The current knowledge status of the genetic population structure of the European sardine (*Sardina pilchardus*): uncertainties to be solved for an appropriate fishery management

To be submitted to: Reviews in Fish Biology and Fisheries

Marta Caballero-Huertas[1], Xènia Frigola-Tepe[2], Marta Coll[3], Marta Muñoz[1], Jordi Viñas[2]*

[1] Institute of Aquatic Ecology (IEA), Department of Environmental Sciences. Universitat de Girona (UdG), Campus Montilivi, 17003 Girona, Spain

[2] Laboratori d'Ictiologia Genètica (LIG), Department of Biology. Universitat de Girona (UdG), Campus Montilivi, 17003 Girona, Spain

[3] Institute of Marine Science, Spanish National Research Council (ICM-CSIC), Passeig Marítim de la Barceloneta, 37-49, 08003, Barcelona, Spain

* Corresponding author: Dr. Jordi Viñas; e-mail: jordi.vinas@udg.edu

ORCIDs: M. Caballero-Huertas 0000-0002-2616-1094; X. Frigola-Tepe 0000-0002-1194-0669; M. Coll 0000-0001-6235-5868; M. Muñoz 0000-0001-5194-9513; J. Viñas 0000-0003-0193-1141

Abstract

To achieve sustainable fisheries implies that resources' management is carried out in accordance with biologically and ecologically relevant processes. In this context, to infer the boundaries of the genetic stocks along their distribution is crucial to avoid the depletion of genetic diversity induced by fishing pressure. Despite its remarkable ecological role and commercial interest, there are still many uncertainties about the genetic population structure and local adaptation processes of the European sardine (*Sardina pilchardus*) along its distributional range. Our analysis revealed that in addition to the uneven genetic study effort throughout its distribution, there are discrepancies when it comes to delimiting populations, especially in the waters surrounding the Iberian Peninsula. Also, powers of the genetic markers applied in the studies were examined, showing that allozymes detected a larger number of significant pairwise values of genetic differentiation, while mtDNA-RFLP detected a greater degree of differentiation among genetic stocks. Moreover, large values of genetic diversity in all the locations were identified regardless of marker type. Thereby, we provide a discussion of updated knowledge, contributing to shape long-term and genetically sustainable harvest strategies for this pelagic

fish, since our findings indicate a mismatch between the genetic stocks and the managed stocks currently defined.

Keywords: Atlantic Ocean, fish stocks, local adaptation, Mediterranean Sea, molecular markers, pelagic

Introduction

An essential requirement for sustainable fish resource management is the overlap of biological processes and management action (Reiss et al. 2009). Thus, efforts for matching populations as biological units with management unities or 'fish harvested stocks' have been persecuted in the need to transfer the knowledge between the scientific sector and the fisheries policy and management sectors. Harvested stocks, as fundamental exploited units in fisheries management, are commonly defined based on morphological and demographic characteristics, fishing patterns, connectivity patterns (adult movement and larval dispersal), and more recently also incorporating genetic variability (Huret et al. 2020). In fact, this has been due to the dissatisfaction with performance of phenotypic methods for stock identification, as genetic approaches potentially allow to assess the level of divergent populations that is required to justify a separate management (Waples et al. 2008).

Available information about biology, evolutionary history, ecology and management of the European sardine (*Sardina pilchardus*, Walbaum 1792) is still fragmented (Coll and Bellido 2019), as the delimitation of populations as biological units is still under debate. This may be surprising since *S. pilchardus* is one of the most important pelagic fish species due to its crucial ecological and economical roles throughout its range of distribution in the North and Central Eastern Atlantic, from the North Sea to the Senegalese coast, including the Mediterranean and the Black Seas (Costalago and Palomera 2014). Several local studies have been carried out to unravel the genetic stocks of *S. pilchardus* (e.g., Tinti et al. 2002; Atarhouch et al. 2006; Deli et al. 2020), but only few works cover a greater extension of the sardine's range of distribution (e.g., Antoniou et al. in press; Fonseca et al. in press). In addition, few reviews have tried to compile the information available to provide a general overview of the existing populations (Kasapidis 2014).

Currently there is no clear consensus on the genetic stock structure of *S. pilchardus*, which is a common circumstance among pelagic fish taxa. Genetic studies on sardines in the Mediterranean employing different types of markers have reported shallow phylogeographic structure with signs of late Pleistocene expansion, low genetic differentiation and weak or non-existent population structure (Kasapidis 2014; Antoniou et al. in press). In fact, it was traditionally accepted that coastal pelagic marine fishes show genetic homogeneity and even panmixia (random mating) over their geographic distributions (Moore and Chaplin 2013). This has to do with several reasons. One is the apparent lack of physical barriers in the marine environment since local patterns of spatial heterogeneity are associated with species diversity patterns (Ayala and Valentine 1979). Also, the high dispersal capabilities in larval and adult

stages of many marine pelagic species (Magoulas et al. 2006; Bierne et al. 2016) prompts to think about weak genetic differences among conspecific individuals of different areas, complicating population delimitation. Another factor is their important effective population size that limits the impact of genetic drift (Laurent et al. 2007). Finally, and closely linked to the current methodology applied based on molecular markers, it has to be considered that molecular methods could present limitations, since if no genetic divergence is detected among samples, the results are ambiguous and inconclusive. In this context, the causes could be the current gene flow among populations, the belonging to a same genetic stock, or that individuals come from different populations but the method is not able to resolve it due to a lack of resolution of markers (i.e., retention of shared ancestral polymorphisms) (Spanakis et al. 1989; Bossart and Prowell 1998; Kasapidis 2014).

This review explores the potential genetic structure of the European sardine by making use of the available data published in the literature. We discuss the different hypotheses raised so far along its distributional range and the selection processes shaping genetic units. We compare methodologies and genetic markers used to date. We also integrate the genetic information available within the context of the present-day fisheries management of this small pelagic fish.

Methodologies to unravel the genetic population structure of European sardine

Different approaches have been applied to untangle the genetic structure of European sardine populations along its distributional range, even though most studies have been carried out to differentiate genetic units at local level (Table 1).

The first attempts to determine European sardine independent stocks aiming to respect the potential existing populations were based on the study of the phenotype, mainly making use of morphometric and meristic analyses (Regan 1916; Fage 1920; Lee 1961; Silva 2003). Also, otolith shape indices (Correia et al. 2014; Jemaa et al. 2015) or the biochemical amino acid composition analysis of eye tissue (Riveiro et al. 2011) were applied for discriminating among areas within *Sardina pilchardus* metapopulation. The first approach to estimate the geographical distribution of genetic variability in sardine was allozyme electrophoresis on starch gel applied to samples from the Eastern Mediterranean (Spanakis et al. 1989). Ramon & Castro (1997) also applied electrophoretic methods to characterize stocks of sardines in the Western Mediterranean Sea.

Studies that used genotype-based approaches implying molecular neutral and non-neutral markers can be applied without the need for the complete sardine genome, since haplotype-resolved draft genome of the European sardine has only been recently obtained (Machado et al. 2018; Louro et al. 2019). Among neutral markers, we can find microsatellites (González and

Zardoya 2007; Kasapidis et al. 2012; Ruggeri et al. 2012) and exon-primed intron-crossing PCR (EPIC-PCR), which combines the advantages of microsatellite DNA analysis, including PCR of alcohol-preserved tissue samples as well as high levels of polymorphism, without the need for specific PCR primers (Atarhouch et al. 2007). In the case of non-neutral markers (i.e., those that allow studying the adaptive or evolutionary potential), allozymes (Spanakis et al. 1989; Chlaida et al. 2005; Laurent and Planes 2007; Chlaida et al. 2009) or mitochondrial DNA (mtDNA) (Tinti et al. 2002; Atarhouch et al. 2006) have been used. Nowadays, the new technologies allow examining more loci than in the past, permitting the simultaneous study of thousands of loci in hundreds of individuals (Allendorf et al. 2008). The last methodologies applied in sardine studies included genome-wide data set of double-digest RAD-derived SNPs (Coll and Bellido 2019; Antoniou et al. in press), which can also be applied without complete genome data. Conversely, one of the most recent studies used whole genome sequence data, incorporating also mitochondrial genomes, which enables a comparison between markers with different modes of inheritance (Fonseca et al. in press).

Genetic variability and population differentiation

Although the studies analysed in this work were developed under different sampling and analytical procedures depending on methodology, we compiled and discussed the available information regarding the genetic variability and population differentiation of *Sardina pilchardus* (Figure 1; Table 1).

Expected heterozygosity values in studies applying nuclear markers ranged from 0.1495 ± 0.1883 in the Siculo-Tunisian Strait in the analysis performed with allozymes by Deli et al. (2020) to 0.9480 ± 0.0000 in the analysis of González and Zardoya (2007) by applying microsatellites to Atlantic and Mediterranean individuals. Both values are larger than average total heterozygosity ($\overline{H}_T$) and subpopulation heterozygosity ($\overline{H}_S$) observed in marine fishes with allozyme markers, determined as 0.064 and 0.059, respectively, by Ward et al. (1994). On the other hand, mitochondrial markers reach an expected heterozygosity (estimated as haplotype diversity, *h*) of 0.997 ± 0.002 in the study of Atarhouch et al. (2006), which applied mtDNA control region as genetic marker to mainly untangle the genetic stocks of sardines from Morocco (coast of Africa), even though it also included other sample areas along the distributional range of *S. pilchardus* (southern Portugal, the Bay of Biscay, the Catalan Coast and the Aegean Sea) (Table 1).

Many marine species are characterized by large population sizes, high levels of genetic variation and weak differentiation (Ward et al. 1994; Ryman et al. 2014). However, the ratio between effective population size (N_e) and census size (N_c) in these organisms has been estimated to be

small, *S. pilchardus* being the species with a lower ratio (of 10^{-8}) of those reviewed by Hauser and Carvalho (2008). In this species, models suggest that high variance in reproductive success can, under some circumstances, produce large effective sizes, while empirical work suggests low effective sizes (Laurent and Planes 2007; Pinsky and Palumbi 2014).

