Fisheries Research 176 (2016) 30–38

Contents lists available at ScienceDirect

Fisheries Research

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

Spatial genetic structure in the saddled sea bream (*Oblada melanura* [Linnaeus, 1758]) suggests multi-scaled patterns of connectivity between protected and unprotected areas in the Western Mediterranean Sea

Antonio Calò*, Irene Muñoz[1], Ángel Pérez-Ruzafa, Carlos Vergara-Chen[2], José Antonio García-Charton

Dept. Ecology and Hydrology, University of Murcia, 30100 Murcia, Spain

ARTICLE INFO

Article history:
Received 17 May 2015
Received in revised form 8 November 2015
Accepted 2 December 2015
Available online 18 December 2015

Keywords:
Genetic connectivity
Marine protected areas
Spatial scale
Sea bream
Mediterranean Sea

ABSTRACT

Marine protected areas (MPAs) and networks of MPAs are advocated worldwide for the achievement of marine conservation objectives. Although the knowledge about population connectivity is considered fundamental for the optimal design of MPAs and networks, the amount of information available for the Mediterranean Sea is currently scarce. We investigated the genetic structure of the saddled sea bream (*Oblada melanura*) and the level of genetic connectivity between protected and unprotected locations, using a set of 11 microsatellite loci. Spatial patterns of population differentiation were assessed locally (50–100 km) and regionally (500–1000 km), considering three MPAs of the Western Mediterranean Sea. All values of genetic differentiation between locations (Fst and Jost's D) were non-significant after Bonferroni correction, indicating that, at a relatively small spatial scale, protected locations were in general well connected with non-protected ones. On the other hand, at the regional scale, discriminant analysis of principal components revealed the presence of a subtle pattern of genetic heterogeneity that reflects the geography and the main oceanographic features (currents and barriers) of the study area. This genetic pattern could be a consequence of different processes acting at different spatial and temporal scales among which the presence of admixed populations, large population sizes and species dispersal capacity, could play a major role. These outcomes can have important implications for the conservation biology and fishery management of the saddled sea bream and provide useful information for genetic population studies of other coastal fishes in the Western Mediterranean Sea.

1. Introduction

Marine protected areas (MPAs) and networks of MPAs are considered effective tools for the restoration and the management of fishery resources both within their borders (Claudet et al., 2008; Pérez-Ruzafa et al., 2008a; Fenberg et al., 2012) and outside, through the export of propagules (eggs and larvae) and the density-dependent spillover of juvenile and adult individuals (Goñi et al., 2010; Grüss et al., 2011a,b; Harrison et al., 2012; Hackradt et al., 2014).

The effectiveness of MPAs and networks would depend on a series of criteria both related to the size, location and zoning of the single MPAs (Pérez-Ruzafa et al., 2008b; Almany et al., 2009) and to the relative positioning and spacing of MPAs among them (Jones et al., 2007; Pérez-Ruzafa et al., 2008b). The latter concepts refer to the spatial arrangement of MPAs within networks and strictly rely on the dispersal potential of marine organisms (Green et al., 2014). Connectivity refers to the demographic link between local sub-populations through the exchange of individuals at whatever life stage (Cowen and Sponaugle, 2009) and is inherently related to species dispersal capacity (Jones et al., 2007; Botsford et al., 2009). Connectivity is recognized to have a fundamental importance for conservation issues because it determines the range of distances over which marine fish populations interact and the geographical scales that should be considered in order to properly manage fishery resources (Leis et al., 2011). From this point of view, the

* Corresponding author.
E-mail address: antonio.calo@um.es (A. Calò).
[1] Present address: Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Portugal.
[2] Present address: Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT-AIP), Ciudad de Panamá, Panama.

http://dx.doi.org/10.1016/j.fishres.2015.12.001
0165-7836/

investigation of connectivity patterns over multiple spatial scales is crucial for the development or the improvement of spatially explicit conservation measures both at local and regional level (Halpern and Warner, 2003; Jones et al., 2007; Almany et al., 2009; Green et al., 2014). At a local spatial scale, the assessment of the level of connectivity between protected and unprotected locations allows to estimate the effectiveness of a MPA to sustain outer non-protected areas or other MPAs within the same network; at a broader scale, it permits the delineation of environmental or anthropogenic barriers to population connections, and allows the characterization of distinct management units.

In spite of its importance, the number of connectivity-based studies in the Mediterranean Sea is still scarce (Calò et al., 2013). Recent studies have focused on the dispersal potential of larvae and/or the movements of juveniles from existing MPAs using, alone or combined, genetic analysis, otolith chemical analysis and biophysical larval dispersal models (Di Franco et al., 2012a,b; Pujolar et al., 2013; Andrello et al., 2013).

Populations genetics is the most frequently adopted approach to assess the structure of fish populations and the gradient of genetic differentiation among spatially distinct units (Palumbi, 2003; Jones et al., 2009). Such information allow to investigate larval dispersal, providing an indirect measure of connectivity (Hellberg et al., 2002; Waples and Gaggiotti, 2006; Jones et al., 2009), and can be used to address specific management issues (González-Wangüemert et al., 2004; Pérez-Ruzafa et al., 2006; Waples et al., 2008). Among the molecular markers currently suitable for these kind of studies, microsatellites have proved to be a powerful tool for investigating population differentiation and gene flow in many fish species (Balloux and Lugon-Moulin, 2002). These markers are highly polymorphic and have fast mutation rates, thus allow to reveal genetic differences even at relatively small spatial scales (Elphie et al., 2012).

