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PhD Thesis

Swordfish, *Xiphias gladius* (Linnaeus, 1758) genetic variability assessment within the Mediterranean Sea

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LIST OF PAPER

I am co-author of the following publications that are not included in this

thesis, but were published during my doctoral studies:

Fioravanti T, Splendiani A, Giovannotti M, Nisi Cerioni P, **Righi T**, Rossetti A, Morandi F, Caputo Barucchi V. 2019. The chamois (*Rupicapra cf pyrenaica*) in central Italy: what ancient DNA tells us. The chamois (*Rupicapra cf pyrenaica*) in central Italy: what ancient DNA tells us *Hystrix, the Italian Journal of Mammalogy*, *30*(2).

Petetta A & **Righi T**, Splendiani A, Virgili M, Giovannotti M, Lucchetti A, Caputo Barucchi V. 2019. A new mtDNA control region haplotype from sea lamprey (*Petromyzon marinus* Linnaeus, 1758: Petromyzontiformes) collected off the Italian Adriatic coast. *Marine Biodiversity*:1–4.

Splendiani A, Fioravanti T, Ruggeri P, Giovannotti M, Carosi A, Marconi M, Lorenzoni M, **Righi T**, Nisi Cerioni P, Caputo Barucchi V. 2019a. Life history and genetic characterization of sea trout *Salmo trutta* in the Adriatic Sea. *Freshwater Biology*.

Splendiani A, Giovannotti M, **Righi T**, Fioravanti T, Nisi Cerioni P, Lorenzoni M, Carosi A, La Porta G, Caputo Barucchi V. 2019b. Introgression despite protection: the case of native brown trout in Natura 2000 network in Italy. *Conservation Genetics* 20:343–356.

1 INTRODUCTION

1.1 The fish stock concept

The main factors that threaten marine fish biodiversity are fishing together with environmental degradation (Dulvy, Sadovy & Reynolds, 2003). Worldwide over 50 local populations of marine fish have gone extinct as a result of overexploitation (Dulvy et al., 2006). Long before a species declines into extinction it will suffer a reduction in the level of genetic diversity within and among its populations Overfishing may lead to the depletion of weak or less productive stocks, resulting in reduced genetic diversity and increased inbreeding but can have substantial consequences for the productivity of fish populations over time (Gascoigne & Lipcius, 2004; Kell, Crozier & Legault, 2004). The *fish stock* concept arose due to the need to define discrete groups of fish, that their internal dynamics could be audited against the effects of fishing (Cadrin & Secor, 2009). The fish stock concept has changed its own significance during the last century and a wide range of stock definitions have been proposed (Carvalho & Hauser, 1995). One of the first definition was purely an operational purpose defined by and defined a fish stock as *"a specific portion of the population that is influenced by anthropic activities that affect population productivity"* (Dahl, 1909 cited in Waldman, 2005). This definition later was identified as *"harvest stock"* as *"a locally accessible fish resources in which, fishing pressure on one resource has no effect on the abundance of fish in another contiguous resource"* (Gauldie, 1988). A biological meaning was added to the stock concept by

Larkin (1972) defining stock as *" a population of organisms which, sharing a common gene pool, is sufficiently discrete to warrant consideration as a self-perpetuating system which can be managed"*. One of the most robust and sufficiently specific definition is that of Ihssen (1981) who defined a stock as *"... an intraspecific group of randomly mating individuals with temporal and spatial integrity"*. This concept covers many of the definitions given by other authors including the concept of spatial and temporal integrity (Secor, 2014). Although the definition of stocks tends to be operational, clearly, the identification of stocks implies the delimitation of the structure of the fish populations. For practical as well as biological reasons, populations are the fundamental biological unit for conservation and management (McElhany *et al. 2*000; Beissinger & McCullough 2002). Delineation of spatial management areas ignoring the spatial distribution and interaction of fish population may result in i) depletion or extinction of the most vulnerable or local subpopulations and ii) loss of genetic diversity that reduces the ability for the species to evolve and to adapt to environmental changes. A frequent mismatch between ecological and/or biological processes and management actions has led, in the last decade, to the decline of many commercial fish stocks (Reiss et al., 2009).

1.2 The genetic approach to stock structure identification

The concept of the 'stock' is fundamental for both fisheries and endangered species management. There are several methods available for stock delineation based on i)

analysis of morphological and morphometric characteristic; ii) analysis of life-history traits (distribution, abundance, age composition, rate of mortality; iii) satellite techniques, marking and recapture of individuals; iv) analysis of the chemical micro-components of otoliths as a natural marker of fish stocks; v) analysis of natural parasites as biological tags; vi) genetic analysis through the use of genetic-molecular markers. Although a holistic approach, involving a broad spectrum of complementary approaches may be appropriate for resolving stock structure (Begg & Waldman, 1999; Waples, Punt & Cope, 2008; Cadrin, Kerr & Mariani, 2013), genetics approach is sensitive and reliable.

The genetic approach to the identification of fish stocks consists of the estimation of the extent of genetic differentiation degree populations belonging to the same species. The changes in the allelic and genotypic frequencies between one generation and the next one are essentially the result of dispersive (inbreeding and genetic drift) and systematic processes (mutation, migration and selection) (Kapuscinski & Miller, 2007). Both processes have a different effect on the genetics of populations. Dispersive processes determine loss of alleles, increased homozygosity and loss of genetic diversity. Contrarily, mutation and migration, increase genetic diversity, counteracting the effects of genetic drift and inbreeding (Allendorf & Phelps, 1981). Finally, selection, whether natural or artificial, can cause changes in allelic and genotypic frequencies, often, reducing diversity. The opposite effect of these two processes could determine several

degrees of genetic differentiation or homogenization among populations. Migration of individuals between populations with different allelic frequencies can homogenize the allelic frequencies reducing population differentiation. Moreover, immigration of reproductive adults may introduce new alleles into recipient populations counteracting the effects of inbreeding and genetic drift on genetic diversity. Relatively low rate of immigration is sufficient to balance loss genetic diversity due to dispersive processes (Allendorf & Phelps, 1981). The impact of immigration on genetic diversity is an important issue for fisheries management because stocking and transplantation of fish are artificial forms of immigration (Kapuscinski & Miller, 2007). On the contrary, genetic drift, inbreeding and selection are involved in the increasing of genetic differentiation between populations. These processes, in the absence of gene flow between populations, could promote the fixation of some alleles and the loss of other ones (Kapuscinski & Miller, 2007).

The marine environment is characterized by a lack of physical barriers, and the broad ranges and high dispersal abilities of marine organisms tend to promote gene flow, showing a shallow population genetic structure made the detection of population subdivision difficult (Waples, 1998). However, the presence of isolating barriers such as landmasses, ocean currents, philopatric instinct might lead to isolation and differentiation of subpopulations that are demographically distinct from others over the species' entire range (Secor, 2014). The genome of each individual record the polymorphism created

by complex interactions between selection, genetic drift and mutation/migration and the resulted genetic variation could be detected through the use of molecular markers (Féral, 2002). Subpopulations, if isolated long enough, may evolve into genetically distinct units, constituting relatively independent demographic units (Steneck & Wilson, 2010). The genetic approach can be extremely powerful tool in fisheries management permit estimates of genetic similarity among organisms and differences among relatively isolated populations within species can be examined.

1.2.1 Molecular markers

Since the discovery of a connection between genetic features and stock concept, the use of genetic tools to identify stock structure has become the approach to choice in this type of studies. The genetic stock identification is based on the use of molecular markers. Molecular markers represent the tools by which it is possible to estimate the extent of genetic differentiation among fish stock. A useful molecular marker should be characterised by a high degree of genetic variability, which is the main feature of molecular marker that allows the detection of genetic differentiation between stocks (Wirgin & Waldman, 2005). Nucleic acids represent universal molecular markers to investigate the identity of stocks and population (Carvalho & Hauser, 1995). According to (Secor, 2014) genetic markers are ideal stock discriminators because they are: i) independent of environmental changes during the course of an individual's lifetime; ii) composed of discrete units of information so that population differences can be readily

quantified; iii) encoded in the universal language of DNA; iv) measurable with reasonable efforts and costs; v) analysed with such statistical procedures. In fisheries, both mitochondrial and nuclear genetic markers are commonly used.

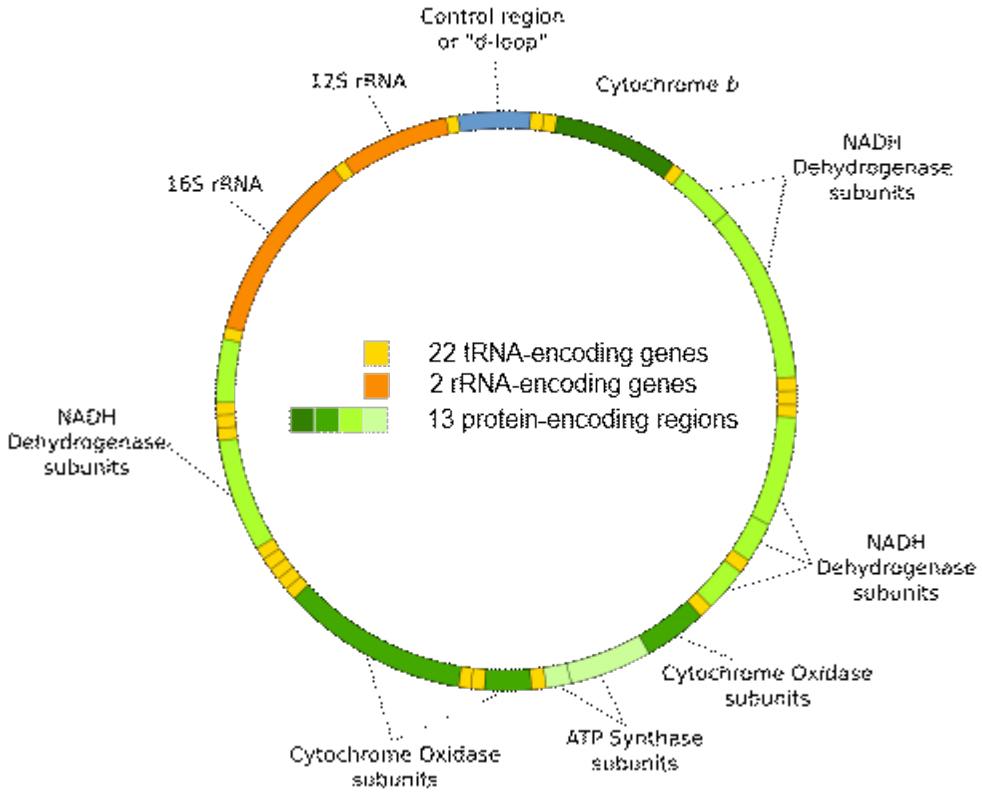


Figure 1.1. Representation of a human mtDNA. (https://www.wikipedia.org/).

1.2.1.1 *Mitochondrial DNA (mtDNA)*

Mitochondrial DNA (mtDNA) is a small (16,000-20,000 base pairs (bp) in fish), double-stranded circular DNA molecule contained in multiple copies in the mitochondria, which are membrane-enclosed organelles found in the cytoplasm of all eukaryotic cells. The

animal mitochondrial genome contains 13 genes encoding proteins, 2 genes coding for ribosomal RNAs (12S and 16S rRNA), 22 genes coding for transfer RNAs (tRNAs), and 1 noncoding control region (called the D-loop in vertebrates). The D-loop is about 1000 bp long and contains the origin of mtDNA replication. The mitochondrial genome has a very compact organization since there are no introns within the coding regions. This molecular marker is almost exclusively inherited directly from mother to offspring without recombination, and, usually, it is homoplasmic. mtDNA is characterized by relatively high mutation rates which generate correspondingly high levels of polymorphism as a consequence of the deleterious effects of oxidative stress and the inefficient DNA repair that continuously generates new mtDNA alleles (Avise, 2009). The basic idea behind using mtDNA for stock structure analysis is that the samples belonging to the same stock contain the same types of mtDNA molecules or haplotypes in similar frequencies. Contrarily, if samples belong to different stocks a substantial difference in the mitotype distribution among the samples are observed, highlighting a certain degree of genetic isolation (Antoniou & Magoulas, 2014).

1.2.1.2 *Microsatellite*

Other most useful and largely used molecular markers are the Short tandem repeats (STR) or, simply, “microsatellite”. The use of microsatellites has increased since the latter half of the 1990s, and they are already extensively applied in conservation and population genetics studies of marine fish. Microsatellite DNA are small segments of

nuclear DNA containing repeat motifs constituted by two to six base pairs (bp) and the levels of variation among individuals are related to the number of repeats (Tautz & Renz, 1984). Dinucleotide, trinucleotide and tetranucleotide repeats are the most common choices for molecular genetic studies. Microsatellites are biparentally transmitted markers that are inherited in Mendelian fashion and they are generally considered to be selectively neutral (Weber & Wong, 1993) Microsatellite loci are analysed by means of PCR amplification, primed by species-specific oligonucleotide sequences (15-25 bp) annealed to the nonrepetitive flanking regions of the locus. Subsequently, the fluorescently labelled fragments amplified are subjected to capillary electrophoresis and their size accurately estimated through laser detection in an automated sequencer. Microsatellites are codominant markers then both parental alleles at each locus are detectable. Even if the study of microsatellites is apparently simple, several drawbacks may affect the analysis. Allele stuttering is the production of multiple peaks for the same allele, caused by strand slippage during DNA synthesis in the PCR; due to polymerase bias, the last of the peaks generally corresponds to the true allele, but heterozygotes with similar-sized alleles can result difficult to score. Allele dropout is the under-amplification of one of the two alleles (often the larger of the two), due to low concentration, poor quality of the template, or simply PCR bias, when one allele is considerably larger than the other. Null alleles are caused by the failed amplification of an allele due to a mutation in the primer region, which prevents the binding of the primer on the site. The likely

presence of null alleles can be statistically inferred (Van Oosterhout et al., 2004) and then verified, if necessary, by sequencing the full fragment of the suspected sample. Finally, homoplasy occurs when two alleles have the same size but different lineages, that is not as a result of common descent but due to random mutation. For instance, point mutations will leave the size of an allele unchanged, and insertions or deletions in the flanking region might create a new allele with the same size as an existing allele (Selkoe & Toonen, 2006). Homoplasy frequency is proportional to the genetic distance of two individuals or populations and can be quite common for high divergent groups, in large populations, over longer timescales and at loci with particularly high mutation rates: in this way it can obscure the signal of population differentiation.

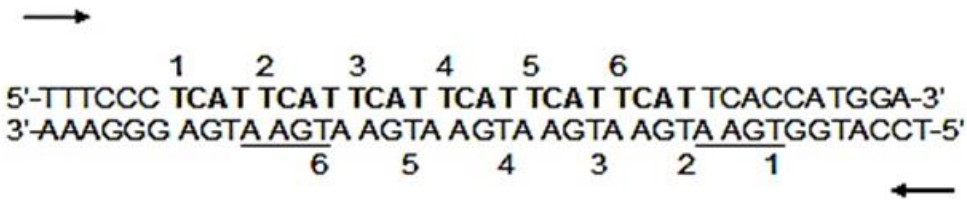


Figure 1.2. Forensic DNA Typing. An example of Short Tandem Repeats 4 base pairs (TCAT) in 6 repeats.

1.2.1.3 *RADseq linked SNPs*

Recent advances in high-throughput DNA sequencing now allow the rapid generation of large amounts of genomic data in non-model species (Davey et al., 2011). NGS-based approaches allow obtaining millions of genomic fragments of known sequence variable among individuals and populations, which can be used as markers in stock identification. The most commonly used variable sites are constituted by single base-pair substitutions, known as single nucleotide polymorphisms (SNPs). SNP markers that exhibit sufficiently high polymorphism can be used, usually in conjunction with many other SNPs, to quantify genetic variation among individuals. SNPs are generally biallelic and located in either coding or noncoding regions of the genome. The development of Restriction site-Associated DNA Sequencing (RADseq) was deemed among the most significant scientific breakthroughs within the last decade (Andrews et al., 2016). RADseq was applied in ecological, evolutionary, and conservation genomics studies thanks to the massive throughput of next-generation sequencing to uncover hundreds or thousands of polymorphic genetic markers across the genome (Davey et al., 2011). The core feature of RADseq techniques is the use of restriction enzymes to obtain DNA sequence at a genome-wide set of loci. Like other reduced-representation sequencing approaches, RADseq targets a subset of the genome, providing advantages over whole-genome sequencing including a greater depth of coverage per locus and allowing sequencing of greater numbers of sample in a single, simple, and cost-effective

experiment. RADseq does not require any prior genomic information for the taxa being studied, then, RADseq has become the most widely used genomic approach for high-throughput SNP discovery and genotyping in ecological and evolutionary studies of non-model organisms (Andrews et al., 2016).

1.2.2 Genetic population structure in large pelagic fish

After the formation of the Isthmus of Panama, the potential contact between Atlantic and Indo-Pacific populations was limited to the waters around southern Africa. Separation of the Atlantic and Pacific Oceans resulted in significant genetic drift for many large pelagic fishes since these subpopulations are now genetically differentiated (Ely et al., 2005).

Mitochondrial DNA has proven to be an extremely powerful marker to reject the hypothesis of panmixia in several worldwide distributed highly migratory pelagic fishes. In several large pelagic fish species, analysis of mitochondrial DNA supported the existence of two distinct mtDNA lineages, one of which ubiquitous, widespread in the Atlantic and Indo-Pacific Oceans, and the other are endemic to the Atlantic Ocean. This asymmetrical distribution of the two mtDNA lineages indicated interoceanic population structuring between Atlantic and Indo-Pacific oceans in bigeye tuna (*Thunnus obesus*) (Bremer et al., 1998; Chow et al., 2000; Martínez et al., 2006), swordfish (*Xiphias gladius*) (Bremer, Baker & Mejuto, 1995; Bremer et al., 1996; Rosel & Block, 1996), albacore (*Thunnus alalonga*) (Viñas, Bremer & Pla, 2004), blue marlin (*Makaira nigricans*) (Finnerty & Block, 1992) and sailfish (*Istiophorus platypterus*) (Graves &

McDowell, 1995). MtDNA also identified genetically differentiated Mediterranean population in bluefin tuna (*Thunnus thynnus*) (Bremer, Naseri & Ely, 1999) swordfish (*Xiphias gladius*) ((Kotoulas et al., 1995; Bremer et al., 1996; Rosel & Block, 1996; Chow et al., 1997; Chow & Takeyama, 2000), albacore (*Thunnus alalunga*) (Viñas, Bremer & Pla, 2004). Significant within ocean differentiation was detected between Northern and Southern swordfish populations in the western Pacific (Reeb, Arcangeli & Block, 2000). Moreover, differentiation between Eastern and Western Mediterranean areas has also been reported for the Atlantic bluefin tuna (Carlsson et al., 2004, 2006) and swordfish (Viñas et al., 2010) using mtDNA.

Population genetic studies based on multilocus microsatellite genotypes revealed genetic differentiation among the Mediterranean and Atlantic and Pacific in albacore (Takagi et al., 2001; Davies et al., 2011; Montes et al., 2012) and swordfish (Kotoulas et al., 2007) populations. Microsatellite revealed also intra-oceanic population structure in some large migratory species. Significant differences were detected between the 2 Pacific hemisphere, as well as between Southwest and Southeast Pacific (Takagi et al., 2001) in albacore tuna. In swordfish, microsatellite revealed small but statistically significant genetic differentiation between South and North Atlantic (Kasapidis et al., 2007). Moreover, within Mediterranean Sea differentiation was detected between western and eastern basin in albacore tuna (Davies et al., 2011; Montes et al., 2012) bluefin tuna (Carlsson et al., 2004, 2006; Riccioni et al., 2010).

The high-throughput SNP genotyping was applied to investigate the global population structure of yellowfin tuna (*Thunnus albacares*) (Pecoraro et al., 2018). Based on neutral loci significant differences were detected among the three oceans. In contrast, analyses based on the outlier SNPs provided evidence for strong differentiation between eastern and western samples collected within the Atlantic and Pacific oceans. Antoniou (2017) analysing genome-wide SNPs from Atlantic bluefin tuna samples collected throughout the Mediterranean Sea did not provide strong evidence of genetic structure. Maroso et al (2016) using 2bRADseq identified weak genetic differentiation within the Mediterranean sea between Western and eastern Mediterranean dolphinfish population.

1.3 Biologic background of Atlantic and Mediterranean swordfish

Swordfish (*Xiphias gladius* Linnaeus, 1758), is the monotypic member of the family *Xiphiidae* and is characterised by a long-flatted bill which makes up a third of its total length. The absence of pelvic fins, teeth and scales, and a single pair of caudal keels are peculiar features that distinguish swordfish from istiophorid billfishes (sailfish, marlins, and spearfishes)(Nakamura, 1985)).

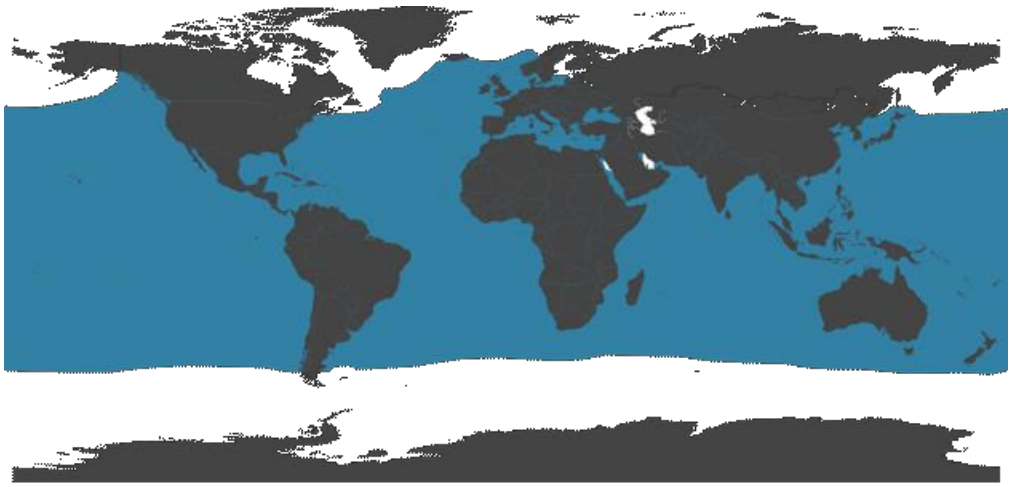


Figure 1.3. Global distribution of swordfish (http://www.fishbase.org/summary/Xiphias-gladius.html).

Swordfish is a migratory and pelagic fish, it is distributed worldwide from 45°N to 45°S in all tropical, subtropical, and temperate waters, including in the Mediterranean Sea, the Sea of Marmara, the Black Sea, and the Sea of Azov (Palko, Beardsley & Richards, 1981). Based on data from commercial catches of longlines, the latitudinal range extends from the coast of Newfoundland (Tibbo, Day & Doucet, 1961) to Argentina (De Sylva, 1962) in the Western Atlantic Ocean; from Scandinavia (Duncker, 1936) along the west coast of Africa down to the Cape of Good Hope (Penrith M.J., 1974) in the Eastern Atlantic Ocean. In the Western Pacific Ocean they are widely distributed from temperate waters off the coast of Japan (Yabe et al., 1959) to the waters of Australia and New Zealand (Webb, 1972) while, in the Eastern Pacific Ocean, from Oregon (Fitch, 1960) to Chile (Lobell et al., 1947); they are also caught off the Hawaiian (Strasburg, 1970) and Galapagos Islands.

Swordfish migration is complex and multi-directional, it is characterised by seasonal patterns in the horizontal movement, to foraging and spawning ground, and diel vertical behaviour, with fish, mainly staying in the mixed layer at night and descending to high depths during the daytime (Abascal et al., 2015). Swordfish is an opportunistic predator that feeds on pelagic vertebrates and invertebrates. Swordfish diet is mainly composed of cephalopod, followed by teleost fishes. Contribution of crustaceans was the lesser extent and it could be considered to be an accidental food (Chancollon, Pusineri & Ridoux, 2006; Gorni et al., 2012). Swordfish generally inhabits waters with sea surface

temperatures (SST) ranging from 10°C to 28°C, however thanks to its thermogenic organ, this species tolerates high changes of environmental temperature ranging from 4° to 28 °C, with daily ranges frequently over 15°C (Neilson et al., 2014; Abascal et al., 2015).

