sea (Compagno, 1984). The population structure and dynamics of Atlantic BS is still poorly known, despite several long-term tagging studies, which revealed extensive movements of BS tagged in the western side of the North Atlantic (henceforth NA), with well documented eastward trans-Atlantic migrations (Kohler, Casey & Turner, 1998; Kohler et al., 2002;

Kohler & Turner, 2008; Vandeperre et al., 2014). Sexual segregation was also evident, with a concentration of mature females in more temperate waters of the northernmost NA, and immature males predominant in the southernmost NA (*Sampaio da Costa, 2013*). Mature BS of both sexes seemed to be distributed in the southern part of NA, while immature individuals of both sexes and sub-adult females are usually distributed in the northern areas (*Kohler et al., 2002*). Conversely, a prevalent occurrence of immature juveniles is reported in the Mediterranean Sea (*Megalofonou, Damalas & De Metrio, 2009; Kohler et al., 2002*). A significant genetic heterogeneity among potential BS nurseries from the Atlantic Ocean (Portugal and Azores) and those from South Africa was detected by *Sampaio da Costa (2013)* from mitochondrial and nuclear marker variation. Their finding indicated a deeper separation between the northern and the southern NA nurseries and supported a male philopatry behaviour to mating areas exclusively contributing to a single nursery ground. Contradictorily, a recent genetic survey (*Verissimo et al., 2017*) carried out on the same dataset (i.e., young-of-year and <2 years juveniles) collected from the same nurseries, enriched with more samples from different areas (i.e., coasts of Brazil), and using the same type of markers, showed a lack of spatio-temporal genetic differentiation, suggesting the presence of a panmictic population in the whole Atlantic.

To date, no genetic data are available for the Mediterranean BS population and population structure and dynamics of BS in the Mediterranean are presently inferred only by Atlantic-Mediterranean integrated tagging studies and fishing data assessments (*Kohler, Casey & Turner, 1998; Kohler et al., 2002; Ferretti et al., 2008; Kohler & Turner, 2008; Megalofonou, Damalas & De Metrio, 2009*).

Irrespective of the small recapture rate (out of the 91,450 BS specimens tagged in the north western Atlantic, only 5.9% were recaptured), extensive tag-recapture surveys carried out from 1962 to 2000, indicated that North Atlantic BS form a single stock and that trans-Atlantic migratory movements were quite frequent, likely favoured by the oceanic current system (*Kohler et al., 2002*). Focusing on the Atlantic–Mediterranean connectivity, the reproductive migratory movements of Atlantic BS towards Mediterranean and the degree of population connectivity between the two areas are still unknown, because only one adult BS male tagged in the north-western Atlantic and one sub adult female tagged in the North-Eastern Atlantic were recaptured in the Mediterranean (*Kohler et al., 2002*). The large majority of BS tagged in the Mediterranean Sea were immature and remained in the tagging area, with the only exception of a subadult female that moved a short distance to the adjacent north-eastern Atlantic area. Most of the BS caught in the Mediterranean (99% and 98% for males and females, respectively) are immature, indicating that the Mediterranean BS stock consists primarily of small immature BS of both sexes, with a sex-ratio skewed toward females or males, depending on different geographical areas (*Kohler et al., 2002; Megalofonou, Damalas & De Metrio, 2009*). A high number of pregnant females was observed in the Adriatic, North Ionian Sea and Ligurian Sea, suggesting potential nursery grounds for BS (*Megalofonou, Damalas & De Metrio, 2009; F Garibaldi, pers. comm., 2017*). On the other hand, the adjacent South-Eastern North Atlantic BS was prevalently

composed by primarily mature individuals of both sexes with male-based sex ratio.

The primary aims of this study is to test the null hypothesis of panmixia between North Atlantic and Mediterranean BS, by comparing the mtDNA genetic variation of two gene regions, the control region (CR) and the Cytochrome b (Cytb) among four population samples collected in the North-Eastern and South-Eastern North Atlantic and in the Western and Eastern Mediterranean. Given the female philopatry observed in other carcharhiniformes (*Mourier & Planes, 2013; Tillett et al., 2012*), mtDNA markers are likely to be useful to spot localised groups due to site-fidelity. Accordingly, this work aims to provide further and needed data on matrilineal genetic structure, female philopatry and demography of Mediterranean BS. These, previously lacking, data will contribute to a better understanding and inclusion of the Mediterranean BS dynamics in the wider North Atlantic population model, to improve assessment and management of BS stocks in the area.

MATERIAL AND METHODS

Blue shark sampling

Sampling was carried out between 2003 and 2016 and tissue specimens and individual data were collected by means of commercial fishermen and scientific surveys. Mediterranean BS were collected from multiple locations in the Eastern (Central Adriatic, CADR, 21; Ionian Sea, IONI, 15; Aegean Sea and Levantine Sea, AEGE, 20) and Western areas (South Tyrrhenian, TYRR, 10; Ligurian Sea, LIGU, 57; Balearic Islands, BALE, 42). North Atlantic BS were caught from the North Eastern Atlantic Ocean off the coasts of Portugal (SNEATL, 33) and Celtic Sea (NNEATL, 16) (*Fig. 1*). A total of 214 BS individuals were collected ($N = 91$ males, $N = 101$ females and $N = 22$ unsexed) (*Table S1*). The BS individuals were grouped according the Total Length (TL) in three size categories (*Pratt, 1979; Vandepierre et al., 2014*): juveniles (J, $TL \leq 120$ cm), young (Y, $TL = 120\text{--}180$ cm) and large (L, $TL \geq 180$ cm).

A unique and transparent sampling documentation tool was developed within the project, in order to render data public. This tool can be used by everyone as an interactive map visiting the website: <https://fishreg.jrc.ec.europa.eu/web/medbluesgen/sampling-data>.

Molecular methods

Individual fin clips or skeletal muscle tissue samples were collected and preserved in 96% ethanol and kept at -20 °C until laboratory analyses. DNA extraction was carried out using the Invisorb[®] Spin Tissue Kit, Invitex (STRATEC Molecular, Birkenfeld, Germany) and the Wizard[®] Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturers' protocols.

