A review of methods to assess connectivity and mobility of fish populations in the Mediterranean Sea

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A review of methods to assess connectivity and mobility of fish populations in the Mediterranean Sea

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Fish populations are linked to each other via dispersal of individuals as eggs, larvae, juveniles or adults. The understanding of this process, known as connectivity, has a pivotal role for the management of overexploited fish stocks and the development of accurate conservation strategies. The correct design of MPA networks is crucial for the achievement of the benefits of protection and can not neglect the knowledge necessary to maximize such benefits, among which, those on connectivity and fish movements are considered fundamental. Connectivity patterns are still largely unknown worldwide. A general lack of knowledge is particularly evident for the Mediterranean Sea where few works dealing with this topic were carried out and some methods, currently available for assessing connectivity, have not been used yet. In this review we present the methods and main results used until now in the Mediterranean Sea for studying connectivity patterns and fish movements at different life history stages. We encompass the pros and cons of each method, and conclude with future perspectives on the use of these methodologies in the Mediterranean context.

Keywords: connectivity; fish; larval dispersal; movements; methods; marine protected areas; Mediterranean Sea
Introduction

Marine populations are generally more open than terrestrial ones [1, 2] as determined by the complex life cycle of marine species, with the prevalence of external fertilization and the production of a huge amount of very small propagules with high dispersive capability. Hence, the concept of connectivity is critical to understand the functioning of marine populations and communities. Connectivity can be defined as the movement of individuals within and among local or subpopulations, provided that the level of exchange is sufficient to impact the demographic rates of the local population(s) [3]. Thus, connectivity in marine populations results from the dispersal of gametes, eggs and larvae, and, in the case of vagile species (fish and some crustaceans and molluscs), from the movement (daily, seasonal and ontogenetic) of juveniles and adults [4-7]. Another facet of connectivity is the exchange of material (nutrients, sediments, organic matter, etc.) among neighbouring areas, modulated by the multiscale spatial arrangement of (and the degree of connexion between) habitat patches [8].

Formerly, it was generally assumed that marine populations present high genetic fluxes, favoured by the inexistence of physical barriers, so that connectivity would be mainly determined by the dispersive capacity of each species. Nevertheless, more recently it has been showed that a number of species present a spatial differentiation which is higher than expected if we only considered their dispersive abilities [9-13]. Oceanographic patterns, the existence of habitat discontinuities, and bottom topography, are some examples of factors likely to influence population connectivity in the marine realm [9, 11, 14-16].

The scales and patterns of connectivity of marine populations determine the management strategies to be implemented in order to conserve marine resources and protect biodiversity, especially in the case of marine protected areas (hereafter MPAs)
[5-7, 17], where spillover of either larvae, juveniles or/adults is crucial to maintain populations outside the protected areas [3, 18]. A number of recent reviews have highlighted the importance and utility of knowing connectivity patterns [4-7, 19, 20], as well as the methods available to estimate connectivity [3, 21-26].

In spite of the above considerations, the degree of knowledge on the connectivity patterns of specific species and particular geographical contexts is still scarce [26]. This is especially true in the case of the Mediterranean Sea, despite being an ideal region for such kind of studies [27]. Moreover, both its importance as a biodiversity hotspot [28] and the cumulative threats by human it suffers [29-31] make this field to be an urgent endeavour in the Mediterranean [32].

Our objective here is to make a comprehensive review on the studies performed to date to estimate population connectivity in the Mediterranean context, with emphasis on the methods used, their pros and cons, and the perspectives open by the combination of different methodological approaches. We will focus particularly on shorefish populations, as they are the main subjects of overfishing and due to their crucial ecological role in coastal ecosystems [30, 33]. This review is directed both to Mediterranean marine scientists and managers, in order to provide a good perspective of what has been done, as well as to identify gaps and future directions of research on this crucial matter.

**Distribution of larvae**

Many researchers have addressed the possible influence of environmental variables on fish during their larval dispersive pelagic phase. Most studies on fish larvae in the Mediterranean were performed at mesoscale (tens to hundreds of kilometres) over the continental shelf and slope, with the aim to characterise the structure of the ichthyoplanktonic assemblage –i.e. their spatial and temporal (daily, seasonal or
interannual) variability in species composition and relative abundance) and ascertain which environmental factors (e.g. vertical and temporal variation in seawater temperature and salinity, primary production, zooplankton abundance, depth and topographic features) and multi-scale hydrographical features (currents, fronts, eddies, gyres, upwellings, run-off water intrusions) shaping different water masses are determining the observed spatial and temporal patterns [34]. Other factors likely to influence ichthyoplankton distribution patterns are biological factors such as mode and time of spawning [35], length of larval phase [36] and behaviour of fish larvae [37]. Typically, these studies are done by sampling in a grid of stations situated along transects, using bongo nets (usually of 40-60 cm in diameter and about 300 µm of mesh size) with calibrated flowmeters mounted in the mouth of the nets, obliquely towed from above the sea bottom to the surface at low speed; hydrographic data are taken using CTD (conductivity, temperature, depth water sampler) and/or using Niskin bottles, often in combination with available information on regional circulation patterns.

The horizontal, vertical and temporal variability in distribution of fish larvae, however, provide little insight into the problem of ascertaining connectivity patterns of fish population, further from the observation that particular hydrographic structures could cause the local retention of fish eggs and larvae, thus influencing the spatial and temporal patterns of larval dispersal [38]. Even so, the usefulness of such mesoscale ichthyoplankton studies to determine connectivity models is limited, due to their weak predictive value.