Several studies have been conducted on the reproductive biology of Atlantic and Mediterranean swordfish including gonad somatic index (GI and RGI), microscopic examination and measurement of whole oocytes and oocyte cytology, and the distribution of eggs and aged young larvae and the characterization of spawning areas (see Arocha, 2007; Neilson et al., 2013 for summary). Swordfish is a gonochoric species but no diagnostic external characters distinguish males from females (Palko, Beardsley & Richards, 1981). Female has asynchronous ovaries showing a long spawning period (>90 days) with a spawning frequency estimated at 2,6 days in the North Atlantic Ocean (Arocha, 2007). The testis is an unrestricted spermatogonial testis type, spermatogenesis appears to be of the cystic type in which all stages of spermatogenesis occur within the cysts. Female swordfish release from 1.6 x 10^6 eggs in the Mediterranean to 3.9 x 10^6 eggs per female in the North Atlantic with a diameter of approximately 1.6 to 1.8 mm (Abid & Idrissi, 2006). The eggs are floating and transparent with a large oil droplet and a very small yolk sac. During the pre-adult phase, swordfish undergo drastic changes in body shape. Juveniles have a long, thin and snake-like body and show teeth and scales that disappear when the animal reaches adulthood. Dorsal and anal fins are continuous

and caudal fin is indented into the fork. Moreover, also the lower jaw is prolonged into a long bill (Nakamura, 1985).

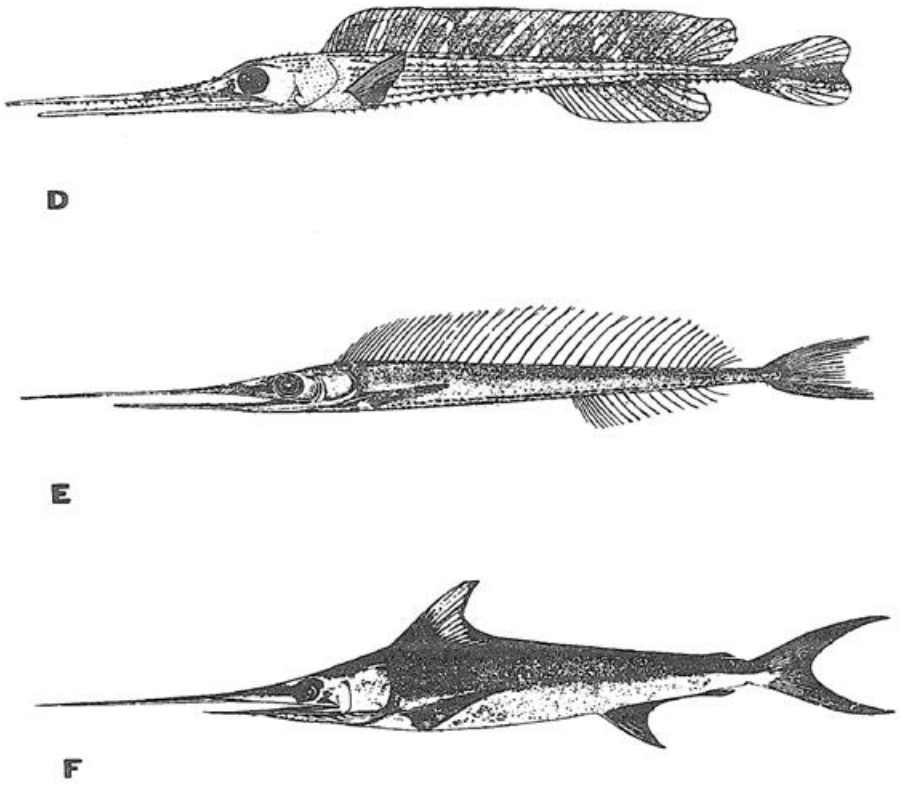


Figure 1.4. Young of the swordfish: D = 68,8 mm SL (Sword Length); E = 252mm BL (length from posterior edge of orbit to base of caudal fin); F = 580mm BL. Specimen D from Aruta (1954), E and F from Nakamura et al. (1951).

Especially during the first year, swordfish growth is relatively rapid and sexually dimorphic. Males have slower growth rates reaching a lower maximum size than females. This characteristic is common in all swordfish stocks in the different oceans, although differences in the maximum size have been observed. Females predominate in catches of fish over 150 cm in the Mediterranean Sea and represent almost 100% of catches of fish over 190cm in the Mediterranean and 225cm in the Atlantic (Abid & Idrissi, 2006).

Sexual dimorphism has been reported for size and age at first maturity. Female maturation, generally, occurs later and at a bigger size than the male. Estimation of L_{50} (length at which 50% of the fish are mature) of swordfish reported differences between stocks and oceans. In north-western Atlantic, females mature at age 5 at the L_{50} size of 178.7 cm LJFL (Lower Jaw Fork length) while males mature very early at ages 1-2 (L_{50}=128.7 cm LJFL). In the South Atlantic, females mature at the ages 2-3 (L_{50}=156 cm LJFL). In the Mediterranean Sea swordfish showed the lowest size compared to Atlantic swordfish (female L_{50}= 142.2 cm, ages 2-3) (Arocha, 2007; Neilson et al., 2013).

Swordfish spawning appeared to be closely related to sea surface temperature (SST) >23-24°C and the displacement of 24°C SST isotherm. Given the strong dependence from the environmental condition, spawning took place in a specific location showing a peculiar seasonal pattern. In the North Atlantic spawning began in December-February southern-west of the Sargasso Sea and continued through the Caribbean Sea passages in

March-May ending in the South-Eastern U.S. in the summer months. In the South Atlantic swordfish, spawning appeared to occur mostly around the equator (5-10°S) and west of 20°W throughout the year. In the Mediterranean sea, spawning took place in a shorter time window from June to the end of August in three main areas, one in each region of the basin (eastern, central and western Mediterranean) between 35° and 40°N.

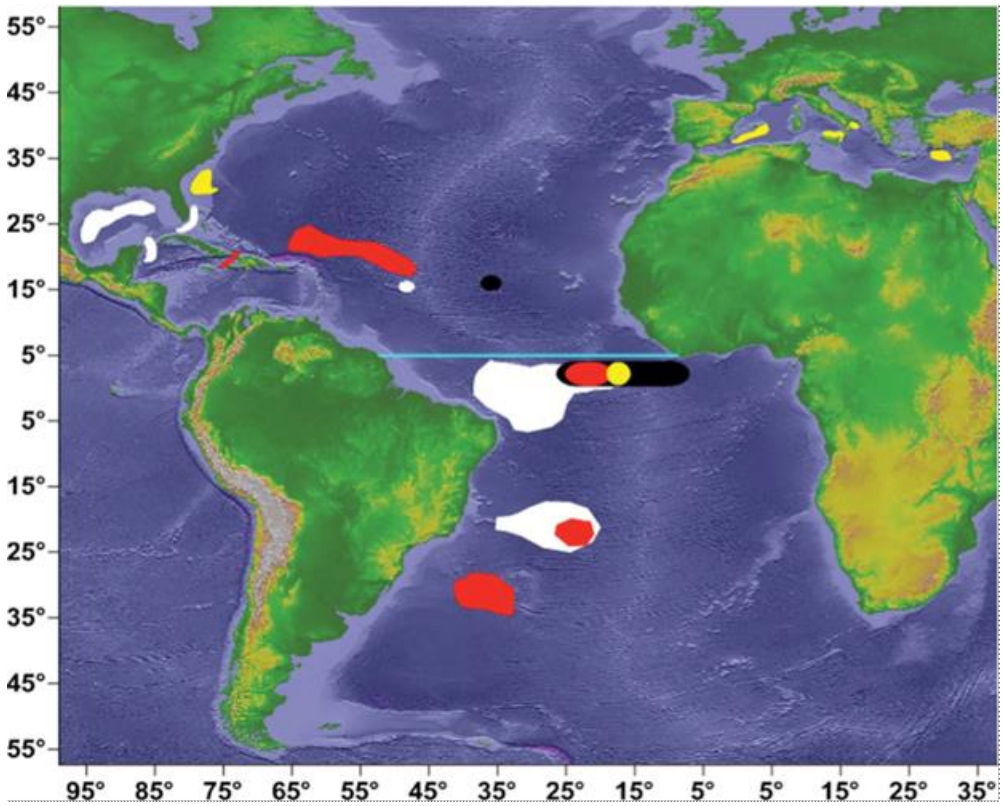


Figure 1.5 Swordfish seasonal spawning areas in the Atlantic Ocean and the Mediterranean Sea. Stock boundary line at 5◦N. (A) North stock, December–February (filled red), March-May (filled white), June–August (filled yellow), and September–October (filled black); (B) South stock, January–March (filled red), April–June (filled white), July–September (filled yellow), and October–December (filled black) (Neilson et al., 2013).

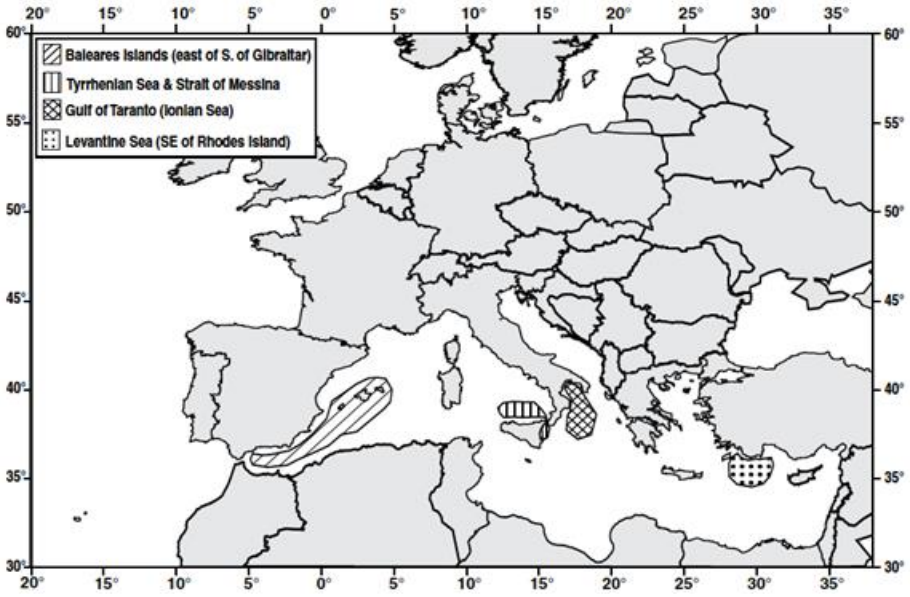


Figure 1.6. Spatial distribution of spawning female swordfish in the Mediterranean Sea, showing the main spawning grounds: one from East of Gibraltar to the Balearic Islands, one in the southern Tyrrhenian Sea and Strait of Messina, one in the Gulf of Taranto and last one in the Levantine Sea (Arocha, 2007).

In particular around the Balearic Islands, in the south of the Tyrrhenian Sea and in the Strait of Messina and in the Ionian Sea (Arocha, 2007). In addition, in the Eastern Mediterranean Sea, swordfish migrates towards the Eastern Levantine for spawning, concentrating in a region between the islands of Cyprus and Rhodes surrounded by persistent eddies and the Rhodes Gyre (Tserpes, Peristeraki & Somarakis, 2001; Tserpes, Peristeraki & Valavanis, 2008).

Evidence of seasonal migration and strong fidelity to spawning grounds and feeding areas have been reported for swordfish. Pop-up satellite archival tags (PSATs) reported

that in North Atlantic swordfish occupy temperate foraging grounds north of 40° N latitude from early June through October, and migrate southward to the tropical and sub-tropical spawning areas (Neilson et al., 2014). Mediterranean swordfish, instead, migrate in winter-feeding grounds in Moroccan Atlantic waters and the waters west of the Strait of Gibraltar (out to 15°W) in the eastern North Atlantic and return toward spawning grounds in the Mediterranean Sea (Pujolar, Roldan & Pla, 2002).

1.4 Swordfish genetic stock structure

Several genetic and molecular studies have demonstrated that, globally, swordfish is divided into discrete populations. Analysis using mitochondrial DNA (mtDNA) (Bremer et al., 1995, 1996, 2005; Kotoulas et al., 1995; Rosel and Block, 1996; Chow et al., 1997; Chow and Takeyama, 2000), allozymes (Pujolar, Roldan & Pla, 2002) and nuclear DNA including single-copy nuclear DNA (scnDNA) (Chow & Takeyama, 2000; Bremer et al., 2007; Chow et al., 2007), microsatellites (Kasapidis et al., 2007; Kotoulas et al., 2007) and single nucleotide polymorphisms (SNPs) (Smith et al., 2015) data have supported inter-oceanic differentiation among Atlantic and Mediterranean swordfish. Intra-Oceanic differentiation between North and South Atlantic was suggested by the heterogeneous distribution of mtDNA lineages (Bremer et al., 2005a). MtDNA swordfish haplotypes can be assigned into two highly divergent Clades (Clade I and Clade II) (Bremer et al., 1996, 2005b). Clade I is further subdivided into two groups: the alpha lineages that contain a nucleotide polymorphism that determines the presence of a

*Rsa*I restriction site that rarely occurs among non-alpha lineages, and the beta lineages that lack this restriction site. Beta-lineages represents the majority of Indo-Pacific fish, are more common in the South Atlantic than in the NW Atlantic and the Mediterranean. An opposite pattern was observed for Clade II showing greatest frequencies in the Mediterranean Sea and lower into South Atlantic and is absent in the Indo-Pacific. Phylogeographic associations support the inferred pattern of unidirectional historical gene flow from the Indo-Pacific into the Atlantic and that the Clade II may be originated in the Atlantic (Bremer et al., 2005a). North and South Atlantic populations differentiation was confirmed by analysis of nuclear locus aldolase B (*aldB*) and lactate dehydrogenase A (*ldhA*) (Greig et al., 1998, cited in Bremer et al., 2007) and locus calmodulin (*Cam*) (Chow & Takeyama, 2000; Bremer et al., 2007). Significant differentiation among Mediterranean, North, and South Atlantic swordfish populations was also corroborated by SNPs data (Smith et al., 2015). Microsatellite data detected differences between Atlantic and Mediterranean swordfish population but no evidence of population structure was detected within the Atlantic oceans (Kotoulas et al., 2007).

Mediterranean swordfish clearly constitutes a genetically distinct population, showing lower levels of genetic variation compared to any other swordfish population worldwide (Bremer, Baker & Mejuto, 1995; Bremer et al., 1996, 2005a; Rosel & Block, 1996; Pujolar, Roldan & Pla, 2002; Kotoulas et al., 2007), suggestive of smaller effective population size for the Mediterranean stock. MtDNA analysis revealed that, contrarily

to the Atlantic populations, the majority of the haplotypes in the Mediterranean swordfish clustered around five major centroids, four of which belong to Clade I, and one centroid to Clade II (Bremer et al., 2005b). The monophyletic origin of subclades to the Atlantic and Mediterranean Clade II lineage and the limited sharing of Clade I haplotypes confirmed genetic isolation between the two stocks (Rosel & Block, 1996; Bremer et al., 2005a). Population differentiation has been corroborated also by nuclear markers (Kasapidis et al., 2007; Kotoulas et al., 2007; Smith et al., 2015). Isolation of Mediterranean swordfish from the Atlantic has been dated during the peaks of at least the last two glaciations which explains the strong signal of population differentiation (Bremer et al., 2005b). Atlantic and Mediterranean populations are in contact through the Strait of Gibraltar. A mixture of Atlantic and Mediterranean individuals was detected in Atlantic waters off the Strait of Gibraltar (Tarifa), dominated by the latter (Kotoulas et al., 1995). Mediterranean individuals carrying out the trophic migration, moving towards the northeast Atlantic remaining in the region between 15° W and Gibraltar (Pujolar, Roldan & Pla, 2002; Viñas et al., 2007). Swordfish migration occurs from the Atlantic to the Mediterranean during the second quarter of the year and a second trophic migration in the opposite direction (De La Serna and Alot, 1990). Contrarily, no Atlantic immigrants have been detected in the Mediterranean basin (Chow et al., 1997). Although intermingle between swordfish populations occurred, seasonally, in feeding and transitional boundary zones (Pujolar, Roldan & Pla, 2002; Smith et al., 2015), strong

philopatric behaviour towards discrete spawning grounds driven this genetic differentiation maintaining an extremely low level of gene flow (Bremer et al., 2005a). Within the Mediterranean, no evidence of genetic differentiation was revealed by Restriction Fragment Length Polymorphism (RFLP) analysis of the whole mitochondrial DNA (mtDNA) (Kotoulas et al., 1995) and by the RFLP and nucleotide sequence analyses of the calmodulin gene and mtDNA Control Region (CR) (Chow & Takeyama, 2000). Despite the apparent genetic homogeneity of swordfish, some evidence does suggest population subdivision, such as the presence of discrete spawning areas in each basin such as the Balearic Sea for the Western Mediterranean, from southern Tyrrhenian Sea to the Ionian Sea for the central part and Levantine Basin for the Eastern Mediterranean. Furthermore, Viñas et al. (2010)(Viñas et al., 2010) suggested swordfish population sub-structuring among West to East Mediterranean basins based on a clinal decrease in mitochondrial DNA control region intra-clade genetic variability from Western and the Eastern basin. This pattern, although present, was less evident when allozyme data were used (Pujolar, Roldan & Pla, 2002)

1.5 Mediterranean swordfish: fishery, management and conservation status

Since 1966, the International Commission for the Conservation of Atlantic Tunas (ICCAT) is an intergovernmental fishery organization responsible for the conservation of tunas and swordfish in the Atlantic Ocean and the Mediterranean Sea. For fishery

management purposes, ICCAT recognizes three unit stocks: the Mediterranean, North and South Atlantic (Anon., 2019) based on biological and genetic evidence and tagging programmes (see the previous paragraph). North and South Atlantic stock are considered separated by an imaginary boundary located at 5°N while the Strait of Gibraltar separates North Atlantic and Mediterranean stocks. Nonetheless, some authors suggest that the current stock boundaries should be refined. Genetic studies suggest that the mixing area between North and South Atlantic stocks occurs much further north, between 10 and 20°N (Chow et al., 2007) and the mixing area between North Atlantic and Mediterranean stock lies beyond 10°W (Viñas et al., 2007).

Swordfish is a high commercial value species, especially in the Mediterranean Sea, where despite this basin represent less than 10% of the swordfish global range, catch levels are relatively high and similar to those of bigger areas such as the North Atlantic (Collette et al., 2011). Larger spawning areas in relation to the area of distribution of the stock, lower abundance of large pelagic predators and higher recruitment were suggested as determining factors the higher abundance of swordfish in the Mediterranean Sea. However, in the last forty years, swordfish in the Mediterranean Sea has been overfished and, to date, is still subject to overfishing and it is the only stock considered to be not well-managed (Anon, 2019). The Spawning stock biomass (SSB) estimated is less than 15% of the maximum sustainable yield (B_{MSY}) estimated and the fish mortality caused by harvesting (F) is almost twice the F_{MSY}. 50-70% of the total yearly catches is

represented by fish of small size often less than 3 years old, with a high level of immature swordfish reported (Anon, 2019).

Recorded landings of swordfish in the Mediterranean Sea showed an upward trend from 1965, reaching the maximum peak of 20,365 t in 1988 and then dropped, fluctuating mostly between 12,000 to 16,000 t. Following the implementation of the fishery closure season (2008) and the establishment of the list of authorized vessels, overall fishing effort has decreased and catches are around 8-10,000 t (Anon, 2019).

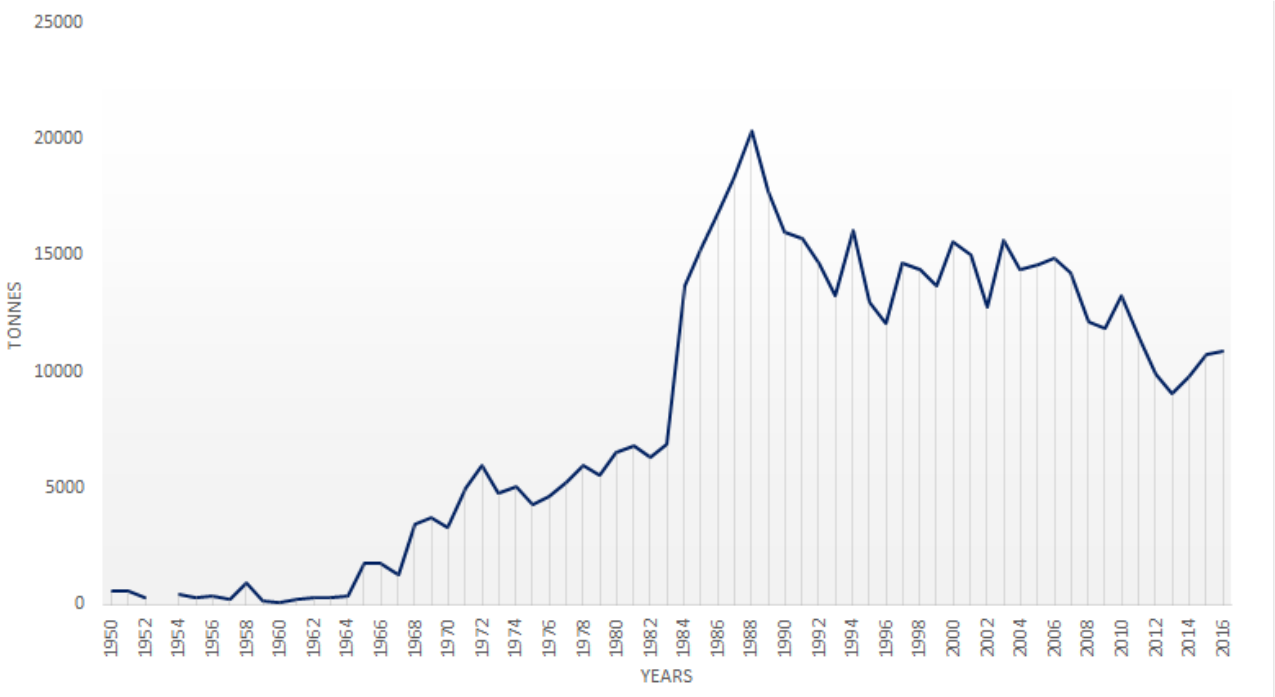


Figure 1.7. Cumulative estimates catch of swordfish in the Mediterranean for the period 1950-2016. (https://www.iccat.int/en/accesingdb.html)

Currently, Mediterranean swordfish fishing management follows the ICCAT Recommendation 16-05 which provides for a 15-year plan aiming to achieve a biomass corresponding to a maximum sustainable yield by 2031 with at least 60% probability. The recommendation establishes: increase of the minimum catching size to 110 cm LJFL (or 11.4 kg round weight, 10.2 kg gutted weight); a Total Allowable Catch (TAC) (10,500 t in 2017) that should be gradually reduced by 3% each year over the period 2018-2022; three months of closed fishing season; and technical characteristics of the fishing gear (2500 maximum number of hooks and smaller than 7 cm of height, 30 NM maximum length of pelagic longline).

During the period from 2003 to 2015, Italy with 45% of production was the greatest exploiter of the Mediterranean swordfish stock, followed by Spain (13%), Greece (10%), Morocco (14%), and Tunisia (7%). Fisheries targeting swordfish in the Mediterranean with little catches have also been reported by Algeria, Cyprus, Malta and Turkey, Albania, Croatia, France, Japan, and Libya. From 2000 the main fishing gear used were: surface longlines (84% of the annual catch) and gillnets, until the driftnet ban in the Mediterranean Sea in 2002. From 2009 the mesopelagic longline gear has been introduced and gradually has replaced the surface longline gear in almost all Italian swordfish fleets (Bertolino et al., 2015; Garibaldi & Lanteri, 2017). In the Straits of Messina, minor catches are also reported from harpoon tradition fishing using a typical vessel called “Feluca”.

Figure 1.8. A drawing of the traditional ship called “Feluca”.

1.6 Reference

Abascal FJ, Mejuto J, Quintans M, García-Cortés B, Ramos-Cartelle A. 2015. Tracking of the broadbill swordfish, Xiphias gladius, in the central and eastern North Atlantic. *Fisheries Research* 162:20–28.

Abid N, Idrissi M. 2006. Swordfish. *Field Manual*:9.

Allendorf FW, Phelps SR. 1981. Use of allelic frequencies to describe population structure. *Canadian Journal of Fisheries and Aquatic Sciences* 38:1507–1514.

Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA. 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics* 17:81.

Anon. 2019. *Report for biennial period, 2018-19 PART I (2018) - Vol. 2*. Madrid, Spain: International Commission for the Conservation of Atlantic Tunas.

Antoniou A, Kasapidis P, Kotoulas G, Mylonas CC, Magoulas A. 2017. Genetic diversity of Atlantic Bluefin tuna in the Mediterranean Sea: insights from genome-wide SNPs and microsatellites. *Journal of Biological Research-Thessaloniki* 24:3.

Antoniou A, Magoulas A. 2014. Application of mitochondrial DNA in stock identification. In: *Stock Identification Methods*. Elsevier, 257–295.

Arocha F. 2007. Swordfish Reproduction in the Atlantic Ocean: An Overview. *Gulf and Caribbean Research* 19. DOI: 10.18785/gcr.1902.05.

Avise JC. 2009. Phylogeography: retrospect and prospect. *Journal of biogeography* 36:3–15.

Begg GA, Waldman JR. 1999. An holistic approach to fish stock identification. *Fisheries research* 43:35–44.

Beissinger SR, McCullough DR. 2002. *Population viability analysis*. University of Chicago Press.