Species-specific primer pairs for the amplification of the mitochondrial control region (CR) and cytochrome b (Cytb) genes were designed. Homologous complete CR and Cytb sequences of *Prionace glauca* available in GenBank were retrieved and aligned using ClusterW algorithm implemented in MEGA ver.7.0 (*Tamura et al., 2013*). Primer pairs were designed using the online software PRIMER3 (ver.0.4.0) (*Untergasser et al., 2012*), minimizing the propensity of oligos to form hairpins or dimers or to hybridize or prime from unintended

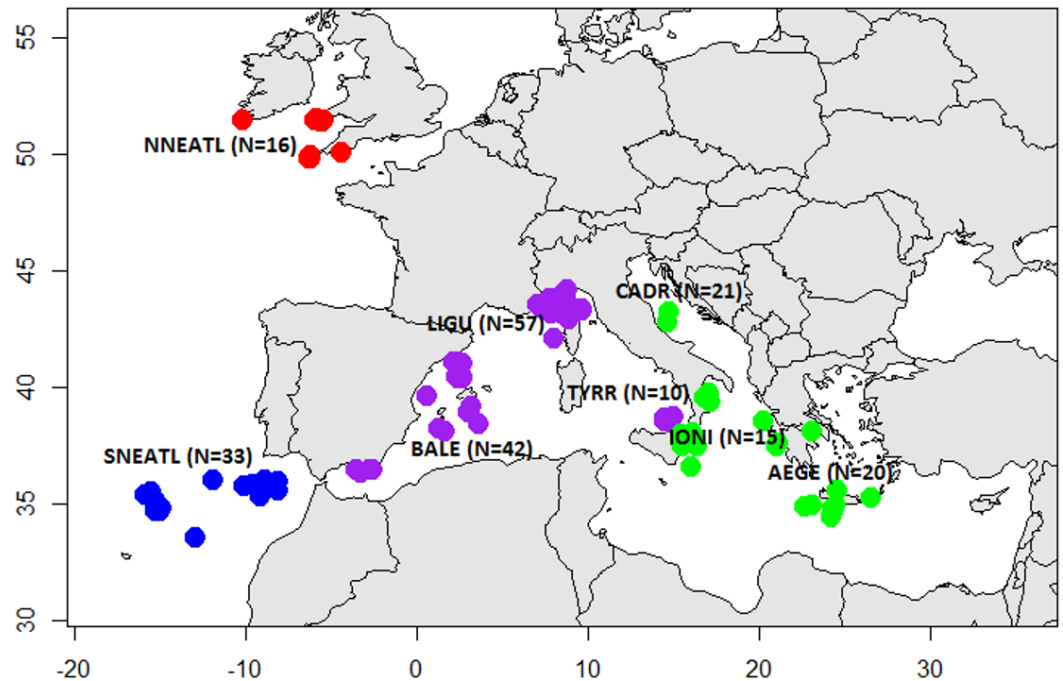


Figure 1 Sampling sites of Mediterranean and North Eastern Atlantic Blue Sharks. North North-Eastern Atlantic (NNEATL, red dots, $N = 16$), South North-Eastern Atlantic (SNEATL, blue dots, $N = 33$), Western Mediterranean (WMED, purple dots, $N = 109$) and Eastern Mediterranean (EMED, green dots, $N = 56$). The map was created using R version 3.4.1 (*R Core Team, 2016; Becker, Wilks & Brownrigg, 2017*).

Full-size DOI: [10.7717/peerj.4112/fig-1](https://doi.org/10.7717/peerj.4112/fig-1)

sites in the full mitochondrial BS genome (Acc. Num. [NC_022819](https://doi.org/10.1016/j.mbs.2013.05.001), *Chen et al., 2013*).

The designed primer pairs (control region: CR-Blues-F 5'AAACACATCAGGGGAAGGA G3', CR-Blues-R 5'CATCTTAGCATCTTCAGTGCC3'; Cytochrome-b: Cytb-Blues-F 5'TCCTCACAGGACTCTTCCTAGC3', Cytb-Blues-R 5'GTCGAAAGATGGTGCTTCGT3') were tested using a temperature gradient to identify the most suitable melting temperatures ($T_m =$ from 50 °C to 60 °C) according to PCR cycling conditions described by *Ovenden et al. (2009)*.

Once the optimal melting temperature was identified, the PCR thermal profile was adjusted and the PCR reactions were performed for both markers in a final volume of 50 μ L containing 31.75 μ L of distilled sterile H₂O, 8 μ L of Buffer 10 \times (Tris-HCl; final 1 \times), 3 μ L of MgCl₂ (25 mM; final 1.5 mM), 2 μ L of dNTPs (10 mM; final 0.37 mM), 2.5 μ L (10 μ M; final 0.46 μ M) of each primer, 0.25 μ L (5U/ μ L; final 1.5U) of *Taq* polymerase and 2 μ L of template DNA (10–20 ng). The temperature profile included an initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, elongation at 72 °C for 30 s and a final elongation step at 72 °C for 5 min. PCR amplicons were sequenced using the external service provider MACROGEN[®] Europe.

Data analysis

The CR and Cytb nucleotide sequences obtained were validated with the homologous gene sequences deposited in the GenBank with the BLASTn search implemented in the NCBI website (Altschul et al., 1990), and aligned using the ClusterW algorithm implemented in MEGA ver.7.0 (Tamura et al., 2013). When aligned to the complete BS mitochondrial genome, Cytb sequences mapped from nucleotide position 14,530 to 15,291 and CR from 15,651 to 16,397.

Given the high potential of geographical dispersal of the species, sequence data were grouped according to the four geographical areas: EMED, WMED, SNEATL and NNEATL (Fig. 1). The software DNAsp v.5.10.01 (Librado & Rozas, 2009) was used to assess the genetic diversity parameters at both markers: the number of haplotypes (Nh), the number of polymorphic sites (S), the haplotype (h) and nucleotide diversity (π) with associated standard deviation (stdev).

Haplotype relationships were inferred using the dnaml program of the PHYLIP package version 3.6 (Felsenstein, 1989; Felsenstein, 2005) implemented in the software program HaploViewer (<http://www.cibiv.at/~greg/haploviewer>).

Partition of molecular variance and its significance was estimated with the AMOVA (Excoffier, Smouse & Quattro, 1992) implemented in Arlequin ver 3.5.2.2 software (Excoffier & Lischer, 2010), testing four alternative groupings of geographical sampling locations (1: no groups; 2: NNEATL+SNEATL vs WMED+EMED; 3: NNEATL+SNEATL vs WMED vs EMED; 4: NNEATL vs SNEATL vs WMED vs EMED). Haplotype frequencies and pairwise Φ_{ST} with the associated p-values were calculated using the software Arlequin ver 3.5.2.2 (Excoffier & Lischer, 2010) after 20,000 permutations, setting up a $\alpha = 0.05$ significance threshold level.

Demographic history was investigated using the mismatch distribution as implemented in the DNAsp software (Librado & Rozas, 2009).

Furthermore, historical demographic trend of the four groups was investigated using Bayesian Skyline Plot (BSP) analysis implemented in the software BEAST v.1.8.2 (Drummond et al., 2005; Drummond et al., 2012), using the best evolutionary models for both Cytb and CR markers inferred using JModelTest 2.1.1 (Darriba et al., 2012), and the average mutation rate for sharks, 0.62% and 0.31% for CR and Cytb respectively (Martin & Palumbi, 1993; Galván-Tirado et al., 2013). The same software and parameters, with associate software TreeAnnotator and FigTree, were used to define the phylogeny of the Mediterranean and Eastern Atlantic BS populations.

