ECOSYSTEM APPROACH TO FISHERIES IN THE MEDITERRANEAN AND BLACK SEAS - ADVANCES IN RESEARCH AND TECHNOLOGIES

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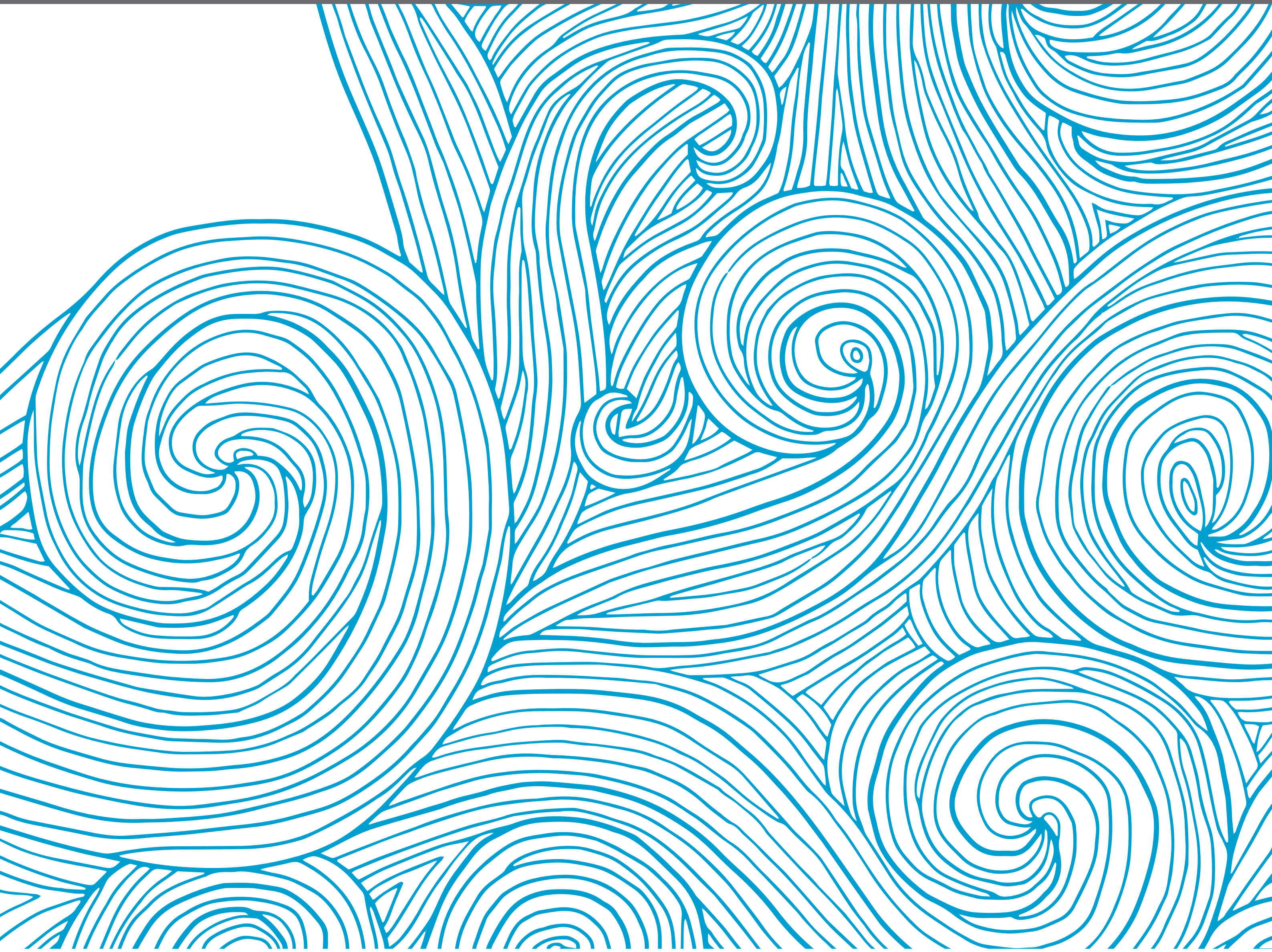

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ECOSYSTEM APPROACH TO FISHERIES IN THE MEDITERRANEAN AND BLACK SEAS - ADVANCES IN RESEARCH AND TECHNOLOGIES

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Table of Contents

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EDITORIAL
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Editorial: Ecosystem Approach to Fisheries in the Mediterranean and Black Seas - Advances in Research and Technologies

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Keywords: management, models, genetic tools, measures, indicators, Black Sea, Mediterranean

Editorial on the Research Topic

Ecosystem Approach to Fisheries in the Mediterranean and Black Seas - Advances in Research and Technologies

INTRODUCTION

The ecosystem approach for fisheries management is a widely accepted concept and has recently been developed to address the failures of traditional fisheries management practices (Morishita, 2008). Various international instruments require its application, improving on single-species fisheries. There is an increasing realization of the importance of species-to-species interactions, genetic tools for fishery stock structure determination, and defining stock boundaries to underpin sustainable fishery management. Thus, ecosystem models play strategic roles by providing an ecosystem context for single-species management decisions. Moreover, tactical ecosystem models can respond dynamically to any ecological and environmental variations. For example, to respond to the lack of comprehensive information about fishing activity catch quantities and composition, and how they affect the current state of Black Sea fish stocks (Raykov and Bikarska, 2011).

The studies included in this Research Topic provide novel sources of information for Mediterranean and Black Sea fisheries, spanning from mass-balance models to new fishing technologies that reduce bycatch of non-target species.

The Ecopath mass-balance model of the central Aegean Sea developed by Dimarchopoulou et al. provides a better understanding of the structure and functioning of a protected ecosystem and the ecological role of the main species of interest. This and results from Russo et al. show that the combined effects of traditional fishing effort regulation with spatial/temporal closures are important in the management action plans toward a reduction of ecosystem exploitation. The study of Darmanin and Vella describes recreational fishing in the Mediterranean as marginal with no impacts on the marine ecosystem. Fishing gears, with all their intrinsic variability, represent the physical link between a fishing management strategy and the target populations directly affected by its application (Sala et al.).

The mean size of the landed catch can be applied as a key ecosystem-based indicator, notably in monitoring exploited marine communities. The evolution of this indicate over time demonstrates long-term exploitation impacts on fishing communities and can help indicate when fishing may be closed as shown in Vasapollo et al.. Genetic structure of species is crucial for effective management of environmental resources, and genetic population data analyses have been proposed as a new

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indicator of biological monitoring. This is demonstrated in Paterno et al. using a genome-wide approach to the phylogeography of the mussel (*Mytilus galloprovincialis*) in the Adriatic and Black Seas. Such studies show the impact of fisheries on genetic diversity and the structure of exploited populations and inform strategies for long-term management and conservation of fisheries resources Turan et al..

Finally, the incidental capture of non-target species is one of the major threats to marine megafauna. Current knowledge on sea turtles-fishing gear interaction and mitigation measures implementation is insufficient to hinder the decline of turtle populations in the Mediterranean. Lucchetti et al. demonstrated this for the sea turtle in the Mediterranean, where incidental catches pose the main threat to its conservation. Furthermore, Pulcinella et al. argued for the introduction of bycatch reducer devices in different Mediterranean fisheries. These two studies are among the first to evaluate incidental capture of loggerhead turtles in a Mediterranean sub-basin fishing métier.

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All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Mean Size of the Landed Catch: A Fishery Community Index for Trend Assessment in Exploited Marine Ecosystems

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Based on fisheries landings data I propose the size-base index (community level) Mean Size of the Landing Catch (MSL). The MSL index was estimated based on high taxonomic resolution data available from auctions (species level) and demographic data acquired during the auction, namely species landed by "size-box" categories, which is assessed mandatorily in all EU members state for fisheries quality and statistic proposes. The MSL was calculated from the average inferred size-box categories of a species weighted by their annual catch. The use of MSL allows determining inter-annual changes in the size of the catch when weighted data is available from the fishery. Using the Portuguese fisheries landing data as an example, the MSL revealed that the landing yield of large fish linearly declined over time while the catch of small fishes increased (i.e., survivors to old age was reduced by fishing). The MSL can be easily used to assess trends in marine exploited commercial communities (community rather than population level) and is fully applicable with any species-size data source (e.g., scientific surveys, visual census data). The MSL can also be applied as a key indicator within the new ecosystem-based Marine Policy Framework Strategy (ecosystem approach to fisheries), which required the use of size-based indicators for the assessment of fisheries trends in exploited marine communities.

Keywords: fishing indicator status, fisheries size-based trends, marine monitoring index, ecological fisheries management, size-based index

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INTRODUCTION

With the collapse of fisheries at a global scale (Pauly et al., 2003; Leitão, 2015) and the growing risk of overexploitation of several marine fish species, it became evident that stock assessments have not accomplished their objectives. Ecosystem-Based Fisheries Management (EBFM) has been proposed as a new paradigm of fisheries management (Garcia et al., 2003) and the new European Marine Strategy Framework Directive (MSFD) requires the implementation of these approaches along with the development of ecosystem indexes to asses ecological trends (No. 2008/56/CE of EU council, 17 de June).

The interactions between species in communities on many spatial and temporal scales, including the distribution, structure, abundance, demography, and interactions between coexisting populations represents a key issue in ecology. Several ecological indexes are used to study community changes over spatial and temporal scales such as species richness, equitability, productivity, species diversity and evenness, dominance, and biomass to describe trophic levels in coastal marine ecosystems (Karydis and Tsirtsis, 1996). These indexes try to assess changes in community structure as estimators of stress, for instance, environmental or anthropogenic factors. More recently, different ecosystem/ecologically based indexes have been developed to asses and monitor exploited fish assemblages including (i) biodiversity indicators such as the marine trophic index (Pauly et al., 1998), (ii) size-based population indicators (reviewed in Shin et al., 2005, 2010), or (iii) environmental-based fisheries indicators, such as the mean temperature of the fishing catch (Cheung et al., 2013).

The usefulness and relevance of size-based indicators (SBIs) to the MSFD has been under discussion for implementation in the EU. As reviewed by Shin et al. (2005, 2010), SBIs (statistics summarizing the size distribution of fish assemblages and populations) may provide a relevant integration of the fisheries impact on the community structure and in biological processes. Several studies (Frank et al., 2005; Hsieh et al., 2006) considered that the size of organisms is a focal factor to critical ecological processes, and changes in size distribution derived from a substantial number of causes, including environment-induced or genetic variability in life history characteristics, predator-prey relationships, or competitive interactions. Nevertheless, more important is the fact that fishing is always size-selective. Targeting larger and more valuable fishes modifies the size structure and functioning of fish assemblages, with direct consequences for the productivity and resilience of those same stocks. The theoretical basis for the relationship between fish size and ecosystem functioning relies on the assumption that (citing: Shin et al., 2005, 2010 and references therein) [. . .*most life history traits are correlated with size, which acts as a constraint on metabolic rates and energy assimilation, so influencing the entire lives of animals, including their growth, reproduction, and survival* (Reiss, 1989). *Species with smaller adult body mass are generally characterized by faster growth rates* (Brey, 1999)*, higher natural mortality* (Beverton and Holt, 1959; Pauly, 1980)*, higher reproductive output* (Gunderson and Dygert, 1988; Charnov, 1993)*, recruitment, and production per spawning adult* (Fenchel, 1974; Denney et al., 2002). . .]. In exploited fish communities there is evidence that indicators based on the size of individuals are appropriate to monitor the community structure. It is therefore arguably that if the use of such indicators to assess ecosystem status, such as if the proportion of large fish (by number or weight), reflects the state of an assemblage. That is if communities subject to intensive fishing will have a proportion of large fish smaller than those not subject to fishing or small-scale fishing. However, the development of marine biological indicators reflecting size-based variability on marine assembles (populations rather than species level) is so far lacking.

Several scientific syntheses have highlighted the connection between size reduction and exploitation in wild marine populations. An approach for estimating the mean catch size was developed by Pauly (access available at FishBase[1]). The FishBase routine considered that fish species targeted by various fisheries change in age and size structure due to exploitation. In a fishery defined as sustainable, such annual changes in catch composition should have no trend. However, the exploitation of multispecies communities has a changing effect on the relative abundance of the different functional groups supporting community ecosystems. Besides that, it is also noteworthy that large, long-lived species with high trophic levels tend to be replaced by smaller short-lived species feeding at lower trophic levels. These trends will ultimately be reflected in catches as the worldwide decline in mean trophic levels showed (Pauly et al., 1998). Therefore, the latter authors have estimated the average maximum size of organisms (fish and invertebrates) based on the mean of the maximum lengths of species or groups. Using the Atlantic blue marlin as an example, Goodyear (2015) showed that in fast-growing, long-lived species, such as the blue marlin, mean length might decline only slightly even with a considerable fishing-induced population decline. When fishing targets a broad age range, the decline in maximum observed size is the result of reduced survival of older ages, which typically include the largest individuals in the population. Therefore, it is assumed that a consequence of fishing is the length reduction of fishes caught over time because: (i) marine trophic ecology is organized in the way that small forage species with short generation time continuously replace large slow-growing and long-lived species in an exploited ecosystem (Pauly et al., 1998); (ii) the selective removal of organisms subjected to fishing because fishing gear (nets) tends to select for large mature individuals, consequently decreasing average length (Ricker, 2011).

Globally, many highly prized commercial species are under assessment, and substantial data exist to predict their status. However, most fisheries worldwide are data-deficient due to insufficient research funding and support. According to Maynou (2015), this situation changed in the last decades for many target species of major fishing fleets due to the resources made available by the Data Collection Regulation (DCR) and the Data Collection Framework (DCF) programs (EU Reg. 1543/2000 and EU reg. 665/2008, respectively). Likewise, stock assessments were carried out under the authority of the Scientific, Technical and Economic Committee for Fisheries (STECF; Cardinale et al., 2010; Colloca et al., 2013). However, sufficient quality data is inevitably tied to important fisheries producing large amounts of landings and falling within DCR/DCF obligations. Landing data (such as demographic aspects of the fishing), continuously collected over the years and combined with limited additional information on the biology of target species, can later be used to develop ecological indexes which will characterize the time series and status of the global fisheries. In Portugal, the DOCAPESCA (entity responsible for Portuguese fishing auctions) has a size-box classification system for fishing catches. DOCAPESCA[2] data collection system follows the EU 1996 regulation (CE No. 2406/96). This regulation establishes the common marketing standards for fishery products. Under this regulation, during the

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[1] http://www.fishbase.org/manual/fishbasefao_statistics00002679.htm

[2] www.docapesca.pt/

auction each box has fish of a size category, thus generating important information for fisheries biologists. Fish price can have a significant variation (elastic product). Therefore, the prices for the different box size-categories can also vary considerably. During auctions, the boxes are ranked from 1 to 6, where boxes labelled with 1 include larger fish (more prized fish) and boxes labelled with 6 have fish of lower commercial size/value (less prized fishes). As the market demand will make prices fluctuate, size categories are kept constant across time (independently of the market price).

Fisheries can change the community of species in the marine environment. Given the general lack of long time-series, only in a few cases a decreasing trend in the population mean size was directly observed related to fishing exploitation of certain commercial species, and not of communities [e.g., sardine in the Aegean Sea (Antonakakis et al., 2011) or hake in the Balearic area (Hidalgo et al., 2009)]. If populations of many commercial species are already characterized by truncated size-structures, than, size-effects should also become evident at a community level. Understanding and monitoring the size-structure of exploited populations is an objective of the EBFM (Froese et al., 2008). This issue has been seldom studied, which can be related to the lack of a more systematic data collection thus hindering the assessment and management of many fishery resources in several areas (Maynou, 2015).

In European countries, most assessed stocks/populations of many commercial species are characterized by truncated size- and age-structures. Generally, owing to the combined effect of high fishing pressure and low size selectivity, the catch composition of most of the Mediterranean commercial stocks is dominated by age 1 and 2 specimens, with a low occurrence of larger individuals (see STECF, 2009). Population SBIs can be used for evaluating, comparing, and communicating the ecological status of exploited marine ecosystems (Shin et al., 2010). The mean size is easily conceptualized for single species, but so far that is not the case for all fished assemblages. Herein, using the size-box categories data obtained from the commercial fish database, the Mean Size of the Landing Catch (MSL) was estimated. Applying this index will allow a comprehensive perspective of all fished species (community level), rather than just individual species analyses. At the same time, time series are expected to reveal biological size-based trends regarding the exploitation status of fishing communities.

MATERIALS AND METHODS

Fisheries and Fish Size-Box Frequency Data

This study only pertained to mainland Portugal landings. Despite the lack of routine biological samplings for many landed species, fishing landings by species and fleet sector (trawl, seine, and multigear) can be compiled daily in Portugal from DOCAPESCA auction database (see footnote 2) for statistical or taxing purposes. The DOCAPESCA monthly landings statistical data were combined into annual periods. The multi-species, or multigear artisanal sector, includes mostly (85.9%) fiberglass open deck local boats (<7 m long). This sector uses static/passive gears, namely gill nets, trammel nets, pots and traps, and hook and line gears (Leitão et al., 2014). The size-box classification system for Portuguese fishing catches follows EU 1996 regulation (CE) No. 2406/96 which establish the common marketing standards for specific fishery products. In this regulation box size categories varied considerably with boxes-size with the higher fish size being scored with 1 (most prized fishes) while lower boxes-size fish scored with 6 (less prized fishes). However, some landed fish boxes can be categorized in the auction as zero size. Underlying reasons for that can be because: (i) species were not yet obliged to be assigned according to freshness or size; (ii) small ports (auction) do not assign them due to logistic technical/technological constraints. Based on yearly landings between 2002 and 2016, the percentage of size-box assigned with score #0 (undetermined) from the Portuguese mainland was 15 ± 1.5% of the total landings in weight, and 15 ± 4, 1.5 ± 0.4, and 50 ± 5.7% were respectively assigned to trawl, seine and multigear fishing sectors. As it was not possible to determine size-box category #0 by species, this was removed from further analyses. Between 2002 and 2016 a total of 74 species (**Supplementary Material**) were landed and sold in the DOCAPESCA auctions, and assigned into box-size categories #1 to #6, contributing to 85% of the total catch in weight. Mix categories such as *Diplodus* spp. (e.g., include *D. sargus*, *D. vulgaris*, *D. bellottii*), *Lophius* spp. (e.g., include *Lophius piscatorius* and *L. budegassa*) or other co-generic groups (at genus taxon) such as *Mullus* spp. (include *Mullus surmulletus* and *M. barbatus*) were used as they appear at auction discriminated by size-box category.

Mean Size of the Landing Catch Index

The number of the size-box category (or box length-class category) corresponds to the label/score provided in auction by the expert technicians to discriminate the length-class of the fish contained within each fish box, based on pre-determined fish size-categories (CE No. 2406/96). That said, the mean size of the box was considered as a proxy to the size-class which means that this index can be used in other circumstances (e.g., scientific cruises with detail data on weight-size frequencies, etc.). The average yearly frequency distribution of landings in weight, by size-box category, was determined using species size-box categories weight information. The frequency distribution of landings by size-box category was plotted and later used to estimate MSL. We estimate MSL trends considering: (i) species mean landings size cumulative contribution and; (ii) relative contribution in weight of each species, by size-box categories, to overall landings. Taken together, the MSL was calculated and inferred from the cumulative contribution of the average size-box categories of each of the exploited species (**Supplementary Material**) weighted by their annual landings, as following:

$$MSL_{yr} = \sum_{i}^{n} \sum_{S}^{m} \frac{L_{i,S} \cdot S}{L_i}$$

Where *yr* is the year, *i* is the species, *n* is the number of landed species, S is the size-box category, m is the number of size-box categories, L_i is the total landing weight of the species i for that year and $L_{i,S}$ is the landing weight of size-box S from species i. Therefore, index values range between 1 and 6 and MSL values close to 1 represent a large percentage of large/size fish in landings while values close to 6 represent a large percentage of small/size fish in the landings. The MLS was estimated for each fleet sector (trawl, seine, and multigear) and overall fleet.

Following the EU 1996 regulation (CE) No. 149 2406/96, the size-categories differ with species and country. However, the systematization process of assigning species size categories

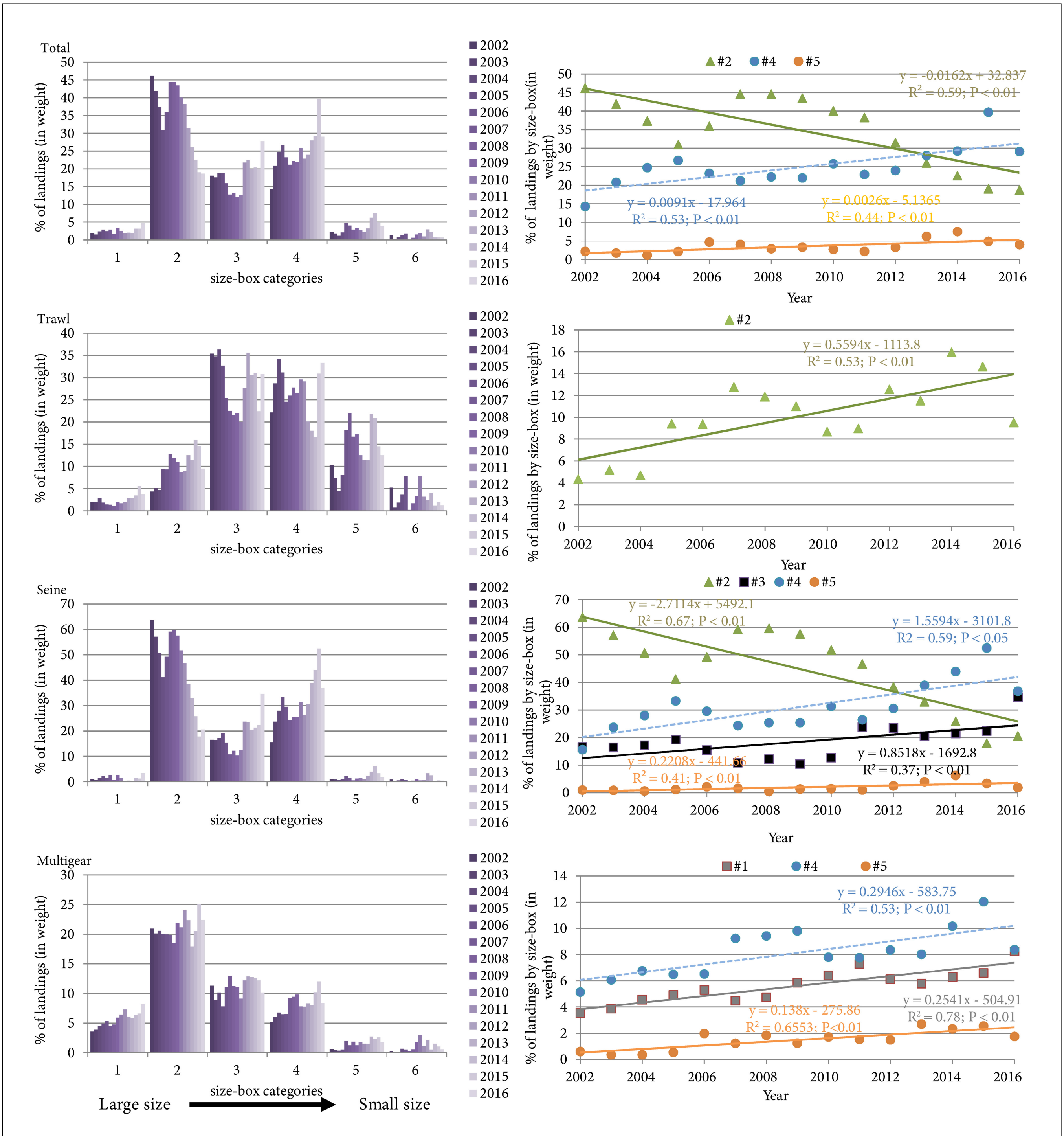

FIGURE 1 | Inter-annual size-box relative frequency distribution for total catch and by fishing sector, Trawl, Seine, and Multigear **(left panel)** and significant linear regression models between time (years) and percentage of landings per size-box category (**right panel**; #1 – large size to #6 – small size). R, Coefficient of correlation; *P*, *P*-value.

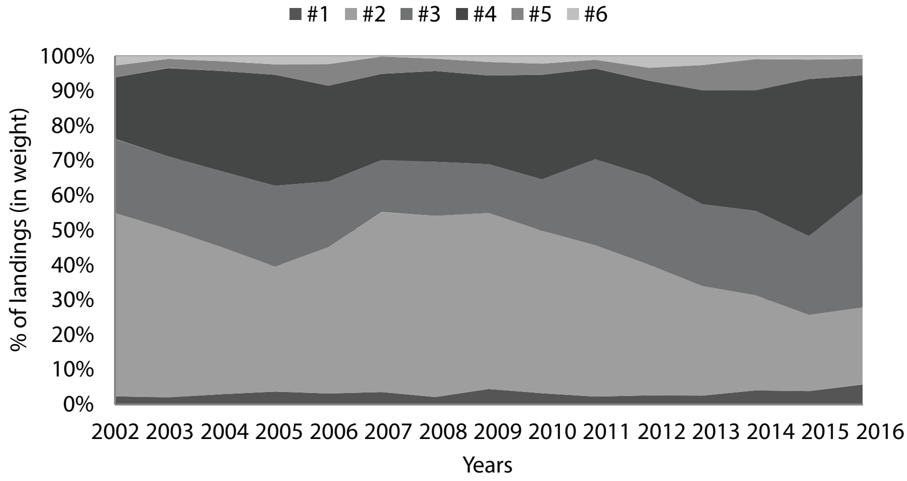

FIGURE 2 | Percentage of landing (in weight) per size-box category (#1 – large size to #6 – small size).

at auction is conservative over time and country, and thus the index can display trends along a time series dataset, allowing an application of this approach specific for each country.

Data Analyses

A linear regression model was used to assess the evolution of the percentage of landings over time for each size-box category. The slope parameter of the regression model was used as a proxy for the variability of the percentage of landings for each size-box category. Therefore, the slope of the linear fitted model was used as a proxy for the trend tendency (upward or downward) and to quantify the rate of change in time series data. The statistical significance of the linear model was assessed via a student t-test (P-value < 0.1). The null hypothesis was formulated as no trend that describes an unchanging landing percentage rate.

RESULTS

The size-box categories #1, #5, and #6 have a small weight contribution (relative contribution) to the overall landings, regardless of the fishing gear (**Figures 1**, **2** and **Supplementary Material**) whereas, the size-box categories #2, #3, and #4 had a higher contribution to overall landings, regardless of the fishing gear (**Figures 1**, **2**).

Overall, the larger fish landings (size-box #2) evidenced a linear decline over time while small size-box categories landings increased out by fishery (size-box #4 and #5). The trends of the relative percentage of landings per size-box categories varied among fishing fleet sectors (**Figure 1**, right panel). For the trawl sector, a significant linear increase ($p < 0.05$) in the landed percentage of large size fish (size-box #2) was recorded.

For the seine sector a significant linear decline ($p < 0.05$) in the landed percentage of large size fish (size-box #2) is recorded while an increase of intermediate size-box categories (size-box #2, 3, 4) was observed.

For the multigear sector a significant linear increase ($p < 0.05$) in the landed percentage of size-box #1 (large size fish landed at auction) was recorded along with the intermediate (size-box #4) and low size-box categories (size-box #5).

The Mean Size of the Landing Catch was lower in the multigear sector and higher and similar in trawl and seine, respectively (**Table 1** and **Figure 3**). The overall MSL, based on auction size-box category, increased significantly at a linear rate of 0.37 units per decade (**Figure 4**). The linear increase of the MSL means that more small size fish were landed over the considered time frame (2002 to 2016). The increase of the MSL, or the decline of large fish in the landings, was recorded in all fishing sectors (trawling, seine, and multigear). A significant linear increase of 0.25, 0.55, and 0.35 units per decade in MSL were observed respectively for the trawling, seine and multigear sectors.

DISCUSSION

The proportion of large fish was selected as a descriptor indicator for the Ecological Quality Status (EcoQs) in the North Sea (OSPAR, 2008; see also Rogers et al., 2010) and for the implementation of the MFPD in EU (Decision COM4031 2010/477/EU). The justification for the inclusion of SBIs for assessment in the OSPAR (OSPAR, 2008) was: "*In exploited fish assemblages, larger fish generally suffer higher fishing mortality than smaller individuals and the size distribution becomes skewed*

TABLE 1 | Average Mean Size of the Landing Catch (MSL) for total catches and by fishing sector (Trawl, Seine, Multigear) between 2002 and 2016.

	Mean Size of the Landings (MSL)			
	Trawl	**Seine**	**Multigear**	**Total**
Mean	2.96	2.90	1.24	2.37
Standard deviation	0.16	0.27	0.18	0.18
Coefficient of Variation	0.05	0.09	0.15	0.08

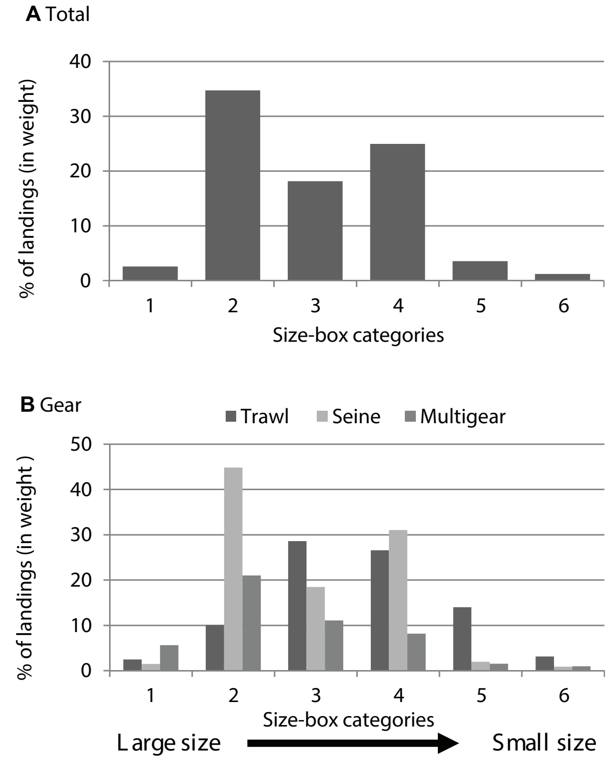

FIGURE 3 | (A) Mean size-box relative frequency distribution for total catch and by **(B)** fishing sector (Trawl, Seine, and Multigear) between 2002 and 2016.

towards the smaller end of the size spectrum. The susceptibility of late-maturing and larger fish species to fishing implies that small and early-maturing species increase in relative abundance. . . . The average weight or maximum length can be expected to be proportional to fishing effort, though natural factors will impact the size distribution as well. From a conservation perspective, appropriate EcoQs would move these metrics towards a larger proportion of large fish and would improve fisheries yields".

The increase of larger fish in landings is the results of the fish growth and reproduction in later years. Assuming this rationale, we can consider that the output information driven from the MSL index based on size-box categories (here considered mostly spawning biomass or adults) can be used as a time series indicator of the exploitation regimes. The MSL index was lower in multigear sector and higher and similar in the trawling and seine sectors. These results imply that multigear landings are composed of a significant percentage of large fish (size-box#2). That is an expected result as the fish size is commonly associated with the fleet type (Leitão et al., 2014). Seine and multigear sectors comprise most of the landings of the Portuguese fishing fleet (Leitão et al., 2014) and both fishing sectors indicate an increase of MSL, revealing a decline in the amount of larger landed fish. The MLS trends for seine seem to be in line with the seine downward trends for traditional commercial species captured by seine nearshore fisheries (medium and small pelagics) that have decreased after the middle 1980s (Leitão, 2015). Indeed, the seine fleet targets mainly sardine, which collapsed in 2017 (ICES, 2017), mackerel and horse mackerel which also decreased in later years (Leitão et al., 2014; Leitão, 2015). The contribution of size-box #1 (large fish sizes) to the total seine catches is ephemeral in the last 14 years (2002–2016). A significant decline in size-box #2 (large fish) was also observed in seine whereas medium to small fish size-box contribution (#3, #4, and #5) increased significantly. The declining catch trend in the later years of small and medium-size pelagic fish mostly caught by the seine sector have changed fleet activity which now target on deep-sea resources and higher trophic levels (cephalopods, large benthopelagics, flatfishes, demersal invertebrates, rays, bathydemersal, shrimps, small benthopelagics, and large sharks) while traditional commercial species captured by nearshore fisheries (medium and small pelagics) have decreased (Leitão, 2015). Trawl and multigear sectors also reveal an increase of MSL thus a decline of large fish in catches. Therefore, a decline of young and large-sized fish in landings over the latter years in the multigear sector is probably due to the overexploitation of many coastal resources. Deep-water fish resources are generally considered to have high longevity, slow growth, late maturity, and low fecundity (Morato et al., 2006).

As 50% of the data (size-box #0) was not available, some caution is required regarding the interpretation of multigear fleet results. However, to our concern, the major scope here is not to debate the Portuguese fisheries situation/report system but rather the usefulness of the indexes as a size-based indicator of the trends of exploited marine communities. The size-box distribution of landed fish, regardless of the gear, reveals the demographic profile with size #3–#4 dominating in weight and sizes #1 and #6 with a minor contribution. Thus, using fish size-box categories, it was possible to determine a proxy (a proxy because discards are not taken into account) of fishing community length-frequency distribution. Overall, based on the size-box information it was possible to see an increase in the MSL (landings decrease of large fish). That means that the amount of large size fish is declining. This information is of great concern as the proportion of large fish (in weight) reflects the state of an assemblage. Communities subject to intensive fishing will have a smaller proportion of large fish rather than those not subject to fishing or just to small-scale fishing. It can, therefore, be used as a measure of the relative abundance status of top predators whose value below certain limits may indicate the disappearance of the higher trophic levels within the trophic web (Shin et al., 2005, 2010). According to Goodyear (2015), the mean and maximum observed sizes tend to decline with increasing fishing mortality, a trait easily recognized by fishermen and scientists. Consequently, such data is an informative indicator of population health and receive particular scrutiny during the fisheries management process.

Attempts to monitor the overall size of catches reveal that a decline in average maximum size of organisms landed by various countries did occur (see footnote 1, Froese and Pauly, 2019). However, as stated by later authors, these results are probably underestimated, given the fact that those do not consider the reduction of mean length within species. Moreover, OSPAR (2008) concluded that [. . .“ *further studies on the metrics*

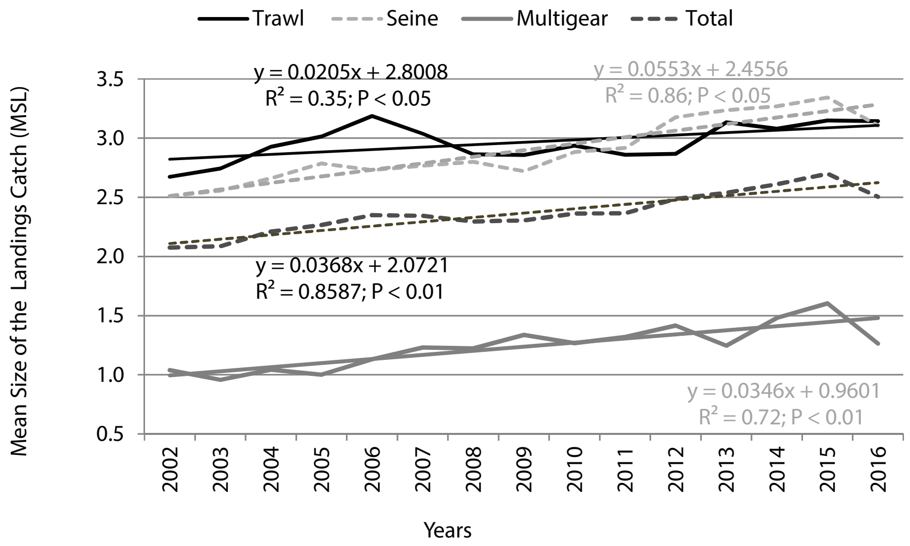

FIGURE 4 | Mean Size of the Landed Catch (MSL) index for total catch and for each fishing sector (Trawl, Seine, and Multigear) with significant adjusted linear regression statistical models. R, Coefficient of correlation; *P*, *P*-value.

"mean weight" and "mean maximum length of fish" are still needed. The latter metrics are closely related to the area fished and the gear used. Reference points that could be developed would therefore also be specific to the surveys and areas"...]. The integrated analyses of all fish assemblages using weighted size-category frequency inferred from auction data consists of a new approach that allows for MSL estimation and can joint to other attempts to access landings size-based information trends in marine communities. The estimation of the MSL index was based on auction data, but it demonstrate that simplistic approaches (relatively easy to understand by non-scientists and those who will decide on their use – stakeholders) can be used to access trends in marine assemblages (even based on landings and comprising mostly adult stock size). Moreover, it can be used as an additional tool within the conservation and management fisheries programs in the EU and elsewhere. For example, for the EU member countries, all are committed to the common fisheries policy, as the legislation is equally enforced in each member state. It can be argued that the procedure to assign landings by size-box category along EU member's state can change across different auctions in the EU. However, size-box assignment routines are surveyed by an expert veterinarian and chef auction inspectors that are obligated to enforce EU regulation. Moreover, commercial fishers are most interested in keeping the fisheries assignment at higher standards, aiming for maximum revenue, and dealers who want to get the best fish price for each quality category (size is an attribute of fish quality). These arguments will allow for consideration of implementation of size-box data analysis over time and space (different EU member states for instance) under a standardized evaluation.

The MSL decrease across years, independently of gear type. Consideration about size changes in community dynamics of harvested stocks should be tracked because biological attributes that affect stock productivity such as fecundity and survival are closely related to organism sizes (De Roos et al., 2006; Conover et al., 2009). The selective removal of organisms with different growth and maturity rates impact fisheries in different ways. Harvested stocks with density-dependent mechanisms can accelerate growth at low stock sizes (De Roos et al., 2006). Premature maturation mechanism can enhance population/communities biomass gain, and recovery from fishery impacts, but this biomass is ultimately reduced by fishing gears size-selective catch. In the future, catch size information provided from auction data can be an additional fisheries management tool to be used as indicators of community size status and not just for trend analyses proposes. The MSL index can be used with other data (e.g., survey data) where multiple species demographic information can be compiled. Such a simple size-based index can induce researchers to look to demographic population aspects when selecting fishing indicators (population size indicators status), within the new MFPE policy to be enforced in the EU. Additionally, MSL can optimize the inclusion of fisheries information in monitoring programmers so that they provide maximum information for EBFM management purposes.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00302/full#supplementary-material

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ORIGINAL RESEARCH
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Reducing Sea Turtle Bycatch in the Mediterranean Mixed Demersal Fisheries

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The sea turtle (*Caretta caretta*) is the most common sea turtle in the Mediterranean, where incidental catches due to fishing activities are considered the main threat to its conservation. Over 50,000 capture events and likely over 10,000 deaths are estimated to occur in the Italian waters alone. However, current knowledge on the interaction of sea turtles with fishing gears and the implementation of mitigation measures are still poor to hinder the decline of turtle populations in the Mediterranean. In this basin, where fisheries are multispecies, multi-gears and multinational, making demersal fishing activities profitable while preserving sea turtles is a challenge. This study aimed to develop bycatch reducer devices (BRDs) and alternative fishing gears to mitigate the impact of demersal fishing gears on sea turtles: (a) hard and flexible turtle excluder devices (TEDs) were tested in bottom trawling to immediately exclude turtles from the net; (b) visual deterrents (ultraviolet LEDs) were used to illuminate set nets and to alter turtle visual cues, avoiding entanglement during depredation activity. The results showed the different devices did not affect the commercial catch, while bycatch reduction was instead evident. Thus, the study highlights that introducing mitigation measures to reduce sea turtle bycatch in the Mediterranean, where the bycatch of vulnerable species seems as a global issue, can be possible at least in certain areas and periods. Considering fishermen reticence to change the gear traditionally used, determining the optimal gear configuration to minimize commercial loss while reducing bycatch, is the main issue while introducing new technologies. Therefore, a global effort should be done to introduce BRDs in different areas and fisheries of the Mediterranean.

Keywords: sea turtle bycatch, bycatch reducer devices, turtle excluder devices, visual deterrents, Mediterranean Sea

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INTRODUCTION

Fisheries in the Mediterranean basin include a wide variety of fishing activities and different gears operating mainly on small and medium scale. The intense fishing pressure is responsible of a general overexploitation status of fish resources (Colloca et al., 2017) and a growing degradation of marine habitats (Lotze et al., 2011). Large vertebrates like sharks (Ferretti et al., 2005), cetaceans (Bearzi et al., 2008), and sea turtles (Casale, 2011) are heavily affected by commercial fisheries, mainly because they are incidentally caught as bycatch. The loggerhead sea turtle (*Caretta caretta*) is the most common species of sea turtle in the Mediterranean, with densities estimated in summer of more than 0.046 individuals km^{-2} (Lauriano et al., 2011).

Caretta caretta is a protected species, included in the list of Annex IV of animals requiring close protection under the Habitat Directive and assessed by the International Union of Conservation of Nature and Natural Resources (IUCN) as "vulnerable" at global scale (Casale and Tucker, 2015) and as "least concern" for the Mediterranean Sea (Casale et al., 2015; Lucchetti et al., 2016b). Lucchetti et al. (2017a) estimated more than 52,000 bycatch events and 10,000 deaths occurring only in Italian waters in 2014, highlighting how fishing activities represent the main threat to the conservation of loggerheads (Lucchetti and Sala, 2010). Individuals frequenting shallow waters in order to feed on benthic species close to the bottom (Tomas et al., 2001; Hochscheid et al., 2013) often interact with fishing gears operating on the seabed (Lucchetti et al., 2016b).

Bottom trawl and set nets fishing are among the most widespread gears targeting demersal stocks, and responsible of high sea turtle bycatch rates in the Mediterranean (Casale, 2011). Bottom trawl is a fishing gear actively towed on the seabed, and turtles accidentally caught during trawling activities have a direct mortality depending on tow duration: the longer the tow duration, the greater the prolonged apnoea and mortality (Sasso and Epperly, 2006). The direct mortality induced by bottom trawling is around 18%. Moreover, delayed mortality due to drowning, metabolic disturbance, decompression sickness upon release (García-Párraga et al., 2014) is suspected to be high. In the North Adriatic Sea (GFCM Geographical Sub-Area 17), where over 1,000 trawlers operate annual turtle bycatch due to bottom trawling has been estimated in 8,600 individuals (Lucchetti et al., 2017a). Passive set nets are among the principal gears used in the small-scale fisheries; set nets targeting demersal fish are fixed on the bottom usually for about 12 h and passively catch fish. An accidentally entangled sea turtle is subjected to forced apnoea due to the long soaking time of the nets and consequent drowning. For the North Adriatic Sea, Lucchetti et al. (2017a) estimated more than 6,200 turtles caught in the west GSA 17 each year with the largest number in summer. The direct mortality caused by passive nets is much higher than bottom trawling and estimated at around 51%.

Possible solutions to avoid bycatch reside in new technological fishing gear improvements and adoption of devices, that may potentially help in reducing turtle entanglement or entrapment and, therefore, animal mortality rates (Casale et al., 2007; Lucchetti et al., 2016b). Recently in Mediterranean some bycatch reducer devices (BRDs) directed at mitigating the fishing impact on sea turtles have been tested. In bottom trawl fisheries, turtle excluder devices (TEDs) consisting in rounded sorting grids with bars (Epperly, 2003), placed before the codend to stop large objects or animals, thus expelling them by an exit, have been successfully experimented (Atabey and Taskavak, 2001; Sala et al., 2011; Lucchetti et al., 2016b). TEDs have become mandatory in several countries in prawn trawl fisheries due to their effectiveness (Lucchetti and Sala, 2010) and preliminary sea trials in the Mediterranean Sea showed good results especially in terms of fishing performance (no loss of commercial catch).

In the last years, the effect of a new prototype of TED, a flexible grid (Flexgrid), on the catching efficiency and performance of a commercial bottom trawl was tested in a gear comparison study for a Mediterranean coastal multispecies bottom trawl fishery (Lucchetti et al., 2016b). Findings demonstrated that this device did not affect neither bottom trawl technical performances (horizontal and vertical net opening and door spread) nor increased the required towing force, hence fuel consumption remained constant. Comparison of commercial catches for the major species showed that the use of this TED did not affect catching efficiency, while it reduced the amount of debris. The device did not influence the size of commercial species, leaving the selective performance of the trawl unmodified. Underwater video camera recordings documented that fish caught in the net swam through the grid and easily reached the cod-end, missing the TED escape opening. Easy storage and handling compared with previous devices tested in this area (Sala et al., 2011) make the flexible TED a practical and valuable solution to reduce turtle bycatch in coastal Mediterranean demersal multispecies fisheries.

In recent years, a potential bycatch reduction strategy for set nets fisheries has been connected to alteration of visual cues with lights. LED lamps and light sticks attached to gillnet float lines (Wang et al., 2010, 2013; Lucchetti et al., 2014; Ortiz et al., 2016; Virgili et al., 2018) have proven to be effective in decreasing turtle bycatch rates while preserving target species catch rates. The bycatch reduction observed ranged from 39.7 to 63.9% for Northern and Southern Pacific coasts by illuminating gillnets with green light (Wang et al., 2010; Ortiz et al., 2016) or UV light (Wang et al., 2013). For the Adriatic Sea (GSA 17), Virgili et al. (2018) observed a bycatch reduction of 100%, using UV light in bottom set-gillnet fisheries in deep waters (>70 m), while the efficiency of commercial catch was maintained.

Taken into account previous BRD tested in the Mediterranean Sea, the present study aims at:

(i) comparing the capture performances of a hard (Supershooter) and a flexible (Flexgrid) TED in bottom trawling;
(ii) assessing the efficacy of UV-LEDs to deter sea turtle bycatch in bottom gillnet fisheries set, to complete what has already been experimented by Virgili et al. (2018).

MATERIALS AND METHODS

Study Area and Period

The study was carried out in the Northern Adriatic Sea, in sandy–muddy bottoms lying south of the Po river mouth (**Figure 1**, Central Mediterranean Sea). This area was selected because its shallow waters and rich benthic communities provide a major feeding habitat for turtles in the demersal stage, especially for the populations nesting in Greece (Lazar et al., 2004; Casale et al., 2012). Since the area is an important fishing ground, the risk of interaction with turtles is high: Lucchetti et al. (2017a,b) estimated more than 6,000 and 8,000 sea turtles are annually caught respectively by set and trawl nets in the North-Western Adriatic Sea, with a mortality rate of 15 and 24%, respectively. Winter time was chosen taking into account that previous studies (Lucchetti et al., 2017a) demonstrated sea turtles heavily interact with fishing gears in this period.

Sea Trials and Gears

Bottom Trawl

Sea trials were conducted in December 2018 aboard a commercial fishing vessel from Porto Garibaldi (142 kW of engine power, 14.7 m length overall, and 12 GT of tonnage), in shallow waters (10–20 m deep) at distance of 5–10 nm off the coast (**Figure 1**). Twin trawl was used to compare directly the traditional trawl (Control) and the nets equipped with the TEDs. Each single net of the twin trawl was a typical "Americana" trawl, which is an asymmetric four-face trawl, generally used in this area to target crustaceans such as mantis shrimp (*Squilla mantis*) and caramote prawn (*Penaeus kerathurus*). This trawl is characterized by having two short bridles (~10 m), high attack angles of the otterboards (40–45°; Lucchetti and Sala, 2012) and tickler chain to increase the fishing performance. A nominal 50 mm diamond mesh codend was mounted in each net [in compliance with the Regulation (Ec) 1967/2006, 2006].

Set Nets

A traditional bottom-set gillnet targeting common sole (*Solea solea*) and mantis shrimp was used for the study. The netting panels, made of transparent polyamide monofilament (diameter, 0.20 mm), were joined in the same gang for a total length of 1,200 m. Each panel was 100 m in length, 2.8 m in stretched net drop and the real vertical opening during fishing was around 1.6 m; the mesh opening was 74 mm. The headline was a 5 mm polypropylene rope with oval floats (15 × 20 mm) 4.6 m apart from each other. The leadline was a 4 mm polypropylene rope weighing 150 g/m. The hanging ratio, i.e., the slack of the netting panel (Lucchetti et al., 2015) was 0.36 and 0.38 for the float line and lead line, respectively.

Experimental trials were conducted on board a fishing vessel (14.7 kW, 6.1 m overall length, 2 gross tonnage) employing gillnets throughout the year in coastal waters (3–5 nm off coast) at a depth of 8–15 m.

BRDs Specifications

Hard and Flexible TEDs

Two different types of TEDs were designed according to the technical specifications suggested by (Mitchell et al., 1995), manufactured and tested at sea. The tested TEDs differed in their material and shape, and were similar for size and bar

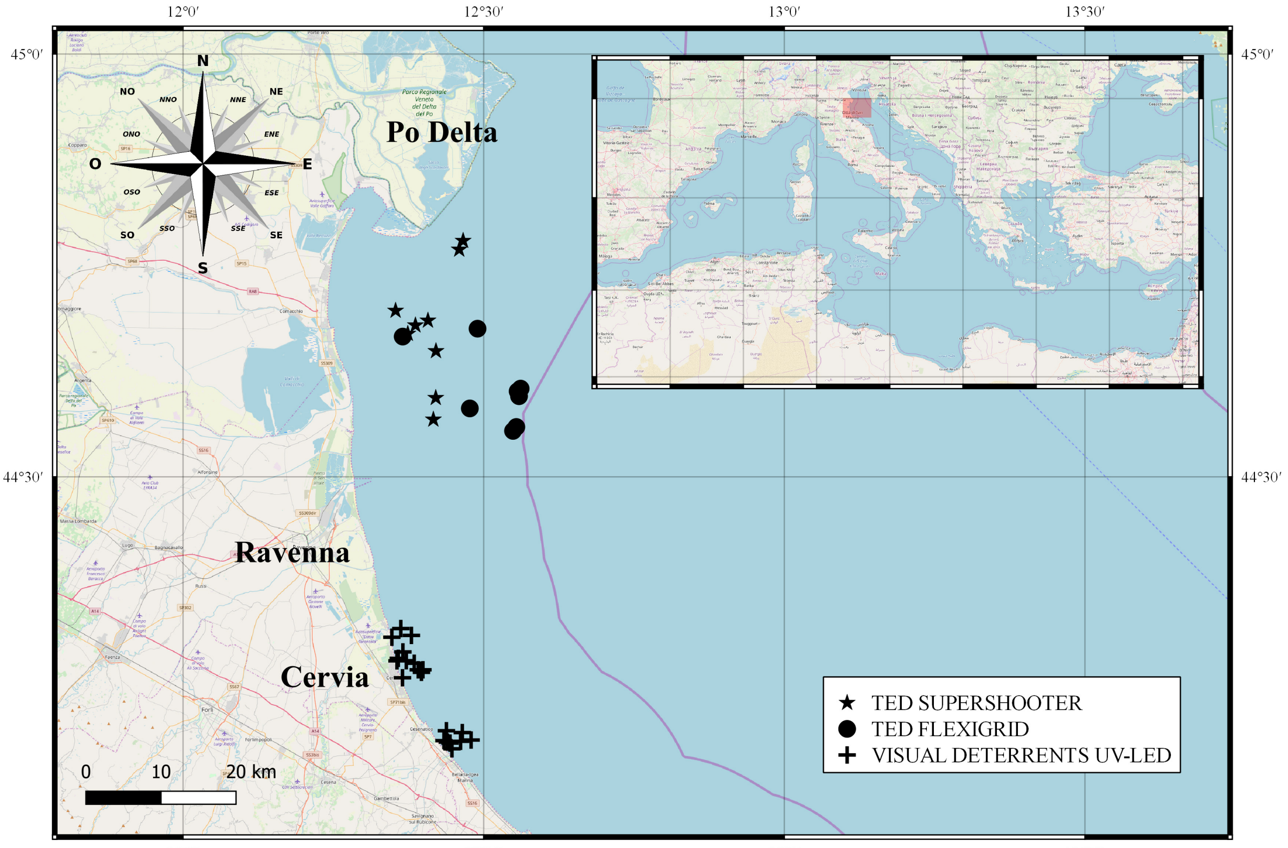

FIGURE 1 | Map of sea trials carried out in North Adriatic Sea with BRDs: sorting grids (TEDs) in bottom trawling and visual deterrents (UV-LEDs) in set net.

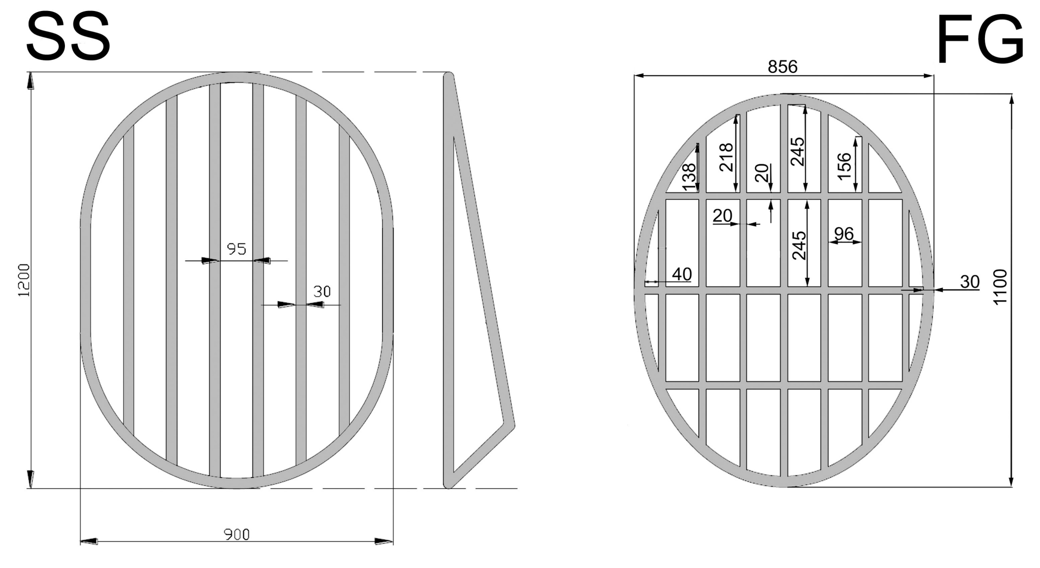

FIGURE 2 | Technical features of the Supershooter (SS) and Flexgrid (FG) TEDs (measurements in mm).

spacing. The hard TED was a classic aluminum Supershooter grid (**Figure 2**), commonly used in shrimp fisheries in several countries. By considering the complex Adriatic fishing composition (crustaceans, molluscs, and fishes caught together), we kept the space between reflector bars greater than in standard models. The Supershooter had the following features: height: 1,200 mm; width: 900 mm; bar diameter: 30 mm; spacing between bars: 95 mm.

The flexible TED (Flexgrid – Comet trawl, Denmark), made of an alloy of high strength plastic material, allowed to maintain a stiff configuration during trawling and to ride on the net drum as the net was recovered. The flexible TED dimensions were: height: 1,130 mm; width: 845 mm; circumference: 3,110 mm; bar diameter: 20 mm; spacing between bars: 96 mm.

Both grids were mounted on a tubular netting section (6 m in length) and placed immediately in front of the codend. An escape opening was cut on the lower portion of the net just before the TED and covered by a netting panel with three sides sewn to the net to prevent loss of commercial species. The fourth side was free and functioned as a valve, as it opened only when it was hit by large and heavy objects, and thus allowing sea turtles and other bycatch species to out the net. In both the TEDs tested, TED angle was set to 45–48°, which is an important factor in preventing commercial losses during the tow (Mitchell et al., 1995; Eayrs, 2007). In fact, an angle less than 40° may involve catch loss due to water diversion through the exit hole. Angles greater than 55° can prevent turtle escape and deflection of trash, clogging the grid.

The use of a twin trawl was the ideal solution to compare directly the catch performance of TED and the traditional net; the net with TED installed was considered as the TEST net, the net without TED was the Control (CTRL) net.

Visual Deterrents

The low water transparency characterizing the study area, which is affected by massive river inputs (Ludwig et al., 2009), prompted the use of UV-LED lamps (Electralume, Lindgren-Pitman, United States). UV-LED lamps perform better than common light sticks, because they provide consistent high-intensity illumination, they last longer, and their light penetrates deeper into the water compared with chemical light sticks. Each lamp is fitted with two batteries that provide approximately 30 days of function. The intensity of the light was checked before each trial and low batteries were replaced as necessary.

The optimal distance between the lamps was established in preliminary tests based on the studies carried out by Wang et al. (2013) and Ortiz et al. (2016), who suggested a distance between lamps of 5 and 10 m, respectively. The negative lamps buoyancy (around 108.6 g each with the batteries) and the low net height required a visual check, to assess that lamp's weight did not reduce the headline floatability, thus impairing fishing performance. Underwater video recordings have shown that setting the distance between UV-LED lamps $\leq$10 m, as suggested by Wang et al. (2013) and Ortiz et al. (2016), would involve an excessive weight on the float line, with a partial closure of the net and a reduction of the fishing surface. Therefore, a distance ~15 m (corresponding to 70 lamps/km) was found to maximize gear performance and illumination as described in Virgili et al. (2018).

Data Analysis

Bottom Trawl

For each haul, the catch was divided into four categories: Commercial species (fish retained and sold), Discards (e.g., fish without commercial value, below the minimum legal size, damaged, skinned, etc.), and debris, which included anthropogenic material (marine litter) and natural material (shells, wood, stones, etc.), PET (Protected, Endangered and Threatened species, including *C. caretta*).

Catches were standardized as:

$$\text{CPUE}_W = W/\left(60'/\text{Tow Duration}\right)$$

where CPUE_W is the catch per unit effort expressed in terms of weight (Kg) per hour of trawling, W is the weight of the catch of each single haul and the Tow Duration is the time the net fished in each single haul expressed in minutes. A One Way ANOVA was applied to compare the commercial, discards and debris CPUE_W among TEDs and control net.

For commercial species, the total length for fish (TL) and the carapace length for crustaceans (CL) were measured on-board the vessels to the nearest 0.5 cm below. To assess the influence of the TED on the size of the species caught, the length frequency distributions (LFD) for the commercial species representing more than 5% of the total catch in weight were analyzed. The generalized linear mixed model – GLMM (with haul as random intercept) was successfully used to fit curves for the expected proportions of the total catch and thus to assess the catch efficiency (at length) of TED relative to CTRL, as suggested by Holst and Revill (2009). The probability of a fish being retained by TED follows from:

$$\Pr\{\text{TED}/(\text{TED}+\text{CTRL})\} = 1/\left(1+e^{-(\beta_0+\beta_1\times\text{length}+\beta_2\times\text{length}^2+\beta_3\times\text{length}^3)}\right)$$

A binomial error distribution was used to calculate the probability of the number of fish caught in the TED gear given they enter both gears by 1-cm size class (1-mm for crustaceans). A probability of 0.5 corresponds to equal catches in both gears. The analyses proceeded as recommended by Holst and Revill (2009), by fitting third order polynomials followed by subsequent reductions until all terms showed significance; this would be adequate for most cases, although in some instances a first or second order would be enough. As suggested by Holst and Revill (2009), the best model is the minimal degree polynomial curve that captures the main trends indicated by the observed proportions. The best model describing the retention probability was decided based on the lowest value of AIC. The species selected correspond to the most important commercial species during the study period: *S. mantis*, *Merlangius merlangus*, and *S. solea*.

Fishing hauls were performed following the fishermen decisions dealing with target species, fishing grounds, tow duration, etc., in order to operate in a commercial situation. According to the fishing practices the Supershooter was used in shallower waters (16 m of depth) than Flexgrid (26 m of depth). Taking into consideration the different fishing grounds, the statistical analysis enabled to directly compare the control net used in coastal waters (TC) against the Supershooter (SS), and the control net used in deeper waters (TO) against the Flexgrid (FG).

All the analyses were performed using the free software R (R Core Team, 2016) and the R packages nlme (Pinheiro et al., 2018) and lme4 (Bates et al., 2015).

The sea turtles eventually caught (as well as other bycatch species) were measured (curved carapace length, CCL, in cm) and weighed, and then rescued as laid down by Italian Ministry of Environment guidelines (Mo et al., 2013).

Set Nets

In each trial, netting panels fitted with UV-LED lamps (*Test* nets) were compared with panels without illumination (*Control* nets). The netting panels with and without the lamps were randomly distributed along the gang, to avoid introducing additional variables. An escape area of about 15 m was left between illuminated and non-illuminated panels, to maximize their separation. The nets were set at sunset and retrieved at sunrise, providing for an average soak time of 10–16 h.

After the net was hauled on board, the catch of the *Test* and *Control* nets was analyzed separately. The catch was sorted into commercial species, discards and PET (including species as *C. caretta, Pteroplatytrygon violacea, Myliobatis aquila, Dasyatis pastinaca, Prionace glauca, Carcharhinus plumbeus*). Commercial, discard, and PET species were classified to the lowest possible taxonomic level, and their number and weight standardized as number and weight per 1000 m of net and 12 h of soak time, as follows:

$$\text{CPUE}_N = N_c/\left[\left(\text{Net Length}/1000\text{ m}\right)\times\left(\text{Net Soak Time}/12\text{h}\right)\right] \quad (1)$$

$$\text{CPUE}_W = W_c/\left[\left(\text{Net Length}/1000\text{ m}\right)\times\left(\text{Net Soak Time}/12\text{h}\right)\right] \quad (2)$$

where CPUE_N is the catch per unit effort expressed as number of individuals and CPUE_W is the catch per unit effort in terms of weight (kg); N_c and W_c are respectively the number and weight of captured individuals.

For commercial species, the total length (TL) of each specimen was measured to the nearest 0.5 cm below. Any sea turtles found in the nets were disentangled after recording their position in the gillnet, measured (curved carapace length, CCL, in cm), weighed, and rescued as above described. Those in good physical condition were released after a period of rest on board (>2 h). The CPUEs of the different categories found in the *Test* and *Control* nets were analyzed using the One Way ANOVA.

Length-frequency distributions (LFD) were analyzed for the target species (*S. solea*). The catch-comparison analysis described for TED vs. Traditional trawl (above described) was applied to assess the influence of LED on the size of common sole caught. The polynomial regression GLMM (with haul as random intercept) was successfully used to fit curves for the expected proportions of the total catch and thus to assess the catch efficiency (at length) of LED relative to CTRL. The probability of a fish being retained by LED follows from:

$$\Pr\{\text{LED}/(\text{LED}+\text{CTRL})\} = 1/\left(1+e^{-(\beta_0+\beta_1\times\text{ length}+\beta_2\times\text{length}^2+\beta_3\times\text{length}^3)}\right)$$

A probability of 0.5 means no difference between the gears.

RESULTS

TEDs in Bottom Trawling

The general details of each haul and the mean catch per gear, categories and species are summarized in the **Supplementary Annex SI**. The two TEDs performed in accordance with their objectives: on average, comparing TC vs. SS and TO vs. FG the discards were reduced and the debris (anthropic litter, shells, stones, etc.) was usually excluded by the escaping window (**Figure 3** and **Table 1**). However, in some hauls both discards and debris showed a great variability, so that the standard deviation was high and the differences were not statistically significant except for the debris excluded by the Supershooter and the discards reduced by the Flexgrid (**Table 1**). The results showed that both TEDs can be used in commercial conditions without negatively affect the commercial catch (**Table 1**).

Constant, Linear, and quadratic models fit catch comparisons for different species (**Table 2**), however, in general, the catch-comparison analysis showed a similar catch performance of TEDs and control nets, because the ratio TED/(TED + CTRL) is almost near the value 0.5 indicating that both nets caught similar number of fishes (**Figure 4**). From the species listed in **Supplementary Annex SII** it is clear there were no differences in the species composition.

During nine hauls performed in coastal waters to assess the performance of Supershooter TED, 10 sea turtles were caught in the control net only. These were juveniles and sub-adults with a carapace length ranging from 33 to 82 cm see **Supplementary Figures S3, S4**. No turtles were observed in the catch of sea trials performed at deeper depth.

UV LEDs in Set Nets

A total of 20 sets (*Test* and *Control* nets) were carried out during June and November 2018. Total net length ranged from 1.2 to 1.8 km (mean 1.53 ± 0.3 km; hereafter mean ± standard error, SE). The mean length of the net portions without and with illumination was 0.76 ± 0.15 km. Mean soak time was 12 ± 1.6 h and mean fishing depth was about 8–10 m.

The mean CPUE_N of the three catch fractions (commercial, discard, and bycatch) neither mean CPUE_W were not significantly different between *Control* and *Test* nets (**Table 3**). Further analysis of the catch of the target species showed that the CPUE was highest for the two target species, *S. solea* and *S. mantis* (**Table 3**), which accounted respectively for 28 and 20% of the catch in terms of number of individuals and for 17 and 9% in terms of weight. Also in this case, the mean CPUEs for the two target species were not significantly different between the nets.

Two loggerheads were caught during the study period (see **Supplementary Figures S1, S2**), both were released in good condition after a period of rest on board. Individuals were juveniles and subadults; their CCL was 23 and 40 cm and weight were 1.4 and 8 kg, respectively. Turtles were caught by the *Control* nets. The mean CPUE_N was 0.14 ± 0.10 and the mean CPUE_W was 0.57 ± 0.45 kg.

Catch-comparison analysis highlights there were no differences by size in the catch performance of net equipped with LEDs and nets in the control configuration; the ratio LED/(LED+CTRL) is almost near the value 0.5 (Intercept: 0.499; **Figure 5**) and a constant model fits the catch comparison for this species (parameter $\beta_0 = -0.065 \pm 0.267$). The list of species reported in the **Supplementary Annex SIII** clearly demonstrates there were no appreciable differences in the species composition.

DISCUSSION

Given the fishermen's reluctance to change their traditional gear, a key aim of this study was to determine the optimal BRDs configuration that would minimize both the loss of commercial

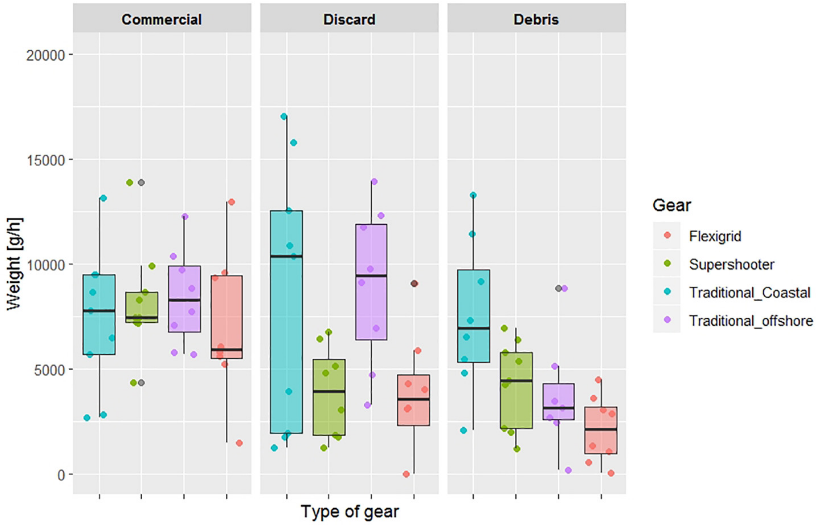

FIGURE 3 | Box plot of the mean catches (g · h^{-1}) and standard deviations of the different categories (Commercial, Debris, and Discards) obtained with the different tested nets.

TABLE 1 | Standardized catch ($g \cdot h^{-1}$) and summary of the One-way ANOVA applied to categories: Commercial, debris, and discard to assess the difference between two types of nets (with and without TED).

Category/Gear	Mean	Ds	ANOVA	Variance component	Sum of squares	df	Mean square	*F*	*p*-value
Commercial									
TC	807.3	1882.8	TC vs. SS	Between net type	3, 615, 155	1	3, 615, 155	0.403	0.534
SS	884.1	2166.1		Within net type	143, 514, 600	16	8, 969, 663		
Debris									
TC	1484.5	2176.8	TC vs. SS	Between net type	111, 304, 929	1	111, 304, 929	5.165	0.037*
SS	673.2	959.5		Within net type	344, 780, 751	16	21, 548, 797		
Discard									
TC	633.7	1450.8	TC vs. SS	Between net type	1, 100, 113	1	1, 100, 113	0.008	0.929
SS	570.4	2989.7		Within net type	2, 152, 522, 760	16	134, 532, 673		
Commercial									
TO	687.9	1462.6	TO vs. FG	Between net type	8, 255, 783	1	8, 255, 783	0.936	0.350
FG	601.3	1413.6		Within net type	123, 484, 567	14	8, 820, 326		
Debris									
TO	888.2	1966.8	TO vs. FG	Between net type	60, 099, 065	1	60, 099, 065	2.344	0.148
FG	484.3	643.6		Within net type	358, 925, 460	14	25, 637, 533		
Discard									
TO	544.0	938.8	TO vs. FG	Between net type	112, 392, 037	1	112, 392, 037	9.809	0.007**
FG	330.4	569.7		Within net type	160, 407, 840	14	11, 457, 703		

TC, traditional net used in coastal waters; SS, net equipped with Supershooter; TO, traditional net used in deeper waters; FG, net equipped with Flexgrid. $^{*}p < 0.05$; $^{**}p < 0.01$.

catch and turtle captures. In the present study, we compared the performance of a rigid Supershooter TED and a flexible TED (Flexgrid) in the Mediterranean bottom twin-trawling. In more detail, we assessed the performances in terms of possible commercial losses and reduction of bycatch, discards, and debris.

Turtle excluder devices are usually designed to reduce sea turtle bycatch but, in the current study, we verified they also reduce the accumulation of debris on the codend, which negatively affects the fish quality by damaging and spoiling fish, crushing crustaceans, etc. Moreover, the presence of a turtle in the codend catch can crush the fish already caught compromising the quality of the catch. In particular, the Supershooter successfully operates by expelling sea turtles from the net and reducing the debris. Innovations of fishing gears can be easily accepted by professional fishermen only if the economic losses are negligible and if the new gears or devices do not involve changes to the on-board procedures. The results show that both tested TEDs reduce debris and discards while keeping the commercial part of the catch unchanged. Discards reduction was statistically significant in the Flexgrid; this is of paramount importance on the light of European Regulation (Ec) 1380/2013 (2013), that introduced a legal framework for discards reduction in the Mediterranean. Present findings confirm the results obtained in the Mediterranean in other studies with Supershooter (Sala et al., 2011) and Flexgrid (Lucchetti et al., 2016b). Atabey and Taskavak (2001) found promising results in the Turkish fishery because their modified Supershooter excluded both loggerhead and green sea turtles (*Chelonia mydas*), as well as unwanted incidental catches such as jellyfish, sharks, and rays.

TABLE 2 | Generalized linear mixed model (GLMM) estimates of the Catch-Comparison.

