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Good practices for common sole assessment in the Adriatic Sea: Genetic and morphological differentiation of *Solea solea* (Linnaeus, 1758) from *S. aegyptiaca* (Chabanaud, 1927) and stock identification

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ABSTRACT

In the Adriatic Sea two cryptic species of sole coexist, the common and Egyptian sole. Soles are one of the most valuable demersal fishery resources in the Adriatic Sea, so a correct species identification is crucial in order to perform stock assessment and implement effective management measures based on reliable and accurate data. In this study specimens collected during fishery-independent and fishery-dependent activities in the Adriatic were analyzed and identified coupling morphological and genetic approaches. A comparison of these two methods for the sole species identification was carried out to assess the most effective, accurate and practical diagnostic morphological key-character(s). Results showed that external characters, in particular features of the posterior dorsal and anal fins, are valid and accurate morphological markers. Based on these traits, a practical identification key of the two sibling species was proposed. Moreover, it was possible to estimate the extent of the error due to species misidentification introduced in the common sole stock assessment carried out in the Northern-central Adriatic Sea (GSA17). A 5% bias in the correct identification of common sole specimens was detected. However, this bias was shown not to affect the common sole stock assessment. Moreover, the genetic profiling of the Adriatic common sole allowed estimating genetic diversity and assessing population structure. Significant divergence between common soles inhabiting the eastern part of the Southern Adriatic Sea and those collected from the other areas of the basin was confirmed. Therefore, the occurrence of genetically differentiated sub-populations supports the need to implement independent stock assessments and management measures.

1. Introduction

In the Mediterranean, common sole represents one of the most valuable flatfish resources; therefore, the monitoring of its status is crucial to achieve management measures for sustainable exploitation. The availability of precise and accurate fishery statistics and abundance indices is extremely important to perform reliable stock assessment (Hilborn and Walters, 2013). Soles are currently distinctly evaluated, as other demersal species, in some of the 27 geographical subareas (GSAs) defined by the relevant RFMO of the area, namely the General Fisheries Commission for the Mediterranean (FAO-GFCM; see http://www.fao.org/gfcm/data/map-geographical-subareas/en/), for management purposes. However only in few cases stock boundaries have been properly defined, and the assessments are usually carried out assuming stock's spatial distribution corresponded to the management subunits' areas (Fiorentino et al., 2014). In such context, the Adriatic Sea

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comprises two fishery management subunits, i.e. the GSA17 (central-northern area) and the GSA 18 (southern area). Common sole is one of the most valuable demersal resources in the GSA17 both in terms of economic value and catches, with average landings of about 2000 tons per year, about 30% of the overall Mediterranean common sole production.

In both GSAs, two cryptic species of soles coexist, *Solea solea* (Linnaeus, 1758) and *Solea aegyptiaca* (Chabanaud, 1927), the most abundant being the former species. In the past, they were considered two subspecies (or forms), *S. vulgaris typica* and *S. vulgaris aegyptiaca* (Chabanaud, 1927; Tortonese, 1946; Pagotto, 1971). These forms were distinguished through inspecting differences in several meristic/morphological characters, i.e. the number of fin rays and vertebrae, and the presence of a reduced or developed membrane that merges the caudal fin to the last dorsal and anal fin rays. According to the latest taxonomic revision of the genus *Solea* (Vachon et al., 2008), *S. aegyptiaca* and *S. solea* are defined as distinct and valid species. Most molecular studies (e.g., She et al., 1987; Borsa and Quignard, 2001; Espiñeira et al., 2008; Boukouvala et al., 2012) also recognized them as distinct species and the use of the above mentioned meristic/morphological features was proposed for their identification (Quéro et al., 1986; Fischer et al., 1987). Even if *S. aegyptiaca* is phylogenetically sister to *S. senegalensis* (Borsa and Quignard, 2001), the common and the Egyptian sole are rather similar in their external morphologies and share similar habitats. Therefore, species misidentification could occur during the collection of fishery data.

The accuracy of different meristic/morphological parameters in distinguishing between the two species has never been tested in parallel with molecular taxonomy. In this context, the evaluation of stock status of common sole in the GSA17 (conducted since 2005; Grati et al., 2013) could be questioned due to a potential bias in species identification.