RESULTS

Among sexed individuals ($N = 192$; Table S1), BS females significantly outnumbered males in the NA samples (sex-ratio 0.34, χ^2 test: 10.256 $P_{2tail} = 0.001$; $P_{1tail} = 0$, *d.f.* 1) while in the two Mediterranean BS groups a weak and not significant predominance of males was observed (WMED: 1.19, χ^2 test: 0.786 $P_{2tail} = 0.375$; $P_{1tail} = 0.188$, *d.f.* 1; EMED: 1.09, χ^2 test: 0.087 $P_{2tail} = 0.768$; $P_{1tail} = 0.384$, *d.f.* 1). Sized BS ($N = 209$) were composed by 63 juvenile, 82 young and 64 large individuals (Table S1). In the NA and WMED the young

Table 1 Mitochondrial gene polymorphism of *Prionace glauca* population samples subdivided according to the four macro areas.

POP	N	Nh	S	h	stdev h	π	stdev π
Cytb							
NNEATL	14	9	6	0.835	0.010	0.00231	0.00046
SNEATL	33	8	10	0.822	0.034	0.00200	0.00038
WMED	105	13	6	0.801	0.023	0.00167	0.00011
EMED	55	10	6	0.784	0.033	0.00151	0.00013
TOTAL	207	23	16	0.821	0.013	0.00184	0.00010
CR							
NNEATL	6	6	15	1.000	0.093	0.00812	0.00106
SNEATL	33	17	13	0.932	0.026	0.00424	0.00038
WMED	79	34	18	0.949	0.011	0.00418	0.00019
EMED	52	19	12	0.894	0.028	0.00382	0.00031
TOTAL	170	55	27	0.951	0.006	0.00453	0.00014

Notes.

N, number of individuals; Nh, number of haplotypes; S, Number of segregating informative sites; h, haplotype diversity and associate standard deviation; π , nucleotide diversity and associate standard deviation; NNEATL, North North–eastern Atlantic; SNEATL, South Northeastern Atlantic; WMED, Western Mediterranean; EMED, Eastern Mediterranean.

BS (TL = 120–180 cm; 48% and 50%, respectively) were predominant, while in the EMED a large predominance of juveniles was observed (TL \leq 120 cm; 63%). Noticeably 67% of the BS sampled in the Ionian Sea and 95% of those sampled in the Adriatic Sea were juveniles. Large BS are similarly represented in the geographical groups with percentages varying from 25% (EMED) to 34% (NA), full details presented in [Table S1](#).

A total of 207 and 170 BS individuals were sequenced for Cytb (762 bp) and CR (747 bp), respectively. Haplotype sequences (Cytb, N = 23 and CR, N = 55) were deposited in GenBank under the accession numbers [MG515900–MG516106](#) and [MG545732–MG545901](#) for Cytb and control region, respectively.

The Cytb sequence dataset exhibited 16 polymorphic segregating sites while CR dataset showed 27 polymorphic segregating sites. The Cytb haplotype diversity ranged from 0.784 to 0.835, and that of the CR from 0.894 to 1.000. The Cytb nucleotide diversity ranged from 0.001 to 0.002, and that of the CR from 0.004 to 0.008. Detailed genetic diversity of BS samples collected from the four macro areas and all sampling locations is presented in [Table 1](#) and [Table S2](#), respectively.

The Cytb and CR haplotype networks highlighted the distribution of haplotypes irrespective of the geographical origin of BS samples, indicating the lack of phylogeographical structure in the Mediterranean and adjacent North Atlantic BS (see [Fig. 2](#), [Fig. S1](#)). In the Cytb network, the four main frequent haplotypes were shared by BS from all the four geographical areas, except for the most frequent haplotype which was shared by BS from the three geographical areas, SNEATL, WMED and EMED. In the CR network, six most frequent haplotypes (No. individuals \geq 10) were observed. Although these six haplotypes were shared by all geographical areas, three of them were shared by Mediterranean and SNEATL, one by Mediterranean and NNEATL, and two within the Mediterranean. In both networks, most of the NNEATL haplotypes were singletons ([Fig. 2](#)).

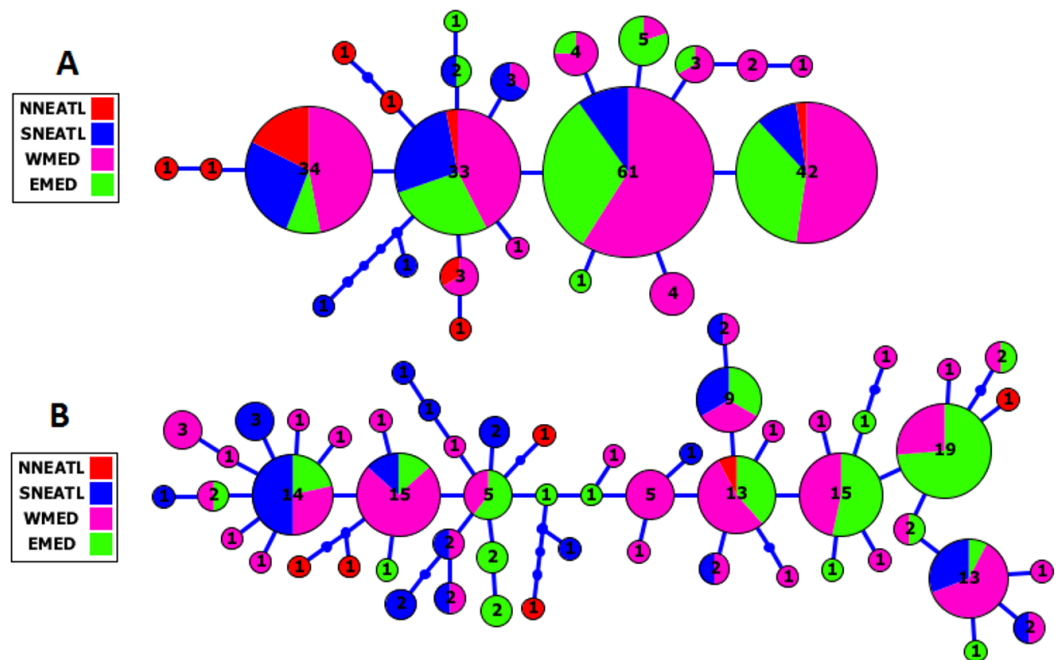


Figure 2 Cytochrome-b (A) and Control Region (B) Maximum Likelihood Haplotype Network of Mediterranean/North East Atlantic Blue Shark collected from the four geographical areas. NNEATL: North North–Eastern Atlantic; SNEATL: South North–Eastern Atlantic; WMED: Western Mediterranean; EMED: Eastern Mediterranean.