Species	Test gear	Model	Parameter	Estimate	SE	*p*
Squilla mantis	SS	Linear	β_0	2.557	0.649	<0.001**
			β_1	−0.083	0.023	<0.001**
Squilla mantis	FG	Quadratic	β_0	14.605	4.397	<0.001**
			β_1	−1.053	0.306	<0.001**
			β_2	0.018	0.018	<0.001**
Merlangius merlangus	SS	Constant	β_0	0.209	0.207	0.311
Merlangius merlangus	FG	Quadratic	β_0	−15.392	6.629	0.020*
			β_1	1.291	0.613	0.035*
			β_2	−0.028	0.014	0.051
Solea solea	SS	Constant	β_0	−0.189	0.254	0.457
Solea solea	FG	Constant	β_0	−0.128	0.223	0.568

SS, net equipped with Supershooter; FG, net equipped with Flexgrid. $^{*}p < 0.05$; $^{**}p < 0.01$.

The selective performance of the net was unchanged with the addition of the TEDs. In fact, there was not any appreciable difference in the size frequency distributions of the fish caught nor in the species composition. Even if with slight differences, the catch-comparison analysis showed that the ratio TED/(TED + CTRL) was almost near the value 0.5, indicating that both nets (control net and net with TED) caught similar number of fishes. The results clearly show that the TEDs designed and tested in the current study can be successfully used in coastal trawling, where the most important commercial species have small sizes. Furthermore, the use of TED would lead to a reduction in debris in the codend in an area (the north-western

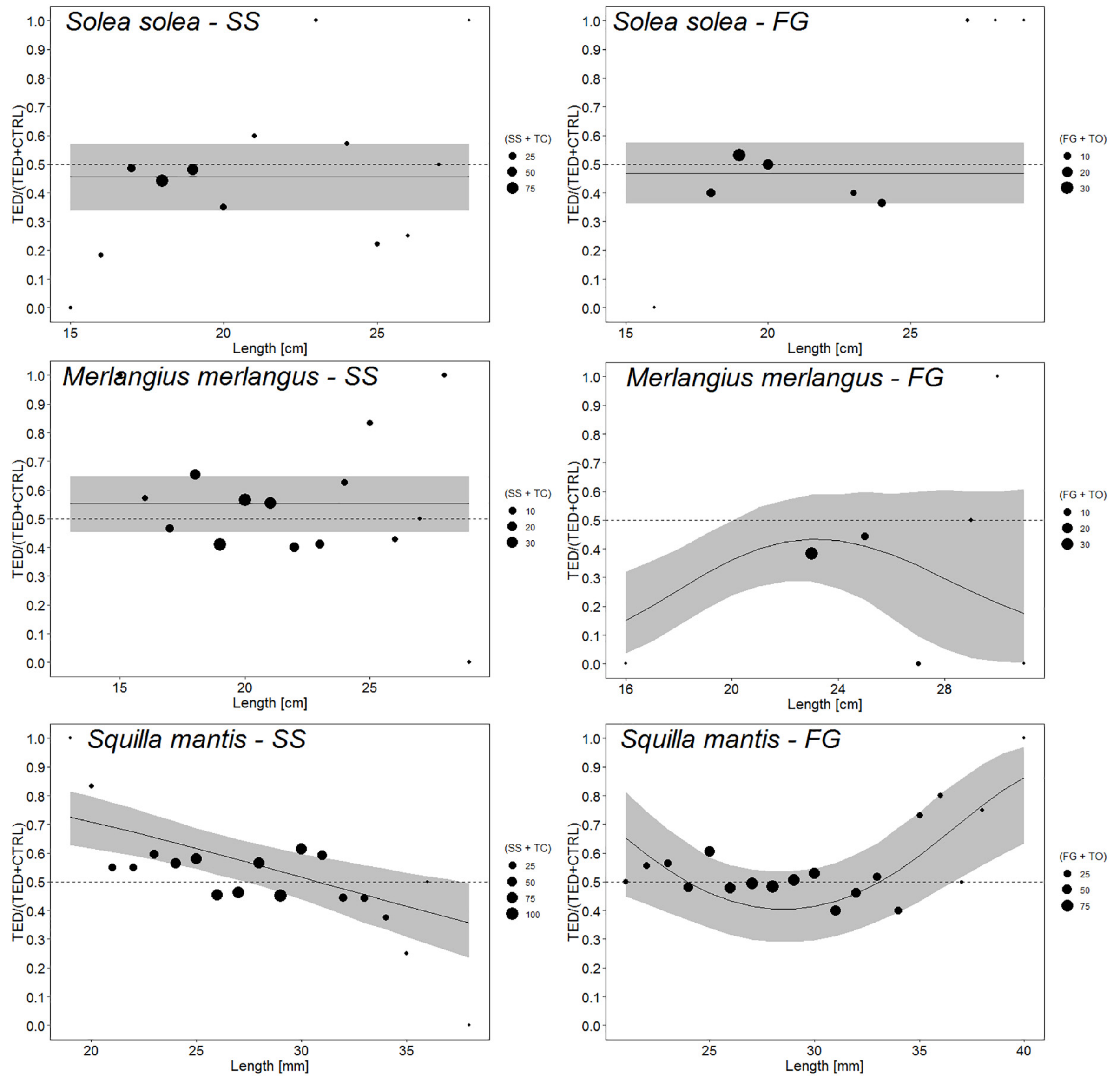

FIGURE 4 | GLMM modeled proportions of the total catches caught by the TEDs. Interpretation: a value of 0.5 indicates an even split between TED and CTRL, whereas a value of 0.25 indicates that the net mounting TED caught 25% of the total fish at that length and 75% were caught in the CTRL net. Shaded area is the 95% confident interval. The size of dots refer to the sum (TED + CTRL) of specimens for each length class.

Adriatic) where the amount of anthropic and natural waste is high due to the massive influx of the Po river (Strafella et al., 2015). Therefore, both TEDs were effective but some logistic and technical aspects could be taken into consideration in choosing the best TED: the Flexgrid was sufficiently stiff to maintain a rigid configuration during towing, and sufficiently flexible for safe winding around a standard net winch. Therefore, it did not require changes to on board procedures and did not induce a loss of time for fishermen during hauling. The Supershooter is made of aluminum and its rigidity would imply a slight change in the procedures on board. On the other hand, two horizontal bars are required in the Flexgrid to maintain the rigidity of the grid during towing; this reduces the space to allow the fish to pass toward the codend compared to the Supershooter.

Sea turtle migrations are strongly linked with sea water temperature and prey availability (Casale et al., 2018). As a possible result of climate changes, the present study confirms that sea turtles winter in the shallow waters of the Adriatic Sea where temperatures fall below 13°C, thus remaining at northern latitudes rather than migrating south and aggregate in small groups, as reviewed by Luschi and Casale (2014) and in accordance with Hochscheid et al. (2007). This is alarming: although sea turtles may adapt to the increasing temperatures, climatic changes in foraging and overwintering habitats will probably negatively affect loggerhead turtle populations nesting

TABLE 3 | Set net catch rates expressed as mean $CPUE_N$ and $CPUE_W$ and summary of the One-way ANOVA applied to categories: Commercial, discard, PET (Protected, Endangered, and Threatened species), and target species to assess the difference between two types of nets (with and without LEDs; Control = without illumination; Test = with UV-LED lamps).

	Category/Gear	Mean	Ds	ANOVA	Variance component	Sum of squares	df	Mean square	*F*	*p*-value
$CPUE_N$	Commercial									
	Control	74.2	96.00	*Control* vs. *Test*	Between net type	406.5	1	406.5	0.061	0.806
	Test	67.8	64.00		Within net type	253,066	38	6659.63		
	Discard									
	Control	4.73	14.00	*Control* vs. *Test*	Between net type	0.07	1	0.07	0.0003	0.986
	Test	4.81	15.43		Within net type	8281.4	38	217.9		
	PET									
	Control	2.21	3.22	*Control* vs. *Test*	Between net type	5.12	1	5.12	0.56	0.459
	Test	1.5	2.77		Within net type	347.44	38	9.14		
$CPUE_W$	Commercial									
	Control	5.98	6.42	*Control* vs. *Test*	Between net type	196,516	1	196,516	0.067	0.798
	Test	6.42	5		Within net type	112,156,000	38	2,951,470		
	Discard									
	Control	0.44	0.67	*Control* vs. *Test*	Between net type	54767.7	1	54767.7	0.129	0.722
	Test	0.36	0.58		Within net type	1,618,750	38	425,986		
	PET									
	Control	4.29	5.77	*Control* vs. *Test*	Between net type	1,682,750	1	1,682,750	0.526	0.473
	Test	2.99	5.5		Within net type	121,573,000	38	3,199,290		
	Solea solea									
$CPUE_N$	*Control*	17.37	25.49	*Control* vs. *Test*	Between net type	366.6	1	366.6	0.324	0.573
	Test	26.93	42.8		Within net type	42989.5	38	1131.3		
$CPUE_W$	*Control*	1.77	2.91	*Control* vs. *Test*	Between net type	2595.43	1	2595.43	0.0004	0.984
	Test	1.76	1.97		Within net type	23,714,000	38	624,054		
	Squilla mantis									
$CPUE_N$	*Control*	16.35	23.43	*Control* vs. *Test*	Between net type	1.12	1	1.12	0.002	0.966
	Test	16.01	25.58		Within net type	22900.3	38	602.64		
$CPUE_W$	*Control*	0.8	1.12	*Control* vs. *Test*	Between net type	332,357	1	332,357	0.199	0.658
	Test	1.01	1.48		Within net type	6,354,450	38	167,222		
	Other species									
$CPUE_N$	*Control*	40.46	77.67	*Control* vs. *Test*	Between net type	1463.33	1	1463.33	0.369	0.547
	Test	28.37	43.48		Within net type	155,031	38	3961.35		
$CPUE_W$	*Control*	3.42	5.2	*Control* vs. *Test*	Between net type	767,872	1	767,872	0.035	0.853
	Test	3.7	4.14		Within net type	83,692,000	38	2,202,420		

in the eastern Mediterranean Basin (Patel et al., 2016). Taking into account the fact that the turtles were only caught in very coastal waters, near mussel farms, these animals are likely to find easy availability of prey near the aquaculture facilities. Lucchetti et al. (2016a) identified the study area as a possible hot spot for bottom trawl-sea turtle interaction, especially at depths shallower than 40 m. In this area, the TEDs tested in the present study were highly efficient. Therefore, the adoption of TED in critical areas and seasons together with other management measures has the potential to provide a substantial contribution to the conservation of *C. caretta* in the whole Mediterranean.

No practical solution to reduce the risk of bycatch due to set nets was available for Mediterranean fisheries until a few years ago, except reducing netting twine thickness or using gillnets instead of trammel nets. The UV-LED lamps tested in the current study did not affect the catch efficiency of the major commercial species. Interestingly, however, they did reduce the interaction with sea turtles, since no loggerheads were found in the illuminated portions of the net. Virgili et al. (2018), tested for

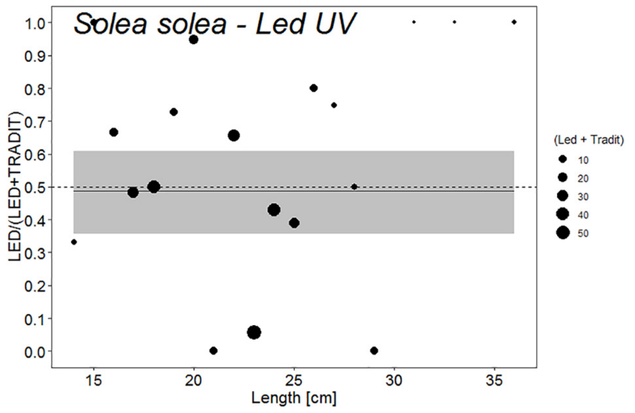

FIGURE 5 | GLMM modeled proportions of the total catches caught by the net equipped with LED. Interpretation: a value of 0.5 indicates an even split between LED and CTRL net. Shaded area is the 95% confident interval. The size of dots refer to the sum (LED + CTRL) of specimens for each length class.

the first time this BRDs applied to gillnets targeting rays in the Adriatic Sea (offshore waters), in an area that has been proven to be a hotspot of sea turtle occurrence (Lucchetti et al., 2017b). The net was illuminated with the same UV-LED lamps used in the present study, which have a longer life and provide greater light intensity than ordinary light-sticks. They obtained the same results, with sea turtle bycatch zeroing and no differences in the commercial catch. Visual deterrents, like chemical light sticks and LED lamps mounted on set nets, have successfully been tested in gillnet fisheries in some areas of the Pacific Ocean, where they have proved effective in reducing the sea turtle bycatch (Wang et al., 2010, 2013; Ortiz et al., 2016). Although further sea trials are needed, UV-LED illumination confirms to be an effective tool to deter sea turtles from approaching set nets in Mediterranean while preserving the commercial catch.

This was a pilot study trying to test the efficacy of TED (in bottom trawl) and lamps (in passive nets) to reduce sea turtle bycatch while keeping unchanged the commercial catch. The study does not claim to be exhaustive and definitive of the problem; however, the results are encouraging and the use of these devices can be replicated efficiently in the main Mediterranean demersal fishing activities that operate with bottom trawls and passive nets. Therefore, before introducing these BRDs in the fisheries mentioned above, it is appropriate to carry out a final distribution of these devices to a reasonable number of vessels.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

Caretta caretta is a protected species, included in the list of Annex IV of animals requiring close protection under the Habitat Directive and assessed by the International Union of Conservation of Nature and Natural Resources (IUCN) as "vulnerable" at global scale (Casale and Tucker, 2015) and as "least concern" for the Mediterranean Sea (Casale et al., 2015; Lucchetti et al., 2016b). In this study, no manipulations were performed on the turtles and no direct interventions were carried out. We only recorded individual capture events. In case of injured turtles observed in the catch, the procedures of the Italian Ministry of Environment guidelines were applied ("*Linee Guida per il recupero, soccorso, affidamento e gestione delle tartarughe marine ai fini della riabilitazione e per la manipolazione a scopi scientifici,*" Mo et al., 2013) and sea turtles were rescued by Authorized Rescue Centres. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed, in particular: Mo et al. (2013). This article does not contain any studies with human participants performed by any of the authors.

AUTHOR CONTRIBUTIONS

AL made the analysis and wrote the manuscript. MV, GB, AP, and CV made the data collection. MV wrote some parts of the manuscript. GB, AP, and CV reviewed the manuscript.

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This research study was authorized by the Italian Coast Guard. It does not necessarily reflect the European Commission's views and in no way anticipates future policy. This support is gratefully acknowledged. We are indebted to the personnel of CNR-IRBIM (Ancona) and CESTHA (Marina di Ravenna), to the crew of FVs Kairos and Stella Maris for their help in fieldwork.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00387/full#supplementary-material

FIGURE S1 | See turtle disentangled from the gillnet during hauling procedures.

FIGURE S2 | See turtle caught in the gillnet tests.

FIGURE S3 | See turtle caught and released by the TED during trawl tests (1).

FIGURE S4 | See turtle caught and released by the TED during trawl tests (2).

ANNEX SI | Details of the hauls performed during the study. TC: Traditional net used in coastal waters; SS: net equipped with Supershooter; TO: Traditional net used in deeper waters; FG: net equipped with Flexgrid.

ANNEX SII | Details of the mean catch and standard deviation (g/h) by categories and gears obtained during the study. TC: Traditional net used in coastal waters; SS: net equipped with Supershooter; TO: Traditional net used in deeper waters; FG: net equipped with Flexgrid.

ANNEX SIII | Details of the mean catch and standard deviation (g/12h*1000m) by categories and gears obtained during the study. CTRL: Traditional net used and UV-LED: set net equipped with visual deterrents.

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Bycatch of Loggerhead Turtle (*Caretta caretta*) in the Italian Adriatic Midwater Pair Trawl Fishery

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Despite the fact that Mediterranean loggerhead turtles are listed as "Least Concern" by the International Union for the Conservation of Nature (IUCN), they are among the most threatened marine megafauna worldwide, because of fishery-related incidental captures. The northern central Adriatic Sea is one of the most overfished basins of the Mediterranean Sea and it supports a very valuable marine biodiversity, including sea turtles. This study assesses the spatial and seasonal impact of the northern central Adriatic midwater pair trawl fishery on loggerhead turtle (*Caretta caretta*) by examining incidental catches recorded between 2006 and 2018 and environmental variables. The model predicted seasonal variation of loggerhead distribution. According to previous studies, data analysis indicates that most bycatch events of loggerhead turtle occurred in the northern Adriatic Sea. The present data confirm that the northern Adriatic may be an important foraging area for loggerhead turtle. They also highlight the urgent need for a better understanding of the interactions between sea turtles and fisheries to develop and apply suitable, *ad hoc* management measures in critical habitats.

Keywords: Adriatic Sea, bycatch, *Caretta caretta*, critical habitats, midwater pair trawl fishery

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INTRODUCTION

The incidental capture of non-target species occurring during fishing operations (e.g., Davies et al., 2009; Ortuño Crespo and Dunn, 2017) is one of the major global threat to marine megafauna of conservation concern (e.g., Worm et al., 2006; Lewison et al., 2014). Among those species, sea turtles are particularly vulnerable to the effects of bycatch given their biological and ecological characteristics (e.g., long life cycle, seasonal distribution patterns, long-distant foraging migrations, etc.) and the intensity of fishing effort across a various range of gears (e.g., Veiga et al., 2016; Lewison et al., 2013; Gray and Kennelly, 2018 and references there in).

The Mediterranean Sea is the world's most overfished sea (Colloca et al., 2017), with the highest bycatch rates of marine turtles (Casale, 2008; Wallace et al., 2013; Casale et al., 2018). The loggerhead turtle (*Caretta caretta*) is the most abundant species regularly found in this area (Tudela, 2004; Lauriano et al., 2011) listed as "Least Concern" by the International Union for the Conservation of Nature (IUCN) Red List (Casale, 2015). Lucchetti and Sala (2010) and Casale (2011) indicated that yearly more than 132,000 sea turtles are unintentionally caught during fishing operations. Hooking, entanglement and capturing by different fishing gears usually intended to catch valuable commercial species are among the major threats to sea turtles from fisheries activities. A number of studies have already investigated the impact of fishing gears on long-lived marine species of conservation concern, like sea turtles (for example, see reviews by Lucchetti and Sala, 2010; Lewison et al., 2013; Wallace et al., 2013). However, more investigations are needed to evaluate how mortality due to interactions with fisheries varies by species and gear type.

The northern central Adriatic Sea is the most heavily impacted basin in the Mediterranean Sea due to a variety of sources of anthropogenic pressure, mainly intense fishing activities, large urbanized and industrialized areas, and environmental pollution (Lotze et al., 2011; Giani et al., 2012; Romano and Zullo, 2014; UNEP-MAP-RAC/SPA, 2014). This basin supports a rich and valuable marine biodiversity including marine megafauna like loggerhead turtle. Interactions between this species and fisheries are therefore unavoidable. Indeed, a moderate-to high bycatch risk of loggerheads has recently been suggested in the northern Adriatic for bottom trawls (Lucchetti et al., 2016) and estimated from interview based approach for set nets (Lucchetti et al., 2017). Nevertheless, data on the extent of incidental catches of sea turtles in other gears in the Adriatic is still limited (Lazar and Tvrtkovic, 1995, Lazar and Tvrtkoviæ, 2003; Lazar et al., 2006; Fortuna et al., 2010).

Since 2006, an extensive monitoring program of bycatch of long-lived species like cetaceans, sea turtles, and elasmobranchs by Italian midwater pair trawlers has been conducted in the northern central Adriatic Sea (Fortuna et al., 2010; Sala et al., 2016, 2018). The information collected in its framework provides a unique opportunity to assess the operational details of capture events and the abundance trends of species over time (Bonanomi et al., 2018).

The present study provides a spatial and seasonal evaluation of the impact of midwater trawling on loggerhead turtles in the northern central Adriatic Sea with the aim to assess the effect of environmental variables influencing the presence of this species and provide a risk map of the interaction between turtles and fishery basing on fishery dependent data. The results of this study can contribute to improve spatial fisheries management in critical habitats and to develop measures aimed at reducing bycatch events of loggerhead turtle in northern central Adriatic Sea.

MATERIALS AND METHODS

Study Area and Data Collection

The Italian midwater pair trawl fishery is based in the northern central Adriatic Sea, Geographical Sub-Area (GSA) 17 (**Figure 1**). The area is characterized by shallow waters, with an average depth of 35 m. The strong influence of the Po river discharge results in low salinity, low water temperature, and high nutrient concentration (Fonda Umani, 1996; Marini et al., 2008; Lipizer et al., 2014). GSA 17 includes the entire northern and central Adriatic Sea as far as the Gargano Promontory in Italy and the city of Kotor in Montenegro.

Between April 2006 and December 2018, a total of 15,975 hauls from 3,975 fishing trips, (see **Table 1**) conducted in the northern central Adriatic Sea, were monitored by independent observers who collected bycatch data of protected species (e.g., cetaceans, sea turtles) and species of conservation concern (e.g., elasmobranchs). The program took advantage of the mandatory monitoring program conducted under permit issued by the Italian Ministry of Agriculture, Food and Forestry, Fishery and Aquaculture directorate in compliance with the Italian obligations to the Council Regulation (EC) 812/2004 and with the EU Data Collection Framework. For each haul, observer recorded operational parameters including coordinates (latitude and longitude in WGS84), date and time of net setting and hauling, haul duration, trawling speed (nm) and water depth (m). For each haul, the observed Catch Per Unit Efforts (CPUEs) of loggerhead turtle were calculated as the number of individuals caught divided by the duration of the haul (in hours).

The influence of environmental variables, potentially affecting the presence of loggerhead sea turtles caught during fishing operations, were investigated. Information regarding the distribution and extension of seabed substrates were obtained from European Seabed Habitat map provided by EMODnet portals[1]. Sea Surface Temperature (SST) and chlorophyll concentration (CHL) data were retrieved from the European Union's Earth Observation Program[2]. Both variables were obtained from satellite-based observations and they were available at different temporal (daily or monthly) and spatial resolution (depending by variable and year). In this study, monthly values were considered for both variables, then, when they were available at daily scale they were averaged at monthly base. Furthermore, to uniform the spatial information, they were rescaled to the same spatial resolution (spatial grid of 0.089°, ∼10 km). For each hauls, the coordinates and date were used to extract the corresponding values of environmental variables.

CPUEs Modeling

Catch Per Unit Efforts represents a common method to summarize fishery data, providing a measure of relative abundance. However, multiple drivers, mainly related to operational and environmental factors, can affect catch rate limiting the relationship between CPUE and abundance (Walters, 2003; Maunder et al., 2006). Generally, CPUE data are modeled to address the effects of these factors affecting catch rate (Maunder and Punt, 2004) but also to analyze species distribution in relation to this fishery's operations (Katsanevakis et al., 2009; Grüss et al., 2014; Parra et al., 2017; Orio et al., 2017). In the case non-target species, which are caught less frequency than target species, modeling problems are related to the excess of zero observation (no accidental captures), due to the low catch rate, rarity of the non-target specie and low interaction with fishing gears (Maunder and Punt, 2004; Minami et al., 2007; Wenger and Freeman, 2008). Furthermore, another modeling problem is related to the over dispersion of the data, mainly caused by the stochasticity of the magnitude of bycatch events (Brodziak and Walsh, 2013).

In this study, spatiotemporal assessment of loggerhead turtles CPUEs was modeled using Generalized Additive Models

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[1] https://www.emodnet-seabedhabitats.eu/

[2] http://marine.copernicus.eu/services-portfolio/access-to-products/

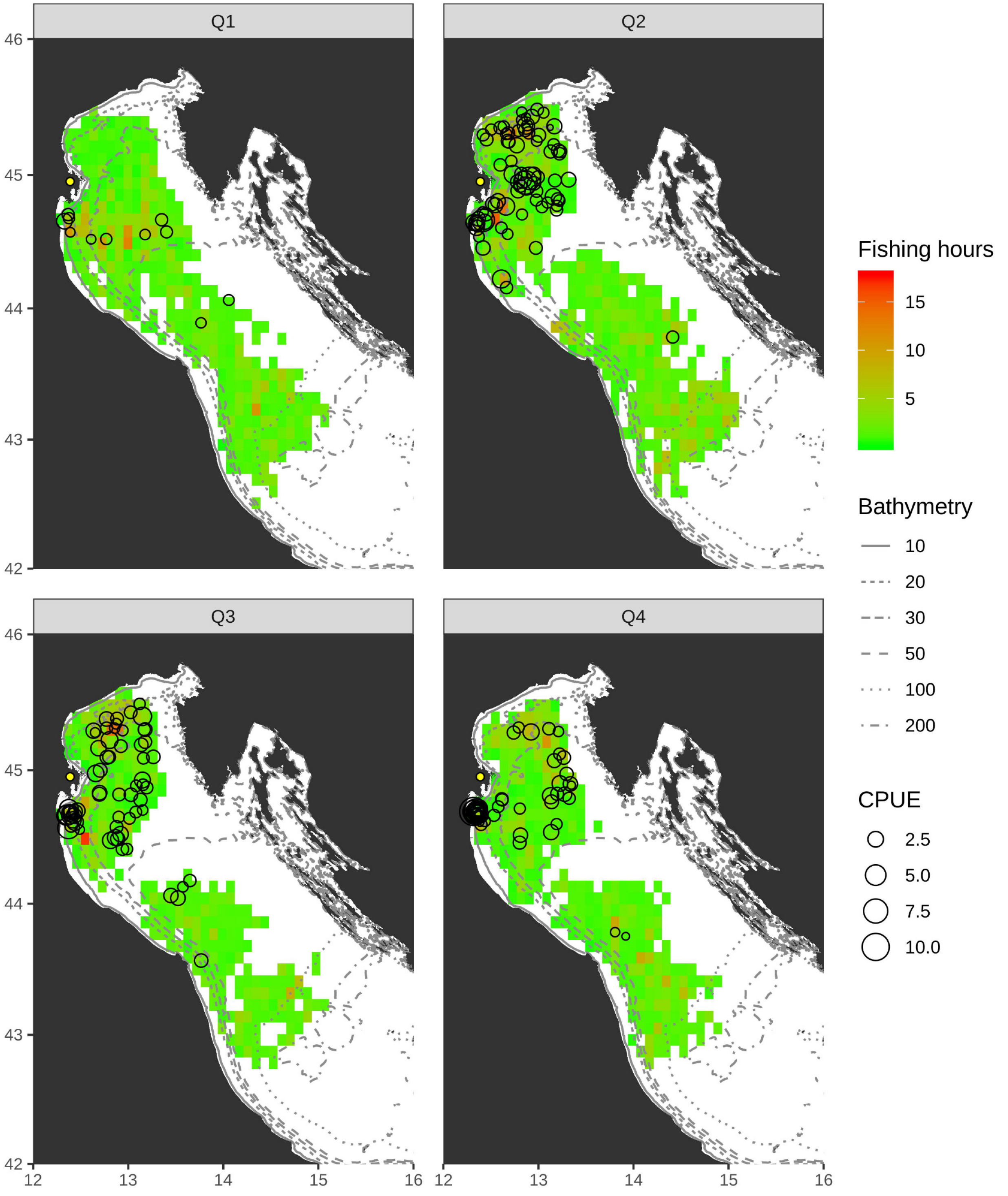

FIGURE 1 | Distribution of hauls and bycatch events of *Caretta caretta* collected between 2006 and 2018 aggregated at quarterly base using a spatial grid with cell of 0.09° (about 10 km). The intensity of fishing hours is showed with a color gradient from green (0–5) to red (10–15). The size of circles indicates the magnitude of bycatch events (CPUE). The River Po delta is illustrated with a red point.

(GAM) (Hastie et al., 2001) in a delta modeling framework. This approach has been proposed as a useful tool in case of zero-inflated data (Maunder and Punt, 2004), and it was successfully used to assess spatiotemporal distribution of marine species worldwide (Punt et al., 2000; Rodríguez-Marín et al., 2003) and, it was applied in other study on the distribution

TABLE 1 | Summary table of the dataset used in the analysis.

				Longitude		Latitude		Depth				Bycatch			
Year	**Quarter**	**Fishing Trips**	**Hauls**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Events**	**Probability**	**Probability (SD)**	**Individuals**	**CPUE**	**CPUE (SD)**
2006	Q3	53	277	12.34	13.24	44.44	45.53	12.4	39.6	8	0.029	0.010	15	0.082	0.542
2006	Q4	92	360	12.36	14.65	42.79	45.48	14.6	96	8	0.022	0.008	15	0.042	0.338
2007	Q1	51	142	12.37	14.84	42.87	44.70	14.8	137.6	1	0.007	0.007	1	0.009	0.112
2007	Q3	35	124	12.37	13.28	44.36	45.26	15	41.4	11	0.089	0.026	13	0.162	0.590
2007	Q4	121	467	12.35	14.28	42.84	45.41	12.4	88.6	13	0.028	0.008	21	0.055	0.358
2008	Q1	137	442	12.34	14.62	42.50	45.30	13	115.4	0	0.000	0.000	0	0.000	0.000
2008	Q2	141	559	12.35	15.08	42.63	45.49	12.8	166.8	6	0.011	0.004	6	0.016	0.162
2008	Q3	91	338	12.35	14.84	42.87	45.53	14.6	106.6	7	0.021	0.008	9	0.048	0.370
2008	Q4	63	226	12.37	14.58	43.19	45.47	13.2	98.2	2	0.009	0.006	3	0.012	0.140
2009	Q1	9	28	12.40	12.96	44.55	45.34	16.4	39.2	0	0.000	0.000	0	0.000	0.000
2009	Q2	9	41	12.35	14.96	43.48	45.02	11.8	102	0	0.000	0.000	0	0.000	0.000
2009	Q3	42	157	12.32	14.38	43.61	44.95	11.6	77	5	0.032	0.014	5	0.043	0.246
2009	Q4	99	314	12.32	14.53	42.85	45.31	11.8	194.4	4	0.013	0.006	7	0.034	0.391
2010	Q1	158	503	12.34	14.98	42.81	45.44	12.8	141.4	0	0.000	0.000	0	0.000	0.000
2010	Q2	143	567	12.33	14.80	42.75	45.53	11.2	131	7	0.012	0.005	8	0.018	0.185
2010	Q3	78	349	12.35	14.25	43.39	45.51	13	84.8	8	0.023	0.008	8	0.034	0.240
2010	Q4	140	475	12.34	14.76	42.88	45.43	12.6	140.2	5	0.011	0.005	7	0.022	0.262
2011	Q1	49	142	12.40	13.69	43.79	45.34	21	58	0	0.000	0.000	0	0.000	0.000
2011	Q2	86	385	12.38	14.86	42.93	45.57	15.2	139.6	4	0.010	0.005	5	0.027	0.343
2011	Q3	54	218	12.37	14.68	42.78	45.52	13.4	150.8	0	0.000	0.000	0	0.000	0.000
2011	Q4	189	794	12.32	14.82	42.74	45.43	11	156.4	8	0.010	0.004	12	0.020	0.232
2012	Q1	143	452	12.33	14.79	42.79	45.29	12.2	110.4	3	0.007	0.004	3	0.007	0.088
2012	Q2	159	644	12.33	15.00	42.83	45.53	11.6	213.8	11	0.017	0.005	12	0.026	0.210
2012	Q3	48	185	12.42	14.44	43.12	45.49	20.6	92.2	7	0.038	0.014	7	0.074	0.414
2012	Q4	153	563	12.40	14.46	43.07	45.47	17.6	100.6	9	0.016	0.005	10	0.030	0.246
2013	Q1	109	411	12.32	14.20	43.53	45.42	11.6	80	2	0.005	0.003	2	0.005	0.080
2013	Q2	142	642	12.32	14.29	43.53	45.51	10.4	83.4	30	0.047	0.008	38	0.098	0.535
2013	Q3	49	230	12.45	14.27	43.24	45.49	14.4	82.6	5	0.022	0.010	6	0.037	0.261
2013	Q4	53	222	12.48	14.44	42.91	45.45	21.6	107	2	0.009	0.006	2	0.013	0.145
2014	Q1	64	187	12.31	15.02	42.74	45.02	10.6	225	4	0.021	0.011	4	0.028	0.215
2014	Q2	19	58	12.31	12.84	44.21	44.70	11	37	1	0.017	0.017	1	0.021	0.161
2014	Q4	1	2	14.17	14.18	43.05	43.12	76	76.6	0	0.000	0.000	0	0.000	0.000
2015	Q1	39	120	12.33	14.67	42.93	45.42	11.6	137.4	1	0.008	0.008	1	0.009	0.101
2015	Q2	151	494	12.31	14.96	42.62	45.49	10.4	126.6	7	0.014	0.005	7	0.027	0.228
2015	Q3	75	267	12.41	14.62	42.95	45.46	16.8	126.6	5	0.019	0.008	6	0.038	0.294
2015	Q4	147	481	12.32	14.85	43.01	45.40	11.4	128	3	0.006	0.004	8	0.042	0.596
2016	Q1	39	137	12.34	14.13	43.31	45.39	12.4	73.2	0	0.000	0.000	0	0.000	0.000
2016	Q2	45	162	12.32	13.96	43.83	45.14	11.6	70	1	0.006	0.006	1	0.011	0.135
2016	Q3	44	142	12.42	14.16	43.30	45.33	15.2	78.6	3	0.021	0.012	3	0.043	0.299
2016	Q4	68	239	12.34	14.43	43.01	45.26	14	90.8	0	0.000	0.000	0	0.000	0.000
2017	Q1	56	171	12.32	15.04	42.62	45.40	11.2	149	0	0.000	0.000	0	0.000	0.000
2017	Q2	71	265	12.32	15.07	42.92	45.47	11.4	149.4	2	0.008	0.005	2	0.011	0.128
2017	Q3	35	138	12.37	15.05	42.90	45.36	13.8	147.2	1	0.007	0.007	1	0.017	0.196
2018	Q2	106	367	12.34	14.57	42.98	45.47	11.8	131.2	10	0.027	0.008	15	0.053	0.356
2018	Q3	72	239	12.33	14.88	42.89	45.46	12.4	123.8	5	0.021	0.009	5	0.039	0.266
2018	Q4	137	449	12.42	14.92	43.03	45.41	14.4	140.8	1	0.002	0.002	1	0.002	0.036

Data collected between 2006 and 2018 were reported by year and quarter. Irregular fishing operations and fishing hauls performed below 43.75° of latitude were excluded from the raw dataset.

of no-target species in this area (La Mesa et al., 2016). Delta modeling procedure allows to modeling the probability of species occurrence and magnitude of catch events (CPUEs) separately, involving two modeling components (Maunder and Punt, 2004). The first component uses the occurrence or non-occurrence of bycatch events to estimate the probability of encountering a loggerhead turtle. Bycatch probability was modeled using a binary response variable (coded as 1/0) with a binomial error distribution and logit link. The second component considers only the hauls with positive catch, to assess the magnitude of bycatch events (CPUE) using a Gamma error distribution model with log link function. The Gamma distribution was commonly used in skewed data, like those occurring in bycatch data (Punt et al., 2000; Maunder and Punt, 2004). Finally, the predicted probabilities of presence were multiplied by the predicted values of positive catch, to obtain predicted CPUEs.

Before proceeding, a preliminary analysis on raw data to select regular hauls (i.e., fishing operations that were successfully completed) and excluding those with erroneous and/or lacking values (e.g., position on land, missing depth values, etc.) was performed. Bycatch events with more than four individuals account for less the 1% of the cases (two events), thus events with more than four individuals were grouped together in extra-group signed as five individuals and CPUE were estimated again. An exploratory data analysis was performed to accommodate the spatio-temporal structure of the data and identify the set of variables to use in CPUEs modeling.

Delta modeling approach allows to model bycatch probability and CPUEs with different set of variables. The initial models used were as follows:

$$\begin{aligned} \mathrm{Y} = {} & \beta_{\mathit{0}} + \beta_{\mathit{1}} \times \mathit{Quater} + \beta_1 \times \mathit{Substrate} + f_1(\mathit{Year}) + \\ & s_1(\mathit{Lon}, \mathit{Lat}) + te_1(\mathit{Lon}, \mathit{Depth}) + te_2(\mathit{Lat}, \mathit{Depth}) + \\ & f_2(\mathit{Depth}) + te_3(\mathit{Lon}, \mathit{CHL}) + te_4(\mathit{Lat}, \mathit{CHL}) + \\ & f_3(\mathit{CHL}) + f_4(\mathit{SST}) + \varepsilon \end{aligned} \tag{1}$$

$$\begin{aligned} \mathrm{Y} = {} & \beta_0 + \beta_1 \times \mathit{Quarter} + \beta_2 \times \mathit{Substrate} + \\ & f_1(\mathit{Year}) + s_1(\mathit{Lon}, \mathit{Lat}) + s_2(\mathit{Lon}, \mathit{Lat})\,\mathit{Depth} + \\ & s_3(\mathit{Lon}, \mathit{Lat})\,\mathit{CHL} + f_2(\mathit{SST}) + \varepsilon \end{aligned} \tag{2}$$

Where Y represents the encountering probability of bycatch event in (Eqs 1), or its magnitude (CPUEs) in (Eqs 2); β_0 is an overall intercept, β_1, $\beta_2$are coefficients estimated for each level of the factors considered in the parametric component of the models; s is an isotropic smoothing function (thin-plate regression spline; Wood, 2003), te represents a tensor product smoothing function used for interaction between variables; f_i are natural cubic line and ϵ are error terms.

Model selection was performed through a backward stepwise selection based the total explained Deviance and on statistical significance (Wood, 2006). Starting from the full models, the predictor with the lowest significant level, was excluded and the model was run again until all remaining predictors were significant. During the model selection procedures, different types of interaction between variables were tested in order to improve models fitting maintaining the initial ecological assumptions. Furthermore, the maximum degree of freedom of the smoothing functions (number of knots k) were limited for smoothers of single variables ($k = 5$) and for interactions ($k = 25$) to avoid overfitting. The χ^2 test was used to estimate the statistical significance of each term. Spatial prediction of CPUEs was performed using a spatial grid of 0.089° (∼10 km) at seasonal scale considering annual quarter (Q1: winter; Q2: spring; Q3: summer, Q4: autum). Excluding depth, the environmental variables associated to grid were averaged in each cell considering the values observed along the whole period.

The statistical significance level assumed in all the analyses was 5%. Data exploration was carried out with R version 3.4.4 (R Development Core Team, 2008), the *mgcv* package (Wood, 2011) was used for modeling data.

RESULTS

A summary of raw data is reported in **Table 1**. Between 2006 and 2018, 14,170 successful hauls were monitored during 3,865 fishing trips. During this period, a total of 291 loggerhead turtles were unintentionally caught; the overall observed annual average probability of occurrence of bycatch events was 0.016 per haul with a mean CPUE value of 0.03 individuals per hour. The 90% ($n = 262$) of loggerheads were released alive without showing any injuries. The geographical distribution of quarterly monitoring effort and bycatch events (with CPUE values) are illustrated in **Figure 1**. The southernmost bycatch event was recorded at 43.56° of latitude. Most events (about 90%) were recorded in the northernmost area of the Adriatic Sea (between 44.5° and 45.5°), in the southern area (below 44.5°) were recorded 10 events in the whole period.

The final model used to predict the probability of the occurrence of bycatch events (binomial) includes 6 variables with the following formula: $\mathit{Presence} \sim \mathit{Year} + s(\mathit{Lon}, \mathit{Lat})\mathit{Quarter} + te(\mathit{Lat}, \mathit{Depth}) + te(\mathit{Lon}, \mathit{Depth}) + te(x, \mathit{CHL}) + f(\mathit{SST})$ (**Table 2**). It explains about 18.4% of the total deviance and the Anova χ^2 test indicates that all terms were significant, except the interaction term between hauls location in the third and fourth quarter. The model-derived effects of covariates used in the binomial model area reported in **Supplementary Figure 1**. All the interactions, latitude-depth, longitude-depth and longitude-CHL, were retained in the model, as well as the effect of SST. In the binomial model, the effect of the year was considered in the parametric component of the model. The final model used to predict abundance of bycatch events (Gamma) includes four variables with the following formula: $\log(\mathit{CPUE}) \sim \mathit{Quarter} + f(\mathit{Year}) + s(\mathit{Lon}, \mathit{Lat})\mathit{CHL} + s(\mathit{Lon}, \mathit{Lat})$ (**Table 2**). It explains about 40.6% of the total deviance and the Anova F test indicates that all terms were significant. The model-derived effects of covariates used in the Gamma error distribution model and residual plots area reported in **Supplementary Figures 2**, **3**. The interaction between hauls position, and longitude-latitude with chlorophyll concentration were retained in the model. The effect of the year was considered as a smoothing function, while the effect of quarter was considered in the parametric component of the model. In the abundance model the global effect of depth and sea surface temperature were not significant and they were discarded.

The prediction of the final models on the spatial grid are showed at quarterly base in **Figure 2**. The predicted values were

TABLE 2 | Results of generalized additive models building for factors affecting the presence/absence and the abundance of loggerhead turtles in the northern central Adriatic Sea between 2006 and 2018.

Model	Parametric terms	df	Chi square	Significance level
Binomial	*Yearf*	12	54.95	<0.01
	Smooth terms	**edf**	**Chi square**	**Significance level**
	s(Lon, Lat)Q1	2.013	16.517	<0.01
	s(Lon, Lat)Q2	7.033	46.663	<0.01
	s(Lon, Lat)Q3	0.231	0.254	0.086
	s(Lon, Lat)Q4	1.451	3.015	0.05
	te(Lat, Depth)	3.816	16.398	<0.01
	te(Lon, Depth)	3.193	11.325	<0.01
	te(Lon, CHL)	5.019	30.31	<0.01
	te(SST)	1.688	11.869	0.001
	Total Deviance explained	18.4%		
	N	14170		
Gamma	**Parametric terms**	**df**	**F**	**Significance level**
	Quarter	3	5.56	<0.01
	Parametric terms	**df**	**F**	**Significance level**
	s(Year)	2.87	3.15	<0.01
	s(Lon, Lat)CHL	9.46	1.05	<0.01
	s(Lon, Lat)	5.88	0.76	<0.01
	Total Deviance explained	40.80%		
	N	291		

divided in four classes: from 0 to 0.009 (very low CPUE, <1 individual caught every 100 fishing hours), from 0.01 to 0.04 (low CPUE, between 1 and 4 individuals every 100 fishing hours), from 0.05 to 0.09 (Medium CPUE, between 5 to 9 individuals every 100 fishing hours) and between 0.1 to the maximum value (High CPUE, ≥10 individuals every 100 fishing hours). In winter (Q1), low CPUEs were predicted in cells in the eastern Adriatic Sea while high values were predicted in cell displaced south of the River Po delta. During spring (Q2), increasing values were predicted in the northern area (above 44° of latitude) with high values predicted in the cell of the western central and eastern central Adriatic. Medium and low values were predicted in cells in the northern and central Adriatic. In summer (Q3), high CPUEs were predicted in the cells south and north of the River Po delta and in a few cells in the western-central Adriatic. Very low values were predicted below 43.5° of latitude. During autumn (Q4), high and medium CPUEs were predicted in few cells in the area south and north of the River Po delta and in the eastern Adriatic, low values were predicted in cells of the northern area and in few cells toward western-central Adriatic.

DISCUSSION

Robust data on spatial patterns of the risk of negative interactions between anthropogenic activities and protected species are required for Environmental Risk Assessments (see, for example, Azzellino et al., 2011). This must be true for fishery management frameworks too, which should be informed by a wide range of information, including spatial patterns of interactions and biological data on concerned species. However, the spatial element is seldom integrated in such frameworks, usually more oriented to consider only aspects related to species biology and population dynamics (e.g., ICES, 2014; Lucchetti et al., 2016). Bycatch Risk Assessments should incorporate the spatial dimension to inform area-based mitigation options, if they were to be effective tools to manage activities within marine regions (e.g., Azzellino et al., 2012). This study is among one of the first to provide an evaluation of the distribution of incidental capture of loggerhead turtle in a fishing métier for a Mediterranean sub-basin. It also provides the first maps of risk that are necessary to inform the process of designation and management of new Natura 2000 sites (Fortuna et al., 2018). In the study area, the interactions between loggerhead turtle and fishing gears have usually been ascribed to bottom trawlers (Casale et al., 2004; Lucchetti and Sala, 2010; Lucchetti et al., 2016), midwater pair trawlers (Casale et al., 2004; Fortuna et al., 2010), rapido (beam) trawlers (Lucchetti et al., 2018), and set nets (Lucchetti et al., 2017). The 13-year data in this study show that loggerheads were caught by midwater pair trawlers with a relative low rate (observed annual mean probability of 0.016 per haul). The monitoring activity demonstrated that a large number of individuals were incidentally caught and released alive without showing any injures. These findings indicate that interactions between loggerheads and midwater pair trawlers is moderate likely with very low mortality rate. In this study commercial CPUEs give an insight on the distribution of interactions between loggerhead turtles and fishing activities of midwater trawlers. They allow the assessment of the effect of operational and environmental variables on the interactions, by highlighting high and low risk areas. To accommodate the large amount of zero observations (about 98% of the records), data were analyzed by separately modeling the probability of occurrence of bycatch events (binomial model) and the positive catch (Gamma error distribution). Overall, the total Deviance explained by models was low and the explained variation was two times higher in the Gamma model. However, spatial and temporal assessment predicted by the final models seems to capture the global patterns present in the data. Different set of variables were used to modeling presence and the abundance patterns. The effects of operational variables, as location (i.e., coordinates of fishing operations) and time (year, quarter), resulted significant in both modeling components, as a consequence of the high spatiotemporal variability of the observed pattern. Depth and chlorophyll concentrations seem to be the most significant environmental drivers affecting the probability of bycatch events and their magnitude. Accordingly, the predicted spatial patterns of bycatch probability were concentrated in the northern Adriatic Sea, above 44.5° of latitude, nearby the River Po delta. This area is characterized by relative shallow waters (average depth of 30 m) and it is strongly influenced by the inter-annual freshwater discharge of the River Po, which is the primary source of nutrients in the whole basin (see **Supplementary Figure 4**; Degobbis and Gilmartin, 1990; de Wit and Bendoricchio, 2001)

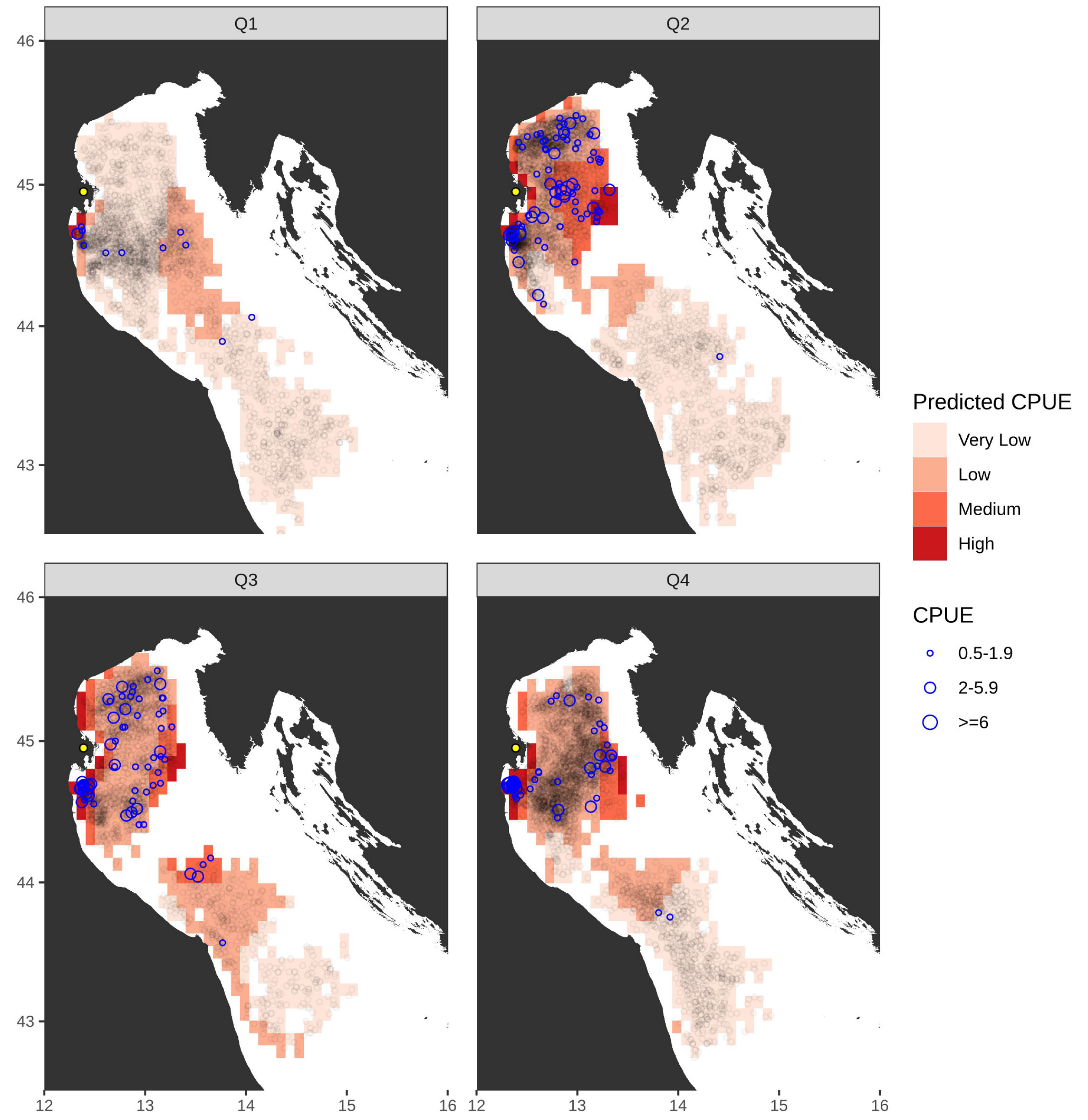

FIGURE 2 | Distribution of predicted CPUEs aggregated at quarterly base using a spatial grid with cell of 0.09° (about 10 km). The predicted CPUEs are showed using tiles with a color a red gradient from light red (very low) to dark red (high). The observed CPUEs are represented with blue circles and their size indicates the magnitude. Position of fishing hauls with zero catches are represented with black circles. The River Po delta is illustrated with a yellow point.

and makes the northern Adriatic one of the most productive area in the Mediterranean. Nutrient inflow, sustaining a high biodiversity community, makes this area important for turtles' trophic interactions with abundant presence of preys like jellyfish, crustaceans and molluscs (Casale et al., 2012). The analyses presented in this study confirmed that the northernmost of Adriatic basin is a suitable foraging habitat for loggerheads according to previous studies (Lazar and Tvrtkovic, 1995, Lazar and Tvrtkoviæ, 2003; Lazar et al., 2006; Casale and Margaritoulis, 2010; Rees et al., 2017; Casale et al., 2018). In this area, the presence of a foraging ground identified nearby the Po delta, was probably the major ecological driver of the presence of this species, increasing the probability of unintentional catches particularly during spring and summer. Accordingly, in the southernmost area few individuals were accidentally caught.

Analysis of the present dataset indicates that probability of bycatch events increases between April and September (Q2–Q3). In accordance with these results, increasing CPUEs were predicted in the northernmost area of the Adriatic Sea, and then in autumn their values decrease in the central zone. These patterns are in accordance with previous investigations suggesting seasonal migration patterns in the Adriatic toward the River Po delta (Casale et al., 2012; Luschi and Casale, 2014; Lucchetti et al., 2016). Based on these studies, along the Italian coast, loggerheads during spring swim from the southern Adriatic toward the Po delta; in summer they frequent the northern area and begin to migrate southward; in autumn loggerheads can be find in the whole Adriatic with a concentration in the coastal area south of the River Po delta. During winter the probability of bycatch events and CPUEs decrease because low temperatures can influence loggerheads behavior, frequenting the northernmost part of the basin, to move southward (Casale et al., 2012).

In addition, the model predicted relative high CPUEs from spring to autumn in the eastern-north of the study area. This predicted patterns, could be explained by factors related to seasonal movement of loggerheads in relation to marine currents. Although sea turtles can travel against sea currents (Meylan, 1995), favorable surface circulation may facilitate their migration. A prevailing surface current enters the Adriatic along the eastern coast moving to the north (Cushman-Roisin et al., 2013), this current may influence the drift of hatchlings and small juveniles, partially directed into the Adriatic (e.g., the eastern Adriatic is a migration corridor for individuals belonging to a population nesting in Greece, Lazar et al., 2004).

Similarly, a number of studies have documented the spatial and temporal impact of fisheries bycatch on sea turtles worldwide (Wallace et al., 2013; Swimmer et al., 2017). In the present study, the observed pattern of loggerhead CPUE is consistent with the one observed in Wallace et al. (2013), which estimated the value of CPUE of loggerheads of different fishing gears, including trawlers, around the world (see tables in Wallace et al., 2013 and references there in).

In this study, the stochasticity of the distribution of loggerheads, and the low interaction with midwater trawlers, make bycatch events very rare reducing the ability of the model to predict correctly the occurrence of an event. Despite delta modeling represents a valid approach to accommodate zero-inflated data (Maunder and Punt, 2004), other limitations of the analyzed dataset could have biased model performance causing the low level of explained deviance and reduced its ability to predict CPUEs in space, in terms of absolute values. However, low level of explained variation of the data, was also obtained in other studies regarding the distribution of different marine species (Grüss et al., 2014; Orio et al., 2017; Parra et al., 2017), particularly with bycatch species using fishery dependent data (La Mesa et al., 2016). An important confounding factor is related to the nature (fishery dependent) of the data collected. Fishery-dependent data suffer of intrinsic bias related to the displacement of monitoring effort in space and time (Fulton et al., 2005; Sims et al., 2008), making difficult to assess the real influence of environmental variables on CPUE. Indeed, the monitoring program that provided these data, lack of a well-defined sampling design and the distribution of monitored activity, in space and time, was dependent to fleets dynamic, that, in the case of midwater pair trawl fishery is high variable in space in the short run, depending on biological (distribution of the target species) and economic drivers (market, fuel price) (Russo et al., 2015). Following the considerations in Fortuna et al. (2010), a non-homogenous distribution of monitoring activity in space would affect both the observation coverage and estimation of bycatch events. The lower number of monitored fishing trips than in the northern area could have an effect on the observed patterns in the southernmost area. However, the great differences between the northern and the southern marine ecosystem (e.g., water depth and quality, species presence and distribution, etc.), which influence the way this fishing gear is used within the study area, seem to support an actual difference in bycatch rates. Annual and inter-annual variability of the monitoring effort, was also conditioned by bureaucratic and administrative delays (e.g., gaps within the end of a project and the following call for tender of up to 6 months, tenders for funding only 1-year or 2-year projects, renewal of all observers contracts every 1–1.5 year), which determine lack of data for certain period and/or areas, and increase the uncertainty around the observed pattern.

The results obtained in this study suggest that management should carefully considered spatial component of ecological drivers of species distribution to design *ad hoc* management measures and conservation strategies aimed to reduce accidental captures (Casale et al., 2018). Knowledge of the biology and ecology of loggerhead turtle is still scanty and comprehensive studies of the spatial pattern of bycatch is lagging behind. However, different technological mitigation measures (e.g., TED and UV-LED lamps) have been tested in the Mediterranean and they seem to be a valuable solutions applied in multispecies fisheries in critical areas and seasons (Lucchetti and Sala, 2010; Sala et al., 2011; Lucchetti et al., 2016; Virgili et al., 2018). Thus, more investigations are clearly needed to understand the real impact and ecological implications of incidental captures of sea turtles in midwater pair trawl fisheries.

DATA AVAILABILITY

All relevant data used in the present study are within the manuscript and within its Supplementary Material. Fishing data (all operational and bycatch information) are from a national monitoring program of protected species and species of conservation concern entitled “BYCATCH” and from the monitoring program of protected species required by the EU Data Collection Framework. These data can be obtained by contacting the General Directorate for Fisheries and Aquaculture of the Italian Ministry of Agriculture Food, and Forestry Policies.

ETHICS STATEMENT

The loggerhead turtle (*Caretta caretta*) is considered as Least Concern under current IUCN Red List criteria. The study was based on incidental catches of protected species and species of conservation concern recorded by qualified observers on board Italian pelagic trawlers in the Adriatic Sea. The data collection was conducted under permit issued by the Italian Ministry of Agriculture, Food and Forestry, Fishery and Aquaculture directorate in compliance with the Italian obligations to the

Council Regulation (EC) 812/2004. No other authorization or ethics board approval was required to conduct the study.

AUTHOR CONTRIBUTIONS

JP and SB developed the ideas within the manuscript. JP analyzed the data. FM curated and maintained the historical dataset. SB and JP wrote the manuscript with inputs from AC, CF, AL, and AS.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00365/full#supplementary-material

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First Central Mediterranean Scientific Field Study on Recreational Fishing Targeting the Ecosystem Approach to Sustainability

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The Maltese Islands have a very active recreational fishing community which may affect the coastal marine ecosystem. Despite this, studies to scientifically document the effects of this activity have been lacking prior to works between July 2012 and June 2017 presented here as a case study. This project, with the aim of collecting long-term data on the characteristics, trends, catches and impacts to fish populations of the recreational shore sport fishery at the national level also involved a pilot study on hobby shore angling. Two thousand five hundred and eighty nine roving-access creel surveys conducted during 132 sport fishing events and 159 catches from hobby fishers were documented with the methodology used also applicable to shore fishing taking place in the Mediterranean and elsewhere. Ninety species belonging to twenty-nine families were documented with the most common being the Sparidae and Labridae. Catch per unit effort was higher for sport fishers with hobby fishers targeting larger fish. Results from this case study go to augment the limited and necessary knowledge on this fishing sector in the Mediterranean. Findings also indicate that recreational fisheries need to be taken into account when considering conservation measures for national, regional and global fisheries management.

Keywords: conservation, Mediterranean Sea, recreational fishing, sport fishing, sustainability, monitoring, fishery management for conservation

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INTRODUCTION

As more reports state that overfishing is mostly found in the Mediterranean, it is essential to consider sustainable management of any fishing activity in the region through research required to guide and monitor its effectiveness (FAO, 2008; Vella, 2009). In fact, under the Common Fisheries Policy, Mediterranean countries are obliged to restore all stocks' sustainability rates by 2020. Until recently, recreational fishing (RF) in the EU and Mediterranean has been considered as small and marginal with no impacts on the marine ecosystem. However, as this activity has increased through the years it has become clear that it requires critical consideration toward targeting its sustainable management (Font and Lloret, 2014) to avoid impacts on marine biodiversity, ecosystem services, and full-time traditional artisanal fishing (Prato et al., 2016).

In the Mediterranean, RF plays important economic, social and cultural role and is a flourishing activity in coastal areas (Font et al., 2012) where it generates a pressure on the service sector in places where it is exercised (Franquesa et al., 2004). It is largely the domain of small-scale concerns operating in coastal areas (Morales-Nin et al., 2005) and involves 10% of the total fisheries production in the area. It principally involves hook and line angling (McPhee et al., 2002; Arlinghaus and Cooke, 2009) where specific methods are used by anglers to pursue selected species or else catch any available species through various means (Griffiths, 2012). RF may also involve activities offshore which focus mostly on deep water resources and big game fishing, typically carried out by fishers who can afford to purchase costly high-quality gear (Thrush et al., 1998; Font et al., 2012) which *de facto* increases fishing efficiency (Lloret et al., 2016). These include electronic equipment, such as GPS, depth sounders, fish finders and sensitive fine tipped graphite rods and invisible fluorocarbon lines.

Despite this, a sound information base and adequate management plan for RF are still lacking (Font et al., 2018). Acquiring sound estimates of vital factors from RF is still challenging but critical for stock assessment and management of ecosystems (Griffiths et al., 2010). Available data on Mediterranean RF catches, caught by hook and line angling or spearfishing show that effects are not to be ignored (Chavoin and Boudouresque, 2004; Cadiou et al., 2009). The removal of biomass in many areas is considerable, especially when one relates it with artisanal fishing, thus confirming the seriousness of the impact on marine resources caused by RF (Font and Lloret, 2014). In Europe an estimated 8.7 million (1.6%) engage in marine recreational fishing (MRF) activities totaling an estimated 77.7 million fishing days annually (Hyder et al., 2018), where besides contributing economically, other benefits such as "relaxation, exercise and experience of nature" (DG MARE, 2017) are gained. The effects of RF therefore merit further investigation to ensure its compatibility with sustainable exploitation of living aquatic resources (European Commission, 2006). Since 2002, there has been an increase in research on European fisheries and their management, since the assessment of recreational catches of some species including bass, cod, salmon, bluefin tuna, eels, and sharks, becoming a requirement through legislation (CEFAS, 2011; Ferter et al., 2013).

USA and Australia are well ahead of Europe when it comes to the collection and use of RF records (EAA, 2016). However, a number of species have been listed for inclusion in data collection for the Mediterranean. They are all highly migratory species falling under ICCAT's mandate: Eels and elasmobranchs (European Commission, 2016). In Europe, some MRF management measures have been employed and should affect future catches. These include prohibition of RF for European eel (*Anguilla anguilla*), bag limits with seasonal variations for cod (*Gadus morhua*) (European Council, 2017a) and only catch-and-release fishing allowed for sea bass (*Dicentrarchus labrax*) by RF in the North Sea and Atlantic (European Council, 2017b). The latter was subsequently changed to allow one specimen of *D. labrax* to be retained per fisher per day (European Council, 2018). Fishing and landing of several shark species has also been prohibited (European Council, 2019) while the European Union habitats directive protects wild Atlantic salmon (*Salmo salar*) and its major spawning sites.

The recreational angling community also holds a wealth of historical data. However, this has seldom been used for monitoring, scientific research and management purposes, mainly because data are provided in diverse formats and are generally not easily accessible (Dedual et al., 2013). Hence, improved data accessibility and better understanding of the different perspectives among all stakeholders including fishery scientists, managers and the recreational fishery sector are also required for RF management (Dedual et al., 2013; Morales-Nin et al., 2015) since these may provide different views on the RF industry (Hasler et al., 2011). Over 6 million Europeans are members of their local fishing clubs and/or a national angling association (Brainerd, 2011). A number of common fishing behaviors exist amongst anglers participating in fishing competitions. These include motives, attitudes and preferences irrespective of the location they are fishing (Wilde et al., 1998). Recreational fishers are often considered to be sensitive to the environment in which they fish and the need to manage aquatic resources they depend on (Gaudin and Young, 2007; FAO, 2012). Thus, while some of the fish caught by anglers are kept for their own consumption (Rudd et al., 2002; Cooke and Cowx, 2004), substantial fish are released shortly after capture. This may be due to the fish caught being different from that targeted (smaller, size, undesirable species etc.) or catch-and-release is practiced by the angler (Cooke and Suski, 2005). Legal sizes [Regulation (EC) No 1967 2006], daily bag limits, minimum hook sizes and support of catch-and-release fishing (Cooke and Schramm, 2007; Alós et al., 2009) have steadily become significant tools to manage RF. Although some fish may perish post-release, there is a great reduction in fishing mortality compared to the mortality associated with planned fish retention (Cooke and Schramm, 2007). However, survival rates and successful reproduction of released fish must be managed to ensure that mandatory or voluntary practices are effective (Bartholomew and Bohnsack, 2005).

Legislation Regulation for Recreational and Sport Fishing

Although many Mediterranean countries have MRF regulations, these vary by country and region (Franquesa et al., 2004). Very few Mediterranean countries have a mandatory system of licenses in place and often not all methods are covered (ICES, 2017). To date the European Commission has introduced some universal advice and some recommendations for sustainability regulation and the collection of data in the Baltic sea and North Sea for salmon and bluefin tuna (European Commission, 2001) managed by international commissions, such as ICCAT. In most European countries, there are only approximate figures of participating recreational fishers, their overall catches, and expenditure, while in others, no information is available (Herfaut et al., 2013).

In the Canary Islands, a fishing license is required. When carrying out RF from the shore or from boats, only a fishing rod or handline with a maximum of 3 hooks per line is permitted with boats also allowed to carry out trolling and use squid-jigs. Both natural bait and lures are permitted while electronic fish attractants are forbidden (Jiménez-Alvarado et al., 2019). In Portugal, a license is also required where a daily bag limit of

10 kg per angler is specified during shore fishing (Presidência do Conselho de Ministros e Ministérios da Defesa Nacional, 2006). The legislation also includes minimum legal sizes (MLS) for both commercial and RF which aims to permit the survival of sufficient juveniles to reach spawning size (Stergiou et al., 2009). Previous legislation (Decree law 246/2000) had already defined allowable RF gears and prohibited selling or displaying of catches for sale (Pawson et al., 2007). Restrictions in Portugal were however implemented with minimal scientific data on the effects of this type of fishing activity on marine stocks and no studies on the demography and figures of the recreational marine fishing population (Veiga et al., 2013). Similarly, Spain presents the most restrictive policies (Franquesa et al., 2004; Gordoa et al., 2019) where a number of regulations regarding classes, tackle and equipment, off-season periods and areas, authorized species and daily bag limits (Decreto, 347/2011) are in force. Sport fishing licenses are only required to participate in official fishing contests (Gordoa et al., 2019). In the Balearic Islands, legislation limits both fishing effort, specifies daily bag limits and stipulates minimum landing sizes and closed seasons for certain species (Morales-Nin et al., 2015). In France, RF is subject to only limited regulation. There is no licensing system or registry of marine recreational fishers (Franquesa et al., 2004; Herfaut et al., 2013). At the other end of the spectrum, Italy is lacking in policies related to RF (Franquesa et al., 2004). Permits are not required for sport or RF activities. Fishing is however subject to a list of permitted gear types, time and area restrictions with sale of catch prohibited (President of the Italian Republic, 1968, 2012; Pawson et al., 2008) and respect of fish minimum sizes required. In addition, for anglers to engage in sportfishing competitions, it is obligatory for them to be enrolled with the national sport fishing federation with reporting of catch data also required (FAO, 2016). Commercial fishing gear is prohibited for use by recreational fishers and only fishing lines are permitted (Pawson et al., 2008), In Albania a license is required only if the individual recreational fisher intends to use a boat while in Greek waters, fishing from the shore does not require a license but is prohibited at night and sale of fish caught is prohibited (Pawson et al., 2008). MRF fishing by sea angling, vertical lines and trolling in Cyprus are exempted from a fishing license. Other categories of MRF including boats with nets (>400 m) and longlines (limited to 100 hooks) and traps, scuba divers, spear fishing, and fishing with nets from the shore all require a license with stipulated daily catch limits for selected species (FAO, 2005; Ulman et al., 2014). Marine recreational fisheries in Turkey also does not require a compulsory license, but a document is given to anglers who wish to certify their activities (Ünal and Göncüoglu, 2012). Tourists are only allowed to practice boat-based and shore-based fishing, with the former requiring a fishing tourism certificate. Policies include prohibition of sale, prohibition of catch of certain species, daily bag limits, length, and weight limits and restriction of gear types (Unal et al., 2010). In Syria and Egypt, an individual RF license is required while in Lebanon, Morocco, and Tunisia, a license is required for recreational underwater fishing (Cacaud, 2005). On the other hand, RF in Algeria is unregulated (Babali et al., 2018). No information was available for Libya.

Case Study: Recreational Fishing in the Maltese Islands

The Maltese Islands are an archipelago located in the Central Mediterranean and the smallest EU member state in terms of territory, population, and economy (Harwood, 2019). The Maltese fishing fleet is predominantly small-scale with 94% of vessels under 10 m in length (NSO, 2018). They employ various modes of artisanal fishing methodologies with seasonal changes in species targeted (Vella and Vella, in press). Very few vessels operate in larger scale or in open seas (Vella, 1999). Maltese small-scale fishers are one of the smallest fisheries in Europe. They face important challenges due to competition with other maritime activities and decline in fishing space (Vella and Vella, 2019) owing to other maritime activities co-occurring in Maltese shallow waters. These include tourism related activities such as pleasure cruises, aquaculture for gilthead bream, sea bass and tuna penning, bunkering activities and yacht marinas. With 2,977 vessels (NSO, 2018), the Maltese fishing fleet accounts for 1.1% of the fishing fleet in the EU (Eurostat, 2017a) with landings of just under 2,000 tons annually (NSO, 2017), equivalent to only 0.1% of the total EU catch (Eurostat, 2017b). The main exploited species include horse mackerel (*Trachurus* sp.), dolphinfish (*Coryphaena hippurus*), swordfish (*Xiphias gladius*), bluefin tuna (*Thunnus thynnus*), and demersal species. The dolphinfish on its own, comprises 11% of the total commercial catch (NSO, 2017) and is of primary economic importance in Malta especially for the Maltese artisanal fishery (Vella, 1999). Although the economic contribution to the national Gross Domestic Product (GDP) at about 0.1% is negligible, the Maltese fishing industry provides important social and cultural influences (FAO, 2015).

Category C vessels (MFC), licensed for RF, comprise 68% of the total fishing fleet (NSO, 2018). Professional fishing operations such as "the use of towed nets, surrounding nets, purse seines, boat dredges, mechanized dredges, gillnets, trammel nets and combined bottom-set nets and longlines for highly migratory species are prohibited" (Government of Malta, 2013). Minor gears including set bottom longlines, traps, trolling lines and jigging (European Commission, 2006) are permitted. The National Maritime Register also registers vessels employed in RF. A fishing license is not required, and only sport fishing gear is permitted (Gaudin and Young, 2007; FAO, 2015). Since 2014, permit applications for boat owners practicing MRF for bluefin tuna have opened annually. The permit is open between June and October. The recreational quota was 1 ton in 2014 increasing to 2 tons in 2015 (Source: ICCAT), equivalent to around 2% of the national total allowable quota (TAC).

MRF in the Maltese islands is an important activity involving both hobby fishing and sport fishing. Hobby fishing is fishing for leisure. Sport fishing is very competitive. Fishers enroll with a sport fishing club and participate in shore fishing competitions organized on a regular basis by their club. Sale of fish caught during RF activities is prohibited (European Commission, 2006; Pawson et al., 2007) with the exception that fish caught from sporting competitions may be sold so long as "*the profits from their sale are used for charitable purposes*" (European Commission, 2006). Since no fishing licenses are required, an absence of data regarding the total figure of recreational shore anglers exists, making data collection challenging. Recently, a recreational fisheries board was set up with the aim of introducing possible management measures such as the reduction of fishing effort (Government of Malta, 2013).

The first shore sport fishing club was set up in 1995 and it is only recently that this sport has picked up. A few years ago, three other sport fishing clubs were instituted, each holding its own leagues and competitions. All clubs are affiliated with the National Federation of Sports Angling Malta (NFSAM) while EFSA-Malta, a branch of the European Federation of Sports Anglers is also present. Catch and release is required during all competitions and use of keep nets (nets for keeping live fish which are hung near the angler and extend partly into the sea) is specified by club competition regulations (KSFA, 2012; Denci Club, 2014; NFSAM, 2018).

