Fisheries Research 154 (2014) 11–16

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Molecular contribution to stock identification in the small-spotted catshark, *Scyliorhinus canicula* (Chondrichthyes, Scyliorhinidae)

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ARTICLE INFO

Article history:
Received 14 May 2013
Received in revised form 10 January 2014
Accepted 27 January 2014
Available online 4 March 2014

Keywords:
Scyliorhinus canicula
COI
Genetic diversity
Genetic stocks
Mediterranean Sea

ABSTRACT

The small-spotted catshark *Scyliorhinus canicula* is a small demersal chondrichthyan distributed on continental shelf and uppermost slope waters of the Mediterranean Sea and north eastern Atlantic Ocean. It has commercial value in some European regions, whereas in other it is considered a bycatch species. Species' genetic structure was analysed by means of 578 bp mitochondrial *COI* sequences. A total of 192 individuals (122 obtained in the present work and 70 retrieved from GenBank) from 11 Mediterranean and 1 Atlantic locations were considered. Overall, we detected 27 *COI* haplotypes, seven of which were newly found. Moreover, a high number of haplotypes were location- and/or region-private. Low values of nucleotide diversity (total $\pi = 0.0027$) and moderate to high haplotype diversity ($h = 0.500–0.920$, total $h = 0.827$) were found. Significant genetic structuring in the study area was highlighted by AMOVA, Φ-statistics and Bayesian assignment analyses. The Atlantic sample was genetically divergent from Western Mediterranean counterparts and the Adriatic samples diverged from Eastern Mediterranean ones. Instead, Western and Eastern Mediterranean were not significantly divergent, suggesting that the Strait of Sicily is not effective in restricting past or current gene flow. No pattern of isolation by distance was detected. From a fisheries perspective, our results represent the first evidence of genetic structuring in *S. canicula* and are consistent with the presence of multiple genetic stocks in the study area. Further genetic analyses coupled with a fine grained sampling design are needed to precisely identify the borders of genetic stocks. These data provide a significant contribution for the planning of a long-term effective management policy, which could ensure sustainability of resource exploitation and stock viability.

1. Introduction

Sustainability of fisheries depends on appropriate management strategies that should rely on a number of biological and ecological factors, such as population genetic structure, connectivity, size, and dynamics of the target species. Since 1950s, genetic tools have offered a valuable contribution to fisheries biology, providing sound data for the identification of stocks (Waples et al., 2008; Ward, 2000 and references therein). Since the advent of mitochondrial DNA (mtDNA) as popular molecular marker, the advancement of population genetic and phylogeographical studies has provided deeper insights into the genetic structure of marine species, in terms of population identification and connectivity among them (Avise, 2000; Ward, 2000). This information is essential for fisheries management, as distinct populations can be considered genetic stocks requiring separate management, in order to ensure long-term sustainability of fisheries (Hauser and Seeb, 2008; Pawson and Jennings, 1996; Waples et al., 2008; Ward, 2000).

The small-spotted catshark *Scyliorhinus canicula* (Linnaeus, 1758) is a small demersal chondrichthyan in the Scyliorhinidae (Carcharhiniformes), one of the most numerous families of sharks. The species is distributed on continental shelf and uppermost slope waters of the Mediterranean Sea and northeastern Atlantic Ocean, from Norway and British Isles south to Senegal (Compagno, 1984). It is an eurybathic species, being distributed from the first few metres to approximately 400 m depth in the Atlantic Ocean (Compagno, 1984) and down to 800 m in the Eastern Mediterranean Sea (Mytilineou et al., 2005). Catshark species has been fished since ancient times, as documented in mosaics of Roman age (earlier than 79 A.D.) in the "House of the Faun" in Pompeii (National Archaeological Museum, Naples, Italy) as well as Arcimboldo's painting *Acqua* (1566, Kunsthistorisches Museum, Vienna, Austria). Early publications on catshark fishing date back to the XVIII century in Atlantic France (Duhamel du Monceau and de la Marre, 1771; Le Masson du Parc, 1727). Currently, *S. canicula* is caught through a number of fishing techniques, such as trawling nets, gillnets and

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http://dx.doi.org/10.1016/j.fishres.2014.01.021

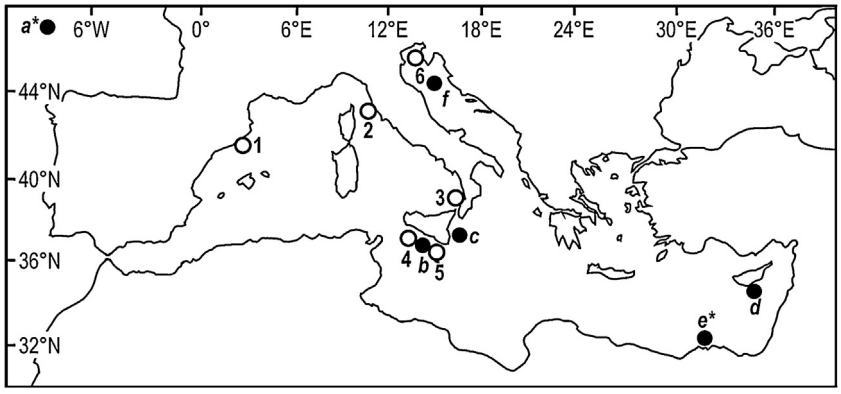

Fig. 1. *Scyliorhinus canicula*. Location of sampling sites of the present work (white circles, numbers) and individual sequences retrieved from GenBank (black circles, letters). 1, Catalan Sea; 2, Ligurian Sea; 3, South Tyrrhenian Sea; 4, Strait of Sicily – site 1; 5, Strait of Sicily – site 2; 6, North Adriatic Sea – site 1; a, Atlantic Ocean; b, Strait of Sicily – site 3; c, Ionian Sea; d, Levantine Sea – site 1; e, Levantine Sea – site 2; and f, North Adriatic Sea – site 2. The position of samples with asterisks is indicative.