Bertolino F, Camolese C, Dell'Aquila M, Mariani A, Valastro M. 2015. Swordfish (Xiphias gladius l.) fisheries using mesopelagic longline in the Mediterranean Sea by Italian fishing fleet. *Collect. Vol. Sci. Pap. ICCAT* 71:2073–2078.

Bremer JRA, Baker AJ, Mejuto J. 1995. Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (Xiphias gladius) populations in the Atlantic Ocean. *Canadian Journal of Fisheries and Aquatic Sciences* 52:1720–1732.

Bremer JA, Mejuto J, Gómez-Márquez J, Boán F, Carpintero P, Rodríguez JM, Viñas J, Greig TW, Ely B. 2005a. Hierarchical analyses of genetic variation of samples from breeding and feeding grounds confirm the genetic partitioning of northwest Atlantic and South Atlantic populations of swordfish (Xiphias gladius L.). *Journal of Experimental Marine Biology and Ecology* 327:167–182.

Bremer JA, Mejuto J, Gómez-Márquez J, Pla-Zanuy C, Viñas J, Marques C, Hazin F, Griffiths M, Ely B, García-Cortés B. 2007. Genetic population structure of Atlantic swordfish: current status and future directions. *ICCAT Collect. Vol. Sci. Pap.* 61:107–118.

Bremer JRA, Mejuto J, Greig TW, Ely B. 1996. Global population structure of the swordfish (Xiphias gladius L.) as revealed by analysis of the mitochondrial DNA control region. *Journal of Experimental Marine Biology and Ecology* 197:295–310.

Bremer JA, Naseri I, Ely B. 1999. Heterogeneity of northern bluefin tuna populations. *ICCAT Coll. Vol. Sci. Pap*. 49:127–129.

Bremer JA, Stéquert B, Robertson NW, Ely B. 1998. Genetic evidence for inter-oceanic subdivision of bigeye tuna (Thunnus obesus) populations. *Marine Biology* 132:547–557.

Bremer JRA, Viñas J, Mejuto J, Ely B, Pla C. 2005b. Comparative phylogeography of Atlantic bluefin tuna and swordfish: the combined effects of vicariance, secondary contact, introgression, and population expansion on the regional phylogenies of two highly migratory pelagic fishes. *Molecular phylogenetics and evolution* 36:169–187.

Cadrin SX, Kerr LA, Mariani S. 2013. *Stock identification methods: applications in fishery science*. Academic Press.

Cadrin SX, Secor DH. 2009. Accounting for spatial population structure in stock assessment: past, present, and future. In: *The future of fisheries science in North America*. Springer, 405–426.

Carlsson J, McDowell JR, Carlsson JE, Graves JE. 2006. Genetic identity of YOY bluefin tuna from the eastern and western Atlantic spawning areas. *Journal of Heredity* 98:23–28.

Carlsson J, McDOWELL JR, DÍAZ-JAIMES P, Carlsson JE, Boles SB, Gold JR, Graves JE. 2004. Microsatellite and mitochondrial DNA analyses of Atlantic bluefin tuna (Thunnus thynnus thynnus) population structure in the Mediterranean Sea. *Molecular Ecology* 13:3345–3356.

Carvalho GR, Hauser L. 1995. Molecular genetics and the stock concept in fisheries. In: *Molecular genetics in fisheries*. Springer, 55–79.

Chancollon O, Pusineri C, Ridoux V. 2006. Food and feeding ecology of Northeast Atlantic swordfish (Xiphias gladius) off the Bay of Biscay. *ICES Journal of Marine Science* 63:1075–1085.

Chow S, Clarke S, Nakadate M, Okazaki M. 2007. Boundary between the north and south Atlantic populations of the swordfish (Xiphias gladius) inferred by a single nucleotide polymorphism at calmodulin gene intron. *Marine Biology* 152:87–93.

Chow S, Okamoto H, Miyabe N, Hiramatsu K, Barut N. 2000. Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (Thunnus obesus) and admixture around South Africa. *Molecular Ecology* 9:221–227.

Chow S, Okamoto H, Uozumi Y, Takeuchi Y, Takeyama H. 1997. Genetic stock structure of the swordfish (Xiphias gladius) inferred by PCR-RFLP analysis of the mitochondrial DNA control region. *Marine Biology* 127:359–367.

Chow S, Takeyama H. 2000. Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of the swordfish. *Journal of Fish Biology* 56:1087–1098.

Collette B, Acero A, Amorim AF, Bizsel K, Boustany A, Ramirez CC, Cardenas G, Carpenter K, LeiteJr NO, Di Natale A. 2011. *Xiphias gladius*. IUCN.

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics* 12:499–510.

Davies CA, Gosling EM, Was A, Brophy D, Tysklind N. 2011. Microsatellite analysis of albacore tuna (Thunnus alalunga): population genetic structure in the North-East Atlantic Ocean and Mediterranean Sea. *Marine biology* 158:2727–2740.

De La Serna JM, Alot E. 1990. Considerations relative to swordfish (Xiphias gladius) movements in Gibraltar Strait and other observations related to biology and reproduction. *Collect. Vol. Sci. Pap. ICCAT* 32:353–359.

Dulvy NK, Jennings S, Rogers SI, Maxwell DL. 2006. Threat and decline in fishes: an indicator of marine biodiversity. *Canadian Journal of Fisheries and Aquatic Sciences* 63:1267–1275.

Dulvy NK, Sadovy Y, Reynolds JD. 2003. Extinction vulnerability in marine populations. *Fish and fisheries* 4:25–64.

Duncker G. 1936. Swordfish. *Flora Fauna*.

Ely B, Viñas J, Bremer JRA, Black D, Lucas L, Covello K, Labrie AV, Thelen E. 2005. Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (Thunnus albacares) and the skipjack tuna (Katsuwonus pelamis). *BMC Evolutionary Biology* 5:19.

Féral J-P. 2002. How useful are the genetic markers in attempts to understand and manage marine biodiversity? *Journal of experimental marine biology and ecology* 268:121–145.

Finnerty JR, Block BA. 1992. Direct sequencing of mitochondrial DNA detects highly divergent haplotypes in blue marlin (Makaira nigricans). *Molecular Marine Biology and Biotechnology* 1:206–214.

Fitch JE. 1960. Swordfish. *Fisheries Resources*.

Garibaldi F, Lanteri L. 2017. Notes about a tagged/recaptured swordfish in the Ligurian Sea (western Mediterranean). *Collective Volume of Scientific Papers, ICCAT* 74:1354–1361.

Gascoigne J, Lipcius RN. 2004. Allee effects in marine systems. *Marine Ecology Progress Series* 269:49–59.

Gauldie RW. 1988. Tagging and genetically isolated stocks of fish: a test of one stock hypothesis and the development of another. *Journal of applied ichthyology* 4:168–173.

Gorni GR, Loibel S, Goitein R, Amorim AF. 2012. Stomach contents analysis of swordfish (Xiphias gladius) caught off southern Brazil: a bayesian analysis. *Collect. Vol. Sci. Pap. ICCAT* 68:1594–1600.

Graves JE, McDowell JR. 1995. Inter-ocean genetic divergence of istiophorid billfishes. *Marine Biology* 122:193–203.

Ihssen PE, Booke HE, Casselman JM, McGlade JM, Payne NR, Utter FM. 1981. Stock identification: materials and methods. *Canadian journal of fisheries and aquatic sciences* 38:1838–1855.

Kapuscinski AR, Miller LM. 2007. Genetic Guidelines for Fisheries. *University of Minnesota Sea Grant Program*.

Kasapidis P, Mejuto J, Tserpes G, Antoniou A, Garcia-Cortes B, Peristeraki P, Oikonomaki K, Kotoulas G, Magoulas A. 2007. Genetic structure of the swordfish (Xiphias gladius) stocks in the Atlantic using microsatellite DNA analysis. *Collect. Vol. Sci. Pap. ICCAT* 61:89–98.

Kell LT, Crozier WW, Legault CM. 2004. *Mixed and multi-stock fisheries*. Oxford University Press.

Kotoulas G, Magoulas A, Tsimenides N, Zouros E. 1995. Marked mitochondrial DNA differences between Mediterranean and Atlantic populations of the swordfish, Xiphias gladius. *Molecular Ecology* 4:473–482.

Kotoulas G, Mejuto J, Antoniou A, Kasapidis P, Tserpes G, Piccinetti C, Peristeraki P, Garcia-Cortes B, Oikonomaki K, De la Serna JM. 2007. Global genetic structure of swordfish (Xiphias gladius) as revealed by microsatellite DNA markers. *ICCAT Coll Vol Sci Pap* 61:79–88.

Larkin PA. 1972. The stock concept and management of Pacific salmon. *The stock concept in Pacific salmon. University of British Columbia, HR MacMillan Lectures in Fisheries, Vancouver*:11–15.

Lobell MJ, Byer AJ, Knake BO, Westman JR. 1947. The fisheries of Chile. *Present status and future possibilities. United States Fishery Mission to Chile, CORFO-US Department of the Interior, Washington*:1–454.

Maroso F, Franch R, Dalla Rovere G, Arculeo M, Bargelloni L. 2016. RAD SNP markers as a tool for conservation of dolphinfish Coryphaena hippurus in the Mediterranean Sea: Identification of subtle genetic structure and assessment of populations sex-ratios. *Marine genomics* 28:57–62.

Martínez P, González EG, Castilho R, Zardoya R. 2006. Genetic diversity and historical demography of Atlantic bigeye tuna (Thunnus obesus). *Molecular phylogenetics and evolution* 39:404–416.

McElhany P, Rucklelshaus MH, Ford MJ, Wainwright TC, Bjorkstedt EP. 2000. Viable salmonid populations and the recovery of evolutionarily significant units.

Montes I, Iriondo M, Manzano C, Arrizabalaga H, Jiménez E, Pardo MÁ, Goñi N, Davies CA, Estonba A. 2012. Worldwide genetic structure of albacore Thunnus alalunga revealed by microsatellite DNA markers. *Marine Ecology Progress Series* 471:183–191.

Nakamura I. 1985. *FAO species catalogue. v. 5: Billfishes of the world. An annotated and illustrated catalogue of marlins, sailfishes, spearfishes and swordfishes known to date*. FAO.

Neilson J, Arocha F, Cass-Calay S, Mejuto J, Ortiz M, Scott G, Smith C, Travassos P, Tserpes G, Andrushchenko I. 2013. The recovery of Atlantic swordfish: the comparative roles of the regional fisheries management organization and species biology. *Reviews in Fisheries Science* 21:59–97.

Neilson JD, Loefer J, Prince ED, Royer F, Calmettes B, Gaspar P, Lopez R, Andrushchenko I. 2014. Seasonal distributions and migrations of Northwest Atlantic swordfish: inferences from integration of pop-up satellite archival tagging studies. *PLoS One* 9:e112736.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4:535–538. DOI: 10.1111/j.1471-8286.2004.00684.x.

Palko BJ, Beardsley GL, Richards WJ. 1981. Synopsis of the biology of the swordfish, Xiphias gladius Linnaeus.

Pecoraro C, Babbucci M, Franch R, Rico C, Papetti C, Chassot E, Bodin N, Cariani A, Bargelloni L, Tinti F. 2018. The population genomics of yellowfin tuna (Thunnus albacares) at global geographic scale challenges current stock delineation. *Scientific reports* 8:13890.

Penrith M.J. CDL. 1974. The Cape of Good Hope: a hidden barrier to billfishes.

Pujolar JM, Roldan MI, Pla C. 2002. A genetic assessment of the population structure of swordfish (Xiphias gladius) in the Mediterranean Sea. *Journal of Experimental Marine Biology and Ecology* 276:19–29.

Reeb CA, Arcangeli L, Block BA. 2000. Structure and migration corridors in Pacific populations of the Swordfish Xiphius gladius, as inferred through analyses of mitochondrial DNA. *Marine Biology* 136:1123–1131.

Reiss H, Hoarau G, Dickey-Collas M, Wolff WJ. 2009. Genetic population structure of marine fish: mismatch between biological and fisheries management units. *Fish and Fisheries* 10:361–395.

Riccioni G, Ferrara G, Landi M, Sella M. 2010. Spatio-temporal genetic patterns in Mediterranean bluefin tuna: population structuring and retention of genetic diversity. *Collect. Vol. Sci. Pap. ICCAT* 65:996–1003.

Rosel PE, Block BA. 1996. Mitochondrial control region variability and global population structure in the swordfish, Xiphias gladius. *Marine Biology* 125:11–22.

Secor DH. 2014. The unit stock concept: bounded fish and fisheries. In: *Stock Identification Methods*. Elsevier, 7–28.

Selkoe KA, Toonen RJ. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology letters* 9:615–629.

Smith BL, Lu C-P, García-Cortés B, Viñas J, Yeh S-Y, Bremer JRA. 2015. Multilocus Bayesian estimates of intra-oceanic genetic differentiation, connectivity, and admixture in Atlantic swordfish (Xiphias gladius L.). *PloS one* 10:e0127979.

Steneck RS, Wilson JA. 2010. A fisheries play in an ecosystem theater: challenges of managing ecological and social drivers of marine fisheries at multiple spatial scales. *Bulletin of Marine Science* 86:387–411.

Strasburg DW. 1970. A report on the billfishes of the central Pacific Ocean. *Bulletin of Marine Science* 20:575–604.

De Sylva DP. 1962. Red-Water Blooms off Northern Chile, April-May 1956, with Reference to the Ecology of the Swordfish and the Striped Marlin. *Pacific Science* 16:271–279.

Takagi M, Okamura T, Chow S, Taniguchi N. 2001. Preliminary study of albacore (Thunnus alalunga) stock differentiation inferred from microsatellite DNA analysis. *Fishery Bulletin-National Oceanic and Atmospheric Administration* 99:697–701.

Tautz D, Renz M. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic acids research* 12:4127–4138.

Tibbo SN, Day LR, Doucet WF. 1961. *The swordfish (Xiphias gladius L.), its life - history and economic importance in the northwest Atlantic*. Ottawa: [s.n.].

Tserpes G, Peristeraki P, Somarakis S. 2001. On the reproduction of swordfish (Xiphias gladius L.) in the eastern Mediterranean. *Col. Vol. Sci. Pap. ICCAT* 52:740–744.

Tserpes G, Peristeraki P, Valavanis VD. 2008. Distribution of swordfish in the eastern Mediterranean, in relation to environmental factors and the species biology. *Hydrobiologia* 612:241.

Viñas J, Bremer JA, Mejuto J, De La Serna JM, García-Cortés B, Pla C. 2007. Swordfish genetic population structure in the North Atlantic and Mediterranean. *Collect. Vol. Sci. Pap. ICCAT* 61:99–106.

Viñas J, Bremer JA, Pla C. 2004. Inter-oceanic genetic differentiation among albacore (Thunnus alalunga) populations. *Marine Biology* 145:225–232.

Viñas J, Pérez-Serra A, Vidal O, Alvarado Bremer JR, Pla C. 2010. Genetic differentiation between eastern and western Mediterranean swordfish revealed by phylogeographic analysis of the mitochondrial DNA control region. *ICES Journal of Marine Science* 67:1222–1229.

Waldman JR. 2005. Definition of stocks: an evolving concept. In: *Stock Identification Methods*. Elsevier, 7–16.

Waples RS. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* 89:438–450.

Waples RS, Punt AE, Cope JM. 2008. Integrating genetic data into management of marine resources: how can we do it better? *Fish and Fisheries* 9:423–449.

Webb BF. 1972. Broadbilled swordfish from Tasman bay, New Zealand (note). *New Zealand Journal of Marine and Freshwater Research* 6:206–207. DOI: 10.1080/00288330.1977.9515418.

Weber JL, Wong C. 1993. Mutation of human short tandem repeats. *Human molecular genetics* 2:1123–1128.

Wirgin I, Waldman JR. 2005. Use of nuclear DNA in stock identification: single-copy and repetitive sequence markers. In: *Stock identification methods*. Elsevier, 331–370.

Yabe H, Ueyanagi S, Kikawa S, Watanabe H. 1959. Study on the life-history of the swordfish, Xiphias gladius Linnaeus. *Rep. Nankai Reg. Fish. Res. Lab* 10:107–150.

2 AIMS AND OBJECTIVES

The aim of this thesis was to explore and describe the population genetics structure of Mediterranean swordfish applying different molecular markers

Specifically, the objectives of this thesis were to:

1. Characterise the presence of different genetic stocks within the Mediterranean Sea. Due to limited and contrasting results population structure of swordfish within this basin remains still uncertain. The presence of discrete spawning grounds in each basin and philopatric behaviour toward these areas could determinate isolation and genetic heterogeneity within the Mediterranean Sea. In this work, the fine-scale genetic structure of swordfish within the Mediterranean Sea was investigated applying multi-locus approach using both microsatellites and genome-wide SNPs sampled by double-digest Restriction Associated DNA sequencing (ddRAD-seq).

2. Determine the temporal stability of genetic variability of the Mediterranean population. Mediterranean swordfish is overfished. Harvest inevitably reduces population size and therefore may cause the loss of genetic variation. The most powerful way to detect loss of genetic variation in exploited populations is the examination of genetic samples collected over time. In this study, a dataset of available mitochondrial sequences of Mediterranean swordfish sampled in the 1990s and early 2000s has been compared with homologous mtDNA data obtained from current samples. This

comparison was carried out in order to investigate temporal genetic variation in the threatened and overexploited Mediterranean swordfish population over twenty years.

3 IS IT POSSIBLE TO IDENTIFY A GENETIC SUBDIVISION IN THE SWORDFISH OF THE MEDITERRANEAN? A SURVEY BASED ON MICROSATELLITE LOCI AND RAD-SEQ GENOTYPING.

3.1 Introduction

Sustainability in harvesting fish stocks to avoid their depletion and to guarantee survival and long-term production is one of the preliminary objectives of fishery management. Fish species are frequently composed of different populations, which are genetically isolated through behavioural and/or distributional differences (Cochrane, 2002). Identification of the population structure, as a fundamental biological unit, is an essential prerequisite for the correct management of the fish resource. Delineation of spatial management areas ignoring the spatial distribution and interaction of fish population may result in i) depletion or extinction of the most vulnerable or local subpopulations and ii) loss of genetic diversity that reduces the ability for the species to evolve and adapt to environmental changes. A frequent mismatch between ecological and/or biological processes and management actions has led, in the last decade, to the decline of many commercial fish stocks (Reiss et al., 2009).

Determining stock structure for marine fish is made difficult by a low level of intraspecific heterogeneity (Ward et al., 1994) as a result of the absence of geographical barriers, long larval periods and their widespread dispersal as well as highly migratory adults that facilitate levels of gene flow. Despite a low genetic population differentiation, demographic history of the ancestral population combined with current ecological

biogeographic factors, such as dispersal potential, spawning behaviour, population size, have led to population differentiation, as revealed by several genetic studies for different fish species (Zardoya et al., 2004; Martínez et al., 2006; Ruzzante et al., 2006; O'Leary et al., 2007; Pecoraro et al., 2016, 2018; Ruggeri et al., 2016a; 2016b).

Swordfish is a pelagic and highly migratory species, distributed worldwide from 45°N to 45°S in the open waters of the Atlantic, Indian, and Pacific oceans. It also occurs in the Mediterranean, Marmara, Black and Azov seas (Palko et al., 1981). According to the International Union for the Conservation of Nature (IUCN), this species has shown a 28% decline in total biomass over the last 20 years globally. The Mediterranean stock is considered the only that is not well-managed (Collette et al., 2011) strongly influenced by the fishery high catch level. The last ICCAT (International Commission for the Conservation of Atlantic Tunas) Mediterranean swordfish stock assessment (Anon., 2019) reported that the Mediterranean swordfish spawning stock biomass (SSB) levels were much lower than those in the '80s and between 50 and 70% of total yearly catches were represented by small-sized individuals. This result indicates that the stock was overfished and today, suffers from overfishing. For management purposes, ICCAT considers populations in the Mediterranean Sea, North Atlantic, and South Atlantic as three separate stocks. The Mediterranean swordfish have different biological characteristics compared to the Atlantic stocks such as a lower growth rate, and sexual maturity reached at a younger age than in the Atlantic populations (Cavallaro et al., 1991;

Ehrhardt, 1992; Tserpes and Tsimenides, 1995; Arocha, 2007). Several genetic studies confirmed the differentiation between these stocks (Bremer et al., 1995, 1996; Kotoulas et al., 1995, 2007; Rosel and Block, 1996; Pujolar et al., 2002; Viñas et al., 2007). Separate analyses using mitochondrial and nuclear markers have highlighted a high level of genetic differentiation among the Atlantic and Mediterranean populations. Geographically discrete spawning areas, as well as different spawning periods, were detected among these basins (Neilson et al., 2014). In the North Atlantic, reproduction takes place in the western subtropical area, with year-round spawning and seasonal peaks (Arocha, 2007). In the Mediterranean Sea, spawning takes place between June and August (Palko et al., 1981; Arocha, 2007) and it is restricted to three main spawning grounds. The first is located in the western basin, the second in the eastern one and the third extends from the southern Tyrrhenian Sea to the Ionian Sea (Cavallaro et al., 1991; Tserpes et al., 2001, 2008; Arocha, 2007).

Philopatric behaviour has been proposed as the driving force behind global swordfish structuring. Maximum levels of genetic differentiation between separated breeding areas, support spawning site fidelity in the Atlantic Ocean (Bremer et al., 2005). Moreover, no evidence of gene flow was observed between Mediterranean and North Atlantic swordfish populations despite mixing in the feeding area to the west of the Strait of Gibraltar (Bremer et al., 2007; Viñas et al., 2007; Smith et al., 2015).

Whilst there are several studies on the global genetic structure of swordfish, very few deals with the Mediterranean Sea, consequently, population structure within this basin remains still uncertain. Some of these studies suggested the occurrence of a homogeneous stock in the Mediterranean Sea. No evidence of genetic differentiation was revealed by Restriction Fragment Length Polymorphism (RFLP) analysis of the whole mitochondrial DNA (mtDNA) (Kotoulas et al., 1995) among Mediterranean and Gulf of Cadìz samples. A similar conclusion was reached by the RFLP and nucleotide sequence analyses of the calmodulin gene and mtDNA Control Region (CR) (Chow and Takeyama, 2000). Mediterranean swordfish genetic homogeneity was, also, detected using a panel of 4 highly polymorphic microsatellites (Kotoulas et al., 2007). Contrarily, Viñas (2010), based on the analysis of mtDNA CR-I sequences, proposed the existence of at least two distinct Mediterranean populations: one in the eastern basin and the other in the western basin. Although less evident, this pattern was detected using allozyme data(Pujolar et al., 2002). The contrasting results regarding the occurrence of a genetic structure for the Mediterranean populations of swordfish suggest the need for more thorough genetic studies focusing on this basin to assess the existence of a genetic structure of this fishery resource.

In the present study, the multi-locus approach using both microsatellites and genome-wide SNPs sampled by double-digest Restriction Associated DNA sequencing (ddRAD-seq) were compared to evaluate their performance to detected genetic differentiation

between Atlantic and Mediterranean swordfish population. Furthermore, for the first time, a large number of markers was used to investigate the fine-scale genetic structure of swordfish within the Mediterranean Sea. Sampling coverage differs from all previous genetic studies by offering a more comprehensive representation of the Mediterranean area.

3.2 Materials & Methods

3.2.1 Sampling and DNA extraction

A total of 298 swordfish were collected from six areas within the eastern, central and western Mediterranean regions, trying to obtain a representative coverage of the basin (Figure 3.1). Moreover, twenty-five swordfish from the eastern coast of Canada have been included in the analysis as a comparison (For details see Table 3.1). Samples were achieved at the fishing landing of commercial longline or from trap bycatch (only in case of Sardinian samples) from May to October in three years 2016-2018. For each sample, a piece of the caudal fin was collected and was stored in ethanol absolute and kept at -20°C until DNA extraction. Total genomic DNA was extracted using specific cartridge 401 in the *MagCore*® automated Nucleic Acid extractor (*MagCore* ®, *Genomic DNA Tissue Kit, n° 401*) following the specific protocol.