With the exception of bluefin tuna catches, national surveys addressing MRF in the Maltese islands are inexistent. Regulation and control over the catches by recreational fishers are absent. Recently, two studies have attempted to estimate RF catches in the Maltese Islands using specific accessible means. A study by Giovos et al. (2018) attempted to identify boat based recreational fisheries in the Mediterranean, including Malta using social media, while Khalfallah et al. (2017) reconstructed the catches for Malta, including recreational fisheries through extensive research of published data. However, both studies fell short of providing an accurate data set and should be considered with caution. In the former, Giovos et al. (2018) used videos from social media, however, most anglers do not upload their catches on social media. Other factors including fishing effort, total catch, date and location cannot be accurately documented from videos. Khalfallah et al. (2017) reconstructed catches based solely on a pilot study taking place in 2005 and ignored non MFC vessels and shore-based anglers. Monitoring of this type of fishery is however essential and catches should be scientifically documented and included with those of commercial fishers for conservation management of affected marine biodiversity and ecosystem.

Objectives of This Study

The present work developed a methodology to monitor sport and hobby fishing in the Maltese Islands through the use of modified creel surveys, which are sampling surveys that target recreational anglers to collect data regarding the quantity and species caught by this type of fishery. It is used to document gear types, preferred locations and assess the adoption and effectiveness of catch and release practices. It aims to contribute to the information of current utilization of coastal fish resources and provide scientific data on which to implement management criteria directed at their protection and conservation.

MATERIALS AND METHODS

Sampling Strategy

The present work involved collection of detailed data through fieldwork in collaboration with sport fishing clubs, federations, and recreational fishers. On-site species catch data for both sport fishing and hobby fishing was carried out between July 2012 and June 2017 as part of a project to monitor the shore sport fishery in the Maltese Islands. All the 4 sport fishing clubs, local federations and tackle shops participated in the data collection. Simultaneously, between April 2013 and December 2017, a pilot study with shore hobby fishers was integrated to document this fishing activity around the Maltese Islands. Roving access creel-surveys (McCormick et al., 2012, 2013) were used in both cases. The authors had no involvement in the choice of fishing locations which were selected by the clubs or anglers generally after consulting the weather forecasts. During competitions, anglers were very briefly interviewed regarding their fishing method, bait and hook sizes used while fishing close to the end of every competitive event to allow them to exhaust all the different fishing methods (Lockwood, 2000) required for that particular competition. The interview aimed to disturb the anglers a little as possible and only comprised the following three questions:

1. Which fishing methods did you use?
2. Which bait did you use?
3. Which hook sizes did you adopt?

At the end of the competition, catches were weighed and quickly placed on a specially designed rectangular catch board with an affixed scale, photographed and the dead ones removed and counted. All live fish were then released. During hobby fishing observations, the same information was documented at the end of each fishing trip. These were also supplemented with catch photographs supplied by hobby fishers who also provided the location fished and the same information collected during the on-site creel surveys. The use of a keep net was noted in both cases. Hook gape width was measured using a Vernier caliper (±0.01 mm) from hooks supplied by tackle shops or the anglers themselves. Sea surface temperature was measured on site (±0.1°C) whenever possible or the mean sea temperature as provided by the Met Office was used when the sea was not accessible (e.g., cliff competitions).

Data Analysis

All catches were standardized to show the catch-per-unit-effort (CPUE: number/weight of fish of a specific species per fishing hour). Club records were used in addition to on-site observations to calculate the mean annual catch by weight based on the complete years documented (2013–2016). The Kruskall Wallis H test was used to determine if there was a difference in the overall mortality across the years. Automatic linear modeling (ALM) was also used to assess the effect of location, duration of competition, time of catch, use of keep net, sea temperature and year of competition on the mortality rate.

The relationship between mortality and the other variables was described in terms of Equation (1):

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots. + \beta_n X_n \tag{1}$$

Where y = dependent variable (fish mortality), β_0 =constant variable, $\beta_{1 \ldots.}\ \beta_n$ are regression parameters, $X_1, X_2, X_3 \ldots\ldots X_n$ = predictors (location, duration of competition, use of keepnet etc.). In the case of categorical variables, the number of estimated parameters was one less than the number of categories, where the last category is the alisaed term (which is set to zero). E.g., For use of keepnet, where keepnet = 1 and no keepnet = 0 resulted in the following equation:

$$y = \beta_0 + \beta_1 \mathrm{D} + \beta_2 X_2 + \ldots. + \beta_n \mathrm{X}_n \tag{2}$$

where D = category variable

If D = 0, then:

$$y = \beta_0 + \beta_2 X_2 + \ldots. + \beta_n \mathrm{X}_n \tag{3}$$

If D = 1, then

$$y = \beta_0 + \beta_1 + \beta_2 X_2 + \ldots. + \beta_n \mathrm{X}_n \tag{4}$$

The model was selected using a forward stepwise method with the corrected Akaike Information Criterion (AICc) and included predictor variables at $P < 0.05$. All outliers from continuous predictor variables (temperature) were removed and categorical variables (locations) were merged to maximize the association to the target variable (fish mortality). The model with the lowest AICc value was selected (Burnham and Anderson, 2002).

The significance threshold was set at an alpha value of 0.05 in this study as for related studies (Gartside et al., 1999; Veiga et al., 2010; Zischke et al., 2012). All statistical analyses were performed using IBM SPSS Statistics ver.24 (IBM Corporation, Somers, NY, USA).

RESULTS

Fishing Effort

Data was collected during 132 sport fishing competitions in 45 different locations. Information from 2,589 roving-access creel surveys was recorded. This represented 60% of the total open competitions taking place during the study period. One hundred and fifty nine catches by hobby anglers were also documented from 44 different locations. Fishing effort totaled to 11,667 h during competitions and 518 h during hobby fishing observations. The mean annual weight of fish caught during fishing competitions was 680.87 kg (SD ± 99.42). Sport fishing was predominantly male oriented with very few female anglers participating in the sport. Average angler participation during fishing competitions was 9.6 (SD ± 9.4) anglers per event and varied during the study period. The average overall number of fish caught per angler per hour ($\mathrm{CPUE_n}$) was 4.52 fish angler h^{-1} (SD ± 4.33) during competitions and 1.97 (SD ± 3.14) fish angler h^{-1} during hobby fishing. During hobby fishing day sessions (47.8% of the sessions documented) 3.02 (SD ± 4.19) fish angler h^{-1} were caught, decreasing to 1.01 (SD ± 0.98) fish angler h^{-1} during the night (52.2% of the sessions documented) and with 7.6% of the fishing trips resulting in no catch. The average weight (kg) of fish caught per angler per hour ($\mathrm{CPUE_{kg}}$) was 0.19 kg angler h^{-1} (SD ± 0.18) for all sport fishing competitions. Sports anglers fished for 4.51 (SD ± 0.64) h while hobby fishers spent 3.26 (SD ± 1.41) h fishing per trip.

Fishing Density

Fishing competitions were held in various locations which were chosen by the clubs a few days before the competitions. Competitions were held in ports, rocky areas facing the open sea, cliff sites and occasionally beaches. The most popular locations for competitions were Manoel Island (9.9%) and Sliema (8.3%), two sheltered locations enclosed within Marsamxett harbor (**Figure 1**). The latter was also popular with hobby fishers (8.2%) followed by Valletta (Foss) (6.9%). Sliema was also the most frequented by hobby fishers for night fishing (10.8%) while Marsa was the favorite location for day fishing (10.5%). The most popular sites for cliff fishing competitions were Mtañleb (6.1%) and Bañrija (3.0%). Both sites were also frequented by hobby fishers (3.6 and 1.2%, respectively) for night fishing.

Tackle and Bait Use

Anglers taking part in competitions invested in various types of rods and tackle. Rods varied from light fine tipped graphite rods used mostly during daytime competitions to more robust equipment used at night or during cliff fishing. The favorite rig for both sport and hobby anglers was the paternoster rig setup using a reel rod (**Table 1**). This involved a sinker attached to the end of the line with two hook traces above and used primarily to target bottom fish. Another popular setup among both types of anglers was the pole rod with a fixed float or a reel rod set up with a running float (**Table 1**). In the first setup, a fixed float is used which can be shifted along the line with varying depth. Here, the maximum depth must not exceed the rod length, which is rarely longer than 6 m. The running float permits fishing in deeper waters since a stopper for the float is used anywhere along the line depending on the depth fished. Hobby anglers also used a very simple setup called the free hook system whereby a line with a hook attached at the end was set up on a fishing pole and baited. Such tackle was used primarily during night fishing. There was no restriction on hook sizes, which varied depending on the type of location and time fishing (**Table 2**). Larger hooks were primarily used during the night and cliff fishing competitions while smaller hooks were used during day competitions. A smaller range of hook sizes were used by hobby fishers where hook sizes at both extremes were not utilized.

Recreational fishers routinely used certain fish species and other bait which varied depending on fishing technique and the target fish. The most popular bait was live polychaete worms, with the widely available Korean worm, *Nereis* sp. being the most popular (**Table 3**) during fishing competitions and also popular amongst hobby anglers. Bristle worms, *Eunice* sp. which are collected locally were frequently used for night fishing, while, the American bloodworm (*Glycera dibranchiata*) was also occasionally used and included with the "other bait." The Korean and American worms were purchased at reasonable prices from tackle shops while Bristle worms were harvested locally by divers and sold at premium prices.

Another class of bait used were cephalopods which were generally used in pieces during cliff fishing by night together with various pieces of fish (*Alosa fallax* and *Auxis rochei,* also included with "other bait"). Crustaceans, notably freshwater crayfish and locally harvested mud shrimp (*Upogebia pusilla*) were popular with sports anglers during competitions. The latter is very scarce and sold at premium prices by individuals who harvest them from areas high in sediment. Some anglers opted for the cheaper

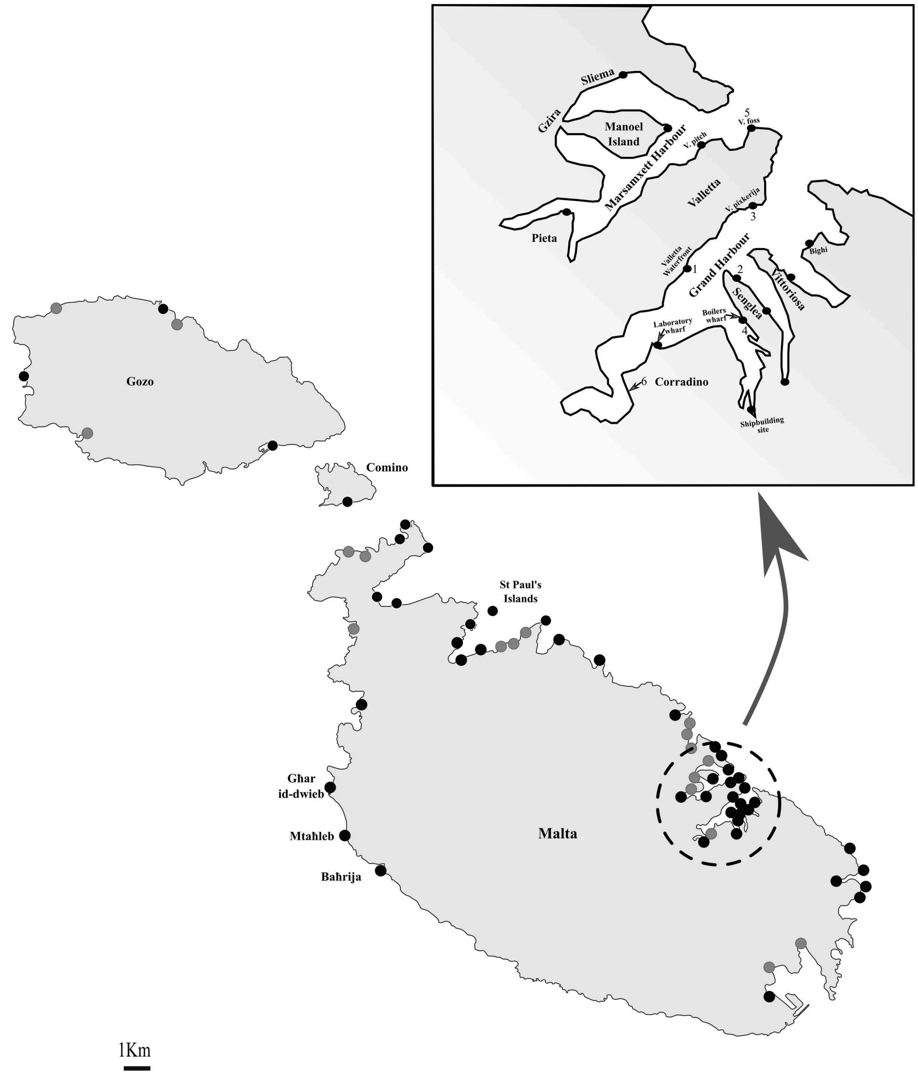

FIGURE 1 | Map of the Maltese islands showing locations where catches were documented. Enlarged area shows competition locations in the Grand Harbor and Marsamxett harbor areas. Black dots denote fishing competitions; gray dots denote locations fished only by hobby fishers; numbers denote locations where non-native species were caught by both sport and hobby fishers. Għar id-dwieb, Mtaħleb, and Baħrija are cliff fishing sites.

commercially available freshwater crayfish instead while two clubs prohibited the use of mud shrimp during competitions.

Catch Composition

A total of 51,822 fish belonging to 90 species from 29 different families were identified from the competition catches (**Figure 1**) while 1,000 fish belonging to 31 species were identified from the catches by hobby fishers (**Table 4**). During both competitions and hobby fishing, the Sparidae and Labridae had the highest species richness albeit lower for hobby fishers. The three most frequently fished species by sport fishers were *Coris julis*, *Diplodus annularis*, and *Diplodus vulgaris* which together comprised 33.31% of the total catch. *Diplodus sargus*, *Oblada melanura*, and *Chromis chromis* were the most frequently caught by hobby fishers. Five non-native species were also recorded during fishing competitions, four of which were recorded locally for the first time. These were the Dusky spinefoot (*Siganus luridus*), the Niger hind (*Cephalopholis nigri)*, the cocoa damselfish (*Stegastes variabilis*), the Dory snapper (*Lutjanus fulviflamma*) and the Indopacific sergeant (*Abudefduf vaigiensis*), with the latter also caught during a hobby fishing observation. All these specimens were caught from the Grand Harbor area which has a very active schedule of cruise liners, grain and cement carrying ships, ship repair, and bunkering activities (**Figure 1**).

Catch and Release

Overall, catch and release (C & R) was practiced by 69% of the anglers during sport fishing competitions while the remaining 31% used water filled buckets. Keepnets were not utilized during cliff competitions since fish could not be released

TABLE 1 | Tackle used during fishing competitions and hobby fishing.

	Sport fishing competitions					Hobby fishing		
Tackle	**All events**	**Day shore**	**Night shore**	**Day cliff**	**Night cliff**	**All**	**Day**	**Night**
Paternoster reel	56.5	54.1	61.2	100	100	31.6	19.5	43.2
Paternoster pole	3.8	4.0	1.5	0.0	0.0	0.6	0.0	1.2
Running ledger	8.0	8.4	7.5	0.0	0.0	4.4	2.6	6.2
Running float	8.8	9.4	3.0	0.0	0.0	12	16.9	7.4
Mullet float fishing pole	0.7	0.6	9.0	0.0	0.0	2.5	5.2	0.0
Mullet bolonaise	5.6	6.0	0.0	0.0	0.0	8.2	15.6	1.2
Fixed float pole fishing	13.1	14.0	9.0	0.0	0.0	13.3	24.7	2.5
LRF	0.1	0.1	0.0	0.0	0.0	1.3	0.0	2.5
Free hook	0.1	0.1	4.5	0.0	0.0	17.1	2.6	30.9
Other tackle	3.2	3.4	4.5	0.0	0.0	8.9	13.0	4.9
Total	100	100	100	100	100	100	100	100

Numbers denote percentage.

TABLE 2 | Mean width of hooks frequently used by anglers and their preference by sport fishing and hobby anglers.

			Fishing competitions					Hobby fishing	
Hook size no	**Mean gape width (mm)**	**SD**	**All events**	**Day shore**	**Night shore**	**Day cliff**	**Night cliff**	**Day**	**Night**
6/0	21.05	0.95	0.1	0.0	0.0	0.0	2.4	–	–
5/0	19.53	1.84	0.1	0.0	0.0	0.6	2.4	–	–
4/0	18.31	1.23	0.4	0.0	1.5	0.0	13.4	–	–
3/0	17.03	1.11	0.3	0.0	3.0	0.6	8.5	–	–
2/0	14.80	1.23	0.2	0.0	1.5	1.1	4.9	–	–
1/0	13.39	1.80	0.3	0.0	1.5	1.7	6.1	–	–
1	12.12	1.61	0.3	0.2	3.0	0.6	3.7	2.3	1.2
2	10.79	1.63	0.8	0.4	6.1	0.6	11.0	2.3	5.8
3	9.04	0.80	0.5	0.2	0.0	2.2	8.5	0.0	7.0
4	8.42	0.62	2.0	0.7	6.1	12.2	36.8	4.7	14.0
5	7.75	0.44	1.0	0.3	3	10.6	3.7	1.2	9.3
6	7.21	0.66	2.7	1.3	7.6	23.9	7.3	8.1	15.1
7	6.67	0.32	1.4	0.8	3.0	11.1	0.0	4.7	18.6
8	6.12	0.32	5.2	4.6	7.6	17.8	1.2	8.1	3.5
9	5.47	0.41	1.2	1.3	0.0	0.0	0.0	1.2	0.0
10	5.55	0.52	24.4	25.9	24.2	10.0	0.0	14.0	10.5
11	4.69	0.11	1.5	1.6	0.0	0.6	0.0	2.3	2.3
12	4.70	0.54	29.2	31.7	13.6	2.8	0.0	30.2	12.8
13	4.52	–	0.1	0.1	0.0	0.0	0.0	2.3	0.0
14	4.34	0.82	18.2	19.6	15.2	3.3	0.0	18.6	0.0
15	3.92	0.20	0.3	0.3	0.0	0.0	0.0	–	–
16	3.64	0.41	5.3	5.7	3.0	0.6	0.0	–	–
17	3.44	–	0.1	0.1	0.0	0.0	0.0	–	–
18	3.25	0.24	2.1	2.3	0.0	0.0	0.0	–	–
20	2.67	0.26	0.8	0.8	0.0	0.0	0.0	–	–
22	2.62	–	0.8	0.8	0.0	0.0	0.0	–	–
24	2.41	–	0.6	0.6	0.0	0.0	0.0	–	–
26	2.26	–	0.4	0.4	0.0	0.0	0.0	–	–

SD denotes standard deviation.

from such heights above sea level. Seventy-five percent of the anglers used keep nets when these competitions were excluded. The overall mean mortality rate was 35.80% (SD ± 39.46), 30.35% (SD ± 36.18) during day competitions when cliff competitions were excluded since the latter had 100% mortality. Only a few night competitions were held and documented so these were not analyzed separately. A total of 32,422 fish were released after competitive events during the study period. Only 23.90% of hobby fishers were observed to use keep nets. A lower mortality rate was observed when keep nets

TABLE 3 | Bait used during fishing competitions and hobby fishing.

Bait	Sport fishing competitions					Hobby fishing		
	All events	**Day shore**	**Night shore**	**Day cliff**	**Night cliff**	**All**	**Day**	**Night**
Korean worm	40.0	40.7	28.0	46.1	3.5	20.3	20.3	20.2
Frozen shrimp	28.6	29.6	19.4	20.7	6.2	8.5	14.5	3.6
Fresh shrimp	3.1	2.7	0.0	14.0	0.0			
Imported crayfish	1.1	1.2	0.0	0.0	0.0	0.7	0.0	1.2
Mud shrimp	6.2	6.7	0.0	0.0	0.0			
Milk bread	10.8	11.5	18.3	0.0	0.0	11.8	23.2	2.4
Bread	1.8	1.9	2.2	0.0	0.0	14.4	12.7	8.3
Maggots	1.5	1.5	4.3	0.0	0.0	8.5	15.9	2.4
Bristle worm	1.7	0.5	15.1	0.7	49.6	26.1	2.9	45.2
Paddled blood worm	0.2	0.2	0.0	0.0	0.0	0.7	0.0	1.2
Pastella (bait mixture)	0.9	0.9	2.2	0.0	0.0			
Chicken	0.0	0.0	0.0	0.0	0.0	7.8	0.0	14.3
Other	4.1	2.4	10.8	18.5	40.7	1.3	1.4	1.2
Total	100	100	100	100	100	100	100	100

Figures denote percentage.

were used [keep net = 35.91% (SD ± 42.97); no keep net = 81.80% (SD ± 37.07)].

Several measures aimed at reducing the mortality rate and catches of juvenile fish were put in place by the NFSAM in 2017 after consultation with the authors. These included a minimum hook size of gape width 5 mm, water changes every hour when keep nets could not be used, and the removal of visibly manhandled fish from the catch before weighing. The latter measure was also adopted by all fishing clubs. One club also adopted the use of a minimum hook size of gape width 7 mm during cliff fishing competitions.

To assess if these changes were significant in lowering the mortality rate, 11 competitions were documented between January and June 2017. To remove the bias in post-capture mortality that may be present when considering a full year, only the period from January to June was used to establish significance (**Table 5**). The Kruskal-Wallis H test showed that there was a statistically significant difference in mortality between the different years, (2013, $n = 105$; 2014, $n = 197$; 2015 $n = 341$; 2016, $n = 445$; 2017, $n = 230$), χ^2 (4, $n = 1{,}318$) = 44.55, $p = <0.001$. The post-capture mortality median score was the lowest in 2013 (Md = 7.14) increasing yearly and reaching a peak in 2016 (2014, Md = 11.11; 2015, Md = 12.5; 2016, Md = 18.18) and then decreasing drastically in 2017 (Md = 7.06). Overall mortality in 2017 was however only significantly different ($P = <0.001$) from that in 2016 and 2015.

The linear regression model used to assess the effect of location, duration of competition, time of catch (day/night), use of keep net, sea temperature and year of competition for overall mortality (excluding cliff competitions) revealed that the use of keep net, sea temperature, capture location, and duration of competition were significant predictors of the fish mortality (**Table 6**). (Mortality: $n = 2{,}067$; mean mortality ± SD: 30.39 ± 36.23; Model AICC: 13,455.28, $r^2 = 0.491$; Intercept Coefficient: 16.89, $P = 0.008$; Keep net coefficient: −44.31, $P < 0.001$; Duration coefficient: 5.60, $P < 0.001$; Sea temperature coefficient: 0.89, $P < 0.001$; Location group 0 coefficient: −5.91, $P < 0.205$; Location group 1 coefficient: 3.09, $P = 0.21$; Location group 2 coefficient: −1.41, $P < 0.15$; Location group 3 coefficient: 1.18, $P = 0.15$; Location group 4 coefficient: 15.79, $P < 0.001$; Location group 5 coefficient: 22.68, $P < 0.001$; Location group 6 coefficient: 4.99, $P = 0.001$. The time of catch and year of competition predictor variables tested did not significantly affect the mortality rate (**Figure 2**).

DISCUSSION

This long-term study documented several shore-based angling options as observed by the diversity of fishing techniques recorded and the numerous fish species caught. Such dedicated and detailed investigations allow an in-depth consideration of the impacts of these activities on the fish communities and the marine ecosystem. The methodology used in this study to document the fish catches can also be applied to fishing competitions taking place outside the Maltese Islands. One example is those organized by FIPS-M (Fédération Internationale de Pêche Sportive—Mer) which has 47 affiliated countries and organizes several international tournaments on a yearly basis. In particular, the European championships of float angling, which follow a very similar modality to fishing competitions documented in this study. Within each participating country including Malta, competitions are also held in which anglers qualify to represent their country in this European championship. The same system of placing fish in keepnets and weighing them at the end of the competition is used, without collecting any information on the fish caught. This method would therefore provide a rapid and cost-effective way to

TABLE 4 | Total catch number and percentage of species caught during fishing competitions and by hobby fishers in this case study.

Family	Species	Common name	Competitions		Hobby fishing	
			Total catch number	**Percentage %**	**Total catch number**	**Percentage %**
Sparidae	*Diplodus annularis*	Annular seabream	5,218	10.08	99	9.91
	Diplodus vulgaris	Two banded seabream	4,397	8.5	64	6.41
	Diplodus sargus	White seabream	333	0.64	146	14.61
	Diplodus puntazzo	Sharpsnout seabream	118	0.23	0	0.00
	Oblada melanura	Saddled seabream	679	1.31	114	11.41
	Lithognathus mormyrus	Sand steenbras	64	0.12	52	5.21
	Pagrus pagrus	Red porgy	565	1.09	0	0.00
	Pagrus auriga	Red banded seabream	3	0.01	0	0.00
	Sparus aurata	Gilt-head bream	45	0.09	69	6.91
	Sarpa salpa	Salema porgy	462	0.89	71	7.11
	Pagellus erythrinus	Common pandora	85	0.16	7	0.70
	Dentex dentex	Common dentex	9	0.02	0	
	Spondyliosoma cantharus	Black seabream	317	0.61	3	0.30
	Boops boops	Bogue	3,478	6.72	55	5.51
Moronidae	*Dicentrarchus labrax*	European seabass	2	<0.01	1	0.10
Labridae	*Coris julis*	Mediterranean rainbow wrasse	7,649	14.78	40	4.00
	Thalassoma pavo	Ornate wrasse	3,656	7.06	15	1.50
	Symphodus tinca	Peacock wrasse	2,467	4.77	5	0.50
	Symphodus roissali	Five spotted wrasse	840	1.62	3	0.30
	Symphodus melops	Corkwing wrasse	223	0.43	0	0.00
	Symphodus mediterraneus	Axillary wrasse	349	0.67	0	0.00
	Symphodus ocellatus	Ocellated wrasse	50	0.10	0	0.00
	Symphodus doderleini	N/A	1	<0.01	0	0.00
	Symphodus rostratus	Pointed-snout wrasse	10	0.02	0	0.00
	Symphodus cinereus	Gray wrasse	2	<0.01	0	0.00
	Labrus merula	Brown wrasse	17	0.03	0	0.00
	Labrus mixtus	Cuckoo wrasse	64	0.12	0	0.00
	Labrus bergylta	Ballan wrasse	19	0.04	0	0.00
	Labrus viridis	Green wrasse	22	0.04	0	0.00
	Xyrichtys novacula	Cleaver wrasse	8	0.02	0	0.00
Scorpaenidae	*Scorpaena notata*	Small red scorpionfish	177	0.34	0	0.00
	Scorpaena porcus	Black scorpionfish	315	0.61	3	0.30
	Scorpaena maderensis	Madeira rockfish	351	0.68	0	0.00
	Scorpaena scrofa	Red scorpionfish	28	0.05	0	0.00
Serranidae	*Epinephelus marginatus*	Dusky grouper	27	0.05	0	0.00
	Epinephelus costae	Goldblotch grouper	39	0.08	0	0.00
	Epinephelus aeneus	White grouper	4	0.01	0	0.00
	Mycteroperca rubra	Mottled grouper	5	0.01	0	0.00
	Serranus scriba	Painted comber	2,917	5.64	16	1.60
	Serranus cabrilla	Comber	967	1.87	1	0.10
	Serranus hepatus	Brown comber	307	0.59	0	0.00
	Anthias anthias	Swallowtail seaperch	818	1.58	0	0.00
	**Cephalopholis nigri*	Niger hind	1	<0.01	0	0.00
Bothidae	*Bothus podas*	Wide-eyed flounder	64	0.12	0	0.00
Muglidae	*Oedalechilus labeo*	Boxlip mullet	1,579	3.05	0	0.00
	Chelon labrosus	Thicklip gray mullet	1,388	2.68	48	4.80
	Chelon ramada	Thinlip gray mullet	5	0.01	0	0.00

(Continued)

TABLE 4 | Continued

			Competitions		Hobby fishing	
Family	**Species**	**Common name**	**Total catch number**	**Percentage %**	**Total catch number**	**Percentage %**
	Mugil cephalus	Flathead gray mullet	103	0.2	14	1.40
Scaridae	*Sparisoma cretense*	Parrotfish	3,846	7.43	27	2.70
Pomacentridae	*Chromis chromis*	Damselfish	4,994	9.65	111	11.11
	**Stegastes variabilis*	Cocoa damselfish	1	<0.01	0	0.00
	**Abudefduf vaigiensis*	Indopacific sergeant	1	<0.01	0	0.00
Mullidae	*Mullus surmuletus*	Striped red mullet	206	0.40	0	0.00
Gobiidae	*Gobius paganellus*	Rock goby	213	0.41	0	0.00
	Gobius niger	Black goby	133	0.26	0	0.00
	Gobius cruentatus	Red-mouthed goby	205	0.40	0	0.00
	Gobius geniporus	Slender goby	102	0.2	0	0.00
	Gobius cobitis	Giant goby	17	0.03	0	0.00
	Gobius fallax	Sarato's goby	6	0.01	0	0.00
	Gobius bucchichi	Bucchich's goby	14	0.03	0	0.00
	Gobius incognitus	Incognito goby	61	0.12	0	0.00
Blennidae	*Parablennius sanguinolentus*	Rusty blenny	29	0.06	0	0.00
	Parablennius pilicornis	Ringneck blenny	17	0.03	0	0.00
	Parablennius gattorugine	Tompot blenny	43	0.08	0	0.00
Centrachanthidae	*Spicara maena*	Blotched picarel	284	0.55	0	0.00
	Spicara smaris	Picarel	146	0.28	0	0.00
Carangidae	*Trachinotus ovatus*	Pompano	75	0.14	10	1.00
	Pseudocaranx dentex	White trevally	39	0.08	0	
	Seriola dumerili	Greater amberjack	4	0.01	1	0.10
	Trachurus trachurus	Atlantic horse mackarel	82	0.16	6	0.60
Synodontidae	*Synodus saurus*	Atlantic lizardfish	29	0.06	0	0.00
Trachinidae	*Echiichthys vipera*	Lesser weever	5	0.01	0	0.00
	Trachinus draco	Greater weever	4	0.01	0	0.00
Apogonidae	*Apogon imberbis*	Mediterranean cardinalfish	58	0.11	0	0.00
Siganidae	**Siganus luridus*	Dusky spinefoot	1	<0.01	0	0.00
Balistidae	*Balistes capriscus*	Gray triggerfish	1	<0.01	0	0.00
Atherinidae	*Atherina hepsetus*	Mediterranean sand smelt	308	0.60	0	0.00
Muraenidae	*Muraena helena*	Mediterranean moray	101	0.20	0	0.00
Congridae	*Conger conger*	European conger	22	0.04	1	0.10
Ophichthidae	*Echelus myrus*	Painted eel	0	0.00	4	0.40
Dactylopteridae	*Dactylopterus volitans*	Flying gurnard	12	0.02	0	0.00
Belonidae	*Belone belone*	Garfish	31	0.06	6	0.60
Phycidae	*Phycis phycis*	Forkbeard	13	0.03	0	0.00
Lutjanidae	**Lutjanus fulviflamma*	Dory snapper	1	<0.01	0	0.00
Sciaenidae	*Sciaena umbra*	Brown meager	1	<0.01	0	0.00
Haemulidae	*Pomadasys incisus*	Bastard grunt	3	0.01	1	0.10
Scyliorhinidae	*Scyliorhinus canicula*	Lesser spotted dogfish	1	<0.01	0	0.00
Zeidae	*Zeus faber*	John dory	3	0.01	0	0.00
Sphryaenidae	*Sphryaena sphryaena*	European barracuda	0	0.00	1	0.10

**Non-native species.*

document such catches and collect data on this important type of fishery.

Results indicate that the biological consequences of shore fishing on littoral fish species cannot be ignored, since these were the most targeted by both sport and hobby angling. Of the 90 species recorded, the annular bream, the two banded bream and the Mediterranean rainbow wrasse were the most frequent fish caught, while *Diplodus sargus* was the most pursued species by hobby fishers. Other studies in Mediterranean coastal areas (Spain, Italy, Portugal, and Turkey) on shore fishing had also identified the Sparidae as being predominant in catches by hobby anglers with species including *D. sargus* and *D. vulgaris* being

TABLE 5 | Mortality rates for day shore competitions from January to June 2017.

Year	Mean % All species	Std. deviation	Mean % study species	Std. deviation
2013	25.66	36.32	26.14	38.75
2014	23.71	30.99	21.87	34.80
2015	34.78	39.88	40.69	45.30
2016	32.35	34.87	36.43	42.61
2017	15.50	24.73	21.30	36.25
Total	28.21	34.97	32.11	41.70

TABLE 6 | Predictive model selection using the corrected Akaike's information criterion for mortality rate (AICc).

Model	R^2	AIC_C	ΔAICc
Year	0.022	14,797.224	1341.94
Keepnet	0.435	13,660.939	205.655
Keepnet + Locality	0.478	13,505.906	50.622
Keepnet + locality + sea temperature	0.486	13,475.665	20.381
Keepnet + locality + sea temperature + duration	**49.1**	**13,455.284**	**0**

Bold denotes model used. Year was excluded from the model.
Fitted regression model: Mortality rate = 5.60 Duration +0.89 Sea temperature −44.31 Keepnet – 5.91 Location 0 + 3.09 Location 1 – 1.41 Location 2 – 1.20 Location 3 + 15.79 Location 4 + 22.68 Location 5 + 4.97 Location 6 + 16.89.

the most targeted, together with the Labridae, especially *C. julis,* and Mugilidae (not always defined by species) (**Table 7**). The number of fish caught by hobby anglers was high compared to studies in Portugal by Rangel and Erzini (2007) and Veiga et al. (2010). This may be attributed to experience or the higher quality fishing equipment also observed in use by sports anglers, which consisted of fine tipped graphite rods and thin transparent fluorocarbon lines since both typologies of anglers employed similar fishing setups.

$CPUE_n$ values were higher for sport fishers. Hobby anglers were however observed to catch a smaller number of larger fish than sport fishers. In the latter, size is not important since competitions are won by anglers scoring the highest weight and not by the number of fish. Therefore, there are no minimum size restrictions, with fish being released at the end of the event. Hobby anglers on the other hand tend to target larger fish for consumption besides enjoyment. Consequently, hobby fishers on average opted for larger hooks than sports anglers with the former in some cases opting for very small hooks when practicing speed fishing. This was especially observed in ports to target small sized fish including damselfish (*Chromis chromis*), small saddled bream (*Oblada melanura*), and sand smelts (*Atherina hepsetus*) in which anglers use a small pole rod with a very small float and small baited hook (size 20 or smaller) at the end. Similarly, in Spain, studies on RF by Font and Lloret (2011) and Gordoa et al. (2019) documented lower $CPUE_n$ values than Guerreiro et al. (2011) with sport fishers. $CPUE_{kg}$ was however lower than sports fishing in both cases. Maltese sports anglers catch more kilos of fish per hour than Portugal (0.12 kg $angler^{-1}$ h^{-1}) (Guerreiro et al., 2011), but less than Spain (0.36 kg $angler^{-1}h^{-1}$) (Morales-Nin et al., 2015). Due to the inability to weigh the catches outside competitions, the $CPUE_{kg}$ values could not be compared.

A few non-native species were also captured and recorded during sport fishing competitions taking place in areas of high shipping activity (Vella et al., 2015a,b, 2016a,b). The European Code of Conduct on Recreational fishing and Invasive Alien species states that "Anglers should make themselves aware of invasive alien species and partake in education programs designed for this" (Council of Europe, 2014). Citizen science therefore has the possibility of contributing to the knowledge about these species and fill present deficiencies in the available data (ICES, 2017). Collaboration with clubs, hobby fishers and scientists can therefore contribute to the monitoring of such non-native species and collect the required data for management.

Since competition sites were chosen by clubs after consulting weather forecasts, this may have led to numerous competitions being held within the same area throughout the year, with increased impacts on the local fish communities. These generally included sheltered areas located in harbor areas (**Figure 1**), allowing them to be fished throughout the year. Cliff competitions were organized by one club and held mostly in the same two sites on the west coast of Malta, probably due to site accessibility. In one of the sites several disputes with hunters/trappers who owned fields extending to the cliff edge were observed, making use of this site problematic. Such practice should not be promoted, and clubs should aim to identify new alternative venues so as to avoid holding competitions in the same venues several times within the year. This may however be a challenge due to a decrease in venues along the years caused by site closures in harbor locations, which then require a special permit to hold a competition that is not always granted. A small number of events took place in marine protected areas (MPAs), Such MPAs are in place to safeguard *Posidonia oceanica* and more offshore for birds. Clubs inquired with the authorities before regarding the holding of competitions in these sites but were allowed to fish since they did not interfere with the main protection goals of the MPAs which are nonetheless without effective management. In all cases catch and release was practiced. Plans within these protected areas should include fishery management provisions in collaboration with fishing clubs holding fishing competitions within them and contemplate introducing management measures for recreational fishers too, such as seasonal closures that coincide with the spawning period and daily bag limits.

Contrary to what was stated by Khalfallah et al. (2017), C & R is indeed practiced by Maltese recreational anglers. In the aforementioned study no creel-surveys were carried out. There was also no actual attempt to scientifically document catch and release practices. Keepnets were indeed used and more popular during fishing competitions with hobby fishers using them to a lesser extent. The use of keepnet, sea temperature, capture location, and duration of competition were significant predictors of the fish mortality with keepnet use being the most important predictor of fish mortality. Although the mortality rate decreased with keepnet use, this increased during the

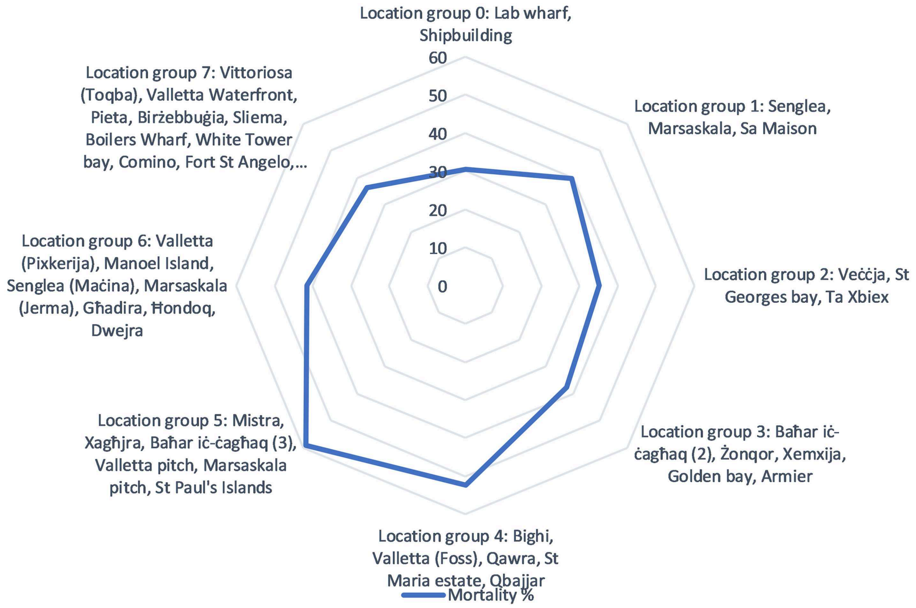

FIGURE 2 | Locations were grouped into 7 categories based on their mean mortality for the linear regression model. Location groups 0, 1, 2 and 3 and 7 were not significant predictors of the mortality rate. The group estimated means from the linear regression model are shown on the web chart.

summer months suggesting that fish are subjected to greater stress warmer periods as also documented in other studies (Bartholomew and Bohnsack, 2005). Competitions of longer duration also contributed to an increase in mortality rate together with choice of locality, where certain localities resulted in higher mortality rates. All these localities were characterized by a rocky shoreline which made keepnet use more difficult since these are easily damaged when knocked by the waves against the pointed rocky shore. While the introduction of conservation methods introduced by clubs is laudable, these have shown to have limited effect without the use of keepnets. Results indicate that the keepnet is the most important contributor to fish mortality therefore the effectiveness of other fishery management measures will be reduced when keep nets are not used. Site selection must therefore account for maximal keepnet use to maintain lower mortality rates.

Some of the sampled dead fish were noted to have angler inflicted injuries caused by hook removal. Research by Palme et al. (2016) observed an improvement in the condition of angler-caught fish after anglers attended education programs. Clubs should therefore be encouraged to hold seminars promoting best practices aimed at reducing fish mortality. Educating anglers with the aim of reducing fish mortality is therefore essential considering the numerous competitive events held annually. While catch-and-release angling is an increasingly popular conservation strategy, whether voluntary or in compliance with legislation, related injuries, stress, and effects in behavior may result in post-release mortality or loss of fitness. The survival of released fish is chiefly determined by angler activities, engaging in "best angling practices" and is critical for sustainable RF. Depending on the fish species targeted, different strategies are used by anglers. A balance must therefore be sought to introduce scientifically backed best practices accordingly. Specific tools and strategies can be unified into RF practices with actual fishing techniques (Brownscombe et al., 2017).

Besides the effects of RF, other possible impacts on coastal fauna may be caused through the use and harvest of exotic live bait (Font and Lloret, 2011). The Korean worm, *Nereis* sp., the American bloodworm (*Glycera dibranchiata*) and imported crayfish used were live-non-native species. In particular, the introduction of the first two species, which are also popular in other Mediterranean countries, may have potential environmental effects particularly due to the lack of awareness amongst anglers and retailers of the harmful effects as a consequence of exotic bait use (Font et al., 2018). In Portugal, a study on *Perinereis aibuhitensis*, an imported polychaete used as bait observed its ability to reproduce in coastal lagoons and estuaries (Costa et al., 2006). Recreational fisheries should however aim to "*prevent the release, spreading and translocation of invasive alien species that can have significant impacts on native fish populations or the environment*" (Council of Europe, 2014). The harvesting of bristle worms, paddled blood worms and mud shrimp is also of concern. The difficulty in obtaining these three species as bait by anglers and their high purchase price should be of concern since it may indicate that all three species are in decline.

Management Measures for Fisheries Sustainability and Safeguard of Ecosystem Services

The exploratory research on hobby fishing using the same methodology as for sport fishing has shown that such methodology can be adopted on a larger scale to collect information on the former. This can also be applied to any shore fishing taking place in the Mediterranean. Surveys should,

TABLE 7 | Species recorded during shore based fishing competitions and hobby fishing in the Maltese Islands and in other countries.

	Sport fishing					Recreational fishing				
Location	**Author**	**Top species**	**No**	**%**	**Fishers surveyed**	**Author**	**Top species**	**No**	**%**	**Fishers surveyed**
Portugal	Guerreiro et al., 2011	*Belone belone*	236	42.2	–	Veiga et al., 2010	*Diplodus sargus*		44.0	1,321
		Muglidae	107	29.0			*Diplodus vulgaris*		14.0	
		Scomber spp.	100	18.0			*Boops boops*		8.0	
		Dicentrarchus labrax	74	13.2			*Scomber japonicus*		5.0	
						Rangel and Erzini, 2007	*Atherina presbyter*	707		2,081
							Muglidae	583		
							Dicentrarchus labrax	554		
							Trisopterus luscus	318		
Spain	Morales-Nin et al., 2005	*Lithognathus mormyrus*	2,122		1,432	Font and Lloret, 2011	*Serranus cabrilla*	137	36.1	250
		Coris julis	1,247				*Coris julis*	111	29.3	
		Diplodus annularis	1,004				*Diplodus vulgaris*	23	6.1	
		Serramus scriba	867				*Conger conger*	20	5.3	
Turkey						Unal et al., 2010	*Spicara smaris*		31.9	190
							Pomatomus saltatrix		20.0	
							Pagellus acarne		16.9	
							Diplodus vulgaris		7.8	
						Aydin et al., 2013	*Dicentrarchus labrax*		32.2	120*
							Mugil cephalus		12.4	
							Mugil soiuy		12.1	
							Pomatomus saltatrix		11	
						Tunca et al., 2012	*Diplodus sargus*		21.0	50
							Sparus aurata		16.4	
							Dicentrarchus labrax		10.5	
							Mugil sp.		7.86	
						Tunca et al., 2016	*Diplodus annularis*			260**
							Diplodus vulgaris			
							Dicentrarchus labrax			
							Mugil cephalus			
						Tunca et al., 2018	*Trachurus trachurus*			874
							Pomatomous saltatrix			
							Mugil cephalus			
							Sarda sarda			
Maltese Islands	This study	*Coris julis*	7,649	14.8	2,589	This study	*Diplodus sargus*	146	14.6	159
		Diplodus annularis	5,218	10.8			*Oblada melanura*	114	11.4	
		Chromis chromis	4,994	9.7			*Chromis chromis*	111	11.1	
		Diplodus vulgaris	4,397	8.5			*Diplodus annularis*	99	9.9	

**16.7% of respondents also attended recreational activity by boats.*
***respondents also included boat based recreational fishers.*

besides documenting catches, angler traits and fishing trips, also aim to collect demographic information to quantify the angling population, both shore-based and boat based, including spearfishing activities together with the economic value of this fishery. This is important to quantify fishing effort and catch rates for the Maltese Islands which must be considered together with commercial catches when devising complex fishery management plans with catch limits. Such limits must be based on data collected from catch surveys to be an effective conservation measure (Veiga et al., 2010). Plans should also include angler education programs and enforcement of the minimum landing sizes, particularly with hobby fishers since these practice C & R to a much lesser extent. This would ensure that the mortality of juvenile fish is reduced allowing each fish to reproduce at least once in its lifetime. A better rotation of competition sites in which competitions are held, together with higher keepnet use should also be encouraged to reduce biodiversity impacts and ensure sustainable use of resources. Other options such as cutting the line before releasing a deeply hooked fish (Alós et al., 2009) and the establishment of minimum hook sizes are also recommended to reduce post-release mortality (Alós et al., 2008). However, since RF activity has major social repercussions, stakeholder participation in the management processes and decision making is essential to ensure successful implementation through research knowledge transfer and participation of stakeholders.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

We declare that we have abided by the University of Malta's Research Ethics Review Procedures.

AUTHOR CONTRIBUTIONS

SA and AV contributed to all aspects of the research work presented here including the conception and design. SA collected the data. Both authors were involved in the drafting of the manuscript and gave approval for publication.

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Trawling in the Mediterranean: An Exploration of Empirical Relations Connecting Fishing Gears, Otterboards and Propulsive Characteristics of Fishing Vessels

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Fishing pressure is often expressed in terms of a vessels physical attributes, like tonnage and engine power, while a common definition of fishing capacity identifies vessel size as a convenient proxy for the size of the gear used. Nevertheless, these definitions remain arguable, and the refinement of these fishing descriptors is increasingly being considered. A stronger understanding of the relationship between the standard measures of effort and capacity and fishing mortality remains a primary objective, followed by the need to overcome a traditional approach that simply describes effort, capacity and mortality as linearly related, conferring a greater fishing power to larger vessels. In this perspective, the analysis of trawlers' technical features in relation to the size and power of the vessel might constitute an essential step. This study specifically investigated a collection of trawling gears' technical specifications collected by CNR-IRBIM, Ancona. The dataset used includes records from several Mediterranean fisheries, and involves three trawling techniques, including single trawling, twin trawling and pair trawling, and diverse trawling gear categories, comprising demersal/bottom 2-panel trawls (OTB2), demersal/bottom 4-panel trawls (OTB4), pelagic 4-panel trawls (PTM4), semi-pelagic 2-panel trawls (OTM2), semi-pelagic 4-panel trawls (OTM4), and a Mediterranean bottom beam trawl (TBB). We analyzed and described the relationships between vessels' technical features (LOA, towing force, and engine power), some among the main trawl-metrics (headline length, footrope length, trawl length, square width; fishing circle) and the otterboard's technical features (height, width, and projected area) in an attempt to enhance fishing capacity definition through the inclusion of the fishing gear deployed. Self-organizing maps (SOM) were used to explore the empirical relationships among different parts of the fishing trawl gears, as well as between some of these parts, the otterboard size and the engine power of the vessel.

Keywords: Mediterranean fishing, fishing gears, trawling, trawls, fishing equipment, gear research, trawl survey, Mediterranean Sea

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INTRODUCTION

Fishing activity regulation is aimed at the management of exploited fish populations while ensuring maximum sustainable yield and maximum economic yield. Mortality management is generally achieved through a couple of competing and alternative approaches, the input control, regulating the extent at which fishing activity is performed, and the output control, with a core concept that revolves around limitations in catches of one or more selected species. The former approach finds its management tools in restrictions in fleet capacity, fishing gears used and the number of licenses, limitations on technological updates and on the spatial and temporal distribution of fishing activity. These may involve the institution of *ad hoc* area closures, MPAs, zoning schemes and the rotation of areas (Veiga et al., 2016; McLachlan and Defeo, 2018). The output control approach instead relies on the definition of total allowable catches per season, daily catch limits, allowance of the capture and retention of a maximum amount (threshold values), and the establishment of minimum legal landing sizes and Individual transferable or non-transferable quotas. In general, fisheries targeting a limited number of species lean toward an output control scheme, while for mixed fisheries, characterized by multiple species and multiple gear types deployed in the same area, the input control strategy represents a more viable solution (Pope, 2009; McLachlan and Defeo, 2018). Management redundancy, namely the simultaneous application of measures pertaining to both strategies, is also sometimes a possibility (Caddy and Defeo, 2003; Gutiérrez et al., 2011; Santiago et al., 2015) in the pursue of profitable, sustainable and long-lasting fishing activity.

Spatial and temporal limitations of activity are strongly related to the concepts of fishing capacity and fishing effort. Fishing capacity can be either measured in number of vessels or in terms of engine power, size and gross tonnage (FAO, 2008). These vessel-metrics are normally used to cluster together all the exponents of a fleet falling within the same category and thus far represents an indirect method of measuring a vessels harvesting potential. Accordingly, the simple association between vessel size and the size of the fishing gear deployed may be used to estimate harvesting potential, and restrictions to a vessels temporal activity may be applied based on the fleet segment it belongs to. The greater the vessel and the larger its engine power is, the fewer the times a year it can practice fishing.

Fishing effort, on the other hand, can be defined as the product of capacity and activity (European Commission [EC], 2002). It represents a vessels time span of activity and can be measured as numbers of days at sea or number of hours fishing. With the recent establishment of VMS (Vessel monitoring System) and AIS (Automatic Identification System) technologies, the ability to gage fishing effort has indefinitely increased. Where needed, the estimate of both capacity and effort is now relatively simple. What remains to be clarified is how these two standard measures are related and how they affect the fishing mortality of different target species. The traditional association between fishing capacity and gear size, remains questionable, since it has already been demonstrated that no simple and clear relationship between a fishing vessels power and the size of the net it tows exists (Reid et al., 2011). Furthermore, the connection between horsepower, gear size (fishing circle) and swept area was found to be non-uniform across vessels, gear types and species targeted (Fiorentini et al., 2004; Eigaard et al., 2011). Furthermore, several studies have described the relationship between the effort deployed, fishing capacity and fishing mortality as weak and variable, underlining the need to include other effects ("skipper effect") to explain the variance observed (Squires and Kirkley, 1999; Marchal et al., 2006, 2007). These knowledge gaps still interfere with a more accurate modulation of fishing pressure and with the achievement of a sustainable fishing mortality level. The outcome is often faulty management restrictions and a general imbalance between the fleets ability to harvest resources and their ability to regenerate.

Fishing gears, with all their intrinsic variability, represent the physical link between a fishing management strategy and the target populations directly affected by its application. Gear type and trawl size do not represent the only measures that affect catch-efficiency. Other gear components may also be of equal importance such as gear geometry, door spread and ground gear. Furthermore, the horizontal and vertical opening of the mouth may also play a significant role in affecting the gear catchability (Eigaard et al., 2011). In this perspective, better knowledge of the geometries and the technical characteristics of fishing gears is an important aspect to consider on par with fishing effort, size class and engine power, while also assessing the fishing mortality induced by a vessel or a fleet belonging to a specific fleet segment. In this paper we specifically investigated a collection of trawling gears' technical specifications collected by CNR-IRBIM, Ancona. The dataset used includes records collected from eight Mediterranean fisheries, and involves three trawling techniques, including single trawling, twin trawling and pair trawling, and diverse trawling gear categories, comprising demersal/bottom 2-panel trawls (OTB2), demersal/bottom 4-panel trawls (OTB4), pelagic 4-panel trawls (PTM4), semi-pelagic 2-panel trawls (OTM2), semi-pelagic 4-panel trawls (OTM4), and a Mediterranean bottom beam trawl (TBB). We analyzed and described the relationships between vessels' technical features, some among the main trawl-metrics and otterboards technical specifications in an attempt to enhance the definition of fishing capacity through the inclusion of the fishing gear deployed. These aspects were explored using Kohonen self-organizing maps (SOM) (Kohonen, 1997) and model-based clustering, based on finite Gaussian mixture modeling. Finally, we tested the predictive capabilities of the trained SOM, observing its ability to predict the size of different trawling gear components, as well as of the otterboards. These parameters were estimated, providing the map with quantitative variables (vessel-metrics) and qualitative descriptors (vessel provenance and gear type), for the observations contained in a test dataset.

MATERIALS AND METHODS

Data Collection

In this paper we analyzed a collection of trawling gears' technical specifications collected by CNR-IRBIM Ancona.

The information collected includes technical specifications of trawling nets (headline length, footrope length, square width, codend circumference and extension, etc.), doors (length, height, and weight) and general fishing vessel features (engine power, LOA, GRT, fleet registry number, bollard pull, base harbor, etc.) collected from eight Mediterranean fisheries, including Italy, France, Spain, Greece, Turkey, Croatia, Tunisia, and Cyprus. Data collection involved a first critical review of diverse literature sources (technical and scientific papers), followed by direct *in situ* measurements of vessels and fishing gears, performed with the help of fishermen, net makers and door manufacturers. The analysis of literature sources proved invaluable in establishing the main gear characteristics required for the evaluation of the overall gear size, while field technical measurements helped in completing the information obtained through literature research, filling the emergent knowledge gaps in all the inspected trawling techniques. The collected data can be subdivided by trawling techniques and gear typologies. Three trawling techniques were described, including single trawling, twin trawling and pair trawling techniques, performed with diverse trawling gear categories, comprising demersal/bottom 2-panel trawls (OTB2), demersal/bottom 4-panel trawls (OTB4), pelagic 4-panel trawls (PTM4), semi-pelagic 2-panel trawls (OTM2), semi-pelagic 4-panel trawls (OTM4), and a Mediterranean bottom beam trawl (TBB). Among the available technical specifications, Vessel Length Overall (LOA), Gross Registered Tonnage (GRT), Engine Power (P) and Total Available Towing force (TAT) were chosen as indicators of vessel size; headline and footrope length (HL, FL), trawl length (TrL), trawl weight (TrW), square width (Wsq), fishing circle (FC) and the primary hanging ratio (E1), were chosen as indicators of gear magnitude; and door height (OBH), length (OBL) and projected area (OBA) were selected as descriptors of the otterboard size. As additional information, vessel and gear provenance (country and base harbor) were also considered in the analysis.

Definition of Vessel Size, Gear-Metrics and Otterboard's Descriptors

The adopted vessel-metrics (LOA, GRT, and P) defined the size of a fishing vessel in terms of the maximum length of its hull (in meters), its internal volume (registered tons), and the power of the main propulsion engine installed onboard (hp). TAT represents an alternative vessel descriptor proposed to overcome the lack of information regarding a vessel's bollard pull, rarely available. The indicator considers installed engine power, propulsion system (nozzle and propeller) and trawling speed, and it was used as an alternative metric of the vessel's actual power in operation. A more extensive description of this indicator can be found in Notti et al. (2013).

Among gear metrics, the headline and the footrope respectively represent the upper frame rope to which netting and floats are attached, and the lower combination rope, carrying the sinkers. Both their lengths are measured in meters. Fishing circle and square width, also measured in meters, are two additional gear descriptors. The fishing circle indicates the perimeter of the net measured at the footrope bosom, while the square width describes the width of the square, the first section of the gear netting, placed in the top panel right behind the wings. Trawl length and weight respectively describe the length of the trawl, codend excluded, along its longitudinal axis (in meters), and the overall trawl weight, expressed in kilograms. The primary hanging ratio indicates the ratio between the length of the rope frame on which a net panel is attached, and the length of the attached net panel stretched. This ratio affects the ability of a net to change shape and area in water, consequently increasing or reducing a trawl's fishing efficiency. Finally, the collection of gear descriptors was completed by two additional calculated indicators, the Horizontal Net Opening (HNO) and the Horizontal Door Spread (HDS), also measured in meters.

As for the otterboards, OBL and OBH respectively represent the length and height of the otterboard, while the projected area indicates the area of its surface, corrected by an otterboard-specific factor to consider the otterboard's shape. A schematic representation of the gear descriptors considered, is reported in **Figure 1**.

Trawling Techniques and Gear Typologies

Each record analyzed was assigned to one of the five gear typologies, based on a classification first described in Eigaard et al. (2011) broadened by the addition of a sixth category, the Mediterranean "*Rapido*" trawl. A brief description of the observed gear categories is provided below, while a short summary of the described gears is available in **Table 1**.

Demersal/Bottom 2-Panel Trawls (OTB2)

Often made entirely of knotless PA netting, the OTB2 trawls present a wide winghead opening attached to long sweeps and bridles, coupled with a narrow vertical opening. Target species of these gears include *Merluccius merluccius*, *Mullus barbatus*, *Merlangius merlangius*, *Trisopterus minutus capellanus*, *Lophius* spp., *Nephrops norvegicus*.

Demersal/Bottom 4-Panel Trawls (OTB4)

These trawls are made by 4 panels, the upper and lower panel and two side panels, which are usually made entirely of knotless polyethylene netting, though sometimes a portion of knotless polyamide netting may appear in the lower panel. The vertical opening of these trawls can reach up to 2 to 4 m in height, increased by a couple of long bridles whose length can easily reach 10 to 15 m. This gear category usually mainly targets crustaceans like *Parapenaeus longirostris*, *Aristaeomoprha foliacea* and *Nephrops norvegicus*.

Pelagic 4-Panel Trawls (PTM4)

Large pelagic gears built with four connected panels, characterized by a wide vertical opening made with very wide meshes or ropes, specifically constructed to flock target species toward the center of the net, where a smaller mesh size collects the catch. These fishing gears are used to mostly target pelagic species like *Sardina pilchardus*, *Engraulis encrasicolus*, *Scomber scombrus*, and *Trachurus trachurus*.

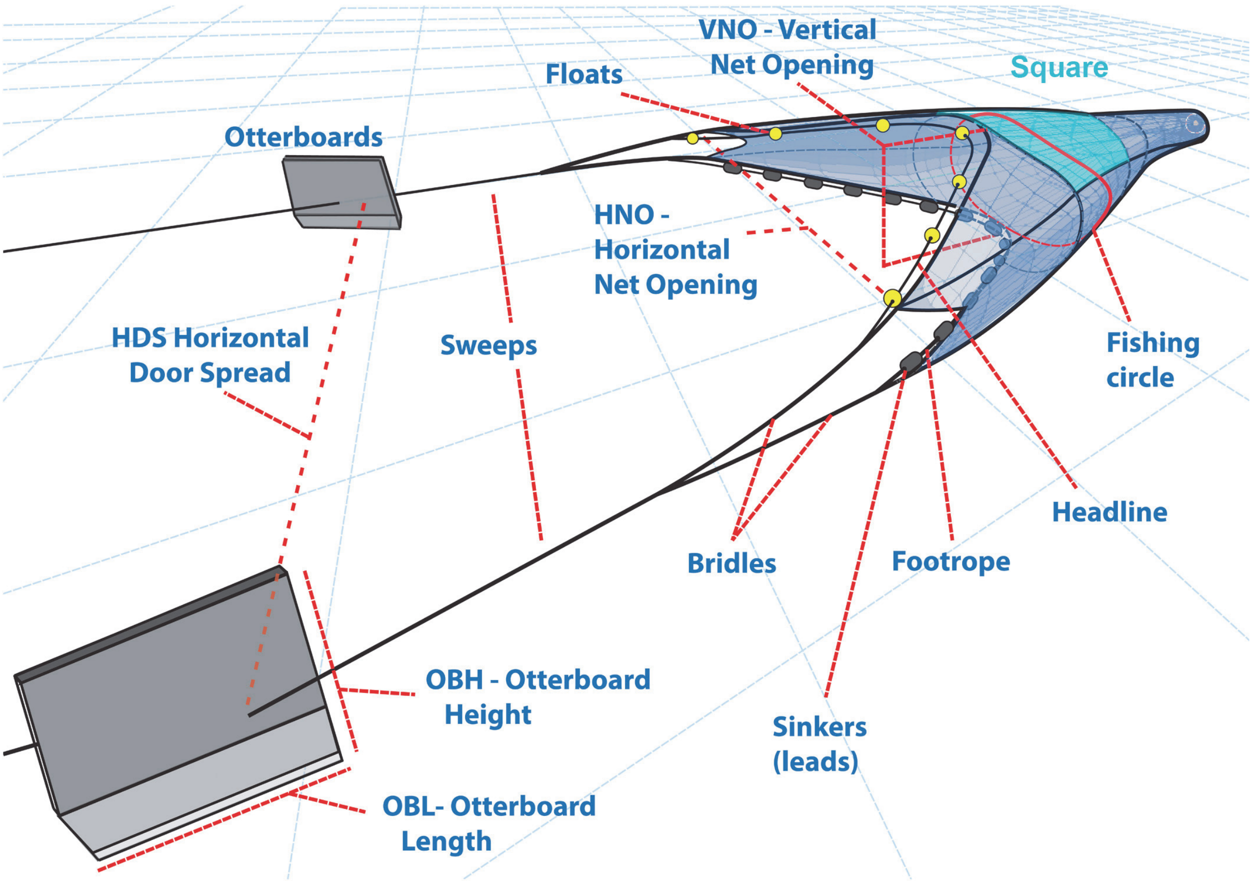

FIGURE 1 | Schematic representation of the main gear-metrics and otterboard's descriptors considered.

Semi-Pelagic 2-Panel Trawls (OTM2)

This fishing gear type is typically used near the seabed in Tyrrhenian fisheries to catch demersal species living a semi-pelagic lifestyle, characterized by frequent ascending displacements. *Lepidopus caudatus, Sparus aurata and Dicentrarchus labrax* are among the major representatives of these trawls' target species. Their main features include relatively large meshes (up to 1600 mm), a mean vertical opening of 3 to 4 m, and a four-cable rigging. In semi-pelagic 2-panel trawls, catching efficiency is determined mainly by the gear's volume.

Semi-Pelagic 4-Panel Trawls (OTM4)

These trawls are similar to semi-pelagic 2-panel trawls but characterized by a wider vertical opening usually ranging within 4 to 10 m. Although this trawl typology mainly targets demersal species, the increased vertical opening also improves their catch efficiency for pelagic species.

Mediterranean Bottom Beam Trawl Typology ("Rapido" – TBB)

This peculiar gear type, mostly used in Northern Adriatic fisheries, is constituted by a cone-shaped net with a mouth opening that attaches itself to a metallic frame, up to 4 m wide, that slides on the sea floor aided by sledges. The trawl uses a rake-like structure, equipped with iron teeth, to dig through the upper layers of sediment and to forcefully displace its target species, herding them toward its body. The Rapido trawl primarily targets flatfish species like *Solea solea*, *Psetta maxima*, *Scophthalmus rhombus*, and bivalve mollusks like *Pecten jacobeus* and *Aequipecten opercularis.*

Data Analysis – Self Organizing Maps

Data analysis was performed using the R language[1]. The collection of technical gears was examined using SOM (Kohonen, 1982, 2001), an unsupervised neural network-based approach, commonly used for classification and association, suitable for non-linear data mining, exploration, clustering and summarization of the variability in a dataset (Park et al., 2018). The SOM were applied as an explorative method to examine and classify vessels and gear records according to similarities in their technical features. Their predictive power was also evaluated observing the map's capability to estimate the major descriptors of gear and otterboards' magnitude on the basis of information regarding the size of the vessel, its geographical provenance and type of trawling gear used.

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[1] http://cran.R-project.org/

TABLE 1 | Summary table of the observed gear types and subtypes followed by an indication of the major species targeted.

Trawl description	*Trawl type*	*Trawl subtype*	*Species targeted*
Demersal/bottom 2-panel trawls	*OTB2*	*Volantina*	*Merluccius*
		Tartana	*Mullus barbatus*
		Huelvano	*Merlangius*
		Fondo duro	*Trisopterus minutus capelanus*
		Cadenero	*Lophius* spp.
		Minifalda	*Nephrops norvegicus*
		Twin trawls	
Demersal/bottom 4-panel trawls	*OTB4*	*Semitangonero*	*Parapenaeus longirostris*
		Cuadrado pescado	*Aristaeomoprha foliacea*
		Tangonero cuchilla	*Nephrops norvegicus*
		Twin trawls	
		Cuadrado gamba (rosso)	
		Tangonero clasico	
		Dos bocas	
		Espada	
		Tangonero	
		Four faces	
		Jumeaux	
		Filet a chaine	
Pelagic 4-panel trawls	*PTM4*	*Pair trawling*	*Engraulis encrasicolus*
		NA	*Sardina pilchardus*
			Scomber scombrus
			Trachurus
Semi -pelagic 2-panel trawls	*OTM2*	*NA*	*Sparus aurata*
			Dicentrarchus labrax
			Lepidopus caudatus
Semi -pelagic 4-panel trawls	*OTM4*	*Semitangonero Alboran*	*Mixed demersal fish*
		Butterfly	
		Pelagic a corde	
		Juge a corde	
		NA	
Mediterranean	*TBB*	*Rapido*	*Solea*
			Psetta maxima
			Scophthalmus rhombus
			Pecten jacobaeus
			Aequipecten opercularis

Self-Organizing Maps algorithms learn from complex multidimensional data and project the multi-dimensional data space onto a regular lower-dimensional grid, usually a two-dimensional space map. The visualization of more than two dimensions is possible, but a bidimensional map is usually preferred since it is closer to human perception. The projection is made preserving the topology (or neighborhood) of the original dataset, with similar records creating neighboring clusters on the grid, and distant records expected to be distant on the map. The distance between sample units and virtual units is calculated by applying a user-defined distance measure, selected to provide an accurate data representation on the map (Brosse et al., 2001). A SOM neural network uses two layers of nodes, an input layer, connected to the original dataset, and an output layer (the Kohonen layer). The output layer, made by n neurons, is a two-dimensional array of virtual units used to represent in an ordered way the distribution of the original dataset. The projection of the sample units of the input layer onto the output layer is achieved through an unsupervised learning algorithm that calculates the components (W_{ik}) of each virtual unit during the training phase. The algorithm starts the learning process, assigning random weights w to the output units, then calculating the distance between each input vector x_{ij} and the weight vectors, identifying the best matching unit (BMU) for every input vector; the unit showing minimum distance from the input vector. A neighborhood is defined around the BMU by the units whose distance from the BMU is less than or equal to the neighbor radius r. The units' weights w are then updated following the rule:

$$w_{ik}(t+1) = w_{ik}(t) + \alpha(t)\, h_{ck}(t)\left[x_{ij}(t) - w_{ik}(t)\right]$$

where w_{ik} is the weight vector of the BMU, x_{ij} is the input vector and $\alpha(t)$ is the learning rate at the time step (t). The function in charge of this update is the Neighborhood function, identified in the equation as $h_{ck}(t)$. During the learning process, the BMU is not the only updated unit, since the units falling within its neighborhood range are updated as well, in inverse proportion of their distance from the BMU. The learning rate and the radius are progressively decreased at each iteration, and the process is iteratively repeated until an ending criterion is met. A more complete descriptions of SOM algorithms can be found in Kohonen (1982, 2001, 2012), Park et al. (2018).

We used two different SOMs, the first for data recovery and the second for the actual exploration of the dataset. The first step of the analysis involved dealing with missing data which, to different degrees, affected the variables describing vessels' characteristics. Missing data can be treated with three possible approaches, they can either be deleted, skipped or replaced by estimated values (Park et al., 2018). SOMs' ability to cluster together in the output space data points showing similar characteristics makes them reliable candidates for data mining and recovery, as outliers and gaps in the original dataset can be replaced by their features in the map (Adeloye et al., 2012). Strictly speaking, when a vector containing gaps is presented to the SOM, its BMU can still be identified according to the other variables available. An estimate of the values for the missing variables can then be obtained as their corresponding values in the BMU (Adeloye et al., 2012). Several studies have already used this approach to recover gaps in datasets with positive results (Kalteh and Hjorth, 2009; Adeloye et al., 2012; Mwale et al., 2012, 2014; Kim et al., 2015; Nkiaka et al., 2016). Following this method, a first SOM was trained to estimate gaps in vessels' description where present, using all the observations available in the dataset (591 records), but

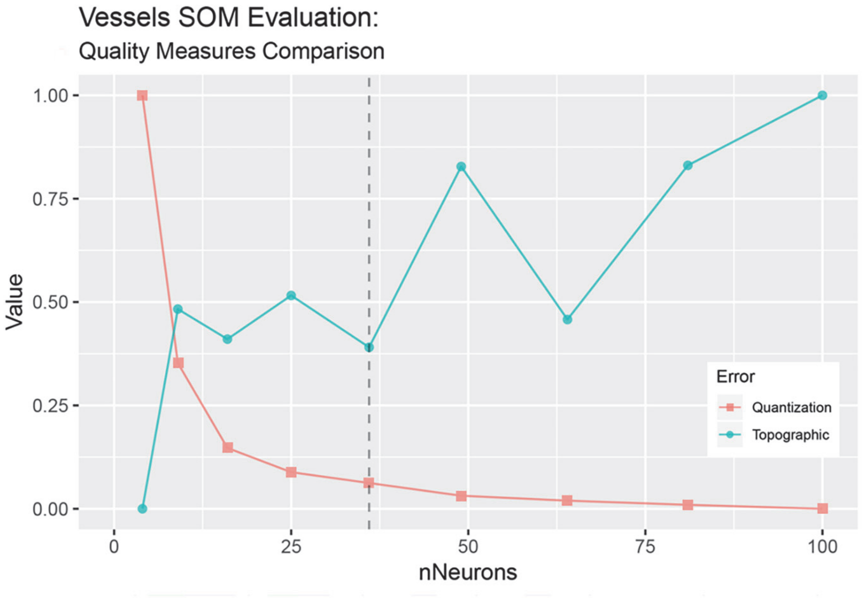

FIGURE 2 | SOM quality evaluation through the optimization of quantization and topographic error.

keeping only the variables that concern the characteristics of the fishing vessels. A 6 × 6 virtual unit map was trained using the "supersom" function from the R package "Kohonen" (Wehrens and Kruisselbrink, 2018). Map size was decided on the observation and comparison of two SOM quality measures, the quantization error and the topographic error. The quantization error (Kohonen, 2001) represents the average distance between the nodes and the training data points, while the topographic error (Kiviluoto, 1996) was calculated as the mean distance in map coordinates between the BMU and the second BMU for all data vectors. Both measures were repeatedly calculated while testing different map sizes with an increasing number of output neurons. The optimal map size was then defined aimed at the best tradeoff that minimizes both quantization and topographic error, as shown in **Figure 2**. SOM Training was performed on two separate data layers, the first containing continuous variables (LOA, GRT, and P) and the second with categorical data (vessels' provenance), coded as a binary variable. The adoption of two separate layers was motivated by the need to select two different distance measures, each one appropriate to the specific data typology. For the first layer the SOM algorithm used Euclidean distance applied to a transformed dataset, normalized between zero and one, in the range of the minimum and maximum values of each variable. Range normalization represented a necessary step to provide the same weight to all variables, otherwise spanning very different ranges. For the second layer, distances were calculated using the Tanimoto distance, which is more suitable for data with binary-valued features. The outcome of the first SOM enabled the completion of vessel data, achieved through the replacement of missing values with their corresponding values in the BMUs. A second SOM was then trained, this time using the updated gapless vessels dataset, joined with the remaining portion of selected variables of interest, describing fishing gears' technical features and otterboards' metrics. The second map, a 10 × 10 unit map, was trained using the same algorithm applied to three layers of data, one for vessel metrics, one for gears and otterboard specifications and a third one carrying categorical data (reporting vessel provenance and trawl type). Euclidean distance was used for the first two layers, along with Tanimoto distance for the third layer. The SOM algorithm was applied to 80% of the collected net observations, from a random 80/20 split of the data, into training and test sets specifically devised to test the map predictive capabilities on a new dataset and to evaluate its ability to infer the dimensions of the gear and otterboards used. The size of the map was decided based on the same optimization method of topographic and quantization errors previously used for the first SOM.