Fine-scale ichthyoplankton surveys around MPAs are likely to provide useful information on the effectiveness of marine reserves to act as source of propagules from fish populations inhabiting protected habitats (but see [39]). In a pioneering study, Vidal-Peñas et al. [40] surveyed the ichthyoplankton assemblage in a grid of 21
sampling stations located in and around the Cabo de Palos – Islas Hormigas marine reserve (Murcia, SW Mediterranean Sea), and distinguished between a coastal and an oceanic larval assemblage; moreover, they suggested the presence of a haline front in the zone, likely to be responsible for limited dispersal (coast-ocean) of larval fishes. For their part, Bordehore et al. [41] studied the ichthyoplankton along a year at two sampling stations within the Tabarca marine reserve (Alicante, SE Spain) by means of fixed ichthyoplankton nets, and found some evidence that this MPA was not concentrating, but rather exporting fish larvae to outside the marine reserve. Sabatés et al. [42] studied the spatio-temporal distribution patterns of fish larvae in a nearshore temperate area off the north-west Mediterranean, located in the vicinity of the Medes islands marine reserve (Girona, Catalunya, NE Spain); for doing so, they sampled monthly in 5 stations in a yearly cycle using a plankton net towed horizontally at a depth of between 2 and 3 m. Their main findings were that larvae of some species could be issued from the MPA, although the effect of protection is not the only factor responsible for the spatial patterns observed; interestingly, the larvae of some species, e.g. *Tripterygion tripteronotus* and Gobiesocidae were able to maintain distributions that remain in the vicinity of the adults, confirming that pelagic larvae are not necessarily at the mercy of passive dispersal (thus suggesting that self-recruitment may occur). More recently, López-Sanz et al. [43] surveyed a grid of 32 stations, placed at intervals of 1.5 km, on seven transects perpendicular to the coast in the same location (Medes is. marine reserve) using bongo nets. In this study, the influence of the type of eggs (pelagic vs. demersal), as well as of the habitat and spawning locations of adults, were evidenced. In addition, the high larval species richness found in the reserve zone, together with a high abundance of shorefish species, would be related to the high fish
abundance and species richness in the MPA, due to the population recovery of certain vulnerable species within the marine reserve.

A further insight into the relationship between MPA location and spatio-temporal distribution of fish eggs and larvae was done within the EU research project BIOMEX (QLRT-2001-00891, 2003-2005). Ichthyoplankton surveys were done by combining obliquely-towed bongo nets and fixed nets in a grid of sampling stations around the Cabrera Archipelago national park [44], Medes islands marine reserve [45], Cerbère-Banyuls natural marine reserve [46-48], Côte Bleue marine park [48, 49] and Cabo de Palos – Islas Hormigas marine fisheries reserve [46-49]. In general, most of these studies did not show a spatial distribution of fish larvae in relation with the location of the MPA; exceptions are the observation of an offshore gradient for eggs and larvae of particular commercial fish species (e.g. *Epinephelus marginatus*, *E. costae*, *Pagellus erythrinus*, *Scorpaena porcus*, *Sciaena umbra*) in Cabrera and Cabo de Palos, thus highlighting the potential role of these MPAs as source of propagules of some species to neighbouring, unprotected sites; however the scale of this effect appears more limited than was previously expected for this type of MPA.

Another way to approach the study of the larval phase of coastal fish is to evaluate the supply to coastal areas by post-larvae – late-stage or competent larvae, which is the transitional form from the pelagic to benthic realm [50] – by means of light traps [51, 52]. This approach is new in the Mediterranean context, although already in the late ‘90s, Deuderó [53] used an experimental purse seine coupled to a light device to capture young fishes (mostly juveniles of *Mullus surmuletus*). In a seminal study performed installing light-traps in the vicinity of the Cabo de Palos – Islas Hormigas marine reserve [54, 55], it has been shown that seasonality exerts a strong influence on larval supply, with greater abundances occurring on warmer months, this process being
also very much influenced by local winds and currents; nevertheless, although it is possible to anticipate the timing of the arrival of post-larvae to specific locations based on environmental information, this approach does not permit to estimate the scale of larval dispersal and population connectivity if it is not combined with other methodological approaches.

**Dispersal models**

The existence of a pelagic larval phase in the life cycle of many coastal fish allowed the development of different models of propagules (i.e. eggs and larvae) dispersion based on models of water circulation. These “physical” models are based on the assumption that fish larvae are passively transported by sea currents and consider the duration of the larval phase as one of the main factors in determining the spatial distribution of fish species [25].

Physical models have been recognized to be useful and powerful tools and different works have been made throughout the world with different purposes: to hindcast/forecast the spatial and temporal variability of spawning events and its effect on connectivity among population [56]; to assess the potential impact of the global warming on larval dispersal [57]; or to help in the design of MPA networks and in their further management [58].

Although models have been used worldwide [3, 25], studies in the Mediterranean Sea are still scarce. However, despite the low number of published papers, the different works performed have considered from small spatial scales to the whole Mediterranean Sea. For example, Catalán et al. [59] developed a small scale model for the North Alborán Sea using the Ichthyop Lagrangian model tool for evaluating the vertical dispersion of the European anchovy (*Engraulis encrasicolus*), in order to assess the larval retention areas and the associated periods of retention. They
found that the area close to the Málaga Bay showed a significantly higher probability of retention than adjacent zones in the North Alborán Sea and that this retention was higher from April to September, coinciding with the spawning period of the anchovies.