Table 3.1 Sampling details for swordfish analysed in this study

Sampling area (FAO fishing area/Geographical subarea)	Sample ID	Sampling date	STR	ddRADseq
Balearic Sea (GSA 5, 6)	SPA	07/16-08/16-09/16 09/19	85	18
Southern Sicily (GSA 15,16)	SIC	07/16 06/17-07/17 06/18	61	22
Aegean Sea (GSA 22)	GRE	08/16	20	7
Southern Adriatic Sea (GSA 18)	ADR	09/16	62	17
Tyrrhenian Sea (GSA 10)	TIR	05/2017	16	-
Sardinian Sea (GSA 11.2)	SAR	06/17-10/17 05/18-06/18	54	7
NW Atlantic (FAO Fishing area 21)	CAN	08/18-09/18-10/18	25	25
Total			323	96

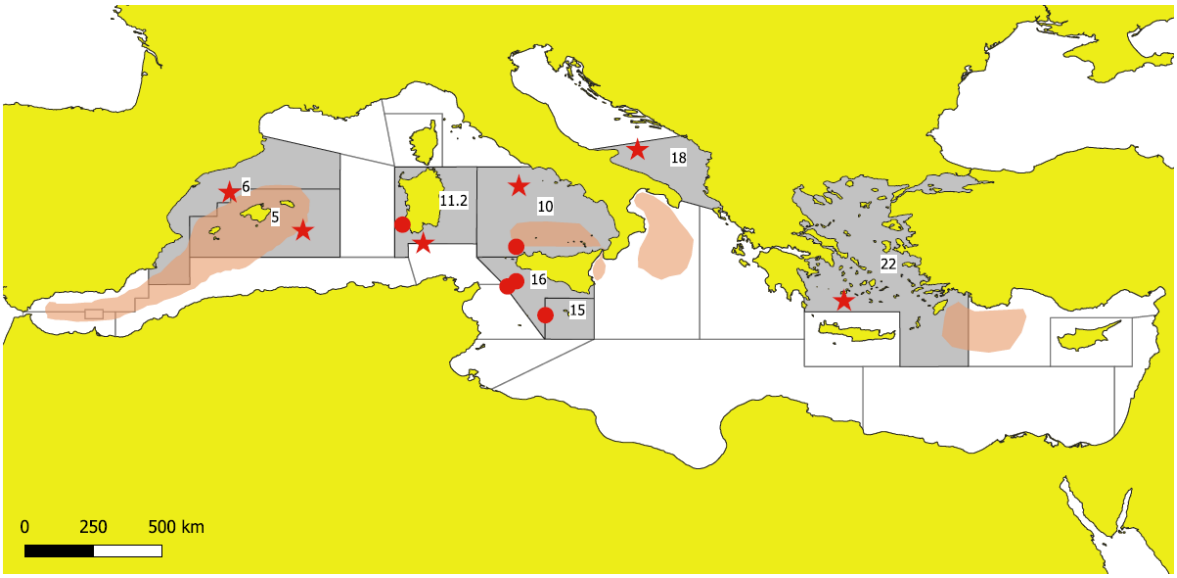


Figure 3.1 Map of the Mediterranean Sea with location of the Swordfish samples collected for the present study. Dots represents samples whose catch coordinates was known, while stars represent samples whose only the GSA were reported. GSA covered by the sampling are coloured in light grey and the respective numbers are reported. The approximate extent of the spawning areas are shaded in orange (Arocha et al., 2007).

3.2.2 Microsatellite amplification and genotyping and diversity analysis

All specimens were genotyped for 20 microsatellite loci: three from Muths et al. (2009): D2A, D2B, C8, eleven from Kasapidis et al. (2009): XgSau98R1, Xgl-14, Xgl-35, Xgl-65b, Xgl-74, Xgl-94, Xgl-106, Xgl-121, Xgl-148b, Xgl-523b, Xgl-561) and six from Reeb et al. (2003): Xg-56, Xg-66, Xg-144, Xg-166, Xg-394, Xg-402). PCR amplifications were performed combining microsatellite loci in five multiplex reactions based on primer's annealing temperature (details are shown in table 3.2). PCR amplification conditions consisted of: 1× MyTaq Reaction buffer (Bioline) (15 mmol·L^{-}

[1]$MgCl_2$, 1,25 mmol·L^{-1} of each dNTP, plus stabilizers and enhancers), 0.3 μM of each primer, 0.2 U Taq DNA polymerase (MyTaq, Bioline) and 40-80 ng of genomic DNA in a final volume of 10 μL for 2- and 3-plex and 15 μL for 4- and 7-plex, respectively. Each forward primer was labelled with a different fluorescent dye (FAM, VIC, NED, and PET) and set up avoiding similar allele size overlapping.

Table 3.2 List of Microsatellite loci used with corresponding repeat motif, fluorescent dye, annealing temperature and multiplex groups.

Locus	Repeat motif	Fluorescent dye	T_a (°C)	Multiplex
Xgl-35	$(CA)_{13}$	NED	58	1
Xgl-121	$(GT)_6(GC)_5(GT)_6$	6-FAM	58	1
Xgl-561	$(CA)_6GA(CA)_7$	VIC	58	1
Xgl-94	$(GGA)_8$	6-FAM	58	1
Xgl-106	$(GA)_{10}$	PET	58	1
Xgl-65b	$(CT)_{16}$	6-FAM	58	1
Xgl-74	$(AGG)_7$	VIC	58	1
Xg-Sau98R1	$(CA)_8$	NED	58	2
Xgl-523b	$(GA)_6AAGG(GA)_6GC(GA)_8$	6-FAM	58	2
Xgl-14	$(CAT)_6CAC(CAT)_3$ $CAC(CAT)_4(CGT)_7$	6-FAM	58	2
Xg-148b	$(GGA)_8$	6-FAM	58	2
D2A	$(CCT)_6$	NED	50	3
D2B	$(CAGT)_8$	PET	50	3
C8	$(CTAT)_{22}$	VIC	50	3
Xg-394	$(TCC)_9$	6-FAM	66	4
Xg-402	$(TCC)_5+(CTT)_2$	6-FAM	66	4
Xg-56	$(CA)_{16}$	NED	53	5
Xg-66	$(CA)_{11}$	PET	53	5
Xg-144	$(GGA)_7$	PET	53	5
Xg-166	$(CAA)_7$	VIC	53	5

PCR conditions were optimized for all loci using the following touchdown protocol: initial denaturation at 95°C for 5 minutes, followed by 10 cycles of denaturation at 92°C for 20 seconds, annealing at the appropriate temperature (Ta) (Table 3.2) for 30 seconds, and extension at 72°C for 45 seconds. At each cycle, the annealing temperature decreased by 0.5°C. After that, others 25 cycles of denaturation at 90°C for 30 seconds, annealing at [Ta - 5°C] for 50 seconds, and extension at 72°C for 55 seconds was performed. The reaction finished with a final elongation at 72°C for 5 minutes. Amplified fragments were separated by electrophoresis using ABI Prism 3130xl genetic analyser executed by BMR-Genomics (Padua). Alleles were scored using GS 500LIZ_3130 size standard using Peakscanner 2 (Applied Biosystems), and the outputs were manually evaluated. To minimize microsatellite alleles miscalling, the binning of alleles was accomplished using Flexibin 2 (Amos et al. 2007).

Micro-Checker 2.2.3 (Van Oosterhout et al., 2003) was employed to detect possible null alleles, and other genotyping errors and FreeNA (Chapuis and Estoup, 2006) was used to estimate the effect of null alleles to the F_{ST} estimation, therefore, global F_{ST} was calculated with both including null alleles (INA) and excluding null alleles (ENA) effects. The bootstrap 95% confidence intervals (CI) for the global F_{ST} values were calculated using 50,000 replicates over loci. Allelic richness (*AR*), which is a standardised index of the mean number of alleles per locus irrespective of sample size was estimated using Fstat 2.9.3 (Goudet, 2001). Observed (H_o) and expected (H_e)

heterozygosity was computed with Arlequin v. 3.5.2.2 (Excoffier and Lischer, 2010). The Fisher's exact test was performed to evaluate deviation from Hardy–Weinberg equilibrium for each population and each locus using Genepop online software (Raymond and Rousset, 1995). The same software was also used to test linkage disequilibrium (LD) for all pairs of loci. Exact P-values were estimated using the Markov Chain algorithm (10,000 dememorization steps, 100 batches and 5,000 iterations) and the significance of HWE and LD values were adjusted by Bonferroni correction.

Effective population size (N_e) was calculated using the method based on the linkage disequilibrium (Waples and Do, 2010) as implemented in NeEstimator v.2.1 (Do et al., 2014). Minimum allele frequency of 0.02 and parametric confidence interval was used.

3.2.3 Sequencing, genotyping and SNPs calling

Libraries preparation and sequencing was performed by IGATecnology (Udine, Italy). Libraries are produced by multiplexing 96 swordfish (see table 2.1) following a modified version of the protocol described by Peterson et al. (2012), using *Sph*I (5' GCATGC 3') and MboI (5' GATC 3') restriction enzymes. Genomic DNA is fluorometrically quantified, normalized to a uniform concentration and double digested. Fragmented DNA is purified with AMPureXP beads (Agencourt) and ligated to barcoded adapters. Samples are pooled on multiplexing batches and bead purified. For each pool, targeted fragments distribution is collected on BluePippin instrument (Sage Science Inc.). Gel eluted fraction is amplified with oligo primers that introduce TruSeq indexes

and subsequently bead purified. The resulting libraries are checked with both Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) and Bioanalyzer DNA assay (Agilent Technologies, Santa Clara, CA). Libraries are processed with Illumina cBot for cluster generation on the flow-cell, following the manufacturer's instructions and sequenced with V4 chemistry paired-end 125bp mode on HiSeq2500 instrument (Illumina, San Diego, CA).

The public server at usegalaxy.eu has been used to analyze the sequencing raw data using tool shed Stacks2 (Catchen et al., 2011, 2013). *Process_Radtags* function was used to demultiplexing, quality filtering and cleaning sequence reads. Reads with quality scores below 20, missing either restriction site or with ambiguous barcodes were discarded. Lacking a reference genome, sequences were assigned to RAD loci and genotypes, using the *denovo_map.pl* component of Stacks2. The maximum mismatches between loci for a single individual (M = 3) and the maximum mismatches (n = 3) between loci for catalogue were selected following *r80* rule (Paris et al., 2017). Population function was run for the SNP calling, only loci present in both Mediterranean and Atlantic samples and in at least 75% of all individuals were retained. In addition, the dataset was filtered setting the maximum threshold for marker heterozygosity to 0.7, and the minor allele frequencies to 0.05. Further, data filtering was done in R (Team, 2015) using *filter_rad* function implemented in the *radiator* v.1.1.2 packages (Gosselin, 2019). Missing genotypes were discarded based on the threshold of 30 %, and markers were filtered

based on, missing data (20 %), coverage (<20, >40) and only one SNP per read was considered. The final RADseq dataset comprised 18,704 loci and 83 individuals. Allelic richness were measured using package *PopGenReport* v.3.0 (Adamack and Gruber, 2014), while observed (H_O) and expected (gene diversity (H_S) (Nei, 1978) heterozygosity and inbreeding coefficient (F_{IS}) were estimated using package *adegenet* v.2.0.1 (Jombart, 2008; Jombart and Ahmed, 2011) in R. To compare microsatellite and RADseq dataset the same genetic index was calculated for microsatellite data from the same individuals in the final RADseq data.

3.2.4 Population structure analysis

The following analyses were conducted on three datasets: i) microsatellite analyses were carried out on the whole samples and specimens (323 fishes), ii) the SNPs analyses emerged from the final RADseq data were carried out on a sub-samples (83 specimens), iii) to compare SNPs with microsatellites outcomes, these latter were analysed by using the same sub-sample used above for the SNPs analysis.

Pairwise and global F_{ST} values (Weir and Cockerham, 1984) were analysed using the package *assigner* (Gosselin et al., 2016). The 95% C.I. was obtained setting 1000 iterations. Furthermore, the Bayesian clustering method implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000) and the Discriminant Analysis of Principal Components (DAPC) (Jombart et al., 2010) were both employed to find the number of genetic discrete populations. Both methods do not require a priori delineation of genetic clusters and are

suitable to identify clusters of genetically related individuals. (Pritchard et al. 2000; Jombart et al. 2010). DAPC, unlike STRUCTURE, does not rely on a specific population genetic model and it is therefore free of assumptions about Hardy-Weinberg equilibrium or linkage disequilibrium (Jombart et al. 2010). The two approaches were used in this study, because various clustering approaches may lead to different conclusions. Moreover, DAPC approach is more suitable to unravel the underlying structuring in more complex population genetic models and generally performs better than Bayesian clustering methods (Jombart et al., 2010). Bayesian methods correct allocation performance decrease when $F_{ST} < 0.03$ (Latch et al., 2006; Waples and Gaggiotti, 2006; Duchesne and Turgeon, 2012).

The Bayesian job (STRUCTURE) carried out by using the microsatellite dataset was based on 10 serial runs for each number of clusters (K) between 1 and 7. The admixture model and correlated allele frequencies model were set. All analyses were run for 5×10^5 generations after a burn-in of 10×10^4 generations. At the same way, the STRUCTURE analysis carried out by using the ddRAD dataset was based on 5 serial runs setting 10×10^4 generations after a burn-in of 5×10^4 generations. The number of clusters that best fitted the observed genotype data was

determined comparing deltaK (ΔK) (Evanno et al., 2005) and the mean logarithmic probability of K, LnP(K), using the StructureSelector website (Li and Liu, 2018). The package *adegenet* was used to execute DAPC analysis. This analysis was also performed

both with and without prior information on individual populations. The first step was an explorative analysis to visualise the relationship between sampling locations. Cross-validation was used to select the number of principal components (PCs) to retain for DAPC, selecting the lowest number of components where the correct assignment probability levelled (19 for SNPs, 17 for both datasets of microsatellites). In the second step the optimal number of clusters (k) was evaluated with the function *find.cluster* which runs successive rounds of k-means clustering with an increasing number of clusters (K) covering K from 1 to 10, with ten runs at each value of K. Bayesian information criterion (BIC) was used to select the optimal number of clusters. Ideally, the lowest BIC value represents the optimal number of clusters, but, BIC values may keep decreasing after the true K value in case of genetic clines and hierarchical structure (Jombart et al. 2010). The axes of Principal Components Analysis sufficient to explain > 90 % of the total variance of data were retained. The result was presented in an ordination plot with the first two axes. Finally, pairwise F_{ST} values among genetic clusters were calculated using Arlequin.

3.3 Results

3.3.1 Microsatellite genetic diversity

Twenty microsatellite loci were amplified for 323 swordfish samples, with a PCR failure per locus ranged between 0 and of 5.9% with an average of 1.1 %.

Considering sample locality separately, significant deviations from Hardy-Weinberg equilibrium were detected in 18 out of 140 single locus exact tests at loci Xgl-94, Xgl-74, Xgl-14, Xg66 and Xg166 (table SM 3.1). Pooling together Mediterranean swordfish, 5 out of 20 loci deviate significantly from Hardy-Weinberg equilibrium (Xgl-94, Xgl-74, Xgl-14, Xg66, Xgl-523). All deviations were towards heterozygote deficit. Micro-checker identified exhibiting null alleles with the estimated frequencies of null alleles > 0.3 for Xgl-74 and Xg-66. These loci deviated in mostly population including Canadian samples thus, these loci were removed from further analysis. Rarer null alleles at Xgl-94, Xgl-14 and Xg166 were detected via Micro-checker at low frequency. Genotype data were retained since population differentiation parameters are not biased by the presence of null allele. In fact, the estimation of F_{ST} both using and without using the ENA correction method gave equal results; F_{ST} = 0.018 with the respective 95% CI [0.011–0.027]. No consistent evidence for linkage disequilibrium was detected between pairs of loci within populations.

All the remaining 18 loci were polymorphic, with the number of alleles per locus ranging from two, at locus Xg-402, to 21, at locus C8. Both, Xg-402 (two alleles) and Xg-394

(three alleles) loci were monomorphic in three samples GRE, SPA and CAN. The Mediterranean samples exhibited a significantly lower number of alleles per locus, allelic richness, and expected heterozygosity, compared to the Atlantic one (Table SM 3.1). The mean number of alleles and the levels of heterozygosity resulted in the same magnitude between different Mediterranean areas in respect of sampling size. Thus, no evidence of geographical pattern was observed for the distribution of genetic variability among Mediterranean samples.

Estimates of effective population size (N_e) based on the linkage disequilibrium method showed N_e = 944.1 (95 % CI: 586.3–2155) grouping all Mediterranean sample together and was infinite for the Atlantic population.

3.3.2 ddRAD SNPs genetic diversity

RAD-Seq sequencing of the 96 individuals generated a total of 164,417,872 reads. In total 159,722,581 reads was retained after discarding reads with low quality, ambiguous barcode and RAD cut-site absent. After STACKS filtering and population tool shed filtering 25,658 polymorphic loci were genotyped and shared across ≥75% individuals and present in all populations. After final data filtering 18,704 SNP loci were identified and 83 individuals were retained. Mediterranean localities exhibited similar values of expected heterozygosity, ranging from H_e = 0.143 in SPA and ADR to Ar = 0.146 in SAR and allelic richness ranged from Ar = 0.136 in GRE to Ar = 0.142 in SIC (table SM 3.2). Polling Mediterranean swordfish in a single population, the values were: H_e = 0.139

and *Ar* = 1.52. The comparison between Mediterranean and Atlantic genetic variability indicated that both H_e and *Ar* were lower in the Mediterranean samples than those observed for the Atlantic population (H_e = 0.149 and *Ar* = 1.64). Estimate inbreeding coefficient values was F_{IS} = 0.015 for Mediterranean and F_{IS} = 0.054 for Atlantic population. Estimates effective population size showed a lower value for whole Mediterranean swordfish population N_e = 2,306 (95 % CI: 2,108 – 2,546) than for Atlantic N_e = 5,554 (95 % CI: 3,195 – 21,105).

3.3.3 Genetics Structure inferred by microsatellite

The global F_{ST} overall microsatellite loci detected a great signal of genetic differentiation between the whole Mediterranean and Atlantic samples (F_{ST} = 0.091; 95 % C.I. 0.056-0.133). The F_{ST} values decreased when considering the Mediterranean samples separately (F_{ST} = 0.018; 95 % C.I. 0.011-0.027). Pairwise F_{ST} across all samples ranged from –0.005 to 0.097. The lower and no statistically significant values were detected between Mediterranean localities, whereas the highest, and significantly different from zero, outcomes were observed in comparisons between the Mediterranean samples and the Atlantic sample (Table 3.3).

From Bayesian clustering analysis performed on the complete microsatellite dataset, the ΔK method (Evanno et al. 2005) suggested the presence of two genetic clusters (K = 2). LnP(K), instead, showed an increase to K = 2 before declining and subsequently increasing to K = 6 (Figure 3.3a). For K = 2 individuals were partitioning according to

their source basins (Mediterranean *vs* North-western Atlantic). Individuals were assigned to the specific cluster with high score showing average q = 0.98 for the Mediterranean group and average q = 0.95 for Atlantic group. Only few individuals showed signs of mixing (Figure 3.3b). Considering K = 6 Atlantic swordfish formed a single cluster, while all Mediterranean swordfish were uniformly assigned to the remaining five groups, failing in population structure identification. In the STRUCTURE analysis performed on a restricted microsatellite dataset (i.e., the same used for RADseq) revealed, with both LnP(K) and ΔK methods, that the most likely number of genetic clusters contained in our samples was two (K = 2). One cluster was composed of all Atlantic swordfish while the second one was composed of Mediterranean individuals, confirming an inter-oceanic genetic differentiation (Figure SM 3.1b).

The exploratory multivariate analysis divided the sampling localities into two groups. The first principal component clearly discriminated the Atlantic and Mediterranean swordfish, clustering all Mediterranean sampling localities together, confirming an inter-oceanic genetic differentiation (Figure 3.2). On the contrary, *a posteriori* assignment of DAPC analysis, despite the absence of a clear best value for the number of clusters, suggested the presence of more than two genetic clusters. Considering the whole microsatellite database, the BIC graph showed a clear decrease until K = 4 reaching a slightly lower value at K = 6 (Figure 3.5). For K = 4, all Atlantic individuals but two,

clustered in a single group, while Mediterranean swordfish were subdivided in remaining three groups. Mediterranean groups were randomly distributed among sampling localities and no geographic pattern of differentiation was observed between localities (Figure 3.4). Increasing the number of K, increased admixture and mixing within the Mediterranean samples. Results from DAPC analysis using restricted microsatellite data from the same individuals in RADseq showed the lowest BIC values for K = 2 and 3 (Figure SM 3.2). In both cases, admixture between Atlantic and Mediterranean populations was detected, although very limited considering the three clusters. If genotypes are partitioned into two distinct clusters ten Mediterranean swordfish clustered into Atlantic group. Partitioning genotypes into three distinct clusters all Atlantic individuals but two clustered in a group and Mediterranean swordfish was divided into two groups randomly distributed among sampling localities. Only one Mediterranean specimen clustered into the Atlantic group (Figure SM 3.2).

3.3.4 Genetics Structure inferred by ddRAD data

The global F_{ST} estimated for RADseq loci highlighted signal of genetic differentiation between Mediterranean and Atlantic population (F_{ST} = 0.092; 95 % C.I. 089-0.095). F_{ST} value drop considering Mediterranean samples separately (F_{ST} = 0.05; 95 % C.I. 0.05-0.05). Pairwise F_{ST} did not detect differentiation among Mediterranean locality (Table 3.3). Bayesian clustering analysis and multivariate analysis performed on RADseq dataset showed concordant results. Both ΔK and mean LnP(K) for STRUCTURE and

BIC value for DAPC identified a partition into two groups as most likely. This clustering clearly separated the Mediterranean and Atlantic swordfish into two genetic groups. No sign of admixture between populations was detected (Figures 3.4,3.6).

Table 3.3 Pairwise F_{ST} calculate among sampling locality. Below the diagonal values estimated on microsatellite data from all 323 individuals, above the diagonal values estimated on RADseq data from 83 individuals. Bold represent values significantly different from zero.

Sampling locality	ADR	SIC	GRE	SPA	TIR	SAR	CAN
ADR		0,001	0,001	0,004	-	0,002	**0,087**
SIC	0,002		0,000	0,002	-	0,002	**0,089**
GRE	-0,005	-0,002		0,006	-	0,000	**0,081**
SPA	0,001	0,000	0,000		-	0,006	**0,089**
TIR	0,001	-0,001	0,000	0,000		-	-
SAR	0,003	0,001	-0,007	0,002	-0,002		**0,088**
CAN	**0,091**	**0,086**	**0,083**	**0,093**	**0,088**	**0,097**	

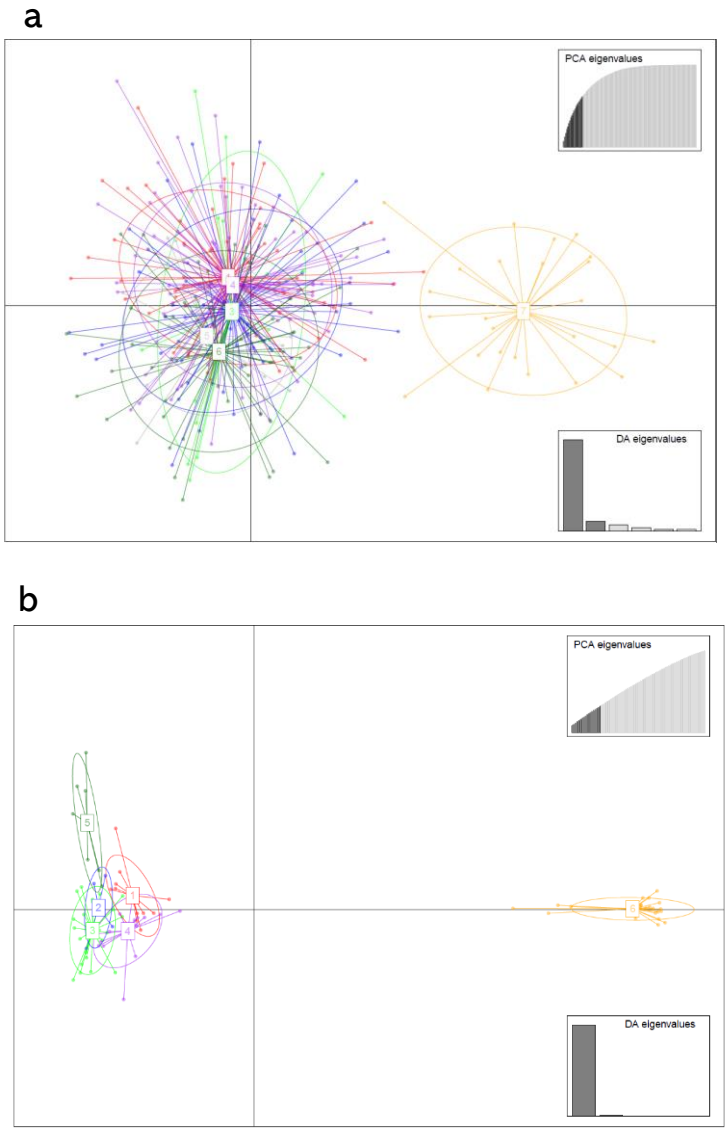


Figure 3.2 Grouping of Swordfish samples on DAPC based on full microsatellite data (a), RADseq data (b). Populations indicated by labelled colours. ADR (red), GRE (blue), SIC (light green), SPA (purple), TIR (grey), SAR (dark green), CAN (orange)

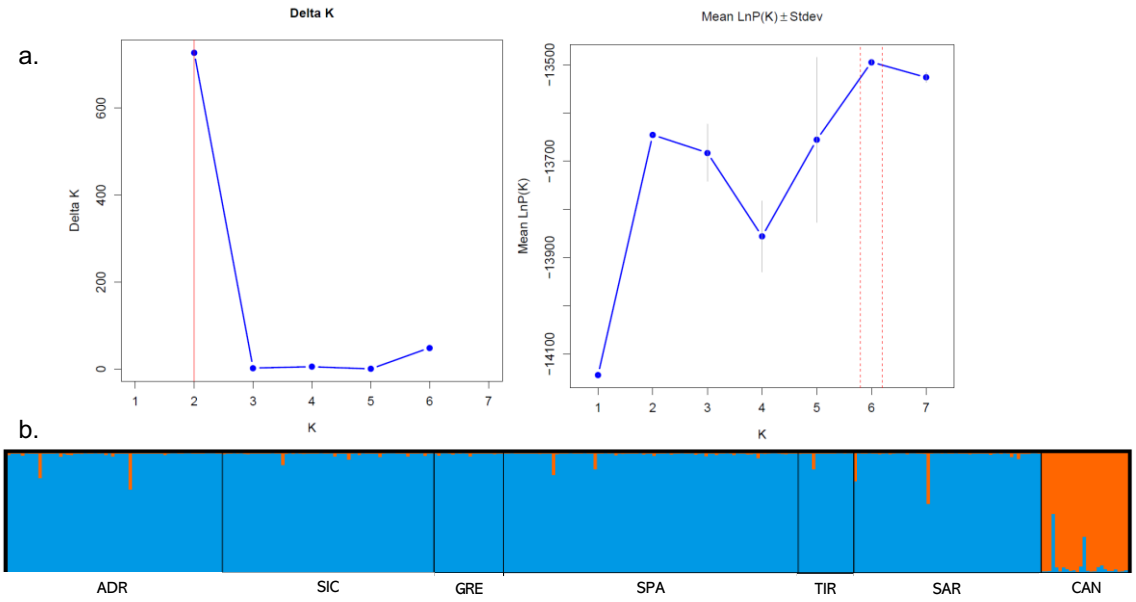


Figure 3.3 a) Plot of Delta K and Mean LnP(K) according to K. b) Structure clustering results obtained at K=2, based on microsatellite data from all 323 individuals. Barplots showing posterior probabilities of swordfish individual genotypes (as bars) assigned to each population. The black lines separate sampling localities.