SOM and Clustering

Model-based clustering, based on finite Gaussian mixture modeling, was performed on the trained map to identify groups of observations with similar metrics. Clustering was obtained using the *Mclust* function from the R "*Mclust*" package (Scrucca et al., 2016). This function estimates the optimal number of clusters and defines the best partition according to the Bayesian Information Criterion (BIC) for expectation-maximization, initialized by hierarchical clustering for parameterized Gaussian mixture models. The function runs several competing models and identifies the best one as the one with the highest BIC. The optimal number of clusters is defined as the point at which adding additional clusters no longer increases the BIC value. This clustering technique is described extensively in Scrucca et al. (2016). Clustering results were optimized excluding the cluster analysis variables that did not show recognizable patterns, therefore contributing less to the final distribution of the variables on the map. Clusters were compared, and between-cluster vessels and gear parameters where further examined with the purpose to improve the description of the relationships between various gear components, gear-metrics and vessel sizes.

Predictive Power and Performance

After the SOM training, the distribution of the variables on the created map remains fixed and can be used to predict values for new observations. This process is very similar to a linear regression, where the parameters of a function are estimated first, then the defined function is used to predict the value of the dependent variable of interest for new observations. A SOM can be considered as a form of non-linear regression without a presupposed form of a defined function. Given the non-linearity of the model, defining the exact contribution to the estimated values could be trivial, but its performance can still be measured using common statistical techniques. The trained SOM was used to infer the dimensions of gears and otterboards used, estimated on the basis of quantitative variables (vessel metrics), and qualitative descriptors (vessel provenance and gear type), for the observations contained in the test dataset. The performance of the obtained predictions was assessed comparing predicted estimates with real observed values. A common measure of the performance of a linear regression model is the coefficient of determination. Given the non-linearity of SOMs, in this case the R^2 coefficient was not the appropriate method of model performance assessment, but still a simulated R^2 coefficient was calculated assuming that the predicted results constituted the outcome of a linear model. We created several scatterplots of predicted vs. observed values, then calculated a linear regression through these values, calculating the associated R^2 measure. The simulated coefficient is not comparable to the R^2 measure in a linear regression model but could still be used to explore model performance and to approximate a description of the fit of predicted values to the observed values. This procedure is described in Tan et al. (2002).

RESULTS

Database Description

The original database contained more than 600 trawling gear records, 589 of which were effectively analyzed during this research study. A few records were excluded from the analysis due to too much missing data, resulting in the inability of the SOM to classify them correctly and properly assigning them to a BMU. Beam trawl data were also omitted due to the small number of records (less than 10) associated with trawl gear metrics that were too different from the rest of the dataset. Their presence, taken into consideration at first, was finally ruled out as they generated anomalous peaks in the trained map. The analyzed dataset is then composed of 55.68% of OTB2, 32.25% of OTB4, 9% of OTM4, 1.69% of PTM4 and 1.32% to OTM2. Trawling gear records belong to eight different countries, with approximately 44% of the records belonging to Italy, 40.77% coming from Spain, 6.26% from Greece, 4.56% from Turkey and 3.55% from France. Croatia, Tunisia and Cyprus are represented in the dataset with less than 1% of the data. **Table 2** shows a summary of the descriptive statistics of gear and vessel metrics observed, without a subdivision per trawl typology.

Self-Organizing Maps and Data Recovery

The first SOM is represented by a map of 36 output units, generated from two information layers, the first one containing normalized vessel metrics (LOA, GRT, and Engine Power) and the second one containing geographical information such as the vessel's nationality and port of origin, coded as binary variables. The map training process went through 18,000 iterations, following the suggestion of Kohonen (2001), which recommends that the number of iterations in the training process should be

TABLE 2 | Summary table of the descriptive statistics of gear, vessel and otterboard's metrics observed.

Item	Acronym	Units	Min	Max	Mean	Median	SD
Vessel length overall	LOA	m	9.01	37.2	21.85	21.84	4.81
Vessel engine Power	P	hp	80	3200	835.8	660.0	499.77
Gross Registered Tonnage	GRT	GT	13.0	285.5	85.17	79.0	55.86
Total available towing force	TAT	kg	581.6	19680	6517.3	5409.3	3704.50
Headline	HL	m	14.0	128–0	53.79	48.5	22.33
Footrope	FL	m	16.0	162.41	69.79	63.00	29.53
Trawl length	TrL	m	2.98	219.86	61.60	54.21	30.01
Square width	Wsq	m	12.44	188.10	41.84	36.97	19.58
Fishing circle	FC	m	16.48	409.6	75.28	58.00	49.97
Trawl weight	TrW	kg	7.94	778	280.6	244.08	182.06
Primary hanging ratio	E_1	–	0.03	0.90	0.37	0.34	0.12
Horizontal door spread	HDS	m	6.29	291.85	89.89	84.92	38.94
Horizontal net opening	HNO	m	1.26	106.24	19.57	17.54	9.66
Otterboard length	OBL	m	0.92	3.37	1.90	1.86	0.39
Otterboard height	OBH	m	0.45	1.87	1.24	1.2	0.32
Otterboard projected area	OBA	m^2	0.57	5.0	2.32	2.15	0.98

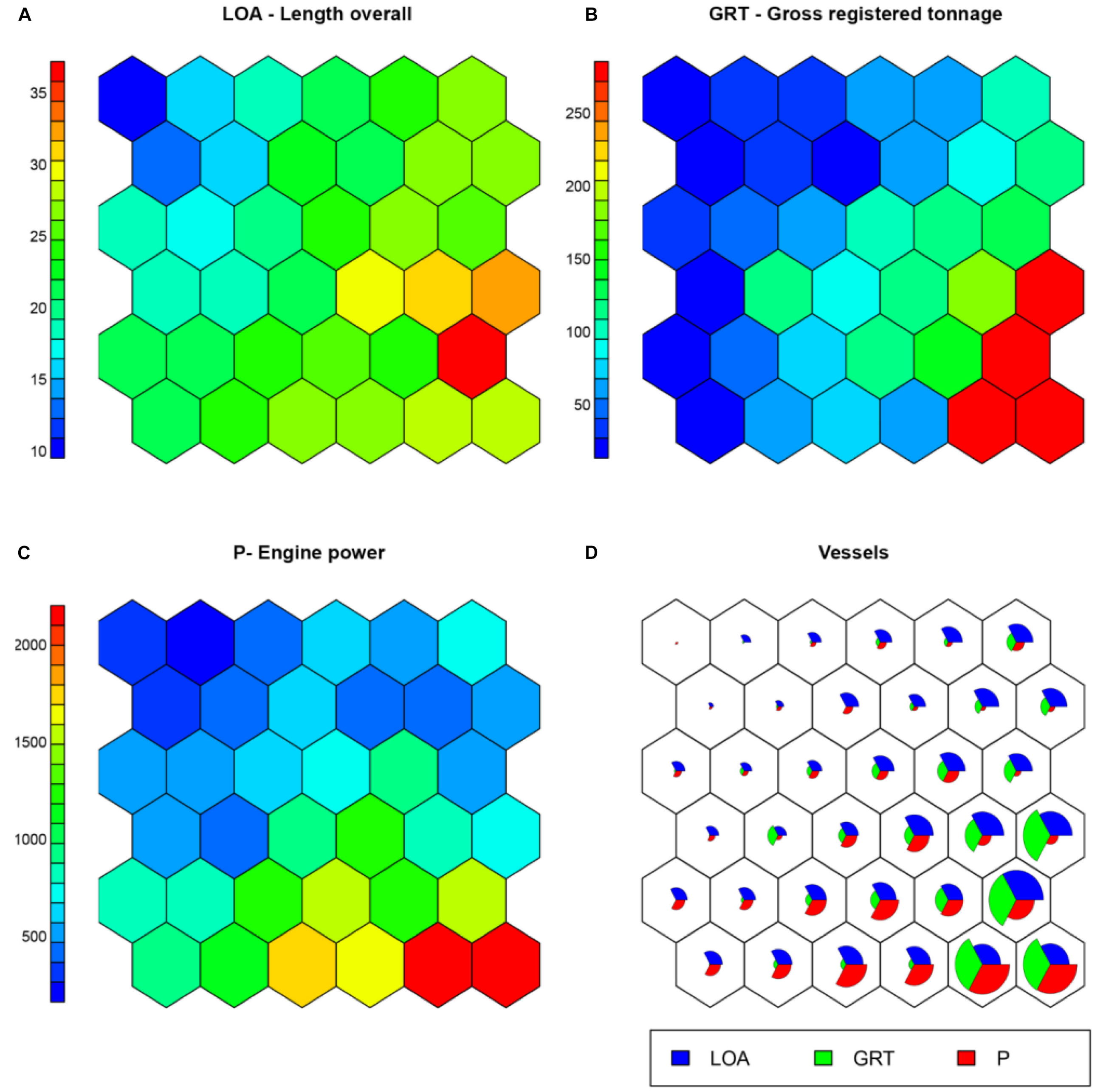

FIGURE 3 | Results of the first trained SOM. From **(A–C)** the patterns of the considered unscaled vessel metrics are represented in a color scale; **(D)** shows the "codes plot," representing the magnitude of each variable per output unit.

at least 500 times the number of network units. All output units were associated with input records in the training dataset, with no empty units emerging at the end of the training process. The number of input records associated with each unit ranged between one and 30 with an average of 14.72 records associated per unit. The main results of the SOM are reported in **Figure 3**. The trained SOM revealed very similar distribution patterns for the continuous variables observed, showing a diagonal gradient that places smaller vessels, characterized by minor LOA, GRT and P, in the upper left corner of the map, and larger vessels in the lower right corner, with values gradually increasing along the diagonal connecting the two corners. According to the gradient shown, vessels characterized by average tonnage, length and engine power are distributed in the central area of the map, following the diagonal connecting the two opposite corners. Although the patterns returned by the map agree in the general distribution of the vessels, the observed gradients diverge slightly from one another in the positioning of the extreme peaks in the units of the map (near but not necessarily overlapping) and for the smoothness in the transition from smaller to higher values, indicating the non-linear nature of the correlation between the observed variables. No further analysis was carried out on the first trained map, which represented only an intermediate step. The estimates delivered by the SOM output units, based on the similarity of the weight vectors, were used as a replacement of the gaps in the original dataset.

Second Map - Analysis of the Observed Patterns

The final trained SOM is a map of 10 × 10 output units, generated from three information layers, the first two containing normalized vessel metrics (LOA, GRT, TAT, and P) and normalized gear and otterboard measurements (HL, FL, TrL, Wsq, FC, TrW, E1, HDS, HNO, OBH, OBL, OBA), and a third one containing categorical variables such as trawl type and gear's nationality, coded as binary variables. The map training process went through 50000 iterations, a number determined following the same procedure adopted during the training of the first SOM. Only four out of 100 units were not associated with input records in the training dataset at the end of the training process. The number of input records associated with each output unit ranged between one and 17 with an average of 4.89 records associated per unit. **Figure 4** shows how the different variables relate to each other within the trained map. A first observation of the patterns delivered by the SOM shows a distribution of the highest values in the upper half of the map, followed by a concentration of the lower values in the lower half. A finer observation highlights a general distribution trend of the highest values in the upper right corner for vessel metrics such as engine power and TAT, gear metrics such as HL, FL, gear length and gear weight, and otterboard descriptors such as otterboard height and the projected area. Associated with the distributions of gear type and country of origin, these higher values can be ascribed to the Spanish gear types OTB2, OTB4 and OTM4 and to the French gear type OTM4.

The vessel variables LOA and GRT showed a second peak in the upper left corner of the map, which however, are associated average values in terms of trawl size, belonging to the fishing gear types OTB2, PTM4, and OTM4, all of Italian origin. The variables HL, TrL, Wsq, FC, TrW showed two evident peaks of maximum values in two units positioned on the left and right of the first line of hexagons in the upper portion of the map. Those units correspond to the larger size category of Italian PTM4 trawls, and to the larger size category of French OTM4 fishing gears, respectively. The minimum values in terms of size of the vessel and the size of the gear used are displayed on the map in the lower left corner, dominated by OTB2 gears of Italian origin. It was not possible to identify any recognizable pattern for the variables HDS and HNO, while the hanging ratio E1 displayed a rather homogeneous distribution gradient, with slightly higher values displayed by gears of a smaller size class (lower right corner of the map). Regarding the distribution patterns of gear types and geographical provenance, the gear type OTB2 clustered on the left side of the map, while the right side of the map was dominated by the OTB4 gear type. Italian and Spanish trawls almost followed the same distribution pattern with a small degree of overlap. The OTM4 trawl type was almost entirely positioned in the upper right portion of the map, among gears belonging to a larger size class, with a second cluster of three units placed in the central-lower portion of the map. Pelagic 4-panel trawls clustered in the upper left corner, in the larger vessel and gear class, while OTM2 records did not show any recognizable pattern. Greek and Turkish trawls clustered on the left side of the map, among the OTB2 gear type and the average and average-to-small vessels and gear classes, respectively. Finally, Tunisia, Croatia and Cyprus had concentrated values within single output units, scattered in the OTB2 portion of the SOM.

Cluster Analysis

The model-based clustering approach based on finite Gaussian mixture modeling evaluated 14 competing models, choosing an ellipsoidal, equal shape and orientation (VEE) model with four components as the best model, indicating an optimal partition of the trained map in four clusters. The HDS and HNO variables were excluded from the cluster analysis as they did not show recognizable patterns, but an approximately homogeneous distribution of values instead, evenly spread all over the map. The outcome of the clustering algorithm and the resulting partition transposed on the trained map are shown in **Figure 5**. The clustering algorithm partitioned the trained map into four subgroups, one for the smaller vessel/gear/otterboard combinations (Cluster 1), another for the heavyweight class (Cluster 3), and two clusters for the average sized vessels (Clusters 2 and 4), which did not necessarily correspond to medium sized gears. Cluster 1, was placed at the bottom of the map, gathering smaller vessels with an average 8.19 m LOA, 368.3 hp and 42.88 GRT; corresponding to the average trawl length of 43.08 m, a square width of 17.77 m, FC of 33.68 and trawl weight of 149.0 kg. Cluster 3, was placed at the top of the map, collecting observations pertaining to the larger LOA and GRT vessels, but characterized by a wide variability in terms of engine power. Vessels belonging to this cluster share an average 19.77 m LOA, 1345.0 hp and 219.1 GRT; corresponding to the average trawl length of 78.43 m, square width of 45.58 m, FC of 110.03 and trawl weight of 318.43 kg. Clusters 2 and 4 represented the average size class vessels and shared very similar characteristics in terms of LOA and GRT. These two clusters were instead very different in terms of engine power, with Cluster 2 vessels characterized by an average 634.1 hp and Cluster 4 vessels characterized by an average 1102 hp. In this case the observed differences in engine power translated into marked differences in the size of the gears deployed. Cluster 2 vessels operated trawls characterized by an average length of 42.3 m, square width of 26.17 m, FC of 36.7 and trawl weight of 135.3 kg. Cluster 4 vessels, on the other hand, deployed trawls characterized by an average length of 88.09 m, square width of 39.55 m, FC of 88.95 and trawl weight of 419.4 kg. A summary of the reported average value for each cluster is reported **Table 3**, while a description of each variable range, median and interquartile ranges divided by cluster is available in **Figure 6**.

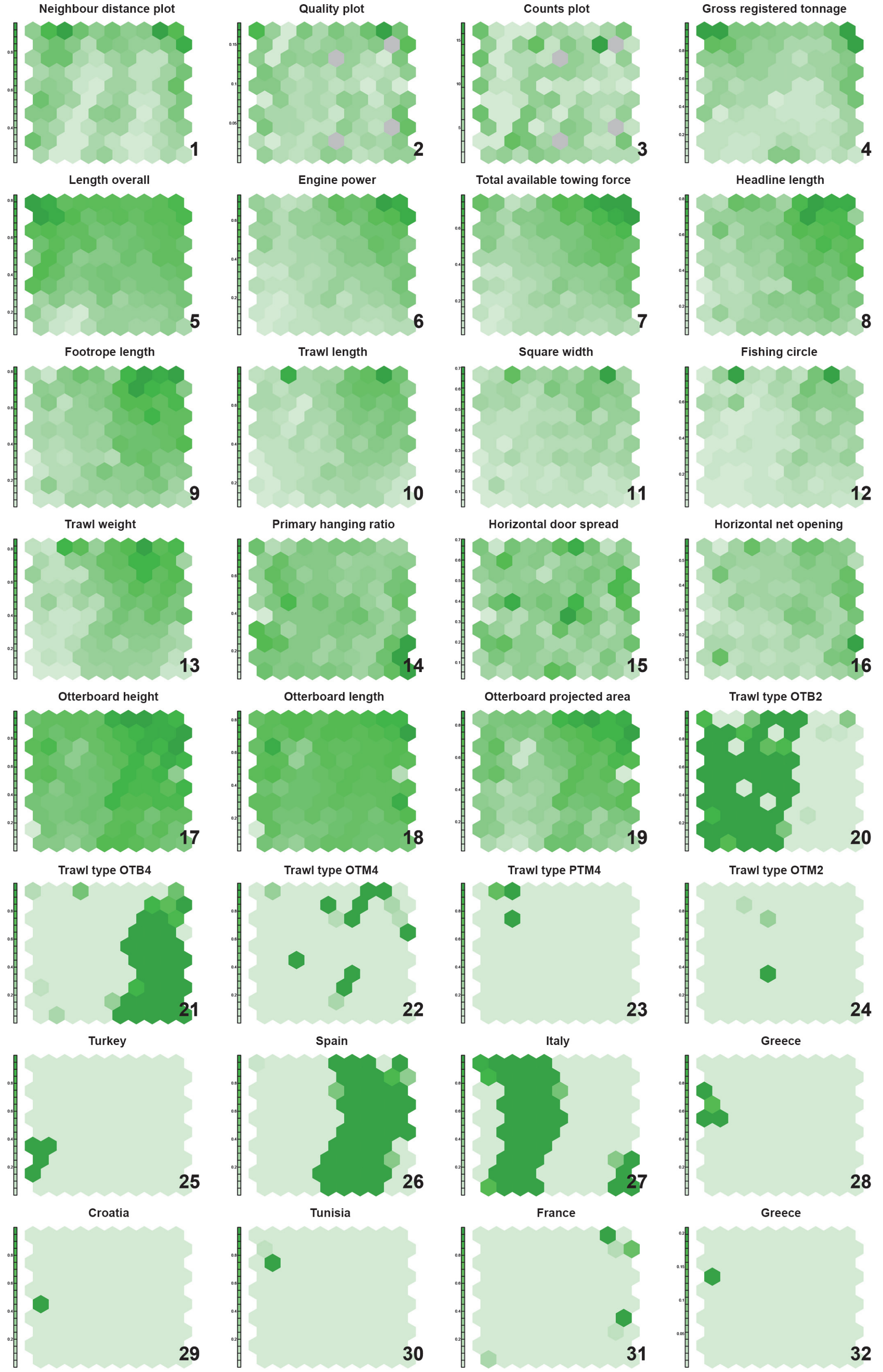

FIGURE 4 | Trained SOM. The distribution pattern of the observed variables, normalized between 0 and 1, is expressed in the SOM using a color scale. Darker areas correspond to the higher values of each variable.

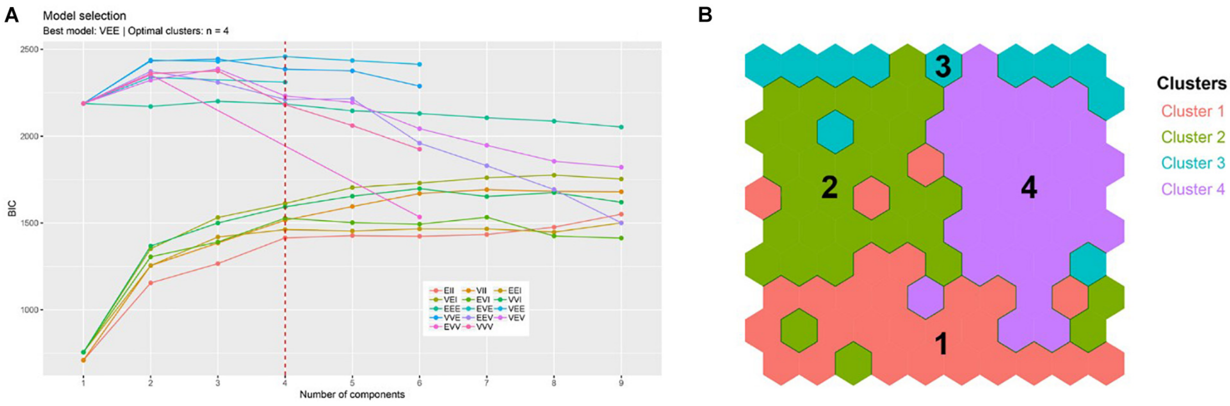

FIGURE 5 | (A) Outcome of the clustering algorithm and **(B)** the resulting partition transposed on the trained map.

SOM Predictive Performance Evaluation

The analysis of 472 observations, corresponding to 80% of the dataset, allowed the investigation of the SOM's ability to predict gears and otterboard sizes for the remaining 20% of the dataset, with a total number of 119 observations. The prediction was based on independent variables describing vessel size and the categorical factors indicating gear type and vessel provenance. A simulated R^2 coefficient was calculated assuming that the predicted results constituted the outcome of a linear model. It was then used to explore model performance

TABLE 3 | Summary table of the observed statistics for the identified clusters.

Item	Acronym	Units	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Vessel length overall	LOA	m	8.19	15.33	19.77	14.44
Vessel engine Power	P	hp	368.3	634.1	1345	1102
Gross Registered Tonnage	GRT	GT	42.88	87.53	219.1	65.11
Total available towing force	TAT	kg	2945	5040	10687	8589
Headline	HL	m	25.44	31.34	49.42	65.15
Footrope	FR	m	35.28	41.28	66.51	87.48
Trawl length	TrL	m	43.08	42.3	78.43	88.09
Square width	Wsq	m	17.77	26.17	45.58	39.55
Fishing circle	FC	m	33.68	36.7	110.03	88.95
Trawl weight	TrW	kg	149	135.33	318.43	419.4
Primary hanging ratio	E_1	–	0.33	0.37	0.31	0.29
Horizontal door spread	HDS	m	74.79	82.15	80.88	98.14
Horizontal net opening	HNO	m	13.11	17.62	20.97	25.15
Otterboard length	OBL	m	1.63	1.82	2.32	2
Otterboard height	OBH	m	1.06	1.1	1.51	1.54
Otterboard projected area	OBA	m^2	1.5	1.69	3	2.88
Country Italy	–	%	56.20	73.88	37.25	0
Country Spain	–	%	30.70	1.49	37.25	100
Country Turkey	–	%	6.52	8.95	0	0
Country Greece	–	%	5.88	12.68	0	0
Country France	–	%	0.65	0	25.49	0
Country Croatia	–	%	0	0.74	0	0
Country Tunisia	–	%	0	1.49	0	0
Country Cyprus	–	%	0	0.74	0	0
Gear type OTB2	OTB2	%	67.97	85.07	35.29	20.45
Gear type OTB4	OTB4	%	23.52	8.95	33.33	62.87
Gear type OTM4	OTM4	%	5.22	4.47	17.64	16.66
Gear type PTM4	PTM4	%	0	0	13.72	0
Gear type OTM2	OTM2	%	3.26	1.49	0	0

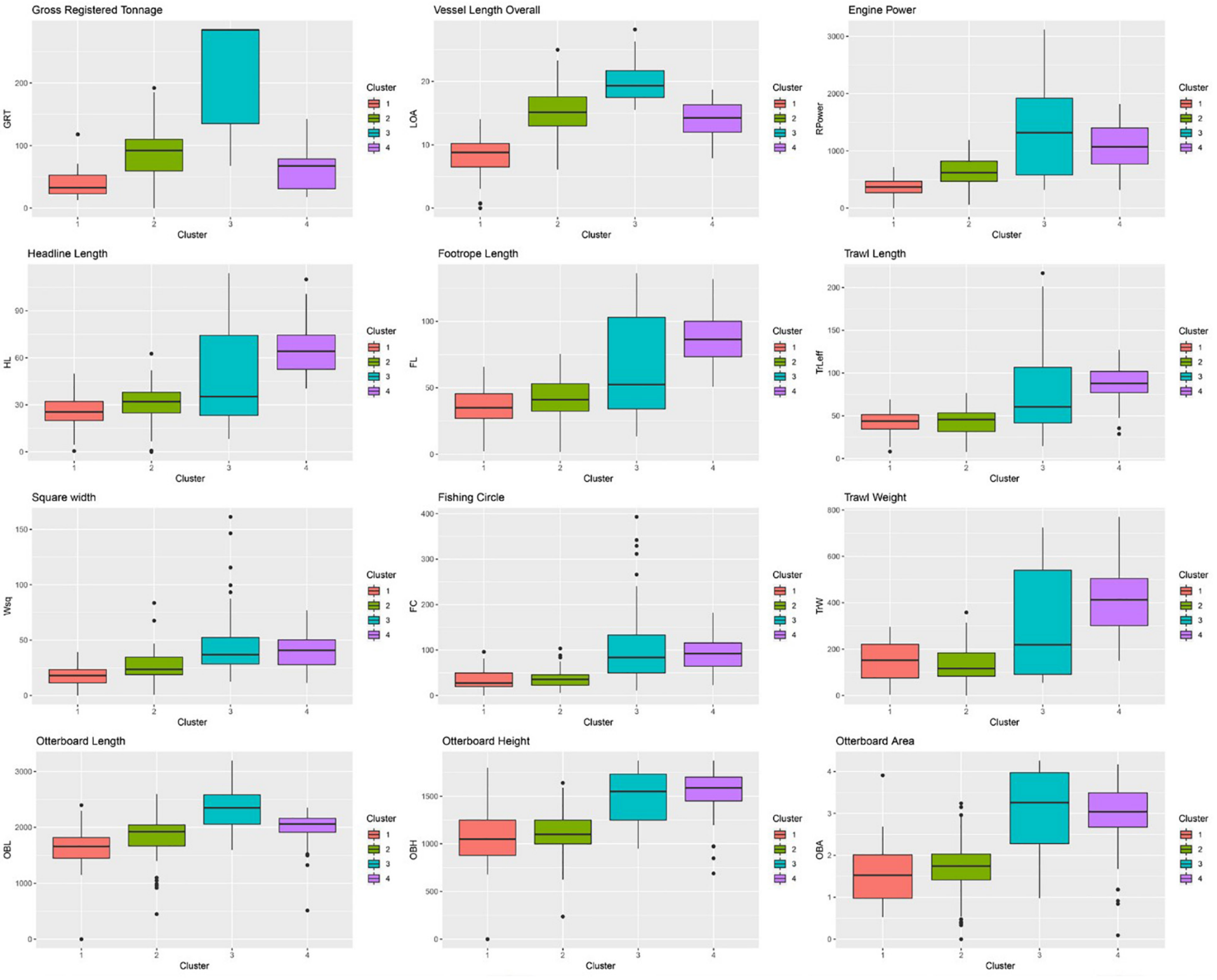

FIGURE 6 | Description of each variable range, median and interquartile ranges divided by cluster.

and to approximate a description of the fit of the predicted values to the observed values. The SOM predictive ability performed differently depending on the variable considered, obtaining the best results in the prediction of trawl length, headline length, footrope length and otterboard area variables. A summary of the evaluation of the SOM predictive performance is available in **Figure 7**.

DISCUSSION

The continuous improvement of fishing efforts and capacity descriptors is an essential element for the adoption of reliable management measures, aimed at the optimization of fishing activity revenues and the concurrent maintenance of sustainable exploitation levels. The purpose of this work was to explore the relationship that connects vessel size to the size of the fishing gear deployed, to facilitate tracing the dimensions of the trawl from readily available information like LOA, tonnage and the engine power of the vessel. If such a relationship were accurately described, it would be extremely useful to tailor specific management measures for specific fleet segments, improving the estimate of the impact exerted on the populations of target species. To the best of our knowledge, this is the first study using SOM applied to the exploration of these technical aspects of fishing activity, taking advantage of the capability of neural networks to perform non-linear data mining, clustering and summarization of the multidimensional variability of a dataset.

The main outcome of the study is that a simple relationship connecting vessels' magnitude and gear size could not be described, confirming the findings produced by other authors in previous studies (Reid et al., 2011). Certainly, the patterns described by the SOM suggests the existence of a general trend that associates the increasing dimensions of the vessel with an increasing size of gears and its components, but the great amount of variability observed in the dataset, reflected in the heatmaps delivered by the SOM, suggests the interaction of multiple factors in determining the size of the fishing gear. A first variable to consider is the distribution of vessel-metrics within the fleet, displayed by the first map, trained using only vessel

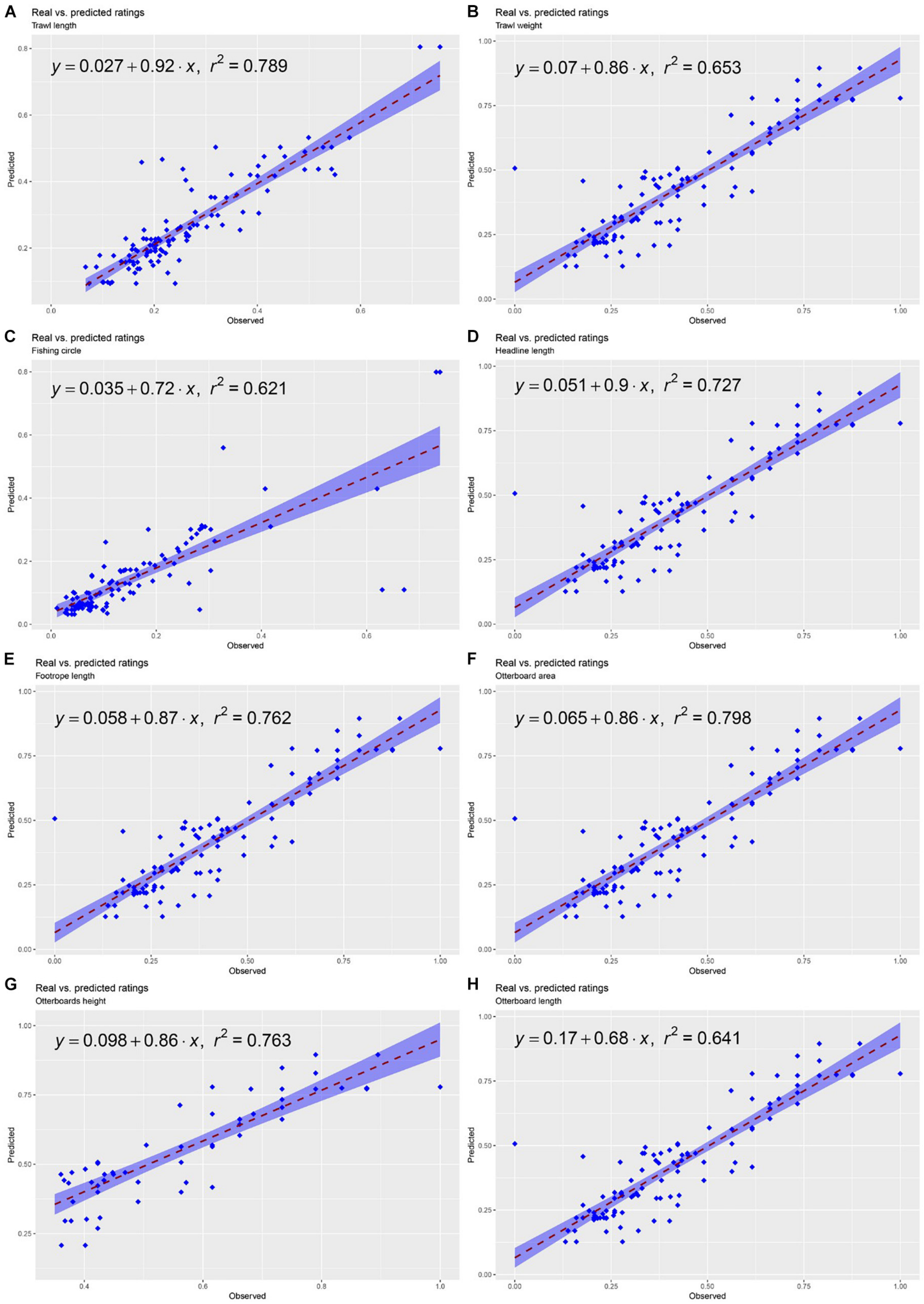

FIGURE 7 | Summary of the evaluation of the SOM predictive performance. **(A)** Trawl length, **(B)** Trawl weight **(C)** Fishing circle, **(D)** Headline length, **(E)** Footrope length, **(F)** Otterboard area, **(G)** Otterboard height, **(H)** Otterboard length.

size descriptors and their geographical origin. Although the first map was only devised for the estimation of missing data in the dataset, it proved useful to observe the distribution of vessel-metrics within the fleet that are not influenced by any additional factor. The patterns displayed by the first SOM suggests a general trend that indicates a correlation between the length of a vessel, its tonnage and the power of the engine. At the same time, however, the positioning of the extreme values in near but non-overlapping units of the map, better defined the nature of this correlation, characterizing it as non-linear. Vessel-metrics *per se* cannot be described by a simple linear relationship, even without the intervention of any additional factor, and this evidence already sets a first level of complexity to consider while studying the relationship between vessels and gears used.

The complexity of the analysis increased with the addition of the parameters pertaining to fishing gears, which complicated the distribution of the patterns in the map. The length of the vessel and gross registered tonnage, described by a unique gradient when only vessel-metrics are considered, resulted divided by the addition of descriptive parameters of the fishing gear, and showed patterns of distribution that associated large-sized boats with different gear sizes, depending on the type of gear, nationality and geographical origin of the vessels. The subdivision of the map into clusters facilitated the identification of extremes in the fleet in terms of vessel and gear size (Clusters 1 and 3), as well as the definition of the intermediate clusters (Clusters 2 and 4), where important differences could be highlighted. In Clusters 2 and 4 vessels characterized by very similar LOA and GRT reported marked differences in terms of engine power. This difference allowed the vessels belonging to cluster 4 (Spanish vessels using gears of the OTB4 type) to operate with much larger trawls, often equal in size to the gears used by the heavyweight class of the analyzed fleet. Among vessel descriptors, engine power was the only variable showing a unique gradient on the map, that associated higher hp values to a larger size gear. This association is visible when comparing the engine power gradient with the distribution patterns of gear descriptors like headline length, footrope length, trawl length, trawl weight, square width, fishing circle and the size of the otterboards used. This result suggests that the engine power, more than the size of the vessel (GRT or LOA), has the greatest influence on the size of the gear deployed. Given two vessels of similar magnitude, a greater engine power grants much more freedom in the choice of the size of the trawl and consequently in the type of fishing that can be practiced. This parameter, more than vessel size, should certainly have a greater relevance when formulating management indications.

The size of the gear used is also dependent on gear type, and varies with the geographical origin of the vessel. In fact, gear type and geographical origin were important variables used by the SOM when estimating gear-metrics and otterboard magnitudes. The inclusion of these factors has indeed contributed to improving the SOM's predictive performance. In many cases the map was able to infer gear and otterboard metrics properly, but its performance can certainly be enhanced through a training phase performed on a larger dataset, and *via* the inclusion of additional descriptors that might be still missing. The geographical variability observed could probably be traced back to a similarity which characterizes vessels belonging to the same local fleet, which usually share similar characteristics and adopt similar solutions in the implementation of a peculiar type of fishing. This speculation, if confirmed, would discourage the adoption of fleet management measures applied on an international scale, in favor of solutions that give greater relevance to local homogeneity/variability.

Future Steps

From this analysis, an excessive variability in the description of the vessels/gears of greater size has emerged, indicating that the dataset should be expanded to provide a more accurate description of this fleet component. Furthermore, the dataset considered provided an adequate description of a portion of the fleet pertaining to two countries (Italy and Spain) but did not allow an equally adequate characterization of the intrinsic variability of other fleets operating in the Mediterranean Sea. Geographical variability has greatly contributed to improving the predictive capabilities of the trained map, suggesting that the dataset should be enriched to consider the regional variability characteristic of Mediterranean fleets even more. A step forward in this direction would also be the inclusion of the species targeted by each fishery/gear combination. The incorporation of these variables, and others to be evaluated, would certainly allow the improvement of descriptive and forecasting models, and will be considered in a future work.

DATA AVAILABILITY

The authors do not have permission to share dataset used.

AUTHOR CONTRIBUTIONS

AC and AS and EN developed research described in the manuscript, curated and maintained the historical dataset, and wrote the manuscript with inputs from SB and JP. AC performed the statistical analyses.

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Simulating the Effects of Alternative Management Measures of Trawl Fisheries in the Central Mediterranean Sea: Application of a Multi-Species Bio-economic Modeling Approach

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In the last decades, the Mediterranean Sea experienced an increasing trend of fish stocks in overfishing status. Therefore, management actions to achieve a more sustainable exploitation of fishery resources are required and compelling. In this study, a spatially explicit multi-species bio-economic modeling approach, namely, SMART, was applied to the case study of central Mediterranean Sea to assess the potential effects of different trawl fisheries management scenarios on the demersal resources. The approach combines multiple modeling components, integrating the best available sets of spatial data about catches and stocks, fishing footprint from vessel monitoring systems (VMS) and economic parameters in order to describe the relationships between fishing effort pattern and impacts on resources and socio-economic consequences. Moreover, SMART takes into account the bi-directional connectivity between spawning and nurseries areas of target species, embedding the outcomes of a larvae transport Lagrangian model and of an empirical model of fish migration. Finally, population dynamics and trophic relationships are considered using a MICE (Models of Intermediate Complexity) approach. SMART simulates the fishing effort reallocation resulting from the introduction of different management scenarios. Specifically, SMART was applied to evaluate the potential benefits of different management approaches of the trawl fisheries targeting demersal stocks (deepwater rose shrimp *Parapenaeus longirostris*, the giant red shrimp *Aristaeomorpha foliacea*, the European hake *Merluccius merluccius*, and the red mullet *Mullus barbatus*) in the Strait of Sicily. The simulated management scenarios included a reduction of both fishing capacity and effort, two different sets of temporal fishing closures, and two sets of spatial fishing closures, defined involving fishers. Results showed that both temporal and spatial closures are expected to determine a significant improvement in the exploitation pattern for all the species, ultimately leading to the substantial recovery of spawning stock biomass for the stocks. Overall, one of the management scenarios suggested by fishers scored better and confirms the usefulness of participatory approaches, suggesting the need for more public consultation when dealing with resource management at sea.

Keywords: vessel monitoring systems, spatial modeling, connectivity, models of intermediate complexity, bio-economics, management strategy evaluation

INTRODUCTION

An overall status of overfishing is reported for most of the demersal resources and related fisheries in the Mediterranean Sea (FAO, 2018). Moreover, in the last decade, several studies documented the poor exploitation patterns of trawl fisheries characterized by high juvenile fishing mortality and high production of discards (Colloca et al., 2013, 2017; Tsagarakis et al., 2014; Damalas et al., 2015; Consoli et al., 2017; Maina et al., 2018). In 2002, the EU Common Fishery Policy[1] (CFP hereafter), followed by the GFCM, forced to reduce the fleet capacity to contrast the overfishing and to reach a fishing effort in balance with the resources productivity. However, in the Mediterranean Sea, also in consideration of the poor exploitation pattern of the demersal resources, the estimated reduction of fishing effort to reach the maximum sustainable yield (MSY) should be very high, e.g., a reduction of the 70% of the current value, for some species such as the hake. Considering the relationship between age/length at first capture and F_{MSY} (Beverton and Holt, 1957), a classical approach to increase the stocks productivity and their profitability is based on moving the size of the first capture toward larger sizes (Froese et al., 2008). To achieve this objective for demersal stocks, the EU earlier and the GFCM later adopted a square mesh of 40 mm or a diamond mesh of 50 mm as minimum mesh size for towed nets in the Mediterranean Sea (EC, 2009). Although these mesh sizes are good compromises for the mixed and the deepwater crustacean trawl fisheries, they do not avoid catches of high quantities of undersized commercial fish, such as hake and horse mackerels (Milisenda et al., 2017). Moreover, since the adoption of larger mesh sizes implies the loss of high-value yield of cephalopods and crustaceans, a possible management option is the reduction of the mortality rate of juveniles by prohibiting trawling when and where recruits and juveniles aggregate. This spatial based approach can achieve similar management targets to those usually linked to mesh size regulations (Caddy, 1999; Frank, 2000; Pastoors, 2000; Colloca et al., 2015).

Marine Managed Areas (MMAs), including marine reserves, marine sanctuaries, no-take zones, closed areas, marine protected areas, and fisheries restricted areas (FRA), are a common tool to achieve both conservation of marine biodiversity and improve fishery sustainability (Hilborn et al., 2004; Sale et al., 2005; Gaines et al., 2010; Mangano et al., 2015; Liu et al., 2018; Cabral et al., 2019). Each of these kinds of MMAs is characterized by a different level of spatial-based restriction of fisheries, which can be in force for limited periods or all year round. There are 681 MMAs covering ∼5.3% of the Mediterranean surface area (Pipitone et al., 2014). However, the advantages/drawbacks of MMAs are largely debated (Liu et al., 2018). On one side, a large part of the literature underlines the theoretical and conceptual value of spatial-based approaches, definitively suggesting that single large closed areas, or better networks of closed areas, could return important successes in terms of age structure recovery and risk reduction toward adverse effects of environmental phenomena such as global warming or pollution (Allison et al., 2003; Gaines et al., 2010; De Leo and Micheli, 2015; Churchill et al., 2016). On the other hand, a growing consensus exists about the need of pre-assessing the medium and long-term consequences, on both stocks and fleets, determined by the entry into force of spatial-based management measures (Abbott and Haynie, 2012; Bartelings et al., 2015; Cabral et al., 2017; Girardin et al., 2017; Mormede et al., 2017). In particular, assessing how much the benefits of closing an area to fisheries are reflected outside the protected area and the magnitude of the spillover from FRAs to adjacent fishing grounds is of crucial importance for the correct understanding of these approaches (Hilborn and Ovando, 2014; McGilliard et al., 2015).

Within this framework, several studies have underlined the effects of the adaptation of fishers, in terms of redistribution of fishing effort, as a consequence of the spatial-based fishing regulation (Abbott and Haynie, 2012; Miethe et al., 2014; Cabral et al., 2017; Girardin et al., 2017).

One of the most widely used approach to take account of fishers' behavior is represented by an individual-based model (IBM) aimed at capturing the strategies applied by individual agents (vessel captains or owners) in order to compensate for the immediate negative economic effects associated to the spatial restrictions (Rijnsdorp, 2000; Bastardie et al., 2010, 2014; Russo et al., 2014b). These effects are related not only to "lost" landings but also to the additional costs to reach, for instance, far fishing grounds when the FRAs are located near the coast.

In order to assess the effects of nurseries protection on fishing mortality and economic performance of demersal fisheries, a modeling approach called SMART (Spatial MAnagement of

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[1] https://ec.europa.eu/fisheries/cfp_en

demersal Resources for Trawl fisheries) was developed (Russo et al., 2014b).

Inside the research project "Marine protected Areas Network Toward Sustainable fisheries in the Central Mediterranean" (MANTIS), supported by the Directorate-General for Maritime Affairs and Fisheries of the European Union, SMART was updated and further developed and distributed as an R package (smartR[2] One of the most innovative characteristics of the new version of SMART is that it accounts for the connectivity, in terms of both larval dispersal and adult migrations, among different spatial units. This aspect is essential to understand how closing a given area (or a set of areas) is reflected outside and how the spillover from FRAs to adjacent areas could contribute to improve both fisheries and status of the stocks in the whole system (Pincin and Wilberg, 2012; McGilliard et al., 2015).

SMART was used, within the MANTIS project, to model the case study of Italian trawlers operating in the Strait of Sicily (Central Mediterranean Sea—SoS hereafter). The targets of this fishery are four species of high commercial value: the deepwater rose shrimp (*Parapenaeus longirostris*—DPS), the giant red shrimp (*Aristaeomorpha foliacea*—ARS), the European hake (*Merluccius merluccius*—HKE), and the red mullet (*Mullus barbatus*—MUT). Using a series of data collected under the umbrella of the European Data Collection Framework in the Fisheries Sector (DCF[3], the spatial and temporal dynamics of resources and fisheries were simulated and used to predict the potential effects of different management scenarios, including (1) the fishing effort regime adopted by the Italian Government and by the EC for demersal fisheries, (2) the FRAs adopted by GFCM for the SoS, (3) a larger network composed of existing and new FRAs, and (4) two different temporal stops. The scenarios 3 and 4 were defined within the framework of activities of the MANTIS project taking into account the Local Ecological Knowledge (LEK) of fishers. The list of simulated effects for each scenario includes the redistribution of fishing effort, the corresponding landings, the economic performance of the fleet, and, finally, the outlook for the status of the target stocks. Finally, all scenarios are analyzed within a framework of Management Strategy Evaluation (MSE) in order to compare the effects of the different management options.

MATERIALS AND METHODS

Case Study

The case study area corresponds to the SoS and adjacent seas and includes the FAO-GFCM Geographical Sub-Areas (GSAs) 12 to 16 and parts of GSAs 19 and 21 (**Figure 1A**). The continental shelf along the southern coasts of Sicily is characterized by two wide banks on the western (Adventure Bank) and eastern side (Malta Bank), respectively, separated by a narrow shelf strip (**Figure 1B**). The African shelf is wide along the Tunisian coasts and becomes narrower along the Libyan coasts with the exception of the Sirte Gulf. The continental slope is generally steeper and more irregular between Sicily and Tunisia and along the eastern side of the Maltese bank than in the area between Malta Island and the Libyan coasts. From a biogeographic point of view, the SoS connects the western and eastern Mediterranean basins, and hosts complex and diversified benthic biocoenosis (Garofalo et al., 2007; Coll et al., 2010) as well as a high diversity and biomass of demersal fish community (Garofalo et al., 2007; Gristina et al., 2013). The high productivity of fishery resources in the SoS can be mainly ascribed to three different factors (Milisenda et al., 2017): (1) the large extension of the continental shelf on both the Sicilian and African side and the occurrence of offshore fishing banks (Russo et al., 2019); (2) the occurrence of stable upwelling and frontal systems enhancing primary and secondary production; and (3) the ecotonal characteristics of the area, which are expected to affect biodiversity by increasing species richness and abundance (Kark, 2017). Bottom trawling is the most important fishing activity in the SoS. Considering only the Italian fishing fleets, two main trawl fishing activities can be identified: (1) inshore trawling, mainly based on the exploitation of the continental shelf, carried out by the fleets of seven ports distributed along the south coast of Sicily and a small portion (about 15%) of trawlers from Mazara del Vallo. Trawlers usually carry out two 4- to 5-h long hauls per day, leaving early in the morning and returning to sell the catch in the afternoon; (2) offshore trawling, generally conducted by trawlers over 24 m LFT and belonging to the Mazara del Vallo port. This fleet exploits fishery resources in international waters working both on the continental shelf and the slope down to 700–800 m depth. Trawlers generally undertake long fishing trips (15–30 days) also exploiting areas in other GSAs inside or adjacent to the Strait of Sicily (i.e., GSA 12, 13, 14, 15, 16, 19, and 21).

The SoS is one of the largest areas of occurrence of demersal-shared stocks in the Mediterranean. These include the stocks of DPS and HKE shared by Italian, Tunisian, and Maltese fisheries. The DPS is the main target species of trawling amounting to about 50% of the total landings of the Italian fleet. European hake is the main commercial by-catch of trawlers targeting DPS, being about 10% of their total landings. Two other economically important species in the SoS are those of ARS and MUT. ARS is fished almost exclusively by the Italian trawlers on slope bottoms of the entire SoS and amounts to about 10% of the landing. According to Gargano et al. (2017), red mullet off the Southern coast of Sicily forms a stock unit that is exploited almost exclusively by Italian trawlers operating on shelf bottoms. Latest assessments carried out within the framework of the GFCM and supported by the MedSudMed FAO regional project revealed an overfishing status for all the stocks with the exception of the red mullet. To improve the exploitation of the stocks, a reduction of fishing mortality, especially on the juvenile fractions of the stock of DPS and HKE, was recommended (SAC, 2018). It should also be remembered that the SoS has been prioritized for conservation (de Juan et al., 2012; Oceana, 2012) with several sites, mainly offshore banks, and seamounts, identified for their future inclusion in a Mediterranean network of marine protected areas. Currently, different areas subject to trawling restrictions are already implemented in the SoS (**Figure 1C**). In the northern sector, these include the Egadi Marine Protected Area and three FRAs established in 2016 (FAO, 2016) in

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[2] https://cran.r-project.org/web/packages/smartR/index.html

[3] https://datacollection.jrc.ec.europa.eu/

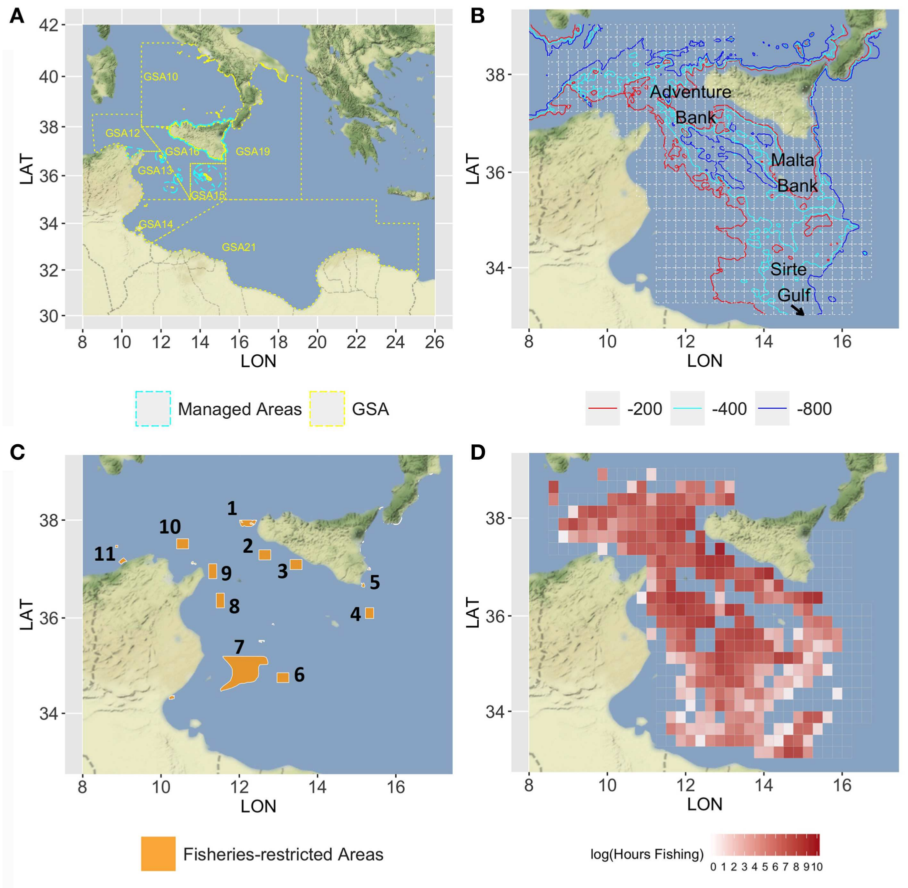

FIGURE 1 | Map of the Strait of Sicily, in which **(A)** the GSAs 12–16, 19, and 21 are represented with the managed areas (in cyan dashed lines) where trawl fishing is forbidden; **(B)** the main bathymetries (−200, −400, and −800) are represented together with the 15 × 15 nautical miles grid (in gray) used to set up the model; **(C)** the network of the nine areas considered for the spatial scenarios are represented by orange polygons, numbered in a clockwise order: 1, Egadi islands; 2, East of Adventure Bank; 3, West of Gela Bank; 4, East of Malta Bank; 5, Capo Passero; 6, "Fondaletto"; 7, "Mammellone"; 8, West of Pantelleria; 9, Cape Bon shoal; 10, Skerki Bank; 11, Galite Bank; **(D)** the mean annual fishing effort, in the period 2012–2016, is represented with a red-scale color (log of total fishing hours). Figures were created using the R package ggmap (Kahle and Wickham, 2013) using Map tiles by Stamen Design, under CC BY 3.0 and data by OpenStreetMap contributors (2017).

correspondence of the main stable nurseries of European hake and deepwater pink shrimp identified along the Italian–Maltese continental shelf. In the southern part of the SoS, the wide area called "Mammellone" subject to trawling restrictions has been implemented by an agreement between Italy and Tunisia with the aim of fish restocking. All these areas were considered as forming a network of spatial closures to be used in the simulation scenarios of SMART. Additionally, four further areas located in the Tunisian platform were considered. They are potential nurseries of European hake as preliminarily identified within the MANTIS project by integrating maps drawn by fishers (LEK) and a predictive model of hake recruits distribution developed in the south-central Mediterranean (Garofalo, 2018).

Data

The fleet of Italian trawlers operating in the SoS during the year 2016 accounted for 395 vessels with length-over-all (LOA) ≥ 12 m. A total of 367 of these are equipped with vessel monitoring systems (VMS) and were considered for this study. The LOA of each vessel was retained from the EU Community Fishing Fleet Register[4] and used as the best proxy of vessel's fishing capacity (Russo et al., 2018). The fishing effort deployed by each Italian trawler operating in the SoS was quantified, for the 60 months in the years 2012–2016, using VMS data provided by the Italian Ministry of Agricultural, Food and Forestry Policies, within the scientific activities related to the Italian national program implementing the European Union Data Collection Framework in the Fishery Sector. VMS data were processed using the VMSbase platform (Russo et al., 2011a,b, 2014a, 2016), an R add-on package providing a complete suite of tools for cleaning, interpolating, and filtering of VMS pings. The amount of fishing effort (in fishing hours) was estimated for each trawler/month/cell of the grid (**Figure 1D**). Although logbook data could represent the main source of information about catch

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[4] http://ec.europa.eu/fisheries/fleet/index.cfm

and landing (Gerritsen and Lordan, 2011), several reasons (see Russo et al., 2018 for a detailed list) supported the adoption, in Italy and other similar Mediterranean countries, of a statistical sampling scheme, based on questionnaires filled by researchers at harbors, to collect vessel-specific monthly landing data (EC, 2008; EUROSTAT, 2015). These data for the Italian trawlers operating within the SoS in the period of interest were therefore used in this study. Catch data by species were derived from the sampling for biological data from commercial fisheries (CAMPBIOL) carried out as part of the Italian plan for DCF. Sampling was performed monthly to evaluate the quarterly length distribution of species in the catches. Data were collected both by scientific observers onboard commercial bottom trawlers and by fishers through self-sampling. These data were used to obtain the age composition of CPUE. The geo-referenced data of abundance at sea of the four target species were collected during scientific bottom trawl surveys: the "Mediterranean international bottom trawl survey" (MEDITS) carried out in the northern part of the SoS from 1994 to 2016 and the Italian national trawl surveys GRUND (Relini, 2000) carried out in a large area covering GSA 16 and portions of adjacent GSAs from 1990 to 2008. The MEDITS data were used for both tuning the catch data and estimating the spatial distribution by age of each species, while the GRUND data were used only for estimating the spatial distribution by age class. The economic data were derived from the sampling for economic data from commercial fisheries as part of the Italian plan for DCF. The raw data are composed of 587 records of costs, for each vessel subject to the economic survey available for the 2-year period of 2014–2015 (301 vessels for 2014 and 286 for 2015). Each record is related to the activity of a single fishing unit and the sample is representative approximately of the 56% of the VMS monitored fleet. The cost data report the amount of expenses sustained by each vessel to perform the fishing operations disaggregated into three main categories of costs: spatial-based, effort-based, and production-based. The spatial-based costs summarize all the economic items proportional to the expenses linked to the spatial pattern; the effort-based costs gather all the fixed costs connected to the daily activity independently from the location choices (i.e., crew salary, maintenance, insurance); the production-based costs summarize the costs incurred by the commercialization of the landed species and it is proportional to the landed quantities.

Model Structure and Workflow

The spatial domain of the SMART model for the SoS was defined as a grid with 500 square cells (15 × 15 nautical miles) (**Figure 1B**). This grid is coherent with the one defined by the GFCM[5] Although several studies demonstrated that large spatial scale could lead to distortions in the analysis of fishing effort and related spatial indicators (Mills et al., 2007; Lambert et al., 2012; Hinz et al., 2013), the spatial resolution applied in this study was selected to harmonize coverage (i.e., number of observation by cell) over different data sources (i.e., VMS, landings and CAMPBIOL) and to limit the number of spatial units (cells), which is a critical parameter affecting computational features of the model. The rationale of the model, as well as the workflow of the smartR package, can be summarized in the following logical steps:

1. Processing landings data, combined with VMS data, to estimate the spatial/temporal productivity of each cell, in terms of aggregated landings per unit of effort (LPUE) by species, according to the method described and applied in Russo et al. (2018);
2. Processing biological data to estimate LPUE by age and by species, for each cell/time;
3. Analyzing VMS data to assess the fishing effort by vessel/cell/time;
4. Combining LPUE by age with VMS data to model the landings by vessel/species/length class/time/cell;
5. Estimating the cost by vessel/time associated with a given effort pattern and the related revenues, as a function of the landings by vessel/species/length class/time (step 4);
6. Combining costs and revenues by vessel, at the yearly scale, to obtain the profit, which is the proxy of the vessel performance. profit could be aggregated at the fleet level to estimate the overall performance;
7. Using estimated landings by species/age, together with survey data, to run mice model for the selected case of study in order to obtain a biological evaluation of the fisheries.

Each of these steps corresponds to a different module of the R package. The relationship between each module of SMART (in gray) and its data sources (box at the center of the image) is represented in **Figure 2A**. The quantities (e.g., LPUE) generated by the different modules, and used in the intermediate steps of the model, are represented in dark yellow.

While the different modules are described in detail in the successive subsections, in **Figure 2B**, the rationale of the simulation approach applied at the level of the individual trawler is summarized. **Figure 2B** also represents the flux diagram for the sequence of steps in the previous bullets points. SMART includes an IBM predicting the allocation of the fishing effort for each vessel under different scenarios. Starting from the observed effort pattern by vessel, several scenarios can be virtually applied in order to predict the pattern resulting from the adaptation of each vessel to the new situation. Firstly, $p_{c,t,v}$, which is the spatial (for each cell c) and temporal (for each time t) distribution of the effort for each vessel v, is reconstructed using VMS data. Afterward, this distribution is modified in space and/or time according to the selected scenario. For instance (scenario with FRA), $p_{c,t,v}$, is set to zero if $c \in FRA$, where FRA is the set of cells closed to fisheries. Otherwise, $p_{c,t,v}$ is set to zero if $t \in B$, where B is the set of times during which a temporal stop of fishing activity is set. Since it is possible to assume that the effort would simply reallocate according to the remaining distribution rescaled to the total effort, candidate configurations were obtained by multinomially sampling points when $c \notin FRA|\ t \notin B$ from this distribution. Checking whether the associated profit is greater than the previous ones will validate this candidate configuration (**Figure 2B**). If the configuration is not valid, it will be discarded and another candidate configuration will be drawn. Otherwise, $p_{c,t,v}$ is updated and the whole procedure is repeated until a convergence criterion is met. These steps are repeatedly carried

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[5] http://www.fao.org/gfcm/data/maps/grid/en/

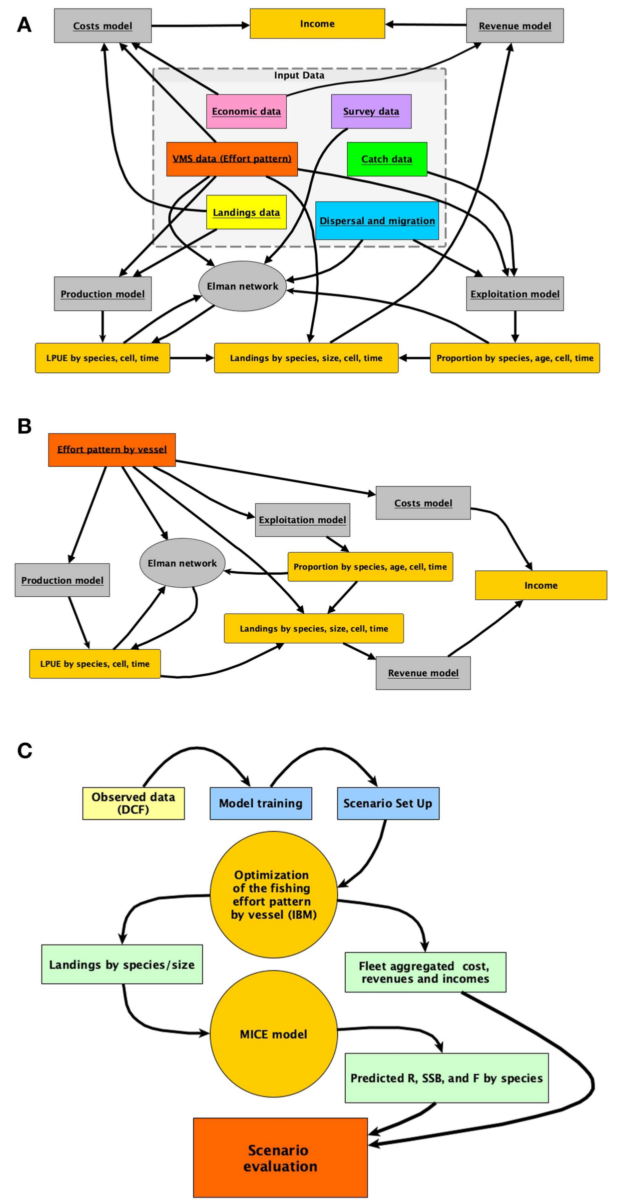

FIGURE 2 | Representation of **(A)** the architecture of the smartR model, showing as the different input data are processed by different modules; **(B)** summary of the IBM implemented to obtain the economic quantities to be optimized in relation to the effort pattern of each vessel; **(C)** the typical workflow, from DCF data to the final MSE evaluation.

out, for each vessel, in IBM optimization (**Figure 2C**). When the optimization ends for all the vessels in the fleet, aggregated revenues, costs, and profit can be computed for the whole fleet. In the same time, the total landings by species/age (or size) are passed to the MICE model devised to assess the biological consequences of the selected scenario. Finally, economic and biological outcomes for the selected scenarios are compared in a Management Scenario Evaluation (MSE). A complete list of input data and related features is provided in **Table S1**.

Spatial LPUE and Age Structure of Landings

Monthly landings were combined with VMS data (using the fishing vessel and temporal range of the fishing activity as references) to estimate the monthly LPUE for each species and cell of the grid (see Russo et al., 2018, for an extensive description of this procedure). The LPUE obtained are initially aggregated by species and across all the different age classes (cohorts). The aggregated LPUE were than transformed in LPUE by size using the biological data (CAMPBIOL) about length composition of catch. The age of each individual was estimated from its length to determine the demographic structure of catch and thus to convert the length–frequency distribution (LFD) of catch into an age–frequency distribution. The growth parameters, according to the Von Bertalanffy model, were estimated internally to the SMART model (see **Table 1** for the estimated values). The Von Bertalanffy model is described by the differential equation:

$$\frac{dL}{dt} = k_1(L_\infty - l) \tag{1}$$

where L is the length at time t, k_1 is the growth rate parameter, and L_∞ is the asymptotic length at which growth is zero. The commonly employed parametrization of the solution is:

$$L\,(t) = L_\infty(1 - e^{-k_1(t-t_1)}) \tag{2}$$

where t_1 is the time at which an individual fish would have had zero length.

Providing the maximum supposed number of components (cohorts) of the mixture, the routine implemented in smartR returns:

1. The estimated age by individual and species;
2. A vector of cohorts proportion by species, time (month), and cell. these proportions are used to split the LPUE by species/month/cell into LPUE by species/age/month/cell, using the length–weight parameters in **Table 1**.

Sex of each individual was not considered and parameter values for the unsexed class were used. The mixture analysis implemented in R is based on the Markov Chain Monte Carlo (MCMC) stochastic simulation engine JAGS (Just Another Gibbs Sampler; Plummer, 2003). An extensive description of this procedure is in preparation.

Connectivity

smartR allows integrating the role of connectivity among the cells or set of cells, composing the spatial model. Two aspects of connectivity were considered: the connectivity due to larval dispersal from spawning to nursery areas and that concerning the reproductive migration from the nursery/feeding grounds to spawning areas. To do this, the original version of the Elman Multilayer Perceptron Network (EMPN) of SMART (Russo et al., 2014b) was modified to predict the LPUE by species/age of each cell i at time t using as input the following variables: (1) the amount of fishing effort at time $t-1$; (2) the time of the system (in months); (3) the LPUE by species/age of cell i at time $t-1$; and (4)

TABLE 1 | Biological parameters not referred to age.

Relationship	Species	k	L_∞ (mm)	t_0	Sources
Von Bertalanffy growth equation	ARS	0.610	67.2	−0.118	This work
	DPS	0.600	44.95	−0.118	This work
	HKE	0.100	926.82	0.0471	This work
	MUT	0.329	229.53	0.0305	This work
Length–weight relationship		α	β		
	ARS	0.0025	2.48		SAC-GFCM
	DPS	0.0033	2.46		SAC-GFCM
	HKE	0.0040	3.15		SAC-GFCM
	MUT	0.0010	3.04		SAC-GFCM

The Von Bertalanffy growth parameters and the length–weight parameters were used to set up the growth model and convert number of individuals by species and length in biomasses. ARS, Aristaeomorpha foliacea; DPS, Parapenaeus longirostris; HKE, Merluccius merluccius; MUT, Mullus barbartus.

the weighted mean LPUE by species/age at time $t-1$ in the set of donor/receiving cells defined for each cell of the grid (**Figure 3**). The set of donor/receiving cells was defined using a connectivity matrix containing the estimated flux, by species/age, for each pair of cells. Fluxes were quantified as positive values for the donor cells and negative for the receiving cells. This connectivity matrix was generated, for the different life stages of each species, using the procedure described in the next subsections. The LPUE by species/age were computed disaggregating the LPUE by species described in the section *Spatial LPUE and Age Structure of Landings* with the proportion by age described in the same section. In this study, it was assumed that the grid defined above defines the boundaries of the system, for the four species. Hence, it was also assumed that immigration and emigration fluxes between the system and the adjacent areas are negligible.

Larval Dispersal From Spawning to Nursery Areas

The connectivity between spawning and nursery areas was investigated by the adoption of numerical modeling (Gargano et al., 2017). The model consists of an off-line larvae transport model that runs with stored ocean model hindcasts (North et al., 2006). The seeding of numerical particles varies with the dimension of the spawning area of each species, and it ranges in between 530 and 934 per day. Particles are passively advected by ocean currents; once they reach the appropriate age for settlement, the model tests the location of particles to determine if they are found inside or outside a nursery area. Advection equation is solved (using, a fourth-order Runge-Kutta scheme) for the current velocities at the particle location using an iterative process that incorporates velocities at previous and future times to provide the most robust estimate of the trajectory of particle motion in water bodies with complex fronts and eddy (Dippner, 2004). A random displacement model (Visser, 1997) is implemented within the larval transport model to simulate sub-grid scale turbulent particle motion in the vertical direction and a random walk model is used to simulate turbulent particle motion in the horizontal direction. The spatial information about the SoS domain that was used to implement the Lagrangian model includes the modeled hydrodynamic variables (i.e., zonal and meridional current velocity at all computed depths) and geographical location and shapes of spawning and nursery areas of the four target species (Colloca et al., 2013). Hydrodynamic variables with daily frequency were retrieved by the Copernicus[6] Marine platform that is responsible for the dissemination of multiannual dataset of the Mediterranean Forecasting System (MFS). MFS reanalyses components are derived by the application of the ocean general circulation model NEMO-OPA (Madec, 2008) that is implemented in the Mediterranean basin at 1/16° by 1/16° (about 6 km) horizontal resolution and 72 unevenly spaced vertical levels (Oddo et al., 2009). Geographical distributions of spawning and recruitment areas were available by observational datasets (Colloca et al., 2013); if not available, they were inferred by integrating substrate and bathymetry information, at a scale of 1:100,000 (EMODnet portal[7], which are typical of juvenile recruits and adults of the target species.

The Lagrangian model uses external time step corresponding to the daily frequency of the released physics products of the zonal and meridional current velocities and an internal time step of 1,800 s, for stability reasons. Turbulent horizontal and vertical components are given by the constant value of 4.9 $m^2\ s^{-1}$ in agreement with a numeric approach based on the computational grid spatial resolution (Okubo, 1971). During each model simulation, a numerical particle is released at the center of each grid cell (2.6 by 3.3 km) with daily frequency, within the edges of the spawning areas and with random depth along the water column. Since we do not model growth explicitly, in the absence of a defined relationship linking temperature and larval phase duration, larvae are assumed to reach the minimum length for settling in two time windows: 10–30 and 40–60 days after spawning. The 10–30 days group can mimic a fast-growing larvae being spawned during summer months, while the 40–60 days group can mimic larvae being spawned during winter months. For this reason, each model simulation includes two different values for the age of settlement and death of numerical particles.