longlines (Ligas et al., 2013); in addition, in some Atlantic regions, it is one of the targets of recreational fishermen (Baeta et al., 2010). Its commercial importance can be regarded as local, being highly appreciated as food in some Mediterranean regions (Capapé et al., 2008) and treated as bycatch in most of the remaining fisheries (Fowler et al., 2005; Rodríguez-Cabello et al., 2004). Nevertheless, trade of skinned individuals is flourishing from countries where *S. canicula* is considered a bycatch species to those in which it is appreciated as food [information gathered by FM at the Alghero fish market (Italy, Sardinia)]. Moreover, its importance is growing due to its use as whelk bait (Griffiths et al., 2012). In a long term analysis of elasmobranch catches carried out in the northern Tyrrhenian Sea (Western Mediterranean), Ligas et al. (2013) observed a decreasing trend of *S. canicula* landings between the 1960s and 1990s; after this period, substantial stock recovery was recorded. In this area *S. canicula* stock is considered abundant and not depleted (Ferretti et al., 2005; Ligas et al., 2013).

The potential for dispersal of *S. canicula* is expected to be low, due to biological traits such as the internal fertilisation, benthic eggs, absence of pelagic larval stages, and adults that show a high degree of habitat fidelity (Rodríguez-Cabello et al., 2004). Hence, the low potential for dispersal may favour population differentiation. Previous studies on the reproductive biology of *S. canicula* highlighted differences among traits in different seas. Geographical variation in reproductive characteristics, such as size at sexual maturity, size of egg-cases, egg-laying rates, and seasonality, has been documented, especially between Atlantic and Mediterranean individuals (Capapé et al., 2008; Ellis and Shackley, 1997; Leloup and Olivereau, 1951; Mellinger et al., 1984 and references therein). A morphometric survey of individuals collected at both sides of Gibraltar Strait lead Muñoz-Chápuli et al. (1984) to hypothesise that Atlantic–Mediterranean differences have a genetic basis. Moreover, analysis on sexual dimorphism revealed significant morphological differences between west African and west European and Mediterranean *S. canicula*, leading to the hypothesis that African catshark is an independent species or subspecies (Litvinov, 2003).

To the best of our knowledge, data on population genetic structure of *S. canicula* do not exist. The present investigation is aimed at studying it in the Mediterranean Sea and providing estimates of levels of connectivity among populations. To accomplish these objectives, we employed the sequencing of a region of the mitochondrial gene coding for the subunit I of the cytochrome c oxidase (*COI*) in individuals of *S. canicula* from six Mediterranean localities. In addition, our dataset was integrated with the *COI* sequences available in GenBank, with the objective of considering a larger number of individuals and a wider spatial scale.

2. Materials and methods

In the present study 122 individuals of *S. canicula* were sampled at the following six Mediterranean localities: Catalan Sea (25 individuals), Ligurian Sea (28), southern Tyrrhenian Sea (7), two sites in the Strait of Sicily approximately 100 km apart (28 and 8), and northern Adriatic Sea (26) (Fig. 1). The specimens from the Strait of Sicily were caught during a scientific campaign carried out by researchers at the Italian National Research Council (CNR) of Mazara del Vallo (Italy), and the remaining 86 individuals were retrieved from fishermen or fish markets. A small piece of muscular tissue (~10 mg) was excised from each individual and stored at −20 °C in absolute ethanol. In addition, 70 sequences were downloaded from GenBank and added to our data set: 18 from the Atlantic Ocean (Costa et al., 2012), 5 from the Ligurian Sea, 4 from the Strait of Sicily, 4 from the Ionian Sea, 25 from the Levantine Sea (Moftah et al., 2011), and 14 from the central Adriatic Sea (Fig. 1, Table S1). Overall, the data set included *COI* sequences from 192 individuals.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fishres.2014.01.021.

Genomic DNA was extracted using the Sigma–Aldrich GenElute™ Mammalian Genomic DNA Miniprep Kit. The mitochondrial *COI* gene was amplified using the specific primers Shark-int (5′-ATC TTT GGT GCA TGA GCA GGA ATA GT-3′) and Fish R2 (5′-ACT TCA GGG TGA CCG AAG AAT CAG AA-3′) (Barbuto et al., 2010). Polymerase chain reaction (PCR) amplifications were carried out in 20 μl reactions using 1× PCR buffer, 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.1 μM of each primer, 1 U of Perfect*Taq* DNA polymerase (5 prime), and ~2.5 ng of template DNA. The profile by Barbuto et al. (2010) was modified as follows: initial denaturing step at 94 °C for 5 min; 35 cycles of denaturing at 94 °C for 50 s, annealing at 54 °C for 50 s and extending at 72 °C for 1 min; and a final extending step at 72 °C for 5 min. A negative control was included for each reaction. PCR products were precipitated with sodium acetate and absolute ethanol and sent to Macrogen Europe for sequencing.

Sequences were scored with Chromas Lite (http://technelysium.com.au) and aligned using CLUSTALX 2.0 (Larkin et al., 2007); subsequently, sequences were checked and edited in BioEdit 7.0 (Hall, 1999). Neither insertions nor deletions were found in the sequenced region. The programme jModelTest 0.1.1 (Posada, 2008), based on the hierarchical likelihood ratio test, was used to assess the best model of evolution for the sequences under the Akaike Information Criterion (AIC). DnaSP v5.10.1 (Librado and Rozas, 2009) was used to calculate values of haplotype diversity (*h*) and