Regarding genetic population differentiation, it can be observed in Table 2 that the largest number of significant pairwise F_{ST} or Φ_{ST} values has been identified by the allozymes approach, with a 70.28 % of the comparisons in which genetic differentiation has been detected, followed by the study incorporating exon-primed intron-crossing PCR (EPIC-PCR), with 61.9% of the values with significance. Comparison of significant pairwise F_{ST} or Φ_{ST} data (if nuclear or mitochondrial markers were applied, respectively) among the locations of the studies (Figure 2) revealed that the work that detected greater genetic differentiation values applied mtDNA-RFLP marker (differences between Adriatic/Ionian pool and Mediterranean Spanish Coast: Φ_{ST} = 0.7414 - 0.9429, $P < 0.0001$) (Tinti et al. 2002). This has been supported by the ICES (2017) report, which showed that earlier studies using mtDNA and allozymes indicated a higher degree of differentiation than recent studies using allozymes and studies applying microsatellite DNA for the stocks of European sardine.

By contrast, studies applying microsatellites were the ones that found the least amount of genetic differentiation, as in Ruggeri et al. (2012), which compared two close areas: the northern and the southern Adriatic Sea (GSAs 17 and 18). The analysis of González and Zardoya (2007) also exhibited weak but significant genetic differentiation, in this case, comparing nine locations in the Atlantic Ocean and the Mediterranean Sea using allelic size variation of eight specific microsatellite loci. Conversely, microsatellite loci have been previously described as especially suitable for stock identification in species showing low levels of detectable variation using allozymes or mtDNA, as they exhibit high levels of length mutation resulting in extensive allelic variation and levels of heterozygosity in fish ranging from 24 % to 90 % (Shaw et al. 1999a, b).

Nevertheless, comparison of the suitability of allozymes, mtDNA, and microsatellites suggested that allozymes and microsatellites are more potent than mtDNA restriction fragment length polymorphism (RFLP) studies for the identification of pelagic fish populations. However, molecular markers which appear to be less powerful may still be more appropriate to reveal population divergence under some premises: markers with different modes of inheritance (e.g., nuclear vs. mitochondrial markers), mutation or selection (e.g., allozymes vs. microsatellites) (Hauser & Ward 1998). This way, in the situation of high levels of gene flow potentially associated with a pelagic species, and therefore low F_{ST}, comparative multi-locus analyses based

on both nuclear and mitochondrial genetic markers are probably the most efficient and informative approach to discerning the relative role of historical events and life-history traits in shaping genetic diversity (González and Zardoya, 2007). This combination of markers with different DNA origin is usually based on the use of several (six to eight) microsatellite loci merged with mtDNA sequence, which has been considered the best procedure (Viñas et al. 2011), applied in the study of sardine population structure of Baibai et al. (2012) but incorporating a lower number of microsatellite loci (four). In this context, to fully establish a population structure for sardine, we suggest to incorporate simultaneously two approaches applied to the samples to be compared: mtDNA (or allozymes) markers as non-neutral markers together with neutral markers like microsatellites, which present higher mutation rates (Goldstein et al. 1999). This way, complementary aspects of the evolutionary history of sardine could be showed, with mitochondrial data reflecting past isolation of sardine populations and nuclear DNA data potentially revealing the present gene flow among populations, and a pattern of isolation by distance (González and Zardoya 2007).

New methods for revealing population structure of European sardine involved whole genome sequencing data, which allow to analyse different parts of the nuclear genome yield population structure and diversity, and also to recover full mitochondrial genomes for comparison with genetic sequences with different modes of inheritance (Fonseca et al. in press). The algorithm based on multiloci testing of allele frequencies between pairs of samples results in the greatest power to detect more accurately the number of populations (Viñas et al. 2011).

Mapping sardine genetic population structure

Delimiting genetic population structure of pelagic fish is a difficult task. In general, no genetic structure is evident in most of the distribution range for neutral markers and genetic differentiation among individuals increases as geographical distance increases (ICES 2017). In fact, results of previous studies have presented inconsistencies and have been non-conclusive determining stock boundaries for European sardine (Figure 3a, b).

Genetic discontinuities between the North-eastern Atlantic and the Western Mediterranean have been reported or suspected in numerous pelagic and demersal fish species applying allozymes, nuclear and mitochondrial DNA markers (mtDNA) (Naciri et al. 1999; Comesaña et al. 2008). In previous decades, some authors have considered the Mediterranean sardine as an independent subspecies of the Atlantic sardine regarding morphometric and meristic characteristics (Regan 1916; Lee 1961): *S. p. pilchardus* on the Atlantic coast of Portugal, and *S. p. sardine* in the Mediterranean and on the Atlantic coast of Africa. Nevertheless, it has not been

supported by most genetic analyses (González and Zardoya 2007; Kasapidis et al. 2012), despite some results that back this structure (Atarhouch et al. 2006). It has to be taken into account that, in general, fish species have greater variance in morphological traits both within and among populations than do other vertebrates, being more susceptible to environmentally-induced morphological divergence (Kinsey et al. 1994), so that the molecular analysis is of special importance at the ichthyological level. A clear example in sardine was reported by Antoniou et al. (in press), who described that individuals of the Mediterranean and the Atlantic respond differently to environmental pressures.

Using the mtDNA control region, Atarhouch et al. (2006) distinguished only the population of the Bay of Biscay from the rest of both Atlantic and Mediterranean populations, unlike other studies that have not found genetic differentiation among sardines in Celtic Seas, English Channel and the Bay of Biscay (ICES 2017). In fact, Laurent et al. (2006) determined that it appears to shape a single panmictic population despite evidence of a gradual change in two loci. Some investigations did not even identify genetic differences among the Bay of Biscay and the Western Mediterranean samples of European sardine (Kasapidis 2012; Fonseca et al. in press). Moreover, preliminary results after applying genotyping through high-throughput showed that the most important component of genetic divergence among populations occurs between the Atlantic and the Western Mediterranean Sea, whereas the Alboran Sea samples are closer to the Atlantic than the Mediterranean samples (Coll and Bellido 2019; Antoniou et al. in press). Preliminary results of Fonseca et al. (in press) after low coverage whole genome sequencing show three genetic clusters: one including individuals from Azores and Madeira, the second corresponding to the whole perimeter of the Iberian Peninsula, involving the Bay of Biscay as well as the Alboran and the Catalan Coast, and the third including the Eastern Mediterranean samples and those from the Canary Islands. Kasapidis et al. (2012) also detected a genetic heterogeneity among the sardine stocks of Madeira and the Azores, and the rest of the Atlantic individuals assessed by using five microsatellite loci.

Some of the most relevant sardine population studies on the southern half of the Eastern Atlantic distribution are those of Laurent et al. (2007) and Chlaida et al. (2005, 2009) performed with allozymes. These authors identified genetic homogeneity, even among populations of northern Morocco and southern Portugal, except for the superoxide dismutase (*SOD*) locus, whose allelic distribution follows an isolation by distance model. Based on their results, two fishing stocks are proposed on the Eastern Atlantic coast: one to the south of the Bay of Agadir, and other to the north, which seems to be genetically related to Mediterranean populations. All these observations were confirmed by Atarhouch et al. (2007) based on the intron

polymorphism of two nuclear genes (CaM-4 and Ops-1), although this study defined as weak the genetic boundary in the Bay of Agadir. In addition, Baibai et al. (2012), who combined microsatellites and mtDNA control region markers, supported genetic differentiation between the sardine population of the Atlantic Moroccan coast and the Spanish coast of Galicia, with a potential contact zone around Cádiz in the Atlantic coast and Málaga for Mediterranean Sea, revealed by the large number of polymorphic sites of the latter.

Local studies in the Mediterranean performed with allozymes described differences between the populations across the Siculo-Tunisian Strait, with the presence of chaotic and complex genetic patchiness (Deli et al. 2020). Besides, different genetic stocks were delimitated in the Aegean and Ionian seas, despite the fact that for some characters the within-area variation was larger than the between-area variation (Spanakis et al. 1989). Kasapidis et al. (2012) also detected differences regarding the Aegean individuals with respect of the Atlantic and other Mediterranean samples by using microsatellite markers, except for samples from Northern Spain (Catalan Coast), thus being grouped into the same genetic stock by the authors.

In addition, Tinti et al. (2002) detected a genetic homogeneity in the distribution of cytochrome b haplotypes between the Adriatic and Ionian populations and clear differences of these with respect to the Mediterranean Iberian populations. Furthermore, Ramon and Castro (1997) and Kasapidis et al. (2012) described population structure along the west coast of the Mediterranean, with notable differences between the population in the Alboran Sea and the rest of the Mediterranean, contradicted by Fonseca et al. (in press).

Selection processes and drivers shaping European sardine's genetic units

Traditionally, population structure in marine species has been explained by 'isolation by distance' in an extended way, which may occur when the distribution of the organisms is larger than the dispersal range of individuals (Wright 1943; Bekkevold et al. 2005). However, marine organisms are permanently exposed to physical-chemical variables as temperature or salinity, among others, as well as to biological agents like parasitism, and to anthropogenic factors such as pollutants, human-induced global warming, or fisheries. The latter contributes to catch-induced selection based on traits related to maturation and growth, eroding functional genetic diversity (Marty et al. 2015), which may be especially relevant to overexploited *Sardina pilchardus*. All the mentioned factors may cause divergent selection by means of differential survival of animals with different genotypes in distinct environment (Kasapidis et al. 2012). On the other hand, oceanographic currents, topography of the ocean floor (Bekkevold et al. 2005),

and other physical and hydrological barriers typical in straits (e.g., Gibraltar) could limit gene flow, contributing to population differentiation.

In addition, intrinsic characteristics of the individuals comprising the population as different habitat preferences and behaviour (i.e., homing or habitat choice), sexual selection or limited dispersal capabilities are also shapers of genetic heterogeneity (Faria et al. 2021; Fonseca et al. in press). In fact, even though clupeids are commonly considered highly mobile, their localized spawning behaviour or migratory patterns may result in restricted gene flow between populations (Bacha et al. 2014).