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[6] http://marine.copernicus.eu/

[7] http://www.emodnet.eu

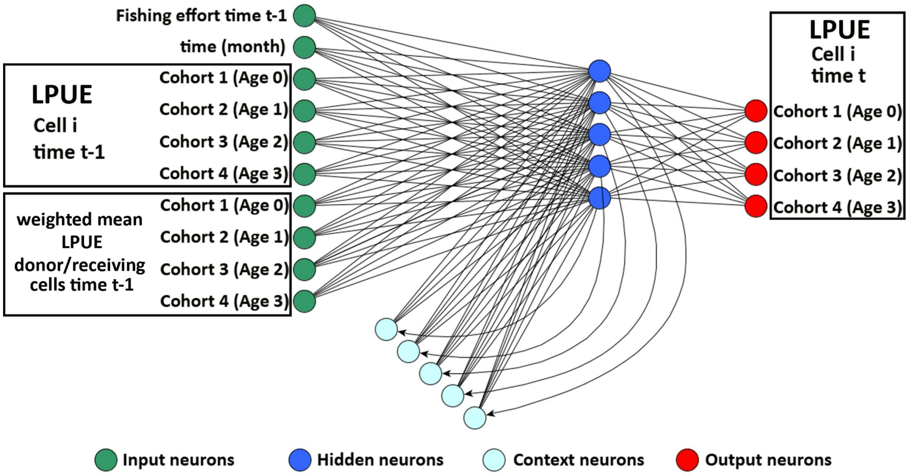

FIGURE 3 | Representation of the Elman network devised to process temporal time series of LPUE for each species in the model. The input layer comprises three blocks of neurons: one for fishing effort and time, one for the temporal series of LPUE, by age, in cell I, and one for the temporal series, by age, of mean LPUE in the donor/receiving cells. The input neurons directly propagate the information to the basic hidden neurons. At each step of the training procedure, the updated pattern of the basic hidden neurons is memorized by the context neurons and, at the successive step, propagated to the basic hidden neurons together with the new information in the input neurons. The output layer contains as many neurons as the number of age classes of the species considered. Modified from Russo et al. (2014b).

The Lagrangian model runs for the period in between 2012 and 2015 for each target species, and the obtained results were processed to evaluate the origin of numerical particles that settle into defined nursery areas, hence providing connectivity information. At this intent, the particle fluxes (PFs) were computed with Equation 3. C_A is the number of numerical particles that settle in a nursery area (A) during the time range of the simulations (j). C_A is normalized by the number of particles (N_s) that are released in the spawning region.

$$PF_s = \frac{1}{N_S} \sum_{j=1}^{n} C_{Aj} \tag{3}$$

Results are organized in connectivity matrices showing for each year and for each time windows the identification numbers of spawning and nursery areas and the ratio between released and recruited particles, representing the success of recruitment of the released particles. For DPS species, the **Supplementary Materials** report a graphical example of connectivity (**Figure S1**) using PFs (Equation 3), between known spawning and recruitment areas of the SoS.

Juveniles' Migration From Nursery/Feeding Grounds to Spawning Areas

The juveniles' migration patterns were investigated for each fish species following an empirical approach. Fish movements were derived by comparing the distribution in space of different age groups at different moments. In order to have complete coverage of the SoS, abundances by species and age were derived by integrating MEDITS dataset with GRUND survey and catch data. The merging procedure was carried out over a common sampling grid and consisted in the sum of the normalized abundances of the individuals from each dataset to obtain a single homogeneous distribution for each age class and fish species.

Although the two datasets slightly differ from each other, with MEDITS and GRUND campaigns occurring during summer and autumn months, respectively, this simplified merging procedure could be applied, with the investigated process being characterized by annual time scale. Furthermore, the use of normalized quantities allowed us to consider data from different sources and to focus the analysis on the relative distribution of the abundances of each age class. According to the species, the abundances by age were grouped distinguishing juveniles from adults, assuming these as individuals belonging to fully mature age groups (**Table 2**). The obtained datasets were adopted to infer the moving of fishes across age classes. Considering the abundance like a tracer, the transport equation for a conservative tracer was evaluated between age class groups (0 and higher). Potential migration from nurseries/feeding grounds to spawning areas for each species was hence computed inverting the conservation equation expressed by:

$$\frac{dA}{\partial t} = \nabla F = -\frac{\partial U}{\partial x} - \frac{\partial V}{\partial x} \tag{4}$$

where A is the abundance, considered as a conservative tracer, and F is a flux that is, in turn, derived by the computation of the spatial gradient between abundances of adjacent age classes.

For each fish species, the abundance distributions of each age class were considered a sequence of steady states. Within this context, Equation 4 was treated following a numerical approach obtaining the following algebraic expression for the horizontal

TABLE 2 | Biological parameters referred to age, used to set up MICE model.

Species	Age	Natural mortality	Proportion of maturity	Selectivity (fisheries)	Selectivity (survey)	Sources
ARS	0	1.42	0.2	0.5	0.5	SAC-GFCM
	1	0.58	0.5	0.75	1	SAC-GFCM
	2	0.44	1	1	1	SAC-GFCM
	3	0.38	1	1	1	SAC-GFCM
	4+	0.35	1	1	1	SAC-GFCM
DPS	0	1.42	0.03	0.5	0.75	SAC-GFCM
	1	1.09	0.98	1	1	SAC-GFCM
	2	1.05	1	1	1	SAC-GFCM
	3+	1.03	1	1	1	SAC-GFCM
HKE	0	1.38	0.01	0.5	1	SAC-GFCM
	1	0.56	0.16	0.75	1	SAC-GFCM
	2	0.27	0.61	1	1	SAC-GFCM
	3	0.22	0.93	1	1	SAC-GFCM
	4	0.19	0.98	0.75	0.75	SAC-GFCM
	5	0.18	1	0.5	0.5	SAC-GFCM
	6	0.17	1	0.5	0.5	SAC-GFCM
	7+	0.16	1	0.5	0.5	SAC-GFCM
MUT	0	1.73	0.0	0.3	0.5	SAC-GFCM
	1	0.90	1	1	1	SAC-GFCM
	2	0.67	1	1	1	SAC-GFCM
	3	0.57	1	1	1	SAC-GFCM
	4+	0.48	1	1	1	SAC-GFCM

	Recruitment (mean of log)	Recruitment (sd of log)	*Z* before maturity	F_{MSY}	
ARS	20.00	0.93	0.8	0.41	SAC-GFCM
DPS	22.00	0.90	1.7	0.88	SAC-GFCM
HKE	19.00	0.88	1.4	0.19	SAC-GFCM
MUT	19.00	0.88	1.7	0.42	SAC-GFCM

Values of F_{MSY} were used as reference for the MSE (Management Strategy Evaluation). ARS, Aristaeomorpha foliacea; DPS, Parapenaeus longirostris; HKE, Merluccius merluccius; MUT, Mullus barbartus.

components of F:

$$U_{x,y} = \left(A^{t+1}_{x+1,y} - A^{t}_{x,y}\right) + \left(A^{t+1}_{x-1,y} - A^{t}_{x,y}\right) \tag{5}$$

$$V_{x,y} = \left(A^{t+1}_{x,y+1} - A^{t}_{x,y}\right) + \left(A^{t+1}_{x,y-1} - A^{t}_{x,y}\right) \tag{6}$$

where $U_{x,y}$, $V_{x,y}$ represent the horizontal components of F for the point x, y of the regular mesh previously described, $A^{t}_{x,y}$ is the number of individuals of age class t at point x, y, the subscript indicates a shift forward or backward in the mesh point of 1 unit, in the meridional or zonal directions, and the superscript $+1$ indicates a shift forward in the age class. Therefore, for each point of the regular sampling grid, the total number of individuals migrated to the nearest points between adjacent ages classes were estimated.

This method assumes that the potential migration among subsequent age classes is based on cell length. Thus, the obtained results only provide qualitative information on the direction pattern between age classes. As an example, in the **Figure S2**, the migration patterns obtained considering as input data for Equations 4 and 5 the juvenile and adult stages distributions of the DPS are depicted.

Economic Models for Costs and Revenues

The economic performance of the fleet results from the balance between costs and revenues of all the vessels actively involved in the fishery. Thus, to evaluate the economic performance of the fishing fleet, it is firstly necessary to model, at the scale of the single vessel, the operational cost linked to the fishing activity with the corresponding revenue and then to aggregate revenues and costs for the whole fleet. For each fishing vessel, the economic performance is determined by the profit resulting from its strategy, which results from the subtraction of the costs from the revenues. Here, the strategy is represented by the fishing grounds selection and the amount of effort deployed. Two blocks of economic parameters were considered to estimate costs and revenues related to the fishing activity of each trawler. Namely, costs were modeled in terms of their "spatial-based," "effort-based," and "production-based" components. Spatial-based costs are a function of spatial locations of fishing operations (i.e., the fishing grounds). Given that, for each vessel, different fishing grounds are characterized by different distances from the harbor of departure (computed as the linear distance between the center of each cell and the positions of the harbors), these costs are mainly related to the fuel consumption. Monthly values of fuel price were provided by the Italian National Institute of Statistics (ISTAT[8]

Accordingly, a spatial index (SI) was computed, for each vessel v and time t (month) as:

$$SI_{v,t} = \sum_{c=1}^{C} d_{v,c} E_{c,v,t} \tag{7}$$

where $d_{v,c}$ is the distance between cell c and the harbor of departure for the vessel v, and $E_{c,v,t}$ is the amount of effort (in hours of fishing) deployed by vessel v in the cell c during the time period t.

The relationship for spatial-based costs (SC), is then defined as:

$$SC_{v,t} = \alpha \times LOA_v \times SI_{v,t} \tag{8}$$

where $SC_{v,t}$ are the spatial-based costs, in Euros, bore by vessel v during the time period t, $SI_{v,t}$ is the spatial index defined above, LOA_v is the length-over-all of the vessel v, and α is the parameter to be estimated. According to Lindebo (2000) and the authors' experience, de-rating practices and the exclusion of auxiliary engines could lead to relevant differences between official Engine Power (KW) and maximum effective KW. Moreover, official records for EKW have proved to be completely skewed, suggesting the adoption of alternative parameters (Sardá, 2000). Thus, LOA was preferred to KW because we consider the official data about LOA more reliable than those about KW. While a detailed reconstruction (e.g., at the scale of single trip) of the fuel consumption is beyond the scope of this paper, the spatial-index we computed includes both the amount of effective time fishing by cell/vessel and the relative distance by cell. In this way, our target is an aggregated estimation of fuel cost ($SC_{v,t}$), mainly driven by the vessel-specific fishing footprint ($SI_{v,t}$).

The effort-based costs are independent of the locations of fishing operations and are defined as a function of the number of days at sea spent by each vessel. This component of the costs is devised to consider the labor costs (e.g., salaries) and the other expenses (repair/maintenance of the vessel) directly linked to the temporal duration of fishing activities. VMS data allow us to easily assess the number of days at sea (DS) for each vessel v during the period t. Thus,

$$EC_{v,t} = \gamma \times LOA_v \times DS_{v,t} \tag{9}$$

where $EC_{v,t}$ are the effort-based costs, in Euros, bore by vessel v during the time period t and γ is the parameter to be estimated. Here, the term LOA_v is aimed at capturing the effect of vessel size in terms of, for instance, the crew size.

The production-based costs are linked to the amount of landings (e.g., commercialization costs). They are defined as:

$$PC_{v,t} = \mu \times LV_{v,t} \tag{10}$$

where $PC_{v,t}$ are the production-based costs, in Euros, beard by vessel v during the time period t, μ is the parameter to be estimated, and $LV_{v,t}$ are the landing value, which is the product of landings by species and size times the respective prices.

The total costs (TC) for vessel v during the period t are:

$$TC_{v,t} = SC_{v,t} + EC_{v,t} + PC_{v,t} + \varepsilon \tag{11}$$

The corresponding Revenues (R) for vessel v during the period t are:

$$R_{v,t} = \sum_{s=1}^{S} \sum_{l}^{L} q_{s,l,t} \times p_{s,l,t} \tag{12}$$

where $q_{s,l,t}$ is the amount of landings for the species s and size class l during the period t by the respective price at the market ($p_{s,l,t}$).

Thus, the Profit (P) for vessel v during the period t is:

$$P_{v,t} = R_{v,t} - TC_{v,t} \tag{13}$$

And, for the whole fleet, during the year y:

$$P_y = \sum_{t=1}^{T} \sum_{v=1}^{V} P_{v,t} \tag{14}$$

Values of prices at the market by species and length class, together with the price of fuel, were partially retrieved by Russo et al. (2014b) and integrated using the public database provided by the "Istituto di servizi per il mercato agricolo alimentare" (ISMEA[9]).

MICE Model

The new version of SMART adopted a MICE approach to model the population dynamics of the exploited resources (Morello et al., 2014; Plagányi, 2014; Punt et al., 2016). The model describes the exploitation of resources by fisheries as well as the main inter-specific and intra-specific trophic interactions (**Figure 4**). Unlike HKE and DPS, MUT, and ARS were considered as stand-alone stocks not characterized by a trophic relationship with other investigated species. The chosen framework models a simple Statistical Catch at Age (SCAA) with a basic population dynamic where the catch-at-age datasets are fitted for multiple cohorts simultaneously and the fishing mortality is split into age and year components (Doubleday, 1976) where the catch-at-age datasets are fitted for multiple cohorts simultaneously and the fishing mortality is split into age and year components. The age-structured population dynamic is designed with a forward projection method, and it is modeled as:

$$\mathbf{N_{ya}} = \begin{cases} \mathbf{R_0 e^{\epsilon y}} \\ \mathbf{N_{y-1a-1} e^{-Z_{y-1a-1}}} \\ \mathbf{N_{y-1x-1} e^{-Z_{y-1x-1}} + N_{y-1z} e^{-Z_{y-1}}} \end{cases} \tag{15}$$

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[8] https://dgsaie.mise.gov.it/prezzi_carburanti_mensili.php

[9] http://www.ismea.it/flex/FixedPages/IT/WizardPescaMercati.php/L/IT

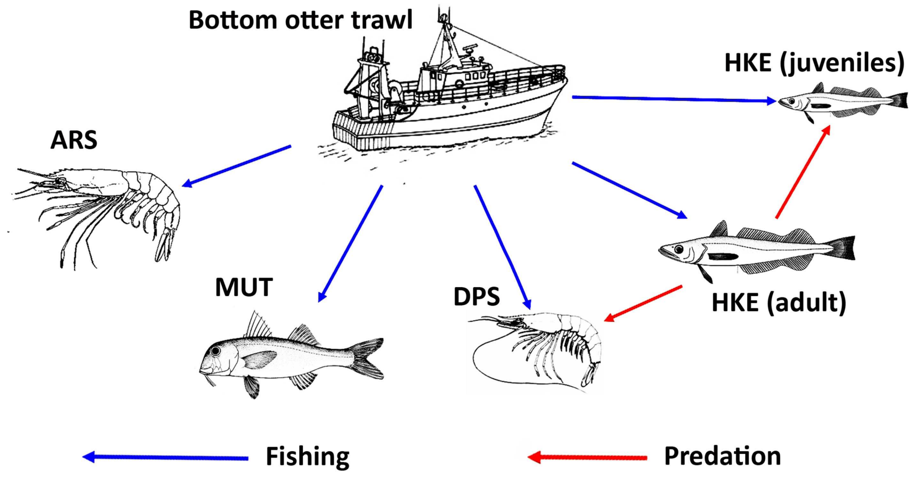

FIGURE 4 | Representation of the relationships between trawl fishing and the main stocks exploited in the Strait of Sicily, together with the main trophic relationships between stocks. Adult HKE is a predator of DPS and HKE juveniles. MUT and ARS were considered as stand-alone stocks with no trophic relationship with other investigated species. The source of the image for ARS is Fischer et al. (1987). The source of the images for DPS, HKE, and MUT is FAO (2017). All these images were reproduced with permission of FAO Copyrigh Office.

where $\boldsymbol{N_{ya}}$ is the number of individual of age a in the year y, $\boldsymbol{R_0}$ is the median recruitment with a yearly deviation of $\boldsymbol{e^{\epsilon y}}$, and x is the maximum age class. Only one source of uncertainty was considered, namely, process error, due to variation in future recruitment. To do this, 100 projections of the model were carried out and, in each projection, the future recruitment is generated as:

$$N_{y,0}^{s} = R_0^s e^{\varepsilon_y^s - (\sigma_R^s)^2/2} \quad \textit{where} \quad \varepsilon_y^s \sim N(0; \left(\sigma_R^s\right)^2) \tag{16}$$

where $\boldsymbol{N_{y,0}^{s}}$ is the number of age-0 animals of species s at the start of year y, $\boldsymbol{R_0^s}$ is the average number of age-0 animals of species s, and $\sigma_{\boldsymbol{R}}^{\boldsymbol{s}}$ is the extent of variation in recruitment for species $\boldsymbol{s}$.

In general, the total mortality Z of the age group during year y is defined as:

$$Z_{ya} = M_a + S_a F_y \tag{17}$$

where $\boldsymbol{M_a}$ is the natural mortality rate at age a, $\boldsymbol{S_a}$ is the fishery selectivity at age a, and $\boldsymbol{F_y}$ is the fishing mortality of the year y. The prey–predator interactions are modeled as a secondary source of mortality. For DPS, this relationship was modified to account the predation by HKE (Carrozzi et al., 2019):

$$Z_{ya} = M_a^{1,DPS} + S_a F_y + M_{y,a}^{2,DPS} \tag{18}$$

where $\boldsymbol{M_a^{1,DPS}}$ is the natural mortality rate at age a, $\boldsymbol{S_a}$ is the fishery selectivity at age a, $\boldsymbol{F_y}$ is the fishing mortality of the year y, and $\boldsymbol{M_{y,a}^{2,DPS}}$ is the rate of natural mortality during year i for DPS of age a due to HKE predation, while for HKE, the total mortality was modified to account for the cannibalism behavior of older HKE that predates younger HKE with age smaller than or equal to 2 years (Carrozzi et al., 2019), giving:

$$Z_{ya} = M_a^{1,HKE} + S_a F_y + M_{y,a}^{2,HKE} \tag{19}$$

The mortality rate for DPS and HKE of age ≤ 2 can be modeled according to:

$$\begin{aligned} M_{y,a}^{2,\,DPS} &= \tilde{M}_a^{DPS}\left(\frac{\alpha^{DPS} B_y^{HKE}}{\beta^{DPS} + B_y^{DPS}} - 1\right) \\ M_{y,Age\leq 2}^{2,\,HKE} &= \tilde{M}_{Age\leq 2}^{HKE}\left(\frac{\alpha^{HKE} B_{y,\,Age>2}^{HKE}}{\beta^{HKE} + B_{y,\,Age>2}^{HKE}} - 1\right) \end{aligned} \tag{20}$$

Additionally, the population dynamic of HKE is also defined in terms of survivability due to abundances of preys:

$$\tilde{S}_{y,Age\leq 2}^{2,HKE} = e^{-M_{y,Age\leq 2}^{2,HKE}} \tag{21}$$

And the survival rate for HKE is modeled as:

$$\tilde{S}_{y,a}^{HKE} = \frac{\alpha^{HKE}(B_y^{DPS} + B_{y,\,Age\leq 2}^{HKE})}{\beta^{HKE} + (B_y^{DPS} + B_{y,\,Age\leq 2}^{HKE})} \tag{22}$$

The parameterization of the secondary mortality $\boldsymbol{M^2}$, for both DPS and $\boldsymbol{HKE^{Age\leq 2}}$, and the survivability of $\boldsymbol{\tilde{S}_{y,a}^{HKE}}$ are constrained by $\alpha^{\boldsymbol{s}}$ and $\beta^{\boldsymbol{s}}$ as the parameters of the interaction functions, $\boldsymbol{\tilde{M}_a^s}$ is a measure of mortality, and $\boldsymbol{B_{y,\,a}^{s}}$ is the start-year biomass for species s, where $\boldsymbol{\alpha = \beta + 1}$ and, for the predator (HKE older than 2 years):

$$\beta = \frac{1 - \chi}{2 * \chi - 1} \tag{23}$$

While for the prey (DPS and HKE younger than 2 years):

$$\beta = \frac{2 * \chi - 1}{1 - \chi} \tag{24}$$

TABLE 3 | Parameters of the trophic relationships considered into the MICE model.

Species	Type	χ	Ω
DPS	Prey of HKE	0.8	0.3
HKE age >2	Predator of HKE < 2	0.9	0.1

ARS, Aristaeomorpha foliacea; DPS, Parapenaeus longirostris; HKE, Merluccius merluccius; MUT, Mullus barbartus. χ indicates the survival coefficients and Ω indicates the predation coefficients.

To ease the input parameterization, we replaced biomass by relative biomass:

$$P_y^{Preys} = \frac{B_y^{DPS} + B_{y,\ Age\leq 2}^{HKE}}{B_0^{DPS} + B_{0,\ Age\leq 2}^{HKE}} \tag{25}$$

The parameters of Equation 20, $\boldsymbol{\tilde{M}_a^{DPS}}$, $\alpha^{\boldsymbol{DPS}}$, and $\beta^{\boldsymbol{DPS}}$ for DPS and $\boldsymbol{\tilde{M}_{Age\leq 2}^{HKE}}$, $\alpha^{\boldsymbol{HKE}}$, and $\beta^{\boldsymbol{HKE}}$ for HKE of age ≤ 2, are then specified by defining the parameters $\chi^{\boldsymbol{DPS}}$ and $\chi^{\boldsymbol{HKE}}$ as the survival coefficients and $\Omega^{\boldsymbol{DPS}}$ and $\Omega^{\boldsymbol{HKE\leq 2}}$ as the predation coefficients, i.e.,

$$\Omega^{DPS} M_a^{DPS} = M_{y,a}^{2,\ DPS} \qquad \Omega^{HKE\leq 2} M_a^{HKE} = M_{y,Age\leq 2}^{2,\ HKE} \tag{26}$$

Values for χ and Ω are reported in **Table 3**. Furthermore, the catch at age in numbers for each year $\mathbf{C_{ya}}$ are estimated as:

$$C_{ya} = \frac{S_a F_y}{Z_{ya}} N_{ya}(1 - e^{-Z_{ya}}) \tag{27}$$

While the catch-in-weight for year y is:

$$\tilde{C_y} = \sum_a w_{a+1/2} C_{y,a} \tag{28}$$

where $\boldsymbol{w_a}$ is the weight of animal of age a. The catch-at-age datasets are assumed to be multinomially distributed, while the survey estimates of abundance by age class are assumed to be log-normally distributed with a standard error of the log that is independent of age and year.

The spawning biomass, for each species and year, is accounted for in the middle of the year as modeled by the expression:

$$SSB_y = \sum_{a=0}^{x} w_a m_a N_{ya} e^{-0.5 Z_{ya}} \tag{29}$$

The model time period runs from 2012 to 2016.

Model Validation

The trained smartR model was checked, for the years 2012 to 2016, by comparing the modeled SSB and total annual landings, by species, with the official DCF ones. The outputs of this comparison are reported in the **Figure S3**.

Simulated Scenarios

The list of management scenarios explored is summarized in **Table 4**. The first one was represented by the *status quo* providing a baseline for assessing the potential effect of the other scenarios. The Effort Regime scenario was based on the Multi-Annual Management Plan adopted by the Italian Governments and by the EC for demersal fisheries in the SoS. Moreover, two sets of scenarios were used to evaluate the effectiveness of spatial and temporal closures of trawling, respectively. Spatial closures corresponded to (1) the year-round closure of trawl fishing in the three FRA identified by the REC.CM-GFCM/40/2016/4 of GFCM; (2) the year-round closure of the full network of FRA (**Figure 1C**) identified within the MANTIS project, which comprises the three GFCM FRA off the Sicilian coast and other nurseries of hake identified with the support of the fishers' LEK off the African coast. Temporal closures corresponded to two scenarios suggested by stakeholders (fishers) within a series of participatory meetings organized by the MANTIS project and held in 2018 in Mazara del Vallo and Portopalo di Capo Passero, two of the main Sicilian harbors hosting trawlers: (1) the so-called "Winter stop," which is the complete stop of trawl fishing in February and March, followed by 2 months of reduced activity (3 fishing days per week instead of 5 as happens in the rest of the year); (2) the so-called "Summer stop," which is the complete stop of trawl fishing in September and October, followed by 2 months of reduced activity (3 fishing days per week instead of 5 as happens in the rest of the year).

For each scenario, a series of 100 simulations were carried out by (1) using the years 2012–2016 to set up and train the model on observed data; (2) simulating the entry into force of management action in 2017; (3) estimating the displacement of the fishing effort and the related landings, costs, revenues, and profit; (4) estimating the new exploitation pattern (F at age for each species) and using it to run the MICE model and projecting its effects on stocks along a 5-year period in terms of Spawning Stock Biomass of each stock.

An MSE was carried out by comparing the overexploitation rate (defined, for each stock, as the ratio between the estimated F and the most recent value of $F_{0.1}$, as proxy of F_{MSY}, available in the literature), the ratio between the mean SSB forecast for the years 2018–2022, the mean SSB for the years 2012–2016, and the forecast profit for the fleet. Thus, nine parameters (four values of $\frac{F}{F_{0.1}}$, four values of $\frac{SSB_{2017-2022}^{Scenario}}{SSB_{2017-2022}^{status\ quo}}$, and one value for profit) were compared across the different scenarios.

RESULTS

Spatial LPUE and Age Structure of Landings

Given that a detailed analysis of LPUE in space and time is beyond the scope of this paper, the description was limited to the mean pattern by species (**Figure 5**). The estimated LPUE for the four species were characterized by different ranges. The highest mean values were associated to DPS, reaching 2 kg per meter of LOA and hour of fishing, followed by ARS (0.7 kg/m/h),

TABLE 4 | Summary of the different scenarios compared through a simulation approach.

Name	Source	Capacity regulation	Effort regulation	Spatial regulation
Status quo	–	None	None	None
Effort regime	Italian Governments and EU	−5% (of fleet GT) with respect to the *status quo*	−8% of total annual effort for each vessel, with respect to the *status quo*	None
GFCM FRA	GFCM	None	None	Year-round closures of areas 2, 3, and 4 (**Figure 1C**)
FRA network	MANTIS (researchers and stakeholders)	None	None	Year-round closures of the all the areas (**Figure 1C**)
Summer stop	MANTIS (stakeholders)	None	Total stop in September and October −40% of effort in November and December	None
Winter stop	MANTIS (stakeholders)	None	Total stop in February and March −40% of effort in April and May	None

GT, Gross Tonnage; FRA, fisheries restricted area; GFCM, General Fisheries Commission for the Mediterranean.

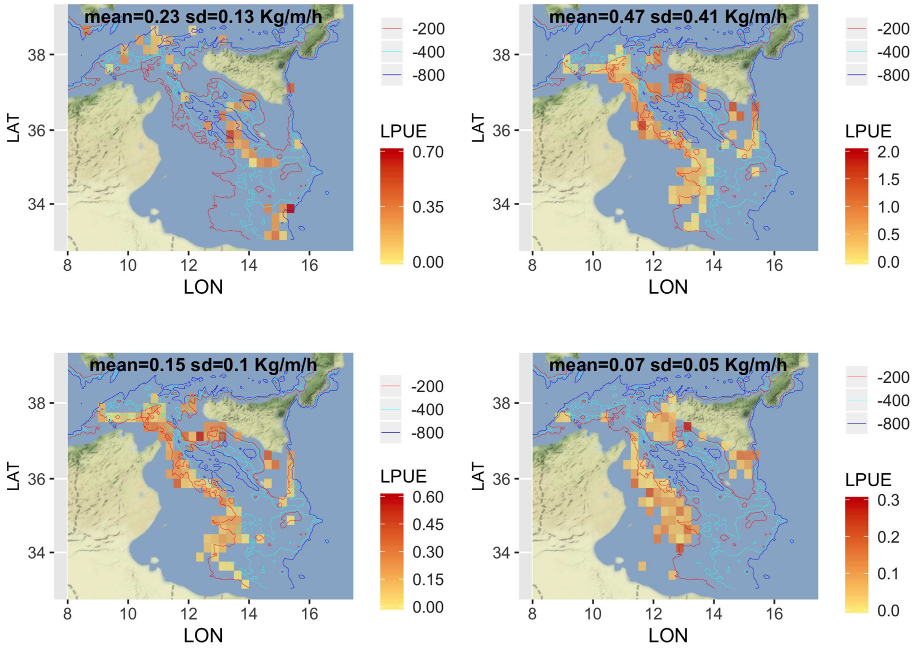

FIGURE 5 | Spatial distribution of LPUE (kg/m/h) by species, as the annual mean for the period 2012–2016. Figures were created using the R package ggmap (Kahle and Wickham, 2013) using Map tiles by Stamen Design, under CC BY 3.0 and data by OpenStreetMap contributors (2017).

HKE (0.6 kg/m/h), and MUT (0.3 kg/m/h). The main fishing grounds of ARS were distributed offshore in the central region of SoS, west of Maltase islands, and in the southern-east corner of the area. Main fishing grounds for DPS along the northern sector of the SoS were distributed in separated areas including the northwest corner of the Sicily, a large area out of Marsala, and the eastern border of the Malta Bank. On the contrary, the DPS fishing grounds off the African coasts were distributed seamlessly between 100 and 400 m depth. This spatial pattern is very similar to the one of HKE, the main commercial bycatch of DPS trawling. Finally, the fishing grounds of MUT were concentrated in three main areas: the whole Adventure bank, the east side of Sicily, and a large area off the Tunisian shelf.

Figure 6 represents, for each species, the mean proportion of catch by age/cell. These closely follow the corresponding patterns of mean LPUE by species (**Figure 5**). It is worth noting that (1) for ARS, the different age classes were consistently overlapped in the three fishing grounds; (2) DPS showed a progressive shift of the spatial distribution, according to the age, toward deeper areas; (3) HKE cohorts occupied the same fishing grounds of DPS, but with different proportion according to age; (4) given the lack of information on the coastal areas off Tunisia and Libya, the first cohort of MUT seems to be present only near the Sicilian coast, whereas older age classes are progressively concentrated in the offshore margin of the Adventure Bank and Tunisian coast.

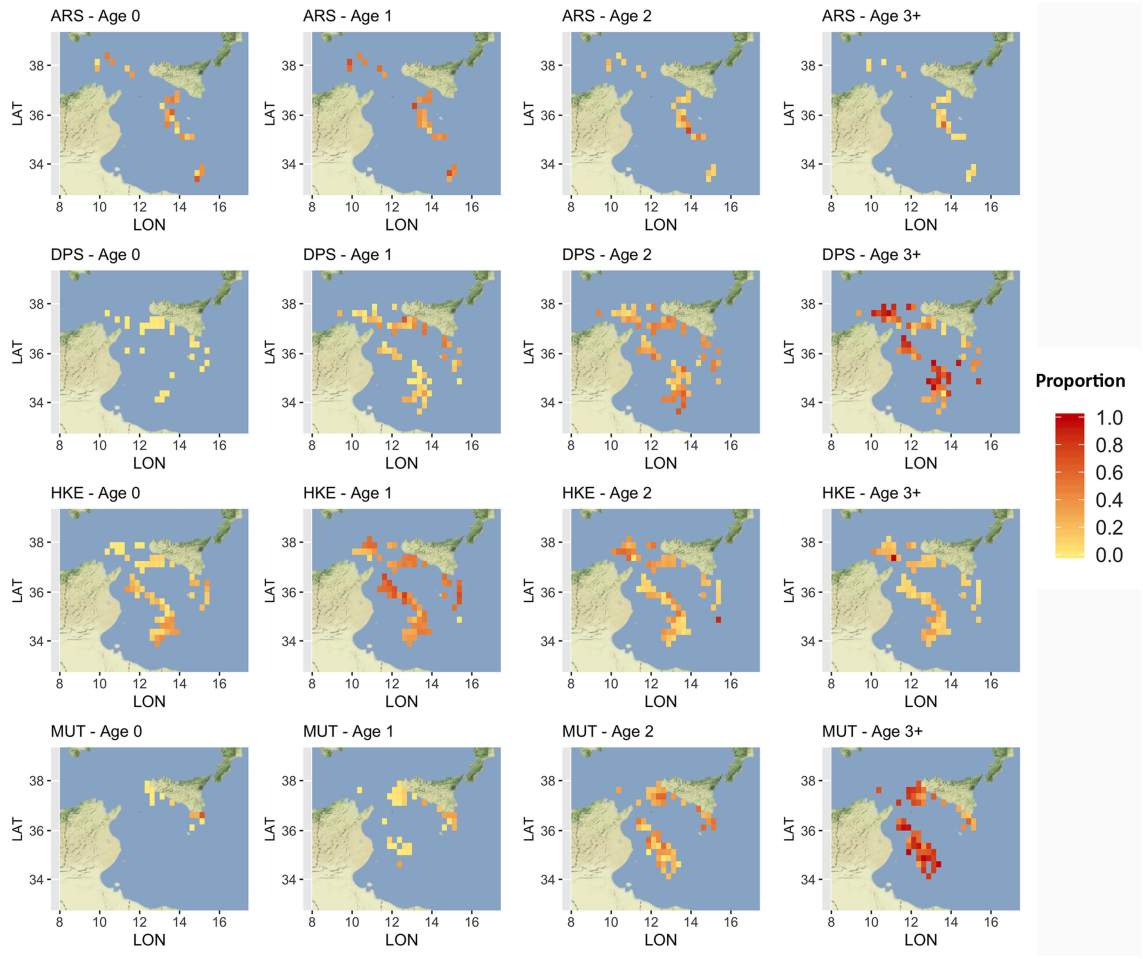

FIGURE 6 | Distribution of the main different age classes, by species, in terms of proportion of landings. Age classes older than 3 years were aggregated to reduce the number of submaps. Figures were created using the R package ggmap (Kahle and Wickham, 2013) using Map tiles by Stamen Design, under CC BY 3.0 and data by OpenStreetMap contributors (2017).

According to the different measures corresponding to each scenario of **Table 4**, SMART returned estimates of the expected fishing effort pattern by vessel, and then at the aggregated level of the fleet, including the fishing effort displacement, if any, under different management scenarios (**Figure 7**). The Effort Regime scenario is likely to determine the abandonment of far fishing grounds, especially those located in the southeast part of the SoS and off Tunisia coasts. The establishment of the network of three FRAs defined by the GFCM is associated to a remarkable increase of the fishing effort around these, and a general increase in the south and southeast region of the SoS. This effort displacement and "border-effect" is even more evident in the FRA Network scenario, in which a kind of "ring" encloses the areas in the network. This scenario also highlights that a remarkable amount of the original effort is forced to be displaced out of the FRAs located near the Tunisian coasts. The temporal scenario represented by the "Summer stop" is associated to a substantial decrease in fishing effort on both the Sicilian and African shelves. In contrast, the "Winter stop" is likely to determine a decrease in effort on the more offshore and deeper grounds, including the slope.

The new fishing effort patterns from the different scenarios were associated to the pattern of fishing mortality by age and as $\overline{F}$ by species of **Figure 8**. The effect for the four species widely varies between scenarios. In general, the Effort Regime scenario determines a flat-cut of the fishing mortality, irrespectively of age. The spatial scenarios are associated to an increase of fishing mortality for ARS, and in particular for the FRA Network scenario. In contrast, these two scenarios are associated to a similar and clear reduction of fishing mortality for DPS and, in a much less remarkable way, for HKE. In the case of MUT, both spatial scenarios correspond to a reduction of fishing mortality, and especially the FRA Network scenario. On the other hand, temporal scenarios are linked to very different patterns of F at age. The Summer stop is always associated to a strong reduction of F_{AAA}, whereas the effects of the Winter stop are less detectable, although present. The variation of $\overline{F}$, expressed as % of the *status quo*, under the different scenarios is summarized in **Table S2**.

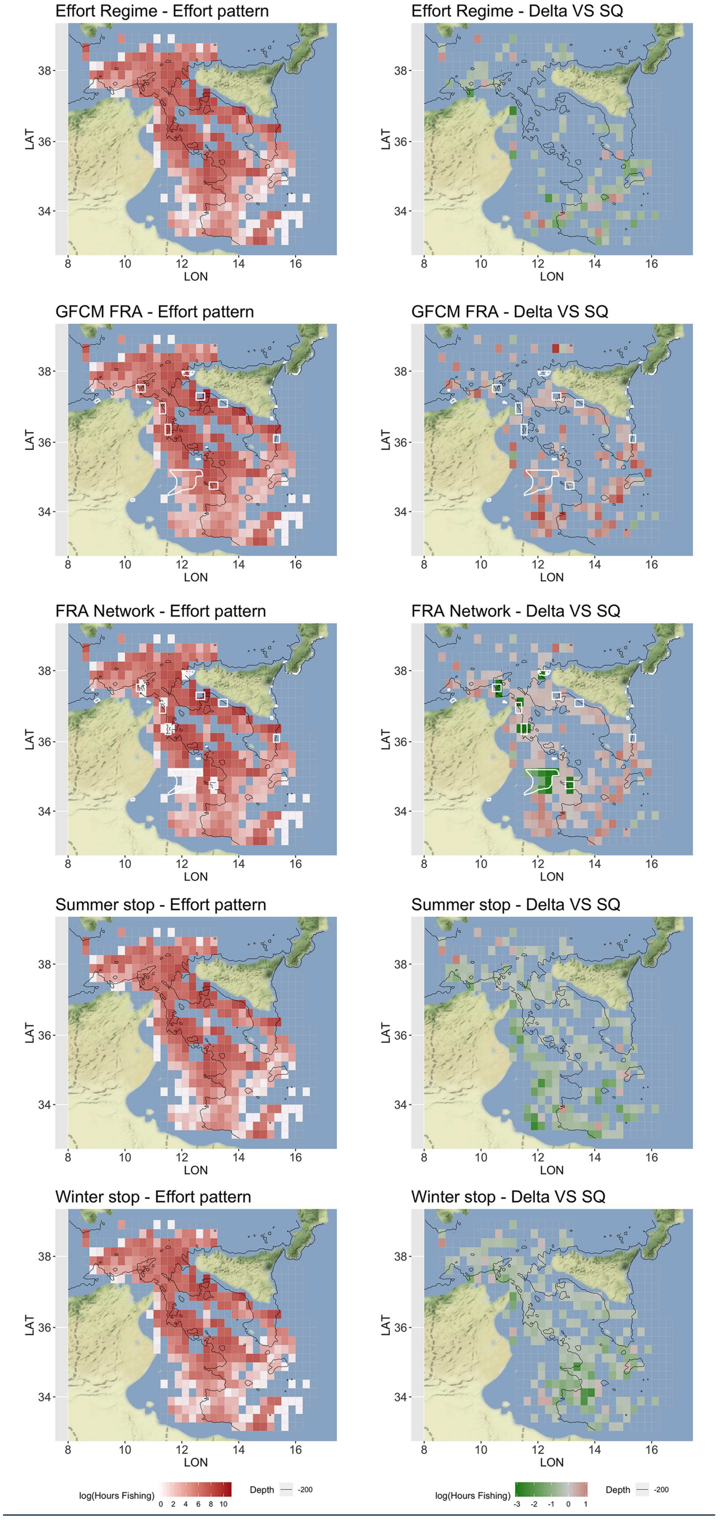

FIGURE 7 | Optimized (mean over 100 simulations) fishing effort pattern, represented in red scale of Log Hours Fishing, and corresponding for the difference (Delta) with respect to the status quo (SQ), represented in green-red scale of Log Fishing Hours, for each scenario. The FRAs are represented as white polygon in the Spatial-based scenarios. These patterns represent the total yearly fishing effort for the Italian trawlers operating in the SoS. Figures were created using the R package ggmap (Kahle and Wickham, 2013) using Map tiles by Stamen Design, under CC BY 3.0 and data by OpenStreetMap contributors (2017).

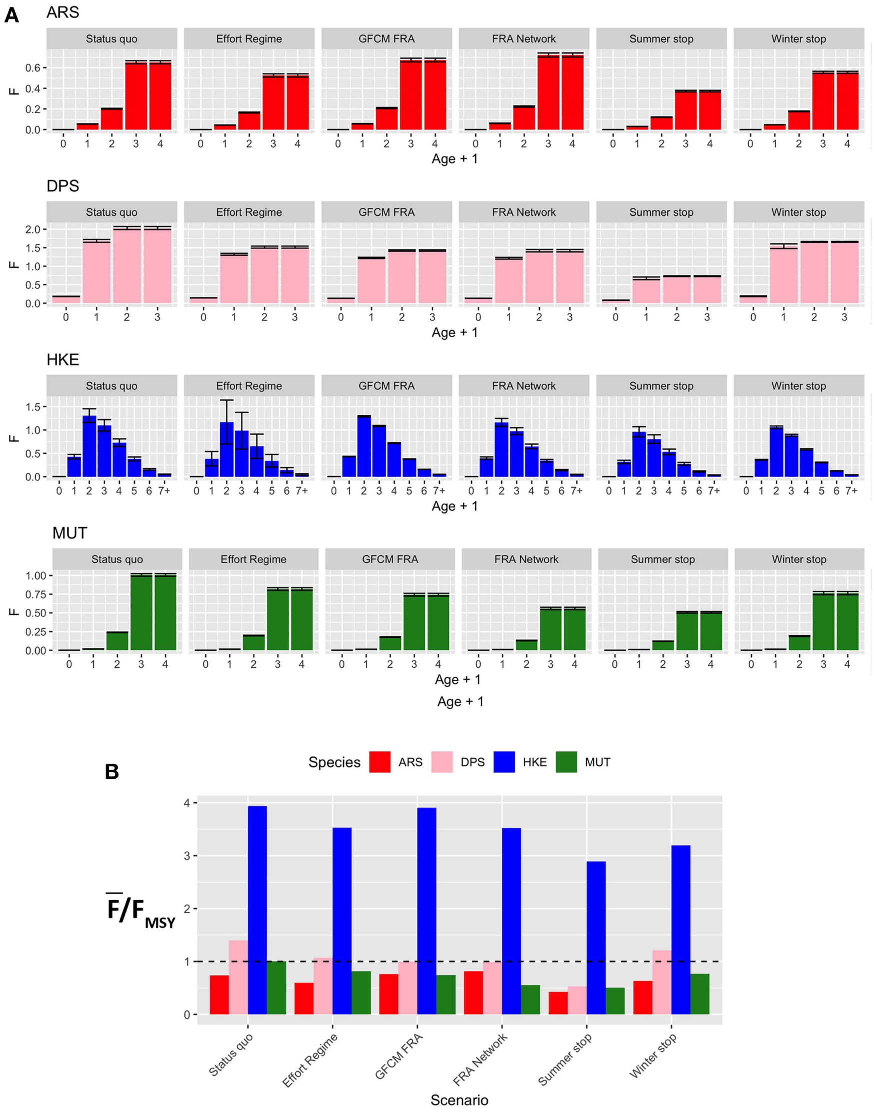

FIGURE 8 | Barplot (mean and MSE) representing **(A)** *F* at age for each species and scenario, corresponding to the new fishing effort pattern after the introduction of a different management measure; **(B)** the corresponding overexploitation rate ($\frac{\overline{F}}{F_{MSY}}$) for the different species and scenarios. Age ranges for the computation of $\overline{F}$ were as follows: 1–3 years (ARS), 0–2 years (DPS), 1–5 years (HKE), and 1–3 years (MUT).

Here, it is possible to observe that the largest reduction in fishing mortalities occurs for the Summer stop scenario, followed by the FRA Network and the GFCM FRA scenarios. The Effort Regime scenario corresponds to a strong benefit for HKE, but less for the other species.

From an economic point of view, the performances of the different scenarios are summarized in **Figure 9**. The revenues related to landings of ARS, DPS, and MUT largely vary between the different scenarios, while those for HKE are quite similar, but always lower than those of the *status quo*. Summer stop and FRA Network are the two scenarios providing the lowest revenues for MUT. Costs by effort (as the number of fishing days) are lower for the temporal stops and the Effort Regime, while they are very similar in the *status quo* and the scenarios based on spatial closures. The pattern is similar for spatial costs (i.e., fuel), given that also this kind of cost is related to the number of fishing days. At an aggregated level, it is worth noting that profit is more or less equivalent under the different scenarios: always lower than in the *status quo*, with the highest values occurring for the GFCM FRA scenario and the other scenarios scoring similar values, around 70% of the *status quo*.

The estimated effects of the different scenarios on the SSB mid-term trends are represented in **Figure 10**. The variation of SSB 2017–2022 in the different scenarios as % of that of *status quo* is summarized in **Table S3**. In this case, the *status quo* represents a reference, and it is characterized by an increasing

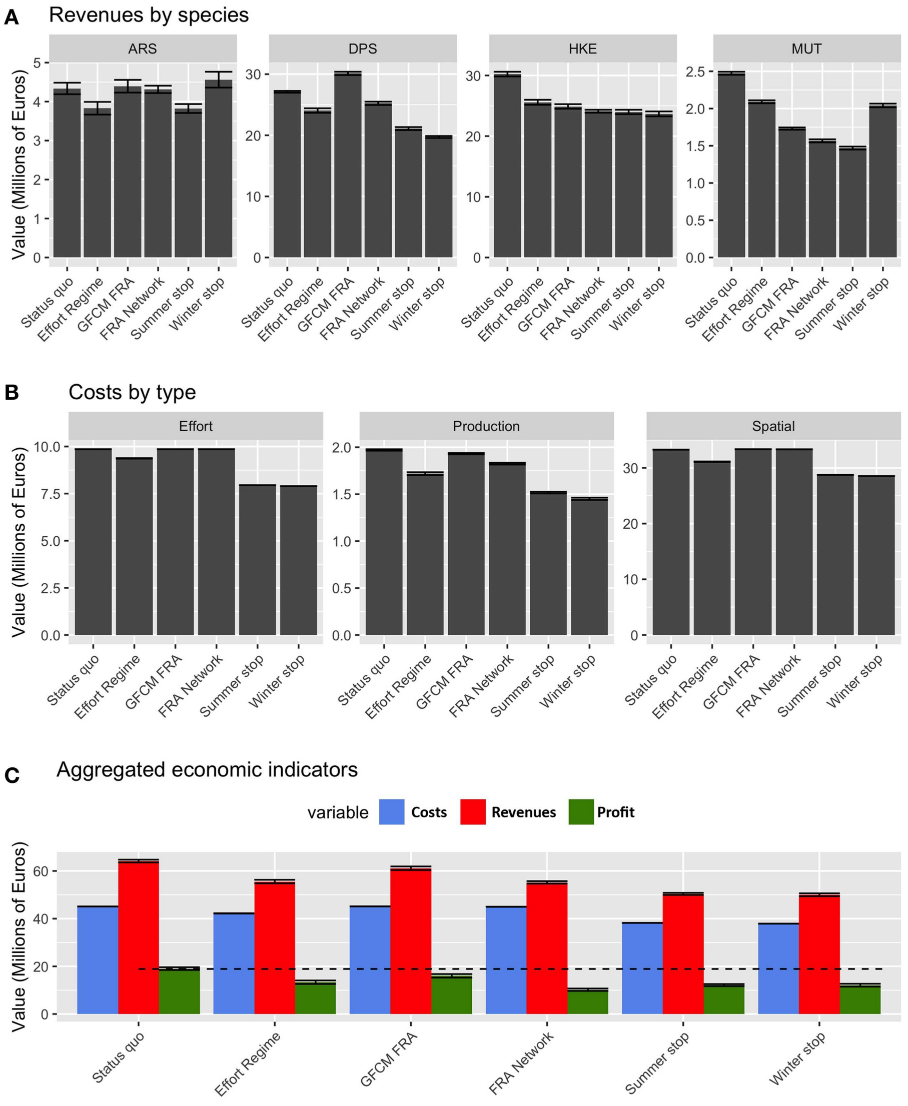

FIGURE 9 | Barplot (mean and MSE) representing **(A)** the values of landings for each species and scenario, corresponding to the new fishing effort pattern after the introduction of the different management measures; **(B)** the corresponding costs by type and scenarios; **(C)** the aggregated costs, revenues, and corresponding profit by scenario, for the whole fleet of Italian trawler.

trend for ARS, a decreasing trend for DPS (rather sharp) and HKE, and a stable trend for MUT. The Effort Regime scenario is associated to positive effects on ARS and a clear decreasing trend for DPS, whereas MUT and HKE seem to be unaffected by this measure. The spatial scenarios (GFCM FRA and FRA Network) are instead characterized by positive effects on all the four species, which show increasing trends (ARS and MUT), or stable trends (DPS and HKE). The FRA Network scenario seems particularly effective for MUT. However, the best results are associated with the Summer stop scenario, in which all four species are expected to increase their SSB to levels twice as high as the *status quo*. In contrast, the Winter stop scenario does not show visible effects and the trends for the four species are very similar to those of the *status quo*.

DISCUSSION

The application of SMART to the case study of trawl fishing in the SoS allowed exploring the possible consequences of six management scenarios in terms of variation from the *status quo*. The results, summarized in **Figure 11**, indicate that all alternative management scenarios are always associated, at least in the year of entry into force, to a decrease of the profit for the fleet with respect to the *status quo*, while the biological consequences on the stocks vary. In particular, the different options of FRAs closure are likely to allow reaching the sustainability targets in terms of fishing mortality for three of the four stocks considered, whereas the effect of the two temporal-based scenarios performed differently. After the *status quo*, the Winter stop scenario shows the worst performance among all the simulated scenarios in

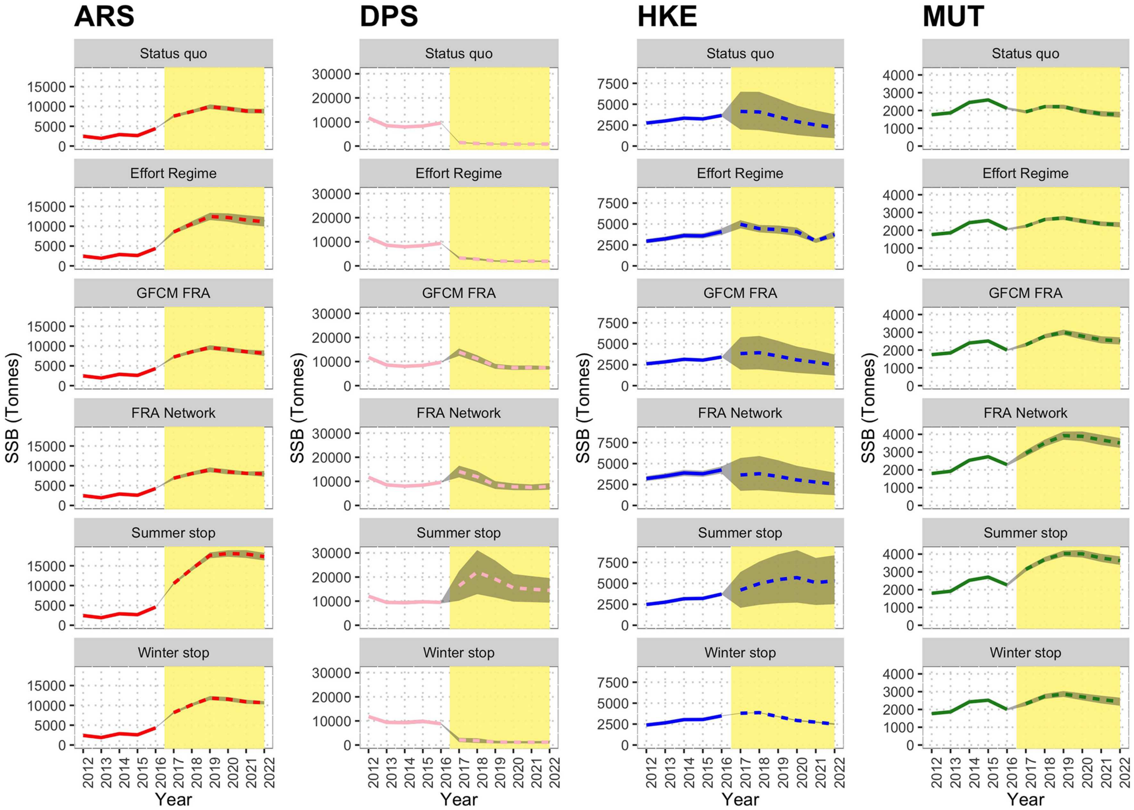

FIGURE 10 | Reconstructed and predicted trends of Spawning Stock Biomass (SSB) by scenario for the different species. The white background identifies the observed time series (years 2012–2016), while the yellow background corresponds to prediction (years 2017–2022). In the predictions, the dashed line marks the mean trend over 100 simulations, while the gray area corresponds to the standard confidence interval.

terms of recovery rate for SSB. Conversely, the Summer stop scenario gives the best biological effects, since it provides a mean SSB recovery rate larger than two for the stocks (**Figure 11**). However, this scenario corresponds also to a relevant reduction (around−40%) of the profit for the fleet, while the loss of gains is very reduced (about −10%) with the Winter stop. The situation is, from an economic point of view, even worse for the FRA Network. Thus, it seems that, overall, the GFCM FRA scenario seems to be the best spatial-based approach.

The consequences in terms of fishing effort displacement are also very different between scenarios. When spatial restrictions are applied (i.e., in GFCM FRA and FRA Network scenarios), effort is re-allocated in unclosed areas (Ba et al., 2019). Indeed, fishing effort is expected to increase around the edges of FRAs, as well as in already exploited fishing grounds. This "fishing-in-the-line" effect has been previously documented in the literature (Wilcos and Pomeroy, 2003; Horta e Costa et al., 2013; Cabral et al., 2017) and it is easily explainable by the fact that FRAs are likely to host higher biomasses and support spillover of resource for the fisheries. This chain of effects also suggests that, in the FRA Network scenario, the establishment of the new FRAs off the Tunisian coast could push the fleet farther (i.e., toward the fishing grounds near the coast of Libya) with larger costs for the fuel (**Figure 9**). Costs are always higher in spatial-based scenarios, even because the spatial component of cost is expected to exceed the value observed for the *status quo*. It is therefore coherent to observe that, for effort-based and temporal-based scenarios, the predicted fishing effort patterns are more or less a puzzle of areas in which the effort is expected to decrease (**Figure 7**).

Under the Effort Regime, the cells at the borders of the SoS (with the exception of those near the Sicilian coast) resulted abandoned, probably due to the cost to reach these fishing grounds. For Winter and Summer stop scenarios, the areas where the fishing effort decrease are much wider and only partially overlapping. The main differences are that under the Summer stop, the shallow bottoms off the Tunisian coast are less exploited, while during the Winter stop, the main reduction occurs on the slope off the Tunisian and the Libyan coast. In other words, the Summer stop is expected to reduce fishing effort in shallow grounds, while the Winter stop would determine an effort reduction in deeper areas. Considering both the effect of F and SSB, the Summer stop scenario showed a better performance than the Winter stop. Furthermore, the Winter stop seems to score similar to the Effort Regime: both scenarios support some improvements for HKE, but not enough to promote a switch of the system toward a sustainable level (**Figure 10**).

Analyzing the results by species, it seems that ARS has its own story, since this stock is the only one showing a sustainable

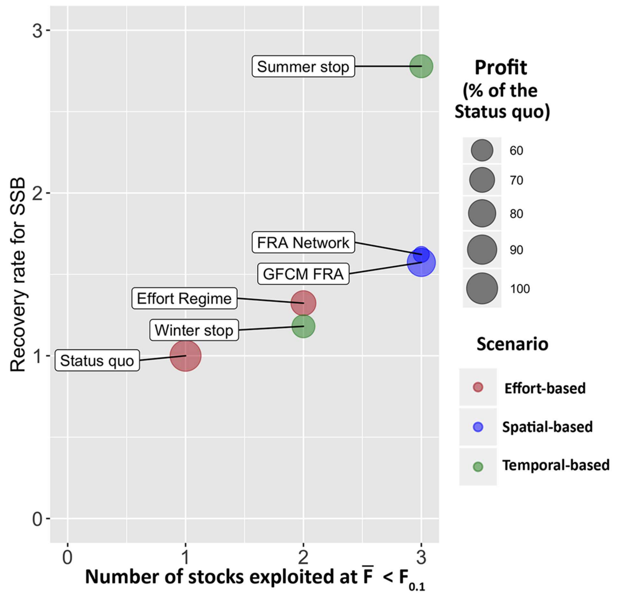

FIGURE 11 | Management Strategy Evaluation of the different scenarios. The *x* axis corresponds to the number of stocks that are expected to be exploited at $F_{0.1}$ after the implementation of the corresponding scenario, the *y* axis corresponds to the mean recovery rate for SSB of the four stocks, computed as $\frac{\mathbf{SSB}^{\mathbf{Scenario}}_{\mathbf{2017-2022}}}{\mathbf{SSB}^{\mathbf{Status\ quo}}_{\mathbf{2017-2022}}}$. The size of the bubble represents the percentage of profit with respect to the *status quo*, for each scenario, in the first year (2017) of application. The color of the bubble groups the scenarios by type.

fishing and an increasing trend in SSB for all scenarios, and in particular for the Summer stop. The pattern is similar for MUT, but this species is very close to $F_{0.1}$ in the *status quo* and only three different scenarios (GFCM FRA, FRA Network, and Summer stop) seem able to strongly improve the SSB for this species. DPS and HKE are the most challenging stocks since the overall performance of the different scenarios is closely dependent on the effects on these stocks. For both species, spatial-based approaches support in the mid-term the current levels of SSB, but only the Summer stop provides an improvement in the longer term (2022). One of the possible explanations is that these two species are linked by trophic relationships in the present modeling approach. This implies that fluctuations of the biomasses of DPS and HKE are not considered a "stand-alone," as in single-species assessments, but non-synchronous trends are likely to occur as the time series expands. This could be observed in the Summer stop forecast for DPS and HKE, where local maxima are followed by a decrease in SSB.

In the new version of SMART, the fishing effort pattern of each vessel is modeled as the best configuration to maximizing individual profit. An emblematic case study on reallocation of fishing effort after the introduction of a large fisheries spatial closure was documented for trawl fishing in the Western Baltic Sea, where fishers redistributed effort to areas that have had relatively high LPUE to compensate for lost landings (Miethe et al., 2014). Within the optimization module of SMART, each vessel is considered as an individual agent that reacts to the different management measures by adapting its spatial configuration of effort to maintain the profit, at a monthly temporal scale, at the maximum level (as the difference between costs and revenues). In this way, the rationale of SMART is consistent with other spatial models (Mahévas and Pelletier, 2004; Bastardie et al., 2014; Miethe et al., 2014; Bartelings et al., 2015; Girardin et al., 2015; Mormede et al., 2017) designed to simulate how fishing effort could be re-allocated following any spatial or temporal closure of fishing grounds (Girardin et al., 2015). In fact the "implementation error," which often impairs the effectiveness of management policies (Wilen, 1979), occurs exactly when fishermen behavior is not considered (Hilborn, 1985).

When comparing the two "scaled" spatial-based scenarios, the FRA GFCM and the FRA Network of 11 FRA, which includes the FRA GFCM, the very similar outputs of SMART suggest that, under the FRA Network scenario, the large displacement of effort is expected to counteract, at least in part, the positive effects of the larger spatial closures. These results support the rule-of-thumb prescribing that "the larger the FRA, the best the effects" is too simplistic and sometimes deceptive (Gaines et al., 2010; Liu et al., 2018).

Similar reasoning could be applied for temporal closures, as the selection of the months/season to close should be carefully evaluated considering not only the life cycle of the different species but also the spatial distributions of the fishing effort in the different periods of the year. This study evidences that, for the case study of trawling in the SoS, the temporal stop of the activity during the late summer, followed by a period of reduced activity, is one of the best options to support the recovery of exploited stocks. This could be explained by observing that the Summer stop scenario is particularly effective to determine a significant reduction of effort in more coastal waters (i.e., the Sicilian and African shelves), hosting most of the nurseries and spawning hotspots (Gristina et al., 2010; Garofalo et al., 2011; Colloca et al., 2015).

Globally considered, these results suggest the critical role of Essential Fish Habitats (i.e., nurseries and spawning areas) and the need to protect them by using modeling exercises to inform more ecosystem-based fisheries management strategies.

It is worth noticing that, in the present modeling approach, the function of these areas is implicitly considered by modeling the connectivity-mediated effects of different patterns of fishing mortality, which is a topic addressed in few studies (McGilliard et al., 2015; Simons et al., 2015; Khoukh and Maynou, 2018). In the present study, larval dispersal from spawning areas to nurseries and the reproductive migration from nurseries to spawning areas migration are described by connectivity matrices that do not consider growth, mortality and vertical migration of larvae, and immigration or emigration of adults from/for adjacent areas. This simplified approach was due to the absence of information on behavior of larvae (Gargano et al., 2017) and movement of adults (Khoukh and Maynou, 2018) of the investigated species. However, comparing different approaches including or not movement patterns to assess the effect of FRAs on simulated stock, some authors evidenced that not considering the movements between the different spatial units in which the stock is distributed can severely overestimated the stock biomass (McGilliard et al., 2015), suggesting that a spatial assessment with estimation of movement parameters among areas was the best way to assess a species, even when movement patterns were not clearly known.

Although we have used the most complete set of data collected within the European DCF in the Strait of Sicily, including trawl surveys, monitoring of commercial catches and VMS, the accuracy of our results will improve as knowledge of the dynamics of resources and the fleet in the area increases.

Beyond the structural difference between temporal and spatial-based scenarios, the results of this study confirm, in agreement with previous similar research (Churchill et al., 2016), that FRAs located over biologically sensitive areas and aiming at protecting critical life stages could work as "lungs" for the system and support fishing activity in far fishing grounds.

According to Khoukh and Maynou (2018), spatial closures of a specific nursery of HKE called Vol de Terra off the Catalan coast, assessed by the spatial explicit bio-economic model InVEST, would be equivalent to a reduction in fishing effort of 20% in the entire study area. The authors reported that this management measure would be easier to implement and would meet with less resistance from the sector than the traditional fishing effort reduction measure.

Unfortunately, none of the scenarios tested in this study seems enough to fully reach sustainability targets for all the investigated species in the short-medium term. Apart from the economic consequences, the stock of the HKE remains overexploited until 2022. This is surely linked to the biology of this species, its distribution, and the inherently "mixed-nature" of trawl fishing in the Mediterranean Sea that makes very difficult to reduce the fishing mortality of HKE. This failure in identifying a fully satisfactory approach is not a novelty in the literature, as other authors previously demonstrated that there is no single management tool capable of satisfying all objectives and that a suite of management tools is needed (Dichmont et al., 2013). In the case of HKE, although it is very difficult to reach a fishing mortality compatible with that corresponding at $F_{0.1}$ without a dramatic reduction of trawler effort, a strategy combining the reduction of fishing effort, the protection of nurseries, and/or the adoption in these critical areas of a selective grid to reduce the catch of undersized fish could be the wise approach to improve the stock status of the species in the Mediterranean while maintaining the profitability of the trawling fisheries (Vitale et al., 2018).

From an economic point of view, the scenarios evaluated in this study are always likely to cause an abrupt loss of profits for the fleet in short–medium terms. This is the logical consequence of reduced activity (for the temporal-based scenarios) and the fishing ground closures for spatial-based scenarios. Both these approaches imply an immediate reduction of landings, at least at their entry into force, which is exactly when the economic indicators of SMART are computed. Actually, SMART does not forecast the values of these economic indicators when resources reach the new equilibrium state, which is when the biological effects are fully achieved and the biomasses of the stocks are recovered. This means that the economic consequences are probably negative only in the short term, whereas the final effects of the management could be economically more sustainable than the *status quo*. The relevant increase of SSB as a consequence of the adoption of GFCM FRAs and the Summer stop suggests an increase of commercial catch rate and a positive effect on the profitability of fisheries. However, the short-term economic impact of temporal-based management measures should be supported through economic incentives for the compliant fishers, so that the revenues lost are in part compensated for by subsidies until the more sustainable state of the fisheries will be reached.

Future perspectives of this study include the exploration of additional scenarios. First of all, it could be interesting to investigate the effects of "hybrid" spatial/temporal-based scenarios, for instance, the combined effects of more traditional regulation of effort with spatial/temporal closures. Given that the reduction of fishing capacity should reduce the competition for fishing grounds, it is possible that some negative effects currently limiting the FRAs performance would disappear or reduce.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

TR and FF wrote the paper. TR, FF, LD'A, SF, and AP developed the model and analyzed the data. AC, GQ, DC, and MS contributed to the development of the connectivity module and provided the connectivity matrices for all the species. PA and RS contributed to the analysis of economic indicators. GG, MG, and SC contributed to the development of the model. All the authors revised the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00542/full#supplementary-material

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frontiers in Marine Science

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A Genome-Wide Approach to the Phylogeography of the Mussel *Mytilus galloprovincialis* in the Adriatic and the Black Seas

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Connectivity between populations shapes the genetic structure of species being crucial for an effective management of environmental resources. Genetic approaches can provide indirect measures of connectivity, allowing the identification of genetically differentiated – unconnected – populations. In this study, we applied a 2b-RAD approach based on hundreds of polymorphic loci to provide the first detailed insight into the population genomics of the Mediterranean mussel *Mytilus galloprovincialis* in part of its native geographical range. We sampled 19 localities within the Mediterranean and Black Seas, and analyzed a total of 478 samples. We detected strong differences between the two seas, whereas no differences were found between samples from the Western and Central Mediterranean and within Western Mediterranean samples. In the Central Mediterranean a significant differentiation emerged comparing Central Adriatic samples with those from South Adriatic and Ionian Seas. Furthermore, an East-to-West genetic structuring was found in the Central Adriatic Sea, which was not present in the Southern Adriatic and Ionian Seas. These results possibly reflect the local oceanography, with a Middle Adriatic gyre unable to prevent genetic differentiation in this species, and a Southern Adriatic gyre that effectively mixes propagules in Southern areas. In the Black Sea, no signal of genetic structure was found, although samples were spaced at similar distances as in the Adriatic-Ionian area. Genetic connectivity patterns of *M. galloprovincialis* reveal peculiar species-specific features respect to other species with similar larval duration, suggesting caution in using genetic connectivity data of single species in defining conservation units. We recommend of using genetic connectivity data of many species representing a variety of life history traits, and we call for new investigations using high resolution population genomics, particularly in the Black Sea, to understand if areas separated by hundreds of kilometers can be considered genetically connected as mussels' data suggest. This information will be critical to ensure "a well-connected system of protected areas" according to Aichi Target 11 of the Convention on Biological Diversity.

Keywords: connectivity, mussel, population genomics, 2b-RAD, SNP markers, Mediterranean, Black Sea

INTRODUCTION

Understanding the distribution of genetic variability is a keystone for environmental resources management and conservation biology of marine species (Moritz, 1994; Palumbi, 2003; Cowen et al., 2006). Population connectivity plays a crucial role in local and metapopulation dynamics, genetic structure and population resiliency, e.g., in response to human exploitation (Hastings and Harrison, 1994; Cowen et al., 2007; Weersing and Toonen, 2009; Puckett et al., 2014). Most marine species release planktonic larvae which disperse over days up to months with the currents and thereby constitute the primary source of the dispersal capacity (Mileikovsky, 1971; Ward et al., 1994; Gilg and Hilbish, 2003). Direct labeling and tracking of larvae is rarely a possibility (Levin, 1990; but see Becker et al., 2007), so genetic data are widely used for the indirect inference of population connectivity (Hellberg et al., 2002; Thorrold et al., 2002; Palumbi, 2003; Broquet and Petit, 2009; Cowen and Sponaugle, 2009; Lowe and Allendorf, 2010).

The FP7 CoCoNET European project aimed at an in-depth analysis of connectivity among present and future Marine Protected Areas (MPAs) in the Mediterranean and Black Seas to instruct the placement of future networks of MPAs (Boero et al., 2016). A major task was the exploration of the population genetic structure of species from different taxonomic and functional groups having different ecological roles and dispersal modes. Specific traits related to the biology of each species, e.g., pelagic larval duration (PLD), reproductive timing, behavioral traits can determine recruitment success and result in different connectivity outcomes (Boissin et al., 2016; Jahnke et al., 2016, 2017; Carreras et al., 2017; Paterno et al., 2017; Boscari et al., 2019). Multispecies genetic analysis is needed to obtain a "collection of networks of genetic variation of all species within a community" (Fortuna et al., 2009).

Mussels of the genus *Mytilus* occur worldwide in all oceans and major seas in both northern and southern hemispheres (Gerard et al., 2008; Kijewski et al., 2011; Zbawicka et al., 2012) showing a wide distribution range. Specifically, in Europe, the presence of 3 distinct *Mytilus* species (*M. galloprovincialis*; *M. edulis* Linnaeus, 1758; *M. trossulus* Gould, 1850) and 2 hybrids (*M. edulis*/*M. trossulus* and *M. edulis*/*M. galloprovicialis*) has been traditionally reported (McDonald et al., 1991; Rawson et al., 1996).

The Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819) was selected in the project CoCoNET as model organism for species with a strong dispersal potential to typify patterns of genetic structure expected within scenarios of high connectivity. *M. galloprovincialis* is a sessile filter-feeder with pelagic larvae very common in both Mediterranean and Black Seas. The species undergoes multiple spawning events per season (Da Ros et al., 1985) with high fecundity per event. In addition, this mussel has long-lived pelagic larvae (i.e., more than a month; Cáceres-Martínez and Figueras, 1998; Bierne et al., 2002) able to settle onto rafts of natural and artificial substrates, thus to arrive at the final recruitment sites even as postlarval stages (Miller et al., 2018; see also Lane et al., 1985). This mussel is therefore considered a species with an exceptionally high potential for dispersion over time and space (Chicaro and Chicaro, 2000; Salinas-de-Leòn et al., 2012). The species is also economically important (Astorga, 2014) since largely utilized in aquaculture, where production is based on natural recruitment, which can further increase genetic mixing, at least at the spatial scale of spat collection. Being a sessile filter-feeder, *M. galloprovincialis* also serves as bioindicator of environmental conditions (Gosling, 1992).

Mytilus galloprovincialis originated in the Mediterranean Sea around 2 million years ago (Daguin and Borsa, 2000) and, at present, it occurs as a pure taxon in the latter and in the Black Sea (Śmietanka et al., 2004; Kijewski et al., 2011). Few studies describe its genetic structuring along the coasts of Mediterranean and Black Seas, and they are characterized by restricted numbers of sampling sites, low within-site replication and by the molecular markers used. High resolution, hypervariable nuclear markers such as microsatellites are typically of low densities and of difficult isolation in molluscs (Cruz et al., 2005; McInerney et al., 2011) and the use of mitochondrial markers is complicated by the doubly uniparental inheritance (DUI) of these organelles in mussels (Skibinski et al., 1994; Zouros et al., 1994). To overcome these limits, single nucleotide polymorphisms (SNPs) have started to be developed as markers in *Mytilus* spp., and they have been successfully used to differentiate species and populations within taxa in native and non-native areas (Zbawicka et al., 2012, 2018; Gardner et al., 2016; Larraín et al., 2018; Wilson et al., 2018).

Regardless of these findings, a consistent and reliable SNPs-based population structure of *M. galloprovincialis* across its native range is still missing. A restricted gene flow was found for this species throughout the Atlantic, Mediterranean and Black Seas (the native range), by using allozyme loci, mtDNA and RFLPs (Quesada et al., 1995; Sanjuan et al., 1996, 1997, Ladoukakis et al., 2002). At a finer geographical scale including only the Aegean Sea and the Central-Eastern Mediterranean Sea, genetic homogeneity was found in contrast to an evident genetic heterogeneity within the Adriatic-Ionian basins (microsatellites, mtDNA F and M; Ladoukakis et al., 2002; Giantsis et al., 2014a,b). Genetic data about this species are still scarce in the Adriatic region because of the restricted number of locations considered (up to 3; Giantsis et al., 2014a,b), while in the Black Sea the genetic diversity of *M. galloprovincialis* is essentially unknown.