Full-size [DOI: 10.7717/peerj.4112/fig-2](https://doi.org/10.7717/peerj.4112/fig-2)

The AMOVA (Table 2) revealed a significant overall Φ_{ST} among population samples for both markers. Significant partition of molecular variance among areas was observed when BS sampling locations were grouped according to the four geographical areas in both markers (AMOVA4), according to three areas (NEATL (NNEATL+SNEATL) vs WMED vs EMED; AMOVA3), and according to two areas (NEATL (NNEATL+SNEATL) vs MED (WMED+EMED)), for both dataset. However, the grouping that best described the partitioning of genetic variance is when the different sampling locations are subdivided into four areas showing the lowest partition of molecular variance among populations within group.

With the Cytb sequence data, all pairwise Φ_{ST} values among the four geographical areas were significant except that between the two Atlantic groups ($\Phi_{ST} = 0.1152$; $p = 0.019$) that became non-significant after the Bonferroni correction for multiple tests (Martin & Douglas, 1995) (Table 3). Unlike the CR dataset, only the pairwise Φ_{ST} values between SNEATL and the two Mediterranean areas and between WMED and EMED remained significant after the Bonferroni correction for multiple tests (Table 3).

AMOVA and pairwise Φ_{ST} analyses were performed on a reduced dataset, selecting only juvenile and immature specimens from each sampling site. Despite the reduced sample sizes and the complete absence of data from the site NNEATL, the results obtained are in agreement with the values observed with the complete dataset (Tables S3 and S4).

Table 2 Analysis of molecular variance (AMOVA) of Cytochrome b (Cytb) and Control Region (CR) of the Mediterranean and North–eastern Atlantic Blue Sharks (*Prionace glauca*).

	Cytb			CR		
	% variation	Φ -Statistics	<i>p</i>	% variation	Φ -Statistics	<i>p</i>
<i>AMOVA1: Overall (all population samples)</i>						
Among populations	8.20			11.25		
Within populations	91.80	ST = 0.0819	0.00000	88.75	ST = 0.11249	0.00000
<i>AMOVA2: 2 groups: (NNEATL+SNEATL vs WMED+EMED)</i>						
Among groups	12.39	CT = 0.1239	0.03496	7.89	CT = 0.0788	0.03471
Among pops within group	2.40	SC = 0.0273	0.02287	7.41	SC = 0.0804	0.00005
Within populations	92.68	ST = 0.1479	0.00000	84.70	ST = 0.1529	0.00000
<i>AMOVA3: 3 groups: (NNEATL+SNEATL vs WMED vs EMED)</i>						
Among groups	7.01	CT = 0.0701	0.02188	5.68	CT = 0.0568	0.03656
Among pops within group	2.84	SC = 0.0305	0.02397	6.78	SC = 0.0719	0.00075
Within populations	90.15	ST = 0.0985	0.00000	87.54	ST = 0.1246	0.00000
<i>AMOVA4: 4 groups: (NNEATL vs SNEATL vs WMED vs EMED)</i>						
Among groups	8.87	CT = 0.0887	0.02073	7.93	CT = 0.0793	0.03726
Among pops within group	1.20	SC = 0.0132	0.13076	4.89	SC = 0.0531	0.00649
Within populations	89.92	ST = 0.1007	0.00000	87.18	ST = 0.1282	0.00000

Table 3 Pairwise Φ_{st} values (below the diagonal) and associated *p*-values (above the diagonal) among the blue sharks of the four geographical areas.

	NNEATL	SNEATL	WMED	EMED
Cytb				
NNEATL		0.01868*	0.00000	0.00000
SNEATL	0.08167*		0.00055	0.00015
WMED	0.23969	0.08633		0.20052
EMED	0.29481	0.12441	0.00658	
CR				
NNEATL		0.0097*	0.0482*	0.0187*
SNEATL	0.1649†		0.0003	0.0000
WMED	0.1061†	0.1049		0.0072
EMED	0.1620†	0.2188	0.0463	

Notes.

*Values that resulted not significant after the Bonferroni correction for multiple tests (a-level of significance after Bonferroni correction: $p = 0.0083$).

The Cytb distribution of sequence mismatch pairwise differences showed a skewed unimodal distribution in all four BS macro areas suggesting a recent bottleneck or sudden population expansion (Fig. S2). A unimodal mismatch distribution was obtained with CR dataset in the NNEATL BS. The CR mismatch distribution of EMED, SNEATL and NNEATL BS resulted to a slightly ragged pattern (Fig. S2) that could suggest a more constant population size of the Mediterranean BS over generations.

Both BSP analyses suggested a constant population size increase of Mediterranean and North–eastern Atlantic BS, starting more recently in the Mediterranean than in

the North–eastern Atlantic (~ 0.02 – 0.15 Mya vs 0.15 – 0.4 Mya; Fig. 3). Divergence time analysis based on both markers (Fig. S3) highlights a similar pattern of separation between two main groups, composed by BS from all regions, without any evidence of separation between defined geographic areas. The separation between the two clades, which is strongly supported of Posterior Probability ($PP = 1.0$) in both markers, is dated back to 1.24 Mya and 0.94 Mya using Cytb and control region, respectively.

DISCUSSION AND CONCLUSIONS

The BS is probably the most mobile shark species in the world (Stevens, 1990) and past research works, using both mitochondrial and nuclear markers, have struggled to find genetic structure at interoceanic scale (Sampaio da Costa, 2013; King et al., 2015; Li et al., 2016; Veríssimo et al., 2017). This high level of gene flow make it difficult to define clear BS population units. In the Pacific Ocean, the lack of structure may be the result of the combination of high potential of migration and the lack of effective barriers to gene flow (Veríssimo et al., 2017).

Experimental data have indicated that no significant genetic structure is detected in spatially distant BS samples (King et al., 2015; Li et al., 2016; Veríssimo et al., 2017). Our results revealed significant signals of geographical structuring for Mediterranean and adjacent Atlantic BS, with several frequent mtDNA haplotypes that are exclusive of the Mediterranean BS and other that are shared with the Atlantic population samples.