Thereby, to develop and use molecular markers that allow characterizing non-neutral genomic regions responding to adaptive variation is gaining value, especially among marine populations, to which erroneous homogeneity has been attributed. This relatively recent ability to identify DNA regions and genes under the influence of selection is closing the gap between molecular scientists and researchers who are interested in addressing the role of local adaptation in shaping biodiversity (Kirk and Freeland 2011). One example of analysis to decipher selection is the assay of allelic frequencies of the allozyme SOD, which has previously been used in *S. pilchardus* to discriminate fish stocks (Ramon and Castro 1997; Chlaida et al. 2005; Laurent et al. 2007; Chlaida et al. 2009). However, Chlaida et al. (2009) refused the idea that *SOD* locus was under selection throughout the Atlantic and Mediterranean Moroccan coastlines. By contrast, Laurent et al. (2007) suggested that genetic analysis of *S. pilchardus* over 15 locations between the North Sea and Mauritania, including samples from the Azores, Madeira, and the Mediterranean Sea, showed both isolation by distance and local selection on the single locus *SOD*, which may be under natural selection pressure with abrupt changes associated with a hydrodynamic barrier in the Gulf of Agadir (Chlaida et al. 2009). Baibai et al. (2012) also identified genetic differentiation following an isolation by distance pattern along the Moroccan Atlantic coast, in addition to some degree of isolation of the northern and southernmost stocks analysed (Galician and Cap Blac, respectively). This was potentially associated with oceanographic barriers (e.g., gyres) or environmental barriers like the presence of several emergence of upwelling in the south of Morocco. A recent study suggested that environmental variables are crucial at all levels of population structuring between populations of the Atlantic (Gulf of Cadiz) and Western Mediterranean and within populations of the Western Mediterranean Sea (Antoniou et al. in press), in particular the ones related to sea surface temperature.

Focusing on the Atlantic genetic heterogeneity, the cluster Azores-Madeira was defined by Kasapidis et al. (2012), as well as by Fonseca et al. (in press) (Figure 3a). The genetic divergence between the stock of Azores-Madeira ecoregion compared to stocks in other Atlantic-Mediterranean regions may be the result of genetic drift due to isolation during the Pleistocene glaciations, the absence of suitable corridors for migrants between the islands and continent, and/or the disruptive effect of the Portugal current, flowing along the East Atlantic coast from northern Portugal southwards to northern Africa (Domingues et al. 2007; Pérez-Portela et al. 2017). Here we should add the effects of other current systems as the Azores and the Canary Currents (Sá-Pinto et al. 2008) or the deep oceanic waters that separate stocks from those of the continental shelf (Kasapidis et al. 2012). These oceanic domains also define to a large extent groups of populations that are genetically distinct from neighbouring population groups in other ocean domains (Magoulas et al. 2006).

On the other hand, and focusing on the genetic Mediterranean stocks, Agostini and Bakun (2002) described five sub-basins based on nutrient enrichment, concentration of larval food distributions, and local retention of eggs and larvae, which could be causing differentiation of clupeoid fish stocks at a genetic level: the Alboran Sea, the Adriatic Sea, the Aegean Sea, the Gulf of Lions and nearby Catalan Coast, and the Straits of Sicily/Tunisian Coast. As it seems, four of these zones overlap with the potential European sardine populations defined in the literature (Figure 3a, b).

The Almeria-Oran Front, a well-defined oceanographic break situated east of the Strait of Gibraltar, is described as responsible for hindering gene flow between Mediterranean and Atlantic fish populations of many fish species (Schunter et al. 2011; Fonseca et al. in press), being the major oceanographic discontinuity in the Western Mediterranean (Naciri et al. 1999). However, hydrological characteristics of the Alboran Sea's surface waters are much closer to those of the North-eastern Atlantic than the Western Mediterranean (Bacha et al., 2014), which matches the similarity between Alboran Sea samples and the Atlantic samples compared to the Mediterranean samples (Coll and Bellido 2019; Antoniou et al. in press), and also supported by hints of gene flow between Alboran and Moroccan Atlantic Ocean coast stocks (Baibai et al. 2012). Studies reported sardine nursery areas within the Bay of Málaga (Würtz 2010; Quintanilla et al. 2020), located in the central part of the northern Alboran coastline, which provides shelter from the large-scale westerly wind flow (Würtz 2010). Besides, the presence of the Alboran gyre could be causing larvae retention (Naciri et al. 1999) and hindering dispersal and migration (Bacha et al., 2014) of the juveniles and reproductively active adults. However, the genetic break inferred with respect to other Atlantic and Mediterranean stocks may include not only several

oceanographic discontinuities but also genetic differentiation because of isolation by distance (Schunter et al. 2011).

Topographic features as continental shelf-brakes, peninsulas and capes, and enclosed gulfs promote the retention of eggs and larvae of clupeids in the productive spawning areas like the shelf-break front in the Western Mediterranean, the land enclosure of the Adriatic Sea and the North Aegean Sea (Somarakis et al. 2019). Genetic differentiation linked to larval retention and confined dispersal is probable in the Adriatic and Aegean Seas, since the peninsulas partially isolate their waters from the rest of the Mediterranean (Magoulas et al. 2006), reducing genetical flow toward other basins. To the south of each of these basins there are mesoscale anticyclonic eddies produced by unstable parts of the gyres (Würtz 2010), which may further enhance the genetic isolation of sardine populations, confirming the results of Spanakis et al. (1989) and Kasapidis et al. (2012).

After what has been discussed, it could be expected that in the Eastern Atlantic, the distribution of European sardine is mostly driven by the phenomenon of isolation by distance since such an extensive body of water shows fewer physical barriers associated with coastal relief, although it should not be forgotten that oceanographic elements such as currents, gyres, upwelling, and oceanic fronts produce physically heterogeneous seascapes that may be obstacles to gene flow and, therefore, may contribute to population differentiation (e.g., a potential explanation for Azores-Madeira genetic stock). On the contrary, in the Mediterranean Sea the selection processes based mainly on physical and hydrological barriers could be potentially shaping the sardine genetic stocks. Homogeneity of Atlantic population groups has been shown in other pelagic and demersal species (Recasens et al. 1998; Souche et al. 2015), with the existence of single large panmictic units. However, in a semi-enclosed Mediterranean with sub-basins, there is likely a greater genetic heterogeneity regarding sardine stocks. In fact, it is considered as a hotspot for fish endemism (Lasram and Mouillot 2009; Coll et al. 2010) characterized by relatively high biodiversity due to its geological evolution and environmental conditions (Granger et al. 2015). This may be the reason why the efforts and discrepancies in the limitations of sardine genetic units are potentially greater in this region. Likewise, it has been observed that a common mapping of the stocks present on the coasts of the Iberian Peninsula remains to be established (Figure 3b), which represents a transition between the Atlantic and Mediterranean waters. Despite a wealth of historical and oceanographic data, in the Atlantic–Mediterranean transition there are still discordant results regarding the biogeographical separation for many species (Patarnello et al. 2007).

To gauge the importance of the different variables influencing genetic structure in a changing environment due to the global change, it is imperative to exhaustively evaluate the impact that the factors previously mentioned have in *S. pilchardus* populations adaptation and, therefore, potential differentiation. This can be helpful not only for the current managing of the biological resource but also to project and develop scenarios taking into consideration genetic units and future directions of change.

Spatial mismatch between biological and fishing management units: implications

Although most of the concern in the literature about genetic changes caused by exploitation has focused on marine and freshwater finfish populations (Allendorf et al. 2008), there are large unknowns about the genetic consequences of harvesting these taxa, partly because temporal investigations that evaluate phenotypic and molecular changes simultaneously are scarce (Pukk et al. 2013). However, it is clear that intense fishing pressures may induce neutral and adaptive evolution affecting life-history traits, with a negative impact on the genetic diversity of exploited populations through selection and genetic drift (Marty et al. 2015; Pavičić et al. 2020). Thereby, with the aim of minimizing these effects and protecting genetic diversity, stock structure and population connectivity data are vital for effective fisheries management (Pavičić et al. 2020).

In EU Atlantic waters, two sardine stocks are considered (Figure 4): Northern stock (ICES Subareas 27.7 and 27.8.a, b, d) fished mainly by France and Spain, and Southern stock (ICES Subareas 27.8.c and 27.9.a) fished by Spain and Portugal (ICES 2017). In this sense, and mainly due to the existing doubts regarding the genetic stocks along the Iberian Coast, it is unknown whether it is necessary to separate these two stocks, or whether they belong to the same genetic pool. Nevertheless, if limits are established for fisheries management in this area, current studies point to the subareas that comprise the Bay of Biscay (ICES Subareas 27.8.a, b, c, d) as a single distinct unit for this species. In addition, it is not clear if there could be an 'Iberian' genetic stock, in which sardines in the Bay of Biscay and the Western Mediterranean are genetically similar, which would cause the need to develop a joint management of Atlantic and Western Mediterranean despite potential practical limitations. On the other hand, there is a large part of sardine distribution (e.g., FAO subareas 27.3, 4, 5, 6) in which its management is not mentioned because sardine as a fishing resource is hardly exploited in these areas.

Also, FAO has delimited four fishing zones with the aim of managing the sardine off the Northwest African coast: Northern Zone: 35°45'N-32°N (Cape Spartel-Eljadida); Zone A: 32°N-29°N (Safi-Sidi Ifni); Zone B: 29°N-26°N (Sidi Ifni-Cape Bojador); Zone C: 26°N-Southwards (Cape Bojador-the southern extent of the species). However, working groups of FAO chose to adopt

three separate stocks: Northern Stock (35°45'N-32°N); Central Stock (32°N-26°N) (Zones A+B); Southern Stock (26°N-the southern extent of the species) (Zone C), with an evaluation limited to two distinct stocks (Zones A+B and Zone C) (FAO 2001). Taking into consideration the genetic stock divisions in accordance with current knowledge, the most appropriate local management approach in African waters should be based on establishing a Northern and Southern African stocks, delimited by the Bay of Agadir (30°48'N) (Figure 4). However, the delimitation by this barrier to gene flow could represent the boundary between two stocks at the Atlantic level in the sardine distribution range: a Northern and a Southern Atlantic stock.

The potential genetic stock of Azores-Madeira defined by Kasapidis et al. (2012) and Fonseca et al. (in press) would currently be out of an independent and specific management. In this context and under the precautionary approach, any partly conflicting conclusions in the matter of the genetic population structure of a species must not prevent management being developed in relation to the potential underlying structure of populations (Reiss et al. 2009) to avoid the loss of genetic variation. Although diversity can be rebuilt through mutation or through immigration from genetic refugia, ignoring differentiated populations in harvest management could hinder genetic diversity recovery, as when an entire species is considered one stock, there are no potential refugia (Pinsky and Palumbi 2014).