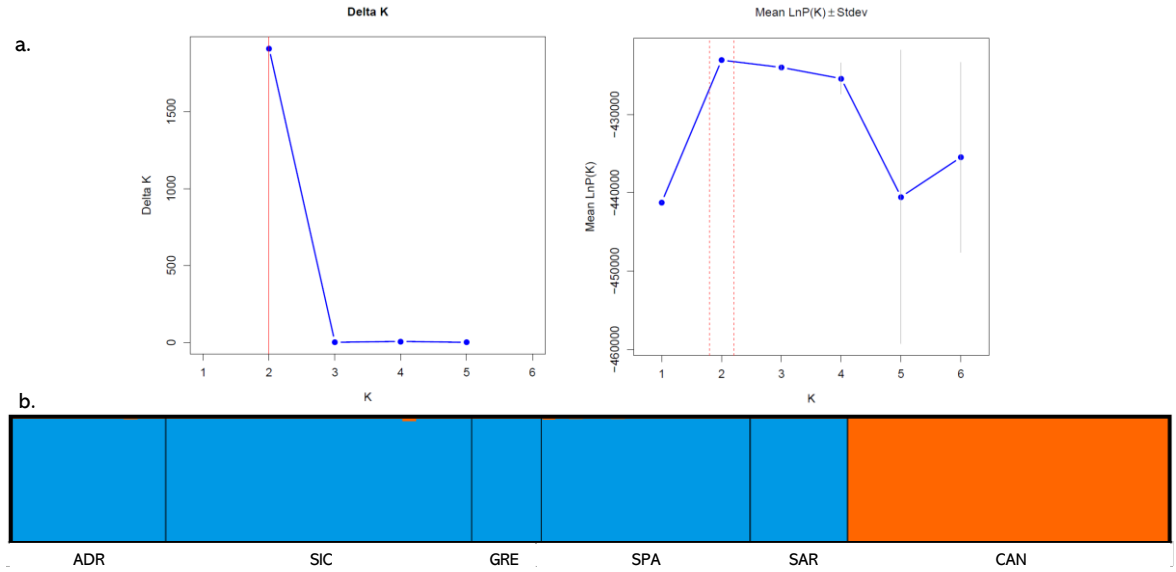


Figure 3.4 a) Plot of Delta K and Mean LnP(K) according to K. b) Structure clustering results obtained at K=2, based on RADseq data from 83 individuals. Barplots showing posterior probabilities of swordfish individual genotypes (as bars) assigned to each population. The black lines separate sampling localities.

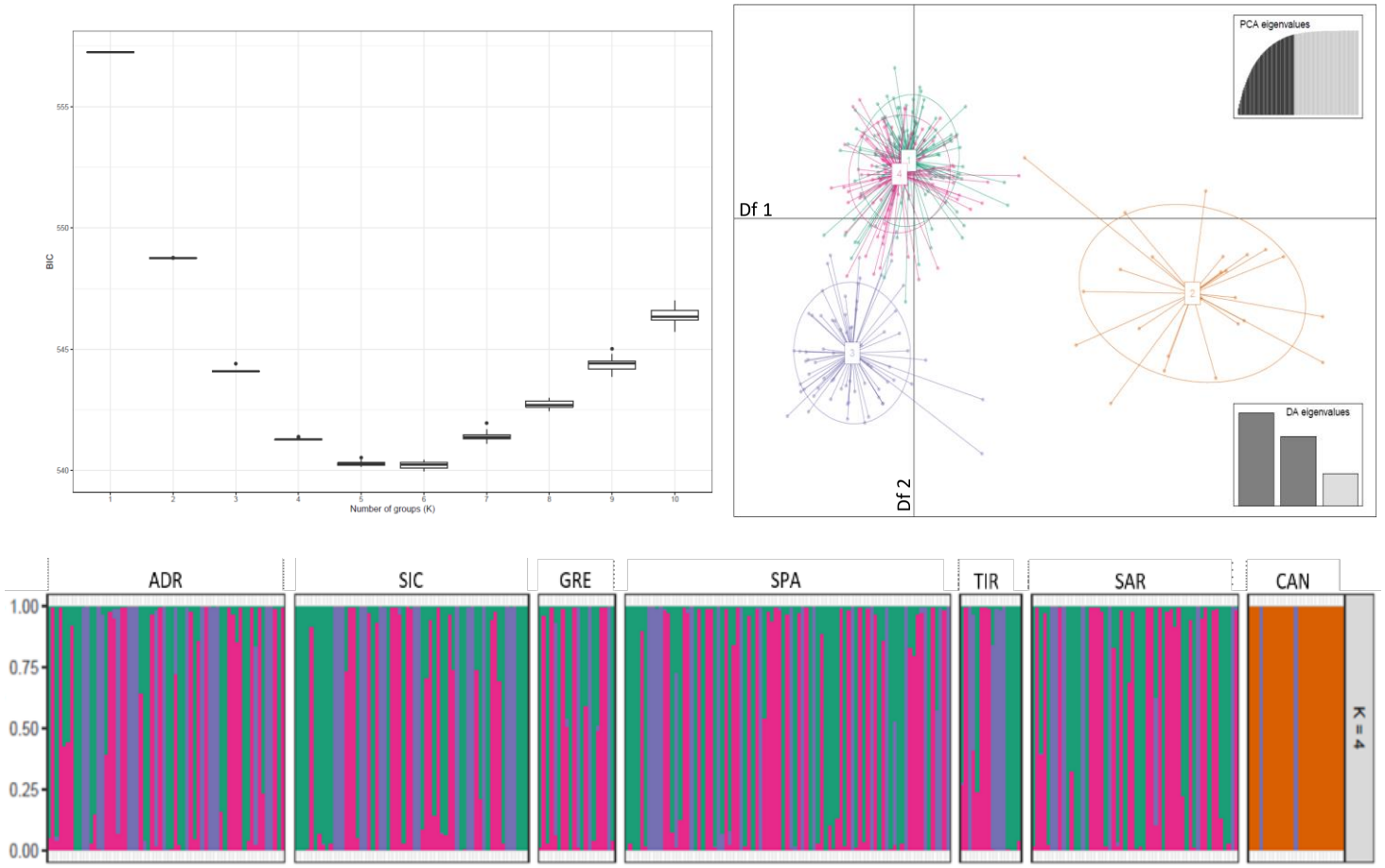


Figure 3.5 Discriminant analysis of principal components (DAPC) dased on microsatellite data from 323 swordfish. a) Inference of the number of clusters in the DAPC. b) Scatterplot based on the linear discriminant functions 1 and 2. Dots represent the individuals and colours represent the clusters identify by find.cluster: cluster 2 (orange) includes Atlantic swordfish, clusters 1,3 and 4 (green, blue and red respectively) include Mediterranean swordfish. The graph above to the right represents the contribution of the eigenvalues of the principal components selected, while the graph below to the right indicates the variance explained by the eigenvalues of the two discriminant functions of the scatterplot. c) DAPC Barplot of group membership probabilities. Each individual is represented as a vertical bar, with colours corresponding to probabilities of membership into the clusters identified according to BIC values.

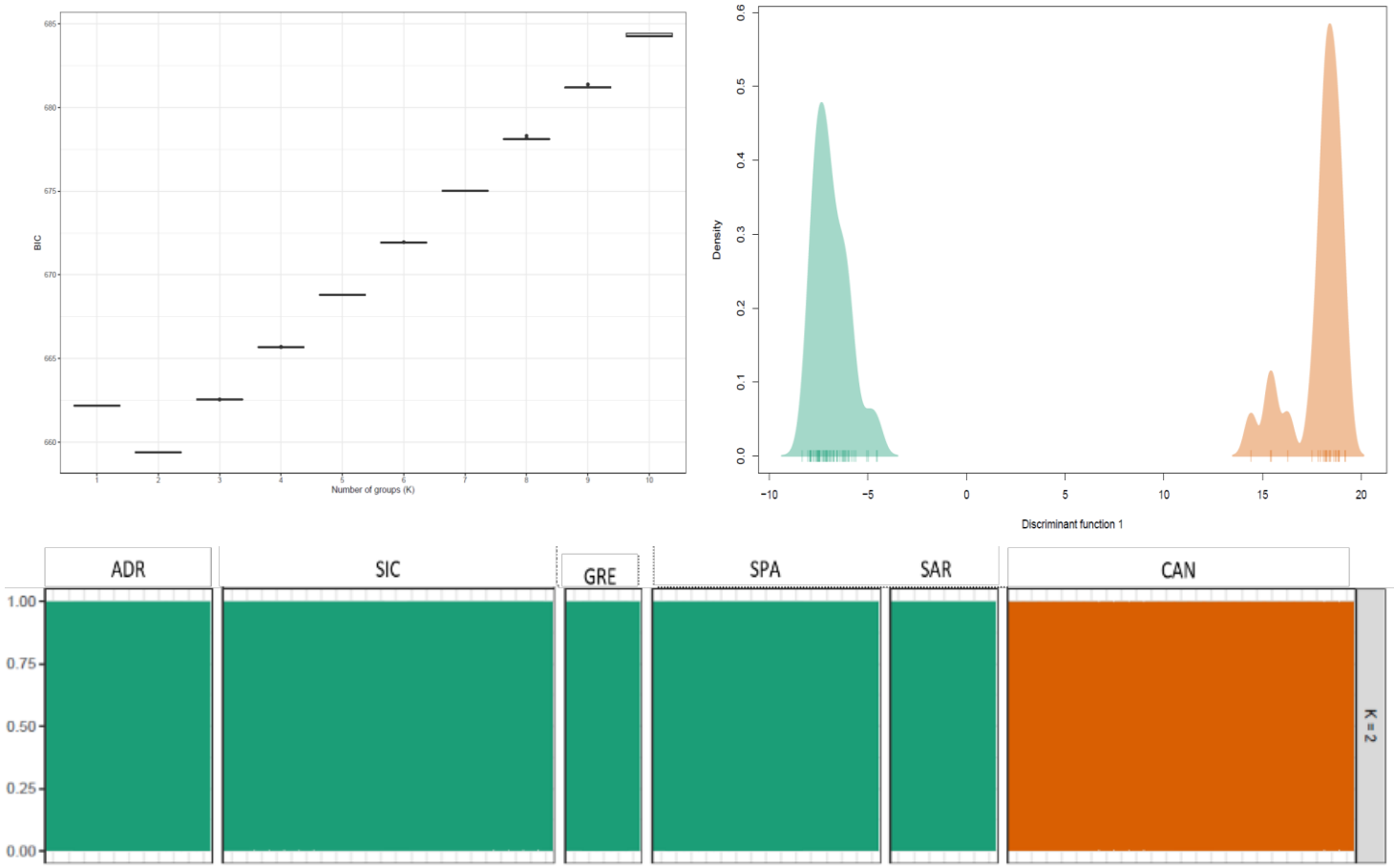


Figure 3.6 Discriminant analysis of principal components (DAPC) based on RADseq data from 83 individuals.. a) Inference of the number of clusters in the DAPC. b) Scatterplot based on the linear discriminant functions 1. Dots represent the individuals and colours represent the clusters identify by find.cluster: cluster 2 (orange) includes Atlantic swordfish, clusters 1 (green) include Mediterranean swordfish. c) DAPC Barplot of group membership probabilities. Each individual is represented as a vertical bar, with colours corresponding to probabilities of membership into the clusters identified according to BIC values.

3.4 Discussion

This study aimed to investigate the genetic structure of the swordfish *Xiphias gladius*, within the Mediterranean Sea and between NW-Atlantic and Mediterranean populations, comparing results from a panel of 18 microsatellite loci and >18,700 SNPs, obtained by RADseq. The first result shows that both marker types were equally able to identify inter-oceanic genetic differentiation among NW-Atlantic and Mediterranean stocks, increasing the ability to assign individuals to their population of origin compared to previous multi-locus works. Secondly, differently from previous studies, microsatellite multivariate analysis suggests the presence of three mixed genetic groups within the Mediterranean Sea.

Swordfish genetic diversity was equally well reflected by both a panel of 18 microsatellite loci and >18,700 SNPs, obtained by RADseq. A similar finding was observed in other studies comparing this marker types in other species (Ryynänen et al., 2007; Fischer et al., 2017). The Mediterranean population showed a lower level of genetic diversity compared to Atlantic ones, corroborating the outcomes of previous studies based on microsatellites (Reeb et al., 2003; Kotoulas et al., 2007; Kasapidis et al., 2009) and mtDNA (Bremer et al., 1995, 1996,2005; Kotoulas et al., 1995; Rosel and Block, 1996). Lower genetic variability of Mediterranean swordfish population is a consequence of genetic isolation and smaller effective population size strongly depends

on the characteristics of the Mediterranean basin (Bremer et al., 2005; Kasapidis et al., 2007).

A clear genetic structure between the Mediterranean and Atlantic populations was identified from both marker types. The presence of these populations is supported by pairwise F_{ST}, multivariate analysis (DAPC) and Bayesian genetic clustering using STRUCTURE. However, the estimates of differentiation (F_{ST}) calculated in this work between the two stocks, similar for both marker types (around 0.09), have turned out to be higher compared to those reported by recent works using a multi-locus approach (Kotoulas et al., 2007; Smith et al., 2015). A very small value ($F_{ST} < 0.03$) was reported between Atlantic and Mediterranean swordfish stocks using four highly polymorphic microsatellite (Kotoulas et al., 2007), while F_{ST} values around 0.07 were reported by Smith (2015) analysing 26 single nucleotide polymorphisms (SNPs) within 10 nuclear genes. Moreover, Smith (2015) managed to effectively distinguish the population of the North Atlantic from that of the South Atlantic that 4 microsatellites could not discriminate (Kasapidis et al., 2007; Kotoulas et al., 2007). This difference is due to the increase in the number of loci used, which could enhance the resolution and improve the precision of estimates of genetic distance (Nei, 1978; Kalinowski, 2005).

Although both cluster analysis of genome-wide SNPs agreed to identify only two populations, the results obtained from the two clustering methods using microsatellites

were not concordant. DAPC in fact provided evidence of a substructure inside the Mediterranean Sea that results more evident when the entire microsatellite dataset was used. The discrepancy between results obtained from the two makers may be imputed to the dataset size used. The ddRAD dataset was composed of only 83 individuals related to the 323 of microsatellite dataset. The addition of individuals, in fact, permits more accurate inferences of population structure (Fumagalli et al., 2013; Benestan et al., 2015), and the detection of significant differences in allele frequencies when divergence is low (Kalinowski, 2005). This hypothesis was corroborated analysing microsatellite from the same individuals used for RADseq. Reducing the sample size, in fact, the number of clusters identified by DAPC decreased.

The results discrepancy between clustering methods on the entire microsatellite dataset may be attributable to the fact that the Bayesian clustering method fails to detect any genetic structure when genetic divergence is very low ($F_{ST} < 0.03$) (Latch et al., 2006; Waples and Gaggiotti, 2006; Duchesne and Turgeon, 2012). Moreover, uneven sampling often leads to wrong estimates of the true number of subpopulations and distinct subpopulations with reduced sampling tended to be merged together (Puechmaille, 2016). The efficiency of DAPC to identify genetic clusters where STRUCTURE failed was also reported by Jombart et al. (2010) and Kanno et al. (2011). Thus, DAPC can outperform the STRUCTURE program when inferring the number of subpopulations weakly differentiated.

Population structure within the Mediterranean Sea is not consistent with previous work based on microsatellite data (Kotoulas et al., 2007). However, despite in the study of Kotoulas et al. (2007) a greater number of specimens (602) have been analysed, as observed above, at the same time, this study used only four microsatellite loci, that are a very low number of loci to detect the presence of genetic differentiation between samples, or low genetic differentiation, as is the case of pelagic fish species. Clusters detected by DAPC, with the exception of the cluster of Atlantic swordfish, were spatially admixed and no geographic pattern was observed. This result was corroborated by pairwise F_{ST} estimation that not detect differentiation among Mediterranean sampling localities. Genetic homogeneity between Mediterranean sampling localities was observed by previous studies based on allozymes data (Pujolar et al., 2002), RFLPs of the entire mtDNA (Chow et al., 1997), analyses of a single-copy nuclear calmodulin gene and PCR–RFLP data of the mtDNA CR (Chow and Takeyama, 2000). However, sample homogeneity does not necessarily equate to population homogeneity (Ward, 2000) and population differentiation may be obscured by population mixture in wintering or feeding areas, especially for highly migratory species (Van Wagner and Baker, 1990; Bowen et al., 1992; Wenink et al., 1993; Bremer et al., 2005). Swordfish is a highly migratory species. In the Atlantic Ocean, it is able to cover annually long distances by horizontal movements as reported by pop-up satellite archival tags analysis (Neilson et al., 2014; Abascal et al., 2015). The same ability was also observed within the

Mediterranean Sea (Canese et al., 2008). Evidence of shared areas has been observed in the feeding area west of the Strait of Gibraltar where Mediterranean and North Atlantic swordfish populations mix together (Bremer et al., 2005; Smith et al., 2015). Furthermore, admixture between North and South Atlantic population occurs over a broader geographic area from Western Sahara to Iberian sea extending west towards the central North Atlantic and then south towards the northern Brazilian coast (Smith et al., 2015). In the Adriatic Sea no evidence of spawning activities was detected (Arocha, 2007) and, due to its semi-enclosed sea characteristic, we can rule out the presence of a mixture of individuals originating from different subpopulations as the consequence of a transitional effect. Therefore, the presence of a mixture of adult individuals using the Adriatic Sea as a feeding ground seems plausible. Regarding the other sampling areas, they could represent transitional zones and then, show mixing between populations as occurs between North and South Atlantic. The hypothesis of admixed population within the Mediterranean Sea is supported, also, by the significant excess of homozygotes genotypes detected in Mediterranean samples. An excess of homozygotes may be due to genotyping errors such as null alleles, allele drop out and stuttering, or a consequence of a not appropriate sample size. However, excluding above technical issues, biological explanations can be proposed to explain the occurrence of Hardy Weinberg disequilibrium (HWD) as the Wahlund effect (WE). WE, instead, is generated when the

sample analysed is composed of a mix of distinct subpopulations, as would be expected by highly migratory and structured species.

Philopatric behaviour has been identified as the driving force behind the structuring of very high migratory pelagic fish *Istiompax indica* (Williams et al., 2016), *Gadus morhua* (Bonanomi et al., 2016) and *Thunnus thynnus* (Rooker et al., 2008; Aranda et al., 2013). As for swordfish, spawning site fidelity is supported by both maximum levels of genetic differentiation obtained comparing separated breeding areas in the Atlantic Ocean (Bremer, Mejuto, et al., 2005; Bremer et al., 2007) and the evidence of the mixing areas but very limited gene flow between Mediterranean and North Atlantic swordfish population (Bremer et al., 2007; Viñas et al., 2007; Smith et al., 2015). Seasonal site fidelity was also suggested into the Mediterranean Sea, thanks to recapturing of tagged individuals, that generally occurred in the same area of tagging also after several years (Garibaldi and Lanteri, 2017). Within the Mediterranean Sea, three main spawning areas were described. The first is located in the western Mediterranean, from the Strait of Gibraltar up to the Balearic Islands; the second one extends from the Southern Tyrrhenian Sea, through the Strait of Messina, to the Gulf of Taranto in the Ionian Sea (Cavallaro et al., 1991; Arocha, 2007), and the last one in the Levantine Sea close to Rhodes and Cyprus islands (Tserpes et al., 2001, 2008). Although, there is no evidence that these spawning aggregations represent discrete stocks, geographical localization of these three discrete spawning areas may explain the three genetic cluster observed in this

study. Already Viñas (2010) suggested population substructure within the Mediterranean sea basing on a clinal decrease in mitochondrial genetic variability from the western to the eastern basins. The authors hypothesised that population differentiation may be the consequence of distinct phylogeographic histories of populations in the eastern and the western Mediterranean basins and is maintained by present-day life-history traits, including homing fidelity to spawning sites.

Rejection of a model of panmixia in a relatively small sea basin is not new for large pelagic species. For example, for the Atlantic bluefin tuna, that share the same spawning ground of swordfish (Bonales et al., 2019), a remarkable salmon-like homing behaviour to Tyrrhenian and Ionian spawning grounds have been detected by satellite tracks (Reeb, 2010). Reproductive isolation explains a fine-scale structure identified among Balearic, Tyrrhenian, and Ionian juvenile bluefin tuna using both nuclear microsatellite loci and the mtDNA control region (Carlsson et al., 2004). Furthermore, spatially and temporally stable genetic structure was observed between Adriatic and Tyrrhenian bluefin tuna (Riccioni et al., 2010).

3.5 Conclusion

This study showed that both a panel of 18 microsatellite loci and >18000 RADseq linked loci have the same ability to identify genetic structure between Atlantic and Mediterranean stocks. A comparison with previous works revealed that the resolution and precision of estimates of genetic distance between swordfish stocks improve increasing the number of loci. Microsatellite or RADseq data may be useful to better delineate the boundary between Atlantic stock and managed correctly the resource. Furthermore, microsatellite data suggest genetic heterogeneity within the Mediterranean Sea. Despite the low differentiation observed, the results obtained provide useful information on the stock structure of the swordfish rejecting the hypothesis of a single Mediterranean population. The discontinuity between Mediterranean spawning areas and philopatry instinct of swordfish towards these areas may have generated a weak degree of genetic differentiation between populations even at the intra-basin scale. Presence of genetically distinct populations would make current single-stock management unit approach used to manage Mediterranean swordfish by ICCAT inappropriate. Large regional scales management regulations considering stock uniformity might overfish some small, but discrete populations. However, the high level of mixing among populations hampers delineation of spatial management areas. In light of these results, further investigations are required to determine the degree of complexity of the Mediterranean swordfish population structure in order to achieve efficient swordfish

conservation. To date, significant gaps still exist regarding Mediterranean swordfish life history and stock structure. Including in the analysis larvae and young-of-the-year (YOY) would assist in the assessment of swordfish population dynamics. Collecting a larger sample size from each population, in spawning ground and during the spawning period maximizing stock discreteness could ameliorate evaluation of geographic genetic segregation. Moreover, including tagging information, currently very limited for Mediterranean swordfish, would be necessary to better resolve the swordfish migratory behaviour.

3.6 Reference

Abascal, F. J., J. Mejuto, M. Quintans, B. García-Cortés, and A. Ramos-Cartelle. 2015. Tracking of the broadbill swordfish, Xiphias gladius, in the central and eastern North Atlantic. *Fisheries Research* 162: 20–28.

Adamack, A. T., and B. Gruber. 2014. PopGenReport: simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution* 5: 384–387.

Anon. 2019. Report for biennial period, 2018-19 PART I (2018) - Vol. 2. International Commission for the Conservation of Atlantic Tunas, Madrid, Spain.

Aranda, G., F. J. Abascal, J. L. Varela, and A. Medina. 2013. Spawning behaviour and post-spawning migration patterns of Atlantic bluefin tuna (Thunnus thynnus) ascertained from satellite archival tags. *PloS one* 8: e76445.