While both haplotype networks failed to evidence a clear geographical structure, either between Mediterranean and North Atlantic BS or within the Mediterranean, the results of AMOVA revealed a significant partition of molecular variance among all population samples and when they were grouped according to the four geographical areas with both mitochondrial markers (8.87% for Cytb and 7.93% for CR). Previous studies carrying out AMOVA on the Atlantic BS using the control region variation, showed a significance variance among groups formed by the North Atlantic BS collected from Portugal and Azores and by the South African (See Table 7 of Sampaio da Costa, 2013) or Brazilian BS (Veríssimo et al., 2017). On the contrary, the global population genetics carried out by Fitzpatrick (2012), using concatenated fragments from: 16S, tRNA, COII, ATPase and control region genes, showed no significance variation among oceans, based upon comparisons between North Atlantic and all sampling locations combined (See Table 5.7 of Fitzpatrick, 2012). Although BS exhibits high potential of dispersal and migration, our results seem to reject an absence of geographical structure in the Mediterranean and adjacent North-eastern Atlantic BS. The pairwise Φ_{st} analysis revealed a geographical structuring between the two Mediterranean groups and Southern North–eastern Atlantic BS, with a closer genetic similarity of the Southern North–eastern Atlantic with the Western Mediterranean BS rather than with the Eastern Mediterranean BS. This pattern of differentiation seems to suggest that reproductive movements, such as female philopatry, may occur between the Western Mediterranean and the Southern North–eastern Atlantic BS. In addition, pairwise Φ_{st} values highlighted that the EMED BS are the more divergent from the NATL BS Given that SNNEATL specimens are from a previously identified

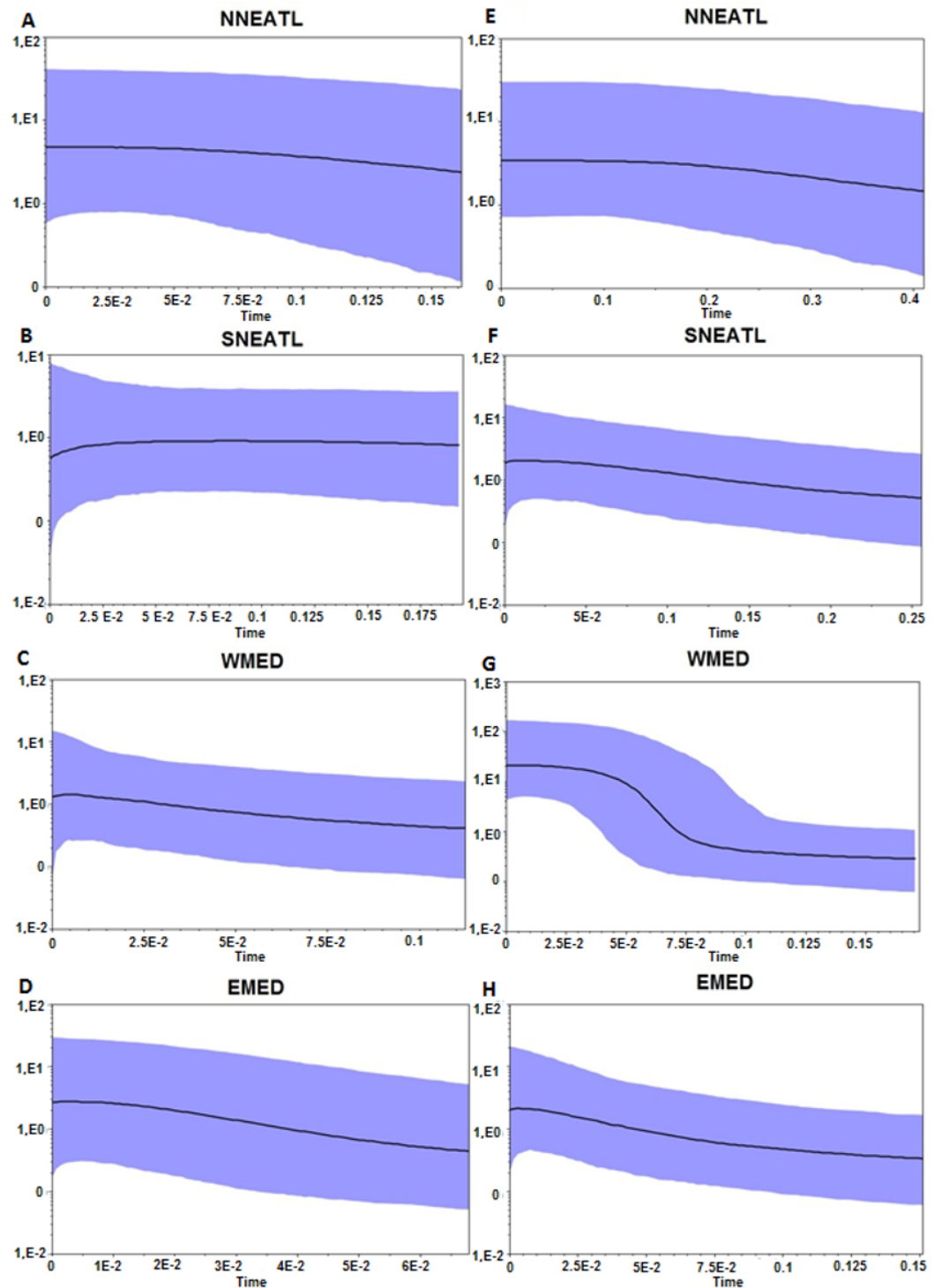


Figure 3 Bayesian Skyline Plot from the Cytb, A–D, and control region, E–H, of the four different geographical areas. NNEATL: North North–eastern Atlantic; SNEATL: South North–eastern Atlantic; WMED: Western Mediterranean; EMED: Eastern Mediterranean. The Y-axis indicates effective population size (N_e) \times generation time, while the X-axis indicates mean time in million of years before present. The thick line represents the average, while the blue band represents 95% highest posterior density (HPD) intervals.

Full-size DOI: 10.7717/peerj.4112/fig-3

nursery site (Veríssimo *et al.*, 2017), the pairwise Φ_{ST} values could suggest that specimens from WMED can be reproductively related to the SNNEATL, while EMED could represent a nursery site in itself (Megalofonou, Damalas & De Metrio, 2009).

Our sampling work has also preliminarily revealed significant differences between North-eastern Atlantic and Mediterranean BS by sex-ratio and size. This pattern could be the result of a sex-biased reproductive migratory behaviour that could contribute to explain the significant phylogeographical structure. Similarly, size differences were observed between WMED and EMED BS, with the large and sexually mature individuals abundant in the easternmost Mediterranean sampling location (Aegean Sea) while the sub-adult and juvenile BS frequent in the Adriatic and Ionian Seas. The great abundance of juvenile BS in the Adriatic Sea seemed to confirm the nursery role of this area for BS (Megalofonou, Damalas & De Metrio, 2009). The biological data reported in Megalofonou and colleagues (2009) describe a larger amount of big female in the easternmost Mediterranean (e.g., Aegean Sea) which is in agreement with the pattern inferred from our dataset. Conversely, using data on size and maturity stages, Kohler and colleagues (2002) observe that the majority of sharks from the Mediterranean Sea were juvenile and immature (99% of males and 98% of females; mean = 65 cm of fork length). The difference may be related to the different sampling design and fishing gear used in the studies. In fact, the majority of the data collected by Kohler and colleagues (2002) came from volunteer recreational fishermen, while the individuals from Megalofonou, Damalas & De Metrio (2009) and from this work, originated principally as by-catch from commercial fisheries, such tuna and swordfish longline.