Focusing on the Mediterranean, scientific advice of sardine fisheries (Working Group on Stock Assessment of Small Pelagic Species) in the framework of the General Fisheries Commission for the Mediterranean (GFCM) has used the geographical subareas (GSAs) as differentiated stocks to diagnose its status as well as to provide advice and recommendations for a better management (FAO, 2019). The exceptions are the Alboran (GSAs 1, 2, 3) and Adriatic (GSAs 17, 18) stocks, each of which have been consider as single units in which sardine stocks are shared (Fiorentino and Vitale 2021). As we have seen, there is no consensus among studies regarding the genetic heterogeneity in this semi-enclosed waterbody, even though some studies coincide in separating an Adriatic-Ionian stock from the rest of the Mediterranean (Tinti et al. 2002; Kasapidis et al. 2012), which also happens with Alboran individuals (Ramon and Castro 1997; Kasapidis et al. 2012). On the last case, the management unit would potentially fit with the genetic unit. However, in the former, a joint management between GSAs 17, 18 (Adriatic) and GSAs 19, 20 (and maybe, 21) (Ionian) should be considered. Likewise, there is the question of the possible existence of a genetic stock in the Aegean, so following this precautionary principle (Reiss et al. 2009), its independent management could be proving appropriate. Thus, all seems to indicate that more assessment and management units have been established than sardine genetic stocks in the Mediterranean. Misspecifying spatial population structure and migration

by the fishery can produce large assessment errors when stock assessment is conducted based on management areas (Guan et al. 2013). Thus, the overestimation of the number of stocks of a species could lead to biases in estimates of fishing mortality and yield, generating the erroneous idea of a more intensive exploitation of a healthier population that actually spreads across several of the management areas, and transferring that pressure to another genetic stock that is actually in worse condition, or misinterpreting the dynamics of areas where the species has been historically depleted (e.g., the case of the Gulf of Lion (GSA 7) and the Catalan Coast (GSA 6). The focus was on the drop in sardine abundance of the former stock (Antoniou et al. in press), although it has been observed that there is genetic homogeneity with respect to individuals from the Catalan Coast (Coll and Bellido 2019)).

Discussion and final conclusions

We showed the difficulty of studying the population structure of a pelagic species. As we have discussed, there are currently gaps of information about the population structure of European sardine. One of the most important unsolved questions is that it is not clear if there are two sardine subspecies (i.e., *S. p. pilchardus* and *S. p. sardina*), although recent genetic studies reject this possibility.

Besides, disparate hypotheses of stock structure have emerged around the Iberian Peninsula and the Aegean Sea. In addition, current methods have limitations, since if homogeneity between stocks is detected, it may be due to real genetic flow or due to a lack of statistical power of the genetic marker, incurring a type II error (i.e., the non-rejection of a false null hypothesis). Counterintuitively, mtDNA-RFLP marker showed higher genetic differentiation values than other markers as microsatellites or allozymes, even though the latter detected a larger number of significant pairwise values of genetic differentiation. Thereby, we suggest the combination of several approaches involving nuclear and mitochondrial information to increase the power of detecting population differences. On the other hand, genetic diversity values among studies were miscellaneous, probably because of the results depend on the areas to be compared in addition to the sample size and the techniques used in each investigation. However, these estimates of diversity were larger than expected in marine fish species. The study of the loss of genetic variation is especially of interest in pelagic fishes, as it can occur when census population sizes are large because the genetically effective population size is often much smaller than the census size (Allendorf et al. 2008), as highlighted in the case of sardine (Laurent and Planes 2007; Pinsky and Palumbi 2014).

Another conclusion is the uneven study effort along the distribution of *S. pilchardus*. Genetic sampling of sardine specimens along the Black Sea, the Southern Ionian Sea and the Levantine Sea, as well as the northern and the southernmost Atlantic distribution is scarce or non-existent. By contrast, it is one of the most caught species in the Mediterranean eastern basin (i.e., Levantine Sea) (Marttin et al. 2006; Eurostat 2008), although that does not occur in the northernmost East Atlantic Ocean, where the target fishes with higher economical value are different. Both the divergence in determining the spatial limits of each genetic stock among the reviewed works and the paucity of studies due to a biased sampling effort have direct implications on fisheries management. Thus, further studies are required to re-evaluate and determine the population structure of the European sardine in order to apply a differential fisheries management approach of sardine genetic stocks. Fishing management areas are not updated based on the current knowledge of the genetic structure and there are areas in which the number of biological units has been overestimated (e.g., the African coast and the Western Mediterranean Sea) and areas that are not managed independently even though they seem to be differentiated entities (i.e., Madeira-Azores).

Declarations

Funding

Authors' research funding is supported by Spanish Ministry of Science, Innovation and Universities (RTI2018-097544-B-I00, 'ConSarVar', I+D+i Retos de Investigación), as well as by the Fons Europeu Marítim i de la Pesca (FEMP) and the Agency for Management of University and Research Grants (AGAUR) of the Generalitat de Catalunya (Ajuts per a la contractació de personal investigador novell (FI-2020)).

Conflicts of interest/Competing interests

The authors declare no conflict of interest/competing interests.

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contributions

The five authors of this work have contributed significantly to its preparation and development and agree with the content it presents. MCH collected the data and drafted the manuscript; XFT integrated the information into the figures; MC, MM and JV revised it critically and provided important intellectual content. JV and MM supervised the work and acquired the financial support for the project leading to this publication.

Ethics approval

Not applicable

Consent to participate

Authors give their explicit consent to participate in this work.

Consent for publication

Authors give their explicit consent for the publication of this work.

References

Agostini VN, Bakun A (2002) Ocean triads' in the Mediterranean Sea: physical mechanisms potentially structuring reproductive habitat suitability (with example application to European anchovy, *Engraulis encrasicolus*). Fish. Oceanogr. 11:129-142. https://doi.org/10.1046/j.1365-2419.2002.00201.x

Allendorf FW, England PR, Luikart G, Ritchie PA, Ryman N (2008) Genetic effects of harvest on wild animal populations. Trends Ecol. Evol. 23:327-337. https://doi.org/10.1016/j.tree.2008.02.008

Antoniou A, Manousaki T, Ramírez F et al. (in press) Sardines at a junction: seascape genomics reveals ecological and oceanographic drivers of variation in the NW Mediterranean Sea. Authorea. https://doi.org/10.22541/au.162854295.58977249/v1

Atarhouch T, Rami M, Naciri M, Dakkak A (2007) Genetic population structure of sardine (*Sardina pilchardus*) off Morocco detected with intron polymorphism (EPIC-PCR). Mar. Biol. 150:521-528. https://doi.org/10.1007/s00227-006-0371-8

Atarhouch T, Rüber L, González EG, Albert EM, Rami M, Dakkak A, Zardoya R (2006) Signature of an early genetic bottleneck in a population of Moroccan sardines (*Sardina pilchardus*). Mol. Phylogenet 39:373-383. https://doi.org/10.1016/j.ympev.2005.08.003

Ayala FJ, Valentine JW (1979) Genetic variability in the pelagic environment: a paradox?. Ecology 60:24-29. https://doi.org/10.2307/1936463

Bacha M, Jemaa S, Hamitouche A, Rabhi K, Amara R (2014) Population structure of the European anchovy, *Engraulis encrasicolus*, in the SW Mediterranean Sea, and the Atlantic Ocean: evidence from otolith shape analysis. ICES J. Mar. Sci. 71:2429-2435. https://doi.org/10.1093/icesjms/fsu097

Baibai T, Oukhattar L, Quinteiro JV, Mesfioui A, Rey-Mendez M (2012) First global approach: morphological and biological variability in a genetically homogeneous population of the European pilchard, *Sardina pilchardus* (Walbaum 1792) in the North Atlantic coast. Rev. Fish Biol. Fish. 22:63-80. https://doi.org/10.1007/s11160-011-9223-9

Bekkevold D, Aandré C, Dahlgren TG et al (2005) Environmental correlates of population differentiation in Atlantic herring. Evolution 59:2656-2668. https://doi.org/10.1111/j.0014-3820.2005.tb00977.x

Bierne N, Bonhomme F, Arnaud-Haond S (2016) Editorial dedicated population genomics for the silent world: the specific questions of marine population genetics. Curr. Zool 62:545-550. https://doi.org/10.1093/cz/zow107

Bossart JL, Prowell DP (1998) Genetic estimates of population structure and gene flow: limitations, lessons and new directions. Trends Ecol. Evol. 13:202-206. https://doi.org/10.1016/S0169-5347(97)01284-6

Chlaida M, Kifani S, Lenfant P, Ouragh L (2005) First approach for the identification of sardine populations *Sardina pilchardus* (Walbaum 1792) in the Moroccan Atlantic by allozymes. Mar. Biol. 149:169-175. https://doi.org/10.1007/s00227-005-0185-0

Chlaida M, Laurent V, Kifani S, Benazzou T, Jaziri H, Planes S (2009) Evidence of a genetic cline for *Sardina pilchardus* along the Northwest African coast. ICES J. Mar. Sci. 66:264-271. https://doi.org/10.1093/icesjms/fsn206

Coll M, Bellido JM (2019) SPELMED, evaluation of the population status and specific management alternatives for the small pelagic fish stocks in the Northwestern Mediterranean Sea. Publications Office of the European Union, Luxembourg. https://doi.org/10.2826/018625

Coll M, Piroddi C, Steenbeek J et al (2010) The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. PloS One 5:e11842. https://doi.org/10.1371/journal.pone.0011842

Comesaña AS, Martínez-Areal MT, Sanjuan A (2008) Genetic variation in the mitochondrial DNA control region among horse mackerel (*Trachurus trachurus*) from the Atlantic and Mediterranean areas. Fish. Res. 89:122-131. https://doi.org/10.1016/j.fishres.2007.09.014

Correia AT, Hamer P, Carocinho B, Silva A (2014) Evidence for meta-population structure of *Sardina pilchardus* in the Atlantic Iberian waters from otolith elemental signatures of a strong cohort. Fish. Res. 149:76-85. https://doi.org/10.1016/j.fishres.2013.09.016

Costalago D, Palomera I (2014) Feeding of European pilchard (*Sardina pilchardus*) in the northwestern Mediterranean: from late larvae to adults. Sci. Mar. 78:41-54. https://doi.org/10.3989/scimar.03898.06D

Deli T, Guizeni S, Ben Abdallah L, Said K, Chatti N (2020). Chaotic genetic patchiness in the pelagic teleost fish *Sardina pilchardus* across the Siculo-Tunisian Strait. Mar. Biol. Res. 16:280-298. https://doi.org/10.1080/17451000.2020.1752921

Domingues VS, Almada VC, Santos RS, Brito A, Bernardi G (2007) Phylogeography and evolution of the triplefin *Tripterygion delaisi* (Pisces, Blennioidei). Mar. Biol. 150: 509-519. https://doi.org/10.1007/s00227-006-0367-4