The need to improve the efficiency of the protection strategies and the increasing propensity to establish networks of MPA has led to the development of dispersion models to predict the best combination of size, position and spacing of the MPAs inside the network, in order to maximize the benefits of protection. Basterretxea et al. [60], for example, assessed the larval dispersion of coastal fish in the southern coast of Mallorca, using a three dimensional density-resolving model based on the Princeton Ocean Model (POM) and a particle tracking algorithm. Their objective was to determine the factors that contribute to a successful recruitment and the level of exchange of individuals inside the network of 4 MPAs along the coast of Mallorca. They showed that at the spatial scale considered, the coastal morphology and stochastic wind forcing can favour local recruitment events. For their part, Di Franco et al. [61] investigated the potential for propagules production and retention/export from a Mediterranean MPA in the SW Adriatic Sea (Italy). They used Lagrangian simulations of dispersal based on an oceanographic model of the region and data on early life-history traits of the white sea bream (*Diplodus sargus sargus*) finding a potential larval dispersal of 100-200 km.

All the previous models considered larvae as passive particles and did not take into account the biological traits of the species under study. In the last decade, some authors demonstrated that larval behaviour seems to influence the dispersal patterns of coastal fish more than previously thought [62] and that settlement could be highly affected by the stochasticity of biological processes [63]. This has recently led to the development of more accurate “bio-physical” models that couple together oceanographic and biological data and allow the assessment of the influence of various
parameters on the dispersal pattern of coastal species. Such models are considered to be highly informative and are used to understand the combined effects of the general marine circulation and larval behaviour on dispersal processes [6]. An example for the Mediterranean Sea is available in Nicolle et al. [64] that used the Europeans anchovy *E. encrasicolus* as model species for understanding the main factors affecting the transport/retention and distribution processes of this species. In a first step they considered a passive transport of the larvae, using the Lagragian tool Ichthyop and the MARS hydrodynamic model. Then they implemented dial vertical migration for larvae > 7 days.

Whereas modelling represent an interesting tool for understanding the importance of various factors on larval dispersal and connectivity among populations, they can not offer a totally realistic picture of the whole natural processes. The accuracy of a model is strictly dependent on the knowledge and precision of the input parameters both for the hydrodynamic models and the biological ones. In the first case, the spatial resolution of the circulation data can strongly influence the output of the model, since in most cases the fine-scale marine circulation is unknown. Moreover, the larval stage of several marine species is still overlooked, and some aspects of fish early life history like behaviour or their natural mortality are hard to be assessed correctly. Coupling models with field and laboratory studies is necessary to better parametrize the model and to test the predictions and the assumptions [58, 65]. Moreover, the combination with other methods could be useful for corroborating the results of the models [66–71].

**Genetics**

Genetic approaches have become widely used in ecological studies due to the development of new technologies which makes equipment and laboratory tools more affordable and user-friendly. Moreover, the non-lethal sampling protocol required for
such studies makes genetic procedures the ideal tool to deal with species under some level of threat, to identify populations at risk, resolve taxonomic uncertainties, define management unities, or understand population dynamics, among others [72].

Allozymes have been the primary method to quantify genetic diversity worldwide as well as in Mediterranean Sea. Allozymes are the direct product of a particular allele [73] and assumed to be neutral to selection [74]. The primary advantages to this method are its simplicity, quickness and its unlimited applicability, as any kind of organism produces some type of enzyme [73]. Nevertheless, a high number of enzyme loci must be employed (minimum of 20) to get a representative sample of population variability [73]. Another technical difficulty is that enzyme visualization is only acquired when it is still active, therefore tissues from which enzymes were extracted must be kept frozen (- 80 °C). In the Mediterranean, allozymes have been used to reveal the existence of significant genetic structure among Sarda sarda [75] and D. sargus [76] populations and also to detect temporal variability among D. sargus cohorts as a result of variation in reproductive success [77, 78]; while some studies, failed to reveal any genetic effect from fishing prohibition on D. sargus populations other than demographic ones [79], other found a significantly higher allelic richness in D. sargus individuals coming from MPAs compared to unprotected sites [15], thus showing the likely importance of MPAs to preserve genetic diversity. Allozymes were also used to confirm the importance of coastal lagoons in shaping genetic structure among populations of Dicentrachus labrax [80].

With the development of restriction enzymes and universal primers, and the advent of PCR methodology for amplification of genetic material [81], the use of mitochondrial DNA (mtDNA) became increasingly popular among researchers working in phylogenetic and population genetics. Mitochondrial DNA is more conservative, not
recombinant, and with only maternal inheritance, which convert it in an ideal candidate for describing evolutionary relationships among species [82]. However, it is less sensitive to estimate diversity in contemporary population, as well as to reveal the impact of short term or episodic events and to describe population connectivity at finer spatial scales [82]. The great majority of mtDNA works done in the Mediterranean context aimed at resolving large scale connectivity patterns, such as the distinction of Atlantic-Mediterranean management stocks. Although this segregation was confirmed for *Dipturus oxyrinchus* [83], populations of other species such as *Scomber japonicus* [84] and *D. sargus* [85] were considered panmitic. However many other species displayed a West-East segregation inside Mediterranean basin as was the case of *S. scombrus* [84], *Pomatochisthus marmoratus* [86] and *Pomatomus saltatrix* [87], in which the Siculo-Tunisia Strait (STS) was identified as the main geographic barrier determining this genetic distinctness, probably due to historical hydrographical regimes (see also [88-90], for examples with other species).