Molecular markers provide species identification and can identify patterns of population structure (Ovenden et al., 2015 and citations therein), thus enabling the definition of biological units (i.e. stock boundaries) and the verification of their match to spatial management units. In the Mediterranean Sea, the common sole populations are genetically structured with deepest divergence shown along the west-to-east axis (Kotoulas et al., 1995; Guarniero et al., 2002; Garoia et al., 2007; Rolland et al., 2007). Based on the mitochondrial DNA variation, Guarniero et al. (2002) concluded that in the Adriatic Sea two near-panmictic populations of common sole exist. The first inhabits the entire GSA17, the western part of the GSA18, and the adjacent northern part of the GSA19 (Western Ionian Sea), while the second population is located along the Albanian coasts (eastern part of the GSA18). However the low resolution power of allozyme markers used in Kotoulas et al. (1995), the small sample sizes used in Guarniero et al. (2002) and the lack of wide and deep geographical sampling design of the latter two studies, may have prevented the detection of genetic differences at small geographical scale (i.e. between adjacent GSAs or within the same GSA). In this framework, the goals of the present study are: i) to assess and validate the most accurate and useful meristic/morphological key (s) for the identification of these two species through the comparison of morphological and molecular analyses; ii) to estimate the misidentification bias in the common sole stock assessment carried out in the GSA17; iii) to assess genetic diversity and population differentiation of the common sole in the Adriatic Sea in relation to the current management units.

2. Material and methods

2.1. Sampling methods

Specimens were collected in the framework of different sampling activities (Fig. 1). A total of 170 specimens was collected during fall 2007 and 2011–2013 onboard the rapido trawl-survey SoleMon (Grati et al., 2013), carried out mainly in the Northern Adriatic Sea (GSA17). Other specimens (307) were collected from commercial fishing activities (fishing gears: otter-trawl, rapido trawl and trammel nets) conducted in 2002 and 2011–2013, mainly in the Northern Adriatic waters and in two lagoons along the western coasts (Venice and Varano Lagoon).

2.2. Meristic and morphological data analyses

The macroscopic identification of individuals was carried out on 433 samples. Specimens were preliminarily classified as *Solea solea* or *S. aegyptiaca* according to the presence of a developed or reduced membrane joining the dorsal and anal fins to the caudal peduncle (Fig. 2).

The number of rays of pectoral, dorsal and anal fins was assessed on the right side of 222 specimens. A specimens' sub-sample ($n = 28$) was dissected to count the vertebrae, excluding the Cunningham rudimental vertebra (Pagotto, 1977). During the laboratory analysis, another character was observed and assessed for 191 specimens: the shape of the posterior dorsal and anal fins, that was classified as R = round-shaped or S = square-shaped.

Meristic (number of pectoral, dorsal and anal fins and number of vertebrae) and morphological characters (membrane's size and fins' shape) of the two species were compared by performing analyses on specimens identified by molecular analysis.

For all meristic characters the outliers, i.e. cases with values below the Q1 (first quartile) – 1.5*IQR (interquartile range) or above the Q3 (third quartile) + 1.5*IQR, were removed from further analyses, resulting in the elimination of three specimens. Since the two species' samples were characterized by different size ranges (common sole: 6.5–29.2 cm, mean length: 15.2 ± 5.5 cm; Egyptian sole: 14.2–37 cm, mean length: 23.4 ± 6.4 cm), a linear regression analysis was carried out to investigate the existence of a relationship between the meristic characters and total length. The analyses showed no such relation in all meristic character ($p > 0.05$, in all linear regressions); therefore, it was not necessary to transform the absolute values to size-independent values (Turan, 2004).

A non-parametric Mann-Whitney *U* test was performed to assess the existence of a significant difference in meristic characters' values and presence/absence of morphological features (membrane's size and fins' shape) between the two species (p level set at $\alpha = 0.05$).

A Principal Component Analysis (PCA) was applied to investigate the contributions of the meristic and morphological characters to species identification. The number of vertebrae was not included due to the limited number of available samples. All data were normalized (the variable's mean was subtracted from each record, then the result divided by the standard deviation) before the analysis, to avoid effects due to the different measurement scales of variables. The PCA was carried out with the statistical package PRIMER-E, version 6 (Clarke and Warwick, 2001).

A Classification and Regression Tree analysis (CRT; Breiman et al., 1984) was performed to derive a species identification key based on meristic/morphological characters. The analysis was carried out by means of the software Statistica, v.8 (StatSoft Inc.). Three meristic characters (number of pectoral, dorsal and anal fins), and the two morphological characters were used as predictors, while species' classification according to genetic results was used as the dependent variable; the number of vertebrae was not included in the analysis. Moreover, to assess the degree of agreement between macroscopic and genetic species classification, the Cohen's kappa coefficient was applied (K; Cohen, 1960). The index ranges between 0 (no agreement) and 1 (perfect agreement) and was calculated by the following equation: $K = (p_o - p_e)/(1 - p_e)$, where p_o is the proportion of observed agreement and p_e the proportion of the expected agreement (caused by chance). According to Landis and Koch (1977), the agreement with K values above 0.80 is classified as almost perfect.