Eurostat (2008) Half of Mediterranean fish catches are by Mediterranean Partner Countries - Issue number 88/2008. Publications Office of the European Union, Luxembourg. Catalogue number: KS-SF-08-088-EN-N

Fage L (1920) Rapport sur la sardine. Notes et mémoires 1:11. Open Access version: https://archimer.ifremer.fr/doc/00016/12772/

FAO (2001) FAO Fisheries Report No. 657. 24-31 March 2001, Nouadhibou, Mauritania

FAO (2019) Report of the Working Group on Stock Assessment of Small Pelagic Species (WGSASP), 9-14 December, Rome, Italy, pp 61

Faria R, Johannesson K, Stankowski S (2021) Speciation in marine environments: Diving under the surface. J. Evol. Biol 34:4-15. https://doi.org/10.1111/jeb.13756

Fiorentino F, Vitale S (2021). How Can We Reduce the Overexploitation of the Mediterranean Resources?. Front. Mar. Sci. 8: 674633. https://doi.org/10.3389/fmars.2021.674633

Fonseca RD, Campos P, Iglesia ARDL et al (in press) Low coverage whole genome sequencing reveals the underlying structure of European sardine populations. Authorea. https://doi.org/10.22541/au.161628445.52373083/v1

Goldstein DB, Roemer GW, Smith DA, Reich DE, Bergman A, Wayne RK (1999) The use of microsatellite variation to infer population structure and demographic history in a natural model system. Genetics 151:797-801. https://doi.org/10.1093/genetics/151.2.797

González EG, Zardoya R (2007) Relative role of life-history traits and historical factors in shaping genetic population structure of sardines (*Sardina pilchardus*). BMC Evol. Biol. 7:1-12. https://doi.org/10.1186/1471-2148-7-197

Granger V, Fromentin JM, Bez N et al (2015) Large-scale spatio-temporal monitoring highlights hotspots of demersal fish diversity in the Mediterranean Sea. Prog. Oceanogr. 130:65-74. https://doi.org/10.1016/j.pocean.2014.10.002

Guan W, Cao J, Chen Y, Cieri M (2013) Impacts of population and fishery spatial structures on fishery stock assessment. Can. J. Fish. Aquat. Sci. 70:1178-1189. https://doi.org/10.1139/cjfas-2012-0364

Hauser L, Carvalho GR (2008) Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish Fish (Oxf) 9:333-362. https://doi.org/10.1111/j.1467-2979.2008.00299.x

Hauser L, Ward RD (1998) Population identification in pelagic fish: the limits of molecular markers. NATO Adv. Stud. Inst. Ser. A. Life Sci. 306:191-224.

Huret M, Lebigre C, Iriondo M, Montes I, Estonba A (2020) Genetic population structure of anchovy (*Engraulis encrasicolus*) in North-western Europe and variability in the seasonal distribution of the stocks. Fish. Res. 229:105619. https://doi.org/10.1016/j.fishres.2020.105619

ICES (2017) Report of the Working Group on Southern Horse Mackerel, Anchovy and Sardine (WGHANSA). 24–29 June 2017 Bilbao, Spain. ICES / CIEM - International Council for the Exploration of the Sea/Conseil International pour l'Exploration de la Mer, Ref. ICES CM 2017/ACOM:17, 684p.https://archimer.ifremer.fr/doc/00415/52665/

Jemaa S, Bacha M, Khalaf G, Dessailly D, Rabhi K, Amara R (2015) What can otolith shape analysis tell us about population structure of the European sardine, *Sardina pilchardus*, from Atlantic and Mediterranean waters?. J. Sea Res. 96:11-17. https://doi.org/10.1016/j.seares.2014.11.002

Kasapidis P (2014) Chapter 2 Phylogeography and Population genetics. In: Ganias K (ed) Biology and ecology of sardines and anchovies. CRC Press, Boca Raton, pp 43-75

Kasapidis P, Silva A, Zampicinini G, Magoulas A (2012) Evidence for microsatellite hitchhiking selection in European sardine (*Sardina pilchardus*) and implications in inferring stock structure. Sci. Mar. 76:123-132. https://doi.org/10.3989/scimar.03366.29B

Kinsey ST, Orsoy T, Bert TM, Mahmoudi B (1994) Population structure of the Spanish sardine *Sardinella aurita*: natural morphological variation in a genetically homogeneous population. Mar. Biol. 118:309-317. https://doi.org/10.1007/BF00349798

Kirk H, Freeland JR (2011) Applications and implications of neutral versus non-neutral markers in molecular ecology. Int. J. Mol. Sci. 12:3966-3988. https://doi.org/10.3390/ijms12063966

Lasram FBR, Mouillot D (2009) Increasing southern invasion enhances congruence between endemic and exotic Mediterranean fish fauna. Biol. Invasions 11:697-711. https://doi.org/10.1007/s10530-008-9284-4

Laurent V, Caneco B, Magoulas A, Planes S (2007) Isolation by distance and selection effects on genetic structure of sardines *Sardina pilchardus* Walbaum. J. Fish Biol. 71:1-17. https://doi.org/10.1111/j.1095-8649.2007.01450.x

Laurent V, Planes S (2007) Effective population size estimation on *Sardina pilchardus* in the Bay of Biscay using a temporal genetic approach. Biol. J. Linn. Soc. 90:591-602. https://doi.org/10.1111/j.1095-8312.2007.00747.x

Laurent V, Voisin M, Planes S (2006) Genetic clines in the Bay of Biscay provide estimates of migration for *Sardina pilchardus*. J. Hered. 97:81-88. https://doi.org/10.1093/jhered/esj005

Lee JY (1961) La sardine du golfe du lion (*Sardina pilchardus sardina* Regan). Rev. Trav. Inst. Peches Marit. 25:418-513. Open Access version: https://archimer.ifremer.fr/doc/00000/4266/

Louro B, De Moro G, Garcia C et al (2019) A haplotype-resolved draft genome of the European sardine (*Sardina pilchardus*). Gigascience 8:giz059. https://doi.org/10.1093/gigascience/giz059

Machado AM, Tørresen OK, Kabeya N et al (2018) 'Out of the Can': A draft genome assembly, liver transcriptome, and nutrigenomics of the European sardine, *Sardina pilchardus*. Genes 9:485. https://doi.org/10.3390/genes9100485

Magoulas A, Castilho R, Caetano S, Marcato S, Patarnello T (2006) Mitochondrial DNA reveals a mosaic pattern of phylogeographical structure in Atlantic and Mediterranean populations of anchovy (*Engraulis encrasicolus*). Mol. Phylogenet 39:734-746. https://doi.org/10.1016/j.ympev.2006.01.016

Marttin F, Barone M, Bizsel C et al (2006) Brief introduction to the Eastern Mediterranean fisheries sector. GCP/INT/918/EC - TCP/INT/2904/TD-6. MedFisis Technical Document No. 6, FAO, Rome.

Marty L, Dieckmann U, Ernande B (2015) Fisheries-induced neutral and adaptive evolution in exploited fish populations and consequences for their adaptive potential. Evol. Appl. 8:47-63. https://doi.org/10.1111/eva.12220

Moore GI, Chaplin JA (2013) Population genetic structures of three congeneric species of coastal pelagic fishes (Arripis: Arripidae) with extensive larval, post-settlement and adult movements. Environ. Biol. Fishes 96:1087-1099. https://doi.org/10.1007/s10641-012-0105-3

Naciri M, Lemaire C, Borsa P, Bonhomme F (1999) Genetic study of the Atlantic/Mediterranean transition in sea bass (*Dicentrarchus labrax*). J. Hered. 90:591-596. https://doi.org/10.1093/jhered/90.6.591

Patarnello T, Volckaert FA, Castilho R (2007) Pillars of Hercules: is the Atlantic–Mediterranean transition a phylogeographical break?. Mol. Ecol. 16:4426-4444. https://doi.org/10.1111/j.1365-294X.2007.03477.x

Pavičić M, Žužul I, Matić-Skoko S et al (2020) Population Genetic Structure and Connectivity of the European Lobster *Homarus gammarus* in the Adriatic and Mediterranean Seas. Front. Genet 11:1540. https://doi.org/10.3390/w13081072

Pérez-Portela R, Rius M, Villamor A (2017) Lineage splitting, secondary contacts and genetic admixture of a widely distributed marine invertebrate. J. Biogeogr. 44:446-460. https://doi.org/10.1111/jbi.12917

Pinsky ML, Palumbi SR (2014) Meta-analysis reveals lower genetic diversity in overfished populations. Mol. Ecol. 23:29-39. https://doi.org/10.1111/mec.12509

Pukk L, Kuparinen A, Järv L, Gross R, Vasemägi A (2013) Genetic and life-history changes associated with fisheries-induced population collapse. Evol. Appl. 6:749-760. https://doi.org/10.1111/eva.12060

Quintanilla JM, Laiz-Carrión R, García A et al (2020) Early life trophodynamic influence on daily growth patterns of the Alboran Sea sardine (*Sardina pilchardus*) from two distinct nursery habitats (bays of Málaga and Almería) in the western Mediterranean Sea. Mar. Environ. Res. 162:105195. https://doi.org/10.1016/j.marenvres.2020.105195

Ramon MM, Castro JA (1997) Genetic variation in natural stocks of *Sardina pilchardus* (sardines) from the western Mediterranean Sea. Heredity (Edinb) 78:520-528. https://doi.org/10.1038/hdy.1997.81

Recasens L, Lombarte A, Morales-Nin B, Tores GJ (1998) Spatiotemporal variation in the population structure of the European hake in the NW Mediterranean. J. Fish Biol. 53:387-401. https://doi.org/10.1111/j.1095-8649.1998.tb00988.x

Regan CT (1916) I.—The British fishes of the subfamily Clupeinæ and related species in other seas. Ann. mag. nat. hist. 18:1-19. https://doi.org/10.1080/00222931608693817

Reiss H, Hoarau G, Dickey-Collas M, Wolff WJ (2009) Genetic population structure of marine fish: mismatch between biological and fisheries management units. Fish Fish (Oxf) 10:361-395. https://doi.org/10.1111/j.1467-2979.2008.00324.x

Riveiro I, Guisande C, Iglesias P et al (2011) Identification of subpopulations in pelagic marine fish species using amino acid composition. Hydrobiologia 670:189-199. https://doi.org/10.1007/s10750-011-0663-y