Overall, Mediterranean and adjacent North-eastern Atlantic BS displayed a complex geographical structure in which weak but significant differences proved that a certain degree of population connectivity across the Strait of Gibraltar occurred. These results are in contrast with those obtained by tagging data in the past (Kohler, Casey & Turner, 1998; Kohler *et al.*, 2002; Kohler & Turner, 2008; Poisson *et al.*, 2015). Similar findings of genetic differences were observed in other shark species, more related to a benthic environment, such the small-spotted catshark, *Scyliorhinus canicula*, and the velvet belly lanternshark, *Etmopterus spinax* (Gubili *et al.*, 2014; Gubili *et al.*, 2016; Kousteni *et al.*, 2015). The reported evidence of genetic structure in the blue shark analyzed in this study are associated with geographical differences in sex-ratio and size. Our results suggest BS in the NE Atlantic and the Mediterranean are not panmictic. There is still no direct observations of mating events take place in the Eastern Mediterranean, but the biological data analysis results support the Eastern Mediterranean as an important nursery area for this species (Megalofonou, Damalas & De Metrio, 2009). Such microevolutionary pattern of differentiation of Mediterranean and North-eastern Atlantic BS prompt the need for a deeper population genetic analysis on the same population samples with more powerful markers for investigating potential subtle structure of BS populations (e.g., microsatellites or SNPs) to provide robust data on BS population structure that are of priority for the BS stock management. High genetic diversity values are usually related to large population size (Frankham, 1996), and the high genetic diversity showed by both Mediterranean and North-eastern Atlantic BS at the two mitochondrial markers advocates in favour of a large

size of these populations. Mediterranean and North–eastern Atlantic BS showed higher Cytb gene polymorphism than Pacific BS (Mediterranean and North–eastern Atlantic: $h = 0.777\text{--}0.814$; $\pi = 0.002\text{--}0.004$; Pacific: $h = 0.517\text{--}0.768$; $\pi = 0.0007\text{--}0.0011$, *Li et al., 2016*).

Based on nuclear markers, similar values of observed heterozygosity were detected between Pacific and North Atlantic BS (*Sampaio da Costa, 2013; King et al., 2015; Veríssimo et al., 2017*). High genetic diversity in abundant species is likely due to a combination of demographic factors, such as local population sizes, fast generation times and high rates of gene flow with other populations (*Hague & Routman, 2016*). The high genetic diversity shown by Mediterranean and North–eastern Atlantic BS could be a consequence of the short time elapsed, in proportion to the relative generation time, since the population started to suffer overexploitation. In fact, the abundance of the Mediterranean BS has declined by $\sim 78\text{--}90\%$ over the past 30 years (*Ferretti et al., 2008*), approximately corresponding to three generations; the BS generation time was estimated at 8.2 and 9.8 years for South African and North Atlantic populations, respectively (*Cortés et al., 2015*). Furthermore, biological characters such as the large size of litters, the low nucleotide substitution rate compared to other vertebrates (*Martin, Naylor & Palumbi, 1992*), the high potential of migration and the high gene flow between geographical distant populations, may have affected the relationship between genetic diversity and population size, masking the sudden potential population bottleneck of the last three decades, without genetic erosion.

Otherwise, the mismatch distributions of the different macro areas appear to be slightly skewed unimodal, related to a recent bottleneck or a sudden population expansion (*Fig. S2*), and given the Bayesian skyline plots (*Fig. 3*), there is overwhelming evidence that the Mediterranean and North East Atlantic populations have undergone a constant population expansion during the last 400–200 Kya, especially within the Mediterranean samples.

The data we show here represent a novelty for the knowledge of Mediterranean blue shark, and our findings highlight the importance of the Mediterranean Sea as nursery area for this species, with direct implication to specific conservation measures for the species.

This work sheds new light on the understudied BS of the Mediterranean Sea, and emphasizes the need of conducting further population genetic surveys on this population. With ongoing efforts, (i.e., <https://fishreg.jrc.ec.europa.eu/web/medbluesgen/>) a greater understanding of the genetic diversity, spatial population structure and gene flow in this species will be achieved, which will enable us to devise more effective strategies for the management of this increasingly exploited ocean predator.

ACKNOWLEDGEMENTS

We are grateful to all MEDBLUESGEN partners that contributed to the fulfilment of project, including both scientific staff and administrative colleagues. We thank Clara Hugon for the valuable technical support.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This research was funded by the European Commission, Joint Research Center (JRC) with the Tender “MEDBLUESGEN—A population genetic study on Mediterranean blue shark for stock identification and conservation” (<https://fishreg.jrc.ec.europa.eu/web/medbluesgen/>; Negotiated call Procedure no. JRC/IPR/2014/G.3/0023/NC and Contract number 259844), and by the Genetics & Genomics of Marine Resources and Environment Laboratory (GenoDREAM), Department of Biological, Geological & Environmental Sciences (BiGeA) of the University of Bologna, Italy. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
European Commission, Joint Research Center (JRC).
University of Bologna, Italy.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Agostino Leone and Ilenia Urso conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Dimitrios Damalas, Jann Martinsohn, Antonella Zanzi and Stefano Mariani analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.
- Emilio Sperone, Primo Micarelli, Fulvio Garibaldi, Persefoni Megalofonou, Luca Bargelloni, Rafaella Franch, David Macias, Paulo Prodöhl, Séan Fitzpatrick and Marco Stagioni contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Fausto Tinti and Alessia Cariani conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.

Data Availability

The following information was supplied regarding data availability:

Sequences are available in Genbank with related biological data under accession numbers [MG515900–MG516106](#) and [MG545732–MG545901](#) for Cytb and control region, respectively, and raw data is presented in the [Supplemental Files](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.4112#supplemental-information>.