To resolve spatial scale restrictions, sequence variants in nuclear DNA (nDNA) were developed to enhanced genetic resolution to fine scales [91]. Microsatellites markers are the most widely used owing to their high polymorphism, neutrality to selection, and relative low cost (if primers libraries are already available) [82, 91]. Such techniques, also referred to as DNA fingerprinting, allow estimation of relatedness of individuals within populations and by this mean are the preferred tool for estimating dispersal in the marine environment. Due to their high resolution power, several connectivity studies have used microsatellites to survey populations all around the Mediterranean Sea, with distinct results among species. Different connectivity patterns were found for closely related species such as *Mullus barbatus* and *M. surmuletus* [54, 92], while significant heterogeneity was found in *M. barbatus* [93]. For its part, a weak
genetic structure was found in samples issued from the endangered species *Epinephelus marginatus* along the Mediterranean basin, differing only from the Atlantic ones [94]. In the case of the migratory bluefin tuna, *Thunnus thynnus*, a strong and chaotic subpopulation segregation was noticed and correlated to distinct demographic histories and human impact, although no evidence of genetic erosion was found [95].

Dispersal distances have been evaluated for a number of species through connectivity studies focusing on adult populations by measuring the rate of gene exchange among distinct groups [96, 97]. The indirect estimation of gene flow relies on the evaluation of the genetic differences among populations (usually using $F_{st}$ parameter) under the assumption of equilibrium. Such method presents limitations due to the fact that they are based on theoretical models of population structure that sometimes in practice could not be applied on true populations, especially those with intermediate to high levels of gene flow ($F_{st} < 0.05$), as it is the majority of marine species (see [98] for a recent review). On the other hand, novel techniques which directly estimate connectivity by the assignment of young individuals (juveniles or larvae) to their putative original populations and parentage analysis are based on fewer assumptions and present higher accuracy. However they demand a great effort on sampling potential sources (parents or populations), and they are effective only in the cases where genetic structure is high (number of migrants < 5 individuals) [98].

The combination of different markers to assess population genetic diversity has numerous examples in Mediterranean Sea, with markers showing concordant (e.g., *M surmuletus* and *M. barbatus* [99, 100], *D. sargus* [85, 101]) or discordant results (e.g. *E. marginatus* [102, 103], *D. sargus* [104]). Additionally, several studies have adopted the inclusion of Bayesian approaches and assignment tests into their analysis, improving by this mean the resolution and power of the analyses power [78, 93, 105, 106]. Although
parentage analysis has been emerging worldwide, mainly in rocky reef environments [107-110], such approach has not been used yet in natural fish populations in the Mediterranean context except for Solea senegalensis and Sparus aurata in aquaculture studies [111, 112].

From the above, it appears clearly that much work has to be done in order to disclose the underlying mechanisms of dispersal. Genetic tools could be per se very informative, and could be even improved when associated to other procedures (e.g. seascape genetic and spatial ecology, see [113, 114]).

Otoliths

Otoliths are mineralised concretions that grow throughout the entire lifespan of fishes [115]; the fact that they are immune to modification once formed makes them unique among fish structures, because they do not undergo the effects of replacement or cellular resorption that can occur in other bony parts [116]. The analysis of an otolith section allows researchers to determine growth and ageing [116], and to estimate the duration of early life stages (both larvae and juveniles) and the settlement date by back-calculation [117]. These potentialities make otoliths one of the most important tools for studying fish biology and ecology.

Although many works using otoliths have been conducted until now on fish populations connectivity worldwide ([118] for a review, [119]), few studies are available for the Mediterranean Sea. As a consequence, some of the available techniques for assessing connectivity using otoliths have never been used in the Mediterranean context.

Otolith microchemistry

Due to the nature of otolith formation, any chemical element, once embedded within the
otolith matrix, remains there permanently, without undergoing any modification [115].

This important property allowed the development of a series of direct marking
techniques based on the inoculation of a chemical tag (fingerprint) in the otolith that can
be recognized after fish catch. Artificial tagging techniques of otoliths include the
incorporation of fluorescent compounds (e.g. tetracycline,[120]), rare elements and
isotopes [121]. This allows the direct estimation of movements made by individuals,
even from their birth [122] which would be impossible using other types of markings
(e.g. T-bars, coded wire bars, visible fluorescent elastomers, etc., see below). On the
other hand, these techniques require the marking of a large number of individuals as the
target life stages are generally subject to high mortality rates (e.g. larvae, [123]).

However, although these techniques have a great potential, proved by their growing use
around the world, they have never been used in the Mediterranean Sea.

Besides these artificial tagging techniques, a second category of methodologies
for assessing connectivity by using otolith chemistry is based on the identification of
natural tags. These techniques require direct observation of otolith micro-structure
during the chemical analysis and the use of instruments, as laser ablators coupled with
ICP-MS (inductively coupled plasma mass spectrometry), able to analyse specific and
extremely small portions of the otolith, and take advantage of a second important
assumption: the elemental composition of otoliths is influenced by the physical and
chemical features of the ambient water (mainly temperature and salinity, [116]).
Moreover, the concentration of trace elements in the otolith is related to the
concentration of the same elements in the environment, especially for those elements
whose incorporation rates are not physiologically controlled (Sr, Ba, Mn, Pb, Li, Mg,
Cu, Ni, [115]). Therefore, the concentration of such elements and isotopes such as
carbon, oxygen, nitrogen or sulphur can be used as a natural tag to investigate fish
movements and connectivity among areas or during different life stages, as isotopic ratios in otoliths, like in other fish tissues (see below), reflect those of the ambient water or the local food webs of the habitat where the fish lives [118, 122]. Coupling together the natural fingerprint with the microstructure of the otoliths it is possible to evaluate the time at which a given chemical fingerprint was deposited in the otolith, and determine whether fish caught in different areas have lived in the same habitat in a certain period of their life [118]. Although few, there are very recent papers that have used the analysis of otolith natural fingerprint in Mediterranean Sea for evaluating connectivity (Table 1). Correia et al [124] used oxygen isotopic ratios (18O/16O) and carbon isotopic ratios (13C/12C) in the otoliths of European conger eel (Conger conger) to discriminate fishes from Mediterranean Sea and Atlantic Ocean. Djerking et al [125] assessed the migration of juveniles of common sole (Solea solea) from lagoons to the open sea by comparing isotopic fingerprints of carbon and oxygen. For the same species the potential to assess connectivity was demonstrated at different life stages (larvae, juveniles and adults, [126]). However, although otoliths are preferable for measuring isotopic ratios, other tissues can be used for the same task [125], in fact, a number of works, performed in the Mediterranean Sea, have analysed dermal and muscle tissues of sharks [127, 128] and muscle tissues of the yellowfin tuna (Thunnus albacares) and the swordfish (Xiphias gladius) [129].