Ruggeri P, Splendiani A, Bonanomi S et al (2012) Temporal genetic variation as revealed by a microsatellite analysis of European sardine (*Sardina pilchardus*) archived samples. Can. J. Fish. Aquat. Sci. 69:1698-1709. https://doi.org/10.1139/f2012-092

Ryman N, Allendorf FW, Jorde PE, Laikre L, Hössjer O (2014) Samples from subdivided populations yield biased estimates of effective size that overestimate the rate of loss of genetic variation. Mol Ecol Resour 14:87-99. https://doi.org/10.1111/1755-0998.12154

Sá-Pinto A, Branco M, Sayanda D, Alexandrino P (2008) Patterns of colonization, evolution and gene flow in species of the genus *Patella* in the Macaronesian Islands. Mol. Ecol. 17:519-532. https://doi.org/10.1111/j.1365-294X.2007.03563.x

Schunter C, Carreras-Carbonell J, Macpherson E et al (2011) Matching genetics with oceanography: directional gene flow in a Mediterranean fish species. Mol. Ecol. 20:5167-5181. https://doi.org/10.1111/j.1365-294X.2011.05355.x

Shaw PW, Pierce GJ, Boyle PR (1999a) Subtle population structuring within a highly vagile marine invertebrate, the veined squid *Loligo forbesi*, demonstrated with microsatellite DNA markers. Mol. Ecol. 8:407-417. https://doi.org/10.1046/j.1365-294X.1999.00588.x

Shaw PW, Turan C, Wright JM, O'connell M, Carvalho GR (1999b) Microsatellite DNA analysis of population structure in Atlantic herring (*Clupea harengus*), with direct comparison to allozyme and mtDNA RFLP analyses. Heredity (Edinb) 83:490-499. https://doi.org/10.1038/sj.hdy.6885860

Silva A (2003) Morphometric variation among sardine (*Sardina pilchardus*) populations from the northeastern Atlantic and the western Mediterranean. ICES J. Mar. Sci. 60:1352-1360. https://doi.org/10.1016/S1054-3139(03)00141-3

Somarakis S, Tsoukali S, Giannoulaki M, Schismenou E, Nikolioudakis N (2019) Spawning stock, egg production and larval survival in relation to small pelagic fish recruitment. Marine Mar. Ecol. Prog. Ser. 617:113-136. https://doi.org/10.3354/meps12642

Souche EL, Hellemans B, Babbucci M et al (2015) Range-wide population structure of European sea bass *Dicentrarchus labrax*. Biol. J. Linn. Soc. 116:86-105. https://doi.org/10.1111/bij.12572

Spanakis E, Tsimenides N, Zouros E (1989) Genetic differences between populations of sardine, *Sardina pilchardus*, and anchovy, *Engraulis encrasicolus*, in the Aegean and Ionian seas. J. Fish Biol. 35:417-437. https://doi.org/10.1111/j.1095-8649.1989.tb02993.x

Tinti F, Di Nunno C, Guarniero I, Talenti, M, Tommasini S, Fabbri E, Piccinetti C (2002) Mitochondrial DNA sequence variation suggests the lack of genetic heterogeneity in the

Adriatic and Ionian stocks of *Sardina pilchardus*. Mar. Biotechnol 4:163-172. https://doi.org/10.1007/s1012602-0003-3

Viñas J, Gordoa A, Fernández-Cebrián R, Pla C, Vahdet Ü, Araguas RM (2011) Facts and uncertainties about the genetic population structure of Atlantic bluefin tuna (*Thunnus thynnus*) in the Mediterranean. Implications for fishery management. Rev. Fish Biol. Fish. 21:527-541. https://doi.org/10.1007/s11160-010-9174-6

Waples RS, Punt AE, Cope JM (2008) Integrating genetic data into management of marine resources: how can we do it better?. Fish Fish (Oxf) 9:423-449. https://doi.org/10.1111/j.1467-2979.2008.00303.x

Ward RD, Woodwark M, Skibinski DOF (1994) A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. J. Fish Biol. 44:213-232. https://doi.org/10.1111/j.1095-8649.1994.tb01200.x

Wright S (1943) Isolation by distance. Genetics 28:114-138. PMC1209196

Würtz M (2010) Mediterranean pelagic habitat: oceanographic and biological processes, an overview. IUCN, Gland and Malaga, ES. pp 88

Figures and tables - Descriptions

Table 1. Summary of the genetic population studies of European sardine (*Sardina pilchardus*).

Figure 1. Studies per subareas along the distribution range (darkest blue) of European sardine (*Sardina pilchardus*). Numbers correspond to study numbers in Table 1. Boundaries of FAO Subareas and GSAs (Geographical Subareas) (in FAO Major Fishing Area 37) of the General Fisheries Commission for the Mediterranean are detailed (white lines).

Table 2. Summary of the population differentiation significant F_{ST} or Φ_{ST} pairwise values in European sardine (*Sardina pilchardus*). Pool of the available data. Reference numbers correspond to study numbers in Table 1.

Figure 2. Range of the available significant F_{ST} or Φ_{ST} values in the studies reviewed (Ramon and Castro 1997; Tinti et al. 2002; Chlaida et al. 2005; Atarhouch et al. 2006; Atarhouch et al. 2007; González and Zardoya 2007; Chlaida et al. 2009; Baibai et al. 2012; Ruggeri et al. 2012; Cannas and Tsigenopoulos 2018; Deli et al. 2020).

Figure 3. Maps of the European sardine (*Sardina pilchardus*) populations according to the current genetic studies. a. Red dotted lines indicate the genetic stocks most robustly defined and probable to date. Diagonal lines represent the areas in which there are more doubts about the definition of the stocks to date. b. Different plausible hypotheses according to the authors along the Iberian Peninsula's coasts. Following the investigations compiled in Table 1.

Figure 4. Map of the potential genetic stocks of the European sardine together with the present-day defined fishing stocks. Dotted lines indicate the genetic stocks determined by the current works (red-dotted lines: robust genetic stocks; white-dotted lines: potential genetic stocks). Colours indicate the current management stocks: Purple: Northern European Stock; pink: Southern European Stock; red: Northern African Stock; green: Central African Stock; orange: Southern African Stock; blue-turquoise transition shades: Mediterranean Stocks managed by GSA (Validated stock assessment forms (SAFs)). Spots represent shared management for multiannual plans (white spots: Alboran area (GSAs 1, 2, 3); black spots: Adriatic area (GSAs 17, 18)).

Figure

Click here to access/download;Figure;Caballero-Huertas et al_Figures_Rev. Fish Biol_Revised.pdf