REFERENCES

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215(3):403–410
DOI 10.1016/S0022-2836(05)80360-2.
- Becker RA, Wilks AR, Brownrigg R. 2017. maps: draw geographical maps. R package version 3.2.0. Available at <http://CRAN.R-project.org/package=maps>.
- Chen X, Xiang D, Ai W, Shi X. 2013. Complete mitochondrial genome of the blue shark *Prionace glauca* (Elasmobranchii: Carcharhiniformes). *Mitochondrial DNA* 26(2):313–314 DOI 10.3109/19401736.2013.825790.
- Clarke SC, McAllister MK, Milner-Gulland EJ, Kirkwood GP, Michielsens CGJ, Agnew DJ, Pikitch EK, Nakano H, Shivji MS. 2006. Global estimates of shark catches using trade records from commercial markets. *Ecology Letters* 9(19):1115–1126
DOI 10.1111/j.1461-0248.2006.00968.x.
- Coelho R, Mejuto J, Domingo A, Yokawa K, Liu K-M, Cortés E, Romanov EV, Da Silva C, Hazin F, Arocha F, Mwilima AM, Bach P, De Zárata VO, Roche W, Lino PG, García-Cortés B, Ramos-Cartelle AM, Forselledo R, Mas F, Ohshimo S, Courtney D, Sabarros PS, Perez B, Wogerbauer C, Tsai W-P, Carvalho F, Santos MN. 2017. Distribution patterns and population structure of the blue shark (*Prionace glauca*) in the Atlantic and Indian Oceans. *Fish and Fisheries* 2017:1–17 DOI 10.1111/faf.12238.
- Compagno LJV. 1984. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. In: *FAO species catalogue. Vol. 4, part 2, FAO fisheries synopsis*. Rome: FAO, 251–633.
- Cortés E, Domingo A, Miller P, Forselledo R, Mas F, Arocha F, Campana S, Coelho R, Da Silva C, Hazin FHV, Holtzhausen H, Keene K, Lucena F, Ramirez K, Santos MN, Semba-Murakami Y, Yokawa K. 2015. Expanded ecological risk assessment of pelagic sharks caught in Atlantic pelagic longline fisheries. *Collective Volume of Scientific Papers ICCAT* 71(6):2637–2688 SCRS/2012/167.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9(8):772 DOI 10.1038/nmeth.2109.
- Drummond AJ, Rambaut A, Shapiro B, Pybus OG. 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution* 22(5):1185–1192 DOI 10.1093/molbev/msi103.
- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and BEAST 1.7. *Molecular Biology and Evolution* 29(8):1969–1973
DOI 10.1093/molbev/mss075.
- Dulvy NK, Fowler SL, Musick JA, Cavanagh RD, Kyne PM, Harrison RL, Carlson JK, Davidson LNK, Fordham SV, Francis MP, Pollock CM, Simpfendorfer CA, Burgess GH, Carpenter KE, Compagno LJV, Ebert DA, Gibson C, Heupel MR, Livingstone SR, Sanciangco JC, Stevens JD, Valenti S, White WT. 2014. Extinction risk and conservation of the world's sharks and rays. *eLife* 3:e00590
DOI 10.7554/eLife.00590.001.

- Excoffier L, Lischer HE. 2010.** Arlequin suite ver3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**(3):564–567 DOI [10.1111/j.1755-0998.2010.02847.x](https://doi.org/10.1111/j.1755-0998.2010.02847.x).
- Excoffier L, Smouse P, Quattro J. 1992.** Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**:479–491.
- Felsenstein J. 1989.** PHYLIP—phylogeny inference package (Version 3.2). *Cladistics* **5**:164–166.
- Felsenstein J. 2005.** “PHYLIP (phylogeny inference package)” Distributed by the author. Version 3. Seattle: Department of Genome Sciences, University of Washington. Available at <http://evolution.genetics.washington.edu/phylip.html>.
- Ferretti F, Myers RA, Serena F, Lotze HK. 2008.** Loss of large predatory sharks from the Mediterranean Sea. *Conservation Biology* **22**(4):952–964 DOI [10.1111/j.1523-1739.2008.00938.x](https://doi.org/10.1111/j.1523-1739.2008.00938.x).
- Fitzpatrick SF. 2012.** Global population genetic structure of the pelagic blue shark (*Prionace glauca*). PhD Thesis, Queen’s University Belfast.
- Fowler SL, Cavanagh RD, Camhi M, Burgess GH, Cailliet GM, Fordham SV, Simpfendorfer CA, Musick JA. 2005.** Sharks, rays and chimaeras: the status of the Chondrichthyan fishes. Status Survey. IUCN/SSC Shark Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK. 461 pp.
- Frankham R. 1996.** Relationship of genetic variation to population size in wildlife. *Conservation Biology* **10**(6):1500–1508 DOI [10.1046/j.1523-1739.1996.10061500.x](https://doi.org/10.1046/j.1523-1739.1996.10061500.x).
- Galván-Tirado C, Díaz-Jaimes P, García-de León FJ, Galván-Magaña FG, Uribe-Alcocer M. 2013.** Historical demography and genetic differentiation inferred from the mitochondrial DNA of the silky shark (*Carcharhinus falciformis*) in the Pacific Ocean. *Fisheries Research* **147**:36–46 DOI [10.1016/j.fishres.2013.03.020](https://doi.org/10.1016/j.fishres.2013.03.020).
- Gubili C, Macleod K, Perry W, Hanel P, Batzakas I, Farrell ED, Lynghammar A, Mancusi C, Mariani S, Menezes GM, Neat F, Scarcella G, Griffiths AM. 2016.** Connectivity in the deep: phylogeography of the velvetbelly lanternshark. *Deep Sea Research Part I Oceanographic Research Papers* **115**:233–239 DOI [10.1016/j.dsr.2016.07.002](https://doi.org/10.1016/j.dsr.2016.07.002).
- Gubili C, Sims DW, Veríssimo A, Domenici P, Ellis J, Grigoriou P, Johnson AF, McHugh M, Neat F, Satta A, Scarcella G, Serra-Pereira B, Soldo A, Genner MJ, Griffiths AM. 2014.** A tale of two seas: contrasting patterns of population structure in the small-spotted catshark across Europe. *Royal Society Open Science* **1**:Article 140175 DOI [10.1098/rsos.140175](https://doi.org/10.1098/rsos.140175).
- Hague MTJ, Routman EJ. 2016.** Does population size affect genetic diversity? A test with sympatric lizard species. *Heredity* **116**:92–98 DOI [10.1038/hdy.2015.76](https://doi.org/10.1038/hdy.2015.76).
- ICCAT (International Commission for the Conservation of Atlantic Tunas). 2015.** Report of the 2015 ICCAT Blue Shark stock assessment session. Lisbon, Portugal SCRS/P/2015/031.
- King JR, Wetklo M, Supernault J, Taguchi M, Yokawa K, Sosa-Nishizaki O, Withler RE. 2015.** Genetic analysis of stock structure of blue shark (*Prionace glauca*) in the north Pacific ocean. *Fisheries Research* **172**:181–189 DOI [10.1016/j.fishres.2015.06.029](https://doi.org/10.1016/j.fishres.2015.06.029).