Trace-elements signatures are particularly efficient for detecting links between habitats with marked differences in environmental features as temperature, salinity and concentration of chemical compounds (for example between fresh/estuarine water and sea water). The analysis of micro-element concentrations along transects from the inner part of the otolith (core) to its edge allows the chronological reconstruction of annual and seasonal migrations made by adult fish between lagoons and coastal waters [130],
Using this technique Mercier et al. [130] assessed connectivity between adult and juvenile habitats for the gilthead sea bream (S. aurata). For their part, Di Franco et al. [132] recorded connectivity between protected and unprotected areas during post-settlement phase in D. sargus sargus by analysing the portion of the otolith formed immediately after the settlement. They proved that otolith geochemistry allows to discriminate among slightly dissimilar chemical fingerprints and to infer on the level of connectivity at relatively fine spatial scale, between groups of fish associated with coastal habitat distant few kilometres from each other.

**Shape analysis**

Otolith (mainly sagittae) morphological characteristics are highly variable, and they are influenced by the environmental features (type of habitat, depth, water temperature) and genetic [133–135]. Otolith shape seems to reflect the local environmental conditions affecting a group of conspecifics at the same location [136–138], and differs among groups of fish that live in distinct geographical areas.

Few studies have used otolith morphometrics and shape to identify fish stocks in the Mediterranean Sea, and none of them has directly inferred connectivity. Some of these studies aimed to discriminate Mediterranean fish stocks from Atlantic ones for different species, e.g. Scomberesox saurus [139], Trachurus trachurus [140], Merluccius merluccius [141]. The results of these studies showed that the otoliths of fish from the Mediterranean Sea were larger in size than the Atlantic ones. This size difference was ascribed to the physicochemical differences between Atlantic and Mediterranean Sea, the latter considered to be a more homogeneous and stable environment [142].

Otolith shape appears to be an efficient tool to distinguish populations at local scales (<200km) in the Mediterranean Sea as demonstrated for E. encrasicolus [143].
and *S. solea* [144]. Morat et al. [145] identified several local populations of *M. barbatus* in the Gulf of Lions (France). Turan [146] found a clear differentiation between *Trachurus mediterraneus* populations of the middle Black Sea and those of the Aegean Sea.

However, although, some works showed that environmental factors seem to influence otolith shape more than genetic factors [134, 147], it remains still difficult to establish which between these two determinants contribute the most to otolith shape [136, 147], this feature representing a constraint for the method.

**Biological tags**

Biological tags include all the different natural characteristics of an individual that allow its inclusion in a defined group that shares the same traits. Two types of biological tags have been used in the Mediterranean Sea up to now: parasites and morphometric characters.

The use of parasites for stock identification started in the first mid of the 20th century and they have been employed worldwide until now for a multitude of species [148]. This method takes advantage of the fact that some species of parasites live in defined geographical areas and can be used as a natural tag. This methodology requires a complete knowledge of the natural distribution of the parasites and their life cycle in order to extrapolate the movements of the infected fish. For this and other reasons not all fish parasites can be used [149, 150]. Despite its global use, the few works that have explored this technique in the Mediterranean Sea focused mainly on pelagic fish species such as the horse mackerel (*Trachurus trachurus*) [151] and the wild albacore (*Thunnus alalunga*) [152].

Meristic and morphometric variability have been applied for stock identification and connectivity studies due to its favourable cost-effectiveness ratio [153]. It consists
in the measurement of diverse morphometric characters on fins, head, and other parts of
the body of the fish. In the last decade its applicability has been improved notably
because of the development of better image processing techniques (e.g. Truss network
measurements) [153]. In the Mediterranean Sea, this technique have been used for the
study of populations of horse mackerels (*T. trachurus*) [154, 155], anchovies (*E.
encrasicolus*) [143], sardines (*Sardina pilchardus*) [156] bluefish (*P. saltatrix*) [157]
and the sparids *D. sargus, D. puntazzo* and *Lithognathus mormyrus* [158].

**Tagging and tracking fish**

**Natural marks**

Natural marks have been used worldwide as an innocuous method for identifying single
individuals inside a population and tracking their movements [159-161]. Broadly
employed in terrestrial animals its use has been scarce in marine research until the last
decades, when the development of new technologies, like digital photography, have
permitted an important increase in the number of scientific works using natural marks as
identification method.

Natural marks include any type of spot, patch or scar which is demonstrated to
remain over time without varying [162]. With the development and improvement of
underwater photography systems this methodology was applied to the study of several
species of elasmobranches and the manta rays [162].