Arocha, F. 2007. Swordfish Reproduction in the Atlantic Ocean: An Overview. *Gulf and Caribbean Research* 19.

Benestan, L., T. Gosselin, C. Perrier, B. Sainte-Marie, R. Rochette, and L. Bernatchez. 2015. RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the A merican lobster (H omarus americanus). *Molecular ecology* 24: 3299–3315.

Bonales, J. V., A. Cañadas, S. Tensek, A. P. García, and F. Alemany. 2019. ICCAT GBYP AERIAL SURVEY FOR BLUEFIN TUNA SPAWNING AGGREGATIONS IN 2018. *Collect. Vol. Sci. Pap. ICCAT* 75: 1525–1544.

Bonanomi, S., N. Overgaard Therkildsen, A. Retzel, R. Berg Hedeholm, M. W. Pedersen, D. Meldrup, C. Pampoulie, et al. 2016. Historical DNA documents long-distance natal homing in marine fish. *Molecular Ecology* 25: 2727–2734.

Bowen, B. W., A. B. Meylan, J. P. Ross, C. J. Limpus, G. H. Balazs, and J. C. Avise. 1992. Global population structure and natural history of the green turtle (Chelonia mydas) in terms of matriarchal phylogeny. *Evolution* 46: 865–881.

Bremer, J. A., J. Mejuto, J. Gómez-Márquez, F. Boán, P. Carpintero, J. M. Rodríguez, J. Viñas, et al. 2005. Hierarchical analyses of genetic variation of samples from breeding and feeding grounds confirm the genetic partitioning of northwest Atlantic and South Atlantic populations of swordfish (Xiphias gladius L.). *Journal of Experimental Marine Biology and Ecology* 327: 167–182.

Bremer, J. A., J. Mejuto, J. Gómez-Márquez, C. Pla-Zanuy, J. Viñas, C. Marques, F. Hazin, et al. 2007. Genetic population structure of Atlantic swordfish: current status and future directions. *ICCAT Collect. Vol. Sci. Pap.* 61: 107–118.

Bremer, J. R. A., A. J. Baker, and J. Mejuto. 1995. Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (Xiphias gladius) populations in the Atlantic Ocean. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 1720–1732.

Bremer, J. R. A., J. Mejuto, T. W. Greig, and B. Ely. 1996. Global population structure of the swordfish (Xiphias gladius L.) as revealed by analysis of the mitochondrial DNA control region. *Journal of Experimental Marine Biology and Ecology* 197: 295–310.

Bremer, J. R. A., J. Viñas, J. Mejuto, B. Ely, and C. Pla. 2005. Comparative phylogeography of Atlantic bluefin tuna and swordfish: the combined effects of vicariance, secondary contact, introgression, and population expansion on the regional phylogenies of two highly migratory pelagic fishes. *Molecular phylogenetics and evolution* 36: 169–187.

Canese, S., F. Garibaldi, L. O. Relini, and S. Greco. 2008. Swordfish tagging with pop-up satellite tags in the Mediterranean Sea. *Collect. Vol. Sci. Pap. ICCAT* 62: 1052–1057.

Carlsson, J., J. R. McDOWELL, P. DÍAZ-JAIMES, J. E. Carlsson, S. B. Boles, J. R. Gold, and J. E. Graves. 2004. Microsatellite and mitochondrial DNA analyses of Atlantic bluefin tuna (Thunnus thynnus thynnus) population structure in the Mediterranean Sea. *Molecular Ecology* 13: 3345–3356.

Catchen, J., P. A. Hohenlohe, S. Bassham, A. Amores, and W. A. Cresko. 2013. Stacks: an analysis tool set for population genomics. *Molecular ecology* 22: 3124–3140.

Catchen, J. M., A. Amores, P. Hohenlohe, W. Cresko, and J. H. Postlethwait. 2011. Stacks: building and genotyping loci de novo from short-read sequences. *G3: Genes, genomes, genetics* 1: 171–182.

Cavallaro, G., A. Potoschi, and A. Cefali. 1991. Fertility gonad-somatic index and catches of eggs and larvae of Xiphias gladius L. 1758 in the southern Tyrrhenian Sea. *Col. Vol. Sci. Pap. ICCAT* 35: 502–507.

Chapuis, M.-P., and A. Estoup. 2006. Microsatellite null alleles and estimation of population differentiation. *Molecular biology and evolution* 24: 621–631.

Chow, S., H. Okamoto, Y. Uozumi, Y. Takeuchi, and H. Takeyama. 1997. Genetic stock structure of the swordfish (Xiphias gladius) inferred by PCR-RFLP analysis of the mitochondrial DNA control region. *Marine Biology* 127: 359–367.

Chow, S., and H. Takeyama. 2000. Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of the swordfish. *Journal of Fish Biology* 56: 1087–1098.

Cochrane, K. L. 2002. A Fishery Manager's Guidebook-Management Measures and Their Application (Tech. No. 424). *Rome: FAO*.

Collette, B., A. Acero, A. F. Amorim, K. Bizsel, A. Boustany, C. C. Ramirez, G. Cardenas, et al. 2011. Xiphias gladius. IUCN.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular ecology resources* 14: 209–214.

Duchesne, P., and J. Turgeon. 2012. FLOCK provides reliable solutions to the "number of populations" problem. *Journal of Heredity* 103: 734–743.

Ehrhardt, N. M. 1992. Age and growth of swordfish, Xiphias gladius, in the northwestern Atlantic. *Bulletin of Marine Science* 50: 292–301.

Excoffier, L., and H. E. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular ecology resources* 10: 564–567.

Fischer, M. C., C. Rellstab, M. Leuzinger, M. Roumet, F. Gugerli, K. K. Shimizu, R. Holderegger, and A. Widmer. 2017. Estimating genomic diversity and population differentiation–an empirical comparison of microsatellite and SNP variation in Arabidopsis halleri. *BMC genomics* 18: 69.

Fumagalli, M., F. G. Vieira, T. S. Korneliussen, T. Linderoth, E. Huerta-Sánchez, A. Albrechtsen, and R. Nielsen. 2013. Quantifying population genetic differentiation from next-generation sequencing data. *Genetics* 195: 979–992.

Garibaldi, F., and L. Lanteri. 2017. Notes about a tagged/recaptured swordfish in the Ligurian Sea (western Mediterranean). *Collective Volume of Scientific Papers, ICCAT* 74: 1354–1361.

Gosselin, T. 2019. radiator: RADseq data exploration, manipulation and Visualization using R.

Gosselin, T., E. C. Anderson, and I. Bradbury. 2016. assigner: Assignment Analysis with GBS/RAD Data using R. *R package version 0.4* 1.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversity and fixation indices (version 2.9. 3). *http://www2. unil. ch/popgen/softwares/fstat. htm*.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24: 1403–1405.

Jombart, T., and I. Ahmed. 2011. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* 27: 3070–3071.

Jombart, T., S. Devillard, and F. Balloux. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC genetics* 11: 94.

Kalinowski, S. T. 2005. Do polymorphic loci require large sample sizes to estimate genetic distances? *Heredity* 94: 33.

Kanno, Y., J. C. Vokoun, and B. H. Letcher. 2011. Fine-scale population structure and riverscape genetics of brook trout (Salvelinus fontinalis) distributed continuously along headwater channel networks. *Molecular Ecology* 20: 3711–3729.

Kasapidis, P., J. Mejuto, G. Tserpes, A. Antoniou, B. Garcia-Cortes, P. Peristeraki, K. Oikonomaki, et al. 2007. Genetic structure of the swordfish (Xiphias gladius) stocks in the Atlantic using microsatellite DNA analysis. *Collect. Vol. Sci. Pap. ICCAT* 61: 89–98.

Kasapidis, P., V. Pakaki, G. Kotoulas, and A. Magoulas. 2009. Isolation and characterization of 18 new polymorphic microsatellite loci for the swordfish, Xiphias gladius. *Molecular ecology resources* 9: 1383–1386.

Kotoulas, G., A. Magoulas, N. Tsimenides, and E. Zouros. 1995. Marked mitochondrial DNA differences between Mediterranean and Atlantic populations of the swordfish, Xiphias gladius. *Molecular Ecology* 4: 473–482.

Kotoulas, G., J. Mejuto, A. Antoniou, P. Kasapidis, G. Tserpes, C. Piccinetti, P. Peristeraki, et al. 2007. Global genetic structure of swordfish (Xiphias gladius) as revealed by microsatellite DNA markers. *ICCAT Coll Vol Sci Pap* 61: 79–88.

Latch, E. K., G. Dharmarajan, J. C. Glaubitz, and O. E. Rhodes. 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics* 7: 295–302.

Li, Y.-L., and J.-X. Liu. 2018. StructureSelector: A web-based software to select and visualize the optimal number of clusters using multiple methods. *Molecular Ecology Resources* 18: 176–177.

Martínez, P., E. G. González, R. Castilho, and R. Zardoya. 2006. Genetic diversity and historical demography of Atlantic bigeye tuna (Thunnus obesus). *Molecular phylogenetics and evolution* 39: 404–416.

Muths, D., P. Grewe, C. Jean, and J. Bourjea. 2009. Genetic population structure of the Swordfish (Xiphias gladius) in the southwest Indian Ocean: Sex-biased differentiation, congruency between markers and its incidence in a way of stock assessment. *Fisheries Research* 97: 263–269.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.

Neilson, J. D., J. Loefer, E. D. Prince, F. Royer, B. Calmettes, P. Gaspar, R. Lopez, and I. Andrushchenko. 2014. Seasonal distributions and migrations of Northwest Atlantic swordfish: inferences from integration of pop-up satellite archival tagging studies. *PLoS One* 9: e112736.

O'Leary, D. B., J. Coughlan, E. Dillane, T. V. McCarthy, and T. F. Cross. 2007. Microsatellite variation in cod Gadus morhua throughout its geographic range. *Journal of Fish Biology* 70: 310–335.

Palko, B. J., G. L. Beardsley, and W. J. Richards. 1981. Synopsis of the biology of the swordfish, Xiphias gladius Linnaeus.

Paris, J. R., J. R. Stevens, and J. M. Catchen. 2017. Lost in parameter space: a road map for stacks. *Methods in Ecology and Evolution* 8: 1360–1373.

Pecoraro, C., M. Babbucci, A. Villamor, R. Franch, C. Papetti, B. Leroy, S. Ortega-Garcia, et al. 2016. Methodological assessment of 2b-RAD genotyping technique for population structure inferences in yellowfin tuna (Thunnus albacares). *Marine genomics* 25: 43–48.

Peterson, B. K., J. N. Weber, E. H. Kay, H. S. Fisher, and H. E. Hoekstra. 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PloS one* 7: e37135.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

Puechmaille, S. J. 2016. The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. *Molecular Ecology Resources* 16: 608–627.

Pujolar, J. M., M. I. Roldan, and C. Pla. 2002. A genetic assessment of the population structure of swordfish (Xiphias gladius) in the Mediterranean Sea. *Journal of Experimental Marine Biology and Ecology* 276: 19–29.

Raymond, M., and F. Rousset. 1995. GENEPOP on the Web (Version 3.4). URL: http://wbiomed. curtin. edu. au/genepop/Updated from Raymond & Rousset.

Reeb, C. A. 2010. Genetic discontinuity of big fish in a small sea. *Proceedings of the National Academy of Sciences* 107: 2377–2378.

Reeb, C. A., L. Arcangeli, and B. A. Block. 2003. Development of 11 microsatellite loci for population studies in the swordfish, Xiphias gladius (Teleostei: Scombridae). *Molecular Ecology Notes* 3: 147–149.

Reiss, H., G. Hoarau, M. Dickey-Collas, and W. J. Wolff. 2009. Genetic population structure of marine fish: mismatch between biological and fisheries management units. *Fish and Fisheries* 10: 361–395.

Riccioni, G., G. Ferrara, M. Landi, and M. Sella. 2010. Spatio-temporal genetic patterns in Mediterranean bluefin tuna: population structuring and retention of genetic diversity. *Collect. Vol. Sci. Pap. ICCAT* 65: 996–1003.

Rooker, J. R., D. H. Secor, G. De Metrio, R. Schloesser, B. A. Block, and J. D. Neilson. 2008. Natal homing and connectivity in Atlantic bluefin tuna populations. *Science* 322: 742–744.

Rosel, P. E., and B. A. Block. 1996. Mitochondrial control region variability and global population structure in the swordfish, Xiphias gladius. *Marine Biology* 125: 11–22.

Ruggeri P, Splendiani A, Di Muri C, Fioravanti T, Santojanni A, Leonori I, De Felice A, Biagiotti I, Carpi P, Arneri E. 2016a. Coupling demographic and genetic variability from archived collections of European anchovy (Engraulis encrasicolus). *PloS one* 11:e0151507.

Ruggeri P, Splendiani A, Giovannotti M, Fioravanti T, Occhipinti G, Nisi Cerioni P, Caputo Barucchi V. 2016b. The role of life-history traits, selective pressure and hydrographic boundaries in shaping the genetic structure of the transparent goby, Aphia minuta. *Marine Ecology* 37:518–531.

Ruzzante, D. E., S. Mariani, D. Bekkevold, C. André, H. Mosegaard, L. A. Clausen, T. G. Dahlgren, et al. 2006. Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring. *Proceedings of the Royal Society B: Biological Sciences* 273: 1459–1464.

Ryynänen, H. J., A. Tonteri, A. Vasemägi, and C. R. Primmer. 2007. A comparison of biallelic markers and microsatellites for the estimation of population and conservation genetic parameters in Atlantic salmon (Salmo salar). *Journal of Heredity* 98: 692–704.

Smith, B. L., C.-P. Lu, B. García-Cortés, J. Viñas, S.-Y. Yeh, and J. R. A. Bremer. 2015. Multilocus Bayesian estimates of intra-oceanic genetic differentiation, connectivity, and admixture in Atlantic swordfish (Xiphias gladius L.). *PloS one* 10: e0127979.

Team, Rs. 2015. RStudio: integrated development for R. *RStudio, Inc., Boston, MA URL http://www. rstudio. com* 42: 14.

Tserpes, G., P. Peristeraki, and S. Somarakis. 2001. On the reproduction of swordfish (Xiphias gladius L.) in the eastern Mediterranean. *Col. Vol. Sci. Pap. ICCAT* 52: 740–744.

Tserpes, G., P. Peristeraki, and V. D. Valavanis. 2008. Distribution of swordfish in the eastern Mediterranean, in relation to environmental factors and the species biology. *Hydrobiologia* 612: 241.

Tserpes, G., and N. Tsimenides. 1995. Determination of age and growth of swordfish, Xiphias gladius L., 1758, in the eastern Mediterranean using anal-fin spines. *Fishery Bulletin* 93: 594–602.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2003. Micro-Checker, version 2.2. 3. *Department of Biological Sciences and Department of Computer Science, University of Hull, Hull, UK*.

Van Wagner, C. E., and A. J. Baker. 1990. Association between mitochondrial DNA and morphological evolution in Canada geese. *Journal of Molecular Evolution* 31: 373–382.

Viñas, J., J. A. Bremer, J. Mejuto, J. M. De La Serna, B. García-Cortés, and C. Pla. 2007. Swordfish genetic population structure in the North Atlantic and Mediterranean. *Collect. Vol. Sci. Pap. ICCAT* 61: 99–106.

Vinas, J., A. Pérez-Serra, O. Vidal, J. R. Alvarado Bremer, and C. Pla. 2010. Genetic differentiation between eastern and western Mediterranean swordfish revealed by phylogeographic analysis of the mitochondrial DNA control region. *ICES Journal of Marine Science* 67: 1222–1229.

Waples, R. S., and C. H. I. Do. 2010. Linkage disequilibrium estimates of contemporary Ne using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evolutionary Applications* 3: 244–262.

Waples, R. S., and O. Gaggiotti. 2006. INVITED REVIEW: What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular ecology* 15: 1419–1439.

Ward, R. D. 2000. Genetics in fisheries management. *Hydrobiologia* 420: 191–201.

Ward, R. D., M. Woodwark, and D. O. F. Skibinski. 1994. A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *Journal of fish biology* 44: 213–232.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. *evolution* 38: 1358–1370.

Wenink, P. W., A. J. Baker, and M. G. Tilanus. 1993. Hypervariable-control-region sequences reveal global population structuring in a long-distance migrant shorebird, the Dunlin (Calidris alpina). *Proceedings of the National Academy of Sciences* 90: 94–98.

Williams, S. M., M. B. Bennett, J. G. Pepperell, J. A. Morgan, and J. R. Ovenden. 2016. Spatial genetic subdivision among populations of the highly migratory black marlin Istiompax indica within the central Indo-Pacific. *Marine and Freshwater Research* 67: 1205–1214.

Zardoya, R., R. Castilho, C. Grande, L. Favre-Krey, S. Caetano, S. Marcato, G. Krey, and T. Patarnello. 2004. Differential population structuring of two closely related fish species, the mackerel (Scomber scombrus) and the chub mackerel (Scomber japonicus), in the Mediterranean Sea. *Molecular Ecology* 13: 1785–1798.

3.7 Supplementary materials

Sampling Location		Locus (total number of alleles)																			
		Xgl-35 (14)	Xgl-121 (8)	Xgl-561 (9)	Xgl-94 (6)	Xgl-106 (18)	Xgl-65b (12)	Xgl-74 (7)	Xg-Sau98R1 (14)	Xgl-523b (3)	Xgl-14 (13)	Xg-148b (4)	D2A (4)	D2B (7)	C8 (21)	Xg-394 (3)	Xg-402 (2)	Xg-56 (13)	Xg-66 (7)	Xg-144 (7)	Xg-166 (8)
ADR	N	62	62	62	61	62	62	60	62	61	60	60	62	62	62	62	62	62	62	60	62
	N_A	5	4	6	5	5	7	6	11	2	6	3	3	4	14	3	2	9	6	4	7
	AR	3,2	3,3	4,8	4,9	3,5	6,7	4,4	9,0	2,0	4,8	3,0	3,0	3,2	9,6	2,1	1,4	7,8	4,1	3,4	6,3
	H_o	0,45	0,55	0,45	0,64	0,56	0,68	0,33	0,87	0,23	0,58	0,39	0,37	0,66	0,94	0,10	0,03	0,82	0,23	0,68	0,77
	H_e	0,44	0,53	0,49	0,76	0,57	0,78	0,65	0,87	0,25	0,69	0,39	0,42	0,62	0,89	0,09	0,03	0,79	0,57	0,68	0,79
	F_{IS}	-0,04	-0,04	0,08	0,16	0,02	0,14	**0,49**	0,00	0,10	0,16	0,00	0,11	-0,08	-0,06	-0,03	-0,01	-0,04	**0,60**	-0,01	0,02
SIC	N	60	61	61	61	60	60	60	61	61	60	61	61	61	60	61	61	56	60	61	58
	N_A	5	5	6	5	5	8	4	11	2	6	3	3	4	13	2	2	8	6	3	7
	AR	3,71	3,88	5,15	4,68	4,16	6,19	3,43	9,12	2,00	4,64	2,92	2,90	3,42	9,07	1,25	1,68	6,81	4,97	3,00	5,97
	H_o	0,42	0,54	0,62	0,43	0,72	0,77	0,32	0,85	0,26	0,28	0,33	0,44	0,59	0,82	0,02	0,07	0,77	0,23	0,52	0,66
	H_e	0,45	0,56	0,59	0,73	0,67	0,72	0,61	0,85	0,36	0,62	0,34	0,44	0,61	0,85	0,02	0,06	0,81	0,63	0,66	0,72
	F_{IS}	0,07	0,03	-0,05	**0,42**	-0,08	-0,07	**0,48**	0,00	0,27	**0,55**	0,03	0,00	0,04	0,04		-0,03	0,05	**0,63**	0,20	0,08
GRE	N	20	20	20	20	20	20	19	18	20	20	20	20	20	20	19	19	19	20	20	20
	N_A	4	4	6	5	5	6	6	8	2	6	3	3	3	9	1	1	6	4	3	5
	AR	3,74	3,50	5,47	4,94	4,74	5,97	5,33	7,81	2,00	5,63	2,93	2,94	3,00	8,38	1,00	1,00	5,97	3,69	3,00	4,98
	H_o	0,50	0,50	0,40	0,75	0,70	0,65	0,42	0,83	0,30	0,55	0,25	0,20	0,45	0,85			0,75	0,20	0,60	0,55
	H_e	0,48	0,56	0,50	0,73	0,68	0,80	0,63	0,86	0,33	0,65	0,23	0,27	0,65	0,86			0,74	0,58	0,67	0,72
	Fis	-0,05	0,10	0,20	-0,03	-0,03	0,19	0,34	0,04	0,09	0,16	-0,08	0,27	0,31	0,02			-0,01	**0,66**	0,10	0,24
SPA	N	85	85	85	85	85	85	83	84	85	83	85	85	85	84	85	85	84	84	82	81
	N_A	6	4	7	5	5	7	6	12	2	5	3	3	3	15	1	1	9	6	4	7
	AR	3,79	3,66	5,14	4,69	3,86	6,29	4,81	9,15	2,00	4,21	2,90	2,98	2,99	9,48	1,00	1,00	7,65	4,66	3,18	6,17
	H_o	0,46	0,55	0,67	0,51	0,49	0,65	0,36	0,79	0,31	0,36	0,28	0,47	0,51	0,90			0,76	0,18	0,67	0,75
	H_e	0,45	0,55	0,62	0,74	0,56	0,76	0,69	0,87	0,28	0,59	0,36	0,49	0,60	0,87			0,78	0,63	0,66	0,77
	F_{IS}	-0,03	-0,01	-0,08	**0,32**	0,12	0,14	**0,48**	0,10	-0,11	**0,38**	0,21	0,04	0,15	-0,04			0,03	**0,72**	-0,01	0,02
TIR	N	16	16	16	16	16	16	16	16	16	16	16	15	16	16	16	15	16	16	16	16
	N_A	4	3	5	5	4	6	2	10	3	4	3	3	3	8	2	1	7	4	4	6
	AR	4,00	2,94	4,93	4,94	3,94	5,87	2,00	9,81	3,00	3,94	3,00	3,00	3,00	7,87	1,94	1,00	6,93	3,94	3,94	5,94
	H_o	0,44	0,56	0,56	0,50	0,88	0,44	0,81	0,81	0,31	0,38	0,38	0,60	0,56	0,94	0,06		0,75	0,13	0,50	0,75
	H_e	0,51	0,49	0,51	0,73	0,66	0,64	0,51	0,88	0,37	0,61	0,33	0,47	0,59	0,84	0,06		0,76	0,54	0,69	0,76
	F_{IS}	0,15	-0,16	-0,11	0,33	-0,33	0,32	-0,61	0,08	0,17	0,40	-0,13	-0,30	0,04	-0,12			0,01	**0,78**	0,28	0,01
SAR	N	**54**	54	54	53	54	**54**	**54**	54	54	**52**	54	54	53	52	51	54	**52**	**53**	52	42
	N_A	6	5	7	5	5	7	6	11	2	7	3	3	3	13	1	2	8	4	4	6
	AR	4,18	4,12	5,27	4,81	3,90	6,16	5,04	9,01	2,00	4,49	2,92	2,98	2,99	9,68	1,00	1,48	7,36	3,57	3,29	5,28
	H_o	0,43	0,50	0,57	0,60	0,63	0,61	0,39	0,89	0,20	0,31	0,28	0,37	0,53	0,81		0,04	0,85	0,15	0,56	0,38
	H_e	0,41	0,61	0,58	0,73	0,63	0,74	0,70	0,87	0,29	0,52	0,32	0,43	0,59	0,86		0,04	0,78	0,59	0,64	0,71
	F_{IS}	-0,05	0,18	0,01	0,17	0,00	0,17	**0,45**	-0,02	0,31	**0,41**	0,14	0,14	0,11	0,06		-0,01	-0,09	**0,74**	0,14	**0,47**
CAN	N	25	25	25	25	25	25	25	25	25	22	25	25	25	25	25	25	25	23	25	25
	N_A	13	6	8	6	15	11	7	12	2	12	4	4	7	17	1	1	13	5	6	7
	AR	11,05	5,60	7,42	5,53	12,32	9,16	6,44	11,24	2,00	10,25	3,59	3,60	5,65	14,32	1,00	1,00	11,24	4,88	5,53	6,36
	H_o	0,84	0,68	0,72	0,68	0,92	0,80	0,36	0,88	0,56	0,68	0,48	0,64	0,76	0,80			0,76	0,35	0,64	0,60
	H_e	0,90	0,79	0,82	0,76	0,92	0,84	0,81	0,92	0,51	0,86	0,43	0,58	0,71	0,94			0,90	0,75	0,72	0,72
	F_{IS}	0,06	0,14	0,12	0,11	0,00	0,05	**0,56**	0,05	-0,10	0,21	-0,12	-0,11	-0,07	0,15			0,16	**0,54**	0,12	0,17

Table SM 3.1 Summary statistics of 20 microsatellite loci for each sampling locality. N number of genotyped individuals, N_A number of alleles, AR allelic richness, H_o observed and H_e expected heterozygotes, F_{IS} deviations from Hardy-Weinberg equilibrium (bold identified significand deviation after Bonferroni correction).