Figure 1

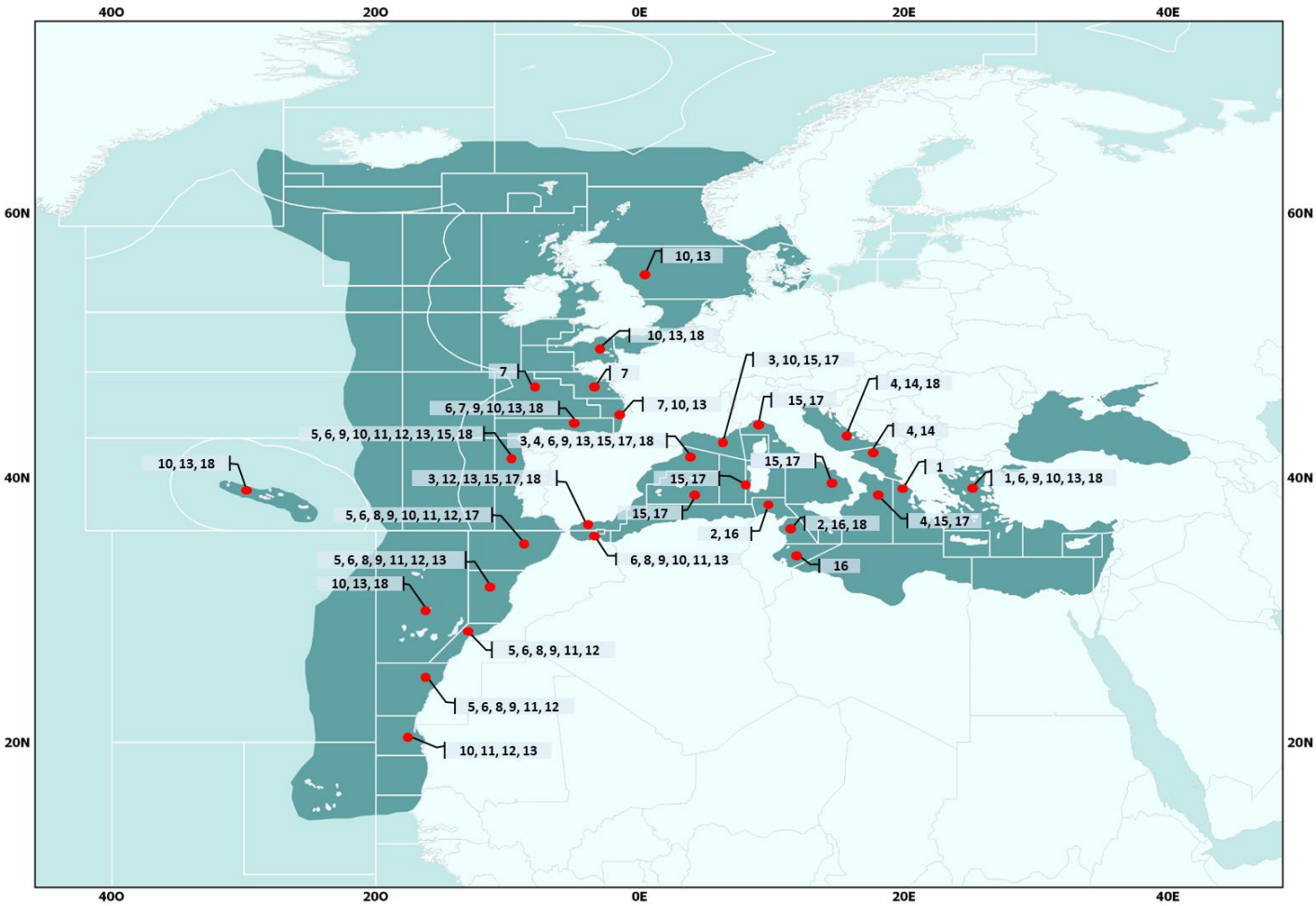


Figure 2

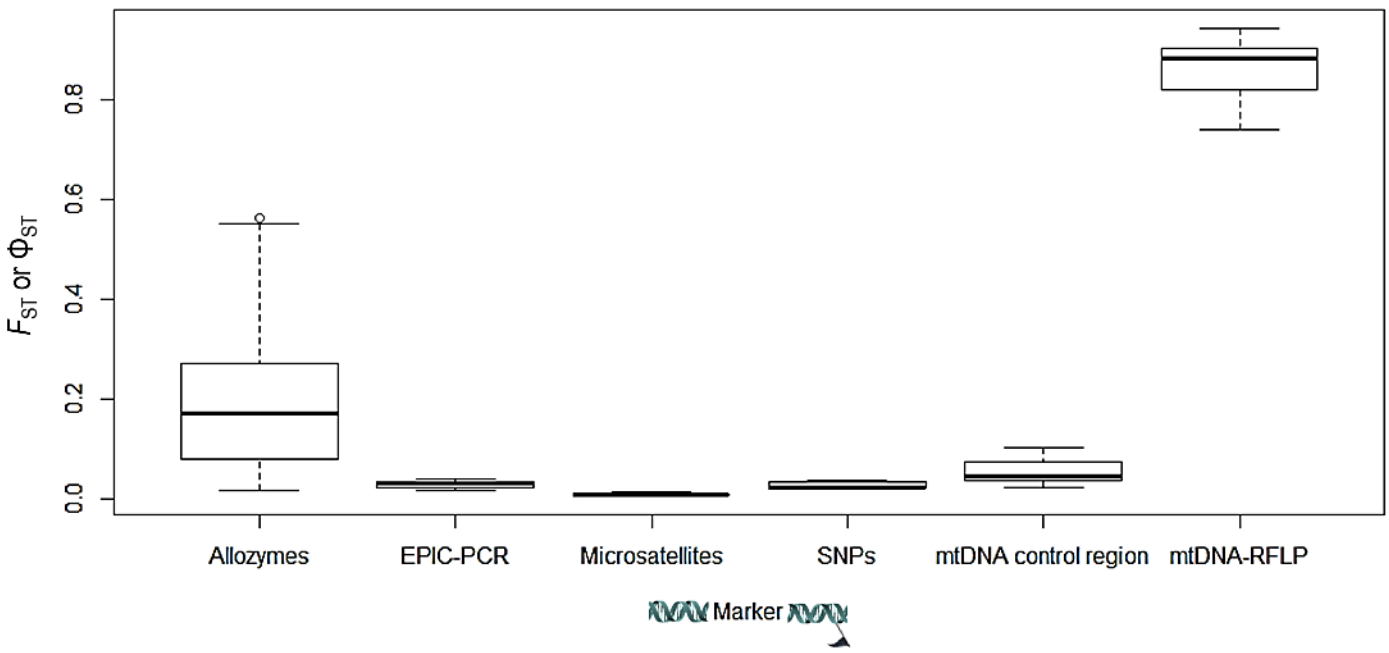
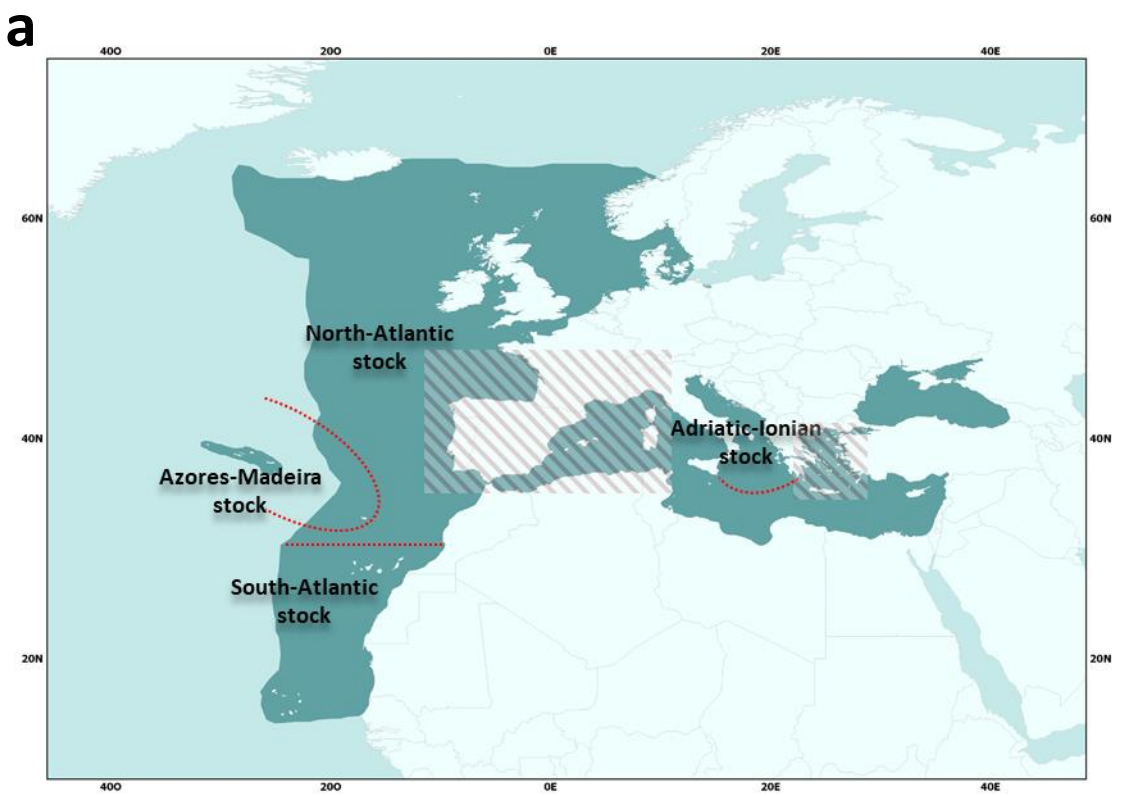

F_{ST} or Φ_{ST}
0.0
0.2
0.4
0.6
0.8
Allozymes
EPIC-PCR
Microsatellites
SNPs
mtDNA control region
mtDNA-RFLP
Marker

Figure 3


a
40O
20O
0E
20E
40E
60N
40N
20N
North-Atlantic stock
Adriatic-Ionian stock
Azores-Madeira stock
South-Atlantic stock