In the Mediterranean, this procedure has not been applied to the study of bony
fishes, except in one case [163], in which a photo-identification technique of individuals
of *E. marginatus* by cephalic blotches was tested. This methodology, although it has
been demonstrated as feasible due to its low interaction effect and its cheap application,
does not seem to be useful for the study of other bony fish species because of the difficulty of founding permanent and identifiable marks [160].

**External tags**

Mark and recapture experiments are commonly used to determine aspects of biology (including trans-oceanic species), migration patterns and parameters of fish stocks of marine fish, hence adding substantial information to understand the overall patterns of spatial utilization [164, 165].

Analysis and interpretation of data generated by these studies may have strong influence on the development of fisheries [166]. The validity of their conclusions relies on the following assumptions [167]: (1) marks do not affect normal biological functions of the fish, i.e. movement behaviour, growth, reproduction, mortality and predation, and (2) marks remain in the animals throughout the study period, or the rate of loss can be described by a mathematical function with known parameters.

Nevertheless, the knowledge that marks produce slower growth rates [168] and can affect swimming performance [169] is calling such assumptions into question.

The main techniques used in mark and recapture studies are Passive Integrated Transponder (PIT) [170], visibly deployed fluorescent elastomers (VIFE) [171] and wire tags [172], dart barbed and T-bars [173]. In Mediterranean waters T-bar technique was applied principally to evaluate migration and connexion between stocks of bluefin tuna (*T. thynnus*) [174] and albacore (*T. alalunga*) within and between Mediterranean Sea and Atlantic Ocean [175]. Pilot studies using external tags in groupers are being done in SW Mediterranean Sea, with the aim to determine ontogenetic movements between juvenile and adult dusky grouper population (*E. marginatus*) (Reñones O., personal communication) and to evaluate small scale movements between rocky shoals and connectivity levels between populations inside and outside marine reserves [176].

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**Electronic tags**

An alternative methodology to external tags is the use of electronic devices in mark-recapture studies. Archival tags work in the same way of external tags but they have the advantage of storing continuously oceanographic data, thus allowing the visualization of trajectories on an intermediate spatial scale during a long time period [177, 178]. The inconvenient of this technique is that tags must be recovered to access data logged, which implies the capture of the marked fish. In the Mediterranean Sea this technique was widely applied, mainly on fishery stock studies, vertical migration patterns and population connectivity of *T. thynnus* and *T. alalunga* [179].

To avoid the problem of catching marked fishes, recent studies have employed pop-up archival tags. Basically they function in the same way as an archival tag with the advantage that they are programmed to be liberated after certain time, and then the tag goes to surface and sends by satellite connection all the information stored [180]. This methodology has helped researchers to better understand the population dynamics of pelagic species in the Mediterranean Sea such as bluefin tuna [179, 181, 182], swordfish (*X. gladius*) [183] and devil manta ray (*Mobula mobular*) [184].

Acoustic telemetry techniques are used to study fish behaviour and movements [185, 186]. They are efficient tools to obtain information about fish home range [187], site fidelity [188] and homing behaviour [189].

Acoustic monitoring is performed by using acoustic receivers (passive or mobile) which record the presence of fishes previously tagged with an acoustic transmitter. Passive monitoring uses moored receivers that record the presence of the tagged fish within a limited range of detection around the receiver. These automated systems can monitor several individual movements simultaneously [186, 190]. Mobile receivers (hydrophones) allow to follow tagged fish continuously but in a short period.
of time (<24h) [189, 191-193]. However, the detection range of these two types of receivers is usually up to hundreds of meters although this range can vary consistently with environmental conditions (habitat, water turbidity) [194]. The acoustic transmitter is a miniature tag (generally ranging 12-98 mm in length and 0.65-34 g in weight) and allows tagging many species at different life stages. This new technology not only increases the species range size which can be tagged but also the potential number of species that can be studied [186].

Studies of movement patterns of adult fish using acoustic tags in the Mediterranean Sea are relatively scarce (Table 2). Five studies have been conducted in the Palma Bay MPA (Mallorca, Balearic Islands) on five different species: Serranus cabrilla [195], Serranus scriba [196], Diplodus vulgaris [197], Diplodus annularis [186] and Xyrichtys novacula [198]. Their results showed that fishes present high site fidelity and spend 95% of their time within an area smaller than 1 km². D'Anna et al. [193] found that D. sargus seems to have a very small home range (0.01-0.17 km²). A similar home range was found for E. marginatus [176, 188], E. costae [176], Sparisoma cretense [190] and Sarpa salpa [191].

These results show that acoustic monitoring can be a useful tool for assessing movement patterns of quite sedentary species. However, this method generally has high costs and the number of species that can be tagged is limited. Moreover, the variability in age, size and sex and the low number of individuals that can be tagged entail some difficulties to bring out a general movement model for a particular species.

Combination of methods

From the above review, it emerges the idea that an optimum strategy consists on investigating connectivity at different spatial and temporal scales and with more than one methodology. Each of these methods has its pros and cons, and it is very hard to
determine which of them is the most accurate. In order to increase the level of accuracy of the analyses, there is worldwide an increasing use of combined approaches for the study of connectivity that allow an improvement of the spatial and temporal resolution of the studies [25].

The main reason to choose integrated methodologies for assessing connectivity is the possibility to validate the estimates of a method by the results from another [25]. In some cases, the use of a multidisciplinary approach is necessary since some methods are only able to forecast/hindcast connectivity patterns. In this regard, predictions from physical/biophysical models can be tested with one or more approaches as genetic or otoliths analyses.