The Next Generation Sequencing (NGS) population genomics approach applied in the present study is based on hundreds of polymorphic loci and takes advantage of the wide scale sampling design within the CoCoNET network, with analyses on 8 localities in the Central Mediterranean (Adriatic-Ionian basins), 2 in the Western Mediterranean and 9 in the Black Sea, providing the first insight into the *M. galloprovincialis* population genomics across a substantial part of its native distribution range. We used the RADseq 2b-RAD protocol (Wang et al., 2012) for the isolation and genotyping of SNPs with a genome-wide coverage.

Specifically, through a big effort of samples collection across both Mediterranean and Black basins, the present study aims to: (1) provide the overall genetic structure of *M. galloprovincialis* at the large scale (i.e., Mediterranean and Black Seas); (2) investigate the presence of genetic differentiation at a smaller scale within basins (i.e., Central Mediterranean and Black Seas); (3) compare our findings to those obtained for other species investigated in the same CoCoNET project. We anticipate that the outcomes provide useful insights for the conservation management and in particular for the design of connected MPAs, within the target areas, but potentially exportable to other geographic regions.

MATERIALS AND METHODS

Sampling and Genomic DNA Extraction

Totally, 478 individuals of *M. galloprovincialis* were collected from 2013 to 2014 in the Mediterranean and Black Seas at 19 sampling locations (9 sites in the Black Sea, 8 sites in the Central Mediterranean, i.e., Adriatic and Ionian Seas), and 2 sites in the Western Mediterranean Sea (see **Table 1** and **Figure 1** for details). At each location, the shell of each sample was opened, the body removed and preserved in absolute ethanol until the genomic DNA (gDNA) extraction from gills (Eurogold Tissue DNA mini kit, EuroClone). For all the samples, the quality of extracted gDNA in terms of fragments size (1% agarose gel electrophoresis), concentration and purity ratio (NanoDrop UV-Vis spectrophotometer) was checked.

Construction and Next Generation Sequencing of 2b-RAD Libraries

The concentration of high-quality RNA-free gDNA obtained from *M. galloprovincialis* samples was optimized for the 2b-RAD protocol (Wang et al., 2012) at about 250–400 ng for each individual. All the collected samples (N = 478) were processed following the 2b-RAD protocol steps described in Paterno et al. (2017), except for (i) the ligation of adaptors with partially (not fully) degenerated overhangs to the restriction fragments and (ii) the 2b-RAD tags amplification for a smaller number of cycles (14). Three pools (about 160 barcoded-samples each) were assembled and the high-molecular weight fragments and primer-dimers removed in two steps as in Paterno et al. (2017). The Next Generation Sequencing (NGS) of each pool was performed on Illumina HiSeq platforms with a SR50 High Output mode by UC Davis Genome Center (CA, United States) and Genomix4Life S.r.l. (Baronissi, SA, Italy); the sequencing services performed also the data demultiplexing and quality-filtering. In order to increase the read depth and ensure an equal number of reads for each sample, each pool was sequenced twice, adjusting the relative volumes of barcoded-samples on the second run (Paterno et al., 2017).

De novo Analysis: *In silico* Identification of Loci and Genotyping

The quality of the raw demultiplexed and quality-filtered reads was verified by using FASTQC 0.11.5[1]. Later, custom-made Python^{TM} scripts were used for trimming the adaptors and filtering the reads for the presence of the IIB restriction enzyme (*CspCI*) recognition site, producing trimmed high-quality reads of uniform 32 bp-length.

STACKS 1.42 (Catchen et al., 2013) was used to carry out the assembly of loci and the individual genotyping (*denovo_map.pl* pipeline). Two technical replicates (one Mediterranean and one Black Sea specimen) were included for the optimization of the *de novo* assembly parameters in STACKS. Parameters were set as follows: m = 15 (minimum stack depth per allele in a sample), M = 4 (number of mismatches allowed between stacks to build a locus in an individual), n = 4 (maximum distance between loci from distinct individuals to be merged in the population catalog), *SNP bound* = 0–0.1 (error rate to call SNP), *alpha* = 0.05 (significance level to call a heterozygote or homozygote).

The employment of SNPs as RAD-Seq markers in population genomics typically implicates a certain level of missing data in the dataset because not all the genotyped markers are shared by all the samples (Chattopadhyay et al., 2014). This implies the need to retain only those loci shared among a reasonable, yet arbitrary, fraction of individuals for downstream analysis. However, if the missing data are not evenly scattered among the samples, a dataset of many individuals collected from distinct areas could lose power in detecting a fine genetic differentiation within areas because of the decrease of the number of shared polymorphic markers usable for the analyses.

For this reason, we performed three independent STACKS runs in order to achieve the best power of resolution according to the geographical scale considered: the wide scale (the entire study area) on one side, and the basin scale (Adriatic-Ionian basins or Black Sea) on the other side. First, an overall catalog of loci for the exploration of the global population structure of the species throughout the entire study area was obtained by running together all 478 samples (with two additional technical replicates) from the 19 population samples. Later, the 207 samples (plus one technical replicate) from the 8 Adriatic-Ionian sites and the 232 samples (plus one technical replicate) from the 9 Black Sea's sites were run separately to obtain basin-specific datasets

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[1] http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

TABLE 1 | Sampling information about the 19 *Mytilus galloprovincialis* population samples collected from the Mediterranean and Black Seas.

Region	Nation	Name of sampling location	Acronym	Coordinates N – E	Sampling Date	N
Ionian Sea	Greece	Corfù island	OTH-A*	39°47.522′ – 19°54.211′	November 2013	28
Ionian Sea	Albania	Karaburun Peninsula	KAP	40°23.568′ – 19°19.498′	June 2013	18
Adriatic Sea	Montenegro	Boka Kotorska Bay	BOK	42°23.252′ – 18°34.178′	June 2013	30
Adriatic Sea	Croatia	Kornati islands	KOR	43°47.535′ – 15°16.889′	June 2013	25
Adriatic Sea	Italy	Tremiti islands	TRE	42°08.315′ – 15°31.437′	June 2013	27
Adriatic Sea	Italy	Torre Guaceto	TOG	40°42.999′ – 17°48.003′	May 2013	21
Adriatic-Ionian Sea	Italy	Otranto	OTR	40°06.554′ – 18°31.153′	May 2013	29
Ionian Sea	Italy	Porto Cesareo	POC	40°11.715′ – 17°55.077′	May 2013	29
Western Med. Sea	Tunisia	Haouaria	TUN	37°03.026′ – 10°58.020′	October 2014	22
Western Med. Sea	France	Banyuls	FRN	42°28.937′ – 03°08.245′	October 2014	17
Black Sea	Ukraine	Karadag	KAR	44°54.370′ – 35°15.330′	July 2013	29
Black Sea	Ukraine	Tharkhankut	TAR	45°20.030′ – 32°33.090′	July 2013	19
Black Sea	Romania	Costinesti Monastery	COS	43°55.534′ – 28°38.442′	July 2013	28
Black Sea	Bulgaria	Cape Kaliakra	KAL	43°24.712′ – 28°21.001′	May 2013	28
Black Sea	Bulgaria	Ropotamo-Kiten	ROK	42°11.706′ – 27°50.163′	June 2013	27
Black Sea	Turkey	Sile	SIL	41°10.986′ – 29°36.736′	July 2013	30
Black Sea	Turkey	Sinop	SIN	42°00.964′ – 35°10.956′	May 2013	22
Black Sea	Georgia	Batumi	BAT	41°41.279′ – 41°42.078′	July 2013	26
Black Sea	Russia	Novorossiysk-Gelendzhik	RUS	44°31.995′ – 38°04.878′	July 2013	23

*For each population samples, we report: area, nation, sampling location, acronym, coordinates (with latitude, N, and longitude, E), date of collection and number of samples (N). *Sampling site alternative to Othonoi Island, where the species was not found, following CoCoNET code.*

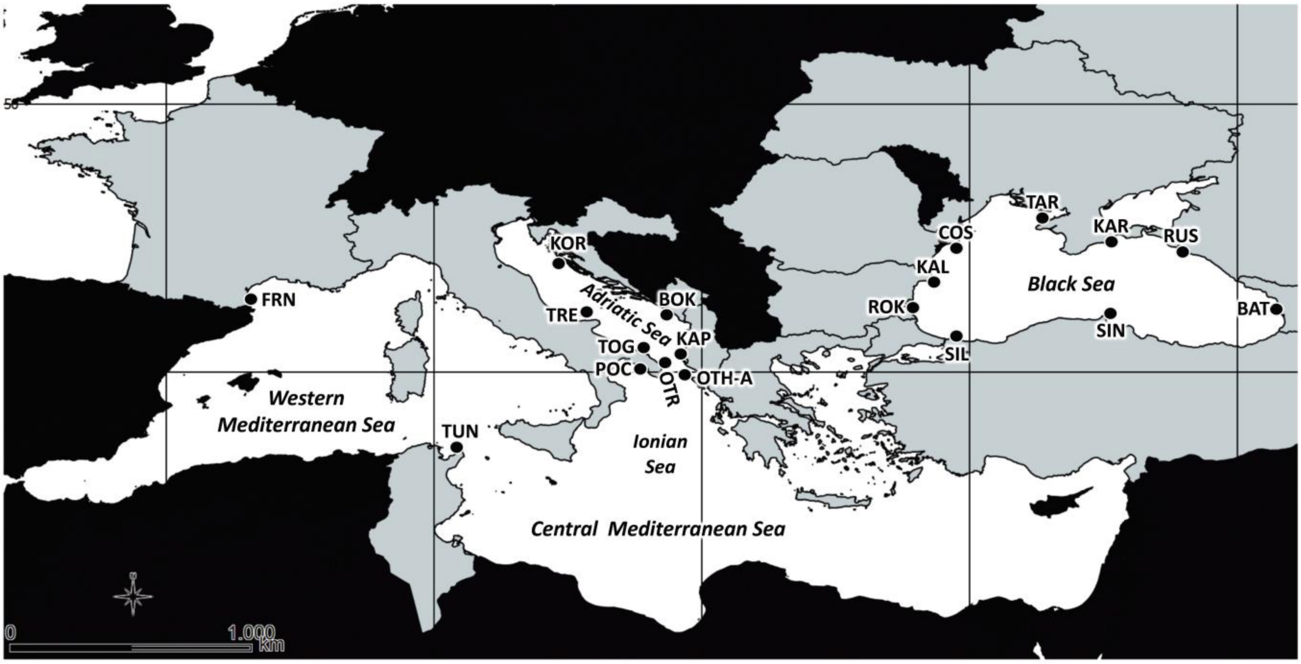

FIGURE 1 | Map of sampling sites of the 19 *Mytilus galloprovincialis* population samples in Mediterranean and Black Seas. See **Table 1** for acronyms.

for the evaluation of the genetic structuring of the species at a small spatial scale.

For each of the three STACKS runs, we selected a final dataset consisting of all the polymorphic loci shared by at least 80% of the individuals with up to 3 SNPs and 6 alleles. When more than one SNP was found at a locus, only the SNP with the highest expected heterozygosity across each dataset was retained (Phillips, 2005). The threshold of missing loci per individual was set to 30%. Since the parameter *m* (minimum read depth for allele) was set to 15, no additional filter for sequencing depth was applied (Paterno et al., 2017). At the end, we obtained 3 distinct datasets (i.e., the overall dataset, the Adriatic-Ionian dataset, and the Black Sea one). The package *Populations* in STACKS was used to generate input file in GENEPOP format, and later CREATE (Coombs et al., 2008) and PGDSPIDER 2.1.0.3 (Lischer and Excoffier, 2012) were used for the conversion in several formats for genetic analyses.

Statistical Power of the Basin-Specific Datasets

The statistical power to detect true levels of genetic differentiation was evaluated and compared for the Adriatic-Ionian and Black Sea's datasets by the simulation method of Ryman and Palm (2006) implemented in POWSIM 4.1 (SNPs executable Powsim_b). POWSIM simulates sampling from populations at various levels of expected divergence under a classical Wright-Fisher model without migration or mutation. Simulations were run using default parameter values for dememorizations (1000), batches (100), and iterations per batch (1000) for a scenario involving 8 and 9 subpopulations, respectively, using a range of effective population size (N_e = 1000–10,000) and of generations of drift (t = 10–100). The statistical power to detect an expected divergence as small as F_{ST} = 0.005 was estimated after 200 replicates as the proportion of statistically significant test ($P < 0.05$).

Population Genomics

The genetic variability within population samples, population structure and genetic differentiation were evaluated separately for the three datasets (overall, Adriatic-Ionian, Black Sea). The observed heterozygosity (H_o), unbiased expected heterozygosity (H_e) and the inbreeding coefficient (F_{IS}) of each population sample were assessed on the polymorphic loci (GENETIX 4.05.2; Belkhir et al., 2000); the allelic richness (A_R) was calculated on the smallest population sample size of each dataset (HP-RARE; Kalinowski, 2005). Non-hierarchical and hierarchical analysis of molecular variance (AMOVA; Excoffier et al., 1992) as well as the pairwise genetic distances between population samples (F_{ST}) were calculated (ARLEQUIN 3.5; Excoffier and Lischer, 2010); due to the presence of missing data, the locus by locus option was set. Benjamini and Hochberg (1995) correction for multiple tests was applied for adjusting the significance level in multiple comparisons. The Discriminant Analysis of Principal Components (DAPC; ADEGENET for R, Jombart, 2008) was used (1) to find the best number of clusters of genetically related individuals (based on the Bayesian Information Criterion, BIC) without the reference of the original populations and (2) for the visual assessment of the between-population differentiation of the original population samples. Bayesian clustering (STRUCTURE 2.3.4; Pritchard et al., 2000) was also performed to infer the most likely genetic clusters. After some trials, a burn-in of 100,000 followed by one million steps, K = 1–8 (depending on the datasets) and 5 iterations per each K value was set; sampling locations as prior (model LOCPRIOR, Hubisz et al., 2009) was used; admixture model and correlated allelic frequencies were assumed. The most probable number of clusters was identified based on delta K (STRUCTURE HARVESTER online; Earl and VonHoldt, 2012).

Detection of Loci Under Selection (Outliers)

For the identification of putative loci under directional selection, two neutrality tests were employed. The first test is the Bayesian method implemented in BAYESCAN 2.01 (Foll and Gaggiotti, 2008), and the second one is the F_{ST}-outlier method implemented in LOSITAN (Antao et al., 2008). Both software were run separately with the three datasets. LOSITAN parameters were set as follows: 1 million simulations under neutral mean F_{ST}, confidence interval of 0.95% and a false discovery rate (FDR) of 0.01; the infinite allele model was applied. For each run, three replicates were performed. BAYESCAN parameters were: burn in = 50,000; thinning interval = 30; sample size = 5000; numbers of pilot runs = 50; length of pilot runs = 5000 and the same false discovery rate (FDR) threshold set in LOSITAN (0.01). The loci detected by both methods were considered outliers under selection.

RESULTS

Sequencing Results, Filtering and Selection of Loci for Genetic Analysis

For all the collected samples (N = 478), high-quality RNA-free gDNA was successfully extracted from the gills. The Illumina sequencing of the three 2b-RAD pools (N = 480; 2 technical replicates included) produced 1,032,760,048 de-multiplexed and quality-filtered reads (mean quality score per base > 37) and more than 92% was retained after trimming and filtering for *CspCI* restriction site (about 1,990,000 reads for each sample). The three independent STACKS runs identified 113,494, 77,459, and 83,000 2b-RAD tags considering, respectively, all the 19 population samples, the 8 Adriatic-Ionian ones alone, or the 9 Black Sea's collections; about the 33% of loci were polymorphic in all 3 runs.

After filtering and the exclusion of the two technical replicates and of those individuals showing more than 30% of missing loci, we obtained three datasets as follows: (1) the overall dataset: 461 individuals, 512 polymorphic loci; (2) the Adriatic-Ionian dataset: 201 individuals, 811 polymorphic loci; and (3) the Black Sea's dataset: 228 individuals, 998 polymorphic loci.

Genetic Analyses

Statistical Power of the Basin-Specific Datasets

The 200 simulations performed by POWSIM showed that the population sample size, number of loci and their allele frequencies have enough statistical power to detect F_{ST} values as small as 0.005 with a power approaching 100% under all the tested conditions of effective population sizes and number of generations of drift.

Genetic Variability

For the three datasets, similar values were obtained among the population samples for H_o, unbiased H_e and A_R, confirmed by one-way ANOVA detecting no significant differences among samples ($P > 0.05$; **Supplementary Tables S1–S3**). A generalized heterozygote deficit was detected, as evident from the positive values obtained for the inbreeding coefficients (F_{IS}) in all the populations (**Supplementary Tables S1–S3**). Locus-by-locus analysis showed that the distribution of F_{IS} values is skewed toward positive values, though the majority of the loci show F_{IS} values very close to 0 and few of them negative values (**Supplementary Figures S1–S3**).

Genetic Structure at the Large Scale

The non-hierarchical AMOVA carried out on the 19 population samples (the overall dataset, 512 polymorphic loci) provided a clear indication of genetic structuring at the large spatial scale, with a statistically significant global multilocus F_{ST} (F_{ST} = 0.03243, $P < 0.0001$). Considering the 171 pairwise F_{ST} (**Table 2**), all 90 comparisons involving a location from the Mediterranean Sea and a location from the Black Sea were statistically significant after correction for multiple tests (F_{ST} range = 0.04208–0.08079; $P < 0.0001$), indicating a clear genetic differentiation between the 2 seas. In addition, among Mediterranean samples 14 comparisons out of 45 were significant after multitest correction (F_{ST} range = 0.00809–0.02075; $P < 0.05$); in this case, pairwise differences were mainly due to the deviation of the sample from Montenegro (BOK, 9 significant comparisons). The North-Western (FRN) and the South-Western (TUN) samples were not genetically different despite the geographic distance, and they differed only weakly from the Central Mediterranean ones (3 significant comparisons, 2 of them involving the BOK sample). No significant pairwise F_{ST} was detected within the Black Sea.

Hierarchical AMOVA showed that the genetic variation of the dataset can be best partitioned into 2 groups, one including the 10 Mediterranean population samples, and the other one the 9 Black Sea's population samples (F_{CT} = 0.05286, $P < 0.0001$), maximizing the genetic variance between groups. Similarly, the DAPC identified two genetic clusters as the optimal clustering solution based on the lowest BIC (**Figure 2A**), with the 2 clusters corresponding to the Mediterranean Sea and the Black Sea population samples (**Figure 2B**). Interestingly, the graphs plotting the density of the inferred clusters and the original populations on the single discriminant function described the same trend (**Figures 2C,D**). The Bayesian clustering carried out by STRUCTURE was consistent with the DAPC, in fact the most probable number of clusters was identified for $K = 2$ with the same groupings (**Figure 3**).

Genetic Structure at the Basin Scale

The lack of genetic structure of the Black Sea and the signal of genetic differentiation in the Central Mediterranean found with the overall dataset were confirmed by the more powerful basin-specific datasets.

The non-hierarchical AMOVA conducted on Adriatic-Ionian dataset (811 polymorphic loci, 8 population samples) pointed out that most of the genetic variation (>99%) arose from the within-populations level and only 0.69% from the among-populations level, with, however, a statistically significant global F_{ST} ($P < 0.0001$). A number of pairwise F_{ST} values were statistically significant after correction for multi-test (9/28; **Table 3**) and, once

TABLE 2 | Pairwise genetic distances (F_{ST}) between all the 19 population samples based on the overall dataset of 512 polymorphic loci.

	OTH-A	KAP	BOK	KOR	TRE	TOG	OTR	POC	TUN	FRN	KAR	TAR	COS	KAL	ROK	SIL	SIN	BAT	RUS
OTH-A		0.3317	**0.0011**	0.0363	0.3853	0.4441	0.3554	0.1260	0.2912	0.1631	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
KAP	0.00370		**0.0022**	**0.0141**	0.6835	0.1098	**0.0006**	0.0326	0.2389	0.2244	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
BOK	**0.01052**	**0.01336**		**0.0171**	**0.0180**	**0.0028**	**<0.0001**	**<0.0001**	**0.0112**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
KOR	0.00739	**0.01161**	**0.00816**		**0.0191**	0.6951	**0.0041**	0.0339	0.3798	**0.0088**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
TRE	0.00280	0.00076	**0.00809**	**0.00933**		0.7108	0.0957	0.2115	0.2599	0.6005	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
TOG	0.00263	0.00819	**0.01113**	0.00042	0.00057		0.3195	0.7797	0.9893	0.4229	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
OTR	0.00297	**0.01740**	**0.01778**	**0.01139**	0.00653	0.00420		0.4884	0.0662	0.0391	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
POC	0.00490	0.00925	**0.01793**	0.00758	0.00412	−0.00060	0.00197		0.5505	0.1634	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
TUN	0.00421	0.00645	**0.01003**	0.00379	0.00518	−0.00432	0.00833	0.00196		0.1336	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
FRN	0.00648	0.00698	**0.02075**	**0.01349**	0.00213	0.00427	0.01080	0.00630	0.00895		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
KAR	**0.04532**	**0.04870**	**0.04821**	**0.05202**	**0.05206**	**0.06536**	**0.05679**	**0.06200**	**0.05029**	**0.06607**		0.2067	0.9196	0.7956	0.4711	0.2631	0.3381	0.4182	0.2252
TAR	**0.05050**	**0.05875**	**0.04982**	**0.05634**	**0.05890**	**0.07106**	**0.05826**	**0.06710**	**0.05445**	**0.07511**	0.00381		0.1642	0.2921	0.0819	0.1958	0.5709	0.4627	0.2647
COS	**0.05049**	**0.05369**	**0.04960**	**0.05279**	**0.05845**	**0.06885**	**0.06117**	**0.06788**	**0.05583**	**0.06765**	−0.00210	0.00443		0.6807	0.6391	0.6952	0.3598	0.9040	0.7359
KAL	**0.04625**	**0.04875**	**0.04376**	**0.05098**	**0.05531**	**0.06514**	**0.05696**	**0.06571**	**0.04784**	**0.06764**	−0.00098	0.00263	−0.00015		0.6112	0.9350	0.4479	0.8022	0.8546
ROK	**0.04421**	**0.04208**	**0.04765**	**0.04723**	**0.04821**	**0.05775**	**0.05212**	**0.05761**	**0.04496**	**0.06467**	0.00125	0.00569	0.00025	0.00014		0.4077	0.1774	0.5060	0.5122
SIL	**0.05210**	**0.05592**	**0.04834**	**0.05712**	**0.06196**	**0.07315**	**0.06131**	**0.07157**	**0.05216**	**0.07275**	0.00278	0.00391	−0.00013	−0.00267	0.00158		0.8118	0.9632	0.9966
SIN	**0.05825**	**0.06367**	**0.05326**	**0.06152**	**0.06260**	**0.08075**	**0.06561**	**0.07556**	**0.05906**	**0.08079**	0.00275	0.00095	0.00272	0.00169	0.00425	−0.00116		0.3621	0.7691
BAT	**0.04646**	**0.04902**	**0.04760**	**0.05093**	**0.05539**	**0.06434**	**0.05638**	**0.06561**	**0.05160**	**0.06558**	0.00205	0.00191	−0.00173	−0.00080	0.00134	−0.00277	0.00311		0.7486
RUS	**0.04962**	**0.05224**	**0.05398**	**0.05856**	**0.06229**	**0.07233**	**0.06432**	**0.07053**	**0.05454**	**0.07173**	0.00360	0.00343	−0.00045	−0.00206	0.00088	−0.00577	−0.00096	−0.00034	

*F_{ST} indices and p-values are reported below and above the diagonal, respectively; significant indices in bold. Benjamini and Hochberg (1995) correction for multiple tests was applied. See **Table 1** for location acronyms.*

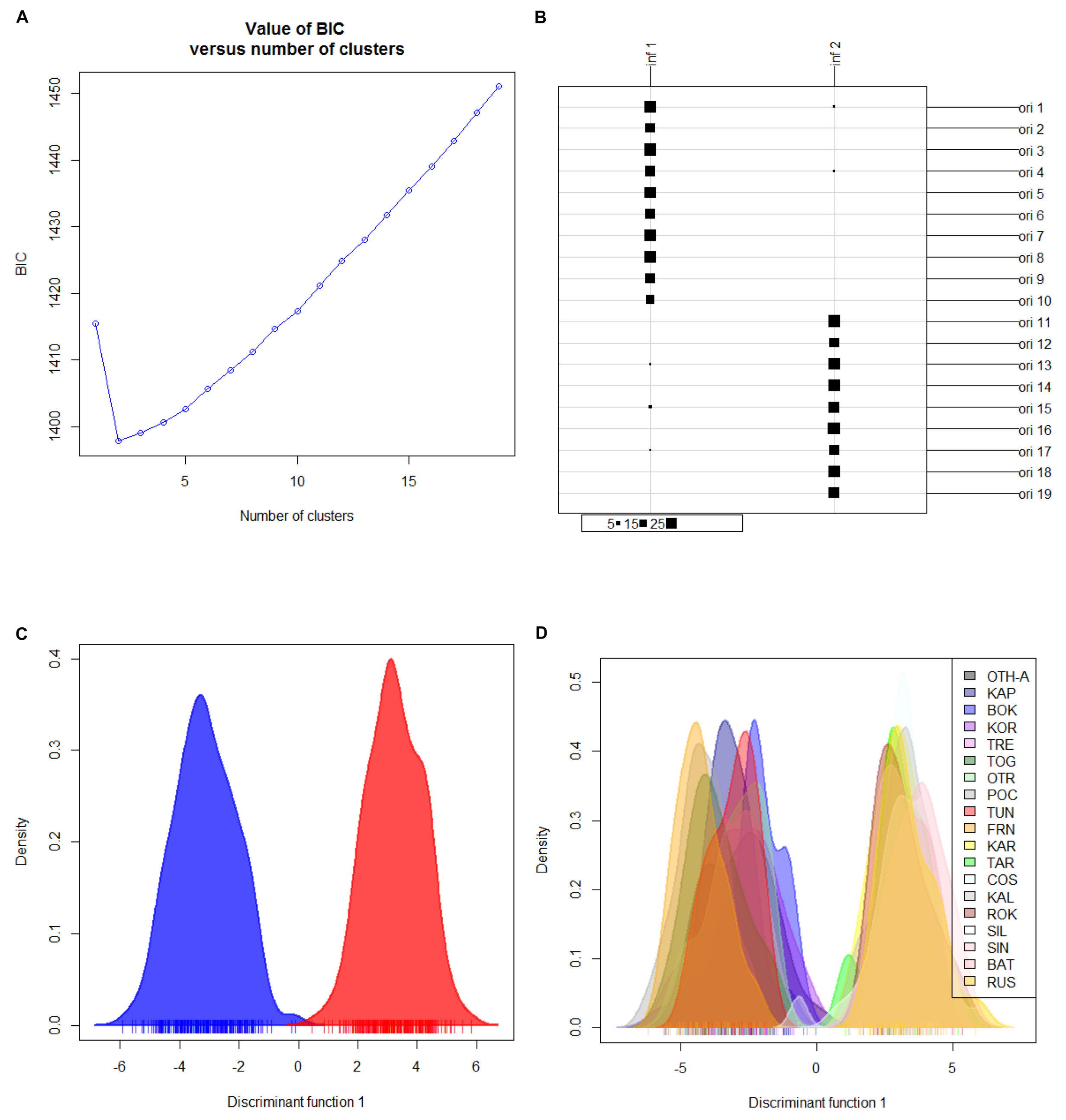

FIGURE 2 | Discriminant analysis of principal components (DAPC) for the overall dataset of 19 population samples from Mediterranean and Black Seas. **(A)** BIC value. Graph of BIC values for increasing value of number of clusters (k). **(B)** Graphical table showing the number of original populations (ori) VS the number of inferred clusters (inf) and the group sizes of each correspondence. Original populations legend: ori 1: Corfù Island, Greece (OTH-A); ori 2: Karaburun Peninsula, Albania (KAP); ori 3: Boka Kotorska Bay, Montenegro (BOK); ori 4: Kornati islands, Croatia (KOR); ori 5: Tremiti islands, Italy (TRE); ori 6: Torre Guaceto, Italy (TOG); ori 7: Otranto, Italy (OTR); ori 8: Porto Cesareo, Italy (POC); ori 9: Tunisia (TUN); ori 10: France (FRN); ori 11: Karadag MPA, Ukraine (KAR); ori 12: Tharkhankut, Ukraine (TAR); ori 13: Costinesti Monastery, Romania (COS); ori 14: Cape Kaliakra, Bulgaria (KAL); ori 15: Ropotamo-Kiten, Bulgaria (ROK); ori 16: Sile, Turkey (SIL); ori 17: Sinop, Turkey (SIN); ori 18: Batumi, Georgia (BAT); ori 19: Novorossiysk-Gelendzhik, Russia (RUS). **(C)** 2D scatterplot representing the density of the inferred numbers of clusters ($k = 2$) on the single discriminant function. **(D)** 2D scatterplot representing density of the 19 original populations on the single discriminant function.

again, most of these comparisons (6/9) involved the Montenegro sample (BOK); 2 significant values were obtained comparing the Croatia sample (KOR) with the Tremiti islands (TRE) and with Otranto (OTR), and the last significant value was between TRE and OTR. Both the genetic clustering methods found out a single cluster including all the population samples (BIC values of the DAPC in **Figure 4**; STRUCTURE plot not provided). Interestingly, a hierarchical AMOVA found the best

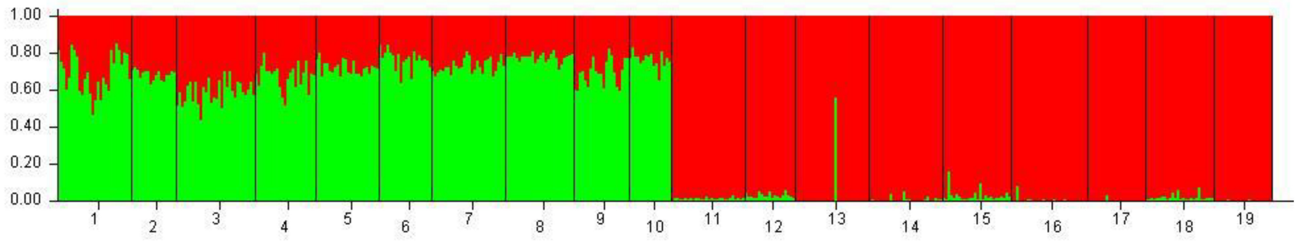

FIGURE 3 | Bayesian clustering performed with STRUCTURE on the overall dataset of 19 population samples from Mediterranean and Black seas. Legend: 1: Corfù Island, Greece (OTH-A); 2: Karaburun Peninsula, Albania (KAP); 3: Boka Kotorska Bay, Montenegro (BOK); 4: Kornati islands, Croatia (KOR); 5: Tremiti islands, Italy (TRE); 6: Torre Guaceto, Italy (TOG); 7: Otranto, Italy (OTR); 8: Porto Cesareo, Italy (POC); 9: Tunisia (TUN); 10: France (FRN); 11: Karadag MPA, Ukraine (KAR); 12: Tharkhankut, Ukraine (TAR); 13: Costinesti Monastery, Romania (COS); 14: Cape Kaliakra, Bulgaria (KAL); 15: Ropotamo-Kiten, Bulgaria (ROK); 16: Sile, Turkey (SIL); 17: Sinop, Turkey (SIN); 18: Batumi, Georgia (BAT); 19: Novorossiysk-Gelendzhik, Russia (RUS).

TABLE 3 | Pairwise genetic distances (F_{ST}) between the 8 Adriatic-Ionian population samples based on Adriatic-Ionian dataset (811 polymorphic loci).

	OTH-A	KAP	BOK	KOR	TRE	TOG	OTR	POC
OTH-A		0.3084	**0.0023**	0.2309	0.1383	0.4631	0.0497	0.0364
KAP	0.00419		**0.0009**	0.0296	0.5433	0.4056	0.1269	0.0375
BOK	**0.00795**	**0.01133**		0.1677	**<0.0001**	**0.0140**	**<0.0001**	**<0.0001**
KOR	0.00407	0.00878	0.00464		**0.0001**	0.7508	**0.0105**	0.0264
TRE	0.00440	0.00265	**0.01154**	**0.01097**		0.7715	**0.0005**	0.0181
TOG	0.00291	0.00422	**0.00787**	0.00138	0.00097		0.2105	0.3810
OTR	0.00609	0.00674	**0.01252**	**0.00843**	**0.00960**	0.00523		0.0863
POC	0.00579	0.00740	**0.01443**	0.00669	0.00655	0.00329	0.00535	

*F_{ST} indices and p-values are reported below and above the diagonal, respectively; significant indices in bold. Benjamini and Hochberg (1995) correction for multiple tests was applied. See **Table 1** for location acronyms.*

TABLE 4 | Pairwise genetic distances (F_{ST}) between the 9 population samples from the Black Sea based on the Black Sea's dataset (998 polymorphic loci).

	KAR	TAR	COS	KAL	ROK	SIL	SIN	BAT	RUS
KAR		0.5039	0.5200	0.9232	0.5624	0.8246	0.3893	0.2206	0.1632
TAR	0.00305		0.2894	0.5105	0.5045	0.8897	0.1232	0.2061	0.3549
COS	0.00247	0.00450		0.5670	0.7575	0.4215	0.0930	0.7892	0.4902
KAL	0.00017	0.00318	0.0023		0.7817	0.7631	0.1285	0.7395	0.5749
ROK	0.00217	0.00318	0.00143	0.00118		0.9188	0.2941	0.1630	0.5437
SIL	0.00104	0.00087	0.00302	0.00147	0.00032		0.7063	0.8643	0.7254
SIN	0.00369	0.00698	0.00609	0.00555	0.00426	0.00225		0.4197	0.6066
BAT	0.00405	0.00536	0.00140	0.00158	0.00458	0.00096	0.00386		0.2842
RUS	0.00475	0.00441	0.00302	0.00243	0.00254	0.00185	0.00281	0.00411	

*F_{ST} indices and p-values are reported below and above the diagonal, respectively. Benjamini and Hochberg (1995) correction for multiple tests was applied. See **Table 1** for location acronyms.*

clustering solution for three groups: the first group with the population sample from Tremiti islands (TRE) alone, the second group including BOK sample and the Croatian sample (KOR), and the last including the three other Italian samples (OTR, POC, and TOG), the Greek (OTH-A) and Albanian (KAP) samples (F_{CT} = 0.00312, $P < 0.05$). The scatterplot of the discriminant analysis set on the original populations reflected the weak genetic differentiation detected among these population samples (**Figures 4B,C**).

The Black Sea dataset (998 polymorphic loci, 9 population samples) revealed a small and not significant global multilocus F_{ST} value (F_{ST} = 0.00278, P = 0.59584) and no pairwise F_{ST} value resulted to be significant within this basin (**Table 4**). Both Bayesian clustering methods conducted by ADEGENET and STRUCTURE found only one genetic cluster (BIC values of the DAPC in **Figure 5**; STRUCTURE plot not reported). As expected due to the lack of genetic structuring, the visual assessment of the between-population differentiation of the original populations showed all the samples strongly overlapping (DAPC plots, **Figures 5B,C**).

Outliers Detection

The outliers detection conducted on the overall dataset identified 25 loci putatively under positive selection.

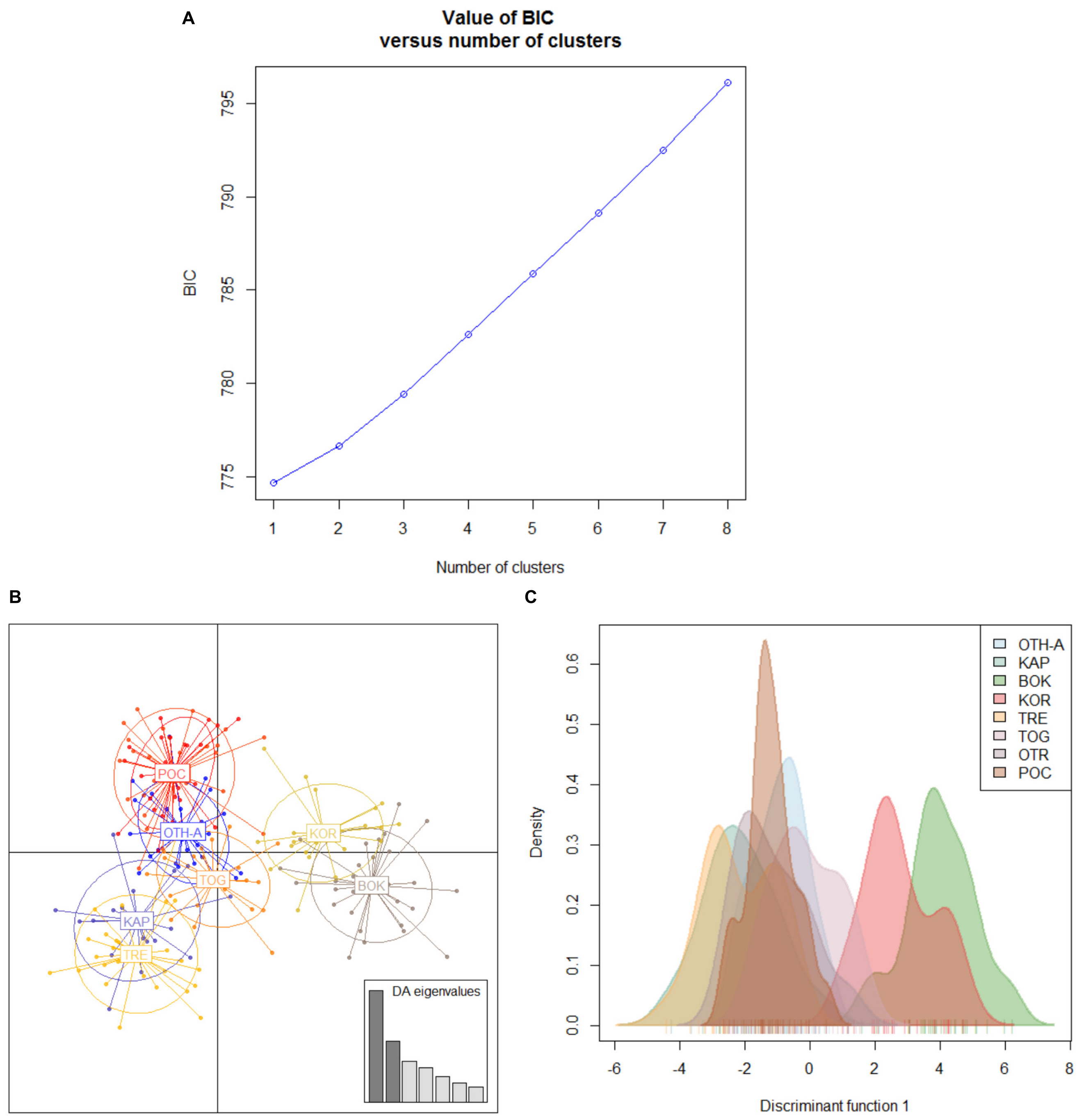

FIGURE 4 | Discriminant analysis of the principal components (DAPC) for the Adriatic-Ionian dataset of 8 population samples. **(A)** Graph of BIC values for increasing value of number of clusters (k). **(B)** Scatterplot of the discriminant analysis set on the original populations. **(C)** 2D scatterplot showing the density of the original populations plotted on the single discriminant. For the acronym locations see **Table 1**.

Fourteen out of these 25 loci were detected by both software, and 11 only by BAYESCAN. The exclusion of these loci resulted in a substantial reduction of the global genetic variance of the dataset (F_{ST} = 0.01152; $P < 0.0001$), but the general pattern of structuring was maintained, with the best clustering solution for two groups (Mediterranean population samples against Black Sea's population samples; F_{CT} = 0.01565; $P < 0.0001$) and with 86/90 significant pairwise F_{ST} distances between the Mediterranean and the Black Sea.

The scan of the Adriatic-Ionian dataset by both software detected 2 putative outliers, whose exclusion caused a small

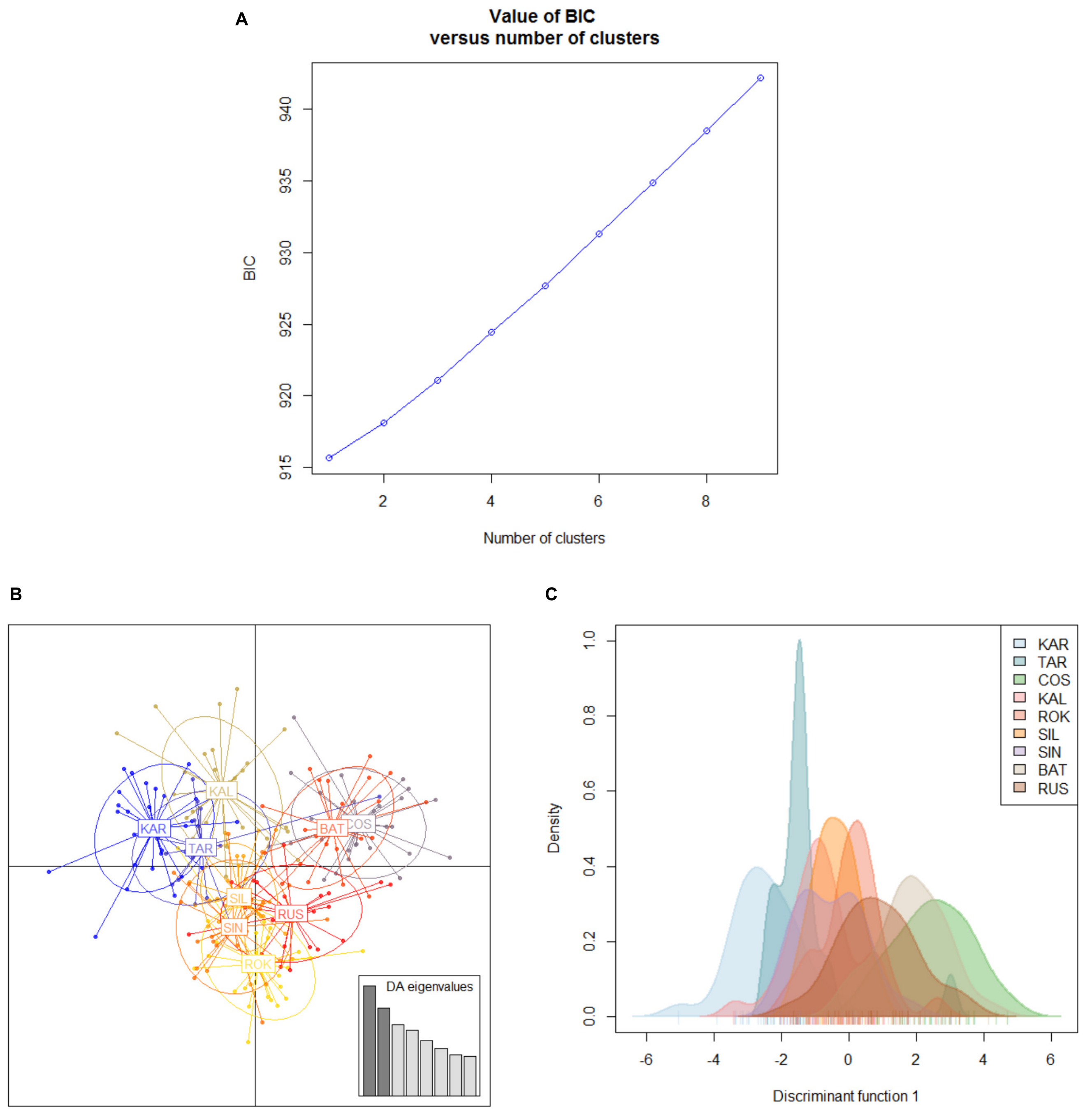

FIGURE 5 | Discriminant analysis of the principal components (DAPC) for the Black Sea's dataset of 9 population samples. **(A)** Graph of BIC values for increasing value of number of clusters (k). **(B)** Scatterplot of the discriminant analysis set on the original populations. **(C)** 2D scatterplot showing the density of the original populations plotted on the single discriminant. For the acronym locations see **Table 1**.

decrease of the molecular variance ($F_{ST} = 0.00591$; $P < 0.0001$) and the loss of 3 significant pairwise F_{ST}, all involving the population sample from Montenegro (BOK). As expected due to the complete lack of genetic differentiation, the scan of the Black Sea's dataset did not identify any locus putatively under selection within the Black Sea.

DISCUSSION

The genomic analysis of the 19 *M. galloprovincialis* populations sampled across different locations: (1) provided the first detailed analysis of the metapopulation structure of this species throughout part of its native range at the large scale; (2) allowed to investigate the presence of genetic differentiation at a smaller scale within basins; (3) enabled the comparisons with other CoCoNET species.

Using hundreds SNPs identified and genotyped with the 2b-RAD protocol (Wang et al., 2012), we detected a clear differentiation between the Mediterranean and the Black Sea samples, the existence of weak genetic structuring within the Adriatic-Ionian Sea and a complete genetic homogeneity in the Black Sea. We also detected a generalized heterozygote deficit caused by an excess of loci showing positive F_{IS} values. This result could be explained by an extensive mixing at the local scale, expected for a species with an exceptionally high potential for dispersion such as *M. galloprovincialis*, leading to a Wahlund effect at all the analyzed locations. However, technical factors linked to genotyping can also lead to loss of heterozygotes, particularly when dealing with Next Generation Sequencing (NGS) approaches (Andrews et al., 2016). Thus, further investigations, with a specific experimental design, are needed to understand the potential biological relevance of the detected heterozygote deficit.

Genetic Structure at the Large Scale

A marked difference in genetic structure comparing the Mediterranean and the Black Seas was found. This result confirms and strengthens the limited genetic data obtained for this species (Ladoukakis et al., 2002), which hinted at reduced gene flow between the two seas. A similar differentiation was previously reported for other species (including anchovies, Magoulas et al., 2006; sticklebacks, Mäkinen and Merilä, 2008; sprats, Limborg et al., 2012; bluefishes, Miralles et al., 2014). The effect of oceanographic features like the narrow Dardanelles and Bosphorus straits and the existence of environmental barriers (salinity, temperature) limit the exchange of water and species between the two seas and are suspected as a probable cause. The extent of isolation of the Black Sea makes it a potential refuge for temperate and cold water species under future warm conditions. The current absence of the harbor porpoise (*Phocoena phocoena*), a cold-water adapted species, from the Mediterranean Sea and its presence in the Atlantic Ocean and in the Black Sea (the latter colonized not before 5000 years ago) seems to confirm such refuge potential (Fontaine et al., 2014). A post-glacial timing of Black Sea colonization has been recently suggested for the black scorpionfish (*Scorpaena* porcus Linnaeus, 1758), another species analyzed in the CoCoNET project, facilitated by the reopening of the connection between the Black Sea and the Mediterranean Sea about 8000 years ago (Boissin et al., 2016). Both time estimates are consistent with timing of the Black Sea postglacial transition from a fresh/brackish-water ecosystem to a fully marine ecosystem allowing new colonizations from the Aegean Sea. This shift followed a period of isolation from the Mediterranean due to glacial advance and sea level retreat, when water salinity was strongly reduced leading to the eradication of the preexisting marine fauna (Aksu et al., 1999; Çağatay et al., 2000). It is worth noting that, besides being obtained with different genetic markers, the level of differentiation found between Mediterranean and Black Seas samples (average SNPs F_{ST} = 0.058, range 0.042–0.081, our study) is comparable to that found for the black scorpionfish (average microsatellites F_{ST} = 0.057, range 0.011–0.126; Boissin et al., 2016), suggesting that a recent colonization of the Black Sea occurred in mussels as well, also considering that microsatellites tend to underestimate genetic differentiation (O'Reilly et al., 2004), due to their higher heterozygosity compared to SNPs.

Within the Mediterranean Sea, no signal of differentiation was found between Western and Central Mediterranean based on mussel population samples. This result is unexpected since the Sicily Channel is known to act as a barrier and seems to prevent the gene flow in many other species (Borsa et al., 1997; De Innocentiis et al., 2004; Zitari-Chatti et al., 2009; Serra et al., 2010; Pascual et al., 2017). The lack of differentiation, however, again agrees with the genetic structure observed for the black scorpionfish (Boissin et al., 2016). A similar lack of differentiation within Western Mediterranean samples from France and Tunisia was found in the present study on mussels despite a geographic separation of more than 1000 km.

Genetic Structure at the Basin Scale

In contrast to the Western Mediterranean, the Central Mediterranean, represented by Adriatic-Ionian mussel population samples, showed a weak but significant genetic differentiation, which was not affected by the presence of a few outliers. Our results confirm and complement previous reports that indicated, despite a limited sampling effort, genetic heterogeneity in the Adriatic for this species (Giantsis et al., 2014a,b). In our study, differences were weak, e.g., Bayesian clustering failed to detect genetically differentiated clusters, and significant pairwise F_{ST} comparisons were found mostly in pairing involving BOK sample (Montenegro). However, hierarchical AMOVA clearly indicated that genetic variation of the dataset can be best partitioned into three groups: a first group consisting of TRE sample (Tremiti Islands) alone, a second including BOK and KOR (i.e., Montenegrin and Croatian) samples, and a last group comprising the remaining three Italian samples (POC, OTR, TOG) together with OTH-A and KAP samples (i.e., Greek and Albanian samples respectively). This pattern suggests an East–West differentiation in the Central Adriatic Sea and a further North–South differentiation between the Central Adriatic and the South Adriatic and Ionian Seas. A general homogeneity was found among localities within the latter ones (i.e., South Adriatic-Ionian area). Considering that the adult stage of mussels species is sessile, their dispersal ability (and thus the gene flow) is mostly due to the passive transport of larvae driven by the complex water circulation of the Adriatic and Ionian Seas (i.e., characterized by the presence of minor gyres in addition to the main currents). Indeed, the differentiation found here seems to reflect the general surface circulation of the area and the need to support this hypothesis with further sampling. The Adriatic is characterized by a large-scale cyclonic meander, with a northerly flow along the East coast and a southerly flow along the West one (Orlić et al., 1992). Within this large system, three cyclonic gyres further divide the basin into three regions named North, Central and South Adriatic Sea (hereafter NAd, Cad, and SAd respectively). The NAd gyre is defined mainly in autumn and located at the surface, while the middle MAd and SAd gyres become more intense in summer and autumn (Artegiani et al., 1997). According to this, the genetic subdivision detected by AMOVA suggests that the boundary between the MAd and SAd gyres can represent an area of genetic discontinuity, with the MAd unable to prevent East to West differentiation in the Central Adriatic and the SAd promoting an effective mixing of the genetic pool in the Southern Adriatic, further extending to the Ionian Sea. Interestingly, also the population of shore crab *Carcinus aestuarii* follow this pattern as highlighted with genetic markers and biophysical models provided by Schiavina et al. (2014).

As far as in the Black Sea we found a complete lack of genetic structure, the current patterns combined with the long PLD probably ensure a good connectivity among the sampled locations. Unfortunately, the lack of baseline information regarding *M. galloprovincialis* population genetic structure in the Black Sea impair us to make comparisons. Further insights can be obtained exclusively by comparison with the black scorpionfish *Scorpaena porcus* populations (Boissin et al., 2016) also showing a remarkably homogeneous genetic structure in the basin Black Sea.

Comparison With Other CoCoNET Species

Striking similarities occur between *M. galloprovincialis* and the black scorpionfish analyzed in the CoCoNET project. Both species show Mediterranean/Black Sea weak but significant differentiation, no differences between Western and Central Mediterranean samples, genetic heterogeneity among Adriatic-Ionian samples and genetic homogeneity in the Black Sea (Boissin et al., 2016). However, when the comparison is extended to other species analyzed in the CoCoNET project in the Adriatic-Ionian basins, the picture becomes more nuanced.

Black scorpionfish are benthic, with a pelagic larval stage life duration of about 4 weeks (Raventós and Macpherson, 2001). Within the Adriatic-Ionian basins they show a clear East to West genetic break between population samples (Boissin et al., 2016), extending to southern localities the pattern found in the Central Adriatic in *M. galloprovincialis*. In this case, the East to West differentiation seems to prevail on the Adriatic sub-basin regionalization based on oceanography, suggesting that both the MAd and the SAd gyres are not able to effectively connect eastern and western localities across the sea, whereas dispersal alongshore and nearby islands (TRE in the West or KOR and OTH-A in the East) is sufficient to prevent differentiation.

The east Atlantic peacock wrasse *Symphodus tinca* (Linnaeus, 1758) is a demersal fish with a larval duration of about 10 days (Raventós and Macpherson, 2001). The evaluation of the population structure of this species in the Adriatic-Ionian basins identified the existence of 2 barriers to gene flow separating as before the eastern shore localities from the western ones, but also differentiating the Tremiti islands's sample (TRE) from all the others (Carreras et al., 2017). Thus, in this case, the Tremiti islands sample seems not connected with the samples collected from the mainland Italian coast, which might be tentatively explained with its shorter larval duration and nearshore larval distribution.

For the marble crab *Pachygrapsus marmoratus* (Fabricius, 1787), a rocky shore crab species with a PLD of about 4 weeks, population samples collected at the same Adriatic-Ionian localities showed a fairly genetic homogeneity, with a weak differentiation only due to a single locus putatively under selection (Marino et al., personal communication). This result could be explained with the existence of random fluctuations of allele frequencies depending on variability in fecundity, mortality or reproductive success, that can lead to a scenario of genetic patchiness in a general context of genetic homogeneity, as was previously reported by different studies on this species (Silva et al., 2009; Fratini et al., 2011, 2013, 2016).

At the extremes of this differentiation range, still considering a propagule duration of about one month, the habitat former *Posidonia oceanica* (Jahnke et al., 2017) and the sea urchin *Paracentrotus lividus* (Lamarck, 1816) (Paterno et al., 2017) showed completely different patterns. The seagrass *P. oceanica* (Linnaeus Delile, 1813) showed a marked genetic structuring within and between Adriatic-Ionian basins, even between the closest localities, with a possible north-to-south subdivision (Jahnke et al., 2017). On the other side, the analysis of *P. lividus* samples indicated a clear genetic homogeneity in the same area, with a broadly consistent prediction of the potential larval exchange among the same sites (obtained with Lagrangian simulations). This latter outcome supports the view of a relatively persistent flux of propagules from each sites to the closest neighbor, mostly in a counterclockwise direction (Paterno et al., 2017).

Implications for MPAs

MPAs have been increasingly considered the most effective tool for conservation and management of marine environment, able to mitigate the alteration of ecosystems and the negative effect of human activities (Gaines et al., 2010; Gabrié et al., 2012). The implementation of networks of MPAs, where the protected areas can cooperatively operate, is the new conservation target, because it is supposed that networks could be more effective and offer more protection than the individual sites alone (Wood et al., 2008; Wabnitz et al., 2010; Fenberg et al., 2012; Grorud-Colvert et al., 2014). Connectivity data obtained so far in the CoCoNET study area, highlighted species-specific connectivity patterns, ranging from the total absence of genetic differentiation (*P. lividus*) to a strong structuring (*P. oceanica*). In the marine environment, historical and evolutionary processes as well as temperature and ocean currents strongly contribute in shaping species distribution at large biogeographical scale and thus determine the different connectivity patterns observed. Altogether, the framework developed in CoCoNET highlighted the importance of evaluating and discussing the genetic data of population structure in the light of those biological, physical and ecological factors that could have a significant role in shaping the genetic variability of these species. In a management and conservation perspective, the implementation of ecological effective networks of MPAs should rely on the knowledge of connectivity patterns of a representative panel of species living in the selected areas.

The different connectivity outcomes so far obtained (i) indicate the importance of evaluating the genetic distribution of the species under a seascape genomics framework (Di Franco et al., 2012; Pujolar et al., 2013; Paterno et al., 2017); (ii) warn against any generalization in defining conservation units based on the results of a single species because they cannot represent the entire community (Melià et al., 2016); (iii) suggest to take into account connectivity data for different species with a variety of life history traits to improve the efficacy of the management and conservation strategy of the marine ecosystem (Pascual et al., 2017). Besides these limitations, on the basis of the study outcomes, we can anticipate that the delineation of Cells of Ecosystem Functioning, ecologically coherent units of management based on connectivity (Boero et al., 2016), will likely result in much smaller units of conservation in the Adriatic-Ionian area than in the well-connected Black Sea, this latter being particularly promising for the establishment of "a well-connected system of protected areas" according to Aichi Target 11 of the Convention on Biological diversity (Brooks, 2014).

DATA AVAILABILITY

The datasets for this study can be found in Dryad at https://doi.org/10.5061/dryad.4177rd5. Short read data are deposited in SRA with BioProject accession number PRJNA564770.

AUTHOR CONTRIBUTIONS

MPat and LZ conceived and designed the experiments, analyzed the data, and wrote the manuscript. MPat performed the experiments. LZ contributed reagents, materials, and analysis tools. All authors equally contributed in reviewing and approving the final version of the manuscript. LB, JS, AC, GG, CK, CP, VM, DM, NM, MPan, SP, VT, and EV contributed to the sampling and logistics.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00566/full#supplementary-material

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Ecosystem Models and Effort Simulations of an Untrawled Gulf in the Central Aegean Sea

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Ecosystem models can be used as fisheries management tools in the context of a holistic approach and view of assessing the status of aquatic ecosystems and proposing plans of action. The Ecopath with Ecosim modeling suite has been widely used to describe exploited marine systems and perform simulations over time. Pagasitikos Gulf is a shallow semi-enclosed gulf in the western coast of the central Aegean Sea that is characterized as semi-protected, with a bottom trawling ban in force since 1966. In this study, an Ecopath model was constructed including 31 functional groups (FGs) of organisms of lower to higher trophic levels, while Ecosim temporal simulations were run for 18 years (2008–2025), including the calibration period (2008–2017). An overall decrease in biomass and catch of the studied marine resources was observed by the end of the simulation period, due to environmental factors as well as fisheries exploitation. To examine the effect of fishing, three different scenarios were investigated, all aiming toward fishing effort reduction by 10, 30, and 50% compared to the initial business-as-usual scenario, applied to both fleets operating in the area (purse seiners and small-scale). All examined scenarios led to higher total biomass compared to the basic Ecosim simulation (the higher the reduction in fishing effort, the higher the increase in biomass), while catches were significantly lower in all cases as a result of less fishing. The most profound biomass increase with reduced fishing effort was observed in other larger pelagics, anchovy, anglerfish, sharks and rays, mackerels, hake and other gadiforms. In conclusion, reducing the exploitation levels of the ecosystem is a key factor that contributes to rebuilding of marine resources.

Keywords: ecosystem modeling approach, mediterranean fisheries, ecopath with ecosim, Pagasitikos gulf, fisheries management, fisheries regulations

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INTRODUCTION

Overexploitation of marine resources in the Mediterranean Sea in general and Greece in particular has long been identified and is well acknowledged, leading to the bad status of exploited fish and invertebrate populations and oftentimes resulting in collapsed stocks and economic loss (Tsikliras et al., 2015; Froese et al., 2018). Traditionally, the methods applied to assess the status and exploitation of marine stocks are single-species taking into account biological parameters and fishing mortality for each stock (Colloca et al., 2013), but failing to provide management insight in the context of the whole ecosystem, including non-target species, on their own (Pikitch et al., 2004). Putting fisheries management decisions into an ecosystem context demands shifting from traditional single-species stock assessments to more complex ecosystem models, which encompass multi-species interactions, environmental effects and human activities, and can therefore test the effect of different fisheries policies on the entire ecosystem, thus qualitatively facilitating management advice (Collie et al., 2016).

Ecopath ecosystem models (EwE: Ecopath with Ecosim[1]) provide a static, mass-balanced snapshot of the trophic flows and interrelationships, energy fluxes and food web structure of marine ecosystems, i.e., the species of a studied ecosystem and their trophic interactions (Christensen et al., 2005). They are used as a tool to analyze exploited aquatic systems while attempting to take into consideration all trophic levels included, from primary producers and lower invertebrates to top predatory species (Christensen and Walters, 2004). The Ecosim module of EwE is a time-dynamic simulation that models the impact of changes in fishing pressure and the environment on the ecosystem. It can be used to simulate the past (Halouani et al., 2016; Corrales et al., 2017a) or to run future simulations (Coll et al., 2013).

The EwE modeling approach is broadly used around the world, being applied to hundreds of ecosystems and counting more than 433 unique models globally, as listed and gathered in the EcoBase model repository (Colléter et al., 2015). The Mediterranean and Black Sea are among the areas with the highest proportion of studies, accounting for 9% (more than 40 models) of the total published models (Coll and Libralato, 2012; Colléter et al., 2015), which are mainly focused on the western (e.g., Coll et al., 2008, 2009a) and central (e.g., Coll et al., 2007, 2009b) Mediterranean; the eastern part of the basin being underrepresented with six models having been developed in Israel (Corrales et al., 2017b) and in Greece (Ionian Sea: Piroddi et al., 2010, 2011; Moutopoulos et al., 2013b; Piroddi et al., 2016; Aegean Sea: Tsagarakis et al., 2010).

Pagasitikos Gulf is located in the eastern Mediterranean Sea, particularly in the western coast of the central Aegean Sea, Greece (**Figure 1**). It is notable that Pagasitikos Gulf is characterized as a semi-protected area where fishing with towed gears, i.e., bottom trawling and boat-seining, has been banned since 1966 (Royal Decree 917/1966). The ecological and economic importance of the area is highlighted by its rich biodiversity, as well as its multi-species and multi-gear exploitation by numerous purse seiners, small-scale coastal vessels and recreational fishers, that has resulted in constantly decreasing catches since the second half of the 20th century (ELSTAT, 2017). This is in line with the overall declining trend in the eastern Mediterranean catches (Tsikliras et al., 2015) but despite the partial protection established for more than 50 years Pagasitikos Gulf.

In this work, a descriptive Ecopath mass-balance base model was developed for the first time in Pagasitikos Gulf (central Aegean Sea, Greece) aiming to describe the structure and functioning of a semi-enclosed and semi-protected ecosystem in terms of trophic flows and biomasses and to determine the ecological role of main species of interest. The temporal dimensions of this model were further extended with the time-dynamic Ecosim module of the EwE methodology, in order to set up and run temporal simulations aiming to quantify the ecosystem impacts of fishing and analyze the role of fishing activity in an area where towed gears have been absent for over 50 years. Fisheries management strategies were explored through fishing effort reduction scenarios and the potential benefits were outlined.

MATERIALS AND METHODS

Study Area

Pagasitikos Gulf is a semi-enclosed shallow gulf in the western coast of central Aegean Sea with a mean depth of 69 m and a maximum depth of 102 m (**Figure 1**). Its eastern part is more than 80 m deep with the sea bottom covered with sediments rich in silt but poor in clay, while its western part is less than 80 m deep with the sea bottom covered with sand and biogenic detritus. Pagasitikos Gulf is in contact with the waters of north Evoikos Gulf and the Aegean Sea through the channel of Trikeri, which is 6 km wide.

About ninety fish species, many of which have high commercial value, spawn in Pagasitikos Gulf (Caragitsou et al., 2001) and the vast majority of them (with the exception of large pelagic migratory fishes) spend their entire life cycle inside the Gulf as they have been collected all year round during surveys and across sizes and life stages (Caragitsou et al., 2001; EPAL, 2008). Pagasitikos is a semi-protected area as fishing with towed gears has been prohibited all year long since 1966. Purse seiners, targeting small and medium pelagics such as European anchovy (*Engraulis encrasicolus*), European pilchard (*Sardina pilchardus*), Atlantic mackerel (*Scomber scombrus*), Atlantic chub mackerel (*Scomber colias*), and numerous small-scale coastal vessels, mainly using nets, longlines and traps to target Norway lobster (*Nephrops norvegicus*), surmullet (*Mullus surmuletus*), red mullet (*Mullus barbatus*), European hake (*Merluccius merluccius*), common pandora (*Pagellus erythrinus*) and anglerfish (*Lophius* spp.) are active in the area (Stergiou et al., 2007). Recreational fishing is rather extensive (Moutopoulos et al., 2013a) and recreational fishers mainly target sparids (Family: Sparidae) and seabass (*Dicentrarchus labrax*). The total fisheries production in Pagasitikos Gulf was fluctuating around an average of about 2500 metric tons before 1982, it then dropped to an average of about 1000 metric tons with an ascending trend from the mid-80s until 2010, and has rapidly been decreasing since then with an average of about 470 metric tons of landed fish and invertebrates (ELSTAT, 2017).

Ecopath Modeling

For the description of the Pagasitikos Gulf ecosystem we used Ecopath with Ecosim (EwE: Christensen and Walters, 2004) to construct an Ecopath mass-balance base model. Ecopath models, either simpler or more complex, represent a static, mass-balanced snapshot of the studied ecosystem, i.e., the species inhabiting

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[1] www.ecopath.org

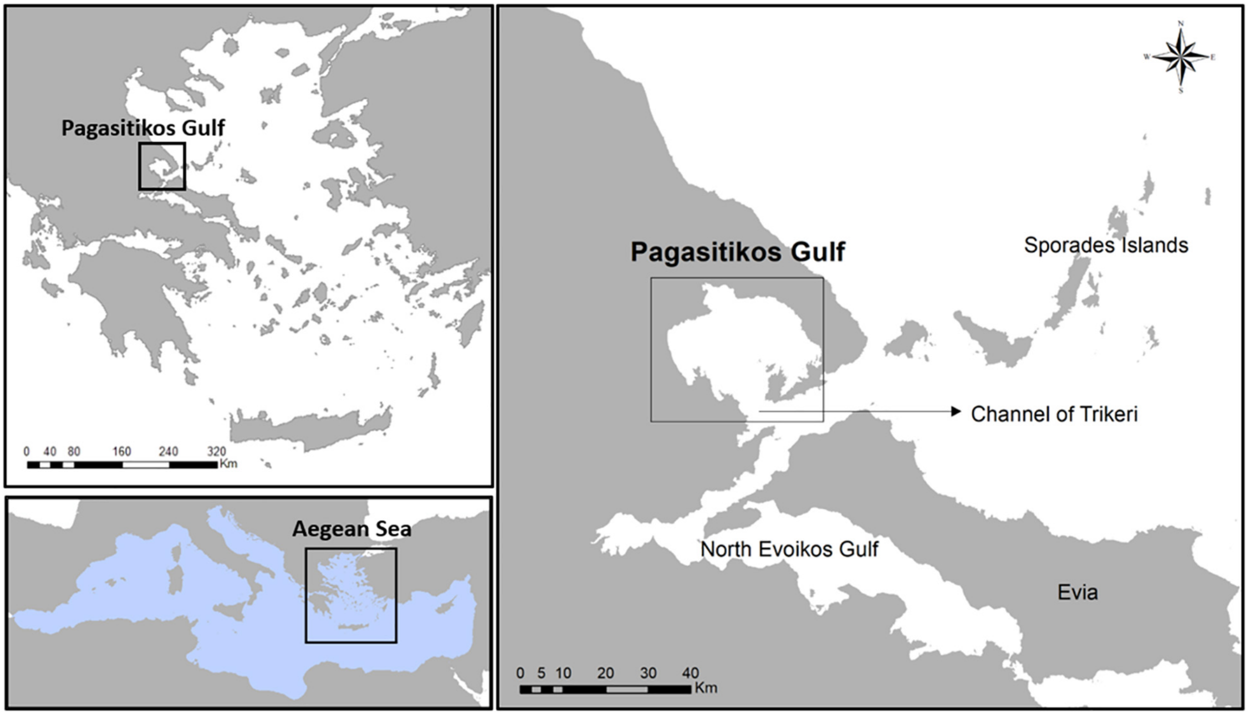

FIGURE 1 | Map showing the location of Pagasitikos Gulf in the central Aegean Sea, Greece, eastern Mediterranean Sea.

it and their trophic relationships. Overall, the EwE software package (see footnote 1) can be used in order to (a) address ecological questions, (b) evaluate ecosystem effects of fishing, (c) explore management policy options, (d) analyze impact and placement of marine protected areas, and (e) model effect of environmental changes.

Ecopath models are designed to describe a specific ecosystem which therefore needs to be explicitly defined by the modeler who sets the spatial boundaries, as well as the time period for the model and defines the functional groups (FGs) of organisms (Christensen et al., 2005). FGs (or ecological compartments) can be single-species, or groups of (ecologically or taxonomically) related species, i.e., species that share similar population dynamics and ecological function, or even size/age groups (stanzas). As Ecopath models are a useful tool for developing a holistic ecosystem approach to fisheries management, the species to be included are not only the commercially important ones but may belong to different trophic modes (Coll and Libralato, 2012), from lower to higher trophic levels, and can be primary producers, heterotrophs or facultative consumers, i.e., organisms which consume part of their food and photosynthesize the other part. Depending on the level of aggregation and therefore complexity of the developed model, studied ecosystems have been described by a minimum of 7 up to a maximum of 67 FGs (Colléter et al., 2015). At least one detritus group must be entered, and optionally discards can be entered as a specific detritus group. Also, the fishing fleet(s) that exploit the resources of the studied ecosystem must be defined in the model (Christensen et al., 2005).

Ecopath assumes mass-balance, i.e., that the energy input and output of all living groups are balanced, usually over a yearly time period, and bases the parameterization on two master equations, one to describe the production and another for the energy balance of each component in the ecosystem (Christensen et al., 2005):

Master equation 1: **Production** = predation mortality + fishery catches + biomass accumulation + net migration + other mortality

Master equation 2: **Consumption** = production + respiration + unassimilated food

The assumption of mass-balance requires that production from any of the groups should end somewhere else in the system while taking into consideration the basic physiological and thermodynamic constraints. Predation mortality is the parameter linking the groups with each other. When balancing the model to achieve mass-balance, one production equation is used for each of the FGs. The diet composition, biomass accumulation, net migration and fishery catches of each group must always be entered (**Table 1**). It is optional to enter any of the rest four parameters in **Table 1** (B, P/B, Q/B, EE), because the set of linear equations can be solved with one unknown value. Most of the times, based on the ease of estimation, EE is left to be estimated by the software (Christensen and Walters, 2004). Biomass accumulation is entered as rate (t/km^2/year) or relative to biomass if the data show change in biomass during the modeled year. Unassimilated food is a function of consumption,

TABLE 1 | Data requirements for Ecopath models.

Input parameter		Unit
B	Biomass	t/km^2
P/B	Production/biomass	year^{-1}
Q/B	Consumption/biomass	year^{-1}
EE	Ecotrophic efficiency = 1-other mortality	Proportion
Diets	Diet composition	Proportion
Catches	Landings + discards	t/km^2/year

production and respiration that represents physiologically non-useful urine and feces. In general, it takes a default value of 20% for carnivorous fishes, with the exception of herbivores and detritivores for which 40% is more appropriate (Winberg, 1956).