Table SM 3.2 Summary statistics of RADseq data loci for each sampling locality. N number of genotyped individuals, AR allelic richness, H_o observed and H_e expected heterozygotes, F_{IS} deviations from Hardy-Weinberg equilibrium.

Sampling locality	N	AR	Ho	He	Fis
ADR	11	1,140	0,139	0,143	0,022
GRE	5	1,136	0,141	0,144	0,003
SIC	22	1,142	0,139	0,144	0,032
SPA	15	1,140	0,151	0,143	-0,033
SAR	7	1,140	0,139	0,146	0,029
CAN	23	1,154	0,145	0,156	0,072

Figure SM 3.1 1 a) Plot of Delta K and Mean LnP(K) according to K. b) Structure clustering results obtained at K=2, based on microsatellite data from the same 83 individuals of RADseq dataset. Barplots showing posterior probabilities of swordfish individual genotypes (as bars) assigned to each population. The black lines separate sampling localities.

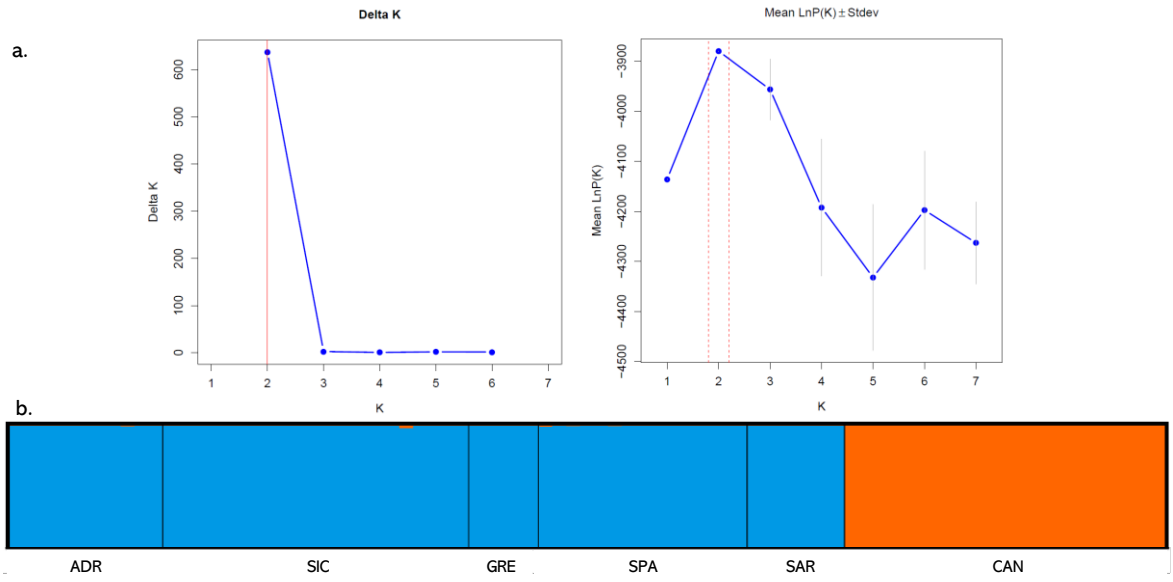
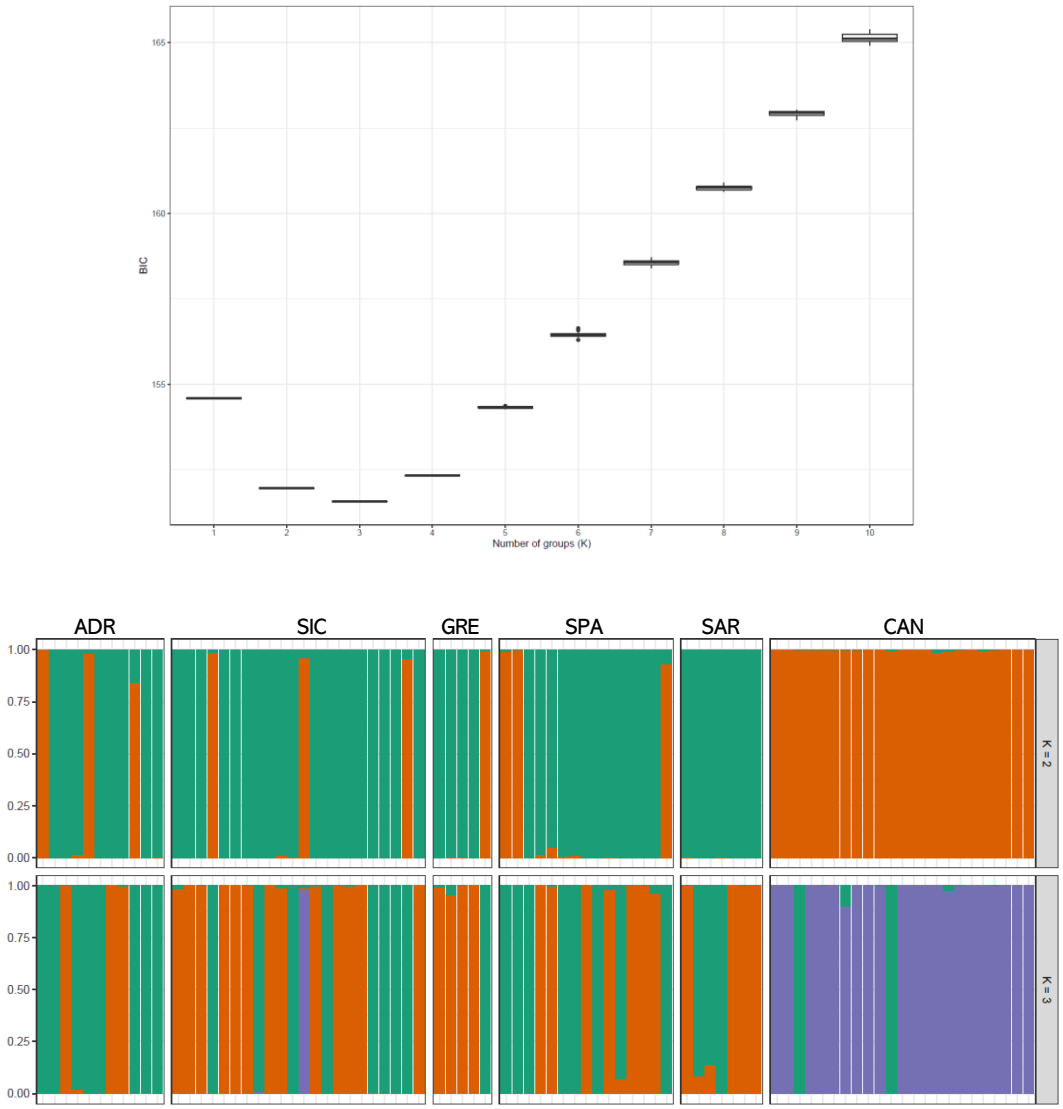


Figure SM 3.2 Discriminant analysis of principal components (DAPC) dased on microsatellite data from the same 83 individuals of RADseq dataset. a) Inference of the number of clusters in the DAPC. c) DAPC Barplot of group membership probabilities for K=2 and K=3. Each individual is represented as a vertical bar, with colours corresponding to probabilities of membership into the clusters identified according to BIC values.



4 LOSS OF MITOCHONDRIAL GENETIC DIVERSITY IN OVEREXPLOITED MEDITERRANEAN SWORDFISH (*XIPHIAS GLADIUS, 1759)* POPULATION

4.1 Introduction

The swordfish (*Xiphias gladius*) is a large migratory fish found in the open waters of all oceans, including the Mediterranean Sea (Palko, Beardsley & Richards, 1981). Mediterranean swordfish consist of a population genetically and biologically distinct from Atlantic ones. Genetic differentiation (Bremer et al., 2007), as well as several differences in biological parameters (Neilson et al., 2013), have been used to manage the Atlantic and Mediterranean swordfish as separated stocks (Anon., 2019). The Mediterranean population showed lowest levels of genetic variation compared to any other population worldwide (Bremer, Baker & Mejuto, 1995; Bremer et al., 1996, 2005; Rosel & Block, 1996; Pujolar, Roldan & Pla, 2002). According to Bremer et al. (2005), the reduced level of variation in Mediterranean swordfish mtDNA is congruent with multiple faunal extinctions and subsequent re-colonization events occurred during the Pleistocene. However, according to Kotoulas et al. (2007), the low genetic variability observed using nuclear markers could be related to the small effective population size of Mediterranean swordfish, probably caused by fishing pressure (Pujolar, Roldan & Pla, 2002). Moreover, according to Arocha (2007), prolonged exploitation of swordfish in the Mediterranean area may have reduced the numbers of older specimens and

consequently explain the lower mean size of the catches and lower size of maturation observed for the Mediterranean population.

Swordfish is a high commercial value species, especially in the Mediterranean Sea, where despite this basin represents less than 10% of the swordfish global range, catch levels are relatively high and similar to those of larger areas such as the North Atlantic (Collette et al., 2011). Some characteristics as the presence of a larger spawning area in relation to the area of distribution of the stock, lower abundance of predators and higher recruitment were suggested as the determining factors explaining the higher abundance of swordfish in the Mediterranean Sea. However, in the last forty years, the swordfish stock of the Mediterranean Sea has been overfished and, to date, is still subject to overfishing (Anon., 2019). The Spawning Stock Biomass (SSB) estimated represents less than 15% of the maximum sustainable yield (B_{MSY}) estimated and the fish mortality caused by harvesting (F) is almost twice the maximum sustainable yield. The 50-70% of the total yearly catches is represented by fish of small size often less than 3 years old, with a high level of immature swordfish reported (Anon., 2019).

The loss of genetic variation caused by population reduction is an important concern in fishery management. Intense and prolonged mortality caused by overexploitation could drive the decay of genetic diversity across a wide range of marine fishes (Pinsky & Palumbi, 2014). Genetic diversity has been defined as the variety of alleles and genotypes present in a population and that is reflected in morphological, physiological

and behavioural differences between individuals and populations (Frankham, 2005). The reduction of genetic diversity may lead to decrease species resilience to environmental changes, thus increasing their extinction risk (Spielman, Brook & Frankham, 2004; Frankham, 2005). Small and isolated populations are more vulnerable to genetic degradation (Frankham, 2005). Moreover, in marine fishes, effective population size (Ne) can be several orders of magnitude smaller than census size (N) (Hauser et al., 2002; Turner, Wares & Gold, 2002; Hutchinson et al., 2003) and species may suffer a loss of genetic diversity under fishing pressure despite large census size.

The most powerful way to detect loss of genetic variation in exploited populations is the examination of genetic samples collected over time (Allendorf et al., 2008). In this study, a dataset of available mitochondrial sequences of Mediterranean swordfish sampled in the 1990s and early 2000s has been compared with homologous mtDNA data obtained from current samples (2016-2018). This comparison was carried out in order to investigate if a temporal genetic variation occurred in the Mediterranean swordfish, where this species is considered endangered and overexploited.

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4.2 Materials and Methods

4.2.1 Sampling and DNA extraction

A total of 287 swordfish were collected from six areas within the eastern, central and western Mediterranean regions, trying to obtain a representative coverage of the basins (For details see Table 4.1). Samples were achieved at the fishing landing of commercial longline or from trap bycatch (only in case of Sardinian samples) from May to October in three years 2016-2018. For each sample, a piece of the caudal fin was collected and was stored in ethanol absolute and kept at -20°C until DNA extraction. Total genomic DNA was extracted using specific cartridge 401 in the *MagCore*® automated Nucleic Acid extractor (*MagCore* ®, *Genomic DNA Tissue Kit, n° 401*) following the specific protocol. Historical mitochondrial data were obtained from a re-analysis of the dataset available from Viñas (2010). That work included specimens sampled in several Mediterranean areas in the 90s and early 2000s (See Viñas et al., 2010 for samples details), allowing us to evaluate the mitochondrial genetic variability between two complementary sample groups of Mediterranean swordfish over a 20-year period.

Table 4.1 Sampling details for swordfish analysed in this study

Sampling area (FAO fishing area/Geographical subarea)	Sample ID	Sampling date	n
Balearic Sea (GSA 5, 6)	SPA	07/16-08/16-09/16 09/19	84
Southern Sicily (GSA 15, 16)	SIC	07/16 06/17-07/17 06/18	59
Aegean Sea (GSA 22)	GRE	08/16	17
Southern Adriatic Sea (GSA 18)	ADR	09/16	62
Tyrrhenian Sea (GSA 10)	TIR	05/2017	16
Sardinian Sea (GSA 11.2)	SAR	06/17-10/17 05/18-06/18	49
Total			287

4.2.2 Mitochondrial DNA amplification and analysis

A PCR-SSCP analysis was performed to screen mitochondrial genetic variability. A 360 bp long portion of the hypervariable L-domain of the mitochondrial control region was amplified using primers L15998 (5'-TAC CCC AAA CTC CCA AAG CTA-3') (Bremer et al., 1996) and SWO 5' CCC TGT GAA ATA TGC TGG TTG 3' (designed in this study). The amplification was carried out in 25 µl reaction volume containing: 1X MyTaq reaction buffer (BioLine), 10 pmol of each primer, 0.5 U of BIOTAQ DNA-Polymerase (BioLine) and approximately 80 ng of the isolated DNA. For thermal cycler has been followed Viñas et al. (Viñas et al., 2004). 5 µl amplicon was added to 4 µl loading buffer (98% formamide, 10 mM, EDTA (0,5 M, pH 8), 0.05% bromophenol

blue, 0.05% xylene cyanol), heated to 95 °C for 5 min and immediately chilled on ice. Vertical electrophoresis run was performed in a nondenaturing polyacrylamide gel (8% acrylamide/polyacrylamide [49:1], 10% glycerol) at 5 W for 12 hours at room temperature, with 1 × TBE as the running buffer. Sanger sequencing of *D-loop* was performed on a subsample for each SSCP pattern on an Applied Biosystems ABI 3730XL DNA. Sequences were aligned using ClustalW (Thompson, Gibson & Higgins, 2003) and checked by eye in BioEdit (Hall, 1999). The number of haplotypes (*N*), and polymorphic site, haplotypic (*h*) and nucleotide diversities (π) were computed in Arlequin v. 3.5 (Excoffier & Lischer, 2010). The analysis was performed for both dataset pooling Clades I and II together, and for each clade separately. To account for the sampling variance of haplotypic richness, rarefaction curves (Heck Jr, van Belle & Simberloff, 1975) were generated for both samples. Rarefaction curves were generated using iNEXT (Chao, Ma & Hsieh, 2016). Median-joining (MJ) networks (Bandelt, Forster & Röhl, 1999) for the entire dataset were built using the software Network v 4.510, using the default settings.

The extent of genetic differentiation between historical and contemporary dataset was investigated with an analysis of molecular variance, AMOVA (Excoffier, Smouse & Quattro, 1992), and modified exact tests of population differentiation (Excoffier & Lischer, 2010). The AMOVA significance levels were determined by 10,000 permutations in Arlequin. The exact tests implemented in Arlequin was conducted by

using 10,000 Markov chain steps and 1,000 dememorization steps. Finally, temporal changes in the relative effective number of females (N_{ef}) was estimated comparing θ_s for each dataset. The θ_s is expected to be equal to 2 $N_{ef}\mu$ for neutral mutations in mtDNA, where μ is the mutation rate per generation and N_{ef} is the effective population size of females. Considering μ constant between two temporal samples, we can treat differences in θ_s among samples as differences in relative female effective population sizes. For the comparison, the Watterson's estimator θ_s (Theta-W), which is based on the number of segregating sites (S) (Watterson, 1975) was calculated in DnaSP (Rozas et al., 2017) for each dataset.

4.3 Results

The SSCP analysis of 360 bp portion of mtDNA D-loop from 287 contemporary Mediterranean swordfish revealed 36 different morphs. For each morph, a subsample has been sequenced for a total of 172 swordfish. The sequence length ranged from 290 to 297 bp due to the presence of 1–3 tandemly repeated copies of the motif TACA near the 5 'end. Sequence alignment revealed 49 polymorphic sites, defining 36 distinct haplotypes (Table 4.2). According to Alvarado-Bremer (1996) the mtDNA haplotypes of swordfish clustered into two highly divergent clades, Clade I and Clade II. The MJ network (Figure 4.1) clearly identified the two mtDNA clades. Clade I included four centroids (Genbank n. AY650768, AY650763, AY650778, AY650781) represented respectively by 61, 55, 14 and 25 fish, while Clade II was characterized by a single star-like formation featuring one major centroid (AY650762) with a total of 73 individuals. These five centroids haplotypes were observed with high frequencies in all Mediterranean localities investigated in this study, as already observed in previous works (Bremer et al., 2005; Viñas et al., 2010). The other haplotypes, instead, were represented in very few numbers of the individual often representing singletons. In this study seventeen, previously unidentified haplotypes were sampled. Fifteen of which belong to Clade I and two belong to Clade II and all were observed at low frequencies (Table 4.2). Sequences are available from the NCBI database with accession numbers MN652595-MN652611.

Table 4.2 Variable sites in the control-region segment of the 36 haplotypes of swordfish mtdna detected in modern sample. Numbers above sites refer to nucleotide position on the L-strand of the control region of the swordfish. Identity with sequence 1 is indicated by dots, and deletions of 1 residue with dashes. Frequences observed have been reported for each sample locality.

Genbank accession number	1 1 1 1 2 2 2 2 2 4 6 6 7 8 9 9 0 1 1 1 2 2 2 2 2 2 2 3 3 3 3 5 5 6 6 6 6 7 7 7 8 8 8 9 9 1 7 6 2 9 2 7 8 9 0 1 2 3 4 6 0 7 7 3 1 2 1 0 1 8 0 2 3 5 6 7 2 4 5 8 8 9 0 1 5 6 2 3 8 0 6 7 0 4 7 5 2	CLADE	ADR	GRE	SIC	SPA	TIR	SAR
AY650768	G T A T A C A - - - - C A A C A T T A G T T A A C T G T T A T C A T T T A A A T C C - T G G T A G	I	13	4	16	15	3	10
MN652597	. . . . . T A C A . . . . . C . . . . . T . . . . . -	I	1	0	0	0	0	0
AY650778	. . . . . C . . . . . T . . . . . -	I	2	2	2	6	1	1
AY650860	. . . . . C . . . . . T G . . . . . -	I	0	0	1	0	0	0
MN652598	A . . . . . -	I	1	0	1	0	0	0
MN652599	. . . . . G . . . . . -	I	0	0	1	1	0	0
EU827759	. . . . . G . . . . . -	I	0	0	0	2	0	0
MN652600	. . . . . T A C A . . . . . G . . . . . -	I	0	0	0	1	0	0
MN652601	. . . . . C . . . . . G . . . . . -	I	0	0	0	0	0	1
AY650836	. . . . . G . . . . . -	I	0	0	3	1	1	0
AY650821	. . . . . C . . . . . -	I	0	0	0	1	1	0
MN652602	. . . . . C . . . . . -	I	1	0	0	0	0	0
AY650858	. . . . . G . . . . . -	I	0	0	0	0	0	1
MN652603	. . . . . G . . . . . -	I	0	0	0	2	0	0
MN652604	. . . . . C . . T . . . . . -	I	0	0	0	0	0	1
MN652605	. . . . . T . . . . . -	I	0	0	0	1	0	0
MN652606	. . . . . G . . . . . -	I	2	0	0	0	0	0
MN652607	. . . . . T . . . . . -	I	0	1	0	0	0	0
MN652608	. - . . . . . C . . T . . . . . G T - . A . . .	I	0	0	0	1	0	0
AY650781	. . . . . C . . T . . . . . G T - . A . . .	I	8	2	6	6	1	2
MN652609	. . . . . A . C . . T . . . . . G T - . A . . .	I	2	0	0	1	0	0
AY650763	. . . . . T . . . . . T . A . C . . T . . . . . C . . - . A . . . A	I	10	4	15	14	0	12
MN652595	. . . . . G . T . . . . . T . A . C . . T . . . . . C . . - . A . . . A	I	0	0	1	1	0	0
AY650855	. . . . . T A C A . . . T . . . . . T . A . C . . T . . . . . C . . - . A . C . A	I	0	1	0	0	0	0
MN652596	. . . . . T . . . . . T . A . C . . T . . . . . C . . - . . . . . A	I	0	0	0	0	1	0
AY650861	. . . . . T . . . . . T . A . C . . T . . . . . G C . . -	I	0	0	0	0	0	2
AY650809	. . . . . T . . T . . . T C . . G G . . A C C G . T G . C . . . . . T C . . . G A	II	1	0	0	1	0	1
MN652610	. . . . . T . . T . . . T C . . G G . . A C C G . T G . C . . . . . T C C . . . G A	II	0	0	0	1	0	0
AY650762	. . . . . T . . T . . . T C . . G G . . A C C G . T G . C . . . . . T C . . A . G A	II	16	2	12	24	2	17
AY650814	. . . . . T . . T . . . T C . . G G . . A C C G . T G . C C . . . . T C . . A . G A	II	2	0	0	1	0	0
AY650805	. . . . . T . . T . . . T C . . G G . . A C C G . T G . C . . G . . . T C . . A . G A	II	0	0	0	2	1	0
AY650761	. . . . . T . . T . . C T C . . G G . . A C C G . T G . C . . . . . T C . . A . G A	II	0	1	0	1	0	0
AY650829	. . . . . T . . T . . . T T . . G G . . A C C G . T G . C . . . . . T C . . A . G A	II	0	0	1	0	1	0
EU827771	. . . . . T . . T . . . T C . . G G . . A . C G C T G . C . . . . . T C . . A . G A	II	3	0	0	0	2	1
MN652611	. . . . . T . . T . C . T C . . G G . . A C C G . T G . C . . . . . T C . . A . . A	II	0	0	0	1	0	0
EU827787	. . . . . T . . T . . . T T . . G G . . A C C G . T G . C . . . . . T C . . A . G .	II	0	0	0	0	2	0

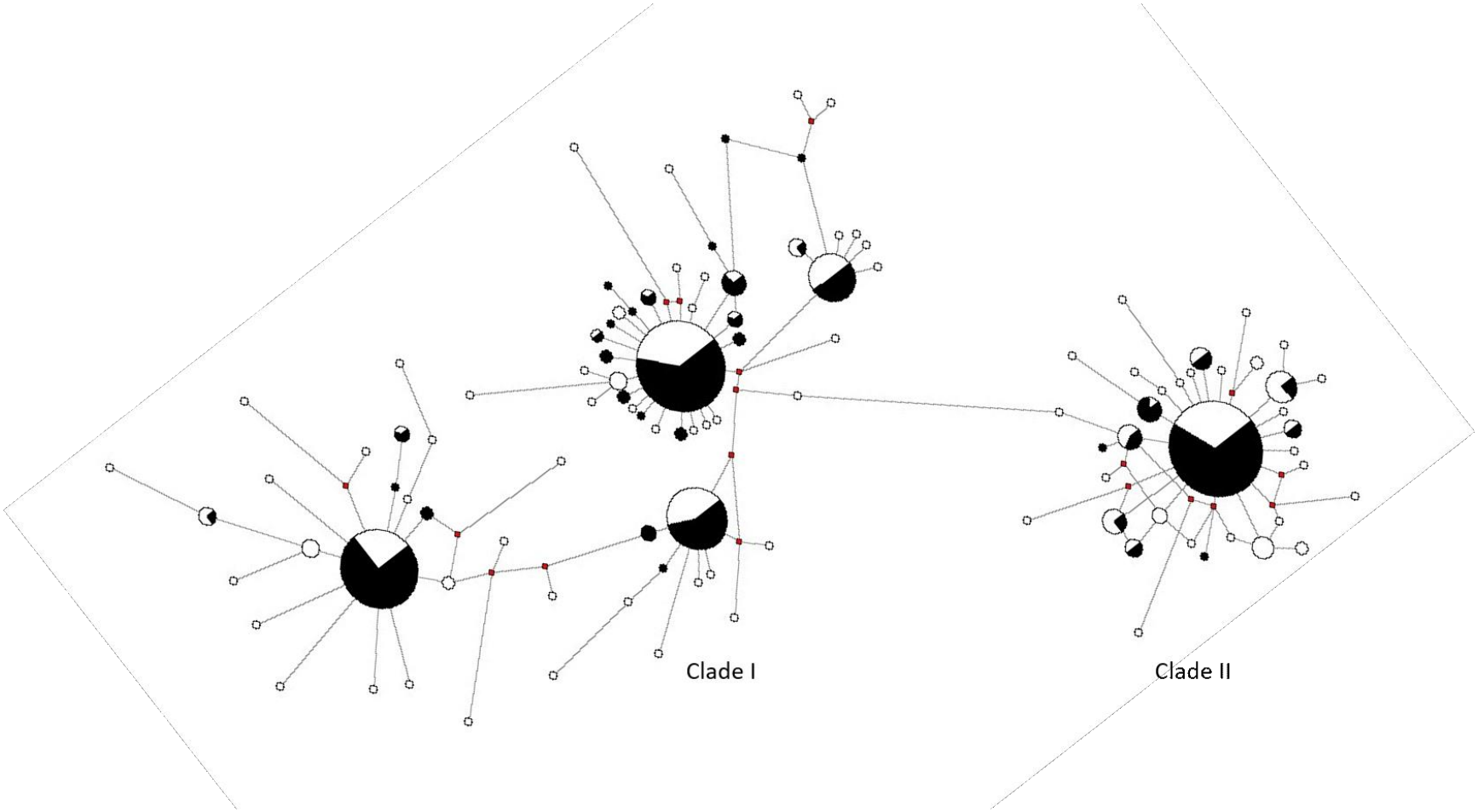

Figure 4.1 MJ network for the complete dataset of Mediterranean swordfish. White slices indicate haplotype observed by Viñas (2010) and black slices recent sample (this work). Small red circles correspond to missing (or hypothetical) haplotypes.