Regarding the Mediterranean Sea, a first study using a combination of approaches was conducted by Schunter et al. [27], who demonstrated that genetic structure shown in S. cabrilla samples matched perfectly with the dispersal simulation models which evidenced the effect of two oceanographic barriers to species dispersal. More recently, Di Franco et al. [61] found that the potential dispersal of D. sargus propagules up to 100 km from the MPA is supported by both Langragian simulations model and genetic homogeneity at the same spatial scale. These results highlight the potential ability of integrated methods to increase the level of certainty on connectivity patterns.

Less common, not only in the Mediterranean Sea, is the work done by Correia et al [199] who assessed connectivity among populations of European conger eel (Conger conger) through the combination of genetic variations in mtDNA and element:Ca ratios in cores and edges of juvenile fish otoliths. Although their study is not limited to the Mediterranean, the results support their hypothesis of a broad-scale dispersal of larvae, with limited connectivity for benthic juvenile fish at large spatial scales, although the
existence of one or multiple spawning areas for the species remains uncertain. The
difference in the “type” of connectivity estimated by genetic methods and otolith
methods (see [25]) and a different spatial and temporal scale at which they should be
properly applied [5], make the results of these two methods hardly comparable in most
of the cases. Selkoe et al. [113] suggested that only in certain circumstances this kind of
integrated approach may have good power for estimation: migration must be high
enough so that migrants are often included in the small samples typical of otolith
microchemistry but low enough that genetic structuring exists to detect migrants by
genotype assignment.

Another example of corroborating results is the study of Boustany et al. [200]
which integrated the use of mtDNAs sampling with electronic tags (archival tags) to
assess bluefin tuna connectivity among three reproductive populations, namely Mexican
Gulf, Eastern and Western Mediterranean Sea. The study reveals that, although
exchange of adult individuals occur among the three populations sampled, it is not
sufficient to homogenize allele frequencies, exhibiting a strong genetic structure.

However, the relatively low number of works on connectivity among fish
populations using multidisciplinary approaches in the Mediterranean Sea reflects the
overall scarce production of papers dealing with connectivity in the same geographical
context. This situation could be due, to some extent, to the technical difficulties posed
by these methodologies. For example, the abovementioned genetic parentage analyses
and the TRAIL (transgenerational isotopic labelling of otoliths), which has been never
used in the Mediterranean context, have demonstrated to be very accurate in
determining connectivity patterns in other ecological circumstances, as showed by
Planes et al. [109] and Almany et al. [201]. These authors studied the connectivity
pattern of a species, the orange clownfish (Amphiprion percula), displaying a very
particular behaviour: they spend their entire life in symbiosis with the same anemone, except for the few days of larval phase, a kind of behaviour which is hard to find in Mediterranean fish species, but that makes “easy” the use of parentage analyses or TRAIL, because it allows to know the precise point in which the eggs will be spawned; they found a 100% agreement between the results of these two methods. Moreover, the use of multiple methods of investigation determines a considerable increase of the cost and the time needed to perform the entire work, especially in the case of integrated otolith and genetic analyses whose individual cost is very high [113].

Conclusions and perspectives

Here we review all the available methods for studying connectivity patterns in Mediterranean fish populations, related both to the larval stage (considered the highest dispersive phase in the fish life cycle) and for assessing fish movements during post-settlement and adult stages. This choice is due to the fact that recent studies have questioned the real influence of the pelagic phase on the dispersal capabilities of fishes and supposed that behavioural factors could lead to self-recruitment or dispersal distances shorter than those predicted from the PLD [107, 120, 202]. In this perspective, adult movements could have a crucial role in determining connectivity patterns among populations.

From the review, it results clear that there is not a flawless method for assessing connectivity patterns and fish movements in the Mediterranean Sea. Each method has its advantages and disadvantages. Patterns of distribution of larvae provide little information on connectivity, as they considered just a small portion of fish life cycle, but can be used to validate the outcomes of dispersal models. Physical and biophysical models are extremely useful to hindcast and forecast dispersal patterns at different temporal and spatial scales (depending on the resolution of the oceanographical data),
but often, a poor understanding of the variables used to model, especially in the case of biophysical models (scarce knowledge of spawning areas, mortality rates or behaviour) is available. Genetic tools can assess connectivity at different temporal scale, although no patterns can be predicted. They seem to be powerful at meso/large spatial scale and are theoretically non lethal even though the equipments and the number of individuals required for the analyses, generally make this method expensive. Otoliths have a greater accuracy at fine-scale and can be used to hindcast connectivity patterns and movements during different life history stages, but the processes that influence the micro-chemical composition as well as the structure of the otoliths are still not clear. Within regard to individual movements, acoustic methods (coupled with other artificial tags) allow to track fish movements during different life stages and periods of the year (e.g. reproductive period), discriminating for sex and among age cohorts, although few individuals can be tracked at the same time and the cost of the instrumentations is generally high. Multidisciplinary approaches are probably the best way to conduct studies on connectivity patterns and fish movements as each method compensates the limits and constrains of the others and can corroborate their results.

In recent years a big effort was made for the establishment of new MPAs throughout the Mediterranean Sea [203], but, as in other parts of the world, many protected areas have not showed the expected results [204]. The correct design of MPAs or networks is considered one of the most important factors in determining the achievement of the benefits due to protection [205] and it can not be separated from the knowledge of the information necessary to maximize such benefits. Among this knowledge, connectivity among populations and individual movements of fish has a pivotal role [18, 206, 207]. For the Mediterranean Sea few works are available on these important topics and almost all were conducted in the NW sector (Fig. 1), with the
greatest part of the Mediterranean Sea not covered until now. For this reason, an
improvement of the use of the available methodologies is desirable in the future years as
well as the development of new techniques that can be applied more easily to the
Mediterranean context.
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Table 1. Studies that have analysed otolith chemistry for assessing connectivity and fish movements in the Mediterranean Sea.