The model of Pagasitikos Gulf was constructed for the year 2008, when reliable empirical biomass data were available. The food web was described by 31 FGs, that encompassed groups of lower to higher trophic levels namely 2 planktonic, 8 invertebrate, 16 fish, and 2 detritus groups, as well as sea turtles, seabirds and dolphins (**Table 2**). The 31 FGs consisted of more than 120 taxa as recorded in survey and landings data and the literature. At first, the listed taxa formed 28 FGs based on their importance to fisheries and management, their phylogenetic or ecological relation and available data. But 51 fish taxa of lower fishing relevance and abundance in the ecosystem remained uncategorized. For 40 of those taxa, quantitative diet information, in the form of stomach content data, were available and were used to perform a cluster analysis (using the Ward's method and Euclidean distances in Statgraphics Centurion XVI) that resulted in forming 3 more FGs (Demersal fishes 1, 2, and 3). The remaining 11 fish taxa were assigned to one of those three FGs according to general knowledge of their feeding preferences, behavior and ecology. Pagasitikos Gulf is being exploited by two fishing fleets that were included in the model, namely purse seiners and small-scale coastal vessels.

Biomass data for fish and main invertebrate FGs (**Supplementary Table A1**) were obtained from local scientific trawling surveys (EPAL, 2008), while for the rest of the FGs the literature and other models were used; landings data were taken from the Hellenic Statistical Authority (ELSTAT, 2017) and were reconstructed based on the literature (Moutopoulos and Stergiou, 2012); diet compositions (**Supplementary Table A3**) were obtained from published reviews regarding fish in the Mediterranean (Stergiou and Karpouzi, 2002; Karachle and Stergiou, 2017); production and food consumption values were calculated using published empirical equations (Pauly, 1980) or the relevant life-history tools in FishBase (Froese and Pauly, 2019). The part of the life-history spent outside the study area was accounted for through the biomass value derived from seasonal biomass empirical data, as in the case of the other larger pelagics, or through import in the diet composition, as in the case of seabirds.

A set of statistics, that describe the studied ecosystem as a whole and can be used as measures to assess its status (Christensen et al., 2005), were included and presented along with other Mediterranean model results to allow for comparisons. The total system throughput represents the sums of all flows in the system, i.e., the total consumption, exports, respiratory flows and flows to detritus and serves as an important indicator of the size of the ecosystem in terms of flows (Ulanowicz, 1986). The system total primary production to total respiration ratio can be used to describe the state of maturity of an ecosystem (Odum, 1971) where immature systems, in their early developmental stages, have production that is expected to exceed respiration and thus the ratio is greater than 1. Fishing exploitation may lead the ecosystem to a less mature state, whereas prohibiting fishing with towed gears is likely to lead in change from disturbed to mature ecosystems in terms of bottom complexity, as well as benthos and fish species composition (Watling and Norse, 1998). The difference between primary production and respiration gives the net system production which is expected to be higher in immature systems and approximate zero in mature ones. Accordingly, the ratio of primary production to biomass declines over time in immature systems where production exceeds respiration for most FGs and biomass accumulation is observed. The system biomass to throughput ratio may take any positive value and it reaches a maximum when the system is at its most mature state. The omnivory index (Christensen and Pauly, 1992) indicates how the trophic interrelations are distributed among trophic levels and is therefore used to characterize the more or less extended web-like features of the studied system. A larger than zero value of the omnivory index suggests feeding on many trophic levels rather than specialization by feeding on just a single trophic level. The pedigree of an Ecopath input categorizes the origin of a given input (the type of data on which it is based), and specifies the likely uncertainty associated with the input, i.e., the reliability of the data (Morissette, 2007). These estimates were then utilized by the Monte Carlo routine to examine model sensitivity and assess the effect of the uncertainty in Ecopath input data on the Ecosim dynamic simulations (Christensen et al., 2005; Heymans et al., 2016).

A couple of network analyses were also performed, namely the mixed trophic impact (MTI) and keystoneness analyses. The MTI plot depicts the relative direct and indirect impact of a very small increase in the biomass of a group on the biomass of another group, thus revealing straight forward predator-prey effects but also indirect cascade effects on a prey's prey or competitor (Christensen et al., 2005). The keystone index is used to identify groups that have considerable impact and play an important role in the studied ecosystem either despite their low biomass (keystone groups) or as a result of their high biomass (dominant groups) (Libralato et al., 2006).

Ecosim Modeling

The Ecopath base model constructed for Pagasitikos Gulf was further used for temporal simulations. Ecosim inherits key initial parameters from the base model to provide temporal dynamic simulations of biomass through a differential equation that calculates the growth rate of an FG during a specific time interval based on the net growth efficiency, the consumption rate of a prey FG by a predatory FG, the immigration and emigration rates and the other natural and fishing mortality rates (Christensen et al., 2005).

TABLE 2 | Input and output (bold) parameters of the Pagasitikos Gulf Ecopath model.

								Landings	
	FG	**TL**	**B**	**P/B**	**Q/B**	**EE**	**P/Q**	**PS**	**SS**
1	Phytoplankton	1.00	6.071	117.3		**0.762**			
2	Zooplankton	2.22	4.98	59.49	177.5	**0.958**	0.335		
3	Benthic small crustaceans	2.21	**2.181**	7.32	54.4	0.870	0.135		
4	Polychaetes	2.10	**14.47**	1.63	12.46	0.920	0.131		
5	Shrimps	3.10	1.25	3.21	7.52	**0.990**	0.427		0.025
6	Crabs	2.96	1.1	2.44	4.94	**0.991**	0.494		0.004
7	Norway lobster	2.87	0.953	1.32	4.76	**0.995**	0.277		0.05
8	Benthic invertebrates	2.05	**25.35**	1.15	3.27	0.950	0.352		
9	Octopuses and cuttlefish	3.32	0.456	2.69	5.53	**0.997**	0.486		0.147
10	Squids	3.83	0.5	2.6	22.15	**0.991**	0.117	0.001	0.055
11	Red mullets	2.89	0.206	1.85	6.896	**0.996**	0.268		0.075
12	Anglerfish	4.29	0.422	1	3.6	**0.930**	0.278		0.102
13	Flatfishes	3.63	0.286	1.5	7.734	**0.992**	0.194	0.009	0.02
14	Other gadiforms	3.93	0.4	1.2	7.15	**0.983**	0.168	0.0005	0.005
15	Hake	4.16	1.157	0.7	3.5	**0.995**	0.200		0.161
16	Demersal fishes 1	3.19	1.747	1.5	8.173	**0.998**	0.184	0.002	0.073
17	Demersal fishes 2	3.79	2.9	1	5.6	**0.990**	0.179		0.0955
18	Demersal fishes 3	3.63	1	0.9	5.51	**0.995**	0.163	0.003	0.044
19	Picarels and bogue	3.22	1.878	1.6	8.237	**0.997**	0.194	0.004	0.07
20	Sharks and rays	4.38	0.454	1.414	3.146	**0.838**	0.449		0.042
21	Anchovy	3.22	3.11	1.642	6.533	**0.999**	0.251	0.67	0.393
22	Sardine	3.06	5.161	1.28	11.39	**0.996**	0.112	0.536	
23	Horse mackerels	3.41	0.354	1.1	6.43	**0.999**	0.171	0.063	0.064
24	Mackerels	3.70	0.33	1.122	6.004	**0.996**	0.187	0.063	0.035
25	Other small pelagics	3.18	1.2	1.205	6.081	**0.995**	0.198	0.021	0.102
26	Other larger pelagics	4.22	0.24	0.698	2.661	**0.982**	0.262	0.007	0.114
27	Loggerhead turtle	3.30	0.02	0.16	2.68	**0.781**	0.060		
28	Seabirds	2.31	0.001	4.78	111.6	**0.000**	0.043		
29	Dolphins	4.46	0.02	0.08	13.81	**0.213**	0.006		
30	Discards	1.00				**0.812**			
31	Detritus	1.00	31.44			**0.677**			

FG, functional group; TL, trophic level; B, biomass (t/km^2); P/B, production/biomass (yr^{-1}); Q/B, consumption/biomass (yr^{-1}); EE, ecotrophic efficiency; P/Q, production/consumption; Landings (t/km^2/year); PS, purse seiners; SS, small scale coastal vessels.

Consumption rates are calculated based on the "foraging arena" theory (Walters et al., 1997), the basic assumption of which is that aquatic organisms are divided in vulnerable and invulnerable to predation risk, as they largely limit predator-prey interactions to spatially restricted foraging arenas. The transfer rate between being vulnerable and invulnerable to predation determines if the biomass of different groups in the ecosystem is controlled by predators (top-down control, i.e., Lotka–Volterra dynamics: prey has no refuge to be protected and is always consumed when encountered by a predator), or preys (bottom-up control: prey is usually protected, by hiding in crevices for example, and becomes available to predators only when it leaves its refuge) or the control is of an intermediate type (Pauly and Christensen, 2002). The level of vulnerability represents the effect that an increase in predator biomass would have on the predation mortality of a given prey and it is an important parameter of the model that can be modified during calibration so that predictions fit better to observed historical data (Christensen et al., 2005).

Since there were no available complete time series of biomass data for the area, the Ecosim model developed for Pagasitikos Gulf was fitted to available historical landings data for the period 2008–2017 as obtained from the Hellenic Statistical Authority (ELSTAT, 2017) and reconstructed with the methodology used in Moutopoulos and Stergiou (2012) to include part of the small-scale coastal fleet and recreational fisheries catches that are excluded from official statistics (Moutopoulos et al., 2016). The recreational catches have not been properly monitored in the area and, apart from a short survey that was conducted on recreational fishing from shore based on questionnaires (Moutopoulos et al., 2013a), there is absolutely no information on their numbers and effort trends. Therefore, the fleet of small-scale coastal vessels is the one that included the scarce data on recreational catches, as the species targeted and many of the gears used are common (Moutopoulos et al., 2013a). Those were complemented with discards data that were estimated as a proportion of the landings for each fleet (Tsagarakis et al., 2014). Time series reference data over a specific historical period, along with estimates of changes in fishing effort by fishing gear type to drive the model over those years, facilitate producing a reasonable fit of the model to observed data (Christensen et al., 2005). Effort data by gear type for the two fleets (purse seiners and small-scale coastal vessels) were extracted from the European Community Fishing Fleet Register (CFR, 2018).

During the calibration of the model, the measure used to assess the goodness of fit was the reduction of the sum of squared deviations (SS) of observed values from predicted ones (Christensen et al., 2005). As in Coll et al. (2009a) and Halouani et al. (2016), the ''Fit to time series" module of Ecosim was used to find the 20 most sensitive to vulnerability changes prey-predator pairs and improve the fit of the model, with a vulnerability search executed to identify those values that would minimize the SS. In order to further minimize the SS, a forcing function (primary producer) was applied to represent a physical or other environmental parameter that might influence the trophic interactions among the components of the food web (Christensen et al., 2005). Primary production anomalies act upon the initial phytoplankton P/B values by adding annual modifiers every year, thus making it more realistic for the model projection (Coll et al., 2009a). The primary production anomaly identified in the model was correlated (Spearman's rank-order correlation test for non-normally distributed data) with the following environmental and climate time series data that have been reported to affect marine populations in the Mediterranean Sea (Tsikliras et al., 2019): sea surface temperature (Aqua-MODIS, 2019), the Atlantic Multidecadal Oscillation index (AMO, NOAA and Climate Prediction Centre, 2019a), the North Atlantic Oscillation index (NAO, NOAA and Climate Prediction Centre, 2019b), and the Mediterranean Oscillation index (MOI, Climatic Research Unit University of East Anglia [CRU-UEA], 2019). The fitting procedure was performed in seven steps as described in Mackinson et al. (2009) and also followed by Piroddi et al. (2016) and the best model with the lowest Akaike's information criterion (AIC) was chosen.

Biomass and catch projections were estimated up to 2025 and three scenarios of reduced fishing effort were examined in order to investigate the response of the studied ecosystem to alternative management schemes. Biomass Monte Carlo simulations for the year 2008 were tested against the projection year 2025 for statistically significant differences with the non-parametric Kolmogorov-Smirnov test (0.05 significance level) in Statgraphics Centurion XVI. Based on the work by Froese et al. (2018) that explores the effect of applying lower levels of fishing mortality on future biomass and catches, three scenarios of fishing effort reduction by 10% (Scenario 1), 30% (Scenario 2), and 50% (Scenario 3) were examined and compared to the baseline scenario 0 (business-as-usual). The fishing effort reduction referred to a reduction in the number of vessels operating in the area and was applied to all fleets equally (purse seiners and small-scale coastal vessels).

RESULTS

The Pagasitikos Gulf model was defined by 31 FGs covering the main trophic components of the ecosystem and including all the professional fishing activities operating in the area, as defined by two fleets (purse seiners and small-scale coastal vessels). The cluster analysis for the unassigned demersal fishes resulted in the formation of five distinct groups of fish species (**Figure 2**). However, because of the low biomass of the species in the fourth and fifth branch of the dendrogram, it was decided to merge branch 4 with branch 2, and branch 5 with branch 1. All in all, branch 1 and 5 formed the FG demersal fishes 1, branch 2 and 4 formed demersal fishes 2 and branch 3 formed demersal fishes 3 (**Figure 2**). The input and resulting output parameters of the balanced model are shown in **Table 2**, while the trophic linkages among the different compartments of the studied ecosystem are depicted in a flow diagram per trophic level and habitat (**Figure 3**).

The model was not initially balanced, so we modified the input parameters of the FGs with EE values greater than 1 (12 in total). The original biomass input data for shrimps, crabs, other gadiforms, mackerels and other small pelagics were unrealistically low and were increased by lowering the catchability factor of the trawler to account for the small shrimps and crabs that aren't caught by the gear as well as for the pelagic nature of the rest three FGs (**Supplementary Table A1**). For shrimps, crabs and other gadiforms, for which the changes were outside of the original range of uncertainty, we trusted more the biomasses of the predators obtained through the trawling surveys that were more focused on measuring fish, as well as the landings data. For flatfishes, hake, octopuses and cuttlefish, red mullets, demersal fishes 1 and 3, horse mackerels and discards we adjusted the diet matrix, since diet composition is the parameter with the highest plasticity (Piroddi et al., 2016). For example, the proportions of the aforementioned unbalanced FGs in their predator's diet were distributed so that consumption was directed toward other appropriate FGs such as anglerfish, demersal fishes 2, picarels and bogue, sharks and rays. Once the model was balanced, most of the FGs showed high EE values due to predation and fishing.

Statistics for the Pagasitikos Gulf ecosystem presented along with the NC Adriatic (Coll et al., 2007) and N Aegean (Tsagarakis et al., 2010) ecosystems for comparison purposes (**Table 3**), indicate a medium sized system in terms of flows and production, with a total system throughput and total production of about 3000 and 1100 t/km^2/year, respectively. The studied ecosystem was shown to be in a more mature stage than the NC Adriatic and N Aegean ones (**Table 3**), but was still characterized as immature, due to high system production, far from zero, exceeding respiration. The estimated omnivory index was higher for Pagasitikos Gulf, indicating more complex web-like trophic interactions among the ecosystem compartments. The model was typical in its uncertainty (**Supplementary Table A4**), with data of reasonable quality used for its construction, as implied by a pedigree index of 0.53. We chose specific models of nearby regions with similar model topology (in terms of number of FGs, aggregation across trophic levels, similar top

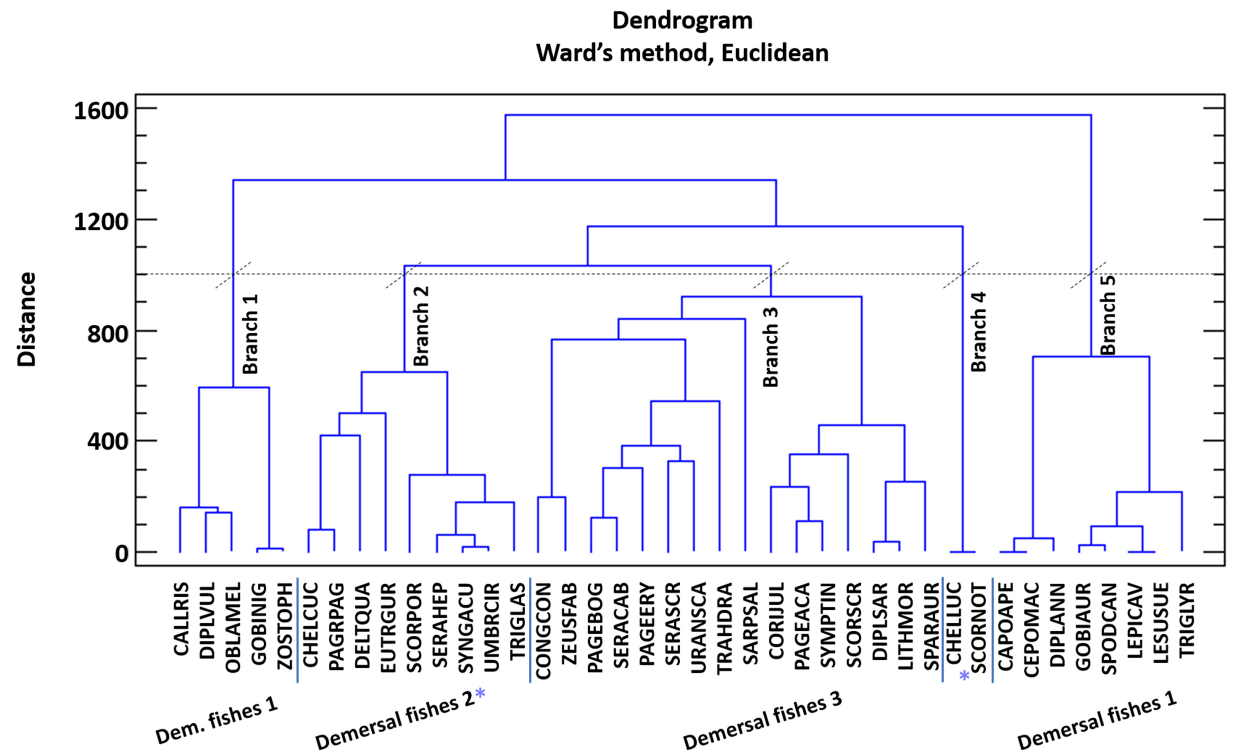

FIGURE 2 | Cluster analysis of the diet composition of 40 fish species for their categorization in functional groups. Species codes are given in **Supplementary Table A2**.

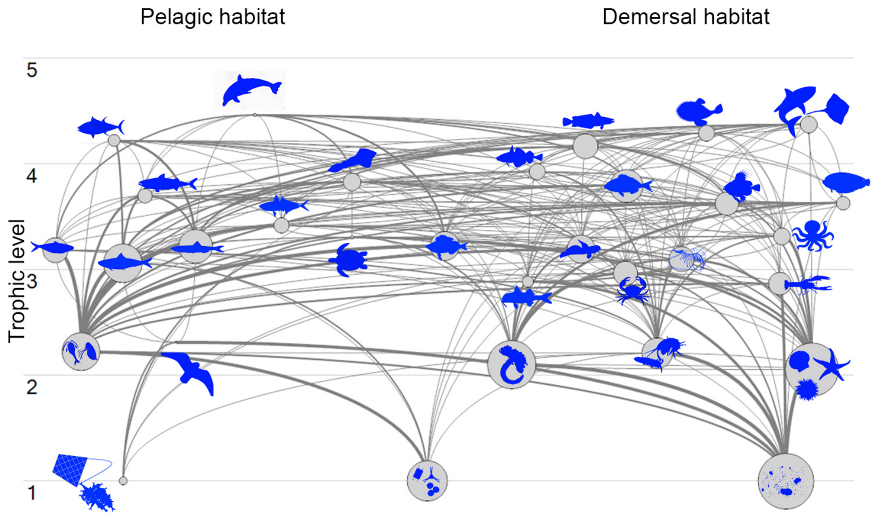

FIGURE 3 | Flow diagram of Pagasitikos Gulf organized by 31 functional groups' trophic levels and by pelagic or demersal habitat (organisms not in scale).

predator specifications, lack of microbial loop) and examined the indicators that are robust to model construction (Heymans et al., 2016). We acknowledge the varying exploitation level and difference in the nature of the system, but we chose to compare with Mediterranean ecosystems of some proximity than with models of ecosystems with completely different FGs and exploitation pattern.

According to the keystoneness graph (**Figure 4**), zooplankton and demersal fishes 2 were the dominant FGs as they had the highest relative total impact and keystone index in the studied ecosystem, however, they could not be characterized as keystone FGs due to their high biomass. On the other hand, squids and other gadiforms seemed to be more important to the survival of their shared ecosystem as their overall impact and keystoneness

TABLE 3 | Statistics, flows, and ecological indicators for Pagasitikos Gulf in comparison to models from other Mediterranean areas (NC Adriatic: Coll et al., 2007; N Aegean: Tsagarakis et al., 2010). Basic descriptive characteristics of the models are also provided.

	Pagasitikos	NC Adriatic	N Aegean	Units
Basic description				
No. of functional groups	31	40	40	
Time period	2008	1990s	2003–6	
State of exploitation	Semi-protected	High	High	
Nature of the system	Semi-enclosed	Shelf	Semi-closed	
Model area	639	55500	8374	km^2
Parameter				
Sum of all consumption	1456	1305	867	t/km^2/year
Sum of all exports	249	730	275	t/km^2/year
Sum of all respiratory flows	486	421	270	t/km^2/year
Sum of all flows into detritus	761	1388	563	t/km^2/year
Total system throughput	2951	3844	1976	t/km^2/year
Sum of all production	1114	1566	791	t/km^2/year
Calculated total net primary production	712	1150	536	t/km^2/year
Total primary production/total respiration	1.47	2.73	1.99	
Net system production	227	729	266	t/km^2/year
Total primary production/total biomass	9.1	8.82	16.21	
Total biomass/total throughput	0.03	0.03	0.02	/year
Total biomass (excluding detritus)	78	130	33	t/km^2
System omnivory index	0.25	0.19	0.18	
Ecopath pedigree index	0.53	0.66	0.61	

were high despite their smaller abundance in Pagasitikos Gulf. The loggerhead sea turtle and seabirds were shown to play the least important role in the studied ecosystem.

The MTI analysis (**Figure 5**) shows the relative direct and indirect impact that a hypothetical very small increase in the biomass of the impacting groups has on the biomass of the impacted groups, thus revealing indirect interactions between groups due to prey availability. Benthic invertebrates had the highest positive impact on octopuses and cuttlefish due to direct trophic interactions, while zooplankton had the highest negative impact on itself. Most groups had a negative impact on themselves, reflecting an increased within-group competition for resources. Predatory FGs, such as anglerfish and sharks and rays, were observed to negatively affect the groups they feed upon, like hake and anglerfish, respectively, while at the same time having a positive impact on their prey's food (squids and demersal fishes 3, and mackerels and horse mackerels, respectively). Regarding fisheries, out of the two fleets exploiting the studied ecosystem, small-scale fisheries had the strongest negative impact on different compartments of the ecosystem with the most pronounced impact on dolphins, loggerhead turtles, other larger pelagics, anglerfish and red mullets.

The model best fitting the observed landings time series data was the one yielding the lowest AICc value and explaining 86.7% of the variance of the data (**Table 4**). The best fit was obtained when trophic interactions, fishing and environmental parameters (in the form of primary production anomaly) were taken into account during the procedure. The combination of trophic relations and environmental drivers could explain most of the variability observed in the ecosystem (85.5%), whereas fishing alone contributed with 11.1%. Although the primary production anomaly resulted in the most profound improvement of the model fit, no significant correlation was found with available environmental and climate variability time series data (**Table 5**). A number of vulnerabilities were estimated by the time series fitting routine, with 20 trophic interactions, of mostly demersal organisms, giving the best improved result (**Table 4**). Eleven out of the twenty (55%) vulnerabilities were low (**Supplementary Table A5**), close to 1, indicating prey control (bottom-up) in the studied ecosystem, in which it is the physiological or behavioral factors of the prey that determine prey mortality rates rather than predator biomass (Christensen and Walters, 2004). The lowest vulnerabilities were estimated for the predator-prey interactions of zooplankton-phytoplankton (1.73), picarels and bogue-zooplankton (1.00), demersal fishes 1-polychaetes (1.09), benthic small crustaceans and polychaetes-benthic invertebrates (1.00 and 1.24, respectively), zooplankton-detritus (1.00).

The catches estimated by Ecosim showed an overall satisfactory match when compared with independent time series data, with some exceptions such as other gadiforms for which the predicted trend did not match the original catch trend (**Figure 6**). The results of the basic Ecosim simulation (scenario 0: business-as-usual) for biomasses and catches for 31 FGs of the Pagasitikos Gulf ecosystem highlighted overall persistent declining trends for many important ecological and commercial groups from 2010 up to 2017, when independent data were available, with a subsequent increase and a following stabilization in the projection years (**Figures 6**, **7** and **Table 6**). The aforementioned pattern up to 2017 was mainly driven by the primary production anomaly estimated during the catch time series fitting procedure, and resulted in the ecosystem balancing in an intermediate more stable state in the projection period. Both the total biomass and total catches were predicted to considerably decrease by the end of the simulation period in 2025, by 42 and 31%, respectively, while the biomass of only four predator groups (anglerfish, hake, sharks and rays, other larger pelagics) showed a marginal increase that varied from 2% to 10%, however, it did not result in a subsequent increase of the catches (**Table 6**). It should be noted that the marginal biomass increase of sharks and rays was not statistically significant. Commercially important FGs like anchovy and sardine showed a decrease in

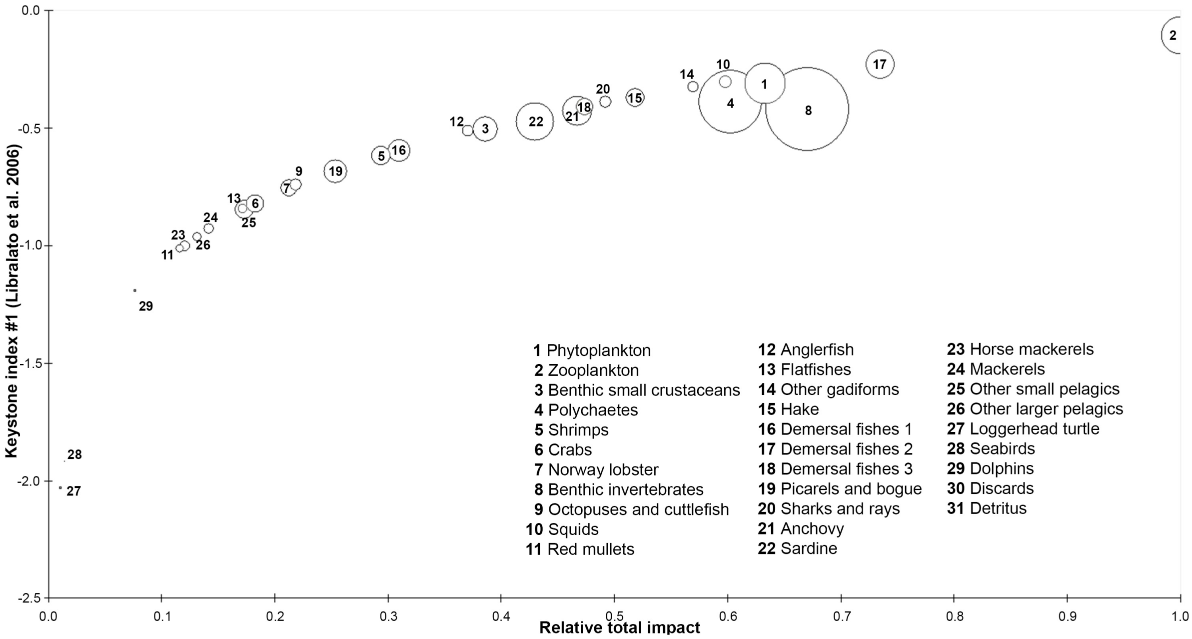

FIGURE 4 | Keystone index and relative total impact of each functional group of the Pagasitikos Gulf model. Circle size indicates the % relative biomass of each group.

biomass in the end of the simulation period, by 21 and 30%. Alongside them, FGs with intermediate consumers like other small pelagics, picarels and bogue and red mullets also had a significant decrease in biomass. Crustacean FGs with important economic and ecological value in the area, like Norway lobster and shrimps decreased in biomass by 27 and 34%, respectively, which subsequently led in a decrease in catches, by 37 and 43%, respectively (**Table 6**).

Sensitivity of Ecosim's outputs to Ecopath input parameters was tested with the Monte Carlo approach. Twenty Monte Carlo trials based on a coefficient of variation (CV) around the input parameters for biomass, P/B, Q/B, EE (**Supplementary Table A6**) gave 20 different outcomes for each FG, with flatfishes being presented here as an example (**Figure 8**). As noted by Steenbeek et al. (2018) – Supplementary File 3, about half of the Monte Carlo simulation trials are accepted and result in alternate mass-balanced Ecopath models that can then be used for Ecosim to run. In line with this, the Monte Carlo simulations failed when all of the parameters were perturbed. Hence, the most certain, according to the pedigree, input values for biomass, P/B and Q/B were not changed (CV = 0), while for the less certain ones, as well as those of FGs with high relative impact and keystoneness in the ecosystem (zooplankton, demersal fishes 2, squids, and other gadiforms), the CV was obtained from the quality of the data as defined in the pedigree routine (**Supplementary Table A4**). The CV used for EE was 0.1. The CVs ranged from 0.05 (which translates into a 10% change around the mean initial value of the parameter) to 0.4 (which translates into an 80% change around the mean initial value of the parameter). None of the trials resulted in a model with lower sum of squares than the baseline model (SS = 172). The best statistical fit out of the 20 runs (SS = 179) was lower for flatfishes than the best estimate based on the AICc (baseline) until 2011 and higher until 2019, with flatfishes biomass being initially underestimated and subsequently overestimated by the model (**Figure 8**).

As far as the examined scenarios of reduced fishing effort are concerned, all three of them resulted in higher total biomass compared to the basic Ecosim simulation (the higher the reduction in fishing effort, the higher the increase in biomass), while catches decreased as a result of less fishing effort (**Table 7**). Only the catches of other larger pelagics were predicted to increase in all three scenarios. The most profound biomass increase with reduced fishing effort was observed in the four predatory FGs (i.e., anglerfish, hake, sharks and rays and other larger pelagics), with other larger pelagics reaching a 114% biomass increase in Scenario 3 (**Figure 7** and **Table 7**). Alongside them, the loggerhead sea turtle, anchovy and mackerels increased by 6.1, 5.6, and 4.1%, respectively, in Scenario 1; by 23.4, 17.1, and 12.2%, respectively, in Scenario 2; and 42.8, 29, and 20.9%, respectively, in Scenario 3. The biomass increase of the abovementioned predatory FGs in the predicted scenarios, led to a subsequent decrease in the biomass of prey FGs, such as shrimps, crabs, Norway lobster, demersal fishes 1, picarels and bogue and sardine thus resulting in a total biomass increase in the entire ecosystem of 0.4% in Scenario 1, 1.1% in Scenario 2 and 1.9% in Scenario 3 (**Table 7**).

DISCUSSION

Shedding light on and understanding the particularities and variability of marine ecosystems to consequently be able to

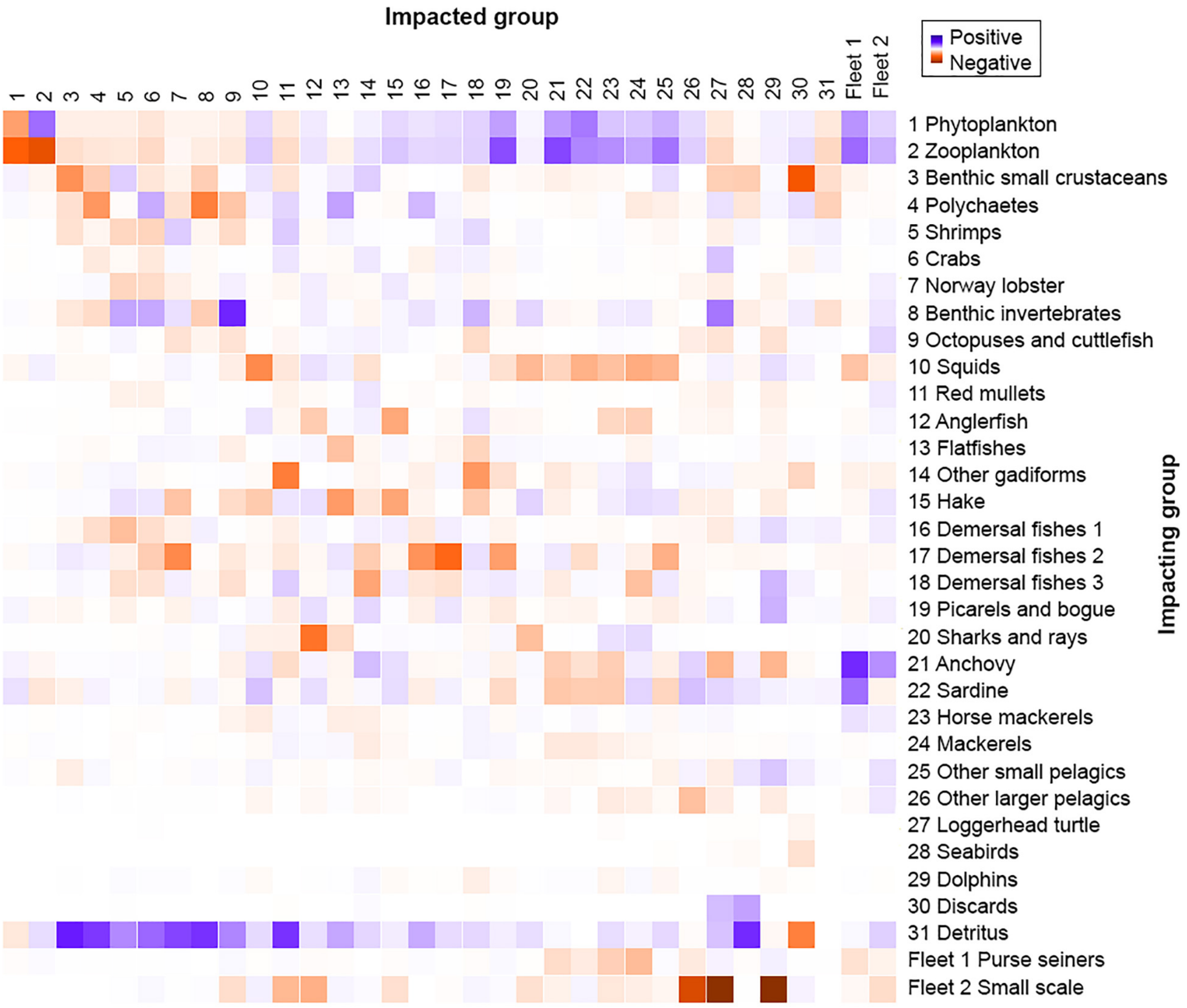

FIGURE 5 | Mixed trophic impact analysis of the Pagasitikos Gulf Ecopath model. Impact on and by the two fishing fleets is also shown (1–2).

TABLE 4 | Model fits according to the seven steps applied by Mackinson et al. (2009) including trophic interactions, fishery, and environmental drivers.

Steps		Vulnerabilities	Anomaly	Min SS	AICc	Improved (%)
1.	Baseline	0	0	417.7	144.7	
2.	Baseline and trophic interactions	30	0	417.7	215.2	−48.7
3.	Baseline and environment	0	6	224.9	27.74	80.8
4.	Baseline, trophic interactions and environment	24	6	164.9	20.9	85.5
5.	Fishery	0	0	386.8	128.6	11.1
6.	Trophic interactions and fishery	30	0	385.2	198.2	−36.9
7.	***Trophic interactions, environment and fishery***	***20***	***6***	***172.2***	***19.2***	***86.7***

The "best" model (shown in bold italics) was the one with the lowest AICc.

predict their future behavior plays a key role in the management of marine resources. The EwE model constructed for Pagasitikos Gulf utilizes at best the available biological and fisheries data to describe the food web structure and complex temporal dynamics of a semi-enclosed gulf in the Aegean Sea, Greece, thus adding to the modeled areas in the vicinity (Tsagarakis et al., 2010) and providing comparative ecosystem information for other coastal enclosed areas (Piroddi et al., 2016). We acknowledge that the lack of a complete biomass time series will add to the uncertainty of the model results, but we believe that ecosystem models

TABLE 5 | Spearman's rank-order correlations of the primary production anomaly with sea surface temperature (SST), the Atlantic Multidecadal Oscillation index (AMO), the North Atlantic Oscillation index (NAO), and the Mediterranean Oscillation index (MOI).

Variables	*n*	Spearman's rho	*p*-value
Anomaly and SST	10	0.09091	0.8114
Anomaly and AMO	10	−0.4303	0.218
Anomaly and NAO	10	0.07879	0.838
Anomaly and MOI	10	−0.4667	0.1782

are very helpful tools in fisheries data-poor areas where, apart from environmental forcing, fishing does remain an important driver of marine populations but only the catch composition and quantity time series are available. Semi-enclosed gulfs are special worth-studying systems, usually shallow and protected, concentrating significant urban and rural development that can disrupt ecosystem functioning due to nutrient overload (Petihakis et al., 2005) and since Pagasitikos Gulf has not been trawled for over 50 years what is evaluated here is the effect of less destructive fishing gears on marine populations and ecosystems. Pagasitikos Gulf is one of the least studied, in terms of fish and invertebrate abundance and population dynamics, marine ecosystems in Greece partly due to its exclusion from the MEDITS bottom trawl survey, which takes place every summer within the framework of the fisheries data collection program (Kallianiotis et al., 2004). Apart from the uncertainty arising from the lack of biomass time series, some uncertainty is associated with the input parameters used to balance the model, including consumption and production rates that were based on empirical equations.

The base model of Pagasitikos Gulf is of a medium-high quality (0.4–0.599) as expressed by its pedigree index of 0.53 which serves as a unique "quality footprint" (Morissette, 2007). The index allows for comparisons with other models even if those have been constructed with different number of trophic compartments (Christensen and Walters, 2004). The current model is shown to be of about the same quality as the model in Amvrakikos Gulf (Piroddi et al., 2016) and of lower quality compared to the ones in the NC Adriatic (Coll et al., 2007) and N Aegean Seas (Tsagarakis et al., 2010), mainly due to the production and consumption input values that were in many

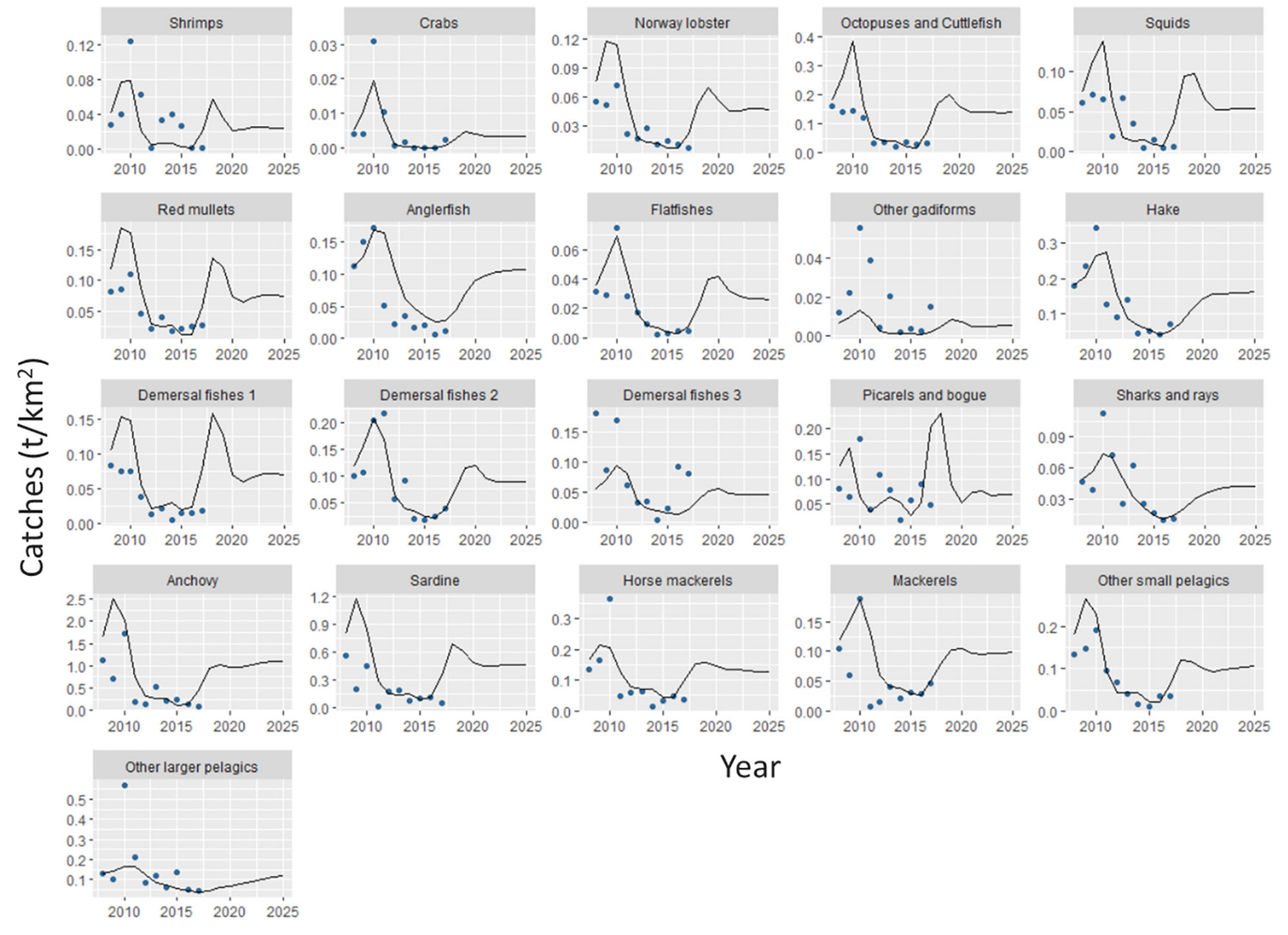

FIGURE 6 | Catches predicted by the Ecosim model (lines) for each functional group of Pagasitikos Gulf from 2008 to 2025, in comparison to reconstructed official catches (points) (based on Moutopoulos and Stergiou, 2012).

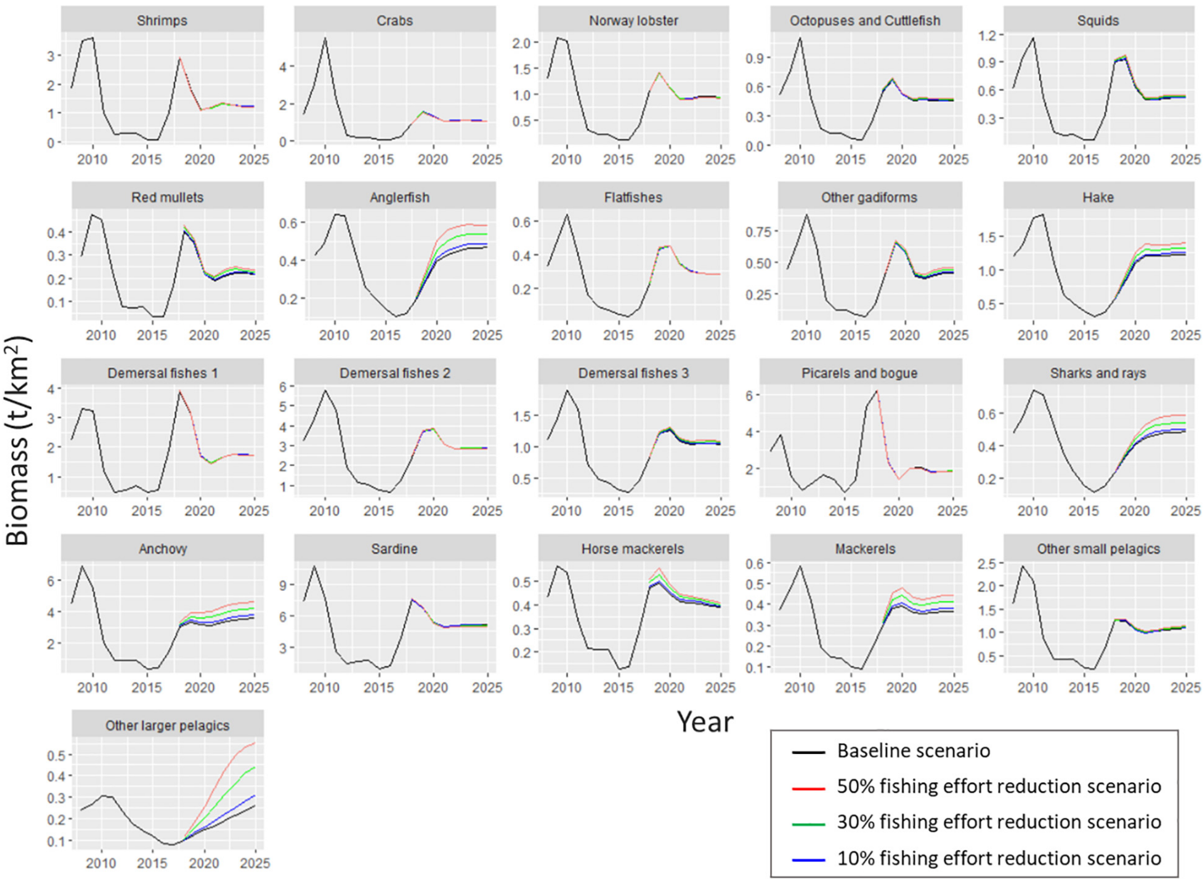

FIGURE 7 | Biomass predicted by the Ecosim model for each functional group of Pagasitikos Gulf from 2008 to 2025, according to the business as usual scenario (black line) and three scenarios of reduced fishing effort by 10% (blue), 30% (green), and 50% (red).

cases calculated from empirical relationships or taken from other models. Comparisons among Ecopath models require that the topology of the models is similar in terms of number of FGs, definition of primary producers and consumers, aggregation across trophic levels, top predator specification, presence or lack of microbial loop (Heymans et al., 2016), but also the level of fisheries exploitation and ecosystem characteristics are important. The Pagasitikos Gulf model was compared with two other models in the Mediterranean Sea that examined similar hypotheses acknowledging that some of the differences may be partly attributed to the inherent uncertainty in ecosystem models, differences in model topology and the different characteristics of the modeled areas.

According to the summary statistics that describe the studied ecosystem as a whole, Pagasitikos Gulf is shown to be an immature system with high system production, far from zero, exceeding respiration (Christensen et al., 2005) probably as a result of the intense fishing pressure exerted on stocks by purse seiners and coastal vessels. Although still high, Pagasitikos Gulf presents the lowest value for system production compared to the other models (NC Adriatic: Coll et al., 2007; N Aegean: Tsagarakis et al., 2010), something that could possibly be attributed to towed gears not operating in the area for over 50 years, as it has been shown that prohibiting fishing with towed gears is likely to lead in change from disturbed to mature ecosystems in terms of bottom complexity, as well as benthos and fish species composition (Watling and Norse, 1998).

The keystone species indicator revealed the high ecological importance of high trophic level organisms, such as other gadiforms and squids, which is indicative of an ecosystem less severely impacted by overfishing (Coll et al., 2009a). However, the absence or low biomass of marine mammals, reptiles, seabirds and sharks from the area shows that, even without trawling, the coastal areas of the Mediterranean are still suffering from historical overexploitation (Lotze et al., 2006), which has caused early food web changes by releasing prey from predation, and are dominated by medium demersal and pelagic fishes, medium and small sharks and rays (Coll et al., 2009a). The ecological importance of squids, which feed upon sardines and anchovies and are mainly responsible for consuming the largest proportion of exploited resources in Pagasitikos Gulf, has been previously highlighted in other models of the Mediterranean Sea (Adriatic Sea: Coll et al., 2007; N Aegean Sea: Tsagarakis et al., 2010).

The decreasing biomass of most FGs by the end of the simulation period (2025) in the baseline business-as-usual (Scenario 0) continues from the previous declining trend, is

TABLE 6 | Ecosim simulation results for Pagasitikos Gulf for the scenario 0: business-as-usual.

	FG	Bi2008	Bi2025	Bi 2025/2008	Ca2008	Ca2025	Ca 2025/2008
1	Phytoplankton	13.15 (12.75 – 13.83)	6.1 (6.09 – 6.1)*	0.46			
2	Zooplankton	8.96 (8.94 – 9.86)	4.94 (4.94 – 4.95)*	0.55			
3	Benthic small crustaceans	5.81 (4.98 – 6.81)	2.19 (1.73 – 2.61)*	0.38			
4	Polychaetes	23.22 (19.82 – 49.77)	14.48 (10.91 – 38.64)*	0.62			
5	Shrimps	1.86 (1.89 – 2.13)	1.23 (1.22 – 1.27)*	0.66	0.04	0.02	0.57
6	Crabs	1.42 (1.38 – 1.63)	1.07 (0.87 – 1.08)*	0.76	0.01	0.00	0.65
7	Norway lobster	1.3 (1.33 – 1.51)	0.94 (0.92 – 0.94)*	0.73	0.08	0.05	0.63
8	Benthic invertebrates	37.54 (28.42 – 62.56)	25.37 (18.1 – 45.52)*	0.68			
9	Octopuses and cuttlefish	0.51 (0.5 – 0.55)	0.46 (0.45 – 0.46)*	0.89	0.18	0.14	0.77
10	Squids	0.61 (0.61 – 0.66)	0.52 (0.52 – 0.52)*	0.85	0.07	0.05	0.73
11	Red mullets	0.29 (0.29 – 0.31)	0.21 (0.21 – 0.22)*	0.73	0.12	0.07	0.63
12	Anglerfish	0.43 (0.42 – 0.43)	0.47 (0.45 – 0.48)*	1.10	0.11	0.11	0.95
13	Flatfishes	0.33 (0.33 – 0.34)	0.28 (0.28 – 0.3)*	0.87	0.04	0.03	0.74
14	Other gadiforms	0.44 (0.45 – 0.47)	0.42 (0.39 – 0.41)*	0.94	0.01	0.01	0.8
15	Hake	1.19 (1.19 – 1.2)	1.23 (1.19 – 1.23)*	1.03	0.18	0.16	0.89
16	Demersal fishes 1	2.23 (2.22 – 2.3)	1.73 (1.72 – 1.83)*	0.78	0.10	0.07	0.67
17	Demersal fishes 2	3.24 (3.29 – 3.41)	2.87 (2.87 – 2.93)*	0.88	0.12	0.09	0.76
18	Demersal fishes 3	1.1 (1.11 – 1.13)	1.04 (1.02 – 1.04)*	0.94	0.06	0.05	0.81
19	Picarels and bogue	2.9 (2.9 – 3.08)	1.88 (1.88 – 1.9)*	0.65	0.13	0.07	0.56
20	Sharks and rays	0.48 (0.48 – 0.49)	0.49 (0.48 – 0.49)	1.02	0.05	0.04	0.87
21	Anchovy	4.52 (4.51 – 4.8)	3.59 (3.27 – 3.51)*	0.79	1.65	1.10	0.67
22	Sardine	7.3 (7.29 – 7.6)	5.13 (5.05 – 5.2)*	0.7	0.79	0.46	0.58
23	Horse mackerels	0.43 (0.43 – 0.45)	0.39 (0.39 – 0.4)*	0.9	0.17	0.13	0.76
24	Mackerels	0.37 (0.37 – 0.38)	0.37 (0.35 – 0.37)*	0.99	0.12	0.10	0.83
25	Other small pelagics	1.61 (1.61 – 1.67)	1.1 (1.19 – 1.45)*	0.68	0.18	0.11	0.59
26	Other larger pelagics	0.24 (0.24 – 0.24)	0.26 (0.24 – 0.28)*	1.07	0.13	0.12	0.92
27	Loggerhead turtle	0.02 (0.02 – 0.02)	0.02 (0.01 – 0.02)*	0.85	0.00	0.00	0.73
28	Seabirds	0 (0 – 0)	0 (0 – 0)*	0.44			
29	Dolphins	0.02 (0.02 – 0.02)	0.02 (0.02 – 0.02)*	0.82	0.00	0.00	0.71
30	Discards	0 (0 – 0)	0 (0 – 0)*	0.93			
31	Detritus	67.91 (67.25 – 71)	31.48 (31.38 – 31.48)*	0.46			
	TOTAL	189.46	110.27	0.58	4.33	2.97	0.69

FG, functional group. Biomass (Bi) and catch (Ca) values (t/km^2) and ratios at the starting year (2008) and the end of the simulation period (2025). Green represents an increase of biomass and catch in 2025 compared to 2008. The confidence intervals of the Monte Carlo simulations (5th and 95th percentile values) are given in parentheses. The significance in the difference of biomass between 2008 and 2025 (Kolmogorov-Smirnov test; at the 0.05 level) is indicated with an asterisk.

related to the ongoing fisheries exploitation pattern in the area and agrees with the general trends for those species in the Aegean Sea (Tsikliras et al., 2013; Froese et al., 2018). Anchovy and sardine that are exploited by purse seiners across Greek waters, account for the vast majority of landings in the northern Aegean Sea and Pagasitikos Gulf (Stergiou et al., 2007), with their abundance also related to climate forcing (Alheit et al., 2014; Tsikliras et al., 2019). Norway lobster is a prime catch of the coastal fleet (netters and potters) and because of its high commercial value it is exploited throughout the year in Pagasitikos Gulf. Netters and potters are heavily competing in the race for Norway lobsters and their intense rivalry has caused the decline in biomass (hence catches) and somatic length of the stock in the area (EPAL, 2008). The biomass decline of most targeted demersal stocks was the main trend of similar models in the South Catalan Sea (Coll et al., 2008) and the Adriatic Sea (Coll et al., 2009b) and was attributed mainly to fishing but also to climate/environmental forcing that degraded these ecosystems.

In the northeastern Ionian Sea (Piroddi et al., 2010) the decline of fish resources was mainly caused by the intensive fishing pressure that occurred in the area until the end of the 1990s and also by changes in primary production that impacted the trajectories of the main FGs. Although environmental drivers played an important role in the fitting of the Pagasitikos Gulf model to historical catch time series, as similarly observed and presented in the study of Alexander et al. (2015), the simulated primary production trajectory could not be correlated with available known climatic environmental drivers in the present study. It can be hypothesized that the primary production anomaly estimated by the model may encompass interactions of various types of primary producers or the microbial loop, compartments not explicitly included in the present model (Alexander et al., 2015). Also, as primary production dynamics are not shaped by a single environmental factor but rather by a combination of factors, it is possible that the identified anomaly does not represent well these dynamics in the studied

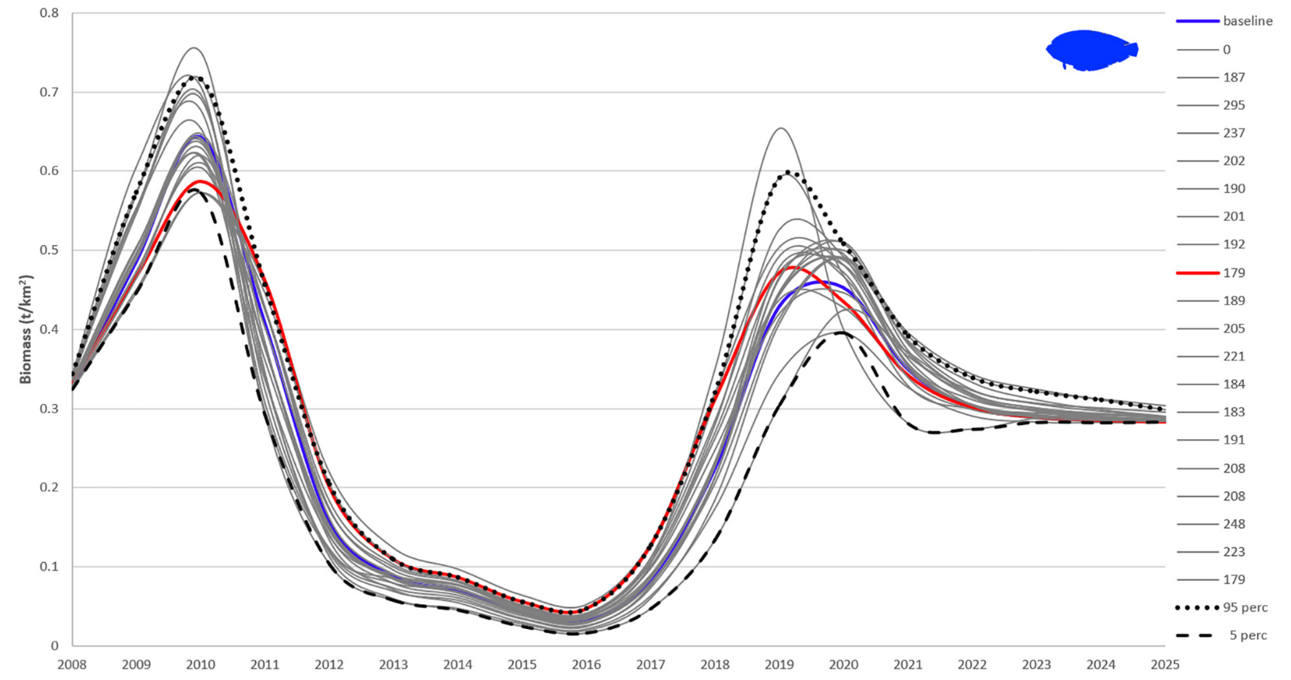

FIGURE 8 | Twenty Monte Carlo simulations of flatfishes biomass with best fitted Pagasitikos model. The blue line (baseline) is the final best fitted model based on the AICc (sum of squares of 172), red line is the model with least sum of squares (179; still higher than the baseline), gray lines are the other 19 fits with legend values representing the final sum of squares for each trial. The 95 and 5% percentile values are also plotted.

system. Salinity, river discharges or nutrients could also be playing a more important role locally and affect enclosed ecosystems, such as Pagasitikos Gulf, more compared to large-scale climatic oscillations such as AMO and NAO, but no time series data were available for those parameters in the study area. Indeed, as analyzed in Christensen et al. (2005), the process of estimating values of a primary production forcing function for the environmental anomalies in the studied ecosystem entails an inherent risk of obtaining a spurious temporal pattern that might not represent any real forcing. However, what one can say is "assuming that primary production was in fact variable and that this did cause changes in relative abundance throughout the food web, then our best estimate of the historical pattern of variation is the one obtained by the fitting procedure" (Christensen et al., 2005).

Measures to reduce overfishing and illegal fishing activities are needed together with the establishment of marine protected areas that will ensure prey survival required to sustain marine predators (Piroddi et al., 2010). In the N Aegean Sea (Tsagarakis et al., 2010), the five artisanal and industrial fishing fleets operating in the area had high impact on vulnerable species and numerous targeted groups while several exploitation indices highlighted that the ecosystem was highly exploited and unlikely to be sustainably fished. In Pagasitikos Gulf it appears that the small-scale coastal fisheries have a stronger negative impact on different FGs of the ecosystem (the impacted groups included target species such as anglerfish and red mullets, but also marine mammals and reptiles) compared to purse seiners that target only small and medium pelagic fishes. Indeed, despite the higher overall contribution of the purse-seining fleet to the national landings compared to all other gears (Stergiou et al., 2007), in Pagasitikos Gulf the catches of the small-scale fleet (1.676 t/km^2/year) exceed those of purse seiners (1.380 t/km^2/year) highlighting the impact of the small-scale fisheries on the ecosystem.

A marginal increase in biomass was observed in four top predator FGs (anglerfish, hake, sharks and rays, and other larger pelagics); however, the difference for sharks and rays was not statistically significant. Anglerfish and hake are targeted by coastal vessels using nets, while sharks (many large sharks are protected and absent from Pagasitikos Gulf) and rays are usually part of the by-catch, as in many areas of the world (Molina and Cooke, 2012) and discarded. However, the predation upon those high trophic level FGs in the area is minimum and the incorporation of trophic interrelations in the model besides fishing pressure (Heymans et al., 2016) may explain their biomass increase in Scenario 0.

All models are simplifications of reality that have an inherent level of uncertainty related to the quality of the input data and should therefore be treated and analyzed accordingly (Steenbeek et al., 2018). As EwE model predictions are generally more sensitive to biomass and production rate input data (Essington, 2007), the lack of a time series of biomass data and the reliance only on catch data in this work increases uncertainty and may limit confidence to model results. However, despite their uncertainty, ecosystem models together with some data-limited approaches that require only catch data (e.g., CMSY: Froese et al., 2018) can be used to evaluate stock status and the effect

TABLE 7 | Ecosim simulation results for Pagasitikos Gulf for three scenarios of fishing effort reduction by 10, 30, and 50% compared to the business-as-usual scenario.

	10%		**30%**		**50%**	
FG	**Bi**	**Ca**	**Bi**	**Ca**	**Bi**	**Ca**
1 Phytoplankton	1.002		1.006		1.010	
2 Zooplankton	0.997		0.991		0.984	
3 Benthic small crustaceans	1.000		1.001		1.002	
4 Polychaetes	1.003		1.008		1.014	
5 Shrimps	1.000	0.92	0.998	0.72	0.998	0.51
6 Crabs	0.997	0.92	0.987	0.71	0.978	0.50
7 Norway lobster	0.996	0.92	0.988	0.71	0.981	0.50
8 Benthic invertebrates	1.001		1.002		1.004	
9 Octopuses and cuttlefish	1.008	0.93	1.029	0.74	1.049	0.54
10 Squids	1.009	0.93	1.027	0.74	1.054	0.54
11 Red mullets	1.016	0.94	1.056	0.76	1.091	0.56
12 Anglerfish	1.044	0.96	1.146	0.82	1.250	0.64
13 Flatfishes	0.999	0.91	0.997	0.71	0.996	0.51
14 Other gadiforms	1.018	0.94	1.055	0.75	1.098	0.56
15 Hake	1.024	0.94	1.079	0.77	1.142	0.58
16 Demersal fishes 1	0.998	0.92	0.994	0.71	0.990	0.51
17 Demersal fishes 2	0.998	0.92	0.995	0.71	0.991	0.51
18 Demersal fishes 3	1.010	0.93	1.030	0.74	1.051	0.54
19 Picarels and bogue	0.997	0.92	0.992	0.71	0.985	0.50
20 Sharks and rays	1.035	0.95	1.121	0.80	1.211	0.62
21 Anchovy	1.056	0.96	1.171	0.83	1.290	0.65
22 Sardine	0.994	0.89	0.978	0.68	0.962	0.48
23 Horse mackerels	1.011	0.92	1.031	0.73	1.056	0.53
24 Mackerels	1.041	0.95	1.122	0.79	1.209	0.61
25 Other small pelagics	1.001	0.92	1.014	0.72	1.028	0.52
26 Other larger pelagics	1.197	1.10	1.704	1.22	2.141	1.09
27 Loggerhead turtle	1.061	0.98	1.234	0.88	1.428	0.73
28 Seabirds	1.007		1.023		1.040	
29 Dolphins	1.010	0.93	1.037	0.74	1.064	0.54
30 Discards	1.000		1.000		1.000	
31 Detritus	1.001		1.002		1.004	
TOTAL	1.004	0.94	1.011	0.79	1.019	0.60

FG, functional group. Biomass (Bi) and catch (Ca) ratios at the end of the simulation period (2025). Green represents higher (ratio > 1) biomass or catch.

of fishing on marine populations in data-poor areas, such as Pagasitikos Gulf, where the lack of biomass and CPUE time series will not allow for the assessment through age based or surplus production models. The Monte Carlo routine in EwE assessed the sensitivity of the Ecosim outputs to the underlying Ecopath input parameters and provided a useful image of the range of possible outputs based on the uncertainty around the input data (Heymans et al., 2016) as shown by the 5th and 95th percentile values plotted (**Figure 8**), thus giving a better understanding of the reliability of the model predictions. Despite the several alternative outputs that may overestimate or underestimate FG abundance, the chosen model was shown to be the best statistical fit with the lowest sum of squares.

The results of Ecosim simulations with decreasing fishing effort comply with the general and common sense rule that less biomass removal by fishing will eventually lead to biomass increase in the sea and stock rebuilding (Froese et al., 2018). It is not argued that the future projections provide absolute quantitative information, but rather an idea of the ecosystem status relative to the past conditions. In the absence of the primary production anomaly in future projections (Heymans et al., 2016), the fishing scenarios indicate the direction of change that is related to fishing and a relative magnitude of this change. Nevertheless, given the contribution of the primary production anomaly in fitting the model to the data during the calibration period, the results regarding the importance of fishing may be modified by environmental factors.

Indeed, the biomass of most FGs that are targeted by both fleets operating in Pagasitikos Gulf increased in all scenarios and the increase was higher for top predators that are not preyed upon in the area and lower for medium to low trophic FGs that are preys for both natural predators and anthropogenic activities. The peculiarity of the lack of bottom trawling in the area complicates the comparison with other ecosystems where trawling is the main biomass removing method and has the highest impact on marine populations and ecosystems (Coll et al., 2007; Hattab et al., 2013). In any case, the main output of no-fishing or decreased fishing effort scenarios in all models always leads to higher biomass of targeted species and decrease in catches. In the South Catalan Sea (Coll et al., 2009a), the no-fishing scenario resulted in biomass increase of higher trophic levels whereas the trophic level of the same groups was substantially lower in exploited food webs. In the northern Gulf of Mexico (Geers et al., 2016), any increase in fishing effort would result in biomass declines and increase of catches leading the ecosystem to immaturity, whereas the decrease in effort resulted in slight increases in overall biomass and substantial decreases in catches. In the coast of Israel (Corrales et al., 2018), fishing effort reductions resulted in significant increasing trends for most ecological indicators including total biomass, invertebrate biomass, predatory biomass and total catch but the trends for individual FGs were mixed because of their interactions and climate effects. There are cases, however, of enclosed areas that are heavily affected by environmental forcing and the impact of fishing is moderate to minor. For example, in Amvrakikos Gulf, which is another semi-enclosed embayment in the Mediterranean Sea sharing morphological similarities with Pagasitikos Gulf, the exact same pattern of how much fishing or the environment improve the fit during the fitting procedure was observed: it was the combination of environmental drivers (mainly riverine input) and trophic interactions that explained the majority of ecosystem variability, with fishing marginally contributing, leading to a degradation of the demersal parts of the food web and a relative stability of the pelagic ones (Piroddi et al., 2016). Similar results have been published in other models (e.g., Coll et al., 2009b, Adriatic Sea) but the effect of each combination to the fitting of the model differs owing to differences in ecosystems, environmental drivers and fishing pressure (Alexander et al., 2015).

The number of vessels has been gradually declining in Greece during the last decade as a result of a retirement of vessels and fishers due to ageing and to a lesser extent as a side effect of the economic crisis that has led to stricter taxation of the fishers (Machias et al., 2016). Despite the retirement of the fleet, the actual fishing effort has remained unchanged if not increased, following the global trend (Anticamara et al., 2011), but also due to technological creep (Marchal et al., 2006). Therefore, although these scenarios may reflect the future of the fishing fleet in terms of numbers, they cannot encompass the true dynamics of the fleet and fisheries in Pagasitikos Gulf and Greek Seas in general. For that reason, in some cases, fishing effort restrictions, the main fisheries management enforcement tool in the Greek Seas (Stergiou et al., 2016), should be complemented with spatial effort closures in essential fish habitats. Spatial effort constraints through the establishment of marine protected areas or spatial fisheries restrictions (Dimarchopoulou et al., 2018) may also have beneficial results in terms of biomass increase and ecosystem function (Fouzai et al., 2012; Abdou et al., 2016).

With the present study, we confirm that stock biomass is shown to increase when fishing effort is reduced, as in all fishing pressure reduction scenarios, the biomass of the FGs increased proportionally to the magnitude of reduction and the catches decreased accordingly. Consequently, in the absence of quotas in the Mediterranean Sea, effort control is the main management tool (Stergiou et al., 2016). Indeed, all recent stock assessments in the Mediterranean Sea suggest that the bad status of most stocks and their declining catches are the results of excessive fishing and clearly suggest that a decrease in fishing mortality to MSY levels is required for the stocks to rebuild (Colloca et al., 2013; Froese et al., 2018). Addressing the negative effects of overfishing through taking measures on reducing exploitation levels has been shown to not only rebuild stocks but also lead to higher catches over time, with considerably higher profits for the fishers in the medium term (Froese et al., 2018). Nevertheless, the urgency of modifying the current behavior of overfishing is pointed out by the fact that the reflection of rebuilding on catches, and thus income for the fishers, takes longer than rebuilding itself. Also, the trophic interactions among and within FGs will not allow biomass to be maximized at the same time for all ecosystem components, while environmental drivers should also be carefully considered, especially in enclosed ecosystems. In any case, effort reduction is the very first step toward sustainability once biomass declines as a result of excessive effort have been noticed.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.statistics.gr/en/statistics/-/publication/SPA03/-.

AUTHOR CONTRIBUTIONS

DD collected the data, created the ECOPATH model, performed ECOSIM simulations, and wrote the manuscript. IK prepared the ECOSIM model. KT created the ECOPATH model, performed ECOSIM simulations, and drafted the manuscript. AT wrote the manuscript.

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Genetics Structure Analysis of Turbot (*Scophthalmus maximus*, Linnaeus, 1758) in the Black and Mediterranean Seas for Application of Innovative Management Strategies

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INTRODUCTION

The turbot, *Scophthalmu maximus,* is a commercially important flatfish and has remarkable attention with respect to fisheries and aquaculture (Iyengar et al., 1999) from Northeast Atlantic to the Arctic Circle and commonly found in the Baltic and some part of north Mediterranean including the Marmara and the Black Sea, but the Aegean Sea (Turan et al., 2007). *S. maximus* has restricted spatial migrations and live at depth of 20 up to 100 m, predate on the small fish, crustaceans and other benthic marine animals (Karapetkova, 1980; Ivanov and Beverton, 1985). The turbot is considered as vulnerable (VU) under the current IUCN Red List criteria (IUCN, 2019). The natural populations of turbot are subject to strong anthropogenic pressure.

The species in the Black Sea is under catch quota following the multiannual management plan for turbot fisheries in the Black Sea. A total allowable catch (TAC) is set at 644 tons for 2018–2019, and each authorized vessel shall not exceed a maximum number of 180 fishing days per year. In addition, the status of the turbot stock must be regularly assessed and the level of current fishing mortality established. Hence, the implementation of a TAC, assuming a 100% curb of IUU fishing, would allow the stock to quickly recover to large biomass values with a relatively low probability of SSB falling below B_{LIM} (11.6% in 2030). These results provide grounds for the continuation of fishing activities at levels that are acceptable for both the population and the fishery, provided the adequate management measures are adopted (FAO, 2018). The total turbot catches in the Black Sea accounted for 661, 1,444 tons IUU included (GFCM, 2018), the prevailing landings belong to Russia and Turkey for 2016. Despite its economic importance and wide range of distribution, limited literature is available on the population structure of *S. maximus* using molecular markers (Atanassov et al., 2011; Nikolov et al., 2015).