In this study, we investigated the genetic structure and patterns of genetic connectivity over multiple spatial scales in a Mediterranean coastal fish, the saddled sea bream, *Oblada melanura* (Linnaeus, 1758) (Perciformes: Sparidae). Two different spatial scales were considered: at a local scale (i.e. 50–100 km) we assessed the level of connectivity between protected and unprotected locations, considering three MPAs of the Western Mediterranean Sea; at a regional scale (i.e. 500–1000 km) we investigated the presence of connectivity breaks possibly indicating the occurrence of barriers to genetic flows. The saddled sea bream is a common and widely distributed gregarious fish that inhabits rocky reefs and seagrass (*Posidonia oceanica*) beds (Bauchot and Hureau, 1986; García-Charton et al., 2004) of Mediterranean coastal ecosystems. It is and an important species both for artisanal and recreational fisheries (Claudet et al., 2008; Lloret et al., 2008) and has a relatively short pelagic larval duration (less than 14 days in the Western Mediterranean Sea; Calò et al., submitted), these characteristics making it a good biological model for genetic an connectivity studies in the considered region.

The outcomes of the present study shall provide useful information on: (1) the effectiveness of already established MPAs in sustaining nearby unprotected areas and (2) the spatial scale that should be considered for the correct conservation of spatially explicit management units in the Western Mediterranean Sea.

2. Materials and methods

2.1. Study area and sample collection

Sampling of *O. melanura* was carried out between September and October 2013. Three sectors (i.e. stretches of coastline of ca. 80–100 km) spaced about 400–600 km from each other were selected along the European coast of the Western Mediterranean Sea (Central France, Northern Spain and Southern Spain) (Fig. 1). In each sector 3 locations were selected (see Fig. 1 for location names and abbreviations). The central location of each sector corresponded to an MPA, respectively: Porquerolles (which became part of the National park of Port-Cros in 2012), Cap de Creus natural park (established in 1998) and Cabo de Palos marine reserve (established in 1995). The other 2 locations of each sector were unprotected and located about 40–50 km northwards and southwards of each MPA (Fig. 1). In each location, both protected and unprotected, 25–32 juveniles (i.e. individuals of 3–4 months of age), for a total of 258 individuals, were sampled during the night, by snorkeling, using a hand net and a torch. Specimens were firstly euthanized immersing them in a water solution with few drops of 95% alcohol for minimizing their suffering (Leary et al., 2013) and, after cessation of opercular movements, preserved in absolute ethanol used for genetic analysis. In the laboratory, caudal fins were dissected from each specimen and stored in absolute ethanol at −20 °C.

2.2. DNA extraction and PCR amplification

Total genomic DNA was extracted from a minute section of caudal fin (~10–20 mg) using Sambrook et al. (1989) protocol.

DNA concentration of each individual was evaluated using NanoDrop 1000 (Thermoscientific) spectrophotometer, using 5 μl of ultra-pure water as blank measure. A dilution with polymerase chain reaction (PCR) ultra-pure water was made to standardize each sample to 50 ng/μl of DNA.

Genotypes were examined at a total of 11 polymorphic dinucleotid microsatellite loci: 7 (Omel primers) specifically developed by Roques et al. (2001) for *O. melanura* and 4 (Dvul primers) cross-validated in *O. melanura* by Roques et al. (2007) from a set originally developed for *Diplodus vulgaris*. PCR products were obtained in a MG96Y PCR Thermocycler (AORI Technology Group) using 2 different multiplex mixes for the 2 sets of primers used. For Omel primers, PCRs were performed in a total volume of 10 μl containing 50 ng of DNA, 2 mM of MgCl2, 0.2 μM of each primer, 0.3 μM dNTP's, 1× reaction buffer [75 mm Tris–Hcl, 20 mm (NH4) 2SO4], 1 mg/ml of BSA and 0.75 U Taq polymerase (BIOTAQ). PCR conditions were as follows: an initial denaturation step of 5 min at 95 °C, eight cycles consisting of 45 s at 92 °C, 45 s at 53 °C annealing temperature, 45 s at 72 °C followed by an additional 24 cycles consisting of 30 s at 92 °C, 30 s at 55 °C annealing temperature, and 20 min at 72 °C. For Dvul primers, PCRs conducted in a total volume of 20 μl containing 50 ng of DNA, 2 mM of MgCl2, 0.25 μM of each primer, 200 μM dNTP's, 1× reaction buffer [75 mm Tris–Hcl, 20 mm (NH4) 2SO4] and 0.5 U Taq polymerase (BIOTAQ). Amplification conditions were the same as for Omel primers. PCR product was run on 1.5% agarose gel stained with safe-DNA® before being viewed under UV light and were visualized by capillary electrophoresis using ABI Prism 3730 automated genetic analyser (Applied Biosystems). Allele scoring was done using GeneMapper v.3.5 software (Applied Biosystems, Foster City, California).

2.3. Data analysis

All loci were tested for the presence of null alleles using the software MICRO-CHECKER v.2.2.3 (Van Oosterhout et al., 2004). The software POWSIM (Ryman and Palm, 2006) was used to assess the statistical power of the markers used in the study using Chi-squared and Fisher's exact tests. A range of predefined levels of expected divergence (Fst = 0.001, 0.005, 0.01, 0.05) was tested using an Ne (effective population size) of 1000 and t (time of divergence) of 10. The total number of alleles (N), the number of private alleles (PA), observed (Ho) and expected (He) heterozygosities for each locus and location were obtained with GenAlex v.6.