<table>
<thead>
<tr>
<th>Method</th>
<th>Species (Family)</th>
<th>Location</th>
<th>Spatial scale</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable isotopes</td>
<td>Conger conger (Congridae)</td>
<td>Mallorca (Spain) and other 3 locations in NE Atlantic Ocean</td>
<td>2500 km (max distance between locations)</td>
<td>Strong differences in isotopic ratios among locations. Low level of connectivity suggested.</td>
<td>Correia et al. 2011</td>
</tr>
<tr>
<td>Stable isotopes and elemental signatures (also stable isotopes in muscles)</td>
<td>Solea solea (Soleidae)</td>
<td>Gulf of Lions (France)</td>
<td>~32 km² (lagoon area)</td>
<td>Lack of migrations between the sea and a coastal lagoon during summer.</td>
<td>Dierking et al. 2012</td>
</tr>
<tr>
<td>Stable isotopes and elemental signatures</td>
<td>Solea solea (Soleidae)</td>
<td>Gulf of Lions (France)</td>
<td>100 km (maximum distance between locations)</td>
<td>Ontogenetic shift between lagoons and sea.</td>
<td>Morat et al. 2012</td>
</tr>
<tr>
<td>Elemental signatures</td>
<td>Sparus aurata (Sparidae)</td>
<td>Gulf of Lions (France)</td>
<td>32-75 km² (area of lagoons investigated)</td>
<td>Migrations between the lagoon and the sea until at least age 4 yr.</td>
<td>Mercier et al. 2012</td>
</tr>
<tr>
<td>Elemental signatures</td>
<td>Diplodus sargus (Sparidae)</td>
<td>Apulian Adriatic coast (Italy)</td>
<td>200 km (maximum distance between locations)</td>
<td>Larval dispersal up to 100-200 km. Post-settlement dispersal occurred at ~30 km.</td>
<td>Di Franco et al. 2012</td>
</tr>
</tbody>
</table>

Table 2. Studies that have used acoustic methods for assessing fish movements in the Mediterranean Sea.

<table>
<thead>
<tr>
<th>Acoustic technique used</th>
<th>Additional technique used</th>
<th>Species (Family)</th>
<th>Location</th>
<th>Maximum area covered</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passive monitoring</td>
<td>External tagging (T-bar)</td>
<td>Serranus cabrilla (Serranidae)</td>
<td>NW Mediterranean (Mallorca Island, Spain)</td>
<td>0.77 km²</td>
<td>Fish spent 95% of time in an area of 0.77 ± 0.17 km².</td>
<td>Alós et al. 2011</td>
</tr>
<tr>
<td>Passive acoustic</td>
<td>No</td>
<td>Serranus scriba (Serranidae)</td>
<td>NW Mediterranean (Mallorca Island, Spain)</td>
<td>1.2 km²</td>
<td>Home range of 1.1 ± 0.2 km².</td>
<td>March et al. 2010</td>
</tr>
<tr>
<td>Passive monitoring</td>
<td>No</td>
<td>Xyrichtys novacula (Labridae)</td>
<td>NW Mediterranean (Mallorca Island, Spain)</td>
<td>0.32 km²</td>
<td>High site fidelity. Fish spent 95% of time in home range of 0.32 ± 0.13 km². Most of activity occurred during the day.</td>
<td>Alós et al. 2012</td>
</tr>
<tr>
<td>Passive</td>
<td>No</td>
<td>Diplodus</td>
<td>NW</td>
<td>1 km²</td>
<td>Night and day activities</td>
<td>Alós et al.</td>
</tr>
</tbody>
</table>
### Table 1: Connectivity and fish movements in the Mediterranean Sea

<table>
<thead>
<tr>
<th>Method</th>
<th>Species</th>
<th>Location</th>
<th>Home Range</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passive acoustic</td>
<td>Diplodus annularis</td>
<td>NW Mediterranean (Mallorca Island, Spain)</td>
<td>0.17 km²</td>
<td>2012</td>
</tr>
<tr>
<td>Passive monitoring and mobile tracking</td>
<td>Diplodus sargus</td>
<td>NW Mediterranean (Italy)</td>
<td>0.043 km²</td>
<td>2011</td>
</tr>
<tr>
<td>Mobile tracking</td>
<td>Sparisoma cretense</td>
<td>NW Mediterranean (coast of Lampedusa, Italy)</td>
<td>0.26 km²</td>
<td>2012</td>
</tr>
<tr>
<td>Passive acoustic</td>
<td>Epinephelus marginatus</td>
<td>NW Mediterranean (Spain)</td>
<td>0.019 km²</td>
<td>2009</td>
</tr>
<tr>
<td>Mobile tracking</td>
<td>Tagging (128 fishes)</td>
<td>Epinephelus marginatus</td>
<td>19.34 km²</td>
<td>2012</td>
</tr>
<tr>
<td>Mobile tracking</td>
<td>Tagging (28 fishes)</td>
<td>Epinephelus costae</td>
<td>19.34 km²</td>
<td>2012</td>
</tr>
</tbody>
</table>

**Figure 1.** Areas of the Mediterranean Sea (dark grey) where the most of the studies on connectivity and fish movements were carried out until now.

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