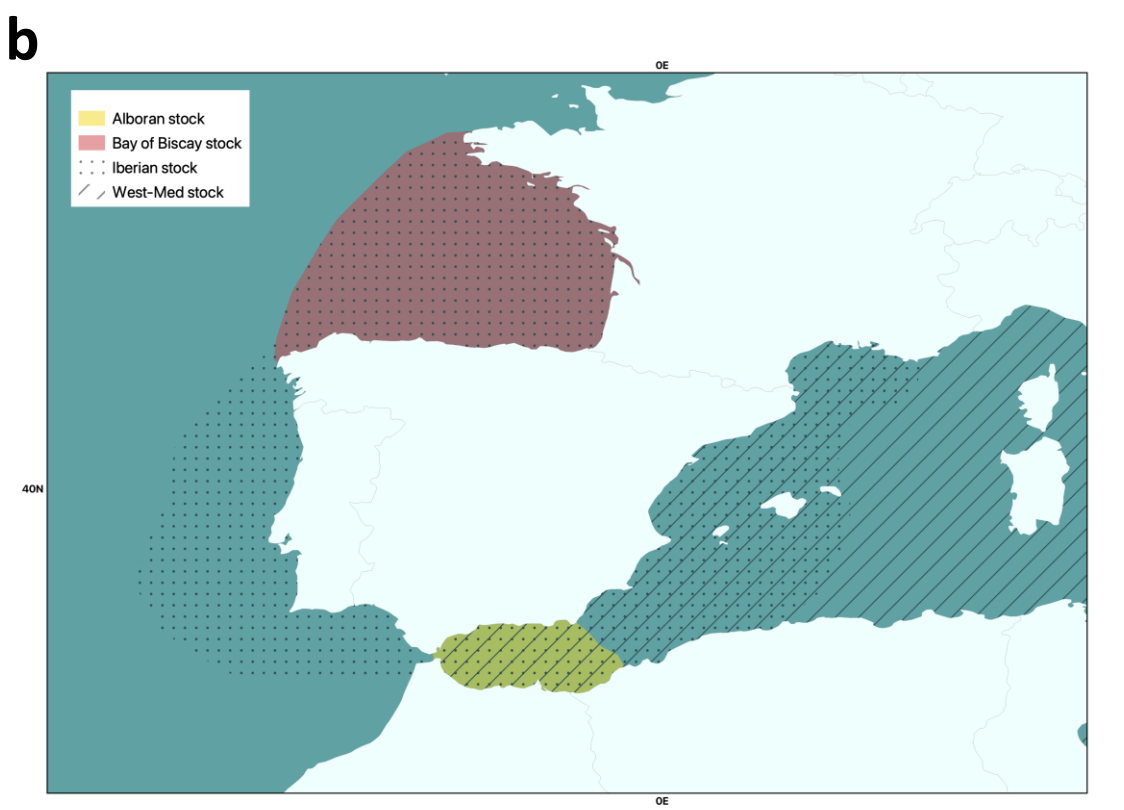

b
0E
40N
Alboran stock
Bay of Biscay stock
Iberian stock
West-Med stock

Figure 4

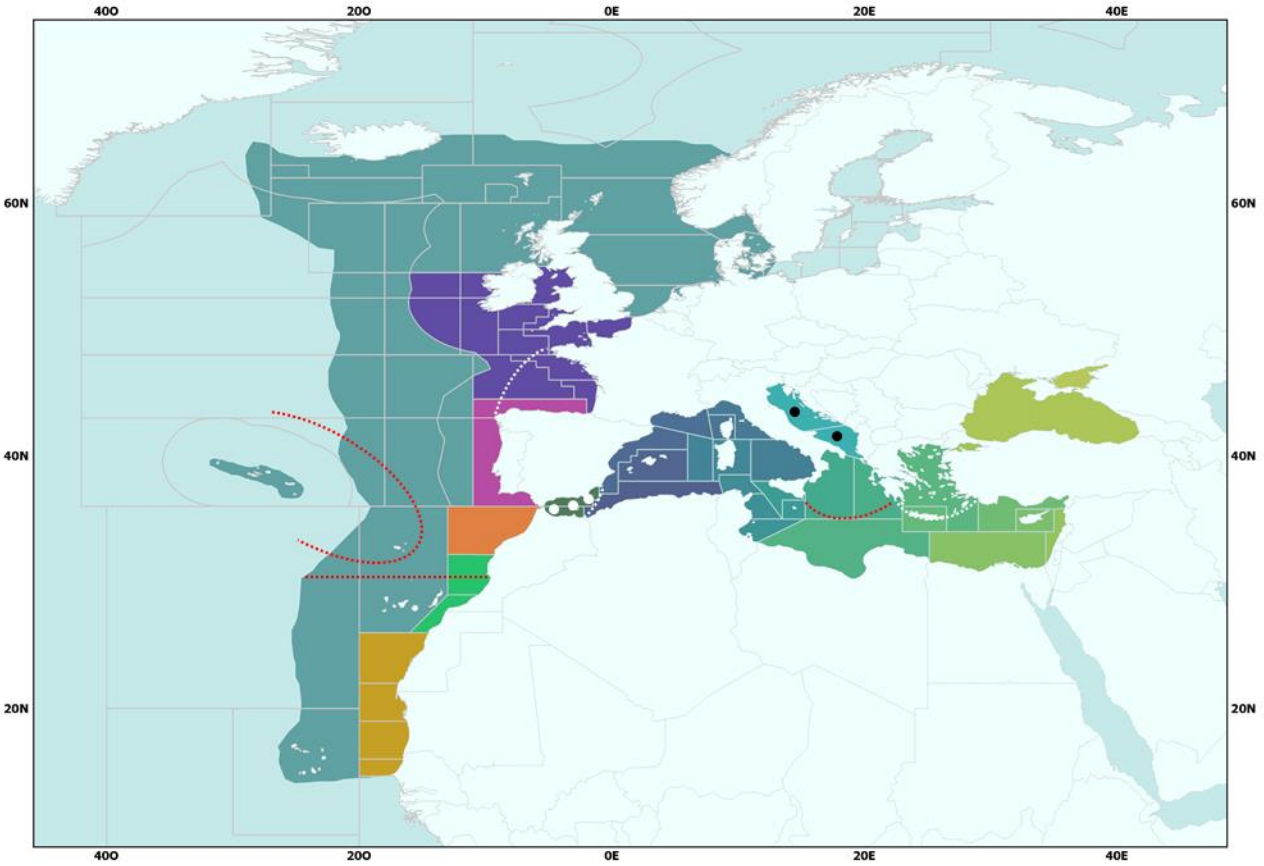


Table 1.

Study	Marker	Area (GSA, FAO)	Comparison	*N*	Ho/h⁺	He	Genetic differentiation	Evidence of natural selection and/or further conclusions
1 Spanakis et al. (1989)	Allozymes	GSA (20,22)	Aegean vs. Ionian seas	2.6	NA	NA	G = 0.0600 – 27.8600, NS	NO
2 Becheikh et al. (1994)	Allozymes	GSA (12, 13)	Tunisian northern coast (Bizerte) vs. eastern seafront (Sousse)	30	NA	NA	NO	NO
3 Ramon and Castro (1997)	Allozymes	GSA (1, 6, 7)	Spanish Mediterranean coast and Gulf of Lion 1990	45	0.130 ± 0.015 0.110 (Valencia-1) - 0.160 (Gulf of Lyon1)	NA	F_{ST} = 0.0749**	North-south cline detected in LDH-1
4 Tinti et al. (2002)	mtDNA-RFLP	GSA (6[a], 17, 18, 19)	Adriatic Sea 1997-1998 vs. Ionian Sea 1997-1998 vs. Spanish Mediterranean Coast	26	NA	NA	Adriatic-Ionian Φ_{ST} = 0.0047, NS Adriatic/Ionian – Spain Φ_{ST} = 0.7414– 0.9429***	NO
5 Chlaida et al. (2005)	Allozymes	FAO (27.9.a, 34.1.11, 34.1.12, 34.1.13, 34.1.31)	All samples 2002-2003	35.86	0.317 ± 0.204	0.309 ± 0.192	F_{ST} = 0.2200	NO
6 Atarhouch et al. (2006)	mtDNA control region	GSA (3, 6, 22), FAO (27.8.c, 27.9.a, 34.1.11, 34.1.12, 34.1.13, 34.1.31)	All samples 2002-2004	29	NA	0.997 ± 0.002	Φ_{ST} = 0.0260 (one gene pool); Φ_{ST} = 0.0590 (three gene pools)	Isolation and genetic drift acting on the Safi population
7 Laurent et al. (2006)	Allozymes	FAO (27.8.a, 27.8.b,	All samples 2002	47.5	NA	NA	F_{ST} = 0.0050 ***	Appears to be composed of a single panmictic population

		27.8.c, 27.8.d.2)						but gradual change in two loci
8 Atarhouch et al. (2007)	Exon-primed intron-crossing PCR (EPIC-PCR)	GSA (3), FAO (34.1.11, 34.1.12, 34.1.13, 34.1.31)	All samples 2001 - 2002	49.4	0.39 - 0.58	0.62 - 0.72	F_{ST} = 0.034*	No correlation between geographic and genetic distances. Potential genetic drift due to climate shifts
9 González and Zardoya (2007)	Microsatellites	GSA (3, 6, 22), FAO (27.8.c, 27.9.a, 34.1.11, 34.1.12, 34.1.13, 34.1.31)	Mediterranean vs. Atlantic	47.3	0.747 ± 0.04	0.948 ± 0.00	R_{ST} = 0.0240 ± 0.0260 – 0.0470***	Significant isolation by distance with R_{ST}
10 Laurent et al. (2007)	Allozymes	GSA (3, 7, 22), FAO (27.4.b, 27.7.e, 27.8.b, 27.8.c, 27.9.a, 27.10.a.2, 34.1.11, 34.1.2, 34.1.32)	All samples 2003-2004	48.6	NA	NA	F_{ST} = 0.0570**	Selective pressure and isolation by distance of coastal populations (from Mauritania to the North Sea)
11 Chlaida et al. (2009)	Allozymes	GSA (3), FAO (27.9.a, 34.1.11, 34.1.12, 34.1.13, 34.1.31, 34.1.32)	All samples 2004	50	0.048 ± 0.023	NA	F_{ST} = 0.2050***	Not possible to evaluate the selection with only one locus showing significant heterozygosity. Genetic cline. Significant relationship between geographic distance and pairwise genetic differentiation among all samples
12 Baibai et al. (2012)	Microsatellites and mtDNA control region	GSA (1), FAO (27.9.a,	All samples	50	0.843 ± 0.11	0.905 ± 0.12	F_{ST} = 0.0017 - 0.0140	Isolation by distance between Cap Blanc and Galician

		34.1.11, 34.1.12, 34.1.13, 34.1.31, 34.1.32)	Maroccan Coast 2006, Iberian Coast 2008			Θ= 51.505 ± 7.336		samples (2,500 km) and oceanographic and environmental barriers contributing to the isolation of the northern and southernmost stocks
13 Kasapidis et al. (2012)	Microsatellites	GSA (1, 3, 6, 22), FAO (27.4.b, 27.7.e, 27.8.b, 27.8.c, 27.9.a, 27.10.a.2, 34.1.12, 34.1.2, 34.1.32)	All samples 1999-2004	73	0.639 ± 0.114 (Sp22); 0.931 ± 0.005 (Sp2,7,I5)	0.674 ± 0.119 (Sp22); 0.924 ± 0.006 (Sp2,7,I5)	F_{ST} = 0.0170	Loci (Sp22) appeared to be under selection and exhibited a geographic pattern
14 Ruggeri et al. (2012)	Microsatellites	GSA (17, 18)	Northern and Southern Adriatic Sea 1978 -2009	47.5	0.765 ± 0.039	0.795 ± 0.015	θ_{ST} = - 0.0033 to 0.0137 2/45 pairwise θ_{ST} comparisons were significant: CH91 vs. VI89 (θ_{ST} = 0.0127**) and CH95 vs. VI89 (θ_{ST} = 0.0137**)	NO
15 Cannas and Tsigenopoulos (2018)	SNPs	GSA (1, 5, 6, 7, 9, 10, 11, 19), FAO (27.9.a)	All samples 2017-2018	35	0.300 ± 0.012	0.335 ± 0.010	F_{ST} = 0.00174*	NO
16 Deli et al. (2020)	Allozymes	GSA (12, 13, 14)	All samples	16.36	0.1628 ± 0.2244	0.1495 ± 0.1883	F_{ST} = 0.1660**	NO
17 Antoniou et al. (in press)	Genotyping through high-throughput sequencing (ddRADseq)	GSA (1, 5, 6a, 6b, 6c, 7a, 7b, 9, 10, 11,	All samples 2017-2018	33.16	0.266 - 0.307	0.266 - 0.296	F_{ST} = 0 - 0.0408	Water temperature, and derived environmental products informing on changing temperature

		19), FAO (34.1.11)						conditions were extremely important driving natural selection and local adaptation
18 Fonseca et al. (in press)	Whole genome sequence	GSA (1, 6, 13, 17, 22), FAO (27.10.a.2, 27.7.e, 27.8.c, 27.9.a, 34.1.2)	All samples	6.5	NA	NA	F_{ST} = 0.9600, highest value	Phylogeographic break between the South of Portugal and Mediterranean populations; Barrier to gene flow between Azores/Madeira and other areas; the population from the Canary Islands has a Mediterranean ancestry

GSA (Geographical Subarea of the General Fisheries Commission) encompassed within FAO Major Area 37

[+] Ho: Observed heterozygosity for nuclear markers / h: haplotype diversity for mitochondrial markers

NA: Data not reported

[a] Data comparison with other studies

***, ** and * indicate significance at $P < 0.001$, $P < 0.01$ and $P < 0.05$ regarding 'Genetic differentiation'

Table 2.

Marker	Methodology	Number of studies included	Percentage of significant values (%)	Smallest significant value	References
Allozymes	Nuclear	4	70.28 (149/212)	0.0180	3, 5, 11, 16
EPIC-PCR	Nuclear	1	61.9 (13/21)	0.017	8
Microsatellites	Nuclear	3	11.71 (13/111)	0.0080	9, 12, 14
SNPs	Nuclear	1	13.64 (9/66)	0.0209	15
mtDNA control region	Mitochondrial	2	30.77 (20/65)	0.0243	6, 12
mtDNA-RFLP	Mitochondrial	1	16.67 (11/66)	0.7414	4