Comparison among historical dataset from Viñas (2010) and the present dataset, respectively composed by 251 and 287 swordfish, revealed the same percentage of individuals belonging to Clade I (about 66 %) and Clade II (about 33 %). However, datasets comparison indicates a reduction in the number of haplotypes from 93 in the historical to 36 in the contemporary samples (Table 4.3). Confirmed by the rarefaction curve that reported the highest haplotype richness values in the older sample (Figure 4.2). Considering the two clades separately, the observed haplotype number decreased from 60 to 26 for Clade I and from 33 to 10 for Clade II. Nineteen haplotypes were shared between datasets (11 for Clade I and 8 for Clade II). Moreover, in the modern sample, a shift towards the fixation of the main five haplotypes (i.e., centroids) was also observed (Table SM 4.1).

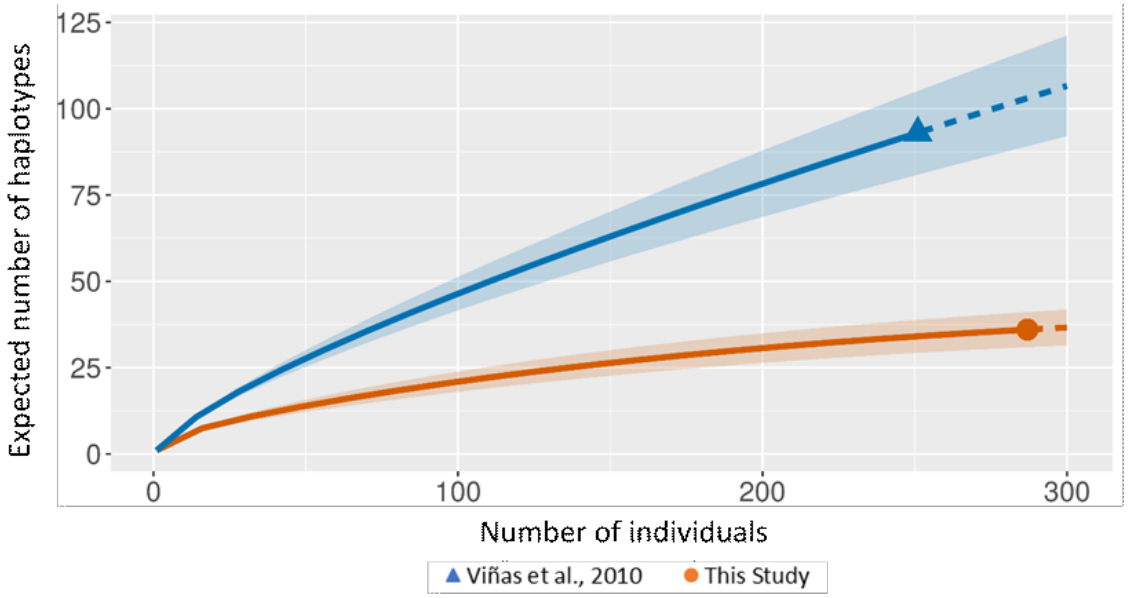

Figure 4.2 Rarefaction curves for Mediterranean swordfish temporal sample groups. Distinct differences in the expected number of haplotypes for ancient (blue) and contemporary (orange) samples.

A reduction of haplotype diversity was observed between historical (h = 0.947 $\pm$ 0.007 s.d.) and modern datasets (h = 0.844 $\pm$ 0.01 s.d.). Haplotype diversity decrease was more evident considering the two clades separately. For Clade I the diversity in historical samples (h = 0.91) was greater than the contemporary (h = 0.793) as well as for Clade II where diversity was h = 0.861 in the historical sample and h = 0.418 in the modern sample. Levels of nucleotide diversity were similar throughout the two databases. All estimates of haplotype and nucleotide diversity are given in table 4.3.

Table 4.3 Summary of molecular diversity indices for Mediterranean swordfish temporal samples for pooled clades and for each clade separately. The number of individuals (N) and haplotypes (H), the haplotype diversity (h), the nucleotide diversity (π), Watterson's estimator θ_s and the standard deviations (s.d.) are showed.

	N	H	*h*	*s.d.*	π	s.d.	θs	s.d.
Viñas et al., 2010								
Clade I + II	251	93	0.946	0.007	0.160	0.080	0.046	0.012
Clade I	156	60	0.910	0.014	0.034	0.020	0.038	0.010
Clade II	95	33	0.861	0.031	0.006	0.004	0.019	0.006
This study								
Clade I + II	286	36	0.844	0.010	0.130	0.070	0.022	0.006
Clade I	190	26	0.793	0.018	0.029	0.015	0.014	0.004
Clade II	96	10	0.418	0.063	0.003	0.002	0.008	0.003

The AMOVA analysis identifies significant genetic differentiation between older and recent dataset. Low levels of differentiation ($F_{ST} = 0.018, p = 0.000$) were detected between samples pooling clades and considering Clade I singularly, while historical and modern sample were moderately differentiated ($F_{ST} = 0.121, p = 0.000$) considering only Clade II (table 4.4). The older sample significantly differed from the modern one when compared through an exact test of sample differentiation. The comparison of the θ_s values showed a reduction of the relative females' effective population size in the recent sample ($\theta_s = 0.022$, S.D. 0.005) compared to the historical one ($\theta_s = 0.046$, S.D. 0.01) (table 4.3).

Table 4.4 AMOVA results of Mediterranean swordfish temporal sample comparison.

	Variance component	Percentage of variation	Fixation index (Φ_{ST})	*p value*
Whole dataset				
Among temporal samples	0.008	1.77	0.0177	0.0000
Within samples	0.446	98.23		
Clade I				
Among temporal samples	0.008	1.85	0.0184	0.0005
Within samples	0.423	98.15		
Clade II				
Among temporal samples	0.044	12.10	0.121	0.0000
Within samples	0.319	87.90		

4.4 Discussion

In this study, a temporal approach has been used to study recent changes in mitochondrial genetic diversity in the threatened Mediterranean swordfish. Comparison between temporal samples allows us to identify short-term changes that happened in the last few decades. Our results revealed that Mediterranean swordfish has undergone genetic depletion during the last twenty years.

The number of mitochondrial haplotypes observed in the recent sample (36 in total; 26 Clade I and 10 Clade II) was extremely lower compared with the very similar older dataset (Viñas et al., 2010) and a previous study (Bremer et al., 2005). The temporal comparison based on the mtDNA genetic variability highlighted as approximately one-third of the haplotypes was lost in the last twenty years. This reduction was noticeable as a lower haplotype diversity observed on modern samples (h = 0.84, this study) respect to those previously observed (h = 0.94) (Bremer et al., 2005; Viñas et al., 2010). When the two mitochondrial clades were analysed separately the reduction of genetic diversity was not uniformly distributed between clades and the reduction in genetic diversity was more evident for the Clade II. Diversity comparison indicates approximately half less haplotype diversity in the contemporary Clade II samples than in the historical. Genetic differentiation between contemporary and historical swordfish was corroborated by both AMOVA and exact test results (Table 4.4).

The reduction in haplotype diversity with an increment in the proportion of identical haplotypes and the loss of rare haplotypes observed in current Mediterranean swordfish suggest that population has undergone a reduction (Allendorf et al., 2008). As far as it's known, nowadays, the Mediterranean swordfish stock is considered threatened by overfishing (Anon., 2019). In fact, the spawning stock biomass (SSB) estimated is less than 15% of the maximum sustainable yield (B_{MSY}). From 2007-2010 the mesopelagic longline was introduced in Mediterranean swordfish fishery, substituting the traditional surface longline (Anon., 2019). The two types of gears differ mainly for the depth of displacement, with the mesopelagic reaching deeper depths (50-800 m) than the traditional longline (15-60 m). Increment in fish size catch using the mesopelagic longline has revealed the presence of a bulk of large spawners, that found their refuge in the deep (Garibaldi, 2015). However, after only six years the mean swordfish size exploited shifted towards smaller sizes, in a situation very similar to that recorded in the past for surface driftnets and surface longline (Garibaldi & Lanteri, 2017). Thus, is possible to assume that the mesopelagic gear catching bigger swordfish may have increased the impact on adults, affecting the spawning stock (Bertolino et al., 2015; Garibaldi, 2015; Garibaldi & Lanteri, 2017). Specifically, mtDNA is maternally inherited therefore, the analysis of mtDNA variability reflects only the history of the Mediterranean swordfish female portion. Swordfish females reach larger sizes than males, therefore an increase in the average size may be related to an increase in the

number of mature females exploited. Furthermore, females mature later than males, at larger age and size (Arocha, 2007; Neilson et al., 2013). The high percentage (50-70%) of small fish, often still immature, reported by the annual catch estimates, may affect the number of recruited new females in the spawning stock. Comparison of θ_s values supports the scenario of a recent population size decrease in Mediterranean swordfish stock, suggesting a reduction in relative effective female population size (Table 4.3). Thus, the fishery may have altered the sex ratio reducing the number of females in the breeding population, therefore leading to the loss in mitochondrial diversity.

Overexploitation and environmental degradation were identified as the main causes of reductions in stocks and of extinction of marine species (Dulvy, Sadovy & Reynolds, 2003; Dulvy et al., 2006). Human activities may reduce the genetic variability of the population in an extremely short time period (Kenchington, 2003). Overexploitation has been related to a decline in genetic diversity across a wide range of marine fish species (Pinsky & Palumbi, 2014). The authors compare neutral genetic diversity in harvested and unharvested fish stocks over 140 species, found it to be lower in the exploited than in the non-harvest stocks. However, a limited number of studies have assessed the temporal reduction of effective population size (N_e) and the loss of genetic variation related to harvesting due to lack of historical data as well as samples. Some of these studies, using DNA extracted from archived otoliths or scales, found a loss of neutral genetic diversity in some fish species. A significant reduction in genetic diversity was

described in New Zealand *Hoplostethus atlanticus* over only 6 years, during which time the exploitation reduced the biomass by 70% (Smith, Francis & McVeagh, 1991). Marked genetic changes were also detected using microsatellite in the *Gadus morhua* Flamborough Head population across a period during which the population exhibited a decline in SSB related to high levels of exploitation (Hutchinson et al., 2003). Moreover, Hauser (2002) detected a significant decline in genetic diversity in a New Zealand snapper population over the 50 years since the onset of exploitation. The number of fish in a population (census population size, N) is often much larger than the fish that are reproducing and helping to maintain genetic diversity (genetically effective population size, N_e). Hauser (2002) using the $\hat{N}_e/\hat{N}$ estimate in snapper suggested that fish stocks of several million individuals may be in danger of losing genetic variability in the long term. Considering that, globally 31% of fish populations are fished unsustainably, with an additional 60% fully fished (FAO, 2016), fishing may have already caused a considerable loss of overall biodiversity. This study reports the first direct measure of reduced genetic diversity for Mediterranean swordfish during a short period, as measured both in the direct loss of mitochondrial haplotypes and reductions in haplotype diversity. Genetic changes observed in this study suggest that reduction in the SSB of Mediterranean swordfish is underlain by significant changes in genetic composition. The significant loss of mitochondrial diversity in Mediterranean swordfish over a short time period is alarming because the rapid loss of genetic diversity has been shown to have

harmful effects. Reduction in genetic diversity, coupled with the low population size (Kotoulas et al., 2007) and reproductive isolation (Neilson et al., 2013), can disfavour Mediterranean swordfish recovery, may result in genetic drift and inbreeding depression and reduction in fitness limiting long-term adaptability (Spielman, Brook & Frankham, 2004; Frankham, 2005), particularly if abundance remains low and diversity continues to decay (Pinsky & Palumbi, 2014). This result underlined the urgent necessity to re-evaluate the management strategies of the swordfish in the Mediterranean Sea.

4.5 Reference

Allendorf, F. W., P. R. England, G. Luikart, P. A. Ritchie, and N. Ryman. 2008. Genetic effects of harvest on wild animal populations. *Trends in ecology & evolution* 23: 327–337.

Anon. 2019. Report for biennial period, 2018-19 PART I (2018) - Vol. 2. International Commission for the Conservation of Atlantic Tunas, Madrid, Spain.

Arocha, F. 2007. Swordfish Reproduction in the Atlantic Ocean: An Overview. *Gulf and Caribbean Research* 19.

Bandelt, H.-J., P. Forster, and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular biology and evolution* 16: 37–48.

Bertolino, F., C. Camolese, M. Dell'Aquila, A. Mariani, and M. Valastro. 2015. Swordfish (Xiphias gladius l.) fisheries using mesopelagic longline in the Mediterranean Sea by Italian fishing fleet. *Collect. Vol. Sci. Pap. ICCAT* 71: 2073–2078.

Bremer, J. A., J. Mejuto, J. Gómez-Márquez, F. Boán, P. Carpintero, J. M. Rodríguez, J. Viñas, et al. 2005. Hierarchical analyses of genetic variation of samples from breeding and feeding grounds confirm the genetic partitioning of northwest Atlantic and South Atlantic populations of swordfish (Xiphias gladius L.). *Journal of Experimental Marine Biology and Ecology* 327: 167–182.

Bremer, J. A., J. Mejuto, J. Gómez-Márquez, C. Pla-Zanuy, J. Viñas, C. Marques, F. Hazin, et al. 2007. Genetic population structure of Atlantic swordfish: current status and future directions. *ICCAT Collect. Vol. Sci. Pap.* 61: 107–118.

Bremer, J. R. A., A. J. Baker, and J. Mejuto. 1995. Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (Xiphias gladius) populations in the Atlantic Ocean. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 1720–1732.

Bremer, J. R. A., J. Mejuto, T. W. Greig, and B. Ely. 1996. Global population structure of the swordfish (Xiphias gladius L.) as revealed by analysis of the mitochondrial DNA control region. *Journal of Experimental Marine Biology and Ecology* 197: 295–310.

Bremer, J. R. A., J. Viñas, J. Mejuto, B. Ely, and C. Pla. 2005. Comparative phylogeography of Atlantic bluefin tuna and swordfish: the combined effects of vicariance, secondary contact, introgression, and population expansion on the regional

phylogenies of two highly migratory pelagic fishes. *Molecular phylogenetics and evolution* 36: 169–187.

Chao, A., K. H. Ma, and T. C. Hsieh. 2016. User's Guide for iNEXT Online: Software for Interpolation and Extrapolation of Species Diversity. User's Guide for iNEXT Online: Software for Interpolation and Extrapolation ….

Collette, B., A. Acero, A. F. Amorim, K. Bizsel, A. Boustany, C. C. Ramirez, G. Cardenas, et al. 2011. Xiphias gladius. IUCN.

Dulvy, N. K., Y. Sadovy, and J. D. Reynolds. 2003. Extinction vulnerability in marine populations. *Fish and fisheries* 4: 25–64.

Excoffier, L., and H. E. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular ecology resources* 10: 564–567.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.

FAO, The State of World Fisheries and Aquaculture 2016, Contributing to Food Security and Nutrition For All (FAO, 2016)

Frankham, R. 2005. Genetics and extinction. *Biological conservation* 126: 131–140.

Garibaldi, F. 2015. Effects of the introduction of the mesopelagic longline on catches and size structure of swordfish in the Ligurian sea (western Mediterranean). *Collect. Vol. Sci. Pap. ICCAT* 71: 2006–2014.

Garibaldi, F., and L. Lanteri. 2017. Notes about a tagged/recaptured swordfish in the Ligurian Sea (western Mediterranean). *Collective Volume of Scientific Papers, ICCAT* 74: 1354–1361.

Gomez-Uchida, D., and M. A. Banks. 2006. Estimation of effective population size for the long-lived darkblotched rockfish Sebastes crameri. *Journal of Heredity* 97: 603–606.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series, 95–98. [London]: Information Retrieval Ltd., c1979-c2000.

Hauser, L., G. J. Adcock, P. J. Smith, J. H. B. Ramírez, and G. R. Carvalho. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (Pagrus auratus). *Proceedings of the National Academy of Sciences* 99: 11742–11747.

Hutchinson, W. F., C. van Oosterhout, S. I. Rogers, and G. R. Carvalho. 2003. Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (Gadus morhua). *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270: 2125–2132.

Kotoulas, G., J. Mejuto, A. Antoniou, P. Kasapidis, G. Tserpes, C. Piccinetti, P. Peristeraki, et al. 2007. Global genetic structure of swordfish (Xiphias gladius) as revealed by microsatellite DNA markers. *ICCAT Coll Vol Sci Pap* 61: 79–88.

Neilson, J., F. Arocha, S. Cass-Calay, J. Mejuto, M. Ortiz, G. Scott, C. Smith, et al. 2013. The recovery of Atlantic swordfish: the comparative roles of the regional fisheries management organization and species biology. *Reviews in Fisheries Science* 21: 59–97.

Palko, B. J., G. L. Beardsley, and W. J. Richards. 1981. Synopsis of the biology of the swordfish, Xiphias gladius Linnaeus.

Pinsky, M. L., and S. R. Palumbi. 2014. Meta-analysis reveals lower genetic diversity in overfished populations. *Molecular Ecology* 23: 29–39.

Pujolar, J. M., M. I. Roldan, and C. Pla. 2002. A genetic assessment of the population structure of swordfish (Xiphias gladius) in the Mediterranean Sea. *Journal of Experimental Marine Biology and Ecology* 276: 19–29.

Rosel, P. E., and B. A. Block. 1996. Mitochondrial control region variability and global population structure in the swordfish, Xiphias gladius. *Marine Biology* 125: 11–22.

Rozas, J., A. Ferrer-Mata, J. C. Sánchez-DelBarrio, S. Guirao-Rico, P. Librado, S. E. Ramos-Onsins, and A. Sánchez-Gracia. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular biology and evolution* 34: 3299–3302.

Smith, P. J., R. Francis, and M. McVeagh. 1991. Loss of genetic diversity due to fishing pressure. *Fisheries Research* 10: 309–316.

Spielman, D., B. W. Brook, and R. Frankham. 2004. Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences* 101: 15261–15264.

Thompson, J. D., T. J. Gibson, and D. G. Higgins. 2003. Multiple sequence alignment using ClustalW and ClustalX. *Current protocols in bioinformatics*: 2.3. 1-2.3. 22.

Turner, T. F., J. P. Wares, and J. R. Gold. 2002. Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (Sciaenops ocellatus). *Genetics* 162: 1329–1339.

Viñas, J., J. A. Bremer, and C. Pla. 2004. Inter-oceanic genetic differentiation among albacore (Thunnus alalunga) populations. *Marine Biology* 145: 225–232.

Vinas, J., A. Pérez-Serra, O. Vidal, J. R. Alvarado Bremer, and C. Pla. 2010. Genetic differentiation between eastern and western Mediterranean swordfish revealed by phylogeographic analysis of the mitochondrial DNA control region. *ICES Journal of Marine Science* 67: 1222–1229.

Watterson, G. A. 1975. On the number of segregating sites in genetical models without recombination. *Theoretical population biology* 7: 256–276.

4.6 Supplementary materials

Table SM 4.1 GenBank accession numbers of the mtDNA CR haplotypes, clade assignment and frequencies observed in each temporal samples.

Haplotype accession number	Clade	Viñas et al., 2010	This study	total
AY650761	II	2	2	4
AY650762	II	33	73	106
AY650763	I	19	55	74
AY650764	I	1	0	1
AY650768	I	36	61	97
AY650776	I	1	0	1
AY650777	I	1	0	1
AY650778	I	13	14	27
AY650781	I	19	25	44
AY650782	I	1	0	1
AY650793	I	1	0	1
AY650794	I	1	0	1
AY650805	II	10	3	13
AY650806	II	1	0	1
AY650809	II	4	3	7
AY650810	I	2	0	2
AY650812	I	2	0	2
AY650813	II	1	0	1
AY650814	I	3	3	6
AY650815	II	2	0	2
AY650816	II	2	0	2
AY650817	II	1	0	1
AY650820	II	1	0	1
AY650821	II	1	2	3
AY650822	I	1	0	1
AY650823	I	4	0	4
AY650825	I	3	0	3
AY650826	I	1	0	1
AY650827	I	1	0	1
AY650828	I	1	0	1
AY650829	I	6	2	8

AY650831	I	1	0	1
AY650832	II	1	0	1
AY650833	I	1	0	1
AY650834	I	1	0	1
AY650835	II	1	0	1
AY650836	I	2	5	7
AY650837	I	1	0	1
AY650839	I	1	0	1
AY650840	I	1	0	1
AY650841	II	1	0	1
AY650842	I	1	0	1
AY650843	I	1	0	1
AY650844	I	1	0	1
AY650846	I	1	0	1
AY650847	I	1	0	1
AY650849	II	1	0	1
AY650851	I	1	0	1
AY650852	I	1	0	1
AY650853	II	1	0	1
AY650854	II	1	0	1
AY650855	I	3	1	4
AY650856	II	1	0	1
AY650857	I	1	0	1
AY650858	I	1	1	2
AY650859	II	1	0	1
AY650860	I	3	1	4
AY650861	II	1	2	3
AY650862	I	1	0	1
AY650863	I	1	0	1
AY650864	II	1	0	1
AY650865	II	1	0	1
AY650866	II	1	0	1
EU827759	I	1	2	3
EU827762	II	1	0	1
EU827763	I	1	0	1
EU827764	I	4	0	4
EU827765	I	1	0	1
EU827766	I	1	0	1
EU827767	II	6	0	6
EU827768	II	1	0	1

EU827769	I	1	0	1
EU827770	I	1	0	1
EU827771	II	1	6	7
EU827772	I	1	0	1
EU827773	II	1	0	1
EU827774	II	1	0	1
EU827775	I	1	0	1
EU827776	I	1	0	1
EU827777	I	1	0	1
EU827784	I	1	0	1
EU827785	I	1	0	1
EU827786	I	1	0	1
EU827787	II	2	2	4
EU827788	I	1	0	1
EU827789	I	1	0	1
EU827790	I	1	0	1
EU827791	I	1	0	1
EU827792	II	1	0	1
EU827794	II	1	0	1
EU827795	I	1	0	1
EU827796	I	1	0	1
EU827798	II	1	0	1
MN652595	I	0	2	2
MN652596	I	0	1	1
MN652597	I	0	1	1
MN652598	I	0	2	2
MN652599	I	0	2	2
MN652600	I	0	1	1
MN652601	I	0	1	1
MN652602	I	0	1	1
MN652603	I	0	2	2
MN652604	I	0	1	1
MN652605	I	0	1	1
MN652606	I	0	2	2
MN652607	I	0	1	1
MN652608	I	0	1	1
MN652609	I	0	3	3
MN652610	II	0	1	1
MN652611	II	0	1	1
Total		251	287	538

5 CONCLUDING REMARKS

Identifying and describing population genetic patterns is a prerequisite for the effective management of species. In this thesis, the basic population structure of swordfish within the Mediterranean Sea was studied and described. Further, temporal changes in the genetic variability of the Mediterranean was investigated comparing genetic samples collected over twenty years.

Despite the low differentiation, genetic heterogeneity within the Mediterranean Sea was detected. The results provide useful information on the stock structure of the swordfish rejecting the hypothesis of a single Mediterranean population. The discontinuity between Mediterranean spawning areas and philopatry instinct of swordfish towards these areas may have generated a weak degree of genetic differentiation between populations even at the intra-basin scale.

Temporal investigation results revealed that Mediterranean swordfish has undergone genetic depletion during the last twenty years. Mitochondrial genetic changes observed in this study suggest that changes in the type of fishing gears may have increased the impact of harvesting on adults, affecting the spawning stock of Mediterranean swordfish, especially in female.

Translating these results into useful concepts of conservation or management has proven to be challenging. The results from this thesis underlined the urgent necessity to re-evaluate the management strategies of the swordfish in the Mediterranean Sea. The presence of genetically distinct populations would make current single-stock management unit approach used to

manage Mediterranean swordfish by ICCAT inappropriate. However, the high level of mixing among populations hampers delineation of spatial management areas. The challenge of incorporating genetics into management has been recognized for decades. In light of these results, further investigations, as tagging analysis, are required to determine the degree of complexity of the Mediterranean swordfish population structure in order to achieve efficient swordfish conservation.