Over-exploitation of this resource is a factor for the complete disappearance of local fish populations worldwide (Dulvy et al., 2003). Therefore, globally increased level of exploitation of fisheries resources requires urgent measures to establish the impact on the genetic diversity and population genetic structure of commercial populations in order to improve the strategy for long-term management, which would ensure the conservation of fisheries resources (Karahana et al., 2014).

The genetic tools deliver significant advances for fisheries management in the short term that the determination of fishery stock structure is important for defining stock boundaries to underpin sustainable fishery management that is one of the priority areas relevant for fisheries management under the E.U. Common Fisheries (Ovenden et al., 2015). The suggestions mentioned above are based on the immediate needs emerging from the CFP regulation (EC, 2013) and genetic approaches that appear to be feasible, practicable and cost-efficient (Casey et al., 2016).

Microsatellite markers have been determined in a large variety of fish species and are commonly used in population genetic studies (Iyengar et al., 2000). Recently, microsatellite markers have progressively been used to get knowledge on population structure and interaction between populations of a given species (Chen et al., 2017). Due to its maternal inheritance mode and relative lack of recombination, mitochondrial DNA symbolizes a useful marker system for use in population genetic studies (Hurst et al., 1999).

Numerous studies of the genetic structure of turbot have been conducted on North-eastern Atlantic and western Mediterranean countries (Pardo et al., 2005), however, there has been limited study conducted on genetic structure of that species in the Black Sea. Therefore, there is a need for elucidation of the population genetic structure of turbot to find appropriate management strategies for Black Sea countries.

Therefore, the aim of this study is to elucidate population genetic structure of turbot (*S. maximus*) from the Black and Marmara Seas and to implement it into management strategies as a key element for rational exploitation and conservation of fish populations.

MATERIALS AND METHODS

Sample Collection

S. maximus samples (fin clips) from 50 individuals in total (10 from each site) were collected at fishing ports (commercial landings) from Turkish marine waters [Trabzon (TRB), Duzce (DUZ), and Marmara Sea (MAR)], from Bulgaria-Varna coastal waters (VAR) and from Russia Sevastopol coastal waters (SVT) between 2013 and 2015 (**Figure 1**). The samples were taken to the laboratory and frozen at −30°C until DNA extraction.

DNA Extraction

The total DNA was isolated by using a phenol–chloroform method (Sambrook et al., 1989). After DNA extractions, DNA product was visualized in 0.7% agarose gels and quantitation of the DNA was completed using a spectrophotometer.

Microsatellites

Gel Analyzer 2010a (1-D Gel Analysis) software was applied to score alleles visualized by electrophoresis. Five microsatellite primers originally developed for *S. maximus* (**Table 1**) were applied after the optimization and determination of polymorphisms. A Polymerase Chain Reaction (PCR) was carried out using reaction volume of 15 µl including 1 U Taq polymerase (Thermo scientific), 2 µM of each primer, 200 mM dNTPs, 25 mM $MgCl_2$, 10 mM Tris-HCI pH 8.8, 50 mM KCl, and 1 µl template DNA (≈10–25 ng). It was performed with two stages: pre-denaturation at 95°C for 1 min followed by five denaturation cycles at 94°C for 20 s, annealing at 55 s, extension at 72°C for 2 s that was followed by the second stage of 25 denaturation cycles in 20 s at 94°C and final extension in 20 s at 72°C.

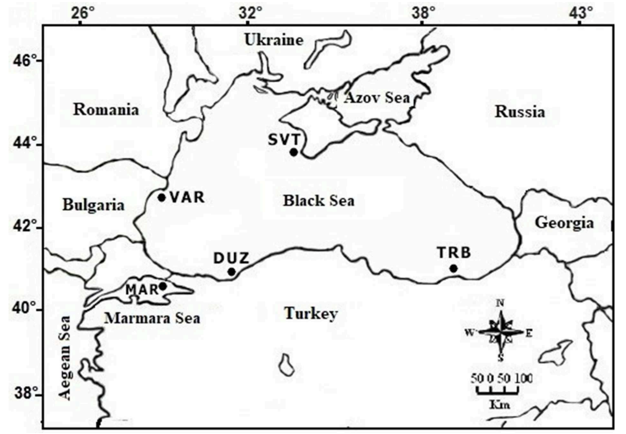

FIGURE 1 | Locations of *S. maximus* sampling sites: TRB (Trabzon coast); DUZ (Duzce coast); VAR (Bulgarian Varna coast); SVT (Russian Sevastopol coast); MAR (the Marmara Sea coast).

Statistical Analyses

Deviations of Hardy–Weinberg genotypic distributions (HWE), observed (HO), and expected (HE) heterozygosities were analyzed with Arlequin *v*3.5.1.3 (Excoffier and Lischer, 2010) to test for excess heterozygosity and deficiency of microsatellite data. A stepwise mutational model based on allelic identity (F_{ST}) was used to elucidate genetic differentiation between populations while the estimation of allelic size (R_{ST}) is less reliable than F_{ST} when <20 microsatellite loci are used (Gaggiotti et al., 1999). The Sequential Bonferroni technique was applied for detected significance levels with multiple tests (Rice, 1989). The Garza–Williamson Index (GWI), which measures the ratio of the observed number of alleles relative to the total number of possible allelic states at the locus over the allelic range, was used to detect bottlenecks (Garza and Williamson, 2001). PCAGEN, SPSS, and SYSTAT software were used for Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) of microsatellite data. A neighbor-joining dendrogram (Saitou and Nei, 1987) was constructed to reveal genetic relationships among the populations (Cavalli-Sforza and Edwards, 1967) with PHYLIP 3.57 (Felsenstein, 1989). Pairwise genetic distance matrices (FST) and geographic distance (km) were tested whether gene flow was geographically restricted and followed the isolation-by-distance model using the Mantel test (Mantel, 1967).

mtDNA Sequence Analysis

The complete mtDNA COIII gene region was amplified via PCR reactions, which was carried out according to the protocol given

TABLE 1 | Set of primers and amplification conditions for the 5th microsatellite loci and universal COIII primers of mtDNA.

Name	Primer	Core sequence	Annealing t°C	References
COIII-F	5′-AGC CCA TGA CCT TTA ACA GG-3′		49°C	Valles-Jiménez, 2005
COIII-R	5′-GAC TAC ATC AAC AAA ATG TCA GTA TCA-3′			
Smax-02F	GGAGGATGTATTGAAAGTGT	$(TG)_{16}$	56°C	Bouza et al., 2002
Smax-02R	AGAGCAGGTCATTATACAGC			
Sma1-125INRA F	CACACCTGACAAAGCTCAAC			Estoup et al., 1998
Sma1-125INRA R	GCTGAACATTTTCATGTTGATAG	(TAGA)11-(TG)4	58°C	
Sma3-12INRA F	CACAATTGAATCACGAGATG	(TG)21	58°C	Estoup et al., 1998
Sma3-12INRA R	GCCACCACTGCGTAACAC			
B12-I GT14 F	GTGATGGAAGATTGTACCAG	(GT)14	56°C	Iyengar et al., 2000
B12-I GT14 R	CACAATAAAGGATAGACCAG			
3/9CA15 F	AGAGTGAAGAACGTACCTGC	(CA)15 60°C		Iyengar et al., 2000
3/9CA15R	CAATGGAGAGGCAGTATCGG			

by Turan et al. (2019). The mitochondrial cytochrome c oxidase subunit III (COIII) gene was amplified using the universal primers (**Table 1**). The mtDNA sequence analysis was performed according to Sanger et al. (1977) on ABI 3130 XL genetic analyzer.

Statistical Analyses

After sequence alignment, the best model for sequence divergences were calculated using Mega v5, and the molecular phylogenetic tree was also constructed using MEGA v5 (Tamura et al., 2011). Neighbor joining (NJ) tree (Saitou and Nei, 1987) was applied to visualize relationship among the populations. The statistical robustness in the nodes of the resulting tree was determined by 1,000 bootstrap replicates. Tajima's D test (Tajima, 1989) was carried out to tests the conformity of DNA sequence evolution to neutrality. The sequences have been deposited in the GenBank with accession numbers, MN062299—MN062348.

RESULTS

Microsatellite

In the microsatellite analysis, a total of 108 alleles were detected from five microsatellite loci and the allele numbers for each microsatellite locus within the *S. maximus* populations ranged from 3 to 14. The expected and observed heterozygosity in *S. maximus* populations varied between 0.32 and 0.88 and 0.07 and 1.00, respectively. The genotypic structuring at overall loci revealed statistically significant deviation from HWE ($P < 0.001$). The highest expected heterozygosity was 0.88 at *3/9CA15* locus while the lowest expected heterozygosity was 0.32 at *Sma02* locus. Alleles numbers within samples at each microsatellite locus ranged from 3 at locus *B12-I GT14* and *3/9CA15* in TRB, DUZ, and MAR sample to 14 at locus *Sma02* and *3/9CA15* in VAR and DUZ samples (**Table 2**).

Average alleles number overall loci ranged from 6.0 at the TRB and MAR populations to 9.60 at the SVT population with an average allele number of 7.32. The Garza-Williamson index was found as the lowest at *3/9CA15* locus in the SVT population, and the highest at *B12-I GT14* and *3/9CA15* loci in the TRB and VAR populations, respectively (**Table 2**). The average GWI across loci was highest at the TRB population and lowest at the SVT population, indicating a possible bottleneck effect.

Genetic diversity values based on microsatellites within populations was the lowest in the TRB population (3.00735). However, the highest genetic diversity was observed in the SVT population (4.17306). Genetic diversity for mtDNA was zero in TRB and DUZ populations (0.0).

The mean pairwise differentiation (F_{ST}) within population was found to be the lowest in the Trabzon population and the highest in the Sevastopol population (**Table 3**). In pairwise comparison of populations, the F_{ST} values ranged from 0.08792 to 0.36059 with an average value 0.249246 (**Table 3**). The Varna and Sevastopol samples showed the lowest genetic distance (0.08792), whereas the Trabzon and Marmara samples showed the highest, 0.36059. The pairwise F_{ST} values showed that all samples were significantly distinct from each other ($P < 0.001$). The Mantel test showed non-significant ($P > 0.05$, $r = 0.43$) isolation-by-distance for the geographically separated turbot populations.

Principal component analysis of microsatellite data revealed five principal components (PCs) of which 31 and 24% of genetic variation were presented in the first and second PCs. Plotting the first two PCs (**Figure 2**) revealed that *B12-I GT14* showed highest contribution on population differentiation on the first PC, and *Sma02* and *Sma03* highly contributed on population differentiation on the second PC. The use of five PCs in DFA revealed overlapping distribution of 95% confidence ellipses of populations (**Figure 3**). The Russia and Marmara populations were overlapped on all the populations, but the Trabzon and Duzce populations clearly separated from each other.

On the other hand, the Neighbor-Joining tree demonstrated that population structuring is associated with geographic separations. The Bulgaria and Russia populations were clustered close to each other (**Figure 4**) while the Marmara Sea population showed the most distinguishing population.

mtDNA Sequencing

After alignment, the COIII region consisted of 565 bp fragments which were contained 2 bp parsimony informative sites. The

TABLE 2 | Descriptive statistics for five microsatellite loci between the turbot populations.

Samples		Locus					
		SMA1	**SMA02**	**SMA3**	**B12-I GT14**	**3/9CA15**	**Average across loci**
TRB	n	10	10	10	10	10	
	a	6	4	7	3	10	6.0
	Ho	0.12***	0.66**	0.58***	1.00***	0.09	0.49
	He	0.59***	0.32***	0.82***	0.39***	0.86***	0.60
	G.W. index	0.12	0.66	0.58	1.00	0.09	0.49
	Allelic size range	49	5	11	2	110	35.40
DUZ	n	10	10	10	10	10	
	a	8	5	4	3	14	6.80
	Ho	0.15***	0.41***	0.80***	0.50***	0.10***	0.39
	He	0.80***	0.54***	0.60***	0.33***	0.88***	0.63
	G.W. index	0.15	0.41	0.80	0.50	0.10	0.39
	Allelic size range	50	11	4	5	130	40.0
VAR	n	10	10	10	10	10	
	a	9	14	9	5	4	8.20
	Ho	0.18***	0.12***	0.60***	0.12***	1.00***	0.40
	He	0.76***	0.78***	0.75***	0.60***	0.74***	0.73
	G.W. index	0.18	0.12	0.60	0.12	1.00	0.40
	Allelic size range	49	114	14	39	3	43.80
SVT	n	10	10	10	10	10	
	a	11	11	11	9	6	9.60
	Ho	0.22***	0.11***	0.64***	0.09***	0.07***	0.23
	He	0.87***	0.84***	0.85***	0.80***	0.78***	0.83
	G.W. index	0.22	0.11	0.64	0.09	0.07	0.23
MAR	n	10	10	10	10	10	
	a	8	7	5	7	3	6.0
	Ho	0.16***	0.08***	0.41***	0.43***	0.42***	0.30
	He	0.84***	0.77***	0.76***	0.61***	0.46***	0.69
	G.W. index	0.16	0.08	0.41	0.43	0.42	0.30
	Allelic size range	47	86	11	15	6	33.0

***Significant probability values ($P < 0.001$) after Bonferroni correction. n, number of samples; a, number of alleles; Ho, observed heterozygosity and He, expected heterozygosity. TRB, Trabzon; DUZ, Duzce; MAR, Marmara Sea; VAR, Bulgaria-Varna coastal waters; SVT, Russia Sevastopol coastal waters.

TABLE 3 | Values of microsatellite pairwise F_{ST} (below diagonal) and average number of pairwise differences within population (diagonal elements).

Samples	TRB	DUZ	MAR	VAR	SVT
TRB	**3.00735**				
DUZ	0.33107***	**3.18367**			
MAR	0.36059***	0.30969***	**3.47592**		
VAR	0.28864***	0.25297***	0.24455***	**3.65551**	
SVT	0.22431***	0.19686***	0.19586***	0.08792***	**4.17306**

***$P < 0.0001$. TRB, Trabzon; DUZ, Duzce; MAR, Marmara Sea; VAR, Bulgaria-Varna coastal waters; SVT, Russia Sevastopol coastal waters.

mean composition of nucleotides for thymine (T), cytosine (C), adenine (A), and guanine (G) were as 28.7, 28.6, 23.0, and 20.5%, respectively. Jukes and Cantor model (Jukes and Cantor, 1969) was chosen as the best method for intra and interspecific variations on our dataset after running ModelTest (Posada and Crandall, 1998). DNA sequencing of the COIII exhibited four different haplotypes. The result of mean haplotype diversity between populations was 0.380 (**Table 4**). The average intra-population genetic diversity was found to be 0.000204. The detected genetic divergence between populations are given in **Table 5**. Average genetic divergence was found to be 0.00081. According to the NJ tree, Trabzon and Duzce populations clustered together as one group with sister clustering the Sevastopol and Varna, respectively. The Marmara population was different from all other populations (**Figure 5**). Tajima's D for the populations of *S. maximus* was found to be 0.045756 (**Table 6**) which indicate that the expected heterozygosity is lower than that observed.

DISCUSSION

In this study, genetic analysis of *S. maximus* populations in the Black and Marmara Seas was investigated by using

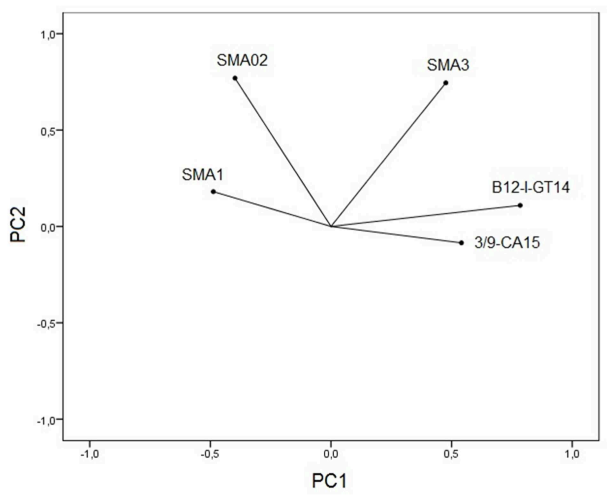

FIGURE 2 | Plotting first two principal component scores shows contribution of five microsatellite loci for population differentiation.

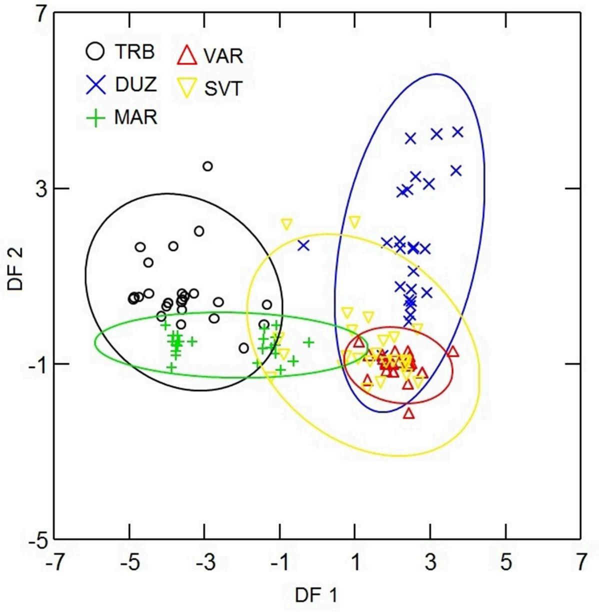

FIGURE 3 | Ninety-five percent confidence ellipses of DFA scores for microsatellite analysis of populations. The abreviations of the samples were given in **Figure 1**.

microsatellite and mtDNA sequencing analysis. On the bases of the two molecular markers, *S. maximus* can be divided into genetically separated populations. For microsatellite analysis, all the geographically separated populations revealed genetic differences from each other. For mtDNA sequencing analysis, only the Marmara Sea (MS) population constitute a genetic unit, while the others comprise genetically different second unit. There is a contradiction between the markers, but the genetic differentiation of the Marmara Sea population was supported by the microsatellite and mtDNA sequencing analyses.

Microsatellite and mtDNA markers indicated that there are restricted gene flows between populations which indicate ongoing processes of genetic differentiation. The Mantel test indicated that the genetic heterogeneity was not related to geographic proximity of the samples for both molecular markers.

Marine species are generally genetically more adaptable than anadromous and freshwater species (DeWoody and Avise, 2000), and therefore, have less genetic differentiation between populations (Ward, 2002). This is thought to mirror their higher effective population size and less restricted migration (DeWoody and Avise, 2000). In the present microsatellite analysis, a total of 108 alleles ranging from 3 to 14 for each locus were detected within the *S. maximus* populations which were similar to the previous studies (Pardo et al., 2005; Florin and Höglund, 2007; Navajas-Pérez et al., 2012). Karan (2015) examined the number of alleles per locus for *S. maeoticus* populations and found to be the lowest in the *3/9CA15* locus as four and the highest in *Sma1* locus as 11. Rodríguez-Ramilo et al. (2007) observed that allele number of *Sma1* locus was eight in *S. maximus* populations. Moreover, pairwise F_{ST} analysis demonstrated that the lowest genetic distance between Sevastopol (SVT) and Varna (VAR) populations for microsatellite (0.08792), whereas mtDNA pairwise genetic differences analysis indicated that the highest genetic distance (0.001522) between the Bulgarian and Marmara populations. Karan (2015) analyzed *S. maeoticus* populations with microsatellite marker and stated that the lowest genetic distance was between the Duzce and Marmara Sea populations (0.19549), whereas the highest was between the Trabzon and Marmara Sea populations (0.21755). Nielsen et al. (2004) reported that the largest pairwise F_{ST} value of *S. maximus* populations between any of the pooled samples was 0.032 (between North Sea and Northern Baltic Sea) even though there was no significant differentiation between the Northern Baltic and the Southern Baltic samples. Florin and Höglund (2007) reported the highest pairwise F_{ST} of *P. maxima* populations in the Baltic Sea as 0.0156 between the Åland and Gotland populations.

The detected expected and observed heterozygosity in each microsatellite locus of *S. maximus* populations are also reported by similar studies, having more sample size (from 22 to 48) than that in our study (Liu and Cordes, 2004; Pardo et al., 2005; Florin and Höglund, 2007; Rodríguez-Ramilo et al., 2007; Navajas-Pérez et al., 2012). Karan (2015) reported that expected and observed heterozygosity of *S. maeoticus* populations varied from 0.64 to 0.88 and from 0.08 to 0.72, respectively.

In the mtDNA sequencing analysis of COIII region, the detected haplotype diversity of *S. maximus* in our study showed four different haplotypes and was found low. Suzuki et al. (2004) found 28 haplotypes for the phylogeographic analysis of *Psetta maxima*. Atanassov et al. (2011) detected 36 haplotypes with average haplotypes diversity of 0.47 among *Psetta maxima* populations from Bulgarian and Romanian in the Black Sea. Karan (2015) identified 10 haplotypes with average haplotype diversity of 0.6345 between *S. maeoticus* populations from the Trabzon, Duzce and Marmara.

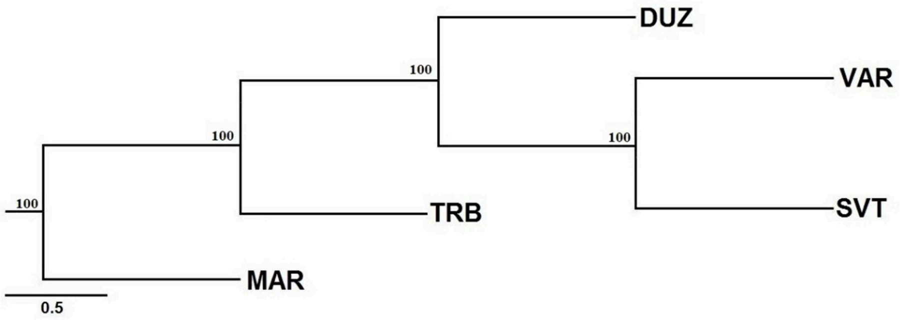

FIGURE 4 | Neighbor-joining tree based on microsatellite variation. Bootstrap values of 1,000 replications are given on nodes in percentages. The abreviations of the samples were given in **Figure 1**.

TABLE 4 | Distribution and frequency of COIII haplotypes of *S. maximus* populations.

Haplotype	TRB	DUZ	MAR	VAR	SVT	Total
Hap 1	10	–	4	7	8	29
Hap 2	–	10	6	–	–	16
Hap 3	–	–	–	2	–	2
Hap 4	–	–	–	1	2	3
Total	10	10	10	10	10	50

TRB, Trabzon; DUZ, Duzce; MAR, Marmara Sea; VAR, Bulgaria-Varna coastal waters; SVT, Russia Sevastopol coastal waters.

TABLE 5 | Pairwise genetic distance based on sequence analysis of the COIII region between populations of *S. maximus* (below diagonal), and genetic diversity within samples (transversal diagonal as given in bold).

Samples	TRB	DUZ	MAR	VAR	SVT
TRB	**0**				
DUZ	0	**0**			
MAR	0.001061***	0.001061***	**0.000943**		
VAR	0.000885	0.000885	0.001522*	**0.001456**	
SVT	0.000354	0.000354	0.001416**	0.001027	**0.000629**

*Statistically significance levels; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.*
TRB, Trabzon; DUZ, Duzce; MAR, Marmara Sea; VAR, Bulgaria-Varna coastal waters; SVT, Russia Sevastopol coastal waters.

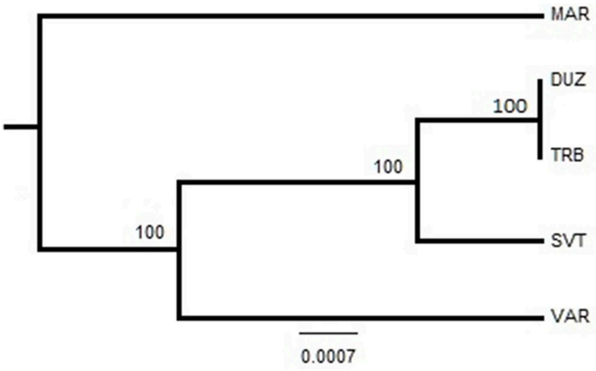

FIGURE 5 | Neighbor-Joining tree based on sequence analysis of the COIII region. Bootstrap values of 1,000 replications are given on nodes in percentages. The abreviations of the samples were given in **Figure 1**.

TABLE 6 | Neutrality tests and the estimated parameters of mismatch distribution for populations of *S. maximus* from Turkish waters.

m	S	ps	Θ	π	D
50	2	0.003534	0.000789	0.000809	0.045756

m, number of sequences; S, number of segregating sites; p_S, polymorphic site rate; Θ, population mutation rate; π, average pairwise distance; D, Tajima's D.

The lowest genetic distance (0.0) was detected between the Duzce and Trabzon populations, and the highest value (0.001522) was found between Bulgarian and Marmara populations based on mtDNA analyses. In the pairwise comparison of the genetic differences of the samples, only the Marmara sample differed significantly from all other samples with different levels of significance (**Table 5**) which is also supported by NJ tree. However, the pairwise F_{ST} and DFA of the microsatellite data indicated genetic separation of Trabzon and Duzce populations (**Figure 3**), and *B12-I GT14* locus plays an important role in this differentiation (**Figure 2**). Moreover, small sized 95% confidence ellipses of the Bulgarian population also indicate its' very low genetic diversity (**Figure 3**) that be important for management purposes. Tajima's D (Tajima, 1989) for the *S. maximus* populations was found to 0.045756, indicating that the observed heterozygosity is higher than that expected (**Table 6**). The detected rare alleles at low frequencies may indicate that the populations were undergone a balancing selection and sudden population contractions. Karan (2015) found Tajima's D for *S. maeoticus* populations as−2.114293 which was related to a recent bottleneck effect while the rare alleles were found at high occurrences. The positive and low D value in the present study may indicate low levels of both low and high frequency polymorphisms, indicating a decrease in population size and/or balancing selection for *S. maximus*.

The overfishing drives the decay of genetic diversity across a wide range of marine fishes (Pinsky and Palumbi, 2014). Reduced population size enhances genetic drift, which in turn causes a higher loss of genetic variability per generation. Increased genetic drift augments the loss of variability and the ability of adaptation (Hauser et al., 2002; Spielman et al., 2004, according to Madduppa et al., 2018). Reductions of genetic diversity in some of the world's most abundant species may lead to a long-term impact of fishing on their evolutionary potential, particularly if abundance remains low and diversity continues to decay (Pinsky and Palumbi, 2014). Therefore, the low genetic diversity observed for both markers may be explained with excessive fishing pressure on Trabzon population and high geographic separation of this population from the other populations which may cause limited interspecific gen flow. Moreover, this case has pointed out possibility of which these populations could have gone through a bottleneck in the recent past.

In the present study, microsatellite and mtDNA results were not congruent to indicate same pattern of differences between the populations which can be explained that mtDNA and microsatellite markers may show different temporal genetic patterns due to differences in their rate and pattern in mutation (Suzuki et al., 2004), and based on the allele frequency distributions, the differences of microsatellite and mtDNA results may be caused by the using of single marker of mtDNA vs. five markers of microsatellite (Larssoni et al., 2009). The results of the present study support restricted gene flow between populations. On the other hand, based on both microsatellite and mtDNA analysis, two major genetic breaks were observed for *S. maximus*; the TRB, DUZ, the VAR, and SVT populations constitute one genetic unit and the MAR population is the second genetically different unit. Different rates of gene flow, mutation or natural selection of mtDNA and microsatellites also could give rise to greater differentiation of microsatellites than mtDNA. Different parts of mtDNA evolve at different rates, thus different parts of mitochondrial DNA could be considered for future studies to find higher-level population differentiation (Avise, 1992).

The current status of the turbot populations in the Black Sea characterize the stocks as unsustainably exploited and at risk of collapse and "overexploited" and "in overexploitation" (GFCM, 2018). The conservation and sustainability of the turbot populations requires knowledge for the population genetic structure and constant monitoring of its biodiversity. Turbot stock identification and stock boundaries are still not well defined and for the time being the turbot population in the Black Sea is assessed as a single stock (GFCM, 2018). According to the GFCM (2018), an adequate scientific monitoring of the status of turbot and associated species in GSA 29 should be provided. Because, data collection is limited in scope and the national systems in the Black Sea region lack of harmonization, existing gaps (GFCM, 2018) including those covering turbot population-genetic monitoring should be appraised.

The results from the present study show consistency of the detected differentiation that may reflect its temporal and spatial integrity and thus would also require its consideration as separate populations for the fishery management purposes. Based on that, we propose to include the genetic population data analyses as a new indicator of biological monitoring.

Genetic approaches to detect stock structure will continue to be an indispensable part of fisheries management. Some developments in this field will increase the analytical power by boosting sample sizes and numbers of DNA markers. In the future, there is likely to be a shift in emphasis toward greater integration of genetics into multidisciplinary assessments of stock structure (Ovenden et al., 2015). The approaches described in the present paper can be of value to the effective integration of genetic information into the fisheries management decision-making process.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in GenBank with accession numbers MN062299 - MN062348.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study, because, the turbot (Scophthalmus maximus) is considered as Vulnerable (VU) under the current IUCN Red List criteria. The species in the Black Sea is under catch quota following the multiannual management plan for turbot fisheries in the Black Sea, approved in 2017 by the General Fisheries Commission for the Mediterranean (GFCM), (COM/2018/710 final). The study was based on landings sampling of species. The tissues needed for genetical analyses were obtained non lethally (e.g., fin clipping) and with minimal handling. No other authorization or ethics board approval was required to conduct the study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Impact on Macro-Benthic Communities of Hydraulic Dredging for Razor Clam *Ensis minor* in the Tyrrhenian Sea

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Bivalve molluscs fishery is of great importance along the Italian coasts, both in economic and landing terms, and different edible bivalve species are harvested both in Adriatic and Tyrrhenian Seas. A medium-term assessment of the impact of the hydraulic dredges targeting razor clam *Ensis minor* on macro-benthic community was made during two surveys carried out in winter 2017 and late spring – summer 2018 in central Tyrrhenian Sea, which represents the main fishing ground for this species. The study area was located between 1 and 4 m depth, within 0.3 nautical miles from the coast. A net sampler (40 cm width, 18 cm height, and 14 mm mesh size) was mounted on a commercial dredge (3 m width) and enabled to collect specimens of the smallest sizes for the entire community present in the areas. A control area was identified where fishing does not occur, in order to compare exploited and not exploited sea bottoms. The results show that benthic assemblages found in dredged areas are characterized by species living in high-energy habitat, due to the closeness to the shore, and thus showing a high resilience at medium-term disturbs. Differences in species richness were not clearly evident both for the entire community and for the mollusc assemblages evaluated over the two surveys, among the control and the impacted areas, with few exceptions mainly depending on local conditions and anthropic pressure. Thus, even if the benthic community is typical of a moderately disturbed environment, the effects of fishing on the community structure are still discernible over and above the natural variation.

Keywords: hydraulic clam dredging, fishing impacts, *Ensis minor*, benthic communities, species diversity

INTRODUCTION

Fishing disturbance on benthic communities is an important issue of fisheries management (Reiss et al., 2009). Effects appear to vary with fishing gear, habitat, and fishing intensity (Thrush and Dayton, 2002). It is therefore important that, as new fisheries and exploitation methods develop, impact studies are undertaken to aid future management decisions. There is now good evidence that commercial fishing has a profound effect on marine ecosystems (Kaiser, 1998; Tuck et al., 2000; Szostek et al., 2015). In the last three to four decades, the problem of the impact of fishing gears on the seafloor has spawned a number of studies that summarize and synthesize the environmental effects of fishing [see Thrush and Dayton (2002) for a review]. The information extrapolated enriched the debate over fisheries management and marine conservation. Disturbance regimes play a fundamental role in influencing biodiversity and generating patchiness (Thrush and Dayton, 2002; Hauton et al., 2003; Morello et al., 2005) and the resultant spatial heterogeneity created by local disturbance events (such as that produced by fishing gears) can increase the presence of opportunistic species in soft-sediment habitats. Although the soft-sediment seafloors are not always seen as highly structured as some marine reef habitats, they actually support extremely high species diversity (Snelgrove, 1999). Soft-sediment communities have a crucial role to many ecosystem processes as well as services that sustain fisheries provisioning food for human consumption. Beside the functional role, benthic organisms are key factors for the sediment stability, water column turbidity, nutrients and carbon cycles, and contaminant sequestering processes (see Thrush and Dayton, 2002). Many fishing gears (trawls and dredges) are dragged over the sea bottom and their physical impact depends on their mass, the degree of contact with the seafloor and the speed of trawling, influencing particle size, resuspensions regimes, and biogeochemical fluxes (Palanques et al., 2001; Lucchetti and Sala, 2012), as well as the microbial activity (Watling et al., 2001). Moreover, the sediment quality is intimately linked to the presence of fisheries target species and to the benthic community supporting it.

Generally, one approach to investigate the impact of fishing gears on benthic invertebrates is to focus on the immediate effects of the passage of the fishing gears over previously undisturbed benthic communities (Morello et al., 2005). An alternative approach comprise the comparison between benthic communities across gradients of fishing effort, focusing on changes over prolonged periods (Frid et al., 1999). Results of the latter approach indicate that persistent disturbance from fishing may alter benthic fauna to more resilient and adapted communities (Kaiser et al., 2000), even hypothesizing an equilibrium disturbed state in which an increase in disturbance has little additional impact (Reiss et al., 2009).

Clams harvesting represents an important fishing activity in the Mediterranean Sea and in the Black Sea, but these fisheries (i.e., dredge fisheries) create a very high share of discards (over 15%) in almost all the subregions where these activities take place. A large proportion of the discarded catch is mainly composed of undersized commercial individuals and non-commercial specimens of molluscs, decapods, and echinoderms (Urra et al., 2017). Furthermore, a significant proportion of target species caught or left on the dredge path have damaged shells (with chipped margins, holed umbos, broken or smashed valves), which causes indirect shellfish mortality and economic loss (Moschino et al., 2003). In the northern Adriatic Sea, where this kind of fisheries is a common practice, discards are estimated to be 50% of the total catch, 30% of which are undersized target species and 20% are other benthic invertebrates (Morello et al., 2005).

One of the most ecologically and economically important superfamilies of marine bivalves is Solenidea which has two families, Pharidae and Solenidae, referred to as razor clams (*Ensis* spp. and *Solen* spp.). In an ecological context, the contribution of razor clams to trophic food webs includes serving as prey to crabs, gastropods, sea birds, and demersal fish. The razor clam fishery is an economically influential industry. Along the European coasts in 2012 more than 33 million Euros have been estimated as the amount of landing value (considering both *Ensis* spp. and *Solen* spp.), corresponding to more than 3500 tons of landing (Eurostat, 2018)[1]. After 2012, both landings and gains suddenly decreased oscillating between 5 and 10 million Euros. This could depend on the European Regulation 1967/2006 that imposed a ban on fishing within 0.3 nautical miles (nm) along the European coasts. In most of the countries (Italy comprised) this closure prevented fishers from catching razor clams, that live mainly inside the 0.3 nm. In regions like those of the northern Adriatic Sea, where razor clams, due to the morphology of the bottom (shallow waters remain at higher distances), can live even more distant than 0.3 nm, thus explaining why an amount of gain after the EU regulation remained.

Along the Italian coasts, historically razor clams fishery grounds were mainly located in the northern Adriatic Sea (Veneto and Friuli Venezia Giulia) and along the central Tyrrhenian Sea (Lazio and Campania), although in several areas artisanal collection by hand of these animals has traditionally taken place at less than 1 m depth. In the last 7 years, the Adriatic razor clams community experienced an exceptional decrease of individuals leading to a collapse of the species *Ensis minor* with a strong crisis of the fishing sector that turned definitively to other bivalve species (*Chamelea gallina* and *Callista chione*). Although it is not clear what caused this clam crisis, most of the attention has been paid to the MOSE (Electromechanical Experimental MOdule) building, a series of mobile dams acting to avoid the flooding of the city of Venice and that radically changed the main current flows and sedimentation (Strozzi et al., 2009; Amos et al., 2010). The works started in 2003 and they are currently completed by 85%.

In the Tyrrhenian Sea the razor clam fishery did not experience the same crisis as in the Adriatic Sea, although the fishery had severe problems linked to the closure of the 0.3 nm from the coast, that, because of the morphology of the seafloor, corresponds to the main habitat for the razor clam *E. minor*. In 2017, the Italian Ministry of Agriculture, Food and Forestry asked for and obtained the extension to fish inside the 0.3 nm from the coast as a 3-year experiment to subsequently ask for an exception to the European Regulation. Since then, a series of annual surveys are being done to assess the status of the resource and of the associated fauna, and to confirm the presence of *E. minor* inside and not further the 0.3 nm.

In the present work the results about the impact of the razor clam dredgers in the Tyrrhenian Sea are reported with a particular emphasis on the bivalve molluscs assemblages, that are the most representative taxon of the entire benthic community (Petetta et al., 2019).

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[1] https://ec.europa.eu/eurostat

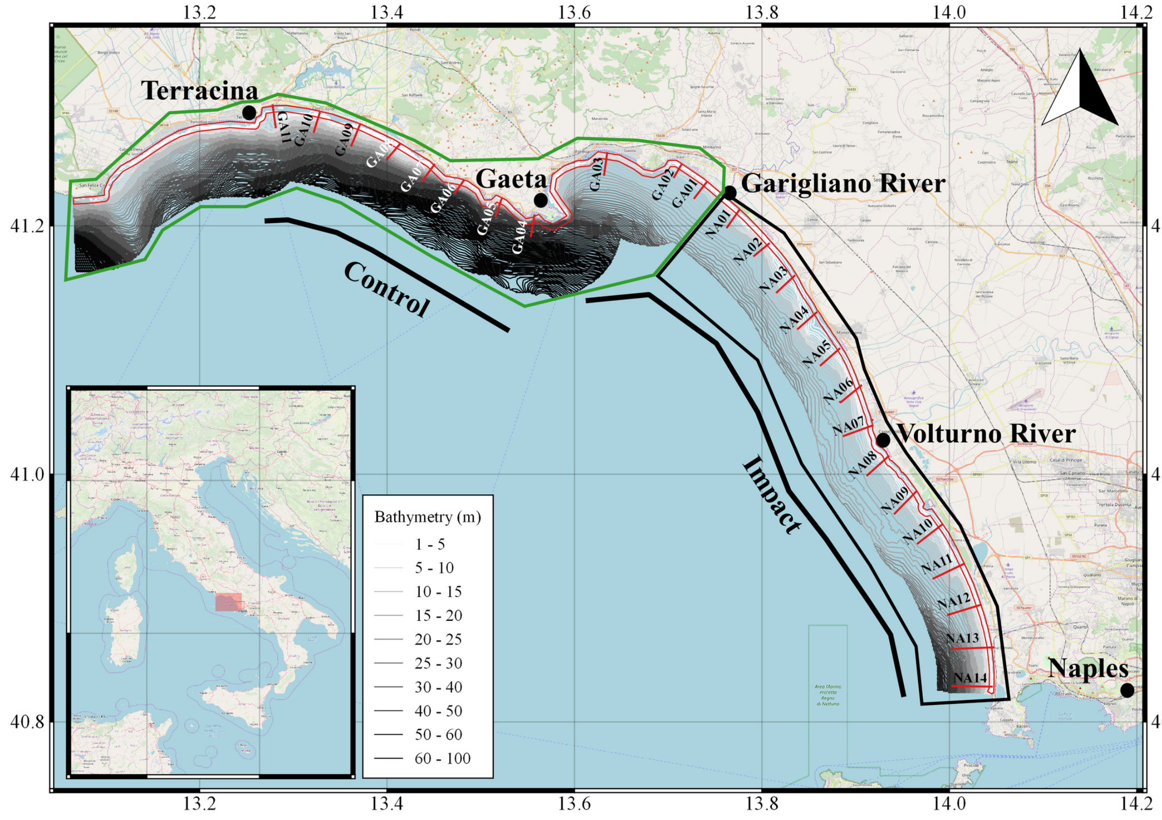

FIGURE 1 | Study area of the dredge.1pc impact surveys. Bars in red represents the transects placed in the two Maritime Compartments of Naples and Gaeta (limited by black and green boxes, respectively). The red polygon represents the 0.3 nm limit within which the surveys were done and that the European Regulation 1967/2006 closed. The bathymetries between the coastline and 4 nm are represented as well.

MATERIALS AND METHODS

Study Area

The study area is located in central Tyrrhenian Sea and includes two compartments, Naples and Gaeta, that are in continuity and are characterized by low and sandy coasts (**Figure 1**). The compartment of Naples extends to about 52 km, while the one of Gaeta extends to more than 150 km, but the area where fishing is permitted extends for about 8 km. The compartment of Naples is characterized by the presence of the Volturno river, the main river flowing in the Tyrrhenian Sea, while the main river in the compartment of Gaeta, is the Garigliano river, which acts as border between the two compartments. Both rivers transport high volumes of organic and inorganic pollutants. The razor clams are harvested within the 0.3 nm (and mainly as close as possible to the coast line) because depth at greater distances suddenly increases and the granulometry deviates toward muddy sediments preventing the animals to sink.

In the two above-mentioned compartments about 17 dredgers are compressively active (3 operating in the Gaeta compartment and 14 in the Naples compartment), mainly fishing the razor clams (*E. minor*), but opportunistically also the striped venus clam (*C. gallina*; in the compartment of Naples only). In both compartments, the Catch per Unit Effort (CPUE; calculated as kg per hour per boat) oscillated between 6 and 14 kg h^{-1} in 2017 and between 6 and 16 kg h^{-1} in 2018 (with a maximum peak of 24 kg h^{-1} recorded in June in Gaeta; data obtained directly from logbooks compiled by fishers on behalf of the Italian Ministry for Agriculture and Fisheries for a scientific survey to request a derogation to the European Regulation 1967/2006 avoiding fishing with dredge within 0.3 nm).

Sampling Procedures

To assess the impact of dredges on benthic communities, two surveys were carried out with commercial fishing vessels made available by the local consortia for bivalve molluscs' fishery management. In each survey, two different areas, named as impact (I) and control (C), were sampled. The I area was placed inside the main fishing grounds of the two compartments, while the C area was placed in the non-fishing area inside the compartment of Gaeta (**Figure 1**). In both C and I, transects were perpendicular to the coast, between 1.5 and 4 m depth and spaced 1.5–2 nm apart from each other. For each transect two to three random hauls were made (considered as replicates). Each haul, having a length of about 100 m, was carried out parallel to the coast to keep the fishing depth as constant as possible and spaced between 30 and 150 m one to each other. In case of impediments at sea (e.g., breakwater barriers or ports) some transects or replicates have undergone a shift, or even deleted, compared to what was at the beginning planned. The first survey was done in winter 2017 (between November and December), for a total of 62 hauls (48 in I and 14 in C), while the second one was done between May and June 2018, for a total of 57 hauls (40 in I and 17 in C). Between the two periods, fishing was closed during April and May 2018, following the Italian Regulation. Moreover, as fishers are obliged to monthly compile logbooks about the razor clam catches, from their data emerged that very few boats were active during the interval between the two surveys (mainly due to bad weather conditions). Thus, the time-lapse between the two surveys could be considered as a recovery time for the macro-benthos communities. The boats were equipped with dredges of 3 m width (respecting the limits of the European Regulation 1967/2006), with a metal rods distance adapt to collect the razor clam and no <7 mm on the inferior side of the cage. The dredge was towed with the recovery of the anchor cable about 100 m long.

Since the dredge is not a well-suited gear to sample macro-benthos, a net sampler with fine meshes (14 mm) and dimensions 40 cm (width) per 20 cm (height) (**Figure 2**) was attached inside the dredge in order to sample the smallest specimens of razor clam and other macro-organisms.

The total weight of the net sampler capture was recorded. Subsequently, the catch was sub-sampled and stored at −20°C to be sorted and analyzed in the laboratory. The macro-benthos sorting consisted in classifying organisms up to the lowest possible taxonomic level, counting the individuals and weighing each species or taxon present (precision 0.1 g). All figures are reported as individual densities in 100 m^2 of swept area.

FIGURE 2 | (a) Placement of the net sampler used during the two surveys to collect macro-benthic organisms; **(b)** a detail of the net sampler with its dimensions in cm; **(c)** a moment after the haul before to open the net sampler on board.

Data Analysis

Prior the analysis, the fauna matrices of densities were simplified by excluding all those species that did not reach the 5% of presence of the total replicates. All data were then forth-root transformed to balance the contribution of very abundant species and maintain intact information on relative abundances (Legendre and Legendre, 2012). The *E. minor* individuals were not considered for the analysis, as it represents the target species.

Biodiversity indices were calculated both for the entire fauna communities and for the molluscs assemblages. Shannon–Weiner's H' (based on $\log_2$) and Pielou's J' evenness values were calculated. One way analysis of variance (ANOVA) was used to assess differences between treatments (I vs. C) for the first period (the entire macrofauna community). Two-way ANOVA was used for the bivalve molluscs assemblages based on the factors treatment (I vs. C) and period (first survey and second survey). In case of significant differences, Tukey's honestly significant difference (HSD) test was adopted as a *post hoc* pairwise analysis to evaluate the pairs among the levels of the significant factors leading to the differences between treatments and years.

To assess the response of the entire communities to the dredges impact, a permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was performed using the macro-fauna on the whole in the first survey and treatment as fixed factor. PERMANOVA was subsequently performed also using the factors treatment and period to assess the response of bivalve molluscs assemblages before and after fishing closure. Treatment and period were both considered as fixed factors. The similarities percentages (SIMPER) procedure was used to identify those species that contributed most to the dissimilarity between I and C.

The unconstrained ordination principal coordinate analysis (PCO) based on Bray–Curtis similarity was used to visualize data regarding the entire fauna communities. Subsequently the constrained ordination of the specific *a priori* hypotheses of interest, concerning differences among treatments over the two periods, was analyzed by means of canonical analysis of principal (CAP) components (Anderson and Willis, 2003). The

TABLE 1 | One-way ANOVA results of the two diversity indices H' (Shannon–Weiner diversity index) and J' (Pielou's evenness) of the two communities, and PERMANOVA result based on the Bray–Curtis similarity matrix of the two communities present in the two treatments (Impact and Control).

ANOVA	**Factor**	**df**	**SS**	**MS**	***F***	***p***	
H'	Treatment	1	0.376	0.376	0.518	0.475	
	Residuals	60	43.583	0.726			
J'	Treatment	1	0.053	0.053	0.976	0.327	
	Residuals	60	3.235	0.054			
PERMANOVA	**Factor**	**df**	**SS**	**MS**	**Pseudo-*F***	***p***	**Perms**
Macro-benthos community	Treatment	1	18,631	18,631	10.563	0.0001	9938
	Residuals	60	105,830	1763.8			

hypotheses were tested by obtaining a *p*-value using permutation procedures (9999 permutations) on the canonical test statistics (squared correlations, δ_1^2) generated by the analysis. In both the ordinations, the relative contributions of each species to the differences found were assessed using the Pearson's correlation coefficient (cut-off 0.5).

All data analysis were performed with PRIMER v.6 with add on PERMANOVA+ (Anderson et al., 2008; Clarke et al., 2014) and the free statistical software R (R Core Team, 2018).

RESULTS

Macro-Benthos

Overall, 54 taxa were found (the complete lists are reported as **Supplementary Tables**), 51 of which classified to the species level: 32 molluscs, 12 crustaceans, 6 echinoderms, and 1 sipunculid. The other ones were not classified and left as generic taxa: cnidarian, nemertean, and polychaeta. As evident, molluscs dominated both in terms of species and in term of individual densities.

TABLE 2 | Simper table of the species that most differentiated the two treatment communities: Impact (I) and Control (C).

Average dissimilarity between I and C = 72.0

Species	Group I Av.Abund	Group C Av.Abund	Av.Diss	Diss/SD	Contrib %	Cum. %
Ec	0.8	3.2	6.4	1.6	8.9	8.9
Dt	3.1	1.7	5.7	1.3	7.9	16.7
Cg	2.0	0.7	4.2	1.5	5.9	22.6
Oo	0.9	2.1	4.2	1.3	5.8	28.4
Te	1.6	0.0	4.2	0.8	5.8	34.2
Dp	1.9	0.8	3.8	1.3	5.3	39.5
Sm	1.5	0.0	3.6	1.3	4.9	44.4
Ms	1.6	0.3	3.4	1.2	4.7	49.1
Lv	1.5	0.3	3.3	1.3	4.6	53.7
Polychaeta	2.1	2.1	3.0	1.1	4.1	57.8
Dl	0.8	0.7	2.2	1.0	3.1	60.9
Ds	1.0	0.2	2.2	0.5	3.0	63.9
Sn	0.6	0.7	2.1	0.9	2.9	66.8
Cc	0.9	0.0	2.0	0.9	2.8	69.6
Nj	0.8	0.4	2.0	0.9	2.8	72.3
Pla	0.5	0.4	1.8	0.8	2.4	74.8
Pl	0.8	0.0	1.7	0.7	2.3	77.1
At	0.3	0.5	1.5	0.7	2.1	79.1
Tt	0.6	0.0	1.3	0.4	1.9	81.0
Pe	0.5	0.3	1.3	0.7	1.8	82.8
Mc	0.5	0.0	1.2	0.5	1.7	84.5
Nm	0.3	0.3	1.1	0.6	1.5	86.0
Ta	0.5	0.0	1.0	0.5	1.4	87.4
Ca	0.2	0.3	1.0	0.5	1.4	88.8
Gg	0.0	0.4	1.0	0.5	1.3	90.1

Cumulative cut-off at 90%. Ec, Echinocardium cordatum; Dt, Donax trunculus; Cg, Chamelea gallina; Oo, Ophiura ophiura; Te, Tellina exigua; Dp, Diogenes pugilator; Sm, Solen marginatus; Ms, Mactra stultorum; Lv, Liocarcinus vernalis; Polychaeta, whole unclassified polychaete individuals; Dl, Dosinia lupinus; Ds, Donax semistriatus; Sn, Sipunculus nudus; Cc, Callianassa candida; Nj, Neverita josephinia; Pla, Portunus latipes; Pl, Pharus legumen; At, Acanthocardia tuberculata; Tt, Tellina tenuis; Pe, Platysquilla eusebia; Mc, Macoma cumana; Nm, Nassarius mutabilis; Ta, Tellina albicans; Ca, Carcinus aestuarii; Gg, Glycymeris glycymeris.

The diversity indices (see **Supplementary Tables** for a detail summary of the indices for each replicate) calculated basing on the entire community of the first survey did not show any differences between I and C (**Table 1**). On average, H' values were 1.8 ± 0.8 (mean ± standard deviation) and 2.0 ± 0.9 for C and I, respectively. Regarding J' values they were on average 0.6 ± 0.3 and 0.6 ± 0.2 for C and I, respectively.

Seventeen species did not reach the cut-off of the 5% of presence overall the samples. The multivariate analysis on the rest of the community showed a difference between the individual densities in I and C, as reported by the PERMANOVA (**Table 1**). SIMPER results showed an average dissimilarity between C and I of 72% and the species that most contributed to this dissimilarity are reported in **Table 2**.

The PCO clearly represented these differences with a good separation between I and C in the bi-dimensional space, with the first axis of PCO explaining 22% and the second axis 19.5% of the total variance (the first four axes alone explained >60% of the total variance; **Figure 3**). The species that were mainly correlated (Pearson's correlation > 0.5) with the first two axes and that mostly contributed to the plot configuration are: *Donax trunculus*, *Tellina exigua*, *Solen marginatus*, *C. gallina*, *Liocarcinus vernalis*, *Mactra stultorum*, and *Donax semistriatus* more associated to the I hauls, while *Ophiura ophiura* and *Echinocardium cordatum* were more related to the C hauls. The

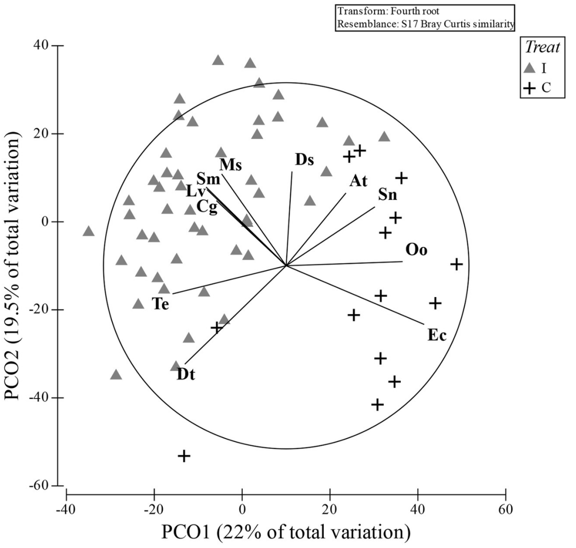

FIGURE 3 | PCO ordination plot obtained by the Bray–Curtis similarity matrix of the communities present in both treatment: impact (I) and control (C). The species that most correlated with two main axis are superimposed (Pearson's correlation index; cut-off 0.5). Dt, *Donax trunculus*; Te, *Tellina exigua*; Cg, *Chamelea gallina*; Sm, *Solen marginatus*; Lv, *Liocarcinus vernalis*; Ms, *Mactra stultorum*; Ds, *Donax semistriatus*; At, *Achantocardia tuberculata*; Sn, *Sipunculus nudus*; Oo, *Ophiura ophiura*; Ec, *Echinocardium cordatum*.

TABLE 3 | One-way ANOVA results of the two diversity indices *H′* (Shannon–Weiner diversity index) and *J′* (Pielou's evenness) of the molluscs assemblages, and PERMANOVA result for the molluscs assemblages based on the Bray–Curtis similarity matrix of the two communities present in the two treatments (Impact and Control) and in the two periods (2017 and 2018).

ANOVA	**Factor**	**df**	**SS**	**MS**	***F***	***p***	
H′	Period	1	6.203	6.203	12.033	0.001	
	Treatment	1	4.309	4.309	8.359	0.005	
	Period × Treatment	1	0.163	0.163	0.316	0.575	
	Residuals	113	58.765	0.516			
J′	Period	1	0.689	0.689	7.284	0.008	
	Treatment	1	0.020	0.020	0.215	0.644	
	Period × Treatment	1	0.015	0.015	0.155	0.695	
	Residuals	113	10.779	0.095			
PERMANOVA	**Factor**	**df**	**SS**	**MS**	**Pseudo-*F***	***p***	**Perms**
Bivalves molluscs assemblages	Period	1	7791	7791	3.876	0.004	9949
	Treatment	1	22,723	22,723	11.305	0.000	9943
	Period × Treatment	1	3561	3561	1.772	0.112	9955
	Residuals	113	229,140	2010			

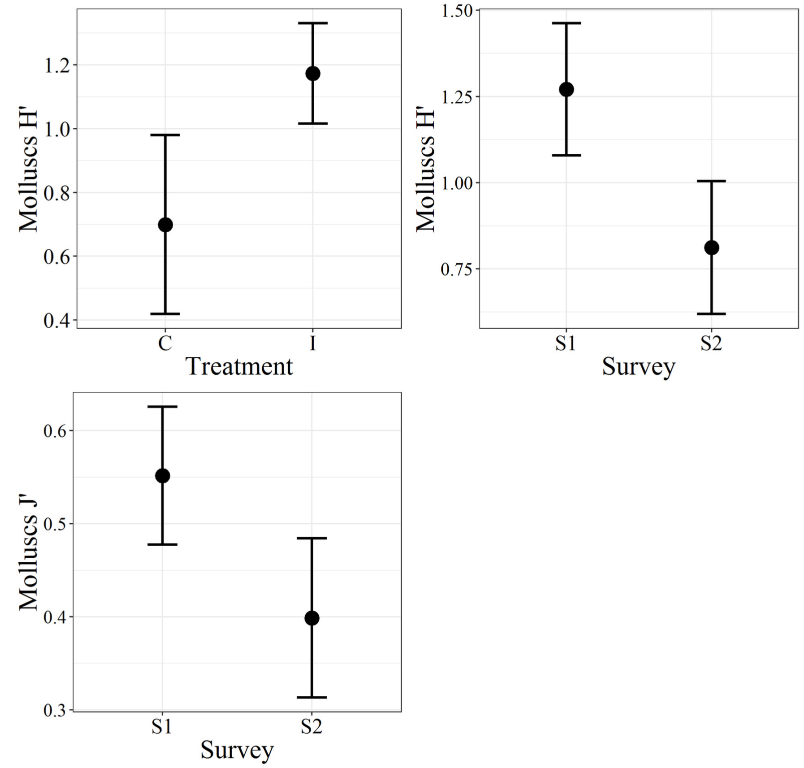

FIGURE 4 | The Plot of the molluscs biodiversity indices. *H′*, Shannon–Wiener diversity index; *J′*, Pielou's evenness; C, control; I, impact; S1, first survey; S2, second survey. The bars represent the 95% confidence intervals.

two species *Acanthocardia tubercolata* and *Sipunculus nudus* were almost borderline in discriminating between the two treatments.

Bivalves Molluscs

In the first survey, 26 species of bivalve molluscs were collected against 13 species found in the second survey. This decrease was significant, as reported by the ANOVA (**Table 3**) applied to the diversity indices (see **Supplementary Material** for a detailed summary of the indices for each replicate). The interaction term of the ANOVA was not significant meaning that, according to the results of the benthic communities, the molluscs assemblages did not show differences in the diversity indices between I and C in both surveys. Notwithstanding, differences were observed between the two surveys both for H' and J' and between treatments only for H' (**Figure 4**). Average values of 1.27 ± 0.75 and 0.81 ± 0.72 were observed for H' in 2017 and 2018, respectively, while for J' 0.55 ± 0.29 and 0.40 ± 0.32 in 2017 and 2018, respectively. Concerning the treatments, average values of 0.70 ± 0.75 and 1.17 ± 0.74 were observed for H' for C and I, respectively. Prior the multivariate analysis, 10 species were discarded because they did not reach the 5% cut-off overall the samples. The PERMANOVA did not highlight any differences in the interaction term, confirming the ANOVA results (**Table 3**). On the other hand, both the two surveys and treatments overall showed significant differences. The SIMPER (**Table 4**) results showed an average dissimilarity between survey 1 and survey 2 of 66.1%, while the dissimilarity between I and C was 70.9%.

The plot resulting by the CAP analysis (**Figure 5**) was coherent with the results obtained by the PERMANOVA, showing a clear separation between I and C in both surveys. The total correct allocation of the points in the plot resulted as 67.8%, thus with a misclassification error of 32.2%. The confusion matrix (**Table 5**) produced by the CAP analysis showed high allocation success percentages of sites inside treatments and surveys. The species that in the plot discriminated most the treatments were *S. marginatus*, *M. stultorum*, *C. gallina*, *T. exigua*, *Pharus legumen* (mainly associated to the horizontal axis and positively related to the I treatment), and *Dosinia lupinus* (mainly related to the vertical axis and I treatment).

TABLE 4 | Simper table of the molluscs species that most differentiated the two periods (2017 and 2018) and the two treatments: Impact (I) and Control (C).

Average dissimilarity between S1 and S2 = 66.1

Species	Group S1 Av.Abund	Group S2 Av.Abund	Av.Diss	Diss/SD	Contrib %	Cum. %
Dt	2.8	2.8	12.4	1.1	18.8	18.8
Cg	1.7	1.0	7.7	1.2	11.6	30.4
Ds	0.8	1.0	6.6	0.8	10.0	40.4
Ms	1.3	0.8	6.2	1.1	9.4	49.8
Sm	1.2	0.8	6.1	1.1	9.2	58.9
Te	1.3	0.0	5.7	0.7	8.6	67.5
Dl	0.8	0.3	4.3	0.8	6.5	74.1
Ta	0.4	0.4	2.9	0.6	4.4	78.5
At	0.4	0.3	2.9	0.5	4.3	82.8
Tp	0.2	0.5	2.8	0.6	4.3	87.1
Pl	0.6	0.1	2.8	0.6	4.3	91.4

Average dissimilarity between I and C = 70.9

Species	Group I Av.Abund	Group C Av.Abund	Av.Diss	Diss/SD	Contrib %	Cum. %
Dt	3.0	2.1	14.2	1.2	20.1	20.1
Cg	1.6	0.7	8.9	1.3	12.6	32.7
Sm	1.3	0.0	7.8	1.1	11.0	43.7
Ms	1.3	0.3	7.2	1.2	10.1	53.8
Ds	1.0	0.5	7.0	0.7	9.8	63.6
Te	0.9	0.0	4.7	0.5	6.6	70.2
Dl	0.6	0.5	4.6	0.8	6.5	76.6
At	0.4	0.2	3.0	0.5	4.3	80.9
Tp	0.4	0.2	3.0	0.6	4.2	85.1
Ta	0.5	0.0	2.9	0.5	4.0	89.1
Pl	0.5	0.0	2.5	0.5	3.6	92.7

Cumulative cut-off at 90%. At, Acanthocardia tuberculata; Cg, Chamelea gallina; Ds, Donax semistriatus; Dt, Donax trunculus; Dl, Dosinia lupinus; Ms, Mactra stultorum; Pl, Pharus legumen; Ta, Tellina albicans; Te, Tellina exigua; Tp, Tellina planata; Sm, Solen marginatus.

DISCUSSION

The impact of fishing activities on shallow benthic communities is hard to detect. Among the difficulties, it is important to stress that there are no well-defined reference data for which comparisons between fished and unfished habitats can be made. Highly dynamic environments, such as those where the razor clam lives in, mask potential community changes caused by fishing gears. Therefore, it can be difficult to separate the effect of fishing effort from natural variability in dynamic shelf-sea systems (Szostek et al., 2015). Moreover, most of the benthic species have multiple life cycle stages that can be influenced by extrinsic disturbance factors that could be considered of more importance (e.g., factors controlling recruitment and settlement; Morello et al., 2005). Thus, the effects of fishing will not depend only on fishing pressure over time, but also on the life history traits of the species within the communities, on interspecific interactions and on the interactions between them and the environment (Thrush and Dayton, 2002). The unpredictability of natural disturbance events of great importance in shallow waters (such as storms) further decrease the discernment between natural and fishing induced impact. Therefore, it is plausible that the assemblages in a fishing ground are already tolerant to physical disturbance (Morello et al., 2006; Szostek et al., 2015).

The results reported here clearly show a distinction of the macro-benthic communities between impact and control sites, although the diversity indices did not show any differences between them. The two treatments were characterized by different species, although molluscs represented the predominant taxon. In both treatments, the main species were opportunistic. At C sites the most abundant species were *E. cordatum* and *O. ophiura*, two echinoderms considered as mobile opportunistic scavengers, and this lets hypothesize that the razor clam shallow waters habitat is characterized by communities of well-adapted species to environmental disturbance. *Echinocardium cordatum* is reported as the most vulnerable species with respect to bottom fishing (Tuck et al., 2000). This fragile echinoderm buries sufficiently deeply into the sediment, between 2 and 20 cm (Tortonese, 1965), but razor clams dredges are able to penetrate even to 15 cm deep or more, thus the catchability of the species is high. Therefore, the fact that both *E. cordatum* and *O. ophiura* drastically decrease in number is probably due to their fragility, having both fragile exoskeletons. In I areas, the two echinoderms are clearly substituted by molluscs (*D. trunculus*, *C. gallina*, *T. exigua*, *S. marginatus*, *M. stultorum*) and crustaceans (*D. pugilator* and *L. vernalis*) that, due to their hard shells, better resist to fishing stresses and mechanical damages. The hermit crab *D. pugilator* is considered as highly correlated with "moderate fishing intensity" (Morello et al., 2006). The moderate fishing activity is consistent with the fishing effort of the study area, because the number of vessels operating is quite low with

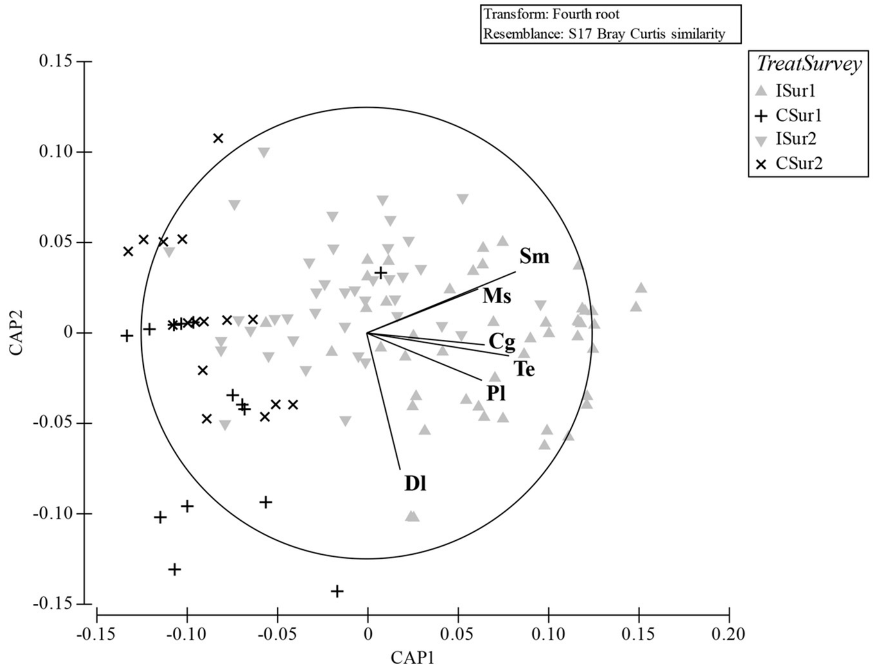

FIGURE 5 | CAP analysis ordination plot based on the Bray–Curtis similarity matrix of the molluscs assemblages sampled during the two surveys with superimposed the species that most differentiated the replicates (Pearson's correlation index; cut-off 0.5). I, impact; C, control; Sur1, first survey; Sur2, second survey. Dl, *Dosinia lupinus*; Sm, *Solen marginatus*; Ms, *Mactra stultorum*; Cg, *Chamelea gallina*; Pl, *Pharus legumen*; Te, *Tellina exigua*.

respect to the entire available fishing ground. Moreover, very often a few boats are active over the year and with an average of days at sea of 24 days/boat/year, thus further reducing the effective effort. *Diogenes pugilator* is not particularly vulnerable to hydraulic dredging because the protection of the gastropod shell prevents any injury. Species like the hermit crab may even benefit from the hydraulic dredging as observed by Hauton et al. (2003) which observed numerous individuals aggregating to feed on dredge discards. The sorting of razor clams of legal size is made on board by hand, thus hard shell molluscs are not damaged by the operation as it could be, e.g., for the striped venus clams that are sorted mechanically (Moschino et al., 2003). For example, Morello et al. (2006) reported a 60% of damage for *M. stultorum* individuals because of the sieving operations carried out to sort the catch, and crushed individuals did not survive after the return at sea.

The great presence of filter and suspension feeder bivalve molluscs in the I samples is potentially linked to the suspension of sediment produced by the regular and constant trawling of the dredges. In fact, when fishers localize a patch of *Ensis*, they cross the area a number of times (as also evident by the logbooks obtained by fishers; data not shown), which may results in profound effects (Tuck et al., 2000; Reiss et al., 2009). In fact, one of the main issue of dredges is the sediment resuspension and subsequent the macronutrient contained in it (Tuck et al., 2000; Kaiser et al., 2002; Lucchetti and Sala, 2012), that would favor the filter and suspension feeding strategy of well-adapted animals. It is then noteworthy even the presence of polychaetes in both I and C sites. Polychaetes are known to be a taxon typically occurring in disturbed sediments and their presence in both I and C confirms the fact that both treatments are effectively two disturbed environments with mix effects of natural and anthropic impacts regarding the I and only natural impact regarding C.

In the second survey the diversity (at least for molluscs) was not an issue between I and C. As a confirmation that a reduced suspension of sediment due to a substantial reduction of dredging activity, in the time-lapse between the two surveys the number of filter feeders decreased. The reduction in species richness in non-impacted areas respect to the impacted ones is not so unexpected. Vasapollo (2010) found a net difference in the polychaete assemblages in two different *Posidonia oceanica* seagrass meadows: the highest diversity was found in a heavily impacted meadow due to a migration of species from bared soft sediments.

TABLE 5 | Confusion matrix derived by the CAP analysis based on the Bray–Curtis similarity matrix of the molluscs assemblages, and indicating the percentage of correct placement of each replicate of the two surveys. I: Impact; C: Control.

Original group	I2017	C2017	I2018	C2018	Total	% Correct
I2017	38	1	8	1	48	79.2
C2017	0	8	1	5	14	57.1
I2018	4	4	24	8	40	60.0
C2018	0	5	1	10	16	62.5
Total	42	18	34	24	118	

Total correct: 80/118 (67.8%) Mis-classification error: 32.2%
$\delta_1^2 = 0.678$; $p = 0.0001$

In the light of the present observations, it is interesting to report the consequences that a diminished number of filter and suspension feeders may have on the target species *E. minor*. It has been observed that at the end of the period of fishing closure, right after May, the number of razor clams fished is extremely high. The reduction of filter feeders may have the effect to allow a high survival rate for the razor clam eggs and larvae, which potentially may grow up to commercial sizes. Therefore, as a paradox, this might happen not because of a biodiversity recovery to pristine conditions but because of a decrease of larvae and razor clam eggs potential predator. Thus, from a management point of view, a longer fishing closure could favor a bigger catch of clams in comparison with the actual 2 months of closure, and consequently this might translate in a better conservation of the species if catches were well managed. The new Italian Management Plan for hydraulic dredges proposed in the last months (DGPEMAC, 2019) that also includes the razor clam fishing management goes in this direction. In the new management plan, it has been proposed a fishing closure of 6 months during the razor clam gonads maturation and larvae development, and a minor daily quota per boat (namely, 100 kg per boat per day). In light of the results obtained, this period could represents a good choice. This is also supported by Thrush and Dayton (2002) and Morello et al. (2006) which highlighted that marine benthic communities in dredged areas suffer from the effects produced by fishing activities for at least 3 months.

CONCLUSION

In conclusion, it is noteworthy that the recovery of benthic communities is important in the view of an ecosystem-based fisheries management. However, it is difficult to implement in practice. The problem relates to the definition of "recovery," since so far, there are no standard recovery reference points for populations or communities (Gilkinson et al., 2005). Presently, our ability to address the functional consequences associated to fishing impact is limited given the complexity of ecosystems, and our limited knowledge of component species and their interactions. Consequently, the prediction of the effects of removing or damaging a large number of benthic animals and species is still almost difficult. Moreover, care must be taken when "standard" monitoring programs carry on with the objective of the mere analysis of the biodiversity based on the diversity indices. As evident, an impact could be masked by substitution of species more tolerant to impacts or conversely, species might be attracted by the impact itself increasing the biodiversity respect to "pristine" areas. Paradoxically, as a consequence, even a small decrease in biodiversity might favor the recovery of fishing target species such as the razor clams that can take advantages by the low densities of their eggs and larvae potential predators that have expanded their habitat ranges due to the impact causes. Finally, to really understand what happens to the benthic communities when an area is closed to fishery, experimental closure studies is recommended to confirm the observations that emerged by the present study.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

CV made the analysis and wrote the manuscript. CV, MV, RD, GB, and AP made the data collection. MV, RD, GB, AP, EP, and AL reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.00014/full#supplementary-material

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