- Kohler NE, Casey JG, Turner PA. 1998.** NMFS cooperative shark tagging program, 1962–93: an atlas of shark tag and recapture data. *Marine Fisheries Review* **60**(2):1–87.
- Kohler NE, Turner P. 2008.** Stock structure of the blue shark (*Prionace glauca*) in the North Atlantic Ocean based on tagging data. In: *Sharks of the open ocean: biology, fisheries and conservation*. Oxford: Blackwell Publishing Ltd, 339–350.
- Kohler NE, Turner PA, Hoey JJ, Natanson LJ, Briggs R. 2002.** Tag and recapture data for three pelagic shark species: blue shark (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*) and porbeagle (*Lamna nasus*) in the North Atlantic Ocean. *International Commission for the Conservation of Atlantic Tunas, Collective Volume of Scientific Papers* **54**(4):1231–1260 SCRS/2001/64.
- Kousteni V, Kasapidis P, Kotoulas G, Megalofonou P. 2015.** Strong population genetic structure and contrasting demographic histories for the small-spotted catshark (*Scyliorhinus canicula*) in the Mediterranean Sea. *Heredity* **114**:333–343 DOI [10.1038/hdy.2014.107](https://doi.org/10.1038/hdy.2014.107).
- Li W, Dai X, Zhu J, Tian S, He S, Wu F. 2016.** Genetic differentiation in blue shark, *Prionace glauca*, from the central Pacific Ocean, as inferred by mitochondrial cytochrome b region. *Mitochondrial DNA Part A* **19**:1–4 DOI [10.3109/19401736.2015.1137903](https://doi.org/10.3109/19401736.2015.1137903).
- Librado P, Rozas J. 2009.** DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**(11):1451–1452 DOI [10.1093/bioinformatics/btp187](https://doi.org/10.1093/bioinformatics/btp187).
- Martin AP, Naylor GJP, Palumbi SR. 1992.** Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. *Nature* **357**:153–157 DOI [10.1038/357153a0](https://doi.org/10.1038/357153a0).
- Martin AP, Palumbi SR. 1993.** Body size, metabolic rate, generation time, and the molecular clock. *Proceedings of the National Academy of Sciences of the United States of America* **90**(9):4087–4091 DOI [10.1073/pnas.90.9.4087](https://doi.org/10.1073/pnas.90.9.4087).
- Martin BJ, Douglas GA. 1995.** Multiple significance tests: the Bonferroni method. *British Medical Journal* **310**(21):170 DOI [10.1136/bmj.310.6973.170](https://doi.org/10.1136/bmj.310.6973.170).
- Megalofonou P, Damalas D, De Metrio G. 2009.** Biological characteristics of blue shark, *Prionace glauca*, in the Mediterranean Sea. *Journal of the Marine Biological Association of the United Kingdom* **89**(06):1233–1242 DOI [10.1017/S0025315409000216](https://doi.org/10.1017/S0025315409000216).
- Megalofonou P, Damalas D, Yannopolous C. 2005.** Composition and abundance of pelagic shark by-catch in the eastern Mediterranean Sea. *Cybium* **29**(2):135–140.
- Mourier J, Planes S. 2013.** Direct genetic evidence for reproductive philopatry and associated fine-scale migrations in female blacktip reef sharks (*Carcharhinus melanopterus*) in French Polynesia. *Molecular Ecology* **22**:201–214 DOI [10.1111/mec.12103](https://doi.org/10.1111/mec.12103).
- Ovenden JR, Kashiwagi T, Broderick D, Giles J, Salini J. 2009.** The extent of population genetic subdivision differs among four co-distributed shark species in the Indo-Australian archipelago. *BMC Evolutionary Biology* **9**:40 DOI [10.1186/1471-2148-9-40](https://doi.org/10.1186/1471-2148-9-40).
- Poisson F, Mitsunaga Y, Kojima T, Torisawa S, Seret B, Demarcq H, Banegue A, Groul JM. 2015.** Satellite tagging of blue sharks (*Prionace glauca*) in the Gulf of Lions:

- depth behaviour, temperature experience and movements—Preliminary results. In: Ceccaldi H-J, Hénocque Y, Koike Y, Komatsu T, Stora G, Tusseau-Vuillemin M-H, eds. *Marine productivity: perturbations and resilience of socio-ecosystems. Proceedings of the 15th French-Japanese oceanography symposium. Part VIII Short and Preliminary Communications*, 367–368. DOI 10.1007/978-3-319-13878-7_41.
- Pratt HL. 1979.** Reproduction in the blue shark, *Prionace glauca*. *Fishery Bulletin* 77(2):445–470.
- R Core Team. 2016.** R: a language and environment for statistical computing. Version 3.4.1. Vienna: R Foundation for Statistical Computing. Available at <https://www.R-project.org>.
- Sampaio da Costa IRF. 2013.** Genetic diversity and population structure of the cosmopolitan blue Shark *Prionace glauca* (Linnaeus, 1758) in the Atlantic Ocean. MSc Thesis, Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO), University of Porto.
- Sims D, Fowler SL, Ferretti F, Stevens JD. 2016.** *Prionace glauca*. The IUCN Red List of Threatened Species 2016: e.T39381A16553182.
- Stevens JD. 1990.** Further results from a tagging study of pelagic sharks in the north-east Atlantic. *Journal of the Marine Biological Association of the United Kingdom* 70(4):707–720 DOI 10.1017/S0025315400058999.
- Stevens J. 2009.** *Prionace glauca*. The IUCN Red List of Threatened Species 2009: e.T39381A10222811. Available at <http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T39381A10222811.en>.
- Tamura K, Stecher G, Peterson D, Stecher G, Filipski A, Kumar S. 2013.** MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30(12):2725–2729 DOI 10.1093/molbev/mst197.
- Tillett BJ, Meekan MG, Field IC, Thorburn DC, Ovenden JR. 2012.** Evidence for reproductive philopatry in the bull shark *Carcharhinus leucas*. *Journal of Fish Biology* 80:2140–2158 DOI 10.1111/j.1095-8649.2012.03228.x.
- Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. 2012.** Primer3—new capabilities and interfaces. *Nucleic Acids Research* 40(15):e115 DOI 10.1093/nar/gks596.
- Vandepierre F, Aires-da Silva A, Fontes J, Santos M, Serrão Santos R, Afonso P. 2014.** Movements of blue sharks (*Prionace glauca*) across their life history. *PLOS ONE* 9(8):e103538 DOI 10.1371/journal.pone.0103538.
- Veríssimo A, Sampaio Í, McDowell JR, Alexandrino P, Mucientes G, Queiroz N, Da Silva C, Jones CS, Noble LR. 2017.** World without borders—genetic population structure of a highly migratory marine predator, the blue shark (*Prionace glauca*). *Ecology and Evolution* 7(13):4768–4781 DOI 10.1002/